

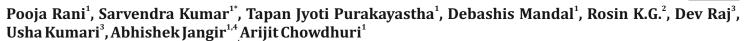
# **Original Research Article**

04 October 2024: Received 07 November 2024: Revised 22 November 2024: Accepted 09 December 2024: Available Online

https://aatcc.peerjournals.net/

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# Enhancing soil microbial health: synergistic effects of long-term manuring and nitrogen fertilization on microbial dynamics in semiarid agroecosystems



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# ABSTRACT

The soil's biological health is an important aspect in sustaining crop production in arable soils. A proven technique for improving soil biological health is adding organic manures to the soil. However, in intensive cropping systems in semi-arid tropical regions, determining the optimal rate of manure application to maintain soil quality while avoiding financial and environmental losses is a major challenge. The impact of the long-term application of graded doses of farmyard manure(FYM) and its integration with mineral nitrogen on soil biochemical properties was investigated in a pearl millet-wheat system. The study involved four levels of FYM (0, 10, 20 and 30 Mg ha<sup>-1</sup>) and two nitrogen levels (0 and 120 kg ha<sup>-1</sup>) and the effects were assessed soil nitrogen availability, microbial activity and nitrogen (N) cycling soil enzymes activities in two soil depths (0-15 and 15-30 cm). The results showed a significant increase (p < 0.05) in soil available nitrogen with addition of FYM and N and it ranged from 169-328 kg ha<sup>-1</sup> in surface soil and 132-238 kg ha<sup>-1</sup> in subsurface soil. The soil microbial biomass carbon (MBC) and nitrogen (MBN) content was significantly increased with increasing FYM and N levels, where MBC ranging from 162-1189 mg  $g^{-1}$  soil in 0-15 cm depth and 91-852 mg  $g^{-1}$  soil in 15-30 cm depth. The substrate substrate-induced respiration (SIR) and soil microbial quotient (SMQ) was also reported increased to increase with FYM and N application irrespective of soil depths. The soil urease activity was increased by 227% in surface soil and 323% in subsurface soil with increased FYM level from 0 to 30 Mg ha<sup>-1</sup>. The activity of arginine deaminase in soil ranged from 4.36-10.3  $\mu$ g NH<sup>+</sup><sub>4</sub>g<sup>-1</sup> soil hr<sup>-1</sup> and 2.60 to 6.34  $\mu$ g NH<sup>+</sup><sub>4</sub>g<sup>-1</sup> soil hr<sup>-1</sup> in 0-15 cm and 15-30 cm soil layer, respectively. Thus, the study emphasizes the importance of FYM and N fertilization for enhancing soil microbial biomass, activity and nitrogen availability in semiarid environment, all of which support better soil health and sustainable crop production.

*Keywords:* Long-term fertilization, Soil Health, Microbial Biomass Carbon, Soil Microbial Quotient, Substrate Induced Respiration, Farm Yard Manure, Soil Urease, Arginine Deaminase

#### **1. Introduction**

The complex agricultural and environmental challenges are properly addressed by long-term field experiments which offer unique insights that short-term studies cannot provide.Both organic and inorganic fertilization are essential components of modern agriculture, each offering distinct benefits and challenges. The most sustainable approach often involves integrating both organic and inorganic fertilizers, optimizing their use to maintain soil fertility, improve crop productivity, and minimize environmental impacts [1]. This balanced approach ensures agricultural systems remain productive and resilient in the face of increasing global food demands and environmental challenges.

The application of manure and nitrogen fertilizers for a longer period in arable soils significantly impacts various soil microbial properties that are essential for maintaining soil health and fertility.

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DOI: https://doi.org/10.21276/AATCCReview.2024.12.04.638 © 2024 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Themicrobial components are crucial for breaking down organic matter and releasing nutrients, thereby playing a vital role in the soil ecosystem. Microbial activity is often assessed through substrate-induced respiration (SIR), which reflects the metabolic activity of microorganisms in response to added substrates [2]. The indices like microbial quotient and MBC/MBN ratio offers a valuable measure of microbial efficiency in converting organic matter into microbial biomass, microbial community structure and nutrient management strategies [3,4]. In addition to these parameters, soil enzymes such as urease and arginine deaminase play fundamental roles in nitrogen cycling, influencing soil fertility and plant nutrient availability [5]. An important stage in the mineralization of nitrogen is facilitated by urease when urea is converted to ammonia, and arginine deaminase breaks down arginine, which contributes to additional nitrogen transformations in the soil. It is crucial to comprehend how long-term manure and nitrogen fertilizer application affect these enzymatic and microbial activities in order to create sustainable soil management techniques that improve soil productivity and quality.Addition of nitrogen irrespective of type of organic manure increased the microbial biomass carbon and nitrogen significantly after 19 years of experimentation. Apart from quality of fertilizers, quantity of fertilizer, climatic conditions, cropping system and

