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Influence of green manure crops on nitrogen mineralization, soil enzyme activities and nitrifying bacterial population in Entisols

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Abstract
The changing climate and increasing population have raised the need for increased food production without compromising the quality of atmosphere as well as soil health. The present opportunities for sustainable agriculture lead the way in reducing these negative effects on soil as well as plant productivity. In view of this, pot culture and incubation studies were conducted to analyze the influence of different green manuring crops on the nitrogen mineralization, soil enzyme activities, and nitrifying bacterial population in Entisols for 75 days with seven treatments, *viz.*, Sunhemp, cluster bean, cowpea, mutant dhaincha, green gram, soybean, and control during *rabi* 2020. The incorporation of green manure crop residues in the soil increased the soil nitrogen mineralization rate. The mineralization rate was slow at the initial stage, which gradually increased to attain a maximum by 30 days of incubation, then eventually decreased till the end of the incubation period. The incorporation also improved the soil urease and dehydrogenase activity along with nitrifying microbial population, *viz.*, *Nitrosomonas* and *Nitrobacter* in the soil. Mutant dhaincha (TSR-1) incorporation exhibited substantial influence on nitrogen mineralization (NH4-N + NO3-N), enzyme activity, and microbial population in the soil.

Keywords: Nitrogen mineralization, urease, dehydrogenase, *Nitrosomonas, Nitrobacter*

Introduction
World food security is facing challenges from the changing climate, increasing population and deteriorating soil quality. The depletion of soil organic matter is a major reason for the degradation of agricultural soils. Entisols are recently developed but highly productive soils, but their environment and chemical fertilizer use make them vulnerable to degradation. The cultivation of suitable green manuring crops and their in-situ incorporation at appropriate growth stage is considered as the most effective means of improving natural supply of organic matter to the soil. It also stimulates soil microbial activity with subsequent mineralization of plant nutrients and improves the physico-chemical and biological properties of soil.

Nitrogen is absorbed by plants in high amounts as it plays an important role in plant metabolism and growth. Despite the high nitrogen contents of soils, most of the N is not in available forms. Nitrogen is fertilized in soils in different ways, including organic residues or mineral fertilizers. Of the mineral nitrogen applied to soil, plants take up 50%, 25% remains in the soil, and 25% gets lost through various mechanisms. Mineralization of organic nitrogen of the added plant material is rapid at first. It can be slowed down by stabilization of the organic residue, as reported by Singh and Singh (2003) [11]. Soil organic matter and texture are two significant factors that affect the rate of nitrogen mineralization in soils. The mineralization of N from crop residues varies with the C: N ratio of residue (Pathak and Sarkar, 1994) [9]. Microbial biomass represents only 5% of soil organic matter, but it is a significant repository of soil nutrients as many nutrient transformations occur in the microbial biomass (Dick, 1992) [3]. During microbial breakdown, enzymes are released into the environment to break down complex organic molecules into simple molecules that plants can absorb (Almeida *et al*., 2015) [1]. Thus nutrients contained within the plant tissues reach the subsequent crops. The activity of soil microorganisms greatly influence the enzyme activity. Analyses of soil enzyme activities provide information on biochemical processes occurring in the soil that are important for soil quality and health (Gianfreda, 2015) [4].

An understanding of the nutrient release dynamics enables management of the organic residues in such a manner that optimizes nutrient uptake and ultimately increases crop Productivity. Therefore, it is essential to have better knowledge of N supplying capacity of the wide variety of green manures to develop feasible and efficient N management strategies. Given this, an investigation was undertaken to study the influence of green manuring crops on nitrogen mineralization, soil enzyme activities, and nitrifying bacterial population in Entisols.

Materials and Methods

Experimental location and soil

The experiment was carried out in Division of Soil Science and Agricultural Chemistry, Pune (18 degree 32" N, 73 degree 51" E) during *Rabi* 2020. The soil type selected for experiment was sandy loam. The peculiar characteristics of the soil were as follows: bulk density 1.45 g cm^{-3}, pH (1:2.5) 7.62, EC 0.14 dS m^{-1}, organic carbon 0.27%, $CaCO_3$ 4%, available N 109.8 kg ha^{-1}, available P 24.85 kg ha^{-1}, available K 257.70 kg ha^{-1}, NH_4-N 5.20 mg kg^{-1} and NO_3-N 6.15 mg kg^{-1}.

