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Nitrogen Dynamics in Soil as Influenced by Split Application of Organic Manures and Fertilizers under Sugarcane Grown on Calcareous Entisol of Bihar

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Effect of nutrient management modules on nitrogen (N) dynamics in sugarcane grown on a Calcareous entisol was studied during 2018-19. The experiment comprised of different levels of NPK fertilizers alone and in combination with Biocompost, Neem Cake Powder, *Trichoderma* inoculated trash and *Rhizobium* inoculated green gram applied at two different crop growth stage (Planting and Earthing Up) was laid out in RBD with three replications. Application of 25% N as inorganic fertilizer + 75% N through organics (Biocompost at planting and Neem cake at earthing up stage split equally) + *Azotobacter* and PSB significantly increased the organic N fractions *viz.*, hydrolysable NH_4^+-N (108.3 mg kg^{-1}), amino acid-N (111.2 mg kg^{-1}), Hexoseamine-N (36 mg kg^{-1}) and Unidentified-N (83.9 mg kg^{-1}). The mineral N (NO_3^--N + exchangeable NH_4^+-N) content (105.6 mg kg^{-1}) was significantly increased with the application of 50% N as inorganic fertilizer + 50% N through organics (Biocompost at planting and Neem cake at earthing up stage split equally) + *Azotobacter* and PSB. The highest contribution of inorganic was 17.65% in treatment receiving 50:50 ratio of inorganic and organic sources of nutrients whereas organic N fractions contribution to total N was highest in treatment receiving 25:75 ratio of inorganic and organic sources of nutrients and comparatively lower contribution of these fractions was recorded in control treatment. The mineralizable N was significantly correlated with all fractions of N, except with hydrolysable unidentified-N and non-hydrolysable N.

Introduction

Crops generally require sufficient quantities of macro nutrients particularly nitrogen during the majority of crop growth period.

Nitrogen (N) is the most vital mineral nutrient which affects the growth and yield of crops. Being the 5th most abundant element in the earth has an important role in increasing food production and sustaining the ever-increasing

human and animal populations (Durani *et al.,* 2016). Nitrogen often limits the primary production in agricultural and natural ecosystems (de Vries *et al.,* 2006) therefore, its availability in adequate amount in plant available form is important for higher crop yields. The availability of nitrogen in soils is the key factor to determine the growth and yield of the crop. Its availability on earth is high ($\square$ 5 x 10^9 Tg) but <2% of it is available to organisms (Mackenzie, 2003). The available N in the soil plays a dominant role in the nutrition of crops.

The two chief forms of nitrogen in the soil are organic and inorganic nitrogen. Organic form of nitrogen accounts for more than 95% of total soil nitrogen and this form plays a significant role in N retention and transformation (Stevenson 1982).The availability of N to the growing plant is closely associated with depolymerization of the N-containing constituents organic forms of nitrogen followed by its subsequent mineralization (Nannipieri and Eldor, 2009). Although depolymerization followed by mineralization takes place of the constituents of organic nitrogen but it becomes slowly available to crop plants due to its diverse nature (Stevenson, 1994). Also, the amount of inorganic form of nitrogen is not adequate to meet the needs of crops; consequently, some external source of readily available form of N in the form of fertilizers is added.

The classical theory of organic nitrogen availability for crops indicates its biochemical transformation to release inorganic N (NO_3^- and NH_4^+), which is generally preferred for uptake. Incorporation of organic materials along with fertilizer-N affects the amount and distribution of organic N-fraction viz. exchangeable NH_4^+-N, hydrolysable NH_4^+-N, hexoseamine-N, amino acid-N, unidentified-N and status of total-N (Santhy *et al.,* 1998) and carbon pool considerably in soil (Sinha *et al.,* 2017). The direct absorption of dissolved

organic nitrogen (DON) is an important source of N nutrition, particularly in sandy soils low in N-supplying capacity and in the absence of external chemical sources (Jones *et al.,* 2005). Moreover, current evidence suggests that roots possess the capacity to take up low molecular weight DON, e.g., urea, amino acids, polyamines, and small polypeptides (Yu *et al.,* 2002).

