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Soil microbial properties influenced with long term application of manures and fertilizers

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ABSTRACT

An ongoing long-term field experiment established in 1995 at CCS Haryana Agricultural University, Hisar (India) under pearl millet-wheat cropping system was selected to examine the impact of organic manures and fertilizers on soil microbiological properties. After 19 years of experiment, the samples were collected after wheat harvest in April, 2014. Under different combinations of treatments, the microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) content in soil ranged from 202–491 and 35.0–79.8 mg/kg, respectively. The lower content of MBC and MBN was observed in farmyard manure (FYM) treated plots as compared to pressmud or poultry manure. Soil microbial quotient (SMQ) ranged from 3.18– 5.61% and higher SMQ was observed with pressmud and poultry manure application as compared to FYM application. The highest dehydrogenase activity (DHA) was reported with FYM_{15} (63.71 µgTPF/g/24 hr) which was statistically at par with $FYM_{15}N_{150}$ (59.75 µg TPF/g/24 h) and $pressmud_{7.5}$ (58.14 µg TPF/g/24 h). Among organic manures applied alone alkaline phosphatase activity (APA) followed the order: poultry manure>FYM>pressmud. The highest urease activity (97.6 µg NH_4^+-N/g/h) was observed with $pressmud_{7.5}$ and this may be attributed to higher N content (3.23%) in pressmud. The dehydrogenase and urease activity decreased in the plots where organic manures were applied in conjunction with NP fertilizers as compared to solitary application of organic manures. However, reverse trend was observed in case of alkaline phosphatase. Overall, pressmud applications exhibited favorable impacts on soil properties under pearl millet wheat cropping. Therefore, continuous application of pressmud could lead to long term maintenance of soil microbial properties in these sandy loam soils.

Key words: Enzymes activity, FYM, Microbial biomass, Sandy loam

In exhaustive cropping system like pearl millet-wheat, the nutrient mining must be replenished by addition of manures and fertilizers for sustaining soil fertility. Addition of organic amendments is a suitable alternative to achieve soil recuperation in these regions, where soil organic matter (SOM) content and soil biological activity is relatively low (Sheoran 2019). In India, FYM as a soil amendment remains the main organic source but poultry manure and pressmud are also used. In addition, inorganic fertilizers especially NPK are added to improve soil fertility, but their application directly and indirectly cause changes in physical, chemical and biological properties of soil (Dhram Prakash *et al.* 2016, 2017). Hence, the use of inorganic fertilizers in combination with organic manures has been found more advantageous than either of the alone for sustainable agriculture on long-term basis (Antil *et al*. 2011, Dhram Prakash 2016, Sheoran *et al.*2018). The SOM acts as a substrate for microbial growth and development. Soil micro-organisms, particularly micro-biota, play an essential role in the cycling of nutrients within soil and involved in the decomposition of organic matter at the ecosystem level via a variety of enzymes (Bastida *et al.* 2012). The response of these soil microbial variables to organic and conventional amendments is often studied in the long-term experiments and can serve as a marker of the soil status (Kussainova *et al.* 2013). However, relatively limited information is available concerning changes in soil biological properties under long- term field conditions of arid and semi-arid regions. The effects of nutrient management practices on SOC pools and soil enzymes have been well documented under different land uses (Benbi *et al.* 2015, Dhram Prakash *et al.* 2018). But the impact of manure and integrated nutrient management on soil microbiological properties and soil enzymes under pearl millet wheat cropping is relatively less known. Hence, the present study was carried out with the objective to assess and compare the long-term influence of different organic manures and fertilizers on soil microbiological properties under semi-arid environment.

