JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2016. Vol. **10**(1), p. 139-150

Long-Term Effect of Organic Manuring and Inorganic Fertilization on Humus Fractionation, Microbial Community and Enzymes Assay in Vertisol

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https://doi.org/10.22207/JPAM.10.1.17

(Received: 17 November 2015; accepted: 01 January 2016)

Investigations were carried out during 2011-12 and 2012-13 to evaluate the long term effect of organic manuring and inorganic fertilization on humus fractions, microbial and enzymatic activities in a Vertisol (*Typic Haplusterts*) by collecting soil samples from long-term fertilizer experiments commenced from 2006-07 at V. N. Marathwada Agricultural University, Parbhani, Maharashtra, India under an intensive soybean-safflower cropping system. Two years pooled results indicated that continuous cultivation with addition of 100% NPK along with organic farm yard manure (FYM) significantly improved the content of soil humin, humic acid, fulvic acid and humic acid: fulvic acid (HA:FA) ratio. The treatment also contributed significantly to the biological properties of soil with respect to CO_2 evolution (56.40 mg 100 g^{-1} soil 24 hr^{-1}), soil microbial biomass carbon (300.10 $\mu g\ g^{-1}$), soil microbial biomass nitrogen (52.32 $\mu g\ g^{-1}$), soil bacteria (229.42 CFU x 10^7 g^{-1}soil), soil actinomycetes (54.53 CFU x 10^6 g^{-1}soil), dehydrogenase enzyme (51.07 μg TPF g^{-1}soil 24 hr^{-1}), acid and alkaline phosphatase (76.08 and 160.65 μg p-NP g^{-1} soil 24 hr^{-1}) activity under the soybean-safflower cropping system. However, soil fungi (8.68 CFU x 10^4 g^{-1} soil) increased significantly due to the application of only 10 Mg ha^{-1} FYM and it was at par with 100% NPK+FYM. Further, treating plots with 150% NPK was found equally good in improving soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN) and soil organic carbon (SOC). It can be concluded from these experimental findings that the balanced use of fertilizers continuously either alone or in combination with organic manure is necessary for sustaining soil health in a soybean-safflower cropping system on Vertisols.

Key words: CO_2 evolution, enzyme activity, fulvic acid, humic acid, humin, microbial biomass carbon.

Soil organic matter is not only important for maintenance of the soil physical conditions but it also supplies essential plant nutrients for successful crop production. Humus, which includes humin, humic acid (HA) and fulvic acid (FA), is the most important and largest constituent of soil organic matter and is formed by decomposition of plant and animal residues by micro-organisms. It is a store house of various nutrients essential for plant growth. Besides, humus also exerts a pronounced influence on physical, chemical and biological properties of the soil (Gathala *et al*. 2007). Soil organic carbon (SOC) level increased markedly in plots receiving farm yard manure and long term continuous use of inorganic fertilizers containing N, P, K, S and Mg but the increase was much less in inorganically treated soils than that brought about by FYM (Santhy *et al*. 2001). Humic compounds are stabilized in the soil against microbial degradation by their complex polymer structure, their physical protection inside dense

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aggregates and by their association with metal ions or clays. Various environmental factors and cultivation practices, however, can affect humus formation and degradation considerably. Among these, temperature, moisture, tillage and cropping systems are very important. Humification is the generic term given to extremely complicated formation and transformation processes in soil where biotic activity is occurring, and polycondensation proceeds under the participation of inorganic-organic catalysts (e.g. OH ion, Fe and Mn oxides). Further, it is a principle of abiotic reaction and the kind of material participating in the formation of immensely diverse products. Various dark colors such as melanin, melanoidins and microbial brown pigments are formed in nature and these may seemingly serve as precursors of soil humus (Stevenson, 1982). Various fractions of humus have been isolated on the basis of their solubility in particular extractants and named as humin, humic acid (HA), fulvic acid (FA) and hematomelaonic acid (Mukherjee and Ghosh, 1984). These fractions differ significantly in elemental analysis, functional groups, total acidity, and found low to high in their molecular weight (Kononova 1966). The humus classify as specific (humic substances) and non specific substances (non-humic fractions). These specific and non-specific materials are important to the soil environment. Non-specific materials provide short-term effects such as a source of food energy for micro-organisms including source of native soil fertility. In a long- term experiment, addition of organic matter through FYM along with fertilizers was found to increase humus fractions slightly due to decomposition of organic matter to form various organic acids and liberation of CO_2 which helps to reduce soil alkalinity (Santhy *et al.* 2001). Organic matter in soil is critical for better soil health and higher productivity. However, maintaining and improving soil organic carbon is difficult in arid and semi-arid regions in view of rapid oxidation due to high temperature. Continuous application of organic manure with inorganic fertilizers is the only way to increase organic matter status and stabilized fertility of soil. A part of nutrient is assimilated by microorganisms and incorporated in microbial biomass. Micro-organisms regulate the nutrient flow in soil by assimilating nutrients and growing soil biomass. The changes of soil organic carbon contents are also directly associated with changes in soil microbial biomass carbon and biological activity in soil (Katkar *et al.* 2011). Soil enzyme activities are 'sensors' of soil degradation since they integrate information about microbial status and physico-chemical condition of soil in relation to nutrient availability. Number of enzymes such as dehydrogenase, acid and alkaline phosphatase provides comprehensive microbial activity. Phosphomonoesterase (PMEase) is a generic name for a group of enzymes which catalyze the hydrolysis of esters of phosphoric acid to release phosphate and is of paramount importance as a soil quality indicator (Trasar-Cepeda *et al.* 2008). Dehydrogenase (DHA) exists as an integral part of intact cells, is involved in oxidative phosphorylation and reflects the total oxidative potential of the soil microbial community (Dick 1997). The present investigation aimed to study the influence of organic manure and inorganic fertilization on soil organic matter fractions and biological activity in Vertisols under a long-term fertilization experiment.

