Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1112-1116
Received: 01-06-2020
Accepted: 03-07-2020

# Effect of long term fertiliser and manure application on Soil Nitrogen Fractions in an Inceptisol under Fingermillet: Maize cropping sequence

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## Abstract
A field experiment was conducted to study the effect of continuous manuring and fertiliser application on soil nitrogen fractions in sandy clay loam soil (Inceptisol) during 2019 -20 in the ongoing Long-Term Fertilizer Experiment with Finger millet - maize cropping sequence. The results revealed that the nutrient availability in soil and nutrient uptake were significantly influenced by FYM along with 100% NPK application. The soil received graded levels of fertiliser from 50% to 150% increased the soil inorganic and organic nitrogen fractions. Comparing the treatments, the treatment which received 100% NPK +FYM recorded the highest values for different fractions of inorganic N (Exchangeable $NH_4$-N, Exchangeable $NO_3$-N & Fixed - N) and organic N fractions (Total hydrolysable N, Total hydrolysable $NH_4$ - N, Hexosamine N, Amino acid N, Unidentified hydrolysable N and Unidentified non hydrolysable N) followed by 150% NPK. Other graded level fertiliser treatments such as 100% NPK, 100% NP and 100% NPK + Hand Weeding, 100% NPK (S free) were on par with each other and the lowest value was observed in control treatment. Significant positive correlations were obtained between N fractions and yield, total N uptake, available N and organic carbon.

**Keywords:** N fractions, $NH_4$-N, $NO_3$-N, fixed N and Hydrolysable - N

## Introduction
Nitrogen is vitally important plant nutrient and is the most frequently deficient of all nutrients. It is absorbed by plants as nitrate ($NO_3$) and ammonium ($NH4^+$) ions. The quantities of $NH_4{}^+$ and $NO_3^-$ available to plants largely depend on the amounts applied as N fertilizers and mineralized from organic soil N. The inorganic forms of soil nitrogen include ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), nitric oxide (NO), nitrous oxide ($N_2O$) and elemental nitrogen. $NH_4^+$, $NO_3^-$ and $NO_2^-$ are important in soil fertility and represent 2 to 5% of total nitrogen. About 90 to 95 per cent of the soil N exists as organic form and cannot be utilised by the plant as it is bound to the clay humus complexes. Organic nitrogen compounds occur as consolidated amino acids or proteins, free amino acids, amino sugars and other unidentified compounds like materials that result from the reaction of $NH_4^+$ with lignin, polymerisation of quinones and nitrogen compounds, the condensation of sugars and amines. Bound amino acids are to the extent of 20-40%, amino sugars (hexosamines) 5 to 10%, purine and pyrimidine derivatives 1% or less.
Soil N availability is credited by two ways such as soil available nutrients and secondarily from fertilisers, manures and green manures etc. Long term application of fertilisers and organic manures has influenced the soil organic, inorganic N pools in soil. $NH_4^+$ is not taken up by plants despite its converted into $NO_3^-$ ions through nitrification process. $NH_4$-N or $NO_3$-N is immobilized into organic fractions and recycled into the inorganic N pool due to soil microbial biomass and this transformation rate is better in no till than tilled soil (Muruganandam *et al.*, 2010).
Nitrate N is found 4.4 times higher in the soil and it is considered as the dominant mineral fraction than $NH_4^+$-N. Significant amount of Hydrolyzable-N is increased by the addition of organic manures and total hydrolyzable-N fractions in soils consists of ammonia -N 25%, amino sugar-N 9.9%, amino acid-N 25.2%, and hydrolyzable unknown-N 39.8%.
Soil $NO_3^-$ ions are soluble and could be leached easily when excess water percolates through coarse textured soil. However, the state of mechanism could different and losses could be low in fine textured soil.
Combined application of organic manures and inorganic fertilizers has significantly improved the N fractions in the soil, which would help increase the rice and wheat yield

(Durani *et al*. 2016) [6]. With this background, the present study has been taken up to study the effect of long term fertiliser and manure application on Soil Nitrogen Fractions in an Inceptisol under Fingermillet – Maize cropping sequence.

**Materials and Methods**

**Site Description**

To evaluate the long term effect of fertilization and manuring on soil fertility and crop productivity under finger millet-maize cropping sequence, a long term fertilizer experiment (LTFE) trial was started during 1972 and being maintained by Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore. The present study was carried out during 2019 -20 in the on going Long-Term Fertilizer Experiment. The Experimental site is located at 11° North latitude, 77° East longitude and at an altitude of 426.7 meter above mean sea level in field No.36 F, Eastern block farm.