The inorganic and organic form of nitrogen exists in equilibrium and is affected by various abiotic and biotic processes. Currently, large amounts of urea are applied to farmland soil, resulting in nitrate leaching, increased soil acidity and other environmental issues (Guo *et al.,* 2010). The alteration of soil properties leads to changes in C and N cycling, but the effects are inconsistent. Results from the long-term experiments envisaged that application of organic or chemical fertilizers alone failed to maintain the productivity of soil and sugarcane. The application of organic fertilizer in combination of chemical fertilizers not only helps sugarcane growing better, but also reduces the cost of cultivation, dependency on the chemical fertilizers, environmental pollution and soil health deterioration. With the raising apprehension on soil conservation and health in the context of depleting traditional organic manures, efforts are required to exploit the potentiality of easily available sources of organics effectively. Thus, this typical combination of nutrients under various nutritional modules proved better option for getting higher profitable cane and sugar yield besides improving soil health for sustaining sugarcane productivity.

Understanding the effect of manuring and fertilization on the N dynamics is prerequisite for precise N management under sugarcane based cropping system in Entisol of Bihar having high free $CaCO_3$. Therefore, the present investigation was carried out to study the effect of different nutrient management

modules on various N fractions and their relative contribution to yield of rice and N uptake.

Materials and Methods

A field experiment was conducted during 2018-19 at Crop Research Centre, Pusa farm, Dr. Rajendra Prasad Central Agricultural University, Bihar. The treatments comprised 7 different combinations of manures and fertilizers (Table 1). The recommended dose of fertilizers for sugarcane was 150: 85: 60 kg N, P_2O_5 and K_2O ha^{-1}. In T_2, *Trichoderma* inoculated sugarcane trash was spread 55 DAP. In T_3, green gram was sown as intercrop and was incorporated in soil at 60 DAP. In T_4, T_5, T_6 and T_7 neem cake powder was applied at earthing up stage at 120 DAP.

Experiment was laid out in randomized block design (RBD) with three replications. Soil samples were collected from 0-15 cm after harvest of sugarcane crop. Collected soil samples were dried in shade and ground with the help of wooden pestle and mortar. These ground samples were then passed through a 2 mm sieve and were mixed thoroughly and stored in polythene bags, properly labeled and preserved for subsequent analysis. fractions of organic-N and total-N in soil samples were estimated as per the methods suggested by Bremner (1965a,b). these fractions were determined: Inorganic-N (2 N KCL extract); total hydrolysable-N (digestion of residue after 2 KCL extract in 6 N HCL); hydrolysable NH_4^+–N (direct distillation of 25 mL of neutralized 6 N HCL extract); hexoseamine-N and hydrolysable NH_4^+–N (direct distillation of 25 ml of neutralized 6 N HCL extract after addition of 25 mL of phosphate borate buffer to give pH 11.2); (hexoseamine-N was calculated as difference); amino acid-N (ninhydrin method); unidentified hydrolysable (total hydrolysable-N—some of hydrolysable NH_4^+–N hexoseamine- N and amino acid-N); non-hydrolysable-N (determined by the same procedure mentioned earlier as in the case of total soil-N determination except that salicylic acid was not included in the digestion); and total-N (modified Kjeldahl method according to Bremner 1965a). The data were analyzed statistically as per Panse and Sukhatme (1971).

Table.1 Treatment details of the experiment

Treatments	Treatments Detail
T_1	100% NPK- RDF (Control)
T_2	100% N as IF + Organic mulching with ST @ 6t ha^{-1} + *Trichoderma*
T_3	100 % N as IF + GM with green gram as intercrop inoculated with *Rhizobium*
T_4	25% N as IF + 75% N through organics; BC, PL + NC, ER (1/2 each) + *Azophos*
T_5	50% N as IF + 50 % N through organics; BC, PL + NC ER (1/2 each) + *Azophos*
T_6	75% N as IF + 25 % N through organics; BC, PL + NC, ER (1/2 each) + *Azophos*
T_7	100% N through organics; BC, PL + NC, ER (1/2 each) + *Azophos*

RDF= Recommended Dose of Fertilizer, IF= Inorganic fertilizer, ST= Sugarcane Trash, BC= Biocompost, PL= Planting, NC= Neem Cake, ER= Earthing up, *Azophos= Azotobacter* + Phosphate solubilising Bacteria

