Indian Journal of Agricultural Sciences **84** (4): 452–7, April 2014/Article
https://doi.org/10.56093/ijas.v84i4.39454

Soil biological properties as influenced by long-term manuring and fertilization under sorghum (*Sorghum bicolor*) -wheat (*Triticum aestivum*) sequence in Vertisols

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Received: 3 March 2012; Revised accepted: 31 February 2014

ABSTRACT

The effect of long-term manuring and fertilization on soil biological properties was studied under the long term fertilizer experiment on sorghum [*Sorghum bicolor* (L.) Moench] -wheat (*Triticum aestivum* L.) sequence conducted on Vertisols. The treatments comprised 50% RDF, 100% RDF, 150% RDF,100% RDF (-S), 100% RDF + 2.5 kg Zn/ ha to wheat crop only, 100% RD of NP, 100% RD of N, 100% RDF + FYM @ 10 tonnes/ha, 100% RDF + S @ 37.5 kg/ ha, FYM @ 10 tonnes/ha, 75% RDF and control. The assessment of soil biological properties at 40 and 70 days after sowing revealed that, significantly highest CO_2 evolution was recorded at 70 DAS of sorghum under 100% RDF + FYM @ 10 tonnes/ ha (66.82 mg/ 100 kg). Soil microbial biomass carbon was also influenced significantly with the combined application of NPK+FYM (278.9 mg/ kg). The soil enzyme activity, viz. dehydrogenase (55.01 µg TPF/g/24 hr), urease (47.9 mg NH_4/kg/ 24 hr) and cellulase (52.23 µg glucose/g/24 hr) were significantly influenced with the application of 100% RDF + FYM @ 10 tonnes/ha. Similarly, the application of 100% RDF + FYM @ 10 tonnes/ha recorded significantly higher grain yield of sorghum.

Key words: Biological properties, Cellulase, Dehydrogenase, Urease, Vertisols.

Soil harbors dynamic population of microorganisms which play major role in decomposition of organic matter and transformation of plant nutrients. The availability of organically bound nitrogen through transformation in soil to the plant mainly depends on the population of microorganisms, which is influenced by the application of inorganic fertilizers and organic manure. The microbial biomass, which is the total mass of bacteria, fungi, actinomycetes, algae and protozoa present in soil, serves as a temporary sink for nutrients including nitrogen and can be considered as an index of soil fertility. Microbial population accounts for only 1-3 per cent of soil organic carbon but it is the eye of the needle through which all the organic material that enters the soil must pass through (Jenkinson 1988). Any management practice that influences microbial communities in soil may be expected to produce changes in soil enzyme level (Perucci and Scarpon 1983). A part of nutrients is assimilated by microorganisms and incorporated into microbial cells (biomass). Microorganisms regulate the nutrient flow in the soil by assimilating nutrients and producing soil biomass (immobilization) and converting C, N, P and S to mineral forms (mineralization) (Jenkinson and Ladd 1981, Wani and Lee 1995). Dehydrogenase activity is often used as a measure of any disruption caused by pesticides; trace element or management practices to the soil (Reddy and Faza 1989). Soil contributes 20 per cent of total emission of CO_2 to the atmosphere through soil respiration. Soil management practices like increasing soil organic carbon concentration, reduction of tillage, manuring, residue incorporation improves enzymes activities, microbial biomass and N-mineralization as compared to inorganic fertilization or unamended control. The production and turnover of the microbes is pivotal to our understanding of nutrient cycling. Activities of enzymes are correlated with microbial respiration and total biomass in the soil (Frankenberger and Dick 1983).

Singh *et al.* (1996) observed that over a period of 5 years the change in soil organic carbon (SOC) was negative under cereal–cereal sequences compared to other cropping sequence with legume component. Continuous application of farmyard manure (FYM) and green manure substantially improved the organic carbon under different soils and cropping systems (Wani and Lee 1995, Manna *et al.* 1997, Singh *et al.* 1996, Swarup 1998). However, neither inorganic fertilizers nor organic manures alone can sustain productivity (Prasad 1996). So judicious uses of organic manures and inorganic fertilizers are essential to safeguard soil health and augment productivity and input use efficiency.

