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Long-term Effect of Nutrient Management on Soil Biochemical Properties in a Vertisol under Soybean–Wheat Cropping Sequence

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The present investigation was conducted during 2013-14, under an ongoing long-term fertilizer experiment (LTFE) with soybean-wheat cropping system, since 1972 at the Research Farm of Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India, under the aegis of All India Coordinated Research Project on Long-term Fertilizer Experiments. The chemical and biological properties of soil under different nutrient combinations were studied in the 41-year old LTFE in a Vertisol. The experiment was conducted in a randomized block design comprising of eight treatments *i.e.* 50% NPK, 100% NPK, 150% NPK, 100% NP, 100% N, 100% NPK+ farmyard manure (FYM) @ 15 t ha^{-1} yr^{-1}, 100% NPK–S and unfertilized-control, with four replications. Soil samples from all treatments were collected from 0–15 cm depth and biochemical properties were determined. Significant increase in soil organic carbon was recorded with 100% NPK + FYM @15 t ha^{-1}. The availability of N, P, K, S, soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) were increased significantly with the integrated application of fertilizers and FYM over control and other fertilizer treatments. Further, the conjoint use of fertilizers and FYM was also significantly superior to other treatments in terms of activities of soil enzymes like dehydrogenase, acid phosphatase, alkaline phosphatase and β-glucosidase. The correlation studies revealed significant positive relationship of available N, P, K and S, SMBC, SMBN, dehydrogenase activity, alkaline phosphatase activity, acid phosphatase activity and β-glucosidase activity with total productivity of soybean and wheat crops.

Key words: Long-term fertilizer experiment, soil microbial biomass carbon, soil microbial biomass nitrogen, enzyme activity, Vertisol

Soybean (*Glycine max* L.) – wheat (*Triticum aestivum* L.) is one of the most prevalent cropping systems followed in a substantial area of Madhya Pradesh. Long-term fertilizer experiments (LTFE) provide valuable information on effect of continuous application of different levels of fertilizer nutrients alone and in combination with organic manure under intensive cropping on soil fertility and crop productivity. These experiments are of paramount help in monitoring soil fertility changes and solving the complex problems related to the soil fertility management (Thakur *et al.* 2011).

Soil microorganisms are important to agro-ecosystems. They are involved in key roles, such as aggregate formation, humus formation, nutrient cycling, decomposition of various compounds and other transformations in soil (Wu *et al.* 2011). Fertilization usually favours the accumulation of bacterial residues and increases soil microbial biomass. In the long-term, repeated fertilization may result in shifts in the functionality and quality of soils by directly or indirectly changing the soil's physical, chemical, and biological properties as it changes available nutrient level and fertility (Murugan and Kumar 2013). In recent years, components like soil microbial biomass carbon, microbial community structures and functions, and enzyme activities have been used to describe soil quality under different agricultural practices (Vallejo *et al.* 2010). Microorganisms regulate the nutrient flow in the soil by assimilating nutrients and producing soil biomass. The changes in soil organic carbon contents are also directly associated with changes in microbial biomass carbon and biological activity in the soil (Katkar *et al.* 2011).

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As information is lacking on the long-term effect of fertilization and manuring on soil biological and enzymatic activity in a Vertisol under soybean-wheat cropping system, this study has been conducted. The hypothesis of the investigation was that the long-term fertilization and manuring under intensive cropping system may influence the soil chemical, biological and enzymatic activities and ultimately the crop productivity. Therefore, the objectives of the present study were: (i) to evaluate the effect of long-term fertilization on organic carbon, biological properties and enzymatic activities (dehydrogenase, acid phosphatase, alkaline phosphatase and β-glucosidase), and (ii) to assess the effect of fertilization on the productivity of soybean–wheat cropping system in a Vertisol.

Materials and Methods

The experiment was conducted during the 2013-14 after forty-one crop cycles under ongoing LTFE started during 1971-72 with soybean-wheat cropping system at the Research Farm of the Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (23°10′ N and 79°57′ E), Madhya Pradesh, India under the aegis of All India Coordinated Research Project on Long Term Fertilizer Experiments. Jabalpur has a semi-arid and sub-tropical climate characterized by dry summers and cold winters. During winter season (November to February), the temperature ranges from 4 to 33 °C and the relative humidity varies from 70 to 90%. Dry and warm weather usually prevails during the months of March to June. The temperature in the month of May rises as high as 46 °C. Monsoon season extends from mid-June to mid-September. The temperature during this period varies from 25 to 35 °C and the relative humidity ranges between 70 to 80%. The total annual rainfall varies from 1000 to 1500 mm with a mean of around 1350 mm. The length of growing period ranges from 150 to 180 days. The experimental soil is medium black belonging to Kheri series of fine montmorillonitic hyperthermic family of Typic Haplustert (Vertisol). The textural class of soil is clay. The key initial soil properties (0-15 cm depth) are presented in table 1.