Treatment details

The seven treatments used for the experimentation were T_1 (sunhemp), T_2 (cluster bean), T_3 (cowpea), T_4 (mutant dhaincha), T_5 (green gram), T_6 (soybean) and T_7 (Control). The green manuring crops were grown in pots in three replications without fertilizer. They were harvested at 50% flowering (i.e. 45 days after sowing) and shredded and used for conducting experiment.

Experiment details

The green manuring crops were sown as per recommended seed rate in respective pots without fertilizer on the basis of weight of soil in pot. After germination, only 5 healthy plants were kept as per treatment. All the crops showed 50% flowering at around 45 days after sowing. So they were harvested at that stage, shredded and incorporated in the same pots for the incubation up to 75 days. The soil samples were drawn at an interval of 15 days up to 75 days (i.e. at 0, 15, 30, 45, 60 and 75 days after incubation) and analyzed immediately for nitrogen mineralization (NH_4-N and NO_3-N) as per standard method. The soil sample from each treatment were drawn at an interval of 30 days up to 60 days (0, 30 and 60 days) for enzyme *viz.*, urease and dehydrogenase analysis, and nitrifying bacterial population. The soil moisture content was regulated at field capacity during incubation.

Results and Discussion

Effect of green manure crop residue incorporation on nitrogen mineralization

Periodical Ammonical Nitrogen (NH_4-N)

Soil treated with green manure crop residues exhibited a significant increase in NH_4-N release throughout the incubation period (Table 1). The NH_4-N release increased slowly during the initial phase, reached a maximum by 30 days, then declined thereafter. At 30 days, NH_4-N release from mutant dhaincha amended soil recorded superior (18.32 mg kg^{-1}) over all other treatments due to high N and narrow C:N ratio. This result conformed the findings of Singh and Kumar (1996) [12].

Table 1: Effect of green manure crop residues on periodical Ammonical nitrogen content in soil

Treatments	Ammonical nitrogen (mg kg^{-1})						Cumulative Total
	0 day	15 days	30 days	45 days	60 day	75 Days	
T_1: Sunhemp	11.25	12.67	16.26	15.31	12.09	9.59	77.16
T_2: Cluster bean	10.47	12.50	15.95	14.55	11.62	8.72	73.80
T_3: Cowpea	10.34	13.25	16.65	15.95	13.31	9.87	79.37
T_4: Mutant dhaincha	12.03	14.17	18.32	18.07	15.22	12.11	89.91
T_5: Green gram	11.21	13.53	17.44	16.15	12.99	10.06	81.38
T_6: Soybean	9.60	11.84	15.25	14.43	11.97	9.44	72.53
T_7: Control	5.24	5.42	5.18	5.37	5.28	5.17	31.66
SE±	0.38	0.38	0.49	0.55	0.60	0.28	
CD at 5%	1.16	1.16	1.51	1.70	1.84	0.86	

Periodical Nitrate Nitrogen (NO_3-N)

Green manure incorporation in soil increased NO_3-N release compared to untreated soil (Table 2). The NO_3-N release exhibited a similar pattern of initial slow-release followed by a sharp rise reaching the peak by 30 days, then declining gradually. The magnitude of NO_3-N amount as per cumulative total follows the order: mutant dhaincha > green gram > sunhemp >cowpea > cluster bean > soybean > control. The low release rate in soybean and cluster beans might be due to their high lignin and polyphenol content. Srinivas *et al.* (2006) [13] and Jude and Odhiambo (2010) [6] discovered that the variation in the quality of organic matter could cause considerable variability and complexity in the N mineralization pattern.