METHODS

The Long-Term Fertilizer Experiment was started in 2006-07 at the Research Farm of Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India (76°46' E longitude, 19°16' N latitude and an elevation 408.46 m above mean sea level). The farm is in a semiarid tropic region with hot summers and mild winters and the annual maximum temperature during study years (2011-12 to 2012-13) ranged from 29.2 to 41.3°C and minimum temperature ranged from 8.8 to 26.9°C in the month of December and May in the year 2011-12, whereas in 2012-13, the maximum and minimum temperature varied between 29.1°C to 42°C and 9.2°C to 27.8°C. Total annual rainfall was 636 and 720.5 mm for 2011-12 and 2012-13, respectively. The soil of the experimental site was a Vertisol, particularly montmorillonitic, hyperthermic family of *Typic Haplustert* having an initial pH 8.18, EC 0.243 dS m^{-1}, organic carbon 5.50 g kg^{-1}, available N 216 kg ha^{-1}, available P_2O_5 18.32 kg ha^{-1}, available K_2O 766.15 kg ha^{-1}, microbial biomass carbon 235.71 µg g^{-1}, microbial biomass nitrogen 42.53

µg g^{-1}, CO_2 evolution 51.18 mg 100^{-1}g soil 24 hr^{-1}, dehydrogenase enzyme activity 42.51 µg TPF g^{-1}soil 24 hr^{-1}, acid phosphatase enzyme activity 65.10 and alkaline phosphatase enzyme activity 65.10 µg p-NP g^{-1}soil hr^{-1} and urease activity (53.20 µg NH_4-N g^{-1} 24 hr^{-1}). Initial status of organic matter fractions found were humin (0.471 g $100g^{-1}$ soil humus), humic acid (0.295 g $100g^{-1}$ soil humus), fulvic acid (0.173 g $100g^{-1}$ soil humus) and humic acid : fulvic acid (HA:FA) ratio (1.705). Though, the experiment conducted during 6th and 7th cycle (2011-12 and 2012-13) for comparative study of residual effect, the initial status with respect to humus fractions (soil humin, humic acid, fulvic acid and humic acid: fulvic acid ratio) (Table 1), organic carbon, bacteria, actinomycetes, fungi, soil microbial biomass carbon, soil microbial biomass nitrogen, dehydrogenase enzyme activity, acid and alkaline phosphatase enzyme activity were found significant (Table 2). The present experiment was framed in randomized block design (RBD) with twelve treatments and four replications in a soybean-safflower cropping system. The treatments comprises T_1–50% NPK, T_2–100% NPK, T_3–150% NPK, T_4–100 % NPK+ hand weeding, T_5–100% NPK + $ZnSO_4$ at 25 kg ha^{-1}, T_6–100% NP, T_7–100% N, T_8–100% NPK+FYM at 5 Mg ha^{-1}, T_9–100% NPK-Sulphur, T_{10}–Only FYM at 10 Mg ha^{-1}, T_{11}–Absolute control and T_{12}-Fallow. The crops soybean (cv. JS-335) and safflower (cv. PBNS-12) were raised during *kharif* (rainy) and *rabi* (post rainy) seasons respectively using recommended practices. Soybean and safflower crops were sown with 45 x 5 cm and 45 x 10 cm spacing between rows and plants respectively. The 100% NPK recommended dose applied to the crops was 30:60:30 kg ha^{-1} for soybean and 60:40:00 kg ha^{-1} for safflower. The fertilizers used were urea, single super phosphate (SSP) and muriate of potash. FYM was applied 15 days before sowing only for *kharif* (rainy) crop and NPK applied were applied as per treatments, whereas in treatment (T_9) diammonium phosphate was used in place of single super phosphate (SSP) to avoid sulphur application. In T_4 treatment only two hand weeding were undertaken for weed control, without use of any herbicide. Inorganic micronutrients were applied through chemical fertilizers ($ZnSO_4.5H_2O$) and FYM was incorporated at 5 Mg ha^{-1} at sowing time in *kharif* (rainy) season only. After harvest of 6th and 7th cycle of soybean-safflower cropping sequence, moist soils were collected from each plotat 0-15 cm depth and kept refrigerated in polythene bags. After processing a suitable amount (500 g) of soil was stored in an incubator at 30^0C for biochemical analysis.