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MATERIALS AND METHODS

A long-term field experiment with pearl millet-wheat cropping sequence was initiated in 1995 on a coarse loamy, *Typic Ustochrept* soil at CCS Haryana Agricultural University, Hisar, India. This long term experiment was selected to study the effect of various combinations of organic manures and fertilizers on soil microbiological properties. The details about experimental site and treatments have been documented by Sheoran *et al.* (2018). Composite soil samples were collected from 0–15 cm depth after the harvest of wheat crop in April 2014. The samples were kept at 4°C in a refrigerator in plastic bag for analysis of microbiological properties and all the results were expressed on dry weight basis. Microbial biomass carbon (MBC) (Vance *et al.* 1987) and microbial biomass nitrogen (MBN) contents (Brookes *et al.* 1985) were estimated by fumigation-extraction method. Soil microbial quotient (SMQ) was calculated as the ratio of MBC to SOC. Urease activity was measured as per the method proposed by Tabatabai and Bremner (1972). Alkaline phosphatase activity (APA) was measured by using the method described by Tabatabai and Bremner (1969). Soil dehydrogenase activity (DHA) was determined by estimating the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC) (Casida *et al.* 1964). The experimental data was statistically analyzed by using randomized block design for the various parameters and subjected to analysis of variance (ANOVA) using the program STATISTCA 6.0 Stat Soft, Inc. (2001). Within the STATISTCA 6.0, we have applied DUNCAN multiple range test to compare significant differences within the treatments.The treatment means were compared at a significance level of 0.05.

RESULTS AND DISCUSSION

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN): Under different combinations of treatments, the MBC and MBN content in soil ranged from 202 to 491 and 35.0 to 79.8 mg/kg, respectively (Table 1). The highest and lowest MBC and MBN content was recorded with the treatment pressmud$_{7.5}$N$_{150}$P$_{30}$ (T$_{10}$) and N$_{75}$P$_{30}$ (T$_1$), respectively. The MBC and MBN content was increased by 52.6 and 38.3%, 58.2 and 30.8%, and 113.6 and 89.6%, respectively, with application of 15 Mg FYM, 5 Mg poultry manure and 7.5 Mg/ha pressmud over the recommended dose of NP fertilizers. Therefore, a significant increase in MBC and MBN content in soil was observed with the application of organic manures compared to the application of chemical fertilizers. The supply of readily hydrolysable C and additional supply of N due to organic manure application resulted in higher microbial activity and their biomass C and N (Tripura *et al*. 2018). As MBC is controlled by availability of C content, addition of manures could have provided more labile C substances needed for maintenance of the larger MBC (Dhram Prakash *et al.* 2016). Increase in MBC content with incorporation of organic manure was also reported by Luo *et al*. (2015), Dhram Prakash (2016), Chen *et al*. (2017). The integrated application of manures and fertilizers (INM) enhanced the MBC and MBN content over organic manures applied alone. The higher MBC content under INM might be due to presence of steady source of readily metabolizable C and better crop yield resulted in higher root biomass and exudates (Liu *et al*. 2016, Wang *et al*. 2016, Ge *et al*. 2017, Tripura *et al.* 2018). Among the organic manures, the MBC content in soil followed the order pressmud>poultry manure> FYM, while MBN content in soil followed the order pressmud>FYM>poultry manure. The distinction in MBC and MBN could be attributed to variation in soil organic matter content in these manures. Higher content with pressmud might be attributed that pressmud is a sugar factory byproduct and may contain more easily decomposable sugar compounds which facilitate the activities of microorganisms and also due to higher amount of N supplied by pressmud. In spite of higher SOC content in FYM treated plots (Sheoran *et al.* 2018), the MBC and MBN content was lower as compared to pressmud treated plots. This could be due to high salt content in FYM treated

Table 1 Long-term effects of organic manures and fertilizers application on MBC and MBN content in soil

Organic manure (Mg/ha)	Dose (Mg/ha)	Fertilizer (kg/ha)		MBC (mg/kg)	MBN (mg/kg)	*SMQ (%)	MBC/MBN
		N	P_2O_5				
No manure	0	75	30	202 a	35.0 a	5.61	5.771
	0	150	60	213 b	38.6 a	4.84	5.518
FYM	15	0	0	325 c	53.4 b	3.18	6.086
	15	150	0	357 e	59.3 c	3.27	6.020
	15	150	30	369 f	64.7 d	3.24	5.703
Poultry manure	5	0	0	337 d	50.5 b	4.81	6.673
	5	150	30	371 f	58.0 c	5.01	6.396
Pressmud	7.5	0	0	455 g	73.2 e	5.61	6.216
	7.5	75	30	470 h	75.1 e	5.46	6.258
	7.5	150	30	491 i	79.8 f	5.34	6.153
CD (P=0.05)				10.53	3.85		

*Soil Microbial Quotient. Different lower case letters within columns indicate significance at P<0.05.

