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Long-Term Effect of Nutrient Management on Soil Microbial Properties and Nitrogen Fixation in a Vertisol under Soybean–Wheat Cropping Sequence

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The present experiment was conducted during 2018-19 under the ongoing All India Coordinated Research Project on Long-term Fertilizer Experiment with soybean-wheat cropping sequence since 1972 at the Research Farm of Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India. The microbial properties and nitrogen (N_2) fixation of soil under different nutrient combinations were studied in a 46-year old long-term fertilizer experiment in a Vertisol. The experiment was conducted in a randomized block design comprising of 10 treatments *viz*., T_1: 50% NPK, T_2: 100% NPK, T_3: 150% NPK, T_4: 100% NPK + Hand weeding (100% NPK+HW), T_5: 100% NPK + zinc (100% NPK + Zn), T_6: 100% NP, T_7: 100% N, T_8: 100% NPK+ 5 t FYM ha^{-1} (100% NPK+FYM), T_9: 100% NPK(–S) and T_{10}: Control, each treatment being replicated four times in a randomized block design. Soil samples from all the treatments were collected from 0–15 cm depth and microbial properties and N_2 fixation of soil were estimated. Significant increase in soil organic carbon, total N and available N were recorded with 100% NPK+FYM. Similarly, the maximum number of nodules and their biomass were also recorded in 100% NPK+FYM treatment at 30, 45 and 60 days after sowing. The bacteria, fungi and actinomycetes population counts in soil were 39.1×10^7, 42.7×10^4 and 39.1×10^5 cfu g^{-1} soil, respectively with the integrated application of inorganic fertilizer and organic manure (100% NPK+FYM) over control (11.7×10^7, 18.5×10^4 and 13.6×10^5 cfu g^{-1} soil, respectively). Further, the conjoint use of balance dose of fertilizers with FYM was also significantly superior in terms of soil microbial biomass carbon and microbial biomass N over other treatments even after 46 years of experimentation. The grain and straw yield of soybean and their N uptake were also significantly increased with graded dose of fertilizers and maximum yield and N uptake were noted in 100% NPK+FYM treatments over control plot.

Keywords: Microbial population, organic carbon, microbial biomass carbon and nitrogen, N_2-fixation, long-term fertilizer experiment, Vertisol

Soybean (*Glycine max* L.) – wheat (*Triticum aestivum* L.) is one of the most prevalent cropping sequences of Madhya Pradesh. Long-term fertilizer experiments (LTFEs) give the valuable information on effect of continuous application of different levels of fertilizer nutrients alone with and without organic manure under intensive cropping on soil fertility and crop productivity. These experiments can be used for precise monitoring of changes in soil fertility and could be of paramount help in solving the complex problems related to the soil fertility management (Dwivedi and Dwivedi 2015). Repeated fertilization under LTFEs may result in shifts of the functionality and quality of soils by directly or indirectly changing the physical, chemical and biological properties of soils as it changes available nutrient level and fertility (Murugan and Kumar 2013).

Microorganisms regulate the nutrient flow in the soil by assimilating nutrients and producing soil biomass. The changes in soil organic carbon (SOC) contents are also directly associated with changes in microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and biological activity in the soil (Katkar *et al.* 2011). The soil microbial diversity is

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the most important functional component of the soil biota (Tate 2000). Microbial adaptation to environmental conditions allows microbial analysis to be discriminating in soil health assessment, and changes in microbial populations and activities may therefore function as an excellent indicator of change in soil health. Soil microorganisms are important to agro-ecosystems. They are involved in key roles, such as soil aggregate formation, soil humus formation, nutrient cycling, decomposition of various compounds and other transformations (Wu *et al.* 2011).