Humus fractions were estimated as outlined by Stevenson (1982). Measurement of soil microbial populations (bacteria, fungi and actinomycetes) was carried out by standard serial dilution plate technique using selective media: agar medium for bacteria; Bengal rose medium for fungi; and Ken knight agar medium (Dhingra and Sinclair 1993) for actinomycetes. Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) determination were made using the chloroform fumigation technique as described by Vance *et al.* (1987) and Brookes *et al.* (1985). CO_2 evolution of soil was determined by the alkali trap method as given by Anderson (1982). Phosphomonoesterases (acid and alkaline phosphatase) activity in soil determines the enzymatic hydrolysis of p-nitrophenyl phosphate to p-nitrophenol which was extracted by $CaCl_2$–NaOH solution (Tabatabai and Bremner 1969). Dehydrogenase enzyme activity in soil (Klein *et al.* 1971) was determined by triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC). Urease activity was determined by assay method as described by Pancholy and Rice (1973). A representative portion of each soil sample was air dried, powdered and passed through 0.2 mm sieve for determination of organic carbon, pH, EC, and available NPK. The analysis was carried out as per methods described by Jackson (1973).

The experimental data were subjected to (one-way directions) analysis of variance (ANOVA) and treatment means were compared, significant differences were tested at P=0.05 using randomized block design (RBD) as described by Panse and Sukhatme (1985) using the statistical package for general block designs analysis MAUSTAT software (MAU, Institute).

RESULTS AND DISCUSSION

Humus fractionation

Humin

The perusal of pooled data on humin content in soil under soybean-safflower cropping system indicates significantly higher humin in treatment T_8 (0.561 g $100g^{-1}$soil humus) receiving optimal dose of organic manure along with chemical fertilizer (i.e.100% NPK+FYM at 5 Mg ha^{-1}) over its before sowing data (Table 1) and initial status of humin in soil (2006-07). However, lower humin content in soil was observed in absolute control treatment T_{11} (0.427 g $100g^{-1}$soil humus). There was a gradual increase in soil humin content with the application of NPK from 50% to 150% of NPK. The improvement in humin fractions with NPK + FYM might be due to high C: N ratio of FYM resulting in higher organic carbon content in soil leading to greater humus formation. Addition of organic manure could serve as source of humus. At the same time, compounds utilized by micro-organism (cellulose, hemicelluloses, mono and disaccharides, glucosides, amino acids etc.) are indirect source of humic substances, being converted in to microbial plasma, which then participate in the formation of humic substances (Kononova 1966). In case of inorganic fertilizer, use of 150% NPK showed pronounced effect on humin in soil due to addition of root residues consequent to higher biomass yield. Gathala *et al.* (2007) in long-term fertilizer experiment noted that the contents of humin, humic acid and fulvic acid in the soil were significantly increased with the application of fertilizer and FYM which could be due to the improved soil organic matter and conducive environment for the formation of humic acid in *Typic Haplusteps*.

Humic acid

Conjoint use of organic manure and fertilizer showed significant improvement in humic acid over its before sowing data (Table 1) and statistically different from initial status of humic acid in soil (2006-07). he pooled data on humic acid content in soil under soybean-safflower cropping system showed significantly (p=0.05) highest value (0.369 g $100g^{-1}$soil humus) with the application of 100% NPK+FYM at 5 Mg ha^{-1} and lowest amount of humic acid (0.249 g $100g^{-1}$soil humus) was noted in absolute control (T_{11}). The application of 100% N alone was noted having higher humic acid in soil than control treatment, whereas significantly lower humic acid in soil as compare to other treatments were also recorded. Significantly more humic acid content in soil after the harvest of soybean-safflower cropping system was noted in T_8 might be due to applied NPK + FYM resulted higher turnover of organic : matter in soil. The continuous application of 100% N alone and 50% NPK caused an increase in humic acid over

Table 1. Humus fractionation as influenced by different nutrient management systems under soybean-safflower cropping sequence