management practices also play a major role in shaping microbial functions [6]. Different cropping systems, such as monoculture and crop rotations, influence soil properties and microbial diversity [7]. The influence of organic and inorganic fertilization on the microbial properties of soil and the transformation of nutrients has been explored in various land use and management systems but a knowledge gap exist in evaluation of effects of different levels of farmyard manure (FYM) application on soil microbial properties in pearlmilletwheat cropping system under semiarid sub-tropical climatic conditions. To fill this gap, this study was carried out with the following objectives: (i) effects of different levels of FYM with or without nitrogen on soil microbial biomass and activity; (ii) effects of different levels of FYM with or without nitrogen on nitrogen transforming enzymes and nitrogen availability in soil; (iii) to investigate any relationship among soil microbial properties and N availability. The intent of the research was to determine the optimum manure and nitrogen level for sustainable soil health and nutrient availability to crops.

#### 2. Materials and Methods

A long-term field study was started in the year of 1967 under the pearl millet-wheat system at CCS Haryana Agricultural University, Haryana, India, to investigate the effects of varying amounts of nitrogen (N) and farmyard manure (FYM) on soil properties. The initial experimental soil was fine sandy loam, classified as TypicUstochrept. The experimental site experiences a semiarid, subtropical climate, with an average annual rainfall of 450 mm, and 90% rainfall remains confined between months of June to September. The highest summer season temperatures varied from 40-46°C, while lower temperatures varied from 1.5 - 4.0°C, with a mean annual temperature of 24°C. At the time of start of the experiment (1967) some important soil properties were measured and presented in Table 1. The experiment consists of eight treatments with four levels of farmyard manure (FYM) and two levels of nitrogen. Initially, FYM was applied at 0, 15, 30, and 45 Mg ha<sup>-1</sup> during summer (June) and winter (October). Since 2008, these rates were modified to 0, 5, 10, and 15 Mg  $ha^{-1}$  (F0, F10, F20, F30), continuing thereafter. There were two sub-plots within each main plot receiving nitrogen doses of 0 and 120 kg N  $ha^{-1}$  (N0 and N120) in each season. The eight treatments were replicated four times, with FYM thoroughly mixed into the 0-15 cm plough layer four weeks before the sowing of Kahrif and Rabi crops. Following the wheat harvest in April 2023, composite soil samples (five from each plot mixed to one) were taken at two distinct depths: 0-15 cm and 15-30 cm. one set of sample were dried and used for the determination of available nitrogen in the soil. The Kjeldhal apparatus was used to distill soil with alkaline 0.32 N KMnO<sub>4</sub> in order to estimate the amount of nitrogen available in the soil [8]. Soil organic carbon (SOC) content was determined using finely sieved (0.2 mm) and homogeneous soil samples through dry combustion in a CHNS analyzer (EurovectorEA3000). Another set of soil samples were air dried, sieved (2mm) and kept at 4°C for biological properties of soil, further and all findings were reported on a dry weight basis. The soil microbial biomass carbon (SMBC) was determined using fumigation extraction method [9] and microbial biomass nitrogen (MBN) was determined by the method suggested by [10]. The activity of urease enzyme was determined using the [11] method. The activity of arginine deaminase was determined using colorimetric method [12]. The substrate induced respiration was measured using [2] method.

The MBC to SOC ratio was calculated and presented as soil microbial quotient (SMQ). Statistical analysis of the experimental data was carried out using a split plot design. The data was subjected to two-way Analysis of variance (ANOVA), performed with R software (Version 4.2.2), utilizing the *Agricolae* package. The Fisher least significant difference (LSD) method was used to compare treatment means at the significance level of p<0.05.