Thus, the present investigation was conducted under ongoing long-term effect of fertilization and manuring on nodule number and its biomass, soil microbial properties and biological N_2 fixation in a Vertisol under soybean-wheat cropping sequence. The hypothesis of the investigation was that the long-term application of balanced fertilization with or without manures under intensive cropping system may influence the soil microbial properties and N_2 fixation and ultimately the crop productivity. Therefore, the objectives of the present study were (i) to evaluate the effect of long-term fertilization on microbial population, MBC, MBN and N_2 fixation, and (ii) to assess the effect of fertilization on crop productivity of soybean–wheat cropping system in a Vertisol.

Materials and Methods

The present study was conducted during 2018-2019 after the 46th crop cycle of soybean-wheat under ongoing All India Coordinated Research Project on Long-Term Fertilizer Experiment (AICRP on LTFE) in a Vertisol which was initiated during 1972 at the Research Farm of the Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (23°10″ N latitude and 79°57″ E longitude). Jabalpur has a semi-arid and sub-tropical climate with a characteristic feature of dry summer and cold winter. In winter season *i.e.* from November to February months, 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 the mean value 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) and had pH of 7.6, electrical conductivity (EC) 0.18 dS m^{-1} (1: 2.5:: soil: water ratio) and SOC 5.7 g kg^{-1}. The available nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) were 193, 7.6, 370 and 15.6 kg ha^{-1}, respectively. Also, at the start of the experiment, the concentrations of soil available zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) in the surface soil were 0.33, 2.47, 16.1 and 0.11 mg kg^{-1} soil, respectively. The experiment includes 10 treatments *viz*., T_1: 50% NPK, T_2: 100% NPK, T_3: 150% NPK, T_4: 100% NPK + Hand weeding (100% NPK+HW), T_5: 100% NPK+ zinc (100% NPK+Zn), T_6: 100% NP, T_7: 100% N, T_8: 100% NPK+ 5 t FYM ha^{-1} (100% NPK+FYM), T_9: 100% NPK(–S) and T_{10}: Control, each treatment being replicated four times in a randomized block design.

Soybean-wheat-maize fodder crop rotation was continued for about 22 years (1972-1993) with the recommended fertilizer applications (100% NPK, 20:80:20, 120:80:40 and 80:60:20 kg ha^{-1}) to the respective crops. However, due to certain constraints, summer maize was discontinued in 1994, and only soybean-wheat cropping sequence could continue further. The sources for N, P and K were urea, single superphosphate (SSP) and muriate of potash (MOP), respectively. In S-free treatment (T9), the diammonium phosphate (DAP) was used instead of SSP as source of P. Zinc was applied @ 20 kg $ZnSO_4.7H_2O$ ha^{-1} to wheat crop in alternate years and its application has been discontinued since *kharif* 1987, as the level of soil available Zn was found to have increased substantially. The application of FYM @ 5 t ha^{-1} yr^{-1} is being practiced only to soybean crop during *kharif* season.

The soil samples were collected after the harvest of wheat crop (2018-19) at 0-15 cm depth from each plot. The SOC was estimated using the method given by Walkley and Black (1934), available N was determined by Subbiah and Asija (1956) and total N was estimated by Bremner (1965). Soil MBC was estimated following Jenkinson and Powlson (1976) and MBC was measured by modified direct extraction method (Jenkinson and Ladd 1981). Total microbial population count (bacteria, fungi and actinomycetes) by serial dilution/pour plate method (Xu and Zheng 1986). Nodulation study was carried out at 30, 45 and 60 days after sowing (DAS) by uprooting 5 plants per plot by placing notch and very carefully taking sample to avoid any losses or damage of nodules. The rhizosphere soil was washed in the running water. Nodules per plant were counted manually. After

Table 1. Effect of long-term application of fertilizers and manure on number of nodules and their dry weight