Treatment details	Before sowing				After harvest (Pooled data)			
	Humin	Humic acid	Fulvic acid	HA: FA ratio	Humin	Humic acid	Fulvic acid	HA:FA ratio
T_1-50%NPK	0.463	0.261	0.140	1.869	0.478	0.299	0.168	1.779
T_2-100%NPK	0.498	0.285	0.169	1.695	0.513	0.323	0.190	1.697
T_3-150%NPK	0.524	0.332	0.218	1.524	0.552	0.362	0.243	1.490
T_4-100%NPK+HW	0.507	0.303	0.181	1.681	0.519	0.331	0.209	1.585
T_5-100%NPK+Zn	0.515	0.318	0.204	1.569	0.529	0.340	0.219	1.552
T_6-100%NP	0.478	0.284	0.154	1.846	0.500	0.315	0.180	1.751
T_7-100%N	0.433	0.242	0.123	1.975	0.448	0.270	0.140	1.929
T_8-100%NPK+FYM	0.549	0.346	0.229	1.513	0.561	0.369	0.250	1.478
T_9-100%NPK-S	0.482	0.283	0.156	1.818	0.497	0.311	0.175	1.778
T_{10}-FYM	0.510	0.301	0.172	1.754	0.522	0.326	0.198	1.649
T_{11}-Control	0.413	0.220	0.103	2.140	0.427	0.249	0.120	2.081
T_{12}-Fallow	0.424	0.231	0.114	2.035	0.448	0.264	0.131	2.013
Mean	0.483	0.284	0.163	1.785	0.500	0.313	0.185	1.732
SE ±	0.0047	0.0026	0.0038	0.045	0.0017	0.0013	0.0011	0.013
CD at (P=0.05)	0.0135	0.0074	0.0109	0.131	0.0051	0.0038	0.0033	0.039

CD, Critical differences at (p=0.05), Humin (g $100g^{-1}$ soil humus); HA, Humic acid (g $100g^{-1}$ soil humus); FA, Fulvic acid (g $100g^{-1}$ soil humus).

control but response exhibited declining humic acid with time due to imbalanced use of fertilizer dose (i.e. 50 % NPK, 100 % N only, 100 % NP respectively) and continuous nutrient mining from soil. However, 150% NPK (T_3) treatment showed quite near values with optimal dose of fertilizer application (NPK+FYM) which may be due to addition of root residues consequent to higher biomass yield and have produced more amount of humic acid and also be due to decomposition of added residue to constitute part such as lignin derived phenolic unit, carbohydrates or amino compounds as building blocks or substrate for humus formation. The content of humic acid was higher than fulvic acid regardless of different treatments. Fulvic acids, although primarily considered to be humic acid precursors, may be humic acid degradation products as well. It is probable that fulvic acid can be absorbed on to clay, but the size of their molecules suggested that the force of attraction would be less than those for longer humic acid constituents (Anderson 1979). Santhy *et al*. (2001) reported that the content of humic acid in soil increased with increasing levels of chemical fertilizers from 50 to 150% and the highest content was recorded with the application of 100% NPK+FYM which could be due to conducive environment for the formation of humic acids in this treatment. In 150% treatment addition of root residue consequent to higher yield have produced more amount of humic acid in *Vertic Ustopept*. Gathala *et al*. (2007) also reported that application of chemical fertilizer alone (150% NPK) and integrated use of FYM and 100% NPK produced more amount of humic acid in *Typic Haplusteps*. Similarly, Bhoye *et al*. (2011) found increased humic acid in soil due to addition of 10 tonnes fully decomposed FYM and 10 tonnes partially decomposed FYM with recommended dose of fertilizer (RDF) and these treatments were significantly superior over rest of the treatments.

Fulvic acid

Pooled data given in Table (1) on fulvic acid content in soil under soybean-safflower cropping system indicates significantly ($p < 0.05$) highest value (0.250 g $100g^{-1}$soil humus) with the continuous application of balanced fertilizers along with organic manure i.e.T_8-100% NPK+FYM at 5 Mg ha^{-1} over control T_{11} (0.120 g $100g^{-1}$soil humus). Application of super optimal dose of fertilizer (150% NPK) was found having considerably nearby values with applied 100% NPK+FYM treatments. It seems that the fulvic acids are simpler in nature and being more stable and found to be more resistance to coagulating action of electrolyte than humic acid (Kononova 1966). Further, significant reduction in fulvic acid was observed under nitrogen alone (100% N) and unfertilized treatments, this may occurred due to heavy removal of nutrients by the crops, therefore less contribution of organic matter in soil and in absence of organic carbon supplementation through external source. Santhy *et al*. (2001) and Gathala *et al*. (2007) noted that the integrated use of organic manures and inorganic fertilizer produced more amount of fulvic acid in *Vertic Ustopept* and *Typic Haplusteps* respectively under long term experiments in India. Application of 10 tonnes fully decomposed FYM and 10 tonnes partially decomposed FYM with RDF significantly improved the fulvic acid in soil (Bhoye *et al*. 2011).