#### **3. Results and Discussion**

#### 3.1. Nitrogen and organic carbon in soil

The soil available nitrogen (N) ranged from 169 kg ha<sup>-1</sup> to 328 kg ha<sup>1</sup> in 0-15 cm soil layer and 132 kg ha<sup>1</sup> to 238 kg ha<sup>1</sup> in 15-30 cm soil layer (Table 2). Higher FYM levels (F30) and nitrogen fertilization (N120) lead to more available nitrogen, but the N content in F30 plots were not statistically different from F20 plots in both the soil depths. No significant interaction effect were noticed between FYM and N with respect to nitrogen availability in soil, it indicated the effects of FYM and nitrogen are largely independent. The improved nitrogen availability upon FYM and N fertilization is well reported as both are direct and indirect source of nitrogen. The nitrogen content increased with FYM application irrespective of different doses, which possibly due to FYM adds organic matter to soil, reducing N losses and microbial activity stimulated by FYM that mineralized organic N into its inorganic form. Inorganic N sources such as urea fertlizer provide rapid but short-term availability, while FYM offers long-term availability due to different types of aliphatic and aromatic compounds present in the FYM.

The soil organic carbon (SOC) ranged from 6.55 - 19.6 g kg<sup>-1</sup> soil in 0-15 cm and 4.25 - 16.0 g kg<sup>-1</sup> soil in 15-30 cm soil depth (Table 2). The SOC content increased by 2.65 and 3.16 times in 0-15 cm and 15-30 cm soil depth respectively, as the FYM level increased from 0 to 30 Mg ha<sup>-1</sup>. The increase in SOCcontent under F0N0 treatment over initial value (in the year 1967) might be due to continuous addition of carbon inputs from the pearlmilletwheat cropping system (56 years) like root biomass, exudates, and other plant inputs [13] which likely contributes to the accumulation of SOC in the soil, even in untreated control plots. The addition of FYM irrespective of its amount, increased the SOC content of soil in both soil depths which might be attributed to: (i) FYM is direct source of carbon (ii) improved root biomass and rhizodeposition (iii) Improved soil aggregation and protection of carbon within these aggregates in manureamended plots [14]. The integration of nitrogen with FYM increased SOC content of soil which probably because of improved above and below-ground biomass of crop plants beside direct addition of organic matter from FYM.

## 3.2. Soil microbial biomass carbon and nitrogen

The microbial biomass carbon (MBC) and nitrogen (MBN) showed significant increase (p < 0.05) with increasing manure and N level irrespective of soil depth. The MBC in 0-15 cm soil, rangedfrom 162-1189 mg g<sup>-1</sup>soil and from 91- 852 mg g<sup>-1</sup>soil in 15-30 cm depth (Table 3). It increased 18% and 20% with the addition of N (N<sub>120</sub>) at 0-15 cm and 15-30 cm soil depth respectively, compared to without nitrogen (N<sub>0</sub>). The interaction between FYM and N was found non-significant at both depths (p < 0.05). The MBNin surface soil ranged from 16.2-138 mg g<sup>-1</sup>soil in 0-15 cm and 8.2-92.0 mg g<sup>-1</sup>soil in 15-30 cm soil depth(Table 3). With each successive addition of FYM dose (10 t ha<sup>-1</sup>) the MBN increased by 267%, 357%, 507% in surface soil and 334%, 487%, 634% in subsurface soil over F0.

The addition of N ( $N_{120}$ ) led to 28% and 37% increase in MBN at 0-15 cm and 15-30 cm soil depth respectively, compared to  $N_0$ . The addition of carbon inputs through FYM is a direct explanation for increasing microbial biomass carbon in soil, beside that FYM also contain N thus contributed towards increased MBN. The integration of FYM with nitrogen also increased MBC and MBN at both soil depths. A similar improvement in MBC and MBN following the addition of organic manure was also documented by [15,16,17]. The mean values of MBC: MBN ratio were significantly differ across different FYM and N levels, however, in both soil depths highest ratio was noticed in F0N0 treatment (10.1 and 11.1) and lowest in F10N120 (7.82 and 8.45) (Table 4). In 15-30 cm soil layer the MBC: MBN ratio was high compared to 0-15 cm soil layer. Under both soil depths nitrogen addition decreased this ratio significantly (p < 0.05). The shifts in the MBC:MBN ratio following manure or fertilizer application may result from alterations in microbial communities and population dynamics. Given that the average MBC:MBNratio is approximately 15 for fungi and 6 for bacteria [18], a decline in this ratio suggests a reduction in the fungal-to-bacterial ratio and vice versa. Thus, in this study the increased ratio in deeper soil indicated fungal dominance over bacteria in 15-30 cm soil layer. An application of 10 Mg ha<sup>-1</sup> of FYM led to the most significant reduction in this ratio and further increase in FYM dose increased the MBC: MBN ratio irrespective of soil depth. This may be due to higher carbon substrate availability which led to higher carbon accumulation in microbial cells. Similar shifts in MBC:MBN ratio on manure and fertilizer integration was also mentioned by [19] and [6].

