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Long-Term Integrated Nutrient Management in the Maize–Wheat Cropping System in Alluvial Soils of North-Western India: Influence on Soil Organic Carbon, Microbial Activity and Nutrient Status

Salwinder Singh Dhaliwal [1], Sandeep Sharma [1], Vivek Sharma [1], Arvind Kumar Shukla [2], Sohan Singh Walia [3], Majid Alhomrani [4], Ahmed Gaber [5,*], Amardeep Singh Toor [1], Vibha Verma [1], Mehakpreet Kaur Randhawa [1], Lovedeep Kaur Pandher [1], Prabhjot Singh [1] and Akbar Hossain [6,*]

[1] Department of Soil Science, Punjab Agricultural University, Ludhiana 141004, India; ssdhaliwal@pau.edu (S.S.D.); sandyagro@pau.edu (S.S.); sharmavivek@pau.edu (V.S.); amardeep@pau.edu (A.S.T.); vermavibha@pau.edu (V.V.); mehakpreet-soil@pau.edu (M.K.R.); lovedeeppandher@pau.edu (L.K.P.); prabh@pau.edu (P.S.)
[2] Indian Institute of Soil Science, Bhopal 462038, India; arvindshukla2k3@yahoo.co.in
[3] Department of Agronomy, Punjab Agricultural University, Ludhiana 141004, India; waliass@pau.edu
[4] Department of Clinical Laboratories Sciences, The Faculty of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; m.alhomrani@tu.edu.sa
[5] Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[6] Department of Agronomy, Bangladesh Wheat and Maize Research Institute, Dinajpur 5200, Bangladesh
* Correspondence: a.gaber@tu.edu.sa (A.G.); akbarhossainwrc@gmail.com (A.H.)

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Abstract: Integrated nutrient management (INM) is a widely recognized tool to ensure sustainable crop productivity while preserving soil fertility. The addition of organic manures in soil has been evidenced to improve soil characteristics, in addition to improving nutrient availability. The soil samples, with five treatment combinations of chemical fertilizers with farmyard manure (FYM), were collected from a 17-year-old field experiment conducted at PAU, Ludhiana to investigate the effect of INM on the buildup of organic carbon (OC), microbial community, soil nutrient status and improvement in soil physical properties under the maize–wheat cropping system. The INM technique enhanced the OC content (0.44 to 0.66%), available N (152.8 to 164.9 kg ha^{-1}), P (22.8 to 31.4 kg ha^{-1}) and K (140.6 to 168.0 kg ha^{-1}) after 17 years. The DTPA-extractable and total micronutrients (Zn, Cu, Fe, and Mn) status also improved significantly with FYM supplementation. The organic source, coupled with inorganic fertilizers, improved the water holding capacity, total porosity, soil respiration, microbial biomass C, microbial biomass N, and potentially mineralizable N. However, pH, EC, and bulk density of soil decreased with the addition of FYM, coupled with chemical fertilizers.

Keywords: organic manure; inorganic fertilizers; cropping pattern; soil physicochemical and biological properties

1. Introduction

In north-western India, the continuous rice–wheat cropping has led to the exhaustion of natural resources and deteriorated soil fertility, producing agricultural outcomes [1]. Thus, a paradigm shift in cropping systems with different crops is required to maintain soil health and sustainable yield. Alternate cropping systems and soil management practices may prove beneficial to improve soil fertility and maintain environmental health. For crop diversification, maize-wheat cropping system has been identified as a suitable alternative to rice–wheat system [2,3]. Moreover, maize accounts for a significant fraction of global food consumption. The acreage under maize has increased in the past few years, as it helps to maintain soil health, in contrast to the rice–wheat cropping system. [4].

Although production, under intensive cultivation, is increasing year after year, it is depleting the huge amount of macro and micronutrients from the soil. Injudicious application of micronutrient fertilizers, declined use of crop residues and organic manures, as well as potential crop harvests in the last few decades, have resulted in micronutrient deficiencies in north-western India [5]. Excessive supplementation of inorganic fertilizers has also deteriorated the soil structure and declined soil organic matter (SOM) and microbial activity. Integrated nutrient management (INM) is the feasible solution for sustaining the crop productivities, as nutrient requirements of both the crops are high and have shown superior response towards higher levels of nutrient application [6]. The balanced use of nutrients is the key to improving the sustainable production of crops [7]. The inorganic fertilizers, through soil or foliar application, have shown tremendous results in terms of agricultural productivity [8,9]. Furthermore, the use of inorganic nutrient sources coupled with organic sources is a feasible approach for higher agricultural productivity and monitoring soil health [10]. The utilization of well-decomposed farmyard manure (FYM) in soil management practices is a well-known practice for enhancing crop yield, enhancing SOM, promoting microbial activities, promoting friendly soil environmental management [11,12], increasing the total organic sources supply, and increasing the plant-available macro and micronutrients in soil. The decomposition of plant residues favors the conversion of unavailable plant nutrients into an available form, increasing their plant absorption [13]. Besides improving the nutrient availability, organic manures also affect the soil physical and biological characteristics, as well as possessing residual effects on the succeeding crops. Previous reports have evidenced the greater residual impact of organic manures on the succeeding wheat crop [14,15].

The organic manures, being low in available nutrients, cannot substitute all nutrients required for yield sustainability [16]. On the other hand, the supplementation of nutrients solely through chemical fertilizers is insufficient to meet the complete nutrient demand of agricultural plants. Hence, INM has been identified as a viable option to improve soil health and sustain agricultural productivity on a long-term basis. For instance, the yield outcomes of the pearl millet–wheat cropping system were improved when nutrients were supplied through both FYM and inorganic fertilizers, over the sole use of inorganic fertilizers [17]. The integrated use of organic and inorganic N fertilizers in MWCS increased the SOM content and microbial activity, and thus improved the soil fertility [18]. The INM system seems to be an environmental-friendly approach that offers an advantage of the least impact on food quality. To date, the in-depth knowledge of build-up of soil carbon status, microbial community, and soil properties with INM under MWCS is scant in alluvial soils of north-western India. Hence, an attempt was made to study the impact of different levels of FYM along with inorganic fertilizers on soil organic carbon status, microbial community, and nutrient status under MWCS.