Humic acid : Fulvic acid (HA: FA) ratio

Applying optimal dose of NPK fertilizer along with organic manure annually (100% NPK + FYM) significantly (p=0.05) reduced HA : FA ratio in soil (1.478) for pooled data of soybean-safflower cropping system over its before sowing (1.513) (Table 1) and initial status of soil (1.705) (2006-07). The considerable improvement in HA : FA ratio with the application of super optimal dose of chemical fertilizer (150% NPK) was also observed. However, wide HA: FA ratio was recorded in absolute control T_{11} (2.081) treatment. A combined application of FYM + fertilizer in the present study provided evident that balanced supply of nutrients and carbon reflected in terms of development in HA: FA ratio of soil. It became narrower due to a decrease of total humic acid content, primarily due to decreased Ca-humate content, an increase in the amount of strongly bound humic and fulvic acids i.e. positive association between fulvic acid and total organic matter (Gathala *et al*. 2007). The increase in magnitude of organic matter fractions might have been due to faster rate of decomposition and mineralization owing to higher temperature of the surface soil in tropical regions (Santhy *et al*. 2001).

Soil organic carbon (SOC)

Two years residual pooled soil organic carbon data (Table 3) on soybean-safflower system

Table 2. Soil biological properties status before conduct of the experiment under soybean-safflower cropping sequence (2011-12)

Treatment details	SOC	Bacteria	Fungi	Actino	SMBC	SMBN	CO_2	DHA	Acid Phosphatase	Alkaline Phosphatase	Urease activity
T_1-50%NPK	5.68	133.40	5.46	33.86	226.66	41.23	42.31	36.97	57.63	131.07	50.1
T_2-100%NPK	5.87	143.86	6.83	38.62	247.60	46.66	46.47	41.59	62.41	132.42	51.96
T_3-150%NPK	6.16	142.53	8.19	45.87	293.23	51.96	57.43	45.56	67.99	149.90	63.2
T_4-100%NPK+HW	5.71	175.20	7.01	39.44	252.58	48.78	48.41	41.93	63.20	137.59	56.31
T_5-100%NPK+Zn	5.93	154.47	7.43	43.69	275.63	51.19	50.29	42.35	66.04	143.63	60.08
T_6-100%NP	5.35	134.95	5.61	36.87	237.02	43.71	44.86	38.98	60.58	131.79	54.61
T_7-100%N	5.26	124.62	5.33	32.53	213.22	38.96	37.27	34.79	56.92	128.83	49.15
T_8-100%NPK+FYM	6.71	211.06	8.22	53.16	295.13	52.71	58.89	50.56	69.06	150.04	64.75
T_9-100%NPK-S	5.63	141.41	5.99	40.24	233.79	50.02	43.45	39.54	65.62	133.35	55.35
T_{10}-FYM	6.11	198.69	11.17	51.36	287.19	43.81	54.49	42.23	60.68	135.59	57.93
T_{11}-Control	5.19	95.26	5.06	30.05	200.97	31.84	37.44	32.78	52.41	126.54	47.65
T_{12}-Fallow	5.60	103.41	5.28	31.38	221.14	32.07	35.85	33.84	53.27	126.78	47.92
Mean	5.77	146.57	6.80	39.75	248.68	44.41	46.43	40.09	61.32	135.63	54.92
SE +	0.219	3.40	0.084	0.80	2.02	0.82	0.81	0.79	0.81	1.02	1.23
CD at (P=0.05)	0.608	9.78	0.242	2.314	5.83	2.36	2.34	2.28	2.34	2.93	3.60

CD, Critical differences at (p=0.05), SOC, Soil organic carbon (g k g^{-1}), Bactria (CFU X 10^7 g^{-1} soil); Fungi (CFU X 10^4 g^{-1} soil); Actino, Actinomycetes (CFU X 10^6 g^{-1} soil); SMBC, Soil microbial biomass carbon (µg g^{-1}); SMBN, Soil microbial biomass nitrogen (µg g^{-1}); CO_2, CO_2 evolution in soil (mg 100^{-1} soil 24 hr^{-1}); DHA, Dehydrogenase enzyme activity in soil (µg TPF g^{-1} soil 24 hr^{-1}); Acid and alkaline phosphatase enzyme activity in soil (µg p-NP g^{-1} soil hr^{-1}) Urease enzyme activity (µg NH_4-N g^{-1} 24 hr^{-1}).

revealed that, the SOC was significantly (p=0.05) increased in treatment T_8 (6.65 g kg^{-1}) receiving 100% NPK + FYM at 5 Mg ha^{-1} and it was found at par with treatment T_3 (6.50 g kg^{-1}) and T_{10} (6.52 g kg^{-1}) treated with 150% NPK and only FYM at 10 Mg ha^{-1} respectively, whereas lowest value of SOC (5.47 g kg^{-1}) was noted in absolute control (T_{11}). In long term fertilizer experiments decline in SOC was observed with application of 50% NPK, 100% N alone, 100% NP and unfertilized plots over the years. Organic carbon build up in T_8 could be ascribed to the contribution from annual use of organic manure at FYM 5 Mg ha^{-1} along with 100% NPK during the period of experimentation and also due to increased levels of fertilizer application which might have helped in increasing the organic carbon content in soil and also due to the increased contribution from the dry leaves, roots, stubbles and other crop biomass. Katkar *et al.* (2011), Arbad and Syed Ismail (2011), Vyas and Khandwe (2012), Shilpa Babar and Dongale (2013) also reported significant improvement in soil organic carbon with the application of organic, inorganic and organic with inorganic sources of nutrients as compared to control treatments in long term fertilizer experiment carried out in different Indian locations.