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Many long-term experiments conducted in India showed increasing yield trends and accumulation of soil organic carbon and biological properties due to combined application of fertilizer and manures (Saini *et al*. 2005, Bhattacharyya *et al.* 2008, Mishra *et al.* 2008, Saha *et al.* 2008, Bedi *et al.* 2009). However, most studies were restricted under irrigated conditions to rice–wheat, soybean –wheat systems and other climatic conditions in India. Relatively limited information is available in Vertisol under semi-arid conditions on sorghum-wheat system.

The present investigation was therefore undertaken to study the dynamics of soil microbial biomass and enzyme activities under sorghum [*Sorghum bicolor* (L.) Moench]-wheat (*Triticum aestivum* L.) system continuously receiving fertilizes and manures in Vertisols of Vidarbha region of Maharashtra.

MATERIALS AND METHODS

The long term fertilizer experiment was initiated at Research Farm, Department of Soil Science and Agricultural Chemistry, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra during 1988-89. The experiment comprised twelve treatments replicated four times in RBD. The soil of experimental site was deep, clayey black soil taxonomically classified as very fine, smectitic, calcareous, hyperthermic family of Typic Haplusterts. The soil is having low hydraulic conductivity and high water holding capacity. The initial analysis indicated that soils are low in organic carbon and available N, very low in available P and high in available K.

The treatments consisted of 50% RDF (T1), 100% RDF (T2), 150% RDF (T3),100% RDF (-S) (T4), 100% RDF + 2.5 kg Zn/ha once in two years to wheat crop only (T5), 100% RD of NP (T6),100% RD of N (T7),100% RDF + FYM @ 10 tonnes/ha to sorghum only (T8), 100% RDF + S @ 37.5 kg/ ha (T9), FYM @ 10 tonnes/ha to sorghum only (T10), 75% RDF (T11) and control (T12). The 23rd cycle of the experiment during 2010-11 was studied in the present investigation. The recommended dose of fertilizer was 100:50:40 kg N, P_2O_5, K_2O/ha applied to sorghum while 120:60:60 N, P_2O_5, K_2O kg/ ha to wheat. Farmyard manure (0.58% N, 0.21% P, and 0.62% K) was added on oven dry basis one month before sowing of sorghum whereas, half dose of N and full dose of P and K was applied at the time of sowing to sorghum and remaining half dose of N was applied 30 days after sowing. The half dose of N and full dose of P and K was applied at the time of sowing to wheat and remaining half dose of N was applied 21 days after sowing.

Treatment wise surface (0-20 cm) soil samples were collected at 40 and 70 days after sowing (DAS) of sorghum and divided into two parts, one for chemical analysis and another part stored at 4°C in refrigerated condition for analysis of biological properties. For CO_2 evolution and soil microbial biomass carbon (SMBC), soil samples were air dried and used for further analysis. The CO_2 evolution was estimated by alkali trap method by adopting methods of Anderson (1982). Soil microbial biomass carbon was estimated by chloroform fumigation - extraction method as per Jenkinson and Powlson (1976) using Kc value of 0.45. Dehydrogenase activity (DHA) was estimated by incubation with triphenyl tetrazolium chloride (TTC) method as per Klein *et al*. (1971).Urease and cellulase activity were determined as per Pancholy and Rice (1973). The microbial population was determined by serial dilution plate technique using respective media for each group as per Dhingra and Sinclair (1993).