Treatments	Number of nodules			Biomass of nodules (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
50% NPK	15.3	38.4	21.6	0.11	0.22	0.14
100% NPK	16.0	42.6	24.7	0.14	0.25	0.17
150% NPK	17.3	45.1	26.1	0.15	0.30	0.19
100% NPK+HW	16.5	41.5	24.6	0.13	0.25	0.16
100% NPK+Zn	16.0	41.8	23.8	0.13	0.25	0.16
100% NP	15.3	36.8	22.4	0.12	0.23	0.15
100% N	14.5	31.7	19.0	0.11	0.20	0.13
100% NPK+FYM	20.3	47.6	27.8	0.18	0.32	0.21
100% NPK(-S)	15.5	40.7	23.1	0.12	0.25	0.15
Control	13.8	29.0	16.3	0.10	0.16	0.12
CD (P=0.05)	2.25	4.38	3.52	0.03	0.03	0.02

counting, the nodules were removed from the roots and kept in small paper bags then kept in hot air oven at 70±2 °C for 24 h (till constant weight) to record their dry weight. Grain and straw samples were analyzed for N uptake by exported biomass in each crop by the Kjeldahl method after adding digestion mixture (K_2SO_4 and $CuSO_4$) followed by concentrated sulphuric acid (Piper 1966). All observations recorded for experimentation were analyzed statistically as per standard method as described by Panse and Sukhatme (1970).

Result and Discussion

Nodule Numbers and their Dry Weight at Different Growth Stages

The number of nodules per plant and their dry weight were comparatively higher at 45 DAS as compared to 30 and 60 DAS (Table 1). The nodule numbers per plant was recorded maximum with the treatment of 100% NPK+FYM at different growth stages 30, 45 and 60 DAS having 20.3, 47.6 and 27.8, respectively and minimum in control plot. Thus, the best nodulation occurred at high levels of N, P and S nutrition. Solanki *et al*. (2018) reported that addition of FYM with 100% NPK showed a maximum and significantly higher nodulation over optimal dose.

However, the highest biomass of nodules was observed at 45 DAS due to maximum nodulation in this growth stage of crop (Table 1). The dry weight of nodules per plant was found superior in 100% NPK+FYM (0.32 g) followed by 150% NPK (0.30 g) at 45 DAS, whereas the lowest dry weight of nodules per plant was recorded in control plot (0.16 g) at 45 DAS.

The nodulation was significantly increased in sub-optimal, optimal and super-optimal doses of fertilizer over control because this nodulation process was very sensitive and it resulted into the available levels of nutrient combinations, thereby extreme imbalances of nutrition reduced the counts. Addition of FYM with 100% NPK showed a maximum and significantly higher nodulation over sub-optimal dose. Similar results were reported by Gupta *et al*. (2018).

Microbial Population (Bacteria, Fungi and Actinomycetes) Counts

The variations in the number of bacteria, fungi and actinomycetes among different treatments are presented in table 2. The colony forming unit (cfu) of bacteria, fungi and actinomycetes in all fertilizer treatments were significantly higher over that of control plot. The data on viable count of bacteria was recorded maximum (39.1×10^7 cfu g^{-1} soil) in optimal dose (100% NPK + FYM) followed by super-optimal (150% NPK) dose (25.1×10^7 cfu g^{-1} soil) and minimum in control (11.7×10^7 cfu g^{-1} soil). The balanced fertilizer application had more ammonifying, nitrifying and cellulose-decomposing bacteria and similar amount of N_2 fixing bacteria and less denitrifying bacteria than the unbalanced fertilizer application treatments (Patel *et al.* 2018; Meshram *et al*. 2018).

However, it was observed that the number of fungi counts were also increased significantly with increasing the rate of fertilizer application. Table 2 indicated that the highest count (42.7×10^4 cfu g^{-1} soil) was recorded in optimal dose (100% NPK + FYM) as compared to that of over-optimal (150% NPK) dose (37.9×10^4 cfu g^{-1} soil). The lowest fungi counts were recorded in control (18.5×10^4 cfu g^{-1} soil) followed by 100% N alone treatments (19.6×10^4 cfu g^{-1} soil).