Microbial population (Bacteria, fungi and actinomycetes)

The continuous use of organic manure along with balance fertilization significantly (p=0.05) increased microbial population in this Vertisol. As per the pooled result recorded in Table (3), the bacteria and actinomycetes population in soil after harvest of soybean-safflower system was found significantly maximum (229.42 CFU x 10^7 and 54.53 CFU x 10^6 g^{-1} soil, respectively) when treated with 100% NPK+FYM at 5 Mg ha^{-1} as compare to other treatments. However, fungal population in soil was noted significantly more (10.04 CFU x 10^4 g^{-1} soil) with addition of FYM at 10 Mg ha^{-1} and it was found statistically at par (8.68 CFU x 10^4 g^{-1} soil) with 100% NPK+FYM at 5 Mg ha^{-1}. The application of 100% N and control treatment recorded lower values of bacteria, actinomycetes and fungi over respective treatments. The organic addition coupled with NPK fertilizer exerted a stimulating influence on the preponderance of soil microbial community viz., bacteria, actinomycetes and fungi. This might be ascribed to the decomposed food material available from organic sources. The relatively higher rate of multiplication of bacteria and actinomycetes was associated with FYM, which might be due to the ready source of carbon from FYM that acts as substrate for stimulation of bacterial and actinomycetes growth. The results indicated that the application of FYM in combination with chemical fertilizers proved better than use of only chemical fertilizers in helping the multiplication of bacterial and actinomycetes population (Selvi *et al.* 2004). However, application of inorganic fertilizers also leads to an improvement in fungal population over control. Such increase was not purely due to nutrient response but it was due to microbial oxidation of ammonium salt present in inorganic fertilizers which leads to formation of nitric acid pre disposing condition for fungal proliferation. Arbad and Syed Ismail (2011), Singh and Dhar (2012) also noted the maximum bacterial, fungal and actinomycetes populations with the combined application of organic and inorganic.

CO_2 evolution

The release of soil CO_2 due to different nutrient management supply system showed significant ($p < 0.05$) effect of these management practices on CO_2 evolution as compare to soil samples before conduct of experiment and their initial status (Table 2, 3). Consistently higher CO_2 evolution as recorded (56.40 mg 100^{-1}g soil 24 hr^{-1}) in the soils from optimal dose of fertilizer along with organics i.e.100% NPK + FYM. However, decline in CO_2 evolution of soil (32.95 mg 100^{-1}g soil 24 hr^{-1}) was noticed in absolute control and also due to the lower soil organic carbon content. Apparently the higher stress due to inadequate nutrient supply in control super optimal fertilizer level restricted crop production and thus carbon substrate (root exudates) with consequent reduction in CO_2 evolution. Soil respiration is evaluation of soil biological activity and extent of organic matter decomposition. Similar results were reported by Malewar *et al.* (1999), Suresh *et al.* (2012) in long term fertilizer experiments in India with the combined application of organic manure and indicated that the nutrient turn over at higher carbon expenses met through added organic carbon. Thus, the increased microbial biomass metabolically

Table 3. Soil biological properties as influenced by different nutrient management system under soybean-safflower cropping sequence (Pooled data of two years)

Treatment details	SOC	Bacteria	Fungi	Actino	SMBC	SMBN	CO_2	DHA	AcidPhosphatase	Alkaline Phosphatase	Urease activity
T_1-50%NPK	5.90	134.60	5.78	32.16	223.03	38.07	39.63	37.97	62.00	137.99	52.51
T_2-100%NPK	6.27	146.60	6.69	35.02	238.98	43.98	43.10	41.76	66.03	142.85	56.26
T_3-150%NPK	6.50	150.52	8.03	43.13	292.20	51.01	54.49	46.50	73.01	157.36	67.22
T_4-100%NPK+HW	6.28	175.57	7.01	37.32	248.89	45.86	45.28	41.13	66.83	145.62	60.59
T_5-100%NPK+Zn	6.28	151.89	7.26	40.08	270.68	48.27	47.82	42.32	68.68	149.10	63.92
T_6-100%NP	5.74	142.24	5.92	33.11	232.09	40.99	41.95	39.18	64.44	141.63	57.98
T_7-100%N	5.58	120.17	5.77	30.12	208.19	35.96	36.49	36.14	58.71	134.09	51.51
T_8-100%NPK+FYM	6.65	229.42	8.68	54.53	300.10	52.32	56.40	51.07	76.08	160.65	68.59
T_9-100%NPK-S	5.86	148.32	5.97	39.43	230.83	48.94	41.16	39.73	63.44	140.44	59.00
T_{10}-FYM	6.52	205.42	10.04	50.43	284.24	42.71	52.16	44.53	66.98	145.48	61.94
T_{11}-Control	5.47	112.16	4.78	26.22	199.95	30.50	32.95	32.99	55.48	131.10	48.87
T_{12}-Fallow	5.62	100.43	5.09	28.39	213.99	32.50	34.82	35.30	57.28	133.30	49.85
Mean	6.06	151.44	6.75	37.49	245.26	42.59	43.85	40.72	64.91	143.30	58.19
SE ±	0.055	3.37	0.48	0.49	1.93	0.55	0.48	0.42	0.62	0.59	1.01
CD at (P=0.05)	0.158	9.69	1.40	1.42	5.55	1.59	1.40	1.23	1.78	1.72	2.91