## 3.3. Soil microbial activity

The soil microbial activities viz. substrate substrate-induced soil respiration (SIR), soil microbial quotient (SMQ) and MBC:MBN were significantly affected by levels of FYM and N in both the soil depths (p< 0.05). The highest SIR value was noticed under  $F_{30}N_{120}$  treatment (26.2 and 22.4 µg CO<sub>2</sub> g<sup>-1</sup> soil hr<sup>-1</sup>in 0-15 and 15-30 cm soil depths, respectively) (S1). The increased level of N from N0 to N120 caused 15% and 22% increment in SIR value in 0-15 cm and 15-30 cm soil layers, respectively (Figure 1). Substrate Induced Respiration (SIR) value provides insights into microbial dynamics and nutrient cycling, which are essential for maintaining soil health and fertility [20].FYM application leads to a notable increase in microbial biomass(Table 3) and activity, as evidenced by higher SIR rates. This is due to the high organic content of FYM and increased availability of nutrients thus become a source of energy for soil microorganisms [21]. The role of nitrogen (N) in influencing substrate-induced respiration (SIR) in soil is multifaceted, and we found that N application increased SIR at both soil depths irrespective of FYM level. Nitrogen addition enhances substrateinduced respiration by improving soil biochemical properties, increasing microbial activity, and elevating soil organic carbon and nutrient contents, thereby stimulating CO<sub>2</sub> production in soil [22]. Nitrogen enrichment alters microbial biomass and substrate quality, enhancing heterotrophic respiration and reducing autotrophic respiration in soils [23]. The highest SMQ was reported in  $F_{30}N_{120}$  treatment (6.08 and 5.34) and lowest in  $F_0N_0(2.47 \text{ and } 2.15)$ , irrespective of soil depth(Table 4). The increasing order with respect to SMQ was F30>F20>F10>F0 in 0-15 cm and 15-30 cm soil layer. The application of nitrogen significantly increased the SMQ and yet the interaction between FYM and N was non-significant at both soil depths (p < 0.05). The SMQ reflects the microbial community's ability to decompose

organic matter and cycle nutrients, where a higher SMQ indicates a robust microbial community that supports essential ecosystem services, such as carbon sequestration and nutrient transformations [24]. Long-term FYM application increased organic matter, total microbial biomass, enzyme activities and thus provide favorable environment for microbial proliferation, which explain the significant positive correlation of SMQ with MBC, SIRand soil enzymes (p<0.01) (Figure 4).