The experimental site belongs to order *Inceptisol*, having calcareous mixed black soil with sandy clay loam texture and comes under periyanaickenpalayam series of V*ertic Ustropept*. The soil is dark greyish brown, deep, fine, imperfectly drained and developed from reddish brown calcareous colluvial materials. It develops cracks during summer and shows their presence of distinct slickenside in sub soil layers. The present experiment was conducted with ten different fertiliser doses and sources such as 50% NPK, 100% NPK, 150% NPK, 100% NPK+ Hand Weeding, 100% NPK + Zinc, 100% NP, 100%N, 100%NPK+FYM, 100% NPK (Sulphur Free Source) along with Control and the trial is replicated thrice. The information regarding the physic-chemical properties of the soil as analysed during 1972 is furnished in Table 1.

**Table 1:** Initial (1972) soil characteristics of experimental site

| Soil Properties | Value |
|---|---|
| **A. Physical properties** | |
| Clay (%) | 32.6 |
| Silt (%) | 11.8 |
| Fine sand (%) | 15.1 |
| Coarse sand (%) | 39.4 |
| Textural class (surface soil) | Sandy clay loam |
| **B. Physicochemical properties** | |
| pH | 8.20 |
| Electrical conductivity ($dSm^{-1}$) | 0.20 |
| Cation exchange capacity ($cmol(p^{+})kg^{-1}$) | 25.2 |
| **C. Chemical properties** | |
| Organic carbon ($g\ kg^{-1}$) | 3.0 |
| Total N ($mg\ kg^{-1}$) | 428.0 |
| Total P ($mg\ kg^{-1}$) | 490.0 |
| Total K ($mg\ kg^{-1}$) | 3964.0 |
| Available nitrogen ($kg\ ha^{-1}$) | 178.0 |
| Available phosphorous ($kg\ ha^{-1}$) | 11.0 |
| Available potassium ($kg\ ha^{-1}$) | 810.0 |
| Available Zn ($mg\ kg^{-1}$) | 2.58 |
| Available Cu ($mg\ kg^{-1}$) | 4.20 |
| Available Mn ($mg\ kg^{-1}$) | 2.74 |
| Available Fe ($mg\ kg^{-1}$) | 2.74 |

**Soil analysis**

The representative soil samples were collected from 0-15 cm depth from all the treatments and the individual soil nitrogen fractions were analysed by the procedure given by Bremner (1965) [4]. The inorganic nitrogen fractions *viz*., exchangeable $NH_4$-N and exchangeable $NO_3$-N were estimated by the preparation of equilibrium extract (10 grams of soil was taken in a shaking bottle and 50 ml of 2M KCL was added and kept in mechanical shaker for one hour and the filtrate collected through whatman No 1 filter paper) For the estimation of exchangeable $NH_4$-N, the above filtrate of about 20 mL was distilled with freshly ignited MgO in Bremner's distillation unit. After the extraction of exchangeable $NH_4$-N from the equilibrium extract, 1mL of sulphamic acid, a pinch of devarda's alloy and 10 mL of 40% NaOH was added and the distillation was continued for the estimation of exchangeable $NO_3$-N.

The organic fractions of nitrogen were estimated by the preparation of hydrolysate which involves transferring of residues after the extraction by 2M KCL into a 250 mL conical flask. 40 mL of 1N HCL (Soil: HCL ratio of 1:4) and two drops of octyl alcohol was added to it. This suspension was kept in the water bath at 100° C and digested for about 12 hours under reflux condenser. Using Whatman No.50 filter paper this hydrolysate mixture was filtered and the residue in the filter paper was washed with distilled water for 3 to 4 times and it was made upto 50 mL of volume. Initially the pH was adjusted to 5.0 by addition of 5N NaOH and finally pH was adjusted to 6.5 with 0.5N NaOH and finally the volume was made upto 100 mL. This neutralized hydrolysate mixture was used for the estimation of organic nitrogen fractions through distillation process.