RESULTS AND DISCUSSION

CO_2 evolution

The CO_2 evolution is an index of microbial activity and fate of decomposition of organic matter in soil. The CO_2 evolution at various treatments ranged from 29.20 to 63.52 mg/100 g at 40 DAS and 31.24 to 66.82 mg/100 g at 70 DAS. Application of 100% RDF + FYM @ 10 tonnes/ ha recorded significantly higher CO_2 evolution, i.e. (63.52 mg/100 g) at 40 DAS and (66.82 mg/100 g) at 70 DAS, however, it was reduced under 150% NPK (T_3) for both stages of 40 (59.40 mg/100 g) and 70 DAS (62.15 mg/100 g) and found at par with 100% RDF + FYM @ 10 tonnes/ ha (Table 1).

The CO_2 evolution was relatively low under all the treatments involving use of only chemical fertilizers and it was lowest under absolute control. As the fertilizer doses increased, the CO_2 evolution also showed increase. The CO_2 evolved under 100 % RDF + FYM was higher, which can be attributed to addition of more root biomass associated with higher grain and fodder yield and nutrient turnover at higher carbon expenses met through added organic carbon. Mishra *et al.* (2008) also reported higher CO_2 evolution in Alfisols of Jharkhand due to application of organic manures in conjunction with chemical fertilizers. The higher CO_2 evolution observed under 150% RDF can be attributed to the fact that accumulation of more root biomass due to higher yield as a result of long term manuring and fertilization. These results are in the conformity with the findings reported by Bedi *et al*. (2009) and Selvi *et al.* (2004).

Soil microbial biomass carbon

The soil microbial biomass carbon at 40 and 70 DAS ranged from 137.81 to 249.01 mg/ kg and 90.99 to 278.95 mg/kg respectively. Application of 100% RDF + FYM @ 10 tonnes/ha recorded significantly higher microbial biomass carbon, i.e. 249.01 and 278.95 mg/ kg, respectively at 40 and 70 DAS followed by FYM @ 10 tonnes/ha. Significantly higher biomass C was observed under 100 % RDF + FYM @ 10 tonnes/ha may be due to the effect of FYM which stimulate microbial growth resulting into higher microbial biomass carbon (Table 1). The integrated use of manures and chemical fertilizers provides a balanced supply of nutrients and carbon, which in turn might maintain the higher population of microbes (Basak *et al.* 2012). These findings are in the line with the results reported by Patil and

Table 1 Long-term effect of nutrient management on CO_2 evolution and microbial biomass carbon

Treatment	CO_2 evolution (mg/ 100 g)		SMBC(mg/ kg)	
	40 DAS	70 DAS	40 DAS	70 DAS
T1 , 50% NPK	48.05	51.77	197.4	153.6
T2 , 100% NPK	52.52	54.45	216.3	169.4
T3 , 150% NPK	59.40	62.15	239.7	236.0
T4 , 100% NPK (S free)	49.50	53.70	206.4	165.4
T5 , 100% NPK + 2.5 kg Zn/ ha	55.00	57.62	218.1	178.6
T6 , 100% NP	48.87	51.90	204.3	161.3
T7 , 100% N	45.30	48.85	180.1	151.5
T8, 100% NPK + FYM @ 10 tonnes /ha	63.52	66.82	249.0	278.9
T9 ,100% NPK + 37.5 kg S /ha	53.47	56.10	221.0	174.8
T10 ,FYM alone 10 t/ ha	55.82	58.02	227.1	216.6
T11 ,75% NPK	49.10	50.45	199.8	159.7
T12 , Control	29.20	31.24	137.8	90.9
SE (m±)	2.49	1.80	5.17	5.94
CD (P=0.05)	6.16	5.18	14.88	15.87

*Zn @ 2.5 kg/ ha to wheat crop once in two year, S**@ 37.5 kg/ ha

Puranik (2001) and Selvi *et al.* (2004). Furthermore the supply of additional mineralizable and readily hydrolysable C due to organic manure application resulted in higher microbial activity and higher microbial biomass carbon. Vineela *et al.* (2008) also reported significant enhancement in SMBC due to NPK +FYM in Vertisols at Coimbatore. The results further indicated that biomass carbon was in general, higher at early stage (40 DAS) and reduced considerably at later stage (70 DAS). The results confirm the findings of Saini *et al*. (2005) in soybean-maize cropping sequence on clay loam soils of Rajasthan.