2. Material and Methods

2.1. Site Specification and Treatment Details

The experiment was planned with the sole objective for the yield sustainability of maize and wheat crops grown in a sequence and maintenance of soil health under the INM technique. The long-term field experiment on MWCS was carried out on permanent plots established since the Kharif 2001 season at the research farm, Department of Agronomy, Punjab Agricultural University (PAU), Ludhiana (30°56′ N, 75°52′ E, and 247 m above mean sea level), India. The experiment comprised five treatment combinations with three replications in a completely randomized block design with plot size 22.5 m × 7.5 m (Table 1).

Different treatment combinations consisted of the addition of nitrogen, phosphorus, and potassic fertilizers, in combination with farmyard manure (FYM), under the conventional tillage system. In brief, the experimental field was subjected to 2 ploughings, followed by planking to get a fine seed bed. Wheat variety PBW 343 was sown in the first week of November, and after harvesting of wheat, the crop maize variety PMH 1 was sown in the last week of June each year. Maize and wheat attained physiological maturity at 90 and 125 days, respectively.

Table 1. Treatment details of the sustainable production system model in the maize–wheat system (2001–2017).

Treatments	Maize				Wheat	
	Practice	N-P_2O_5-K_2O (kg ha^{-1})	FYM (t ha^{-1})	$ZnSO_4$ (kg ha^{-1})	Practice (Row to Row Spacing)	N-P_2O_5-K_2O (kg ha^{-1})
T_1	FP * (55,000 plants ha^{-1})	100-30-0	6	0	FP (22.5 cm)	150-60-0
T_2	RP ** (75,000 plants ha^{-1})	120-60-30	10	25	RP (15 cm)	120-60-30
T_3	RP (75,000 plants ha^{-1})	180-60-30	10	25	RP (15 cm)	150-60-30
T_4	RP (75,000 plants ha^{-1})	Fertilizer on soil test basis (100-0-30)	6	25	RP (15 cm)	120-60-30
T_5	RP (75,000 plants ha^{-1})	120-60-30	10	25	Wheat is replaced with Gobhi sarson followed by mungbean	Gobhi Sarson-100:30:0, mungbean-0:0:0

FP *—farmers practice; RP **—recommended practice.

Treatments differed in terms of nitrogen and FYM levels. The crop residues of previous crops were removed. The well-rotten FYM was obtained from PAU dairy shed, which was decomposed for 6 months in a pit. The FYM was added 15 days prior to sowing of the maize crop. The pH, EC, and OC of FYM were 7.21, 1.52, and 203.81 g kg^{-1}. The nutrient content in FYM was recorded as N = 1.16%, P= 0.48%, and K = 0.56%, on dry weight basis. Farmers add 6 t ha^{-1} FYM, whereas, under RP, 10 t ha^{-1} FYM is added. The urea (46% N), diammonium phosphate (DAP; 18% N, 46% P_2O_5), and muriate of potash (MOP; 60% K_2O) were used as a source of N, P, and K respectively. Nitrogen was applied in three equal splits to both the crops. One-third N was applied at the time of sowing; whereas, the remaining doses were applied with first and second irrigation. Four irrigations were applied to the maize crop; whereas, 5 irrigations were applied to the maize crop. Whole P and K fertilizers were applied at the time of sowing of maize and wheat crops, respectively. The FYM and Zn were applied only during the maize crop.

2.2. Initial Physicochemical Characteristics of the Experimental Soil

The physicochemical and biological properties of initial soil samples in 2001 at 0–15 cm (D1) and 15–30 cm (D2) depth have been given in Table 2. The soil of the experimental field was determined in 2001. The soil was loamy sand in texture (Typic Ustochrept), lying in an Ustic soil moisture regime, with bulk density 1.72 g cm^{-3}, total porosity 30.5%, water holding capacity (WHC) 48.6%, and organic carbon (OC) 0.40%.

2.3. Soil Analysis

In total, 30 composite soil samples from each block (5 treatments $\times$ 3 replications $\times$ 2 depths) were collected after 17 years with a screw auger after maize crop harvest in October 2017 (experiment terminated). Immediately after collection, the samples were separated into two halves. One half of the sample was immediately stored at 4 °C to assay soil microbiological properties, and the other half was air-dried, sieved through a 2.0 mm plastic sieve, and stored for physicochemical analysis. Among soil characteristics, bulk density and WHC were estimated, employing the weighing bottle method and Keen's box method [19,20]. Total porosity was determined using the procedure given by Prihar and Verma [21]. The pH and EC of soil samples were estimated using pH meter and EC meter [22]. The available N, P, and K were determined using the alkaline $KMnO_4$ method, Olsen extractable P method, and neutral ammonium acetate method, respectively [23–25]. Diethylene triamine-pentaacetic acid (DTPA)-extractable soil micronutrients (Zn, Cu, Fe, and Mn) were determined by using DTPA–TEA buffer in the ratio of 1:2 and then their concentration was estimated in atomic absorption spectrophotometer (AAS) [26]. Total macro and micronutrients were estimated by using the method given by Page et al. [27]. Total N in soil was estimated by the micro-Kjeldahl method. The total P, total K, and micronutrients in soil were determined by digesting the soil samples with diacid (i.e., HNO_3 and $HClO_4$ in the ratio of 9:4) and these digests were analyzed for total P, K, and DTPA extractable soil micronutrients after appropriate dilutions. Total P and K content were measured by employing the molybdenum blue method and flame photometric method, respectively. For micronutrients estimation, total Zn, Cu, Fe, and Mn contents were measured using AAS (Varian AAS FS 240 Model).

Table 2. Physicochemical and biological properties of initial soil samples (2001).

Soil Properties	Depth (D1)	Depth (D2)
Bulk density (g cm^{-3})	1.72	1.69
Total porosity (%)	30.5	29.9
Water holding capacity (%)	48.6	48.3
pH (1:2 soil: water suspension)	7.60	7.80
EC dSm^{-1} (1:2 soil: water suspension)	0.30	0.24
Organic carbon (g kg^{-1})	4.0	3.5
Available N (Kg ha^{-1})	119.7	102.3
Available P (Kg ha^{-1})	14.4	12.8
Available K (Kg ha^{-1})	128.6	124.7
Total N (%)	0.12	0.08
Total P (%)	0.25	0.19
Total K (%)	0.27	0.21
DTPA-Extractable Zn (mg kg^{-1})	1.26	0.68
DTPA-Extractable Cu (mg kg^{-1})	0.30	0.22
DTPA-Extractable Fe (mg kg^{-1})	3.83	2.56
DTPA-Extractable Mn (mg kg^{-1})	3.48	2.65
Total Zn (mg kg^{-1})	112.5	86.5
Cu (mg kg^{-1})	13.5	10.4
Fe (%)	2.6	1.8
Mn (mg kg^{-1})	132.8	97.6
PMN (mg kg^{-1} 7 d^{-1})	8.6	6.7
MBC (mg kg^{-1})	82.9	65.4
MBN (mg kg^{-1})	23.4	12.9
CO_2-C (mg kg^{-1} 10 d^{-1})	1.8	0.8

PMN—potentially mineralizable nitrogen; MBC—microbial biomass carbon; MBN—microbial biomass nitrogen; CO_2-C—soil respiration.