## 3.4. Soil enzymes activity

At same level of FYM, urease activity was higher in the N120 treatment compared to the N0 treatment. In soil of both the depths, urease activity increased with increasing FYM application rate from F0 to F30 under both the N levels (Figure 2). The urease activityin surface soil ranged from 11.8-58.6µg  $NH_{4}^{+}g^{-1}$  soil hr<sup>-1</sup>in 0-15 cm and 5.89-39.5µg  $NH_{4}^{+}g^{-1}$  soil hr<sup>-1</sup> in 15-30 cm soil depth (S2). With each successive addition of FYM dose the urease activity increased by 133%, 181%, 227% in surface soil and 154%, 279%, 323% in subsurface soil over F0. The addition of N led to 38% and 48% increase in urease activity at 0-15 cm and 15-30 cm soil depth respectively, compared to N0. Increased urease activity in manure and nitrogen-amended soil may be caused by the enzyme's constant access to substrates, which can be either organic (FYM) or inorganic (urea) sources [22]. Our results align with [25]. where they also found that urease activity increased with increased amount of manure. The integrated application of manure and nitrogen fertilizer further improved urease activity which was also reported by [26]. A highly significant positive correlation was reported between MBCand urease activity in both the soil depths (Figure 4) which confirms the role of microbes in nutrient cycling in soil. The increasing FYM levels and nitrogen application positively influence arginine deaminase activity across both soil depths (Figure 3). Its activity in 0-15 cm soil, ranged from 4.36-10.3  $\mu$ g NH<sub>4</sub><sup>+</sup>g<sup>-1</sup> soil hr<sup>-1</sup>and from 2.60-6.34  $\mu$ g  $NH_4^+$ g<sup>-1</sup> soil hr<sup>-1</sup>in 15-30cmsoil depth(S3). The highest level of FYM (F30) led to 104% and 127% increment in arginine deaminase activity in surface and subsurface soil, respectively compared to F0 treatments. The  $N_{\scriptscriptstyle 120}$  dose caused 15% and 4% increment in arginine deaminase activity in 0-15 cm and 15-30 cm soil layers, respectively. The enzyme arginine deaminase (ADA) is responsible for catalyzing the transformation of arginine into ammonia. In soil ecosystems, this enzymatic activity is essential to the cycling of nitrogen, particularly influencing microbial metabolism. Higher ADA activity often correlates with increased microbial biomass and diversity and the application of FYM, can stimulate microbial growth and provides additional nitrogen sources that can be metabolized by microbes, leading to increased arginine deaminase activities. Similar A similar increase in arginine deaminase activity due to FYM and N addition was also reported by [27]. The depth depthwise decrease in both enzyme activities may be attributed to lower organic matter content, reduced microbial activity, restricted oxygen supply and limited nitrogen uptake compared to the upper soil layer.

## Conclusion

The soil microbial biomass, microbial activity, and nitrogentransforming enzyme activities across both soil depths were significantly improved with the application of FYM and nitrogen on a long term basis. Apart from the increase in content of microbial biomass carbon and nitrogen, their addition also enhanced the microbial activity, as evident evidenced from elevated SIR and SMQ values. In addition to offering a direct source of organic carbon, FYM application also improved soil biological health, whereas nitrogen fertilization increased nutrient availability and further improved microbial metabolism by lowering microbial C:N ratio. Although FYM and N had significant individual effects on soil properties but their interaction was found non-significant. Thus, this work has highlighted that integrated manure and nitrogen management is involved in maintaining long-term soil fertility and optimizing nutrient cycling for agricultural sustainability. The molecular level of microbial communities changes and greenhouse gas emissions with long-term manuring and nitrogen fertilization under different agroclimatic region may be required for further indepth analysis of the results.

**Acknowledgement:**The authors are extremely thankful to ICAR-Indian Agricultural Research institute, Delhi and CCS Haryana Agricultural University, Haryanafor providing all the necessary infrastructure and facilities for carrying out this research. The authors also acknowledge the anonymous reviewers who helped with the manuscript review.

**Disclosure statement:** The authors disclosed no possible conflicts of interest.

Author Contribution: Pooja Rani- field experimentation, soilsampling and analysis, draft manuscript; Sarvendra conceptualization, final manuscript editing; TapanJyotiPurakayastha and Debashis Mandal-supervision: Rosin K.G.- review and editing; Dev Raj and UshaKumari- field experimentation; Abhishek JangirandArijitChowdhuri- data analysis, manuscriptediting.

Table1: Initial physicochemical properties of the experimental soil in the year of 1967.

Properties	Value					
Texture	Fine Sandy loam					
Taxonomic classification	TypicUstochrept					
Soil pH (1:2.5)	8.20					
Soil electrical conductivity (dS m <sup>-1</sup> )	0.43					
Organic carbon (%)	0.47					
CaCO3 (%)	1.12					
Available N (mg kg <sup>-1</sup> )	100					
Available P (mg kg <sup>-1</sup> )	13.0					
Available K (mg kg-1)	249					

 $Table 2: Impact of long-term application of FYM and N on available nitrogen (kg ha^4 soil) and organic carbon content (g kg^4 soil) of soil.$ 