The experiment was designed and conducted with eight treatments having four replications arranged in the randomized block design. Two blocks are separated with a gap of 2 m, whereas individual plots (17 m×10.8 m) were separated with a permanent bund. The amounts of nutrients applied in different treatments are given in table 2.

Soybean (var. JS 97 52) was sown in the last week of June to first week of July as a rainfed crop during *kharif*, and wheat (var. GW 366) in the first week to second week of November as an irrigated

Table 1. Initial properties of the experimental soil (1972)

Soil properties	Value	Method
pH (1 : 2.5)	7.60	Jackson (1973)
Electrical conductivity (1 : 2.5) (dS m^{-1})	0.18	Jackson (1973)
Organic carbon (g kg^{-1})	5.7	Walkley and Black (1934)
Alkaline permanganate N (kg ha^{-1})	193	Subbiah and Asija (1956)
Olsen-P (kg ha^{-1})	7.60	Olsen *et al.* (1954)
Ammonium acetate extractable K (kg ha^{-1})	370	Muhr *et al.* (1965)
$CaCl_2$ extractable S (mg kg^{-1})	7.80	Williams and Steinbergs (1959)

Table 2. Treatment details and nutrient application rates (kg ha^{-1}) in soybean and wheat

Treatments	Soybean				Wheat			
	N	P	K	S	N	P	K	S
50 % NPK	10	17.6	8.3	10.2	60	17.6	16.6	10.2
100 % NPK	20	35.2	16.6	20.4	120	35.2	33.2	20.4
150% NPK	30	52.8	24.9	30.6	180	52.8	49.8	30.6
100 %NP	20	35.2	NA	20.4	120	35.2	NA	20.4
100 % N	20	NA	NA	NA	120	NA	NA	NA
100 % NPK+FYM*	110	54	95.1	56.6	120	35.2	33.2	20.4
100 % NPK (-S)#	20	35.2	16.6	NA	120	35.2	33.2	NA
Control	NA	NA	NA	NA	NA	NA	NA	NA

*FYM was applied 15 t ha^{-1} to soybean every year 15–20 days before sowing; # Diammonium phosphate (DAP) was used instead of SSP as source of P; NA : Not Applied.

crop during *rabi*. Wheat was irrigated 3 – 4 times at critical stages of crop growth *i.e.* crown root initiation, tillering/jointing, booting/flowering, and milk and dough stages. Insects and diseases were kept under check following suitable control measures. Soybean and wheat crops were harvested at maturity and yield data were recorded after threshing. Total productivity of crops was estimated by the sum of grain yield of soybean and wheat. The surface soil samples (0 – 20 cm depth) were collected after harvest of wheat crop during 2013-14 and were analyzed for different parameters (OC, available N, P, K and S) by standard procedures. The fresh soil samples collected during grand growth stage of wheat were used for estimating biological properties. Soil microbial biomass carbon (SMBC) was estimated following Jenkinson and Powlson (1976) and soil microbial biomass nitrogen (SMBN) was measured by modified direct extraction method (Jenkinson and Ladd 1981). The soil enzymatic activities like dehydrogenase activity (DHA) was estimated by incubation with triphenyle tetrazolium chloride (TTC) as described by Burns (1978), phosphatase following Tabatabai and Bermner (1969), and β-glucosidase following Eivazi and Tabatabai (1988).

Results and Disccusion

Soil organic carbon

The organic carbon (OC) content ranged between 4.9 to 9.4 g kg^{-1} (Table 3). Soil organic carbon content increased significantly and attained a maximum value of 9.4 g kg^{-1} in the treatment that received 100% NPK along with FYM over initial value of 5.7 g kg^{-1} (1972). This could be ascribed to the organic manure (15 t FYM ha^{-1}) application in combination with fertilizers that increased total N and soil organic matter contents compared with sole fertilizer treatments (Chakraborty *et al.* 2011). Increasing levels of fertilizer application helped in increasing the organic carbon content, which may be ascribed to increase in productivity and incorporation of larger residual biomass through root, leaves, stuble and rhizodeposition (Singh *et al.* 2012). Further, it may also be attributed to addition of FYM which stimulated the growth and activity of microorganisms and better root biomass. Thakur *et al.* (2011) also observed that soil OC levels in Vertisol increased considerably due to long-term fertilization and manuring over a period of 36 years.