Results and Discussion

Soil Inorganic Nitrogen

The distribution of soil inorganic nitrogen in the surface soil depth (0–15 cm) is presented in table 2.The highest value of NO_3^--N contributed 3% of total- N was observed in treatment T_1 (16.5 mg kg^{-1}) receiving 100% NPK and being lowest in treatment T_7 (13.2 mg kg^{-1}) receiving 100% N through organics (fig 1). The result indicated that application of organic manure reduced NO_3^--N content in soil. This might be attributed to the denitrification and losses of NO_3^- -N. Treatment T_5 (90.6 mg kg^{-1}) gave highest value of NH_4^+-N which received N in 50:50 ratio from inorganic and organic sources along with biofertilizers and lowest was recorded in T_1 (56.8 mg kg^{-1}) receiving 100% RDF in inorganic form. The increase in NH_4^+-N over control was followed the decreasing order T_5 (90.6 mg kg^{-1}) > T_4 (84.6 mg kg^{-1}) > T_6 (74.8 mg kg^{-1}) > T_3 (66.4 mg kg^{-1}) > T_2 (60.8 mg kg^{-1}) > T_7 (60.0 mg kg^{-1}). The NH_4^+-N contributed 13% to total N. Combined application of inorganic fertilizers along with organic manures increased both the inorganic forms of N over their individual application. Manivannan and Sriramachandrasekharan (2009) also reported increase in inorganic N with integration of manures and fertilizers.

Soil organic nitrogen

The distribution of nitrogen in hydrolyzable-N (HN) and non-hydrolyzable-N (NHN) fractions of soil organic nitrogen in the surface soil depth (0–15 cm) is presented in table 2 and the contribution of different forms of soil organic nitrogen to total soil nitrogen is presented in figure 1. Total hydrolysable-N fraction contributed maximum (55%) towards total N in soil. On an average, different component of total hydrolysable-N *viz.,* hydrolysable NH_4^+-N, hexoseamine-N, amino acid-N and unidentified-N contributed 29.0,

9.3, 35.4 and 26.3% respectively to total hydrolysable N. The maximum value of amino acid-N was obtained in treatment T_5 (112.6 mg kg^{-1}) receiving 50% N through inorganic fertilizer + 50% N as organic manure along with biofertilizers while, lowest value of 94.9 mg kg^{-1} was recorded in control (100% RDF). However, combined application of organic + inorganic nutrient sources did not produce any significant difference and therefore, treatments T_2 (104.7 mg kg^{-1}), T_4 (111.2 mg kg^{-1}), T_5 (112.6 mg kg^{-1}), T_6 (109.2 mg kg^{-1}) and T_7 (103.7 mg kg^{-1}) receiving combination of organic + inorganic nutrient sources were at par with each other.

The variation in hydolysable NH_4^+-N was found to be significantly affected (68.6 – 108.3 mg kg^{-1}) due to different nutrient combination. The maximum hydrolysable NH_4^+-N was found for treatment T_4 (108.3 mg kg-1) receiving 75 per cent N through organics (biocompost + neem cake) along with biofertilizers. However, treatments T_4 (108.3 mg kg^{-1}), T_5 (102.2 mg kg^{-1}) T_6 (95.1 mg kg^{-1}) and T_7 (82.1 mg kg^{-1}) was found to be at par with each other and significantly superior over treatments T_1 (68.6 mg kg^{-1}), T_2 (73.5 mg kg^{-1}) and T_3 (74.2 mg kg^{-1}). The extent of increase in hydrolysable NH_4^+-N due to application of different nutrient was 6.67, 7.55, 16.44, 27.86, 32.87 and 36.66 % in treatments T_2, T_3, T_7, T_6, T_5 and T_4 respectively over control.

The unidentified hydolysable-N contributed 26.3% to total hydrolysable-N fraction. It was found highest for treatment T_5 (84.3 mg kg^{-1}) receiving 50% N through inorganic fertilizer + 50% N through biocompost + neem cake along with biofertilizer with an increment of 5.1% over control (T_1; 79.8 mg kg^{-1}). The data further revealed that lowest value of unidentified hydolysable-N was recorded in treatment T_3 (73.8 mg kg^{-1}) receiving 100 % NPK + *Rhizobium* inoculated green gram as green manure.

Hexoseamine-N (9.3%) contributed lowest to total hydrolysable-N fraction. Highest value was obtained for treatment T_4 (36.0 mg kg^{-1}) followed by T_5 (32.1 mg kg^{-1}) T_6 (27.7 mg kg^{-1}) T_2 (25.8 mg kg^{-1}), T_7 (25.2 mg kg^{-1}) and T_3 (24.5 mg kg^{-1}) which were significantly higher over control (T_1; 21.7 mg kg^{-1}).