2.4. Soil Carbon and Soil Microbiological Analysis

The OC content in soil was estimated by using the wet combustion method [28]. The potentially mineralizable nitrogen (PMN) in the soil was estimated by following the procedure described by Keeney [29]. The microbial biomass nitrogen (MBN) was determined from the soil, as described by Keeney and Nelson [30], and the mineral nitrogen released by the microbial component was measured. The chloroform fumigation and incubation procedure was employed for the estimation of microbial biomass carbon (MBC) in the soil [31]. Soil respiration was measured by the chloroform fumigation and incubation procedure (CFIM) [31]. The amount of CO_2-C produced by soil microorganisms during respiration was measured and CO_2-C (soil respiration) was expressed as mg per kg of soil over a 10 day period [32].

2.5. Statistical Analysis

The data were analyzed by using statistical analysis software (SPSS software, 19.0; SPSS Institution Ltd., Chicago, IL, USA). One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was performed to determine the treatment effects at 0.05 level of probability [33].

3. Results

3.1. Impact of INM on Soil Carbon and Microbiological Composition

The maximum OC build-up was obtained in T_3 treatment and showed non-significant variation with all other treatments except treatment T_1 (Figure 1).

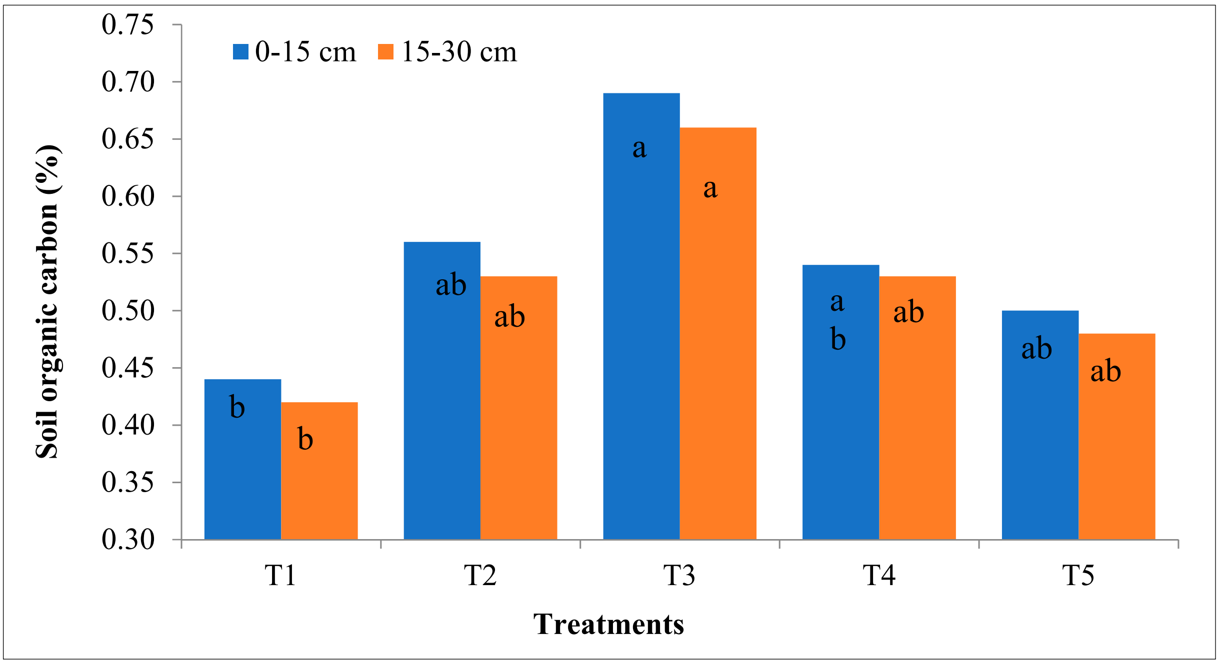

Figure 1. Effect of INM technique on soil OC under the maize–wheat system. In bars, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

The buildup of OC content was observed under all treatments over their initial level. The PMN ranged from 10.3 to 13.7 mg kg^{-1} 7 d^{-1} in D1 and from 7.2 to 10.6 mg kg^{-1} 7 d^{-1} in D2. Among different treatments, PMN was significantly greater in treatment T_3 as compared with the other treatments and was lowest in treatment T_5. The soil MBC varied from 116.3 to 132.8 mg kg^{-1} in D1 and from 42.7 to 56.6 mg kg^{-1} in D2 (Figure 2). Application of chemical fertilizers with FYM enhanced the MBC content over their initial levels which were reported to be 82.9 and 65.4 mg kg^{-1} in D1 and D2, respectively. Among different treatments, T_3 treatment resulted in maximum content of MBC followed by treatments T_2, T_4, T_1 and T_5, respectively. The MBN showed a similar trend as MBC and it ranged from 42.7 mg kg^{-1} in T_5 to 56.6 mg kg^{-1} in T_3 in D1 and from 36.8 mg kg^{-1} in T_5 to 44.7 mg kg^{-1} in T_3 in D2 (Figure 2). The addition of chemical fertilizers with FYM improved the CO_2-C content to a significant extent in all treatments over its initial levels, which were reported to be 1.8 and 0.8 mg kg^{-1} 10 d^{-1} in D1 and D2, respectively. It was found maximum in treatment T_3 (4.9 mg kg^{-1} 10 d^{-1}) and showed non-significant variation with treatments T_2 (4.4 mg kg^{-1} 10 d^{-1}) and T_4 (4.1 mg kg^{-1} 10 d^{-1}) and lowest variation in T_1 (3.7 mg kg^{-1} 10 d^{-1}) in D1. In D2, it was highest in treatment T_3 (3.7 mg kg^{-1} 10 d^{-1}) and showed non-significant variation with treatments T_2 (2.9 mg kg^{-1} 10 d^{-1}) and lowest in T_5 (2.1 mg kg^{-1} 10 d^{-1}).