**Results and Discussion**

**Effect of continuous application of manures and fertilisers on soil N fractions**

**Inorganic Nitrogen Fractions**

**Exchangeable $NH_4$ –N ($mg\ kg^{-1}$)**

Continuous application of graded level of fertilisers and manures increased the exchangeable $NH_4$ - N significantly upto 150% NPK which accounts to the direct influence of high amount of N applied and also attributed to the organic carbon present in the soil (Table 2). Application of 100% NPK + FYM @10t $ha^{-1}$ shows the maximum value for exchangeable $NH_4$-N as 9.5 $mg\ kg^{-1}$ followed by 150% NPK (8.4 $mg\ kg^{-1}$) plots. This might be due to the application of manures which supplies the quantum of N with other rich mineral nutrients. The applied manures with Nitrogenous fertilisers along with P and K has enhanced the microbial dynamics, boosting the mineralization rate of fertiliser N into $NH_4$-N, available to plants. The other treatments such as 100% NPK+$ZnSO_4$, 100% NPK, 100% NPK + Hand Weeding, 100% NPK (-S free) are on par with each other. The plots which received 50% NPK observed the low amount of $NH_4$-N (6.2 $mg\ kg^{-1}$) as the N applied through fertilizers is significantly reduced which resulted in poor formation of $NH_4$-N due to inadequate amount of N supply. The least amount of exchangeable $NH_4$ - N was observed in control plots (3.6 $mg\ kg^{-1}$) resulted by the absence of external supplementation of nitrogenous sources. This result was supported by Sharma *et al.* 2014, who revealed that, incorporation of organic residues could help to enhance the N content in an alfisol and also balanced application of NPK along with FYM increased the labile N pools in rice - rice cropping systems (Bhattacharyya *et al*. 2013) [1].

**Exchangeable $NO_3$ - N ($mg\ kg^{-1}$)**

Total N constitutes 0.7 to 2.7% of Nitrate - N and the significant amount of $NO_3$ - N was observed by the application of manures and different graded level of fertilizers (Table 2). It was ranged from 9.6 to 15.5 $mg\ kg^{-1}$ in which, the highest amount of $NO_3$-N was observed in 100% NPK+FYM

(15.5 mg $kg^{-1}$) followed by 150% NPK (14.7 mg $kg^{-1}$) and the lowest in control plots (9.6 mg $kg^{-1}$). This was due to the improved microbial activity by the addition of organic manures which caused enhanced nitrification process and the end result is maximum $NO_3$ - N in soil. The increase in the $NO_3$ - N in soil is also attributed to the simultaneous reduction in the $NO_3^-$ leaching. This was supported by Khankane and Yadav (2002) who revealed that, soil $NO_3$-N content increases by addition of FYM and nitrification process in the soil is enhanced as the application rate of inorganic N in soil is increased due to the conversion of the applied inorganic N (Yadav and Singh, 1991) [20].

**Fixed $NH_4$-N (mg $kg^{-1}$)**

Ammonium fixation is relatively high in soils having 2:1 and 2:2 clay minerals and the experimental soil is Vertic Ustropet which has high capacity to fix N in soil. Continuous addition of fertilisers has significantly increased the fixed N in soil and the highest fixed N was noticed in INM (100% NPK +FYM @ 10 t $ha^{-1}$) as 39.9 mg $kg^{-1}$ followed by 150% NPK (39.2 mg $kg^{-1}$) and lowest in control (24.5 mg $kg^{-1}$) plots (Table 2). This may be due to applied fertilisers has increased the mineralization rate of $NH_4^+$ and the $NH_4^+$ ions goes to the solution phase and it get exchanged to the other ions or fixed in the inter lattice of clays. The experimental soil have vertic properties like high CEC and continuous addition of manures induced the formation of clay humus complex which also reduces the N losses from leaching, nitrification etc. The fixed N as a residual N in clay lattice further utilised by the plant and equilibrium has been cycled in soil. This was supported by Stevenson (1982) [16] who revealed the fixed $NH_4$-N generally found high in soil containing high micaceous clay minerals and hence the fixed $NH_4$-N in soil depends on the type and amount of clay minerals contained in the soil.

**Table 2:** Effect of long term application of fertilizers and manures in soil inorganic nitrogen fractions (mg $kg^{-1}$)

| Treatments | Exchangeable $NH_4$ –N | Exchangeable $NO_3$-N | Fixed $NH_4$ –N |
|---|---|---|---|
| 50%NPK | 6.2 | 10.7 | 31.5 |
| 100%NPK | 7.3 | 13.1 | 35.6 |
| 150%NPK | 8.4 | 14.7 | 39.2 |
| 100%NPK+HW | 7.7 | 13.6 | 36.1 |
| 100%NPK+Zn | 7.4 | 13.8 | 36.2 |
| 100%NP | 7.2 | 13.9 | 36.5 |
| 100%N | 7.5 | 13.5 | 35.9 |
| 100%NPK+FYM | 9.5 | 15.5 | 39.9 |
| 100%NPK(S free) | 7.7 | 13.5 | 35.9 |
| Control | 3.6 | 9.6 | 24.5 |
| SEd | 0.15 | 0.26 | 0.84 |
| CD (P = 0.05) | 0.32 | 0.54 | 1.76 |