From the fig 1 it can be inferred that non-hydrolysable-N contributed 29 % to total-N. Non-hydrolysable-N fraction varied significantly from 140.5 - 164.1 mg kg^{-1} due to different nutrient management practices. The extent of augmentation due to different treatment over control was 10.39% (T_2 & T_3), 10.63% (T_6), 12.08 % (T_7), 13.11% (T_5) and 14.38% (T_4). Highest value of non-hydrolysable N was recorded in treatment T_4 receiving 75% N through organics + 25% N through inorganics while lowest in control (140.5 mg kg^{-1}) receiving 100% NPK through inorganic fertilizers.

Total-N

Total-N calculated as sum of NO_3^--N + Exchangeable NH_4^+-N + Total hydrolysable-N + Non-hydolysable-N was found to be highest for treatment T_4 (603.1 mg kg^{-1}) receiving 75% N through organics + 25% N through inorganics. However, treatments T_4 (603.1 mg kg^{-1}), T_5 (598.5 mg kg^{-1}) and T_6 (554.4 mg kg^{-1}) were at par with each other and significantly superior over rest other treatments. The extent of increment in total-N due to application of INM modules were 6.32, 6.70, 7.35, 13.63, 20.0 and 20.61 % in treatments T_3, T_7, T_2, T_6, T_5 and T_4 respectively over control (100% NPK).

Application of mineral fertilizers alone or in combination with organic manures might have significantly increased concentration of mineral N (NO_3^--N + NH_4^+-N) in the soil. The lower mineral N in control plot as compared to organic plot might be due to higher losses, such as volatilization, leaching and denitrification. The effect of mineral fertilizers and manures on the interplay between different fractions of organic N is a prerequisite for managing N inputs in a given soil. The changes in these fractions provided an assessment that additional N provided by organic fertilization was primarily concentrated in hydrolysable organic N fractions, which are considered the major source of plant available N.

The increase in hydrolysable N fraction with combined application of organic manures and inorganic fertilizer might be due to the mineralization and release of N contained in manure on their decomposition caused by a favourable environment and presence of consortium of microbes. Among the hydrolysable fraction, increase in hydrolysable NH_4^+-N may be attributed to decomposition of proteins, nucleic acids and large number of other organic compounds, while higher amino acid-N might be due to rate of mineralization of the protein fraction of added manures. Also, at higher level of organic manure application, the decrease in non-hydrolysable fraction of N may be ascribed to its conversion into hydrolysable-N.

The application of inorganic fertilizer and organic manure resulted increase in soil organic carbon which is turn increased the non hydrolysable-N content (Durani *et al.,* 2016). Similar findings were elucidated by Santhy *et al.,* (1998), Eagle *et al.,* (2001), Sarawad and Singh (2005), Zhong *et al.,* (2015) and Sinha *et al.,* (2017)

Table.2 Effect of INM modules on soil nitrogen fractions after sugarcane harvest

Treatments	N fractions (mg kg^{-1})								Total N
	Soil Inorganic N		Soil Organic N						
	NO$_3^-$-N	Exch. NH$_4^+$-N	Hydrolysable N						
			NH$_4^+$-N	Hexose-amine-N	Amino acid-N	Unidentified-N	Total Hydrolysable-N	Non Hydrolysable-N	
T$_1$	16.5	56.8	68.6	21.7	94.9	79.8	265.0	140.5	478.8
T$_2$	15.2	60.8	73.5	25.8	104.7	80.0	284.0	156.8	516.8
T$_3$	15.8	66.4	74.2	24.5	99.5	73.8	272.0	156.9	511.1
T$_4$	14.7	84.6	108.3	36.0	111.2	83.9	339.7	164.1	603.1
T$_5$	15.0	90.6	102.2	32.1	112.6	84.3	331.2	161.7	598.5
T$_6$	14.8	74.8	95.1	27.7	109.2	75.6	307.6	157.2	554.4
T$_7$	13.2	60.0	82.1	25.2	103.7	69.2	280.2	159.8	513.2
SEm(±)	0.5	1.8	2.2	0.6	2.1	2.3	6.8	4.4	13.1
LSD$_{(0.05)}$	1.5	5.4	6.7	1.9	6.3	6.7	20.9	13.6	40.4