Further, the actinomycetes count was significantly increased according to fertilizer rate. It

Table 2. Effect of long-term application of fertilizers and manure on microbial population count (bacteria, fungi and actinomycetes) and soil microbial biomass carbon and nitrogen under soybean-wheat cropping sequence

Treatments	Microbial population count			Microbial biomass	
	Bacteria ($\times10^7$ cfu g^{-1} soil)	Fungi ($\times10^4$ cfu g^{-1} soil)	Actinomycetes ($\times10^5$ cfu g^{-1} soil)	Carbon ($\mu g\ g^{-1}$ soil)	Nitrogen ($\mu g\ g^{-1}$ soil)
50% NPK	19.8	24.4	17.2	234	27.7
100% NPK	23.9	33.2	26.2	292	35.2
150% NPK	25.1	37.9	29.0	315	40.5
100% NPK+HW	23.5	34.1	26.1	284	33.4
100% NPK+Zn	23.4	34.0	26.2	286	34.1
100% NP	18.3	23.6	16.3	238	26.9
100% N	14.3	19.6	15.3	212	25.1
100% NPK+FYM	39.1	42.7	39.6	344	44.3
100% NPK(-S)	23.3	32.4	25.2	286	33.2
Control	11.7	18.5	13.6	168	21.3
CD (P=0.05)	2.32	1.58	1.69	17.0	2.71

varied from 13.6×10^5 cfu g^{-1} soil (control plot) to 39.6×10^5 cfu g^{-1} soil (100% NPK+FYM). Meshram *et al*. (2016) reported that the actinomycetes population in soil after harvest of soybean-safflower system was found significantly maximum when treated with 100% NPK+FYM as compared to other treatments. The treatments 100% NPK, 100% NPK+HW, 100% NPK+Zn and 100% NPK(-S) were found at par with regard to population of soil actinomycetes.

Soil Microbial Biomass Carbon and Nitrogen

The integrated use of inorganic fertilizers and organic manure (100% NPK+FYM) significantly increased soil MBC and MBN as compared to 100% NPK and 150% NPK through inorganic fertilizers without addition of organic manure (Table 2). This can be ascribed due 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 MBC and MBN. Application of FYM @ 5 t ha^{-1} only to soybean crop during *kharif* season significantly increased MBC and MBN over control which might be due to a steady source of SOC to support the microbial community (Bhattacharyya *et al.* 2008). The lowest value of MBC 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. Similar, findings have also been reported by Nagwanshi *et al*. (2018) from their studies.

The soil MBN decreased at 100% NP and 100% N to the tune of 23.6 and 28.7 per cent as compared to 100% NPK indicating necessity of balanced fertilizer application for enhancing soil microbial activity. It was further observed that combined use of 100% NPK+FYM increased MBN by 25.9 per cent compared to NPK indicating augmented effect of organics in microbial activities. High SOC, more root proliferation and additional supply of N by farmyard manure to microorganisms might be responsible for increasing the level of MBN. Similarly, Patel *et al.* (2018) found that the soil MBC and N were significantly increased with the integrated application of 100% NPK+FYM over that from control and other treatments after 40-years of experimentation in a Vertisol under soybean-wheat cropping system.

Soil Organic Carbon Content

The SOC content significantly increased with increasing levels of fertilizer application (Table 3). The lowest value was noted in control (4.7 g kg^{-1}) which was increased to 6.2, 7.2 and 8.0 g kg^{-1} due to application of recommended dose of 50% NPK, 100%

Table 3. Effect of long-term application of fertilizers and manure on soil organic carbon, total N and available N

Treatments	Soil organic C (g kg^{-1})	Available N (kg ha^{-1})	Total N (kg ha^{-1})
50% NPK	6.2	227	1263
100% NPK	7.2	295	1450
150% NPK	8.0	332	1696
100% NPK+HW	7.2	295	1438
100% NPK+Zn	7.2	294	1431
100% NP	6.6	265	1398
100% N	5.0	217	1059
100% NPK+FYM	8.6	351	1798
100% NPK(-S)	7.1	277	1420
Control	4.7	191	986
CD (P=0.05)	0.4	34.8	117
Initial value (1972)	5.7	193	845