Table 2 Long-term effects of organic manures and fertilizers application on soil enzymes activity

Organic manure (Mg/ha)	Dose (Mg/ha)	Fertilizer (kg/ha)		DHA (µg TPF/g/24 h)	APA (µgPNP/g/h)	Urease activity (µgNH_4^+-N/g/h)
		N	P_2O_5			
No manure	0	75	30	36.53 ab	572.25 a	58.81 a
	0	150	60	32.32 a	580.75 a	65.02 ab
FYM	15	0	0	63.71 e	684.00 c	87.72 e
	15	150	0	59.75 e	742.75 ef	76.27 cd
	15	150	30	50.48 d	733.50 e	71.25 bc
Poultry manure	5	0	0	48.62 d	704.45 d	85.20 e
	5	150	30	40.13 bc	756.00 f	75.75 cd
Pressmud	7.5	0	0	58.14 e	664.63 b	97.60 f
	7.5	75	30	44.86 cd	675.65 bc	83.49 de
	7.5	150	30	39.44 bc	673.20 bc	71.28 bc
CD (P=0.05)				5.69	17.91	7.88

Different lower case letters within columns indicate significance at P<0.05.

plots as recorded during the experimentation. The higher values of MBC under pressmud treatments indicated that microbial biomass size depended more on quality of organic matter rather than quantity. This fact was supported by non-significant correlation between SOC and MBC (Table 3). Several studies indicated that higher salinity resulted in a smaller and more stressed microbial community which was less metabolically efficient (Barin *et al.* 2015).

Soil microbial quotient (SMQ) ranged from 3.18–5.61% and higher SMQ was observed with pressmud and poultry manure application compared to FYM application (Table 1). The ratio of MBC to SOC reflects the contribution of microbial biomass to soil organic matter and could act as a useful and sensitive indicator of soil quality. The higher MBC/SOC ratio under pressmud application indicated the higher microbial biomass pool per unit SOC over the other manures. The lower values of their ratio under FYM treatments showed the lower conversion efficiency of SOC into microbial biomass carbon even after their higher quantity (15 Mg/ha) addition compared to pressmud and poultry manure. The microbial biomass carbon comprised a small fraction of total OC and further the OC content is influenced by several other factors for example nutrients ratio, composition and temperature etc. The higher MBC/SOC ratio under pressmud could be ascribed to the supply of readily metabolisable C and favorable environment for microbe proliferation.

Ratio of MBC to MBN ranged from 5.52–6.67 and the lowest MBC/MBN ratio was observed under chemical fertilizer treatment (Table 1). This ratio in fungal hyphae and bacteria usually ranges from 10–12 and 3–5, respectively (Jenkinson and Ladd 1981). Therefore, the present experimental soil might have dominated by bacterial population and this ratio was quite high when compared with the study of Mandal *et al.* (2007). Application of organic manures alone or in combination with chemical fertilizers showed higher MBC/MBN ratio over solitary application of chemical fertilizer. However, their combined application resulted in lower MBC/MBN ratio compared to organic manures applied alone. The decrease in ratio of MBC to MBN under conjoint use of manures and fertilizers could possibly be due to changes in microbial community composition as this ratio could represent the relative proportion of fungal to bacterial population in soil (Horwath 2007).

Soil enzymes: The values of DHA ranged from 32.32–63.71 µg TPF/g/24 h under different treatments (Table 2). The highest DHA was reported with FYM_{15} (63.71 µg TPF/g/24 h) which was statistically at par with $FYM_{15}N_{150}$ (59.75 µg TPF/g/24 h) and $pressmud_{7.5}$ (58.14 µg TPF/g/24 h). This might be attributed to more addition of substrate (i.e. FYM) because of higher application dose. Application of organic manures alone or in combination with N or NP fertilizers significantly enhanced the enzyme activity as compared to application of fertilizers alone. This might be due to higher microbial activity and availability of carbon substrate as compared to solitary application of fertilizers. Increase in DHA with addition of FYM was also reported in several studies (Bastida *et al*. 2012, Srinivasan *et al*. 2016). Application of NP fertilizers recorded lowest enzyme activity

Table 3 Correlation matrix depicting relationship among soil carbon and soil microbiological properties