Treatments	Available nitrogen							Organic carbon							
Treatments	0-15 cm			15-30 cm				0-15 cm		15-30 cm					
	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean			
Fo	169	224	197°	132	161	147 <sup>c</sup>	6.5	7.7	7.1 <sup>d</sup>	4.2	5.4	4.8 <sup>d</sup>			
F <sub>10</sub>	260	314	287 <sup>b</sup>	179	206	192 <sup>b</sup>	14.5	16.0	15.2°	11.8	12.8	12.3 <sup>c</sup>			
F <sub>20</sub>	279	328	304 <sup>ab</sup>	193	218	205 <sup>ab</sup>	16.8	17.9	17.4 <sup>b</sup>	13.9	14.4	14.1 <sup>b</sup>			
F <sub>30</sub>	291	328	310ª	205	238	221ª	18.3	19.6	19.0ª	14.7	16.0	15.3ª			
Mean	250 <sup>B</sup>	298 <sup>A</sup>		177 <sup>B</sup>	206 <sup>A</sup>		14.1 <sup>B</sup>	15.3 <sup>A</sup>		11.1 <sup>B</sup>	12.1 <sup>A</sup>				
					L	SD (p<0.05	)								
Factor	FYM- 21; N-22			FYM- 21; N-9			F	YM- 0.7; N-	).4	FYM- 0.4; N-0.2					
Intonation		FYM×N- NS	5;	FYM×N- NS;			FYM×N- NS;			EVINCIAL O 22 NUEVING 0 42					
Interaction		N×FYM- N	S	N×FYM- NS				N×FYM- NS	5	FYM×N- 0.32; N×FYM- 0.42					

Note: The same letter(s) after the means indicate no significant difference at p < 0.05. LSD, Fisher Least Significant Difference.  $F_{ov}$  Control (without farmyard manure);  $F_{10}$ , farmyard manure application @ 10 Mg ha<sup>-1</sup>;  $F_{20}$  farmyard manure application @ 20 Mg ha<sup>-1</sup>;  $F_{30}$ , farmyard manure application @ 30 Mg ha<sup>-1</sup>;  $N_{ov}$  without nitrogen;  $N_{120'}$  nitrogen @ 120 kg ha<sup>-1</sup>.FYM×N, FYM at same level of N; N×FYM, N at same level of FYM.

 $Table 3: Impact of long-term application of FYM and N on microbial biomass carbon (mg kg^4 soil) and microbial biomass nitrogen (mg kg^4 soil) of soil.$ 

Treatments		Ν	licrobial bio	mass carbo	n	Microbial biomass nitrogen						
	0-15 cm			15-30 cm				0-15 cm		15-30 cm		
	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean	No	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean
F <sub>0</sub>	162	217	190 <sup>d</sup>	91	128	110 <sup>d</sup>	16.2	25.0	20.6 <sup>d</sup>	8.25	13.3	10.8 <sup>d</sup>
F10	551	660	605°	362	459	411 <sup>c</sup>	66.9	84.4	75.6°	39.2	54.5	46.94
F <sub>20</sub>	746	893	819 <sup>b</sup>	567	644	605 <sup>b</sup>	81.5	107.1	94.1 <sup>b</sup>	54.7	72.1	63.4 <sup>k</sup>
F <sub>30</sub>	1041	1189	1115ª	720	852	786 <sup>a</sup>	111.1	138.2	125ª	66.6	92.0	79.3ª
Mean	625 <sup>B</sup>	740 <sup>A</sup>		435 <sup>B</sup>	521 <sup>A</sup>		69.0 <sup>B</sup>	88.5 <sup>A</sup>		42.2 <sup>B</sup>	58.0 <sup>A</sup>	
					LSD	( <i>p</i> <0.05)				•		
Factor	FYM- 96; N-68			FYM- 47.; N-45			FYM- 12.5; N-11.1			FYM- 2.9; N-6.1		
Interaction	FYM×N- NS;			FYM×N- NS;			FYM×N- NS;			FYM×N- NS;		
		N×FYM- NS			N×FYM- NS			N×FYM- NS		N×FYM- NS		

Table 4: Impact of long-term application of FYM and N on soil microbial quotient and MBC:MBN ratio of soil.