Table 2: Effect of green manuring crop residue on periodical nitrate nitrogen content in soil

Treatments	Nitrate nitrogen (mg kg^{-1})						Cumulative Total
	0 day	15 days	30 Days	45 days	60 days	75 days	
T_1: Sunhemp	18.54	25.13	46.73	41.14	40.42	28.25	200.20
T_2: Cluster bean	16.52	25.37	42.09	37.78	35.20	23.22	180.18
T_3: Cowpea	19.73	27.29	46.69	40.44	36.52	25.52	196.19
T_4: Mutant dhaincha	23.99	33.14	59.68	50.37	47.74	36.88	251.82
T_5: Green gram	18.02	27.50	49.50	45.63	41.14	27.58	209.36
T_6: Soybean	17.70	23.90	40.55	37.96	35.54	23.53	179.18
T_7: Control	6.07	5.66	6.10	6.23	6.00	6.33	36.39
SE±	1.10	1.30	1.70	3.77	1.58	1.44	
CD at 5%	3.36	3.99	5.22	11.54	4.85	4.41	

Periodical Nitrogen Mineralization (NH_4-N + NO_3-N)
The incorporation of various green manuring crops significantly influenced the periodical nitrogen mineralization (NH_4-N + NO_3-N) rate. The mineralization rate was slow initially but attained its peak within 30 days. Eventually, the rate decreased till the end of incubation (Table 3). These findings were analogs to the observations of Millar and Baggs (2004) [8] and Srinivas *et al.* (2006) [13]. At 30 days, mutant dhaincha recorded a significantly higher mineralization rate (78.0 mg kg^{-1}) due to high nitrogen content and narrower C: N ratio than other crops.

Table 3: Effect of green manuring crop residues on periodical nitrogen mineralization in soil

Treatments	Nitrogen mineralization (NH_4-N +NO_3-N) (mg kg^{-1})						Cumulative total
	0 day	15 days	30 days	45 Days	60 days	75 days	
T_1: Sunhemp	29.79	37.80	62.99	56.45	52.51	37.83	277.37
T_2: Cluster bean	26.99	37.87	58.04	52.32	46.82	31.94	253.98
T_3: Cowpea	30.07	40.54	63.34	56.40	49.83	35.39	275.57
T_4: Mutant dhaincha	36.02	47.32	78.00	68.45	62.96	48.99	341.73
T_5: Green gram	29.22	41.02	66.94	61.78	54.13	37.64	290.74
T_6: Soybean	27.30	35.74	55.80	52.39	47.50	32.97	251.71
T_7: Control	11.30	11.08	11.28	11.60	11.28	11.50	68.05
S.E.±	1.48	1.68	2.20	4.32	2.18	1.72	
CD at 5%	4.52	5.15	6.73	13.24	6.69	5.27	

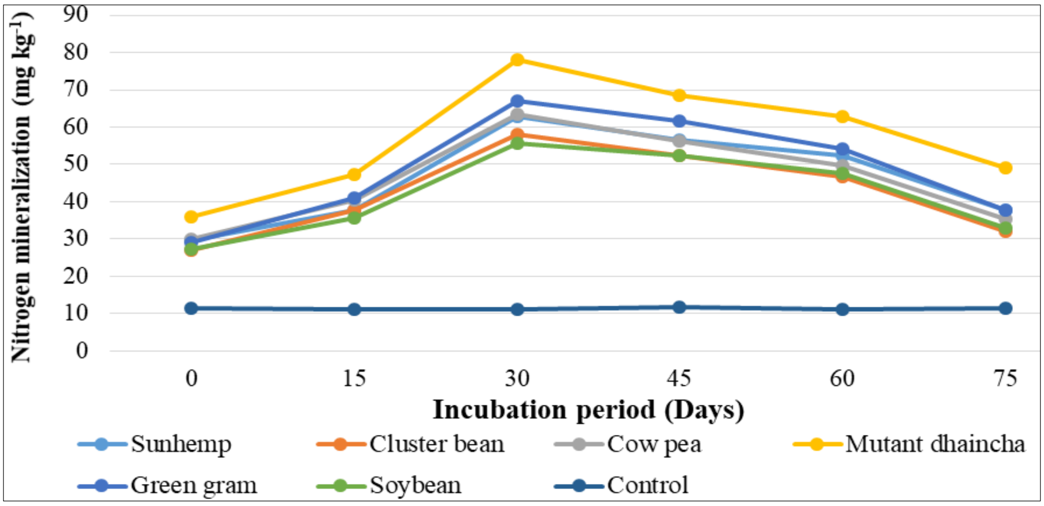

Fig 1: Effect of green manuring crop residues on periodical nitrogen mineralization in soil

Cumulative Nitrogen Mineralization
The cumulative nitrogen mineralization followed an identical trend in all green manure crop residues, and the magnitude follows the order: Mutant dhaincha > green gram > sunhemp > cowpea > cluster bean > soybean > control. The result conforms to the findings of Dey and Jain (1997) [15] and Jude and Odhiambo (2010) [6], which indicate the influence of C: N ratio, chemical composition and quality of organic matter in predicting the nitrogen mineralization rate.