Dehydrogenase activity

The dehydrogenase activity which has been widely used as a generalized comparative index of microbial activity, increased with the increase in the dose of fertilizers from 50 to 150 per cent RDF. Application of 100% RDF + FYM @ 10 tonnes/ha recorded significantly highest dehydrogenase activities at 40 and 70 DAS (53.45 and 55.01 µg /TPF g/soil 24 h) than all other treatments. The application of FYM @ 10 tonnes/ha increased dehydrogenase activity as compared to optimal dose of fertilizer (Table 2). This increase in dehydrogenase enzyme activity is associated with the organic sources acting as the sole sources of carbon and energy for heterotrophs. A significant increase in dehydrogenase activity in the plots with organic treatments, especially with NPK was also observed by Saha *et al.* (2008). The results are in line with the findings by Bhattacharyya *et al*. (2008) wherein four folds increase was observed in dehydrogenase activity due to farmyard manure application along with NPK. Imbalanced and inadequate application of fertilizers (100 % RD of NP or N) caused significant decline in the dehydrogenase activity. The dehydrogenase activity was slightly increased at 70 DAS as compared to 40 DAS which might be due to higher root biomass at this stage. The dehydrogenase activity was significantly lowest under 100% N as compared to all other treatments except control, which can be attributed to the presence of nitrate and nitrite that serve as alternative electron acceptors (Kukreja *et al.*1991). The decreased DHA under 100% N alone is associated with the redox potential of soil. The redox potential (Eh) of the soil might have been increased due to accumulation of nitrate over the year of cropping (23rd year) resulting into positive and high redox potential of the soil, thereby decreasing dehydrogenase activity.

Urease activity

Application of RDF + FYM @ 10 tonnes/ha recorded significantly highest urease activity (45.15 and 47.90 mg NH_4N kg/ 24 hr) than any other treatments (Table 2). Similarly, slight increase in the urease activity was observed at 70 DAS of sorghum as compared to 40 DAS, may be due to solubilization of complex organic compounds at steady rate. The increase in urease activity under RDF and RDF + FYM as compared to other treatments may be due to higher root biomass as a result of vigorous growth, thereby increasing urease activity in these treatments. The increased microbial population under organics and INM was responsible for increase in urease activity of the soil; this can be ascribed to the organic manure which acts as a source of carbon and energy for heterotrophs and provides sufficient nutrition for the proliferation of microbes and their activities in terms of soil enzymes (Rai and Yadav 2011). Chhonkar and Tarafdar (1981) found that the activities of enzymes were significantly and positively correlated with organic carbon and microbial population in the soil. The urease activity was increased with the increasing level of RDF, which could be attributed to the increased rate of nitrogen application and root exudates which promote the production of nitrogenous substances that induce the urease activity (Elayaraja and Singarvel 2011).

Cellulase activity

Application of 100 % RDF + FYM @ 10 tonnes/ha

Table 2 Long term effect of nutrient management on enzymatic activity at 40 and 70 DAS of sorghum

Treatment	DHA (µg TPF/g/24 hr)		Urease activity (mg NH_4/kg/24 hr)		Cellulase activity (µg glucose/ g/ 24 hr)	
	40 DAS	70 DAS	40 DAS	70 DAS	40 DAS	70 DAS
T1 , 50% NPK	43.90	45.83	32.82	34.55	26.45	27.68
T2 , 100% NPK	47.14	49.16	37.62	39.25	36.66	41.63
T3 , 150% NPK	51.53	52.00	40.95	41.95	43.46	50.60
T4 , 100% NPK (S free)	46.32	48.23	35.10	38.35	34.36	36.70
T5 , 100% NPK + 2.5 kg Zn/ ha	48.13	51.47	38.85	40.65	40.20	41.63
T6 , 100% NP	41.25	43.46	31.10	32.15	32.46	34.53
T7 , 100% N	37.94	42.55	25.67	28.19	21.40	24.03
T8,100% NPK+ FYM @ 10 tonnes /ha	53.45	55.01	45.15	47.90	45.36	52.23
T9 ,100% NPK + 37.5 kg S /ha	47.47	51.11	38.01	39.85	38.53	40.98
T10 ,FYM alone 10 t/ ha	50.15	51.02	35.00	39.20	43.40	45.30
T11 ,75% NPK	46.04	47.02	36.75	38.00	30.92	32.15
T12 , Control	34.75	35.35	19.05	20.30	12.26	12.95
SE (m±)	0.91	1.06	1.97	1.42	1.07	1.25
CD (P=0.05)	2.61	3.06	5.68	4.10	2.51	2.98