3.2. Impact of INM on Soil Physical Characteristics

Bulk density, total porosity, and WHC ranged from 1.59 to 1.68 g cm^{-3}, 31.4 to 37.6%, and 50.9 to 59.6%, respectively, in D1 (Table 3). In D2, these ranged from 1.52 to 1.62 g cm^{-3}, 29.2 to 36.3%, and 47.7 to 56.9%, respectively. The maximum bulk density was reported in T_5 and was lowest in T_3, However, total porosity and WHC followed the opposite trend, with maximum values in T_3 and the lowest in T_5 in D1, while the lowest values were found in T_1 in D2.

The pH values in soil samples of D1 ranged from 7.33 to 7.48 and from 7.30 to 7.47 in soil samples of D2, under all treatments. The pH values decreased from their initial levels in all treatments. Lower pH values were reported under treatments in which 10 t ha^{-1} FYM had been added (T_2, T_3, and T_5) as compared to treatments in which 6 t ha^{-1} FYM was added (T_1 and T_4). A similar trend was followed in soil samples of depth D2. The soil EC values varied from 0.21 to 0.27 dS m^{-1} and 0.18 to 0.25 dS m^{-1}, respectively. The higher magnitude of EC was recorded in treatment T_3, while lower values were reported in treatments T_1 and T_5.

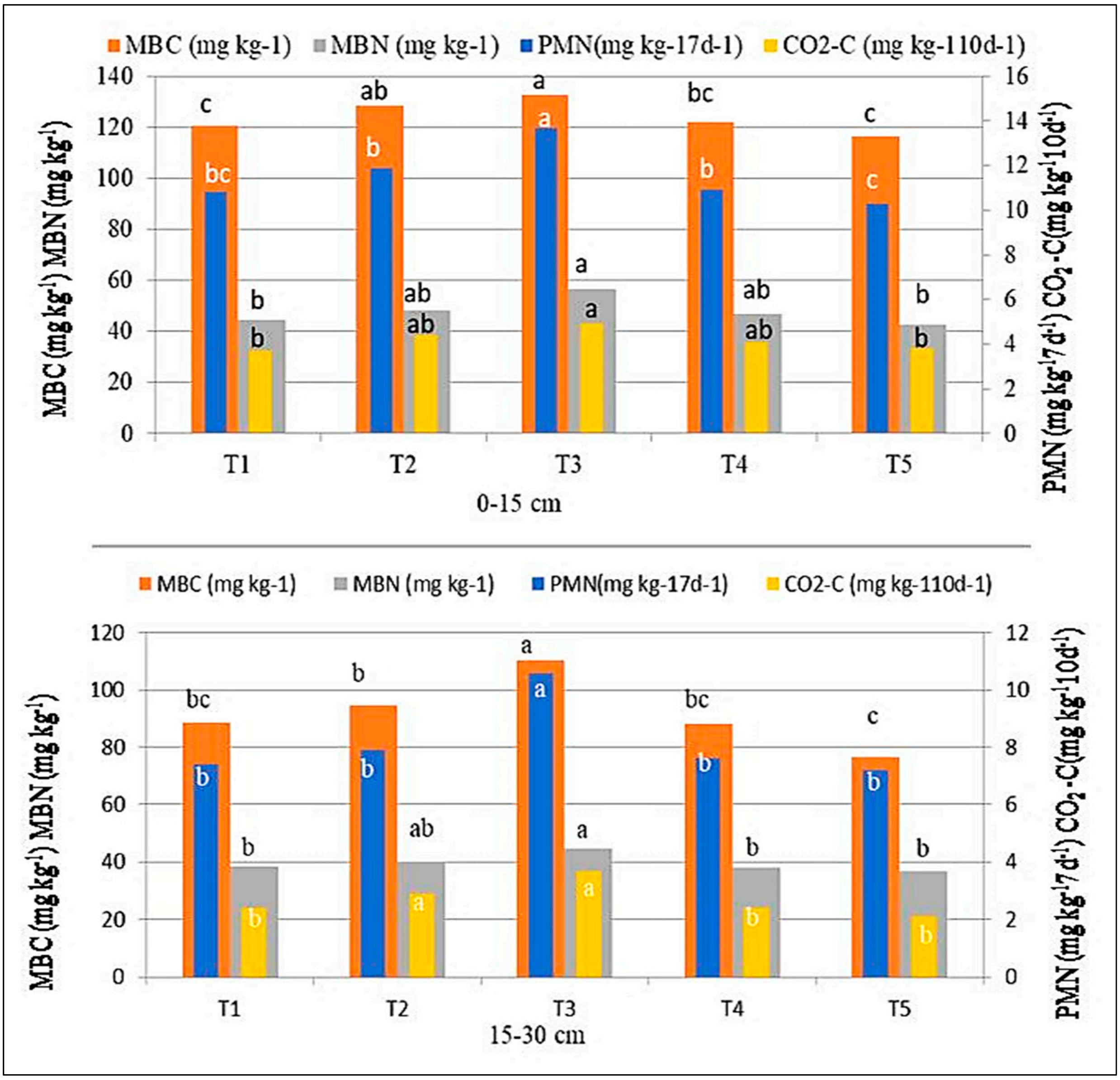

Figure 2. Effect of INM on soil microbiological properties under the maize–wheat system at 0–15 (**top**) and 15–30 cm (**bottom**) depth. PMN—potentially mineralizable nitrogen; MBC—microbial biomass carbon; MBN—microbial biomass nitrogen; CO_2-C—soil respiration. In bars, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

Table 3. Effect of INM technique on soil's physicochemical properties under the maize–wheat system.