Table.3 Correlation coefficient (r) among different fractions of soil nitrogen

|	NO$_3^-$-N	Ex-NH$_4^+$-N	Hydrolysable-N					Non-Hydrolysable-N	Total-N
			Hydrolysable NH$_4^+$-N	Hexose-amine-N	Amino acid-N	Unidentified-N	Total Hydrolysable-N		
NO$_3^-$-N	1								
Ex-NH$_4^+$-N	-0.149	1							
Hydrolysable NH$_4^+$-N	-0.414	**0.921****	1						
Hexoseamine-N	-0.329	**0.896****	**0.944****	1					
Amino axid-N	-0.506	**0.862***	**0.914****	**0.883****	1				
Unidentified-N	0.414	0.615	0.472	0.611	0.428	1			
Total Hydrolysable-N	-0.306	**0.941****	**0.975****	**0.974****	**0.929****	0.634	1		
Non-Hydrolysable-N	-0.680	0.655	0.722	**0.763***	**0.828***	0.075	0.703	1	
Total-N	-0.320	**0.965****	**0.969****	**0.970****	**0.940****	0.586	**0.990****	**0.766***	1

*Correlation is significant at the 0.05 level;   **Correlation is significant at the 0.01 level.

Fig.1 Percent contribution of different fractions of N to total N
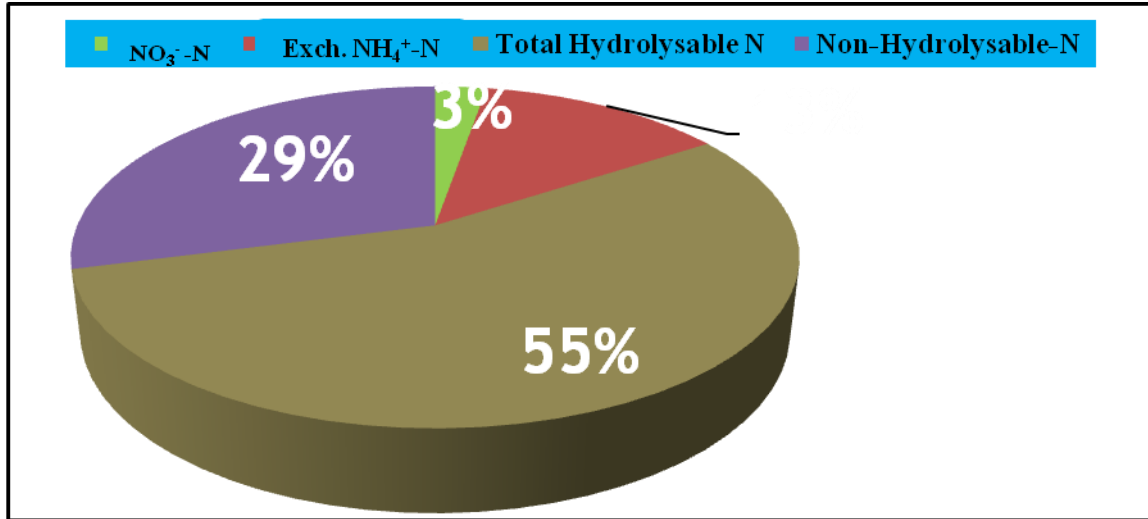
Fig.2 Percent contribution of different hydrolysable fraction of N to total Hydrolysable-N
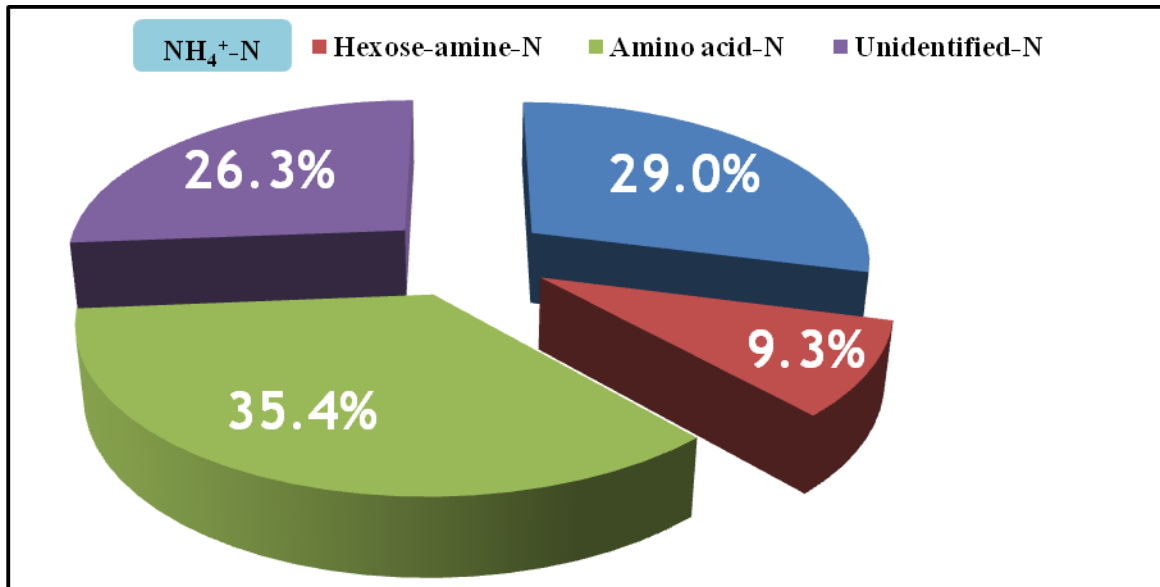
Correlation coefficient (r) among different fractions of soil nitrogen