Variable	SOC	MBC	MBN	DHA	APA	Urease activity
SOC	1.00					
MBC	0.62^{NS}	1.00				
MBN	0.67^{*}	0.99^{**}	1.00			
DHA	0.72^{*}	0.31^{NS}	0.31^{NS}	1.00		
APA	0.77^{**}	0.53^{NS}	0.50^{NS}	0.51^{NS}	1.00	
Urease	0.44^{NS}	0.58^{NS}	0.51^{NS}	0.73^{*}	0.41^{NS}	1.00

*Correlation is significant at P<0.05 level (2-tailed); **Correlation is significant at P<0.01 level (2-tailed); NS, non-significant.

due to lack of sufficient substrate. Addition of NP fertilizers along with organic manures significantly decreased the DHA as compared to application of organic manures alone. The probable reason for this type of results was that the peak of maximum decomposition rate of organic matter was attained during crop season due to sufficient availability of mineral-N and P along with C under integrated treatments. After that decomposition rate decreased gradually due to scarcity of easily decomposable substrate at the end of crop season (Bastida *et al.* 2012). However, in organic manures treatments, the peak stage of organic matter decomposition was not achieved during crop season due to lack of mineral N and P. Higher decomposition rate of organic matter was still going on and thus the DHA was higher at the end of cropping season as compared to combined application of organic manures and NP fertilizers. This type of results are in conformity with the outcomes of several studies (Basak *et al*. 2017, Jia *et al*. 2018, Tripura *et al*. 2018).

The highest APA (756.00 µgPNP/g/h) was reported with poultry manure$_5N_{150}P_{30}$ that was statistically at par with $FYM_{15}N_{150}$ (733.50 µgPNP/g/h). The higher APA observed with application of poultry manure followed by FYM and pressmud could be due to higher P content of poultry manure. The APA was higher with the application of conjunctive use of manures and NP fertilizers over solitary applications of organic manures or NP fertilizers. This increase in activity may be due to the release of more organically bound P due to faster decomposition of organic matter in presence of mineral N and P which stimulated the synthesis of the enzyme. Under balanced fertilization, higher microbial population also resulted into more secretion of enzyme and increased activity. The higher APA under conjoint use of organic manures and NP fertilizers over NP fertilizer alone was also observed by Srinivasan *et al*. (2016), Jia *et al*. (2018). The lowest APA (572.25 µgPNP/g/h) was observed in plots treated with chemical fertilizers may be attributed to less substrate availability. It indicated that APA was not only regulated by P supply but also by C supply.

The values of urease activity ranged from 58.81–97.60 µg NH_4^+-N/g/hr under different treatments (Table 2). The highest urease activity (97.6 µg NH_4^+-N/g/h) was observed with pressmud$_{7.5}$. Urease activity of soil was found higher when recommended dose of NP fertilizers was applied as compared to half of recommended dose of NP fertilizers. It may be due to increase in urease activity with addition of urea-N. Higher urease activity in manure amended soils might be due to increased population of microorganisms with increased availability of substrate through production of diverse extra cellular enzymes. Highest urease activity observed in pressmud amended plots may be attributed to higher N content (3.23%) in pressmud and higher MBC in these plots (Table 1). Like dehydrogenase, urease activity also decreased in the treatments where organic manures were applied in conjunction with NP fertilizers as compared to organic manures applied alone. Nitrogen was given by urea in INM treatments, which stimulated the microbial activity and resulted in rapid decomposition of organic matter during early crop growth stage and thereafter rate of decomposition of organic matter slowed down which resulted in lower urease activity at the end of crop season.

Continuous application of organic manures for 19 years significantly increased the MBC, MBN and enzymes activity in soil as compared to solitary applications of chemical fertilizers. Enhanced microbial activity due to abundant food availability also accelerated organic matter decomposition that in turn improved MBC content in soil. The higher values of MBC under pressmud treatments indicated that microbial biomass size depended more on quality of organic matter rather than quantity. The lower values of MBC/SOC ratio under FYM treatments showed the lower conversion efficiency of SOC into microbial biomass carbon even after their higher quantity addition compared to pressmud and poultry manure. The lower urease and phosphatase activity along with low microbial quotient under FYM applied plots may be due to high EC and deposition of soluble salts. Therefore, pressmud could lead to a greater accrual in soil microbial properties in these semi-arid soils.

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