*Zn @ 2.5 kg/ ha to wheat crop once in two year, S**@ 37.5 kg/ ha

recorded significantly highest cellulase activity (45.36 and 52.23 µg glucose released/ g soil/24 hr) followed by 150% RDF (43.46 and 50.60 µg glucose released/g soil/24 hr) and FYM @ 10 tonnes/ha (43.40 and 45.30 µg glucose released/ g soil/24 hr). Application of FYM @ 10 tonnes/ ha was found beneficial in maintaining higher cellulase activity at 40 DAS (43.40 µg glucose released/g soil/24 hr) and 70 DAS (45.30 µg glucose released/g soil/24 hr) (Table 2). The increased cellulase activity in FYM treated plot might be due to incorporation of organics resulting into increase in microbial activity of soil. Balanced fertilization (100% RDF) over the years of cropping resulted into higher cellulase activity as compared to imbalanced application of fertilizers. Saha *et al.* (2008) also recorded highest cellulose activity in NPK+FYM treatment.

Microbial population

The highest bacterial (30.5 cfu × 107 /g soil), fungal (15.5 cfu × 10^4 /g soil) and actinomycetes (16.25 cfu × 106 /g soil) population registered in the treatment, where 100% RDF + FYM @ 10 tonnes/ha (Table 3). This could be ascribed to the FYM which supplied large amount of readily available carbon, resulting in more diverse and dynamic microbial system than in inorganically fertilized soil over the year of experimentation. Further, most of the soil microorganisms are chemoheterotrohs, which require organic source of carbon as food and oxidation of organic substances provides energy, this might be the reason in improving microbial population in INM. Similarly, application of 50% RDF or imbalanced nutrition to sorghum-wheat sequence caused decline in microbial population as compared to balanced fertilization or conjunctive use of fertilizer. This was attributed to the fact that continued cropping exhausted the native pool of nutrients, which might have caused reduction in microbial population (Suresh *et al.* 1999).

The results thus suggest that the application of organics in combination with inorganics favourably helps in augmentation of beneficial microbial population and their activities such as organic matter decomposition, biological nitrogen fixation, phosphorous solubilization and availability of plant nutrients. Increase in microbial population with application of fertilizer nutrients is indicative of the improvements in biological soil health.

Soil organic carbon

The organic carbon content was significantly highest (7.81 g/kg) at harvest of sorghum (2010-11) with the application of 100% RDF + FYM (Table 3). This might be due to addition of FYM which stimulated the growth and activity of microorganisms and also due to better root growth. This could also be ascribed to the contribution from organic manure (10 tonnes/ha) during the long term period of experimentation (23 year) which stimulated the growth and activity of microorganism and better root biomass (Katkar *et al.* 2011). The effect was further enhanced by the addition of RDF resulting in the improvement in root and shoot growth thereby increased contribution from biomass. Vineela *et al.* (2008) also observed that soil organic carbon levels increased considerably due to long-term fertilization and/or manuring applied for 16-29 years in Vertisol under semi-arid climatic condition.