Treatments	Bulk Density (g cm^{-3})	Total Porosity (%)	Water Holding Capacity (%)	pH	EC (dS m^{-1})
			D1		
T1	1.64 ^{ab}	31.8 ^{cd}	51.7 ^{c}	7.45 ^{ab}	0.22 ^{b}
T2	1.61 ^{ab}	34.7 ^{b}	56.8 ^{ab}	7.34 ^{c}	0.23 ^{ab}
T3	1.59 ^{b}	37.6 ^{a}	59.6 ^{a}	7.33 ^{c}	0.27 ^{a}
T4	1.65 ^{ab}	33.5 ^{bc}	52.5 ^{bc}	7.48 ^{a}	0.24 ^{ab}
T5	1.68 ^{a}	31.4 ^{d}	50.9 ^{c}	7.37 ^{bc}	0.21 ^{b}
Mean	1.63	33.8	54.3	7.39	0.23
Initial	1.72	30.5	48.6	7.6	0.3
LSD ($p \leq 0.05$)	0.08	1.9	4.8	0.09	0.04
			D2		
T1	1.58 ^{ab}	29.2 ^{d}	47.7 ^{c}	7.44 ^{a}	0.18 ^{b}
T2	1.53 ^{ab}	33.6 ^{b}	53.6 ^{ab}	7.33 ^{b}	0.20 ^{ab}
T3	1.52 ^{b}	36.3 ^{a}	56.9 ^{a}	7.30 ^{b}	0.25 ^{a}
T4	1.57 ^{ab}	32.1 ^{bc}	50.4 ^{bc}	7.47 ^{a}	0.21 ^{ab}
T5	1.62 ^{a}	30.6 ^{cd}	49.2 ^{bc}	7.34 ^{b}	0.18 ^{b}
Mean	1.56	32.4	51. 6	7.38	0.2
Initial	1.69	29.9	48.3	7.8	0.24
LSD ($p \leq 0.05$)	0.09	1.9	5.4	0.08	0.05

Treatments detail in Table 1; two depths, i.e., D1 (0–15 cm) and D2 (15–30 cm). In the column, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

3.3. Impact of INM on Available and Total Macronutrients (NPK) in Soil

The observations regarding available N content indicated that the N content was low in both D1 and D2 and the maximum value in soil samples of D1 was 164.9 kg ha^{-1} in treatment T_3. The result of treatment T_3 was statistically different from treatment T_1, in which the least available N (150.8 kg ha^{-1}) was recorded. The concentration of available N in soil reduced with depth (D2), where N contents ranged between 150.6 and 163.4 kg ha^{-1}. The available P levels in soils of depths D1 and D2 enhanced significantly from their initial values of 14.4 and 12.8 kg ha^{-1}, respectively. The available P content in soil improved when nutrients were supplemented through the combined use of chemical fertilizers with organic FYM (T_1, T_2, T_3, and T_5) and also in treatment T_3, which favored the significantly higher buildup of P content (31.4 kg ha^{-1}) more than all other treatments. Soil supplemented with FYM and chemical fertilizers recorded a higher level of available K content over its initial level (Table 4). However, a maximum increase (168.0 kg ha^{-1} in D1 and 166.2 kg ha^{-1} in D2) was observed in treatment T_3. The lowest available K was observed in treatment T_1 in which K was not added through chemical fertilizers but only 6 t ha^{-1} FYM was incorporated in the soil.

Total N content in the present study ranged from 0.16% in T_5 to 0.25% in T_3 treatments in soils of depth D1 and from 0.15% in T_5 to 0.21% in T_3 treatments in D2 (Table 5). Initially, the value of total N in soil was 0.12% in depth D1 and 0.08% in depth D2. All the treatments recorded a decline in total N content with the increase in soil depth. The highest content was observed in treatment T_3, followed by T_2 and T_4, and the lowest content was found in T_5. A similar trend was followed in the soils of depth D2. Total P content of soil ranged from 0.38–0.53% in D1 and 0.34–0.50% in D2 soil samples (Table 5).

A significant buildup of P was observed in all treatments that received chemical fertilizers with FYM. Total P content decreased in soils of depth D2, as compared with the soils of sample D1 under all treatments. In soil samples of D1, treatment T_3 recorded maximum content of total P and showed non-significant variation with all other treatments, except T_1. However, in soil samples of D2, treatment T_3 was significantly superior to all other treatments. The treatments which included the application of FYM coupled with chemical fertilizers recorded a significant buildup of total K in soil, which varied from 0.32

to 0.39% in D1 and from 0.25 to 0.31% in D2 soil samples (Table 5). The results of total K content recorded a higher level in D1 than in D2 soil samples. The maximum total K content was reported in treatment T_3 and lowest in T_2 in both soil layers.

Table 4. Effect of INM technique on available N, P, and K in soil under the maize–wheat system.

Treatments	Available					
	N (kg ha^{-1})		**P (kg ha^{-1})**		**K (kg ha^{-1})**	
	D1	**D2**	**D1**	**D2**	**D1**	**D2**
T_1	152.8 ^{b}	150.6 ^{c}	22.8 ^{b}	20.4 ^{b}	140.6 ^{b}	138.5 ^{b}
T_2	161.2 ^{a}	158.8 ^{ab}	25.4 ^{b}	22.6 ^{b}	151.2 ^{ab}	148.9 ^{b}
T_3	164.9 ^{a}	163.4 ^{a}	31.4 ^{a}	30.2 ^{a}	168.0 ^{a}	166.2 ^{a}
T_4	159.8 ^{ab}	155.3 ^{bc}	24.2 ^{b}	21.9 ^{b}	148.8 ^{b}	145.6 ^{b}
T_5	158.9 ^{ab}	156.4 ^{bc}	23.2 ^{b}	20.7 ^{b}	145.0 ^{b}	143.7 ^{b}
Mean	159.5	156.9	25.4	23.2	150.7	148.6
Initial	119.7	102.3	14.4	12.8	128.6	124.7
LSD ($p \leq 0.05$)	7.9	6.3	4.7	5.5	18.4	16.8

Treatments detail in Table 1; two depths, i.e., D1 (0–15 cm) and D2 (15–30 cm). In the column, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

Table 5. Effect of INM technique on total N, P, and K in soil under the maize–wheat system.