NPK and 150% NPK, respectively. However, the highest value (8.6 g kg^{-1}) was recorded with conjoint use of inorganic fertilizers (100% NPK) and FYM @ 5 t ha^{-1} treatment. The value of SOC was found to be slightly changed at harvest of crop. Similarly, the continuous application of FYM alone or in combination with inorganic fertilizer resulted in higher SOC content as compared to inorganic fertilizer application after harvest of Kutki was reported by (Dwivedi *et al.* 2015). This increase in SOC content could be due to enhanced root development of crop resulting in higher residues as a result of intensive farming with continuous fertilizer applications. Thus, FYM addition had a pronounced effect on SOC build-up in the soil (Meshram *et al*. 2018), thereby, showing that chemical fertilizer along with organic manure would be beneficial for sustaining the soil health and crop productivity (Dwivedi *et al.* 2019).

Available and Total Nitrogen Content in Soil

The soil available and total N contents as affected by continuous application of different treatments are presented in table 3. The available N content ranged from 191 to 351 kg ha^{-1}. However, due to addition of fertilizer dose (sub-optimal, optimal and super-optimal) N content was correspondingly improved indicating an impact of fertilizer application on enrichment of available N in soil. The highest N content was found in 100% NPK+FYM (351 kg ha^{-1}) treatments followed by 150% NPK (332 kg ha^{-1}) which could be due to better biological activities in the presence of FYM (Kushwaha *et al.* 2017; Suman *et al*. 2017). In general, the lowest values of available N contents were found in treatments associated with low level of inputs. Under all sets of inputs, the highest values were associated with 100% NPK+FYM, the treatment in which all resources were integrated and the better proportions, consumption, utilization with maximum fixation of N_2 from natural sources is expected.

Further, the value of total N content in soil was ranged from 986 to 1798 kg ha^{-1}. The total N contents increased with increasing fertilizer levels with sub-optimal, optimal and super-optimal, which showed an impact of fertilizer application on enrichment of N pools. The highest total N content was estimated with 100% NPK+FYM (1798 kg ha^{-1}) treatment followed by 150% NPK (1696 kg ha^{-1}) which could be due to better biological activities in the presence of FYM. The positive effect of FYM in soil N improvement with a widening of the C/N ratio had been seen in other classical experiments (Jones 1971). The total N in soils differed significantly by application of manure and fertilizer reported by Kushwaha *et al.* (2017).

Nitrogen Fixation by Soybean

Biologically N_2 fixed by soybean was computed from change in total soil N after 46 crop cycles of soybean–wheat by taking into account the N removed by the crops and total N applied through fertilizers and manures as per the procedure suggested by Singh *et al*. (1999) are computed and illustrated in figure 1. It was observed that the amount of N_2 fixed by soybean increased with the application of fertilizers alone and integrated use of fertilizers with FYM. The lower contribution of N_2 fixed to soil in control and 100% N alone treatments resulting poor growth of soybean which is due to poor development of roots and root nodules, compared with 100% NPK. The literature reviewed by Khandagle *et al.* (2020) clearly indicated that application of N decreased the N_2 fixation by soybean and concluded that application of N at beginning as a starter dose increased N_2 fixation. Integrated use of fertilizers and organic manure enhanced the N_2 fixation by soybean as compared to control. This may be due to improvement in soil condition conducive to root growth and nodule formation (Singh *et al.* 2012). Similar findings in a Vertisol were also reported by Patel *et al.* (2018).

Grain Yield of and Nitrogen Uptake by Soybean–Wheat System

The grain yields of soybean and wheat of 46 crop cycles (2018-19) are illustrated in figure 2 and 3. The lowest grain yields of soybean and wheat were found in control. While, grain yield obtained in 100% NPK+FYM treatment was significantly higher than 150% NPK treatment, the latter being at par with the application of 100% NPK+FYM. Thus, instead of applying of 150% NPK, it is better to use FYM with 100% NPK, since the yields obtained with 100% NPK+FYM are superior to 150% NPK application. The beneficial effect of FYM can be due to steady supply of all nutrients including the micronutrients and improvements in physical condition. Similar beneficial effects of FYM along with NPK have also been reported by Suman *et al.* (2017) and Dwivedi *et al.* (2016). They observed that the continuous use of chemical fertilizers either singly or in combination with FYM had a marked effect on grain yields of soybean and wheat.