Table 3. Effect of continuous application of fertilizers and FYM on soil chemical properties under soybean-wheat cropping system

Treatments	OC (g kg^{-1})	Soil available nutrients ($kg\ ha^{-1}$)			
		N	P	K	S
50% NPK	6.3	214	21.3	217	23.4
100% NPK	7.2	287	32.2	264	37.1
150% NPK	8.1	310	40.2	281	44.8
100%NP	6.7	256	29.2	223	32.9
100% N	5.1	202	12.6	188	11.1
100% NPK+FYM	9.4	329	40.5	312	48.6
100% NPK-S	6.6	259	28.2	213	11.6
Control	4.9	191	11.7	176	11.3
Initial Value (1972)	5.7	193	7.6	370	15.6
CD (P=0.05)	0.82	24.0	3.2	23.0	3.3

Available nutrients

Continuous application of fertilizers and FYM for 41 years under soybean-wheat cropping system led to significant increase in available N, P and S content in soil (Table 3). The highest value of available nutrients (N, P, K and S) was found under conjoint the application of recommended fertilizers and organic manure (100% NPK+15 t FYM ha^{-1}), and the availability of N, P and S increased over their initial content (1972). The increase in available N observed under NPK+FYM may be due to direct addition of organic matter through FYM, helping multiplication of soil microbes and ultimately enhancing the conversion of organically-bound N to mineral form (Singh *et al.* 2012). Availability of P increased to the extent of 51.2 per cent under 100% NPK compared with 50% NPK, and was higher by 24.9 per cent under 150% NPK compared with 100% NPK. The maximum build-up of soil P was observed under NPK+ FYM. This build-up of P in soil is attributed to its addition every year and also solubilization of native P through greater release of organic acids under FYM treated plots (Thakur *et al.* 2011). However, continuous cropping without addition of K and imbalanced fertilization (N and NP) reduced the availability of K compared with initial soil K status, obviously continuous mining of native K pools that also caused reduction in crop yields under these treatments (Sawarkar *et al.* 2013). The availability of S was increased with the addition of S. Thakur *et al.* (2011) found that regular supply of P through single superphosphate since 1972 increased available S content in soil. This increase was further accentuated with inclusion of FYM in fertilization schedule. A significantly higher content of available S was observed with successively higher addition of S from optimal to super-optimal dose which might be

Table 4. Effect of continuous application of fertilizers and FYM on soil microbial biomass carbon and nitrogen, C:N ratio and enzyme activities under soybean-wheat cropping system

Treatments	Soil microbial biomass carbon	Soil microbial biomass nitrogen	C:N ratio	Dehydrogenase activity (μg TPF g^{-1} 24 h^{-1})	Acid phosphatase activity	Alkaline phosphatase activity	β-glucosidase activity
	(μg g^{-1} soil)				(μg p nitrophenol g^{-1} h^{-1})		
50% NPK	232	26.4	8.8	7.8	6.9	16.7	30.1
100% NPK	293	34.2	8.6	8.9	8.2	21.6	35.3
150% NPK	303	36.2	8.4	9.7	8.3	24.7	38.1
100%NP	247	26.7	9.3	8.5	6.9	18.8	31.3
100% N	218	22.3	9.8	7.1	6.2	15.9	29.8
100% NPK+FYM	335	40.2	8.4	10.0	8.5	27.9	45.1
100% NPK-S	282	28.9	9.7	8.8	7.3	19.8	34.2
Control	165	19.3	8.6	6.6	5.6	14.4	25.9
CD (P=0.05)	27.4	4.77	-	1.07	0.95	2.69	3.20

due to the differential conversion of applied S in available S form as a result of varying transformation (Birla *et al.* 2015).

Soil microbial biomass carbon, soil microbial biomass nitrogen and C:N ratio

The conjoint use of fertilizers and organic manure recorded significant increase in biological parameters *viz.* SMBC, SMBN and C:N ratio compared with 100% NPK and 150% NPK through fertilizers without organics (Table 4). This may be ascribed to direct addition of organic matter through FYM and increase in root biomass which helped in growth and development of soil microorganisms causing beneficial effect on SMBC, SMBN and C:N ratio. Application of FYM to soybean during *kharif* season significantly increased SMBC, SMBN and C:N ratio over control which might be due to a steady source of organic carbon to support the microbial community (Bhattacharyya *et al.* 2008). The lowest value of SMBC was observed in the control obviously due to unfavourable environment arising out of depletion of nutrients due to continuous cropping without any fertilization or manuring. The highest SMBC in the integrated nutrient management treatments was due to additional mineralizable and readily hydrolysable carbon from FYM (Verma and Mathur 2009). Similar, findings have also been reported by Chakraborty *et al.* (2011).