Treatments	% Total					
	N		**P**		**K**	
	D1	**D2**	**D1**	**D2**	**D1**	**D2**
T_1	0.19 ^{ab}	0.16 ^{b}	0.42 ^{bc}	0.40 ^{bc}	0.33 ^{b}	0.29 ^{ab}
T_2	0.22 ^{ab}	0.19 ^{ab}	0.48 ^{ab}	0.43 ^{b}	0.32 ^{b}	0.25 ^{c}
T_3	0.25 ^{a}	0.21 ^{a}	0.53 ^{a}	0.50 ^{a}	0.39 ^{a}	0.31 ^{a}
T_4	0.21 ^{ab}	0.18 ^{ab}	0.45 ^{abc}	0.42 ^{b}	0.36 ^{ab}	0.28 ^{b}
T_5	0.16 ^{b}	0.15 ^{b}	0.38 ^{c}	0.34 ^{c}	0.35 ^{ab}	0.29 ^{ab}
Mean	0.21	0.18	0.45	0.42	0.35	0.28
Initial	0.12	0.08	0.25	0.19	0.27	0.21
LSD ($p \leq 0.05$)	0.07	0.04	0.09	0.06	0.04	0.02

Treatments detail in Table 1; two depths, i.e., D1 (0–15 cm) and D2 (15–30 cm). In the column, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

3.4. Impact of INM on DTPA-Extractable and Total Micronutrients (Zn, Cu, Fe, and Mn) in Soil

Among micronutrient cations, DTPA-extractable Zn varied from 2.92–3.88 mg kg^{-1} in the D1 and 2.34–3.48 mg kg^{-1} in D2 soil samples in different treatments. A significant increase in Zn was observed in treatments T_3 (3.88 mg kg^{-1}), T_2 (3.70 mg kg^{-1}), T_4 (3.54 mg kg^{-1}), and T_5 (3.38 mg kg^{-1}) as compared with T_1 (2.92 mg kg^{-1}), in which no additional dose of Zn was added through $ZnSO_4$. The improved Cu content (0.44–0.84 mg kg^{-1} in D1 and 0.34–0.62 mg kg^{-1} in D2 soil samples) was recorded in all treatments over their initial levels (Table 6).

The maximum content of DTPA-extractable Cu was recorded in T_3 treatment showed non-significant variation with T_2 and T_4 treatments in D1 and with T_2, T_4, and T_5 treatments in D2 soil samples. On the contrary, the DTPA-extractable Fe contents in soil recorded a significant improvement in all the treatments over its initial value of 3.88 mg kg^{-1} (Table 6). The DTPA-extractable Fe content varied from 10.12 to 19.66 mg kg^{-1} and 8.48 to 14.58 mg kg^{-1} in D1 and D2 soil samples, respectively, under different treatments. The DTPA-extractable Mn in the current study increased in D1 and D2 soil samples from 11.16 to 18.38 mg kg^{-1} and 9.24 to 15.08 mg kg^{-1}, respectively, as compared with its initial value (3.48 mg kg^{-1} and 2.65 mg kg^{-1}, respectively). The treatments T_2, T_3, and T_4 showed non-significant variation with reason to DTPA extractable Mn in both layers of soil (Table 6).

Table 6. Effect of INM technique on DTPA-extractable micronutrients in soil under the maize–wheat system.

Treatments	Zn (mg kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mn (mg kg^{-1})	
	D1	D2	D1	D2	D1	D2	D1	D2
T_1	2.92 ^{b}	2.34 ^{b}	0.44 ^{b}	0.32 ^{b}	11.74 ^{bc}	10.26 ^{ab}	11.16 ^{b}	9.24 ^{b}
T_2	3.70 ^{a}	3.22 ^{a}	0.60 ^{ab}	0.47 ^{ab}	14.02 ^{b}	12.36 ^{ab}	16.34 ^{a}	13.12 ^{ab}
T_3	3.88 ^{a}	3.48 ^{a}	0.84 ^{a}	0.62 ^{a}	19.66 ^{a}	14.58 ^{a}	18.38 ^{a}	15.08 ^{a}
T_4	3.54 ^{a}	3.38 ^{a}	0.58 ^{ab}	0.46 ^{ab}	10.12 ^{c}	9.68 ^{b}	14.94 ^{ab}	12.42 ^{ab}
T_5	3.38 ^{ab}	3.12 ^{a}	0.48 ^{b}	0.36 ^{ab}	10.76 ^{bc}	8.48 ^{b}	11.82 $_{b}$	9.64 ^{b}
Mean	3.48	3.11	0.59	0.45	13.26	11.07	14.53	11.90
Initial	1.26	0.68	0.30	0.22	3.83	2.56	3.48	2.65
LSD ($p \leq 0.05$)	0.57	0.60	0.32	0.28	3.27	4.71	3.93	4.11

Treatments detail in Table 1; two depths, i.e., D1 (0–15 cm) and D2 (15–30 cm). In the column, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

The results for total Zn content demonstrated the superior level of total Zn in all the treatments over treatment T_1 (Table 7). The total Zn content ranged from 160.0 to 196.7 mg kg^{-1} and 134.8 to 176.9 mg kg^{-1}, respectively, under all treatments. The highest Zn content was recorded in the T_3 treatment and showed non-significant variation with treatments T_2 and T_4. The total Zn content was reduced with soil depth. The variation in Cu content was found from 18.0 mg kg^{-1} in T_1 to 26.8 mg kg^{-1} in T_3 in D1 and from 15.4 mg kg^{-1} in T_1 to 24.3 mg kg^{-1} in T_3 in D2 soil samples. Soil supplemented with FYM and chemical fertilizers recorded an increased total Cu over its initial levels. The total Fe concentration ranged from 2.7 to 3.9% in D1, in which it increased in all treatments over its initial value (2.6%). Its higher content was reported in T_2, T_3, and T_4 treatments, while lower content was found in T_1 and T_5 treatments. Total Mn content of soil varied from 170.3 to 224.3 mg kg^{-1} in D1 and 148.4 to 202.9 mg kg^{-1} in D2 soil samples. Total Mn content in soil showed an appreciable increase over its initial levels.

Table 7. Effect of INM technique on total micronutrients in soil under the maize–wheat system.