CD, Critical differences at (p=0.05), SOC, Soil organic carbon (g k g^{-1}), Bactria (CFU X 10^7 g^{-1} soil); Fungi (CFU X 10^4 g^{-1} soil); Actino, Actinomycetes (CFU X 10^6 g^{-1} soil); SMBC, Soil microbial biomass carbon (µg g^{-1}); SMBN, Soil microbial biomass nitrogen (µg g^{-1}); CO_2, CO_2 evolution in soil (mg 100^{-1} soil 24 hr^{-1}); DHA, Dehydrogenase enzyme activity in soil (µg TPF g^{-1} soil 24 hr^{-1}); Acid and alkaline phosphatase enzyme activity in soil (µg p-NP g^{-1} soil hr^{-1}, Urease enzyme activity (µg NH_4-N g^{-1} 24 hr^{-1}).

active could have resulted in increased soil respiration rate in Vertisols (Haider and Martin, 1988).

Soil microbial biomass carbon (SMBC)

As per the pooled data recorded in Table 3, application of organic manures, chemical fertilizers and their combinations also significantly ($p=0.05$) affected the soil microbial biomass carbon (Table 2). Long term integrated use of organic manure and fertilizer (NPK + FYM) considerably influenced the SMBC (300.10 $\mu g\ g^{-1}$). However, continuous growing of soybean-safflower without application of any fertilizers (absolute control) caused decline in SMBC (199.95 $\mu g\ g^{-1}$). The readily available carbon fraction of farm yard manure supported the development of microbial biomass. The increase in SMBC might be due to the supply of additional mineralizable and readily hydrolyzable carbon due to organic manure application resulted in higher microbial activity and in turn higher microbial biomass carbon. Selvi *et al.* (2004), Padmavathi *et al.* (2012) and Suresh *et al.* (2012) also reported that soil microbial biomass carbon was gradually increased with the graded levels of NPK from 50% to 150%. They also reported that among the treatments, the highest soil microbial biomass C was noted with integrated nutrient management treatment i.e. NPK+FYM.

Soil microbial biomass nitrogen (SMBN)

The results of pooled data(Table 3) indicated that the mean soil microbial biomass nitrogen (SMBN) was found significantly($p < 0.05$) increased (52.32 $\mu g\ g^{-1}$) with 100 % NPK + FYM at 5 Mg ha^{-1} which was closely followed by super optimal dose of fertilizer i.e. 150% NPK (51.01 $\mu g\ g^{-1}$) and these treatments were found to be at par with each other. The decrease in soil microbial biomass nitrogen (SMBN) was recorded in treatment T_{11} (30.50 $\mu g\ g^{-1}$) absolute control. However, graded dose of NPK and NPK with hand weeding also significantly increased the soil microbial nitrogen over absolute control. The availability of nutrients to crop from FYM is generally lower than nitrogen from inorganic fertilizers because of the slow releases or organically bound N and the volatilization of NH_3 from the manure, especially in alkaline soil. Therefore, a combine use of organic manure and inorganic fertilizer (i.e. 100% NPK +FYM at 5 Mg ha^{-1}) in the present study apparently provided supply of nutrients in balanced proportion resulted in greater amount of soil microbial biomass nitrogen. Application of only N, NP alone and 50% NPK recorded declining trend due to the use of unbalanced nutrients. Thus, it is quite apparent that there exist greater turnover and microbial activity at harvest period when nutrient assimilation is optimum. In contrast biomass nitrogen contributes to crop productivity by releasing nutrients and application of manures with fertilizers promoted this effect more strongly than application of NPK alone (Rita Patil and Puranik 2001). Similar results were also reported by Selvi *et al.* (2004), *Padmavathi et al.* (2012), Suresh *et al.* (2012) in long-term experiments under different cropping systems.