**Organic N fractions**

**Total Hydrolysable $NH_4$ -N**

Organic N fractions contribute the major portion of total N present in the soil and total hydrolysable N constitutes more than 75% of the total N in soil. Significant increase of total hydrolysable N was noticed with application of graded level of fertilisers and the highest total hydrolysable N was observed in INM (321.9 mg $kg^{-1}$) followed by $T_3$ (305.5 mg $kg^{-1}$) and the lowest was observed in control plot (218.4 mg $kg^{-1}$) (Table 3). This was due to the application of manures and fertilisers that increase the organic N fraction by the decomposition of amides, amino sugars, amino acids and other inorganic N fractions. This was supported by Bird *et al.* (2002) [2], who revealed that the balanced fertilization of organic and inorganic fertilization records the maximum value of organic nitrogen fraction over the optimal NPK fertilization. This may be due to the intrinsic characteristic of organic matter in enlarging the effects of inorganic fertilizers which resulted in higher organic N fractions. The combined application of FYM with NPK as balanced fertilization has increased the organic N accumulation in a Long term fertilization of 39 years in rice –rice cropping system (Bhattacharyya *et al.*, 2013) [1].

**Hydrolysable $NH_4$-N and Hexosamine N**

Hydrolysable $NH_4$-N and Hexosamine N was significantly increased by the application of manure with fertilisers in a long run. Among the treatments, INM showed the higher amount of Hydrolysable $NH_4$-N (145.6 mg $kg^{-1}$) and Hexosamine N (35.6 mg $kg^{-1}$) followed by 150% NPK (140.2 and 34.2 mg $kg^{-1}$) (Table 3). The balanced fertilization of organic and inorganic fertilization records the maximum value of organic nitrogen fraction over the optimal NPK fertilization. This may be due to the intrinsic characteristic of organic matter in enlarging the effects of inorganic fertilizers which resulted in higher organic N fractions. This result was supported by Huang *et al.* (2009) [8], who noticed that addition of organic manures with or without inorganic fertilizers shown significant boost up in the organic N fractions.

**Table 3.** Effect of long term application of fertilizers and manures in Organic soil nitrogen fractions (mg $kg^{-1}$)

| Treatments | Total hydrolysable N | Hydrolysable $NH_4$-N | Hexosamine N |
|---|---|---|---|
| 50%NPK | 263.6 | 119.5 | 28.1 |
| 100%NPK | 281.3 | 135.7 | 32.6 |
| 150%NPK | 305.5 | 140.2 | 34.2 |
| 100%NPK+HW | 279.6 | 131.5 | 32.2 |
| 100%NPK+Zn | 275.2 | 133.3 | 31.5 |
| 100%NP | 281.5 | 126.5 | 30.7 |
| 100%N | 278.1 | 121.6 | 30.1 |
| 100%NPK+FYM | 321.9 | 145.6 | 35.6 |
| 100%NPK(S free) | 273.5 | 130.3 | 31.4 |
| Control | 218.4 | 95.3 | 24.6 |
| S.Ed | 6.58 | 2.59 | 0.50 |
| CD (P = 0.05) | 13.83 | 5.44 | 1.05 |

**Amino acid N, Unidentified hydrolysable N and Unidentified non hydrolysable N**

Application of fertilisers and manures in the long run has increased the organic N fractions such as Amino acid N, Unidentified hydrolysable N and Unidentified non hydrolysable N (Table 4). The maximum Amino acid N, Unidentified hydrolysable N and Unidentified non hydrolysable N recorded are 110.5, 92.8 and 237.5 mg $kg^{-1}$ respectively in INM plots followed by 150% NPK (108.4, 90.5 & 232.4 mg $kg^{-1}$) and the minimum was noticed in control (77.5, 42.6 & 84.5 mg $kg^{-1}$). This may be due to addition of manures along with fertilisers has enhanced microbial population which maintains the N equilibrium in the soil. This was supported by Guldekar and Ingle (2009) who reported that FYM along with fertiliser N, sulphur and Zinc had improved the N fraction status in soil.