Treatments	Zn (mg kg^{-1})		Cu (mg kg^{-1})		Fe (%)		Mn (mg kg^{-1})	
Depth	D1	D2	D1	D2	D1	D2	D1	D2
T_1	160.0 ^{c}	134.8 ^{c}	18.0 ^{d}	15.4 ^{c}	2.7 ^{c}	2.1 ^{b}	170.3 ^{c}	148.4 ^{b}
T_2	182.3 ^{ab}	152.6 ^{bc}	24.3 ^{ab}	21.6 ^{ab}	3.6 ^{ab}	2.9 ^{a}	190.0 ^{b}	166.2 ^{b}
T_3	196.7 ^{a}	176.9 ^{a}	26.8 ^{a}	24.3 ^{a}	3.9 ^{a}	3.1 ^{a}	224.3 ^{a}	202.9 ^{a}
T_4	176.7 ^{abc}	158.5 ^{ab}	22.0 ^{bc}	19.8 ^{abc}	3.4 ^{ab}	2.6 ^{ab}	184.0 ^{bc}	156.6 ^{b}
T_5	163.3 ^{bc}	139.6 ^{c}	20.0 ^{cd}	16.8 ^{bc}	3.1 ^{bc}	2.2 ^{b}	174.0 ^{c}	151.2 ^{b}
Mean	175.8	152.5	22.2	19.6	3.3	2.6	188.5	165.1
Initial	112.5	86.5	13.5	10.4	2.6	1.8	132.8	97.6
LSD ($p \leq 0.05$)	21.2	18.8	3.7	5.4	0.6	0.5	14.1	29.1

Treatments detail in Table 1; two depths, i.e., D1 (0–15 cm) and D2 (15–30 cm). In the column, means with similar letter(s) are statistically identical, as per $LSD_{0.05}$.

3.5. Correlation Analysis among Different Soil Parameters

The correlation analysis of OC and microbiological characteristics with other soil characteristics have been presented in Figure 3. The soil OC content showed a strong positive correlation with soil porosity, water holding capacity, and soil EC; however, it was negatively correlated with soil pH and bulk density. Similarly, the soil microbiological properties suggested a positive correlation with soil porosity, WHC, and soil EC to a greater extent. The soil pH and bulk density showed a non-significant correlation with soil microbiological properties. Among different soil characteristics, soil OC showed the highest correlation (i.e., (r = 0.95, $p \leq 0.05$)) with soil porosity, which was followed by a correlation of CO_2-C with soil pH and soil EC (r = 0.90, $p \leq 0.05$).

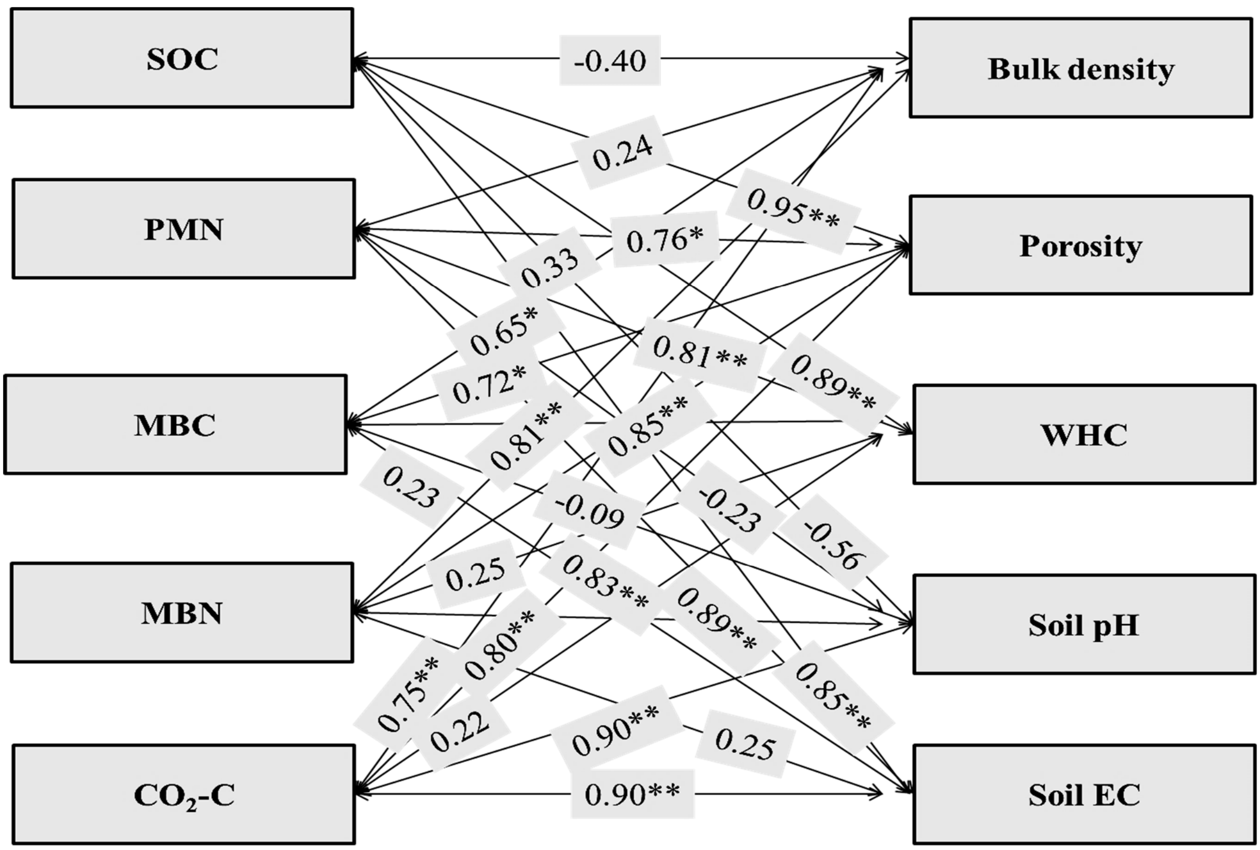

Figure 3. The correlation coefficient of soil OC and microbiological community with soil properties (**-correlation is significant at the 0.01 level; *-correlation is significant at the 0.05 level).

4. Discussion

4.1. Impact of INM on Soil Carbon and Microbiological Composition

Combined supplementation of fertilizers with FYM showed a notable impact on the OC contents of the D2 (15–30 cm). Similar improvement in OC content with combined addition of FYM and chemical fertilizers over inorganic fertilizer alone under MWCS in an Alfisol has also been reported [34]. An improvement in OC content might be associated with the SOM supplementation in the form of FYM, improved root anatomy, and more plant residue addition, with the higher application of nutrients through manure and chemical fertilizers [35].