The organic carbon content was considerably declined due to imbalanced application of fertilizers (50 % NPK, NP and N). However, balanced use of NPK improved status of organic carbon content, which is associated with increased crop productivity. At the end of 30 years, application of balanced fertilization of NPK showed significantly higher organic carbon (7.51g/kg) compared to unfertilized control (6.01 g/kg) in 0-45 cm soil layer (Kundu *et al.* 2007). This has clearly indicated that INM followed over a long-term was beneficial in maintenance of organic carbon levels in Vertisols of semi-arid regions of central India.

Table 3 Long term effect of nutrient management on microbial population

Treatment	Microbial population			Organic carbon	Sorghum yield (tonnes/ha)	
	Bacteria (cfu ×10^7)	Fungi (cfu ×10^4)	Actinomycetes (cfu × 10^6)	(g/kg)	Grain	Fodder
T1 , 50% NPK	9.5	7.25	6	4.47	1.53	3.72
T2 , 100% NPK	15.5	11.25	11.75	4.97	2.49	5.71
T3 , 150% NPK	22.75	13.25	13.5	6.30	3.31	8.22
T4 , 100% NPK (S free)	14.75	12	10.5	4.94	2.23	4.95
T5 , 100% NPK + 2.5 kg Zn/ ha	17.75	11.5	12	6.10	2.73	6.48
T6 , 100% NP	13	8.25	8.75	4.74	1.92	4.23
T7 , 100% N	8.75	6.25	5.75	4.82	1.36	2.97
T8,100% NPK+ FYM @ 10 tonnes /ha	30.5	15.5	16.25	7.81	3.71	9.17
T9 ,100% NPK + 37.5 kg S /ha	20.25	9	12.25	6.14	2.61	6.26
T10 ,FYM alone 10 t/ ha	22.5	12.5	13	7.31	1.05	2.29
T11 ,75% NPK	12.5	7.75	10	4.55	2.04	4.61
T12 , Control	6.25	4.5	5	3.03	0.15	0.36
SE (m±)	0.71	0.67	0.60	0.16	0.13	0.18
CD (P=0.05)	2.05	1.94	1.74	0.45	0.38	0.53

*Zn @ 2.5 kg/ ha to wheat crop once in two year, S**@ 37.5 kg/ ha

Productivity of sorghum

The application of 100% RDF + FYM @ 10 tonnes/ ha recorded significantly higher grain yield of sorghum (3.71 tonnes/ ha) (Table 3). FYM along with 100% RDF directly added an appreciable amount of major nutrients to the soil which could contribute to enhance the grain and fodder yield. The improvement in physical properties as result of application FYM provide desirable condition for plant growth and nutrient uptake might be the reason to sustain productivity of crop. These results are in conformity with the findings of Ravankar *et al*. (2005). Application of 150% RDF through chemical fertilizers (3.31 tonnes/ha) was found second higher treatment in respect of sorghum grain yield. The yield obtained from 100% N, 100% NP and 100% NPK showed significant increasing trend from N to NPK. This suggests the importance of balance fertilization for achieving productivity of crop.

Moreover, the responses to the treatment NPK +FYM were as good as NPK at 100 /150 % alone but continuous use of these treatments after some years started showing less responses to NPK alone than NPK+FYM treatment over the year indicating that micronutrients and secondary nutrients especially Zn and S were becoming yield limiting factors. These nutrients are provided by FYM besides supplying additional quantities of NPK and it has beneficial effect on physical properties and biological condition of soil (Swarup 2010).

It was observed that the crop productivity was sustained under integrated nutrient management indicating that the favourable conditions caused due to improvement in physical and biological properties of soil due to INM, helped in sustaining crop productivity. The significant improvement in soil biological properties observed at INM is certainly beneficial to improve the crop productivity through its effect on nutrient availability and enhancement in fertilizer use efficiency creating good physical environment for plant growth.

It can thus be concluded that, long term application of chemical fertilizers with organics is beneficial for improving soil biological properties which is further useful for sustaining crop productivity in Vertisols. These biological properties can serve as soil biological indicators for deciding management.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the financial support from ICAR and University for implementation the AICRP on LTFE.

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