It is fact that plant utilizes the nutrient which is available in soil pool concentration with successive addition of fertilizer and manure. In this regard, the

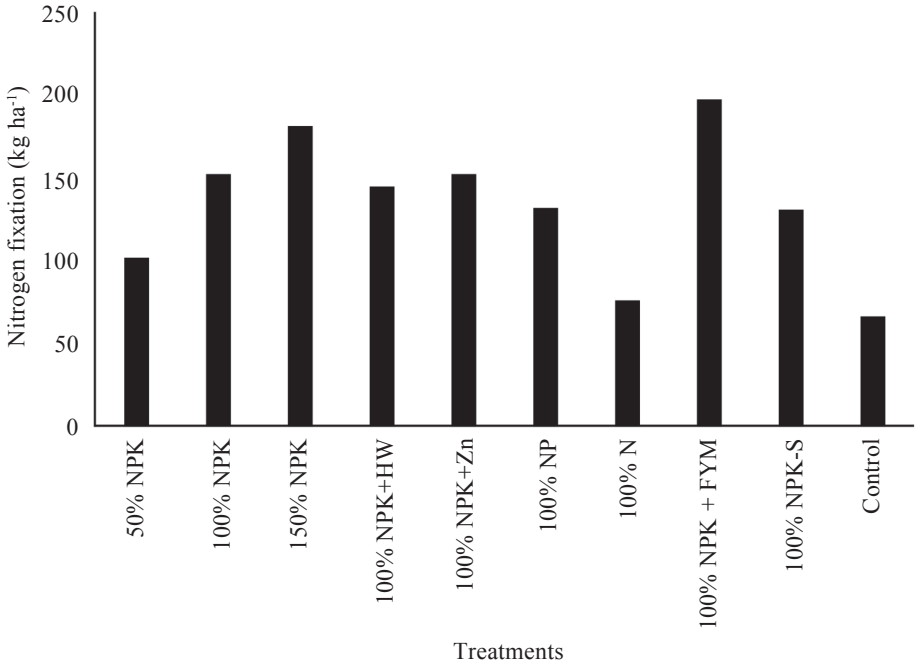

Fig. 1. Impact of different treatments on nitrogen fixation

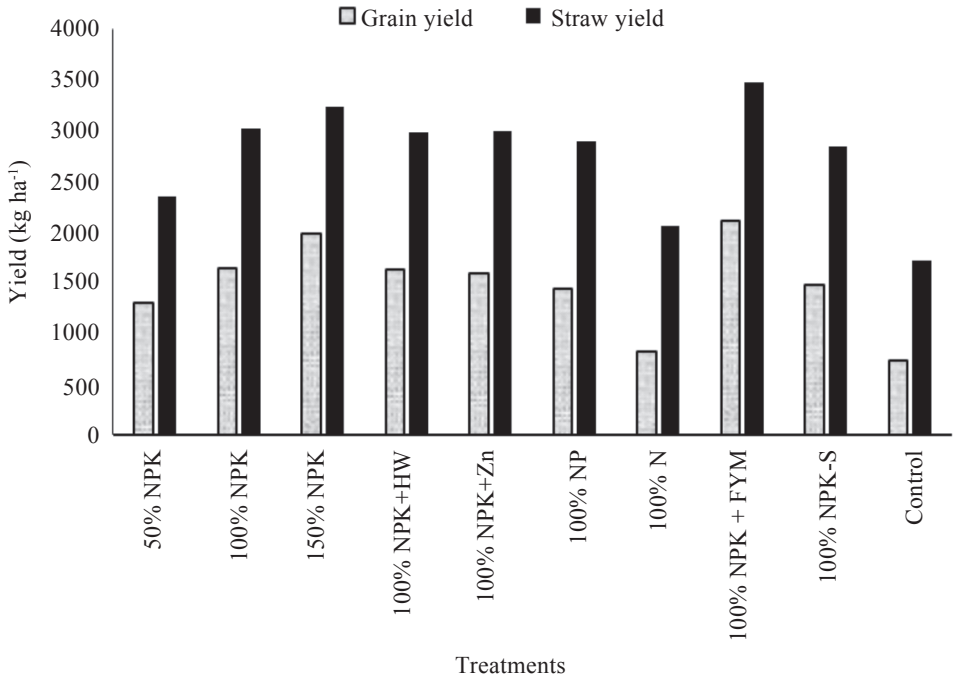

Fig. 2. Impact of different treatment on grain and straw yield of soybean

data illustrated in figure 2 revealed that N uptake was recorded highest with conjoint use of 100% NPK+FYM followed by 150% NPK and the lowest N uptake was observed in control plots in both crops. Similar findings were also reported by Thakur and Sawarkar (2009) and Sharma *et al.* (2015) who found maximum N uptake with the treatment of integrated use of organic manure and recommended fertilizer dose as compared to control plot.

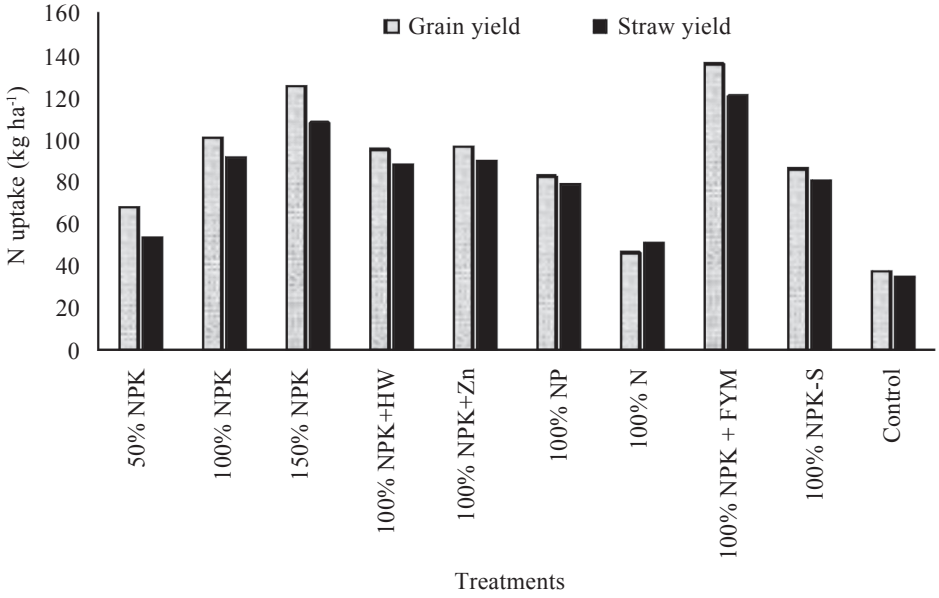

Fig. 3. Impact of different treatments on N uptake by soybean

Conclusions

Thus, it could be concluded that the long-term application of balanced and integrated use of nutrient (100% NPK, 150% NPK and 100% NPK+FYM) to soybean and wheat significantly improved the soil microbial properties, nitrogen fixation and crop productivity. This study has shown that the microbial population counts, microbial biomass carbon and nitrogen were highest in integrated use of balance dose of inorganic fertilizers with FYM (100% NPK+FYM) treatment. While, 100% N alone (imbalance dose) and control plot (without fertilizers) degraded the soil quality and produced a deleterious effect on microbial properties of soil as well as nitrogen fixation in soil. Thus, conjoint and judicious use of organics and mineral fertilizers found promising in long run.

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