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Impact assessment of zero-tillage on soil microbial properties in rice-wheat cropping system

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ABSTRACT

Soil health which is deteriorating at the fast pace due to excessive use of fertilizers can be restored by the adoption of resource conservation technologies (RCT) which can affect the soil properties. The soil health can be measured by the ability of the microorganisms which can serve as early warning signals. In a farmers' participatory field located in Karnal, Haryana, India, soil was sampled spatially and temporally at different stages of the wheat crop. The fields measuring one acre were under conventional tillage (CT), zero tillage (ZT) for the last five years under rice wheat cropping system. As the crop season of wheat progressed, standing stubble from the previous rice crop decomposed, resulting in higher microbial biomass carbon (MB-C) in zero tillage treatment than the conventional tillage. MB-C ranged from 70–269 µg C/g soil as compared to 62–200 µg C/g of soil. At the most vegetative phase of cropping season (i.e. 60 and 90 days after sowing) the MB-C showed a 103 and 46.6% increase over the conventional tillage. In general MB-C and MB-N account for 0.8–7% of total C and total N in the surface layers of arable soils, we observed similar values for the microbial quotient- C (1.16–5.38%) and for the microbial quotient- N (1.72–2.77%) in zero tillage fields. Zero tillage had a greater effect on microbial quotient values under the stubble retained systems and it reflected the seasonal changes and crop growth in the same way as microbial biomass.

Key words: Microbial quotient, Microbial Biomass Carbon, Microbial Biomass Nitrogen, Phospholipid fatty acid **(**PLFA), Zero tillage

In India, the demand for the most staple food, i.e. rice and wheat has been estimated to be 225 mt in the year 2020 in the scenario of declining agricultural productivity in developing countries and climate change (Gornal 2010). Moreover, concerns are also being expressed about the environmental degradation due to rice-wheat cropping system, leading to unsustainability (Bhatt *et al.* 2016). Early warning signals of environmental degradation are becoming visible in the form of declining soil fertility, degradation of irrigation water quality, shift in water table, rising salinity and resistance of many pests to pesticides (Singh *et al.* 2010). Thus, for sustainable agriculture, agriculture scientists are looking towards the resource conservation strategies, such as no tillage, bed planting, etc. These technologies are known for reduction in soil erosion, maintenance/increase of soil organic matter content (SOM) and conservation of soil water (Jat *et al.* 2009).

Till now, emphasis has been laid only on the physical and chemical constituents, whereas the biological components which are highly sensitive to disturbance and perturbation and performs multiple functions in soil sustenance has been ignored. Since the assessment of soil quality in terms of measurement of rate of change in SOM is very difficult and time consuming process, therefore, soil microbial biomass, which has been shown to quickly respond to changes in soil management, is being used. Although, the measurement of microbial biomass (SMB) can provide the comparison of microbial C and N content between soils under different management practices, however, the measurement itself doesn't reflect the degree of microbial activity. Moreover, microbial biomass levels are generally influenced by climatic variables therefore the proportion of total organic C or N within the microbial biomass, i.e. microbial quotient has been suggested as useful indicators of soil processes as the microbial quotient is related to the changes in the quantities of soil C (Schloter 2018). Microbial properties related to total organic carbon content have been considered as the biological indicators of soil quality (Nogueira *et al.* 2006). The objective of this study was to evaluate the influence of zero tillage practice on microbial dynamics and to explore the feasibility of utilizing microbial quotient for capturing the land use/management options.

MATERIAL AND METHODS

The experimental site was located at Taraoi village in Karnal, Haryana, India at 29.7ºN and 76.97ºE. This area receives 700 mm annual rainfall and the mean annual maximum and minimum temperature is 35°C and 18°C

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respectively. The soil is sandy loam in texture. Conservation agricultural practices like zero tillage and zero tillage along with residue application were practiced in wheat crop. Although a rice-wheat cropping sequence had been followed in this field for last 10 years, the conservation practices such as zero tillage (shredded stalks of the previous crop amounting to 0.75 tonne of residue/tonne of wheat grain yield and disking with the no till machine with 20 cm wide spacing), along with conventional tillage practice (measuring one acre each) started way back in 2000. The soil texture of the experimental field was sandy loam in nature (68.2% sand, 18% silt and 13.8% clay) with a bulk density of 1.4 g/cm^3; *p*H 7.1; electrical conductivity 0.22 dS/m; organic carbon 0.3–0.4%, total N 0.04%, Olsen P 16.4 kg/ha; ammonium acetate extractable K, 190 kg/ha. During crop season (2015–16), the soil samples for microbial and chemical analysis were collected every month from 0–15 cm and stored at 4°C until analyzed. Soil *p*H and conductivity were measured (Smith and Doran 1996). Standard methods were used for the determination of available N, extractable P and total organic carbon (Page *et al.* 1982). Soil microbial biomass C and N were estimated using chloroform fumigation extraction method (Witt *et al.* 2000). Extractable organic C in fumigated and un-fumigated soil extracts was determined using dichromate method and SMBC was calculated using:

$$SMBC = EC/kEC$$

where, EC = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and *k*EC = 0.45.