The SMBN decreased under 100% NP and 100% N to the tune of 15.5 and 25.5 per cent, respectively compared with 100% NPK indicating necessity of balanced fertilizer application for enhancing soil microbial activity. It was further observed that combined use of 100% NPK + 15 t FYM ha^{-1} increased SMBN by 14.6 per cent compared with NPK indicating beneficial effect of organics in augmenting microbial activity. High soil organic carbon, greater root proliferation and additional supply of N by FYM to microorganisms might be responsible for increasing the level of SMBN (Verma and Mathur 2009). Selvi *et al.* (2004) reported that FYM is not only rich in carbon but also in N and other macro and micronutrients. Similarly, Katkar *et al.* (2011) found that SMBC and SMBN were significantly increased with the integrated use of 100% NPK+10 t FYM ha^{-1} over control and other treatments under sorghum-wheat cropping system after 20 years of experimentation in a Vertisol.

Dehydrogenase activity

The DHA increased with graded levels of fertilizers from 50 to 100% NPK (Table 4). Application of FYM with 100% NPK recorded significantly higher DHA (10.0 μg TPF g^{-1} 24h^{-1}) compared with other treatments. The increase in DHA was to the extent of 12.3 per cent under INM over 100% NPK. The results are in agreement with the findings of Bhattacharyya *et al.* (2008) that reported 4-5 folds increase in DHA due to FYM application along with N or NPK. The addition of FYM coupled with fertilization exerted a stimulating influence on the preponderance of bacteria (Selvi *et al.* 2004). It was significantly higher under 100% NPK (8.9 μg TPF g^{-1} 24 h^{-1}) compared with control (6.6 μg TPF g^{-1} 24 h^{-1}), suggesting importance of balanced fertilisation. Mandal *et al.* (2007) showed that easily decomposable components of crop residues may have a strong effect on DHA and metabolism of soil microorganisms. Similarly, Katkar *et al.* (2011) also reported that the DHA increased by 18.6 per cent due to integrated nutrient management over 100% NPK under 20-year old long-term fertilizer experiment in a Vertisol.

Acid and alkaline phosphatase activity

The data showed significant effect of graded level of fertilizers (50 to 100% NPK) on acid phosphatase activity (Table 4). The highest value for acid phosphatase activity was found in 100% NPK + FYM (8.5 µg *p*-nitrophenol g^{-1} h^{-1}) and lowest in control (5.6 µg *p*-nitrophenol g^{-1} h^{-1}). With regard to alkaline phosphatase activity in soil (Table 4), 100% NPK+FYM treatment showed significantly highest value (27.9 µg *p*-nitrophenol g^{-1} h^{-1}) compared with 100% NPK (21.6 µg *p*-nitrophenol g^{-1} h^{-1}), whereas control plot showed the lowest value (14.4 µg *p*-nitrophenol g^{-1} h^{-1}). Activity of phosphatases is important in studying the P cycle because this can provide a route for P mineralization and plant uptake. However, activities of these enzymes were not persistent, and sometimes appeared contrasting. The acid phosphatase activity was much lower than alkaline phosphatase activity, irrespective of the treatments, which may be due to the alkaline reaction of the soil. Earlier studies also proved that phosphatase activity was strongly influenced by soil pH (Dick 1994). The significantly greater activity of alkaline phosphatase in the FYM treated plots may be due to enhanced microbial activity and perhaps diversity of phosphate solubilising bacteria consequent to manure input over the years (Mandal *et al.* 2007).

β-glucosidase activity

The β-glucosidase activity as influenced by fertilization options is presented in table 4. The highest value of β-glucosidase activity (45.1 µg *p*-nitrophenol g^{-1} h^{-1}) was found associated with 100% NPK + FYM and lowest value (25.9 µg *p*-nitrophenol g^{-1} h^{-1}) was recorded in control. The activity of β-glucosidase increases with organic matter content and this is why it is considered as a very sensitive biological indicator of the effect of soil management practices. The β-glucosidase enzyme activity was greater in the FYM-treated plots compared with the plots receiving sole fertilizers. The application of FYM significantly increased β-glucosidase activity, as the enzyme activity are strongly correlated with soil organic carbon content (Blaise and Rao 2004).