Effect on Soil Enzyme Activities
Urease Activity: Soil urease activity shows a positive correlation with the soil organic carbon status. Increased organic carbon and nitrogen via organic matter addition and biological nitrogen fixation by the crop residues stimulate urease activity in the soil (Roldan *et al.*, 2003) [10]. So, green manure crop residue incorporation in the soil significantly favored the urease activity (Table 4). The enzyme activity increased during the initial stage and reached the maximum by 30 days. It eventually decreased till 60 days of incubation. At 30 days, the urease activity followed the order: Mutant dhaincha > green gram > sunhemp > soybean > cowpea > cluster bean > control. At 30 days of incubation, the significantly higher activity of soil amended with mutant dhaincha (39.77 mg NH_4-N 100g^{-1} hr^{-1}) might be due to high nitrogen and narrower C:N ratio.

Table 4: Effect of green manuring crop residues on urease activity in soil

Treatments	Urease (mg NH_4-N 100 g^{-1} hr^{-1})		
	0 day	30 days	60 days
T_1: Sunhemp (*Crotalaria juncea*)	27.84	36.29	33.87
T_2: Cluster bean (*Cyamopsis tetragonoloba*)	26.34	33.68	31.58
T_3: Cowpea (*Vigna unguiculata*)	27.98	33.75	32.61
T_4: Mutant dhaincha (*Sesbania rostrata*)	30.79	39.77	35.33
T_5: Green gram (*Vigna radiata*)	27.61	36.44	33.40
T_6: Soybean (*Glycine max* L. *Merill*)	26.56	35.65	32.61
T_7: Control	22.46	21.27	22.02
S.E.±	0.61	0.83	1.05
CD at 5%	1.87	2.55	3.22

Dehydrogenase Activity: Dehydrogenase activity is a good indicator of soil microbial activity because it reflects the range of soil microbial oxidation activity. The addition of green manuring crops significantly surged dehydrogenase activity in the soil. It is due to the increased microbial decomposition of organic matter as more organic carbon is available. The dehydrogenase activity showed a significant increase during the initial 30 days and then a slight rise up to 60 days of incubation (Table 5). The dehydrogenase activity in different treatments follows the order: Mutant dhaincha > sunhemp > green gram > cowpea > cluster bean > soybean > control throughout incubation study.

Table 5: Effect of green manuring crop residues on dehydrogenase activity in soil

Treatments	Dehydrogenase (Sμg. TPF g^{-1} soil 24 hr^{-1})		
	0 day	**30 days**	**60 days**
T_1: Sunhemp (*Crotalaria juncea*)	37.56	45.17	46.64
T_2: Cluster bean (*Cyamopsis tetragonoloba*)	31.90	36.70	38.43
T_3: Cowpea (*Vigna unguiculata*)	32.00	39.02	40.97
T_4: Mutant dhaincha (*Sesbania rostrata*)	37.70	45.52	46.80
T_5: Green gram (*Vigna radiata*)	34.65	41.41	42.83
T_6: Soybean (*Glycine max* L. *Merill*)	29.74	34.86	38.05
T_7: Control	20.71	21.30	21.04
S.E.±	1.23	1.24	1.20
CD at 5%	3.77	3.81	3.67

Effect on Nitrifying Bacterial Population

***Nitrosomonas* Population:** *Nitrosomonas* are a group of ammonia-oxidizing bacteria that are important in the biogeochemical nitrogen cycle. They increase the bioavailability of nitrogen to plants. The addition of green manure crop residue in the soil increased the *Nitrosomonas* population (Table 6). The increased amount of organic carbon and ammonium ions (substrate), adequate aeration, and an optimum supply of required nutrients proliferated the *Nitrosomonas* population in the soil. The result confirms the findings of Kibunja *et al.* (2010) [7] and Bhadoria *et al.* (2011). The population growth rate followed an identical trend in all green manure crops. It increased till 30 days and then decreased till the end of incubation. The *Nitrosomonas* population in different treatments at 30 days of incubation follows the order: mutant dhaincha > sunhemp > cowpea > green gram > cluster bean > soybean > control.