The result presented in table 3 indicated that total-N was highly positively and significantly correlated with exchangeable NH_4^+-N (r=0.965**), hydrolysable NH_4^+-N (r=0.969**), hexoseamine-N (r=0.970**), amino acid-N (r=0.940**) and total hydrolysable-N (r=0.990**), while the value of correlation coefficient for non-hydrolysable-N was (r=0.766*). Also, no significant correlation was found between total-N and NO_3^--N and unidentified-N. The correlation coefficient value for non-hydrolysable-N with amino acid-N and hexoseamine-N was 0.828* and 0.763* respectively, while no correlation was found with exchangeable NH_4^+-N (r=0.655), hydrolysable-NH_4^+-N (r=0.722), NO_3^--N (r= -

0.680), unidentified-N (r=0.075), and total hydrolysable-N (r=0.703). Total hydrolysable-N had highly positive and significant correlation with all fractions except NO_3^--N and unidentified-N. The correlation value for total hydrolysable-N with exchangeable NH_4^+-N, hydrolysable NH_4^+-N, hexoseamine-N and amino acid-N was found 0.941**, 0.975**, 0.974**, and 0.929** respectively. Amino acid-N was positively and significantly correlated with exchangeable NH_4^+-N (r=0.862*), hydrolysable NH_4^+-N (r=0.914**) and hexoseamine-N (r=0.883**). It was also observed that hexoseamine-N showed highly positive and significant correlation with exchangeable NH_4^+-N (0.896**) and hydrolysable NH_4^+-N (0.944**). Also, hydrolysable NH_4^+-N was positively and significantly correlated with exchangeable NH_4^+-N (r=0.921**). The data indicated that since NO_3^--N failed to produce significant correlation with any of the other N fraction and this fraction is not in equilibrium with other nitrogen fractions of soil. This might be due to highly mobile nature of NO_3^--N. The other fractions were in dynamic equilibrium indicating interchangeable behavior of these N Fractions. The present findings are in accordance with Umesh *et al.,* (2014). Schomberg *et al.,* (2009), Durani *et al.,* (2016), and Liu *et al.,* (2018) reported similar findings.

The above study conducted in calcareous soil of Bihar revealed that due to integrated application of organic and inorganic sources different fractions of soil N *viz.,* NO_3^--N, exchangeable NH_4^+-N, total hydrolysable-N, non-hydolysable-N and total-N varied significantly. The contribution of different fractions of soil N to total-N was 3% for NO_3^--N, 13% for exchangeable NH_4^+-N, 55% for total hydrolysable-N and 29% for non-hydolysable-N. The total-N was highly positively and significantly correlated with

exchangeable NH_4^+-N, hydrolysable NH_4^+-N, hexoseamine-N, amino acid-N and total hydrolysable-N. The NO_3^--N did not produce significant correlation with any of the other N fractions which indicated that NO_3^--N fraction was not in equilibrium with other soil nitrogen fractions. The maximum increment in cane yield by 20.67% was recorded in treatment T_5 receiving 50 per cent N through inorganic + 50 per cent N through organic fertilizer along with biofertilizer and lowest in T_1 receiving 100% NPK (control). Application of 100% N through organics resulted cane yield similar to application of recommended dose of fertilizer (100% NPK). Nitrogen uptake followed the similar trend of cane yield.

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