Dehydrogenase enzyme activity

The pooled data on dehydrogenase activity in soil (Table 3) after harvest of soybean-safflower system indicates that the conjoint application of inorganic fertilizer and organic manure (i.e. 100% NPK + FYM at 5 Mg ha^{-1}) significantly ($p=0.05$) increased dehydrogenase enzyme activity in soil (51.07 μg TPF g^{-1}soil 24 hr^{-1}). But, the enzyme activity was drastically reduced in absolute control (32.99 μg TPF g^{-1}soil 24 hr^{-1}) and improved slightly with increasing fertilizer dose. The greater enzymes activity reflected due to increased organic carbon content in soil. The activity of enzymes can be attributed to microbial origin developed during decomposition of organics which acts as good source of carbon and energy to heterotrophs by which their population increased with increase in enzyme activities. The values of enzyme activity were significantly lower in all the treatments of imbalanced fertilization (N alone and 50% NPK). Manna *et al.* (1996) reported significantly positive correlation between soil organic carbon and dehydrogenase activity in *Typic Haplustert*. The stronger effects of FYM on dehydrogenase activity might be due to more easy decomposition of organic matter and due to the increase in microbial growth with addition of carbon substrate (Nath *et al.* 2012; Singh and Dhar 2012).

Acid phosphatase enzyme activity

After harvest of soybean-safflower cropping sequence the soil samples were analyzed for acid phosphatase activity and the pooled data of two years is presented in (Table (2, 3). The results indicate that there was a statistically significant difference in acid phosphatase enzyme activity of soil with the application of NPK + FYM and significantly increases over it have before conducted the experimental data (2012) and initial status (2006-07). The significantly (p=0.05) maximum acid phosphatase enzyme activity (76.08 µg p-NP g^{-1}soil hr^{-1}) was recorded with the combined application of organic manure and fertilizer (100% NPK + FYM) treatment, whereas minimum acid phosphatase enzyme activity was noted in absolute control (55.48 µg p-NP g^{-1}soil hr^{-1}). The applied organic manure along with mineral fertilizer were able to get nutrient mineralized more rapidly, hence there was more mineralization than immobilization which consequently provide sufficient nutrition for the proliferation of microbe and their activities in terms of acid phosphatase enzyme. Significantly more phosphatase enzyme activity in soil was recorded in u balanced fertilized plot (i.e. T_8). Similar result was reported by Santhy *et al.* (2004), Rai and Yadav (2011) in long-term fertilizer experiments with the combined application of NPK + FYM. Long term effect of incorporation of organic source along with fertilizers resulted greater amount of carbon input, root exudation to the soil, enhanced humus content, abundance of carbohydrates coupled with greater microbial activities which pronounced increase in phosphatase enzyme activities in soil (Vandana *et al.* 2012).

Alkaline phosphatase enzyme activity

Application of FYM in combination with inorganic fertilizer also enhanced the alkaline phosphatase enzyme activity in Vertisol. As per pooled means of two years data (Table 3), significantly (p=0.05) highest enzyme activity was recorded in treatment T_8 (160.65 µg p-NP g^{-1}soil hr^{-1}) receiving 100% NPK + FYM at 5 Mg ha^{-1}. However, the lowest value of alkaline phosphatase enzyme activity was noticed in existing T_{11} (131.10 µg p-NP g^{-1}soil hr^{-1}) i.e. control treatment. Among all the treatments, balance application of organic manure with inorganic fertilizer (NPK+FYM) showed superiority as compare to application of fertilizer alone. In case of inorganic applications, super optimal dose of fertilizer (150% NPK) showed its significantly superior effect over rest of the treatments might be due to addition of higher amount of root residue for building of soil organic carbon. Similar results were reported by Kanchikerimath and Dhyan Singh (2001) in long term fertilizer experiments, they found that alkaline phosphatase activities were increased significantly with the addition of balanced nutrients and manure in *Typic Haplusterts*. Rai and Yadav (2011) also reported the higher activities of alkaline phosphatase in the organically treated soils due to manure input over the years.

Urease enzyme activity

Long-term balance applications of organic manuring and inorganic fertilization have been consistently significant improvement in urease enzyme activity in soil over its initial status and control (Table 3). The higher (68.59 µg NH_4-N g^{-1} 24 hr^{-1}) urease activity 1, 3 were recorded with the application of 100% NPK + FYM@ 5 Mg ha^{-1} followed by 150% NPK (67.22 µg NH_4-N g^{-1} 24 hr^{-1}) which were found to be at par with each other. It might be due to the improvement in organic matter status of soils which was in turn reflected in the higher enzymatic activity (Kanchikerimath and Singh 2001). Mishra *et al.* (2008) also observed that a gradual enhancement in urease enzyme activity as recommended fertilizer dose increased from 50% to 100% over control.

CONCLUSION

It can be concluded from the above findings that in continuous cropping with soybean-safflower system over 6th and 7th years, conjoint use of organic manure along with 100% NPK was found significantly superior over rest of the treatments with respect to humus fractions (humin, humic acid, fulvic acid and HA: FA ratio), organic carbon, bacteria, actinomycetes, soil microbial biomass carbon, soil microbial biomass nitrogen, dehydrogenase enzyme activity, acid and alkaline phosphatase enzyme activity and urease enzyme activity in soil to buildup biological fertility of soil. Amongst different nutrient management supply systems, application of only chemical fertilizers 150% NPK was also noted better option for nutrient management through inorganics.

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