Treatments	Soil microbial quotient							MBC:MBN ratio						
	0-15 cm			15-30 cm				0-15 cm		15-30 cm				
	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean	N <sub>0</sub>	N <sub>120</sub>	Mean		
F <sub>0</sub>	2.47	2.80	2.64 <sup>d</sup>	2.15	2.35	2.25 <sup>d</sup>	10.1	8.73	9.40ª	11.1	9.54	10.3ª		
F <sub>10</sub>	3.78	4.13	3.95°	3.08	3.60	3.34 <sup>c</sup>	8.25	7.82	8.04 <sup>c</sup>	9.24	8.45	8.84 <sup>b</sup>		
F <sub>20</sub>	4.44	5.00	4.72 <sup>b</sup>	4.08	4.48	4.28 <sup>b</sup>	9.18	8.43	$8.81^{b}$	10.4	8.94	9.68 <sup>a</sup>		
F <sub>30</sub>	5.67	6.08	5.87 <sup>a</sup>	4.92	5.34	5.13 <sup>a</sup>	9.37	8.69	9.03 <sup>ab</sup>	10.8	9.28	10.1ª		
Mean	4.09 <sup>B</sup>	4.50 <sup>A</sup>		3.56 <sup>B</sup>	3.94 <sup>A</sup>		9.22 <sup>A</sup>	8.42 <sup>B</sup>		10.4 <sup>A</sup>	9.05 <sup>B</sup>			
					LSD	( <i>p</i> <0.05)								
Factor	FYM- 0.58; N-0.38			FYM- 0.38.; N-0.35			FYM- 0.52; N-0.40			FYM- 0.75; N-0.49				
Interaction	FYM×N- NS;			FYM×N- NS;			FYM×N- NS;			FYM×N- NS;				
Interaction		N×FYM- NS			N×FYM- NS			N×FYM- NS	5	N×FYM- NS				

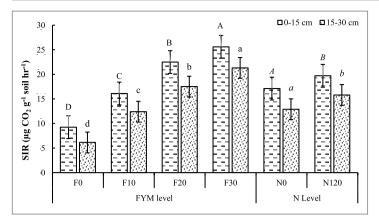


Figure 1: Mean effects of long-term application of FYM and N application on substrate induced respiration (SIR) ( $\mu g CO_2 g^{-1}$  soil  $hr^{-1}$ ) of soil.

Note: The same letter(s) indicate no significant difference at p < 0.05. LSD, Fisher Least Significant Difference. F<sub>0</sub>, Control (without farmyard manure); F<sub>10</sub>, farmyard manure application @ 10 Mg ha<sup>-1</sup>; F<sub>20</sub>, farmyard manure application @ 20 Mg ha<sup>-1</sup>; F<sub>30</sub>, farmyard manure application @ 30 Mg ha<sup>-1</sup>; N<sub>0</sub>, without nitrogen; N<sub>120</sub>, nitrogen @ 120 kg ha<sup>-1</sup>.FYM×N, FYM at same level of N; N×FYM, N at same level of FYM.

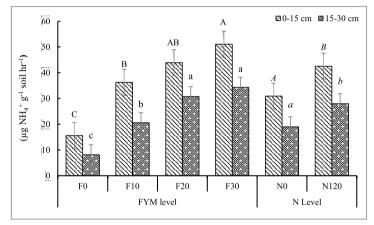


Figure 2: Mean effects of long-term application of FYM and N application on soil urease activity ( $\mu g NH_4^* g^{-1} soil hr^{-1}$ ).

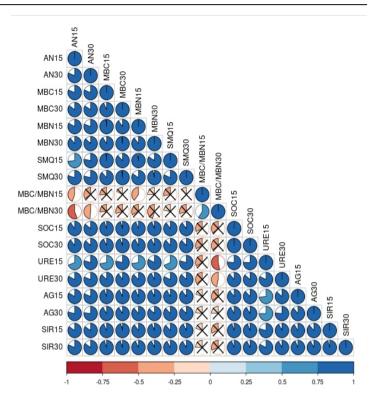


Figure 4: Correlation matrix of different soil properties at two different soil depths (0-15 cm) and (15-30 cm).

Note: Crossed correlation coefficients werenon-significant at p<0.05 level. AN, soil available nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SMQ, soil microbial quotient; MBC/MBN, ratio of MBC and MBN; SOC, soil organic carbon; URE, urease activity; AG, arginine deaminase activity; SIR, substrate induced respiration.

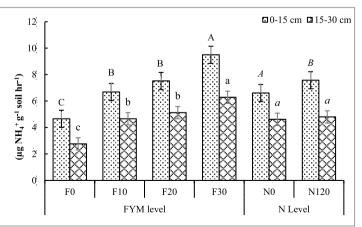


Figure 3: Mean effects of long-term FYM and N application on arginine deaminase activity ( $\mu g NH_4^* g^{-1} soil hr^{-1}$ ) of soil.

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