The PMN reduced with the soil depth in all treatments and increased over its initial levels in D1 and D2 soil samples. The PMN is widely associated with the potential N supplying capability of soil [36]. Higher PMN in all treatments suggests the accumulation of mineralizable N pools in the soil through organic manure addition [37]. The combined addition of FYM and chemical fertilizers enhanced the MBC content over their initial levels in D1 and D2 soil samples, which may be related to improved root growth and crop residues addition after harvesting [38]. Additionally, the addition of organic matter through manure application may provide a favorable environment for enhanced microbial activity and transformations of micronutrients in agricultural soils [39]. The results are concordant with the results reported by Nath et al. [40].

The reduced MBN content with soil depth might be associated with the low OC content in D2 soil samples. The balanced supplementation of organic manure and FYM resulted in the appropriate nutrient availability, which further improved the rhizosphere activity and growth parameters of the plant. The improvement in these parameters resulted in a higher mineralization rate of N and also higher OC content in the soil. The results corroborate the findings of Chang et al. [41]. The increase in CO_2-C (soil respiration) in integrated treatments could have resulted from available carbon substrate through manure, easily mineralizable organic compounds, and other essential nutrients (N and P) for soil microorganisms, available through chemical fertilizers and manure [42]. Higher soil respiration suggested the higher metabolically activity of microbial biomass in soil.

4.2. Impact of INM on Soil Physicochemical Characteristics

Soil bulk density reduced, compared with its initial levels, under all the treatments and total porosity and WHC increased over their initial level. Similar results have already been reported for bulk density and total porosity, with the addition of FYM either alone or integrated use of NPK and FYM in soil samples collected after wheat harvest [43]. This could be ascribed to the produced soil particle binding agents such as polysaccharides and bacterial gums from the microbial breakdown of organic manures. These molecules decrease the soil bulk density by promoting soil aggregation and hence improve the porosity [44]. The improvement in the structural characteristics of soil with FYM supplementation influenced the WHC of soil positively [45].

The soil pH values reduced with an increase in soil depth. Soil pH is also reduced with FYM application, which might be associated with the release of organic acid during microbial decomposition of FYM [46]. The changes in soil pH with FYM supplementation may be owed to oxidation of organic matter and release of carbon dioxide in the soil [47]. The addition of NPK fertilizers resulted in higher EC, which increased the salts accumulation in the soil. This was also due to the decomposition of organic matter added through FYM [48].

4.3. Impact of INM on Available and Total NPK in Soil

The use of INM demonstrated a significant improvement in available N contents as compared with their initial level, which might be related to the N mineralization from the applied fertilizers during decomposition. Higher N availability in the treatments applied with FYM might be due to the slow-release of organically bound nutrients from FYM. It improves the complexation of metal ions, and, thus, increases the bioavailability of nutrient elements to plants [1]. The FYM also provides a favorable environment for the conversion of non-available plant nutrient form to available plant nutrients and slowly release available carbon [49]. The trend for total N followed a similar trend of OC level as the soil-internal cycling is associated with OC; thus, an increase in total N has been recorded with the increase in organic carbon content [37]. Higher content of total N in plots supplemented with organic sources and 50% of recommended NPK fertilizers has been observed in the literature [50].

The addition of FYM to the soil resulted in increased available P content in the soil by mineralization or solubilizing the native P reserves. The elevation in available P content with the application of FYM, along with chemical fertilizers under MWCS, was also reported by Rajneesh et al. [51]. The organic manure increased the nutrient retention capacity of the soil by enhancing the SOM; thus, the available nutrient level of soil required for optimum crop productivity was improved [52]. Mani et al. reported an increase in total P content in soil under treatment in which FYM had been added with NPK, Zn, and phosphate solubilizing bacteria [7]. The application of FYM increased total P in the soil as it acts as P source and also facilitates the retention of P in soil [53]. The increase in available K on FYM addition may be related to the reduced K fixation and release of K, due to the interaction of FYM with clay [54]. Another possible reason for the improvement in total K content might be based on the fact that FYM retains K ions on the exchange sites of its decomposed products, which reduces its leaching loss [55].

4.4. Impact of INM on DTPA-Extractable and Total Micronutrients in Soil

Extractable DTPA increased under all treatments over its initial level as FYM had been added in all treatments at different rates. This could have been due to the fact that FYM supplies an extensive amount of Zn to the soil as well as facilitates the biological and chemical changes that favor the dissolution of non-available Zn [56]. The increase in total Zn content among different treatments might be associated with Zn supplementation through chemical fertilizer and organic manure [18].

The increment in available Cu contents in soil with FYM supplementation might be attributed to its reduced redox potential, which resulted in an increased release of bioavailable micronutrients in the soil over the sole use of synthetic fertilizers. The improved DTPA-extractable Cu content may be due to its complexation with organic molecules released during FYM decomposition, which increased its availability by prohibiting fixation, oxidation, precipitation, and leaching. Nutrient supplementation through FYM in conjugation with chemical fertilizers increased total Cu in soil over its initial level. Addition of FYM to the soil forms organic chelates in soil, which decrease the probability of retaining Cu ions and encourage the increase in microorganism populations, which enhance the plant accessibility of soil micronutrients [38].

The increased availability of Fe with the addition of FYM may be attributed to its increased availability due to the decrease in soil pH by the virtue of organic manure [57]. The enhancement in the soil redox potential with the addition of FYM increased total Fe content [58]. The application of FYM resulted in the buildup of DTPA-extractable Mn in soil which may be attributed to the supply of Mn in the soil through manure. The DTPA-extractable Mn content was greater in the FYM-treated plots, due to Mn release during FYM decomposition. Apart from that, organic acids and humic substances released from FYM decomposition encourage the Mn mobilization from solid phase to soil solution [59]. The micronutrients levels decreased with an increase in soil depth under all treatments. Similar observations were recorded by Sharma and Shweta [60].

5. Conclusions

The long-term study concluded that the integrated use of farmyard manure, coupled with chemical fertilizers in maize–wheat cropping system, had significant improvement in soil organic carbon and soil microbiological community of soil. The data on the build-up of macronutrients (N, P, and K) and DTPA-extractable micronutrients (Zn, Cu, Fe, and Mn) also remarkably improved when the balanced amount of nutrients was supplied through the integrated application of mineral and FYM. Among different treatments, the treatment in which an additional 50% dose of nitrogen was added over its recommended value of soil was found best to sustain the agricultural outcomes of the maize–wheat system in the loamy sand soil of Punjab.

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