**Table 4:** Effect of long term application of fertilizers and manures in Organic soil nitrogen fractions (mg $kg^{-1}$)

| Treatments | Amino acid N | Unidentified hydrolysable N | Unidentified non hydrolysable N |
|---|---|---|---|
| 50%NPK | 89.5 | 63.4 | 112.3 |
| 100%NPK | 103.6 | 78.5 | 189.7 |
| 150%NPK | 108.4 | 90.5 | 232.4 |
| 100%NPK+HW | 102.5 | 76 | 192.2 |
| 100%NPK+Zn | 99.8 | 80 | 193.6 |
| 100%NP | 95.6 | 79.2 | 186.6 |
| 100%N | 93.5 | 77.2 | 139.2 |
| 100%NPK+FYM | 110.5 | 92.8 | 237.5 |
| 100%NPK(S free) | 100.3 | 78.5 | 182.1 |
| Control | 77.5 | 42.6 | 84.5 |
| SEd | 1.94 | 1.17 | 3.86 |
| CD (P = 0.05) | 4.08 | 2.47 | 8.12 |

**Correlation between Yield, total N uptake, available N, organic carbon and Soil N fractions**

Simple correlation was worked out between various N fractions and yield, total N uptake, available N and organic carbon. The correlation studies revealed a positive relationship with all the parameters evaluated (Table.5.). Significant positive correlations were obtained between N fractions and yield, total N uptake, available N and organic carbon. Among the inorganic N fractions, exchangeable $NH_4$ – N was highly correlated with grain yield (r = 0.87**). With respect to organic N fractions Hydrolysable $NH_4$-N recorded maximum correlation with grain and straw yield (r = 0.95**). The correlation studies indicated strong positive relationship between soil organic carbon content and yield, total N uptake and available N.

**Table 5:** Matrix of simple correlation coefficients between Maize Yield, total N uptake, available N, organic carbon and Soil N

| Parameters | Grain Yield | Straw Yield | Total N uptake | Soil Available N | SOC (%) | Exh. Amm. N | Exh. Nitrate. N | Fixed Amm. N | Total hydrolysable N | Hydrolysable Amm. N | Hexosamine N | Amino acid N | unidentified Hyd. N | unidentified Non Hyd. N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grain Yield | ** | 0.97 | 0.96 | 0.85 | 0.93 | 0.87 | 0.78 | 0.86 | 0.88 | 0.95 | 0.89 | 0.90 | 0.86 | 0.87 |
| Straw Yield | | ** | 0.94 | 0.9 | 0.95 | 0.90 | 0.89 | 0.91 | 0.89 | 0.96 | 0.93 | 0.92 | 0.92 | 0.94 |
| Total N uptake | | | ** | 0.94 | 0.95 | 0.87 | 0.83 | 0.86 | 0.90 | 0.96 | 0.94 | 0.94 | 0.89 | 0.94 |
| Soil Available N | | | | ** | 0.9 | 0.91 | 0.93 | 0.90 | 0.93 | 0.92 | 0.95 | 0.93 | 0.93 | 0.96 |
| SOC (%) | | | | | ** | 0.87 | 0.84 | 0.84 | 0.84 | 0.93 | 0.92 | 0.93 | 0.85 | 0.93 |
| Exh. Amm. N | | | | | | ** | 0.95 | 0.98 | 0.97 | 0.95 | 0.96 | 0.94 | 0.98 | 0.89 |
| Exh. Nitrate. N | | | | | | | ** | 0.97 | 0.92 | 0.90 | 0.94 | 0.91 | 0.97 | 0.94 |
| Fixed Amm. N | | | | | | | | ** | 0.96 | 0.95 | 0.95 | 0.93 | 0.99 | 0.91 |
| Total hydrolysable N | | | | | | | | | ** | 0.94 | 0.96 | 0.93 | 0.96 | 0.90 |
| Hydrolysable Amm.N | | | | | | | | | | ** | 0.98 | 0.98 | 0.96 | 0.94 |
| Hexosamine N | | | | | | | | | | | ** | 0.99 | 0.96 | 0.97 |
| Amino acid N | | | | | | | | | | | | ** | 0.95 | 0.96 |
| unidentified Hyd. N | | | | | | | | | | | | | ** | 0.93 |
| unidentified Non Hyd. N | | | | | | | | | | | | | | ** |

## Conclusion

Results of the present study revealed that the application of manures along with fertilisers increased the N-fractions *viz*. Exchangeable $NH_4$-N, Exchangeable $NO_3$-N, Fixed $NH_4$-N, Total hydrolysable-N, Hydrolysable $NH_4$-N, Hexosamine N, Amino acid-N, Unidentified hydrolysable-N, Unidentified non-hydrolysable-N. Exchangeable $NH_4$-N, Exchangeable $NO_3$-N and Fixed $NH_4$-N were significantly influenced by the incremental additions of N. Significant positive correlations were obtained between N fractions and yield, total N uptake, available N and organic carbon. Among the various organic N fractions, the total hydrolysable-N contributed much towards organic N pools. These results showed that INM is the best way to sustain the soil nutrient status.

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