Table 6: Effect of green manuring crop residues on Nitrosomonas population in soil

Treatments	*Nitrosomonas* (×10^{-4} *cfu* g^{-1} soil)		
	0 day	**30 days**	**60 days**
T_1: Sunhemp (*Crotalaria juncea*)	9.37	14.05	11.31
T_2: Cluster bean (*Cyamopsis tetragonoloba*)	8.54	11.91	10.14
T_3: Cowpea (*Vigna unguiculata*)	8.13	13.04	10.27
T_4: Mutant dhaincha (*Sesbania rostrata*)	9.45	14.82	11.21
T_5: Green gram (*Vigna radiata*)	8.85	12.54	10.68
T_6: Soybean (*Glycine max* L. *Merill*)	8.60	11.78	9.56
T_7: Control	6.44	7.08	6.81
S.E.±	0.391	0.677	0.521
CD at 5%	1.196	2.073	1.594

***Nitrobacter* Population:** *Nitrobacter* is obligate aerobes that are a significant part of the nitrogen cycle in oxidizing nitrite to nitrate in the soil. These organisms use energy from the oxidation of nitrite ions to fulfill their energy needs. *Nitrobacter* also fixes carbon dioxide via the Calvin cycle for their carbon requirements. The green manure crop residue incorporation into the soil caused an increase in the *Nitrobacter* population (Table 7). The increased organic carbon supply and improved physical conditions proliferate the growth of *Nitrobacter*. The pattern of population growth recorded a similar fashion of initial increase till 30 days and then gradual decrease till 60 days of incubation. The *Nitrobacter* population in different treatments follows the order: mutant dhaincha > sunhemp > green gram > cowpea > cluster bean >soybean > control. The results obtained confirm the findings of Kibunja *et al.* (2010) [7] and Bhadoria *et al.* (2011) [2]. The addition of mutant dhaincha, sunhemp, and green gram recorded similar Nitrobacter growth in the soil.

Table 7: Effect of green manuring crop residues on *Nitrobacter* population in soil

Treatments	*Nitrobacter* (×10^{-4} *cfu* g^{-1} soil)		
	0 day	**30 days**	**60 days**
T_1: Sunhemp (*Crotalaria juncea*)	12.12	17.85	14.54
T_2: Cluster bean (*Cyamopsis tetragonoloba*)	9.68	11.92	10.59
T_3: Cowpea (*Vigna unguiculata*)	10.04	12.31	10.93
T_4: Mutant dhaincha (*Sesbania rostrata*)	13.46	18.64	14.84
T_5: Green gram (*Vigna radiata*)	11.91	15.56	13.84
T_6: Soybean (*Glycine max* L. *Merill*)	8.56	10.68	9.41
T_7: Control	6.95	8.62	7.80
S.E.±	0.84	1.38	1.08
CD at 5%	2.56	4.22	3.31

Conclusion

The nitrogen mineralization increased slowly in the initial phase and then rose drastically to attain a maximum by 30 days of incubation. It then decreased eventually till the end of the incubation period. The magnitude of the rate of N mineralization followed the order: mutant dhaincha > green gram > sunhemp > cowpea > cluster bean > soybean > control. Urease and dehydrogenase activity also improved significantly by the green manure residue incorporation. Urease activity increased up to 30 days and then decreased till 60 days of incubation. The dehydrogenase activity increased rapidly up to 30 days and then slightly till the end of incubation. Nitrifying bacterial population, *viz*., *Nitrosomonas* and *Nitrobacter*, also elevated after green manure addition. The population increased till 30 days and then decreased. The incorporation of mutant dhaincha (TSR-1) enhanced nitrogen mineralization, enzyme activity, and nitrifying bacterial population in the soil.

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