Simultaneously, absorbance (E280) of the K_2SO_4 extracts from the fumigated and un-fumigated soils was measured at 280 nm (Nunan *et al.* 1998) using a UV-VIS Spectrophotometer. The data used for building the relationship between the absorbance and the dichromate method, were the means of triplicate determinations ± standard error.

Total extractable N was measured as NO_3^- after oxidation with $K_2S_2O_8$ (Cabrera and Beare 1993) and SMBN was calculated as

$$SMBN = EN/kIN,$$

where, EN = (extractable NO_3^- N due to fumigation) - (extractable NO_3^- N in the non-fumigated soil extract) and *k*IN = 0.57.

All results reported are averages of duplicated analyses and are expressed on a moisture free basis. Statistical tests were performed using the general linear models procedure of SPSS (SPSS, ver. 10.0).

Phospholipid fatty acid (PLFA) analysis: PLFA analysis was performed as described by Frostegard *et al.* (1993) with slight modification. Briefly, soil samples (5 g dry weight) were extracted for 2 h in one-phase extraction mixture containing chloroform:methanol:phosphate buffer (1:2:0.8 v/v/v). The chloroform phase containing lipids was filtered and concentrated with flash evaporator. Neutral lipids, glycolipids and phospholipids were eluted from the silicic acid (100–200 mesh, Merck) column using chloroform, acetone and methanol, respectively. Following a mild alkaline methanolysis of the phospholipids, the resulting Fatty acid methyl esters (FAMEs) were separated and quantified by gas chromatography with GC-17A (Shimadju Ltd., Japan), equipped with a flame ionization detector and a BP-5 capillary column (Sigma-Aldrich, USA). Peak areas were quantified using methyl nonadecanoate fatty acid (19:0) as an internal standard and the characterization of the unknown FAMES were done on the basis of comparison of relative retention times of standards (Supelco Bacterial Acid Methyl Esters #47080-Usupelco, Belleforte, Pa, USA). Gram positive bacterial abundance was calculated using PLFA i15:0, a15:0, i16:0, i17:0 and gram-negative bacterial abundance using PLFA 16:1ω9, cy17:0, 18:1ω9, cy19:0. The change in relative abundance of fungal and bacterial PLFAs abundance was determined by an index of fungal to bacterial ratio which was estimated using PLFA's i15:0, a15:0, 15:0; 16:0/i15:0, 16:1ω9, i17:0, 17:0, cy17:0, 18:1ω7/18:1ω9 and cy19:0 for bacteria, 18:2ω6 for fungi.

RESULTS AND DISCUSSION

The Zero tillage systems in which crop residues got mixed and integrated within surface soils showed an increase

Table 1 Comparison of Temporal Dynamics of Microbial Quotient – C (expressed as percentage of total carbon) and Microbial Quotient – N (expressed as percentage of total nitrogen) in Zero Tillage with Conventional Tillage Practices during growing Season of wheat (2015–2016)

Days after sowing	Zero-tillage (ZT)					Conventional tillage (CT)				
	Microbial Biomass C (µg C/g soil)	Microbial Quotient–C (%)	Microbial Biomass N (µg C/g soil)	Total N (%)	Microbial Quotient - N	Microbial Biomass C (µg C/g soil)	Microbial Quotient–C (%)	Microbial Biomass N (µg/g soil)	Total N (%)	Microbial Quotient - N
0	126a	2.21a	50a	0.022a	0.227a	69a	1.40a	46a	0.022a	0.209a
30	155b	2.92b	48a	0.021a	0.228a	88b	1.95a	38b	0.021a	0.180b
60	269c	5.38c	58b	0.021a	0.276b	111c	2.64b	35b	0.022a	0.159c
90	242d	4.84d	61b	0.022a	0.277b	142c	3.30c	39b	0.019a	0.205a
120	257c	4.75d	51a	0.022a	0.229a	200d	3.50c	40a	0.018a	0.222b
150	70e	1.16e	38c	0.022a	0.172c	62a	1.02d	21c	0.018a	0.116c

Different letters in a line denote significant differences among treatments at P <0.05.