Crop productivity

The grain yield of soybean and wheat and total productivity (grain yield of soybean+wheat) for the year 2013-14 are illustrated in fig. 1. Continuous application of N alone resulted increase in yield over control but the response declined with time due to imbalance use of nutrients (Thakur *et al.* 2011). The grain yields of soybean and wheat were reduced in plots receiving 100% NP compared with 100% NPK treatment, apparently due to continuous omission of K in fertilizer schedule that resulted in mining of this nutrient from the soil reserves. In initial years, however, grain yields under 100% NP and 100% NPK did not differ much, but later on continuous cropping without K caused deficiency of this nutrient, leading to reduction in crop yields (Fig. 1). This advocates for inclusion of K in the fertilizer schedule. The highest grain yield of soybean and wheat (1.49 and 5.27 t ha^{-1}, respectively) was obtained under 100% NPK+ FYM, indicating positive role of FYM

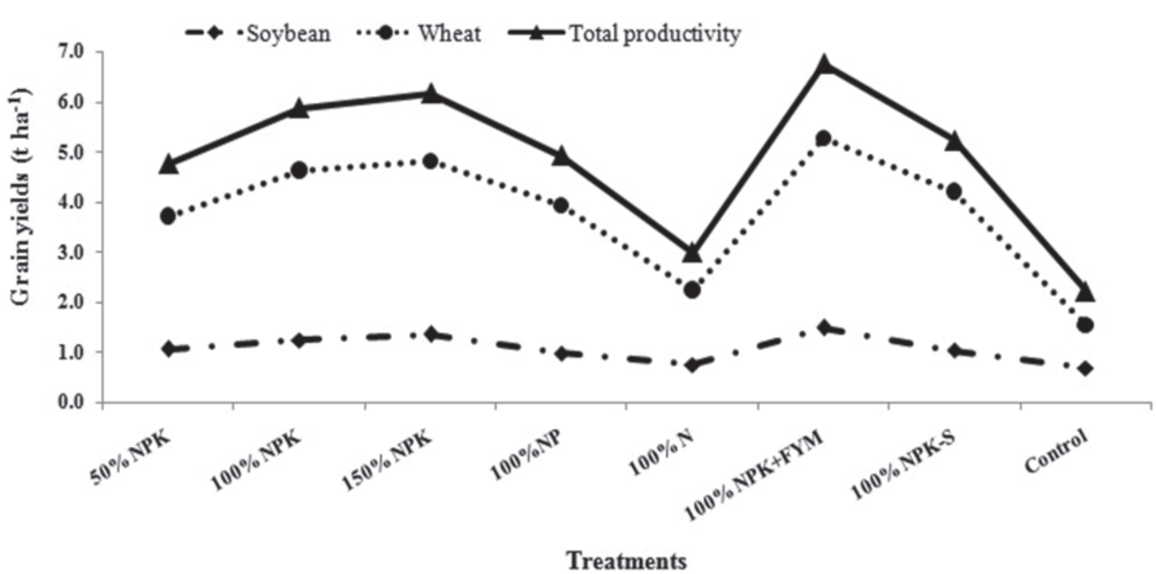

Fig. 1. Effect of nutrient supply options on grain yield of soybean and wheat and total productivity

application in improving the overall soil environment such as improvement in microbial activity and soil physical conditions. This further suggested that the productivity of soybean and wheat could be sustained by integrated use of organics and fertilizers. However, use of FYM alone during *kharif* was unable to sustain high yield levels in the long-run. Similar findings on crop productivity were also reported by Singh *et al.* (2012) and Sawarkar *et al.* (2013).

Relationship of soil properties with crop productivity

A highly significant positive correlation of total productivity was observed with available N (r=0.913**), available P (r=0.981**), available K (r=0.936**) and available S (r=0.944**). Further, the biological parameters *i.e.* SMBC (r=0.949**), SMBN (r=0.952**), dehydrogenase assay (r=0.910**), alkaline phosphatase activity (r=0.909**), acid phosphatase activity (r=0.946**) and β-glucosidase activity (r=0.868**) were also significant and positively correlated with total productivity. This indicates that total productivity of soybean and wheat grown in Typic Haplusterts was enhanced and controlled by these soil parameters (Mandal *et al.* 2007; Katkar *et al.* 2011).

Conclusions

It could be concluded that the long-term application of balanced and integrated use of nutrients (100% NPK, 150% NPK and 100% NPK+FYM) to soybean and wheat significantly improved the soil chemical and biological properties as well as crop productivity. The microbial biomass and soil enzyme activities were controlled by the long-term manure and fertilizer treatments, as the same were highest under NPK+FYM treatment. On the other hand, imbalanced use of nutrients (100% NP and 100% N alone) produced a deleterious effect on chemical and biological properties of soil. The conjoint use of organics and fertilizers was found promising.

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