in soil microbial biomass carbon (SMBC) and N levels (SMBN) as compared to conventional tillage (Table 1). During the growing season, changes in microbial biomass can be accredited to fluctuations in crop residue availability, soil moisture, and temperature and rhizosphere effects. As the crop season progressed, standing stubble decomposed, resulting in higher SMBC in zero tillage treatment than the conventional tillage. SMBC ranged from 70–269 µg C/g soil as compared to 62–200 µg C /g of soil. Better reflection of changes in zero tillage can be seen in the values of Microbial quotient- C and N. At the most vegetative phase of cropping season (i.e. 60 and 90 days after sowing) the SMBC showed a 103 and 46.6% increase over the conventional tillage. In general SMBC and SMBN account for 0.8–7% of total C and total N in the surface layers of arable soils, we observed similar values for the microbial quotient-C (1.16–5.38%) and for the microbial quotient-N (1.72–2.77%) in zero tillage fields. Zero tillage had a greater effect on microbial quotient values under the stubble retained systems and it reflected the seasonal changes and crop growth in the same way as microbial biomass. The increase in microbial quotient values in both the systems clearly depicted the differences in the availability of the substrate and the efficiency of its conversion into SMBC. Use of the microbial quotient data normalized the differences in microbial biomass due to spatial variability of soil properties, allowing the better interpretation of results. Similarly, the net mineralization rates increased during the growing season as evidenced by the increased microbial quotient-N (Table 1). Mineralization of N was greater under zero tillage than in the conventional tillage systems.

The increased variation of SMBC in the zero tillage, during the earlier phases of the crop, probably could be due to the incorporation and mixing of the crop residues within the surface layer. Doran (1987) also showed 54% higher microbial biomass in the surface layer of ZT, than the ploughed soils showing a close association of the microbial biomass with soil organic carbon and moisture content as influenced by the tillage practices. Zero tillage also increased the amount of microorganisms in soil (Groenigen *et al.* 2010). The greater stratification of SMBC under the ZT is in consistent with the results reported by (Salinas- Garcia *et al.* 1997, Jensen *et al.* 1997, Franzluebbers *et al.* 1999). Soil microbial communities encourage the accumulation of the carbon through microbial carbon and this in turn regulates the soil organic carbon with in soil aggregates (Zhang *et al.* 2013, Wang *et al.* 2017).

On the other hand, Haider (1992) emphasized no significant differences in the specific metabolic efficiency of soil microbes and the microbial C assimilation between integrated and conventional soil managements. Similarly, no significant effect of the double zero tillage on soil properties was noticed in the two-year study on the sandy loam soil (Bhatt and Kukal 2015). Conservation tillage practices such as zero tillage significantly reduces physical disturbance in soil and hence improves soil biological health (Guo *et al.* 2015, Guo *et al.* 2019). Furthermore, it has been observed that there is an improvement in soil porosity in the zero tillage system and the root development is quite good, thereby leading to more root exudates consisting of carbonic acids, amino acids and sugars (Colonego and Rosolem 2010). The management practices can alter the environmental conditions of the soil, which may lead to more C and N mineralization (Minoshima *et al.* 2007)

Table 2 Impact of tillage practices on the PLFA biomarker in conventional tillage and zero tillage soils

Days after sowing	Treatment	Total PLFA (nmol/g)	Bacterial PLFA (nmol/g)	Fungal PLFA (nmol/g)	Fungal/ Bacterial PLFA
0	Conventional tillage	81.2a	40a	3.1a	0.078a
30		79.3a	54b	4.3b	0.080a
60		84.3b	61c	4.4b	0.072a
90		90.4b	55b	3.1a	0.056b
120		72.3c	42a	2.1c	0.050b
150		39.1d	38d	2.5c	0.066c
0	Zero tillage	90.1a	78a	5.4d	0.069c
30		96.4b	82a	6.1e	0.074a
60		99.1c	73b	6.8e	0.093d
90		82.3d	71b	7.3f	0.103e
120		73.1e	60c	5.2d	0.087f
150		44.1f	55d	4.3b	0.078a

Different letters in a line denote significant differences among treatments at $P < 0.05$.

PLFA analysis of the microbial communities revealed that total PLFAs in the zero-till surface soil were much higher than the conventional tillage (Table 2). It ranged from 39.1–84.3 nmol/g in the conventional tillage and from 44.1–99.1 nmol/g in zero tillage. The fungal biomass (18:2ω6) was highest in zero-till surface soils (73 nmol/g) as compared to conventional tillage (61 nmol/g). There was increase in fungal and bacterial biomass ratio under zero tillage system (Frey *et al.* 1999). Moreover, soil microbial growth and activity has also shown to be affected by the different environmental factors such as temperature and moisture etc. In zero-till management, the organic matter resources are confined to the soil surface, which generally leads to the development of a higher biomass in the surface layer as this practice leads to warm temperature and abundant precipitation, which affects the decomposition of plant residues.

In the rice–wheat rotation, results of the present study indicate that zero tillage improved microbial biomass in the upper 0−15 cm soil profile as compared to the conventional tillage. Results of microbial quotient–C and microbial quotient–N revealed that zero tillage enhanced microbial metabolic activity in the upper soil layer, thus increasing SMBC of aggregates.

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