Soil Mineral Nitrogen Concentration and its Influence on Biological Nitrogen Fixation of Grain Legumes

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Abstract

Surveys in farmers' fields in Bangladesh, India, and Vietnam showed that there was mineral N up to 70.7 μg g^{-1} soil (and several fields had about 30 μg N g^{-1} soil) in the surface 15-cm soil profile at or before sowing of a legume crop. Such high concentrations at sowing have been reported to suppress nodulation and N_2 fixation by some legumes. A high level of soil mineral N (31.2 μg mineral N g^{-1} soil) at sowing reduced nodulation of chickpea in a field experiment by at least 14%, and proportion of fixed N by 63%, compared with that in the control plots (7.3 μg mineral N g^{-1} soil). In a pot trial with Alfisol, application of five levels of fertilizer N up to 200 kg N ha^{-1} equivalent much before sowing was used to simulate range of soil mineral N concentration at sowing. It was observed that mean nodule number and nodule mass per plant in three of the five legume species studied were substantially reduced in the presence of a soil mineral N concentration of 31 μg g^{-1} soil (the other two species showed reduction at 43 and 66 μg N g^{-1} soil), compared with a control (no fertilizer) having 23 μg N g^{-1} soil at sowing. In the case of pigeonpea, suppression of N_2 fixation was recorded at 43 μg N g^{-1} soil, and in cowpea, at 66 μg N g^{-1} soil. A direct relationship between nitrogenase activity and different soil N pools at sowing and at flowering was observed in all the five legumes (R^2= 0.56–0.80) except in mung bean. Based on the available data, it seems that the general recommendation of applying a starter N dose of 20–30 kg ha^{-1} to legumes in the tropics cannot be applicable in all situations, and native soil mineral N should be considered to effectively harness N_2 fixation by legumes.

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Introduction

Grain legumes have been grown as intercrops or in cropping rotations in the tropics since ancient times. Biological nitrogen fixation (BNF) by legumes is an important and an integral component of sustainable agriculture in the tropics. Most grain legumes can derive up to 80% of their total N need from BNF (Wani et al. 1995), and in situations where plant residues are returned to fields, legumes improved soil N fertility (Peoples and Crasswell 1992). However, in most cases in the tropics, along with legume grains, plant biomass is often also removed from the field to feed animals. In such cases, invariably, with many grain legumes except medium- and long-duration pigeonpea, net N balances (kg ha^{-1}) were negative, for e.g., –63 to –77 for chickpea, –42 to –127 for groundnut (Wani et al. 1995).

Biological nitrogen fixation does not become functional immediately after sowing, and taking into account net negative N balances in the tropics, to sustain productivity of grain legumes, a starter dose of 20–30 kg N ha^{-1} has been recommended. Several studies have indicated significant responses to an application of 20–30 kg N ha^{-1} as a starter dose for legumes under good growth conditions. At an application rate of 20 kg N ha^{-1}, the overall grain yield response rate (kg grain kg^{-1} N) was 14.2 in pigeonpea, 14.4 in groundnut, and 15.4 in chickpea under rainfed conditions (Tandon 1992). In general, high soil nitrogen levels, applied or residual, reduce nodulation and N_2 fixation (Wani et al. 1995). Based on several studies (Wu and Harper 1991, Buttery et al. 1988, Buttery and Gibson 1990), nodulation and/or BNF was reduced by approximately 50% in different legumes, when N concentration in root environment was between 20 and 90 mg kg^{-1} in the growth medium. The suppression in BNF was particularly due to the nitrate fraction in the root growth environment (Streeter 1988). Groundnut grown on an Alfisol without fertilizer N application in India derived 61% of its N requirement through BNF (Pfix), and application of 100 kg N ha^{-1} reduced Pfix to 47% (Yoneyama et al. 1990). This paper reports the occurrence of high soil mineral N on farmers' fields, and discusses: (1) the effect of crop and soil management practices on changes in soil mineral N concentration, and (2) the effects of different mineral N concentrations on BNF by five different legumes, based on greenhouse and field experiments. It also highlights why and where the commonly recommended starter N application to legumes potentially suppresses BNF by legumes.

Mineral N Dynamics in Soil and its Concentration in Farmers' Fields

Mineral N concentration in soil is always in a dynamic state, and is influenced by several factors. It is important to note that an internal N cycle exists in soil that is distinctly different from the overall N cycle, but that interfaces with it. A key

feature of the internal cycle is the biological turnover of N through mineralization-immobilization processes, in which organic N is mineralized through decomposition, producing NH_3 and NO_3, and mineral N is immobilized as organic N through microbial assimilation.

Biological turnover of N in soil is influenced by the factors that affect microbial activity, such as temperature, pH, organic matter content, rate of residue application, and quality of the applied residues (Lee and Pankhurst 1992, Wani and Lee 1995). In addition to the mineralization-immobilization process, mineral N level in soil is influenced by plant N uptake, leaching, denitrification, and ammonia volatilization. Thus, mineral N concentrations in soil samples from farmers' fields or research farms vary widely and could affect BNF differently.

Generally in the tropics, after the long dry spell before sowing of the rainy-season crop, high mineral N concentrations are observed in the top 15-cm layer, which is mainly due to the upward movement of nitrate and ammonia in the profile due to capillary movement of water in the soil (Nye and Greenland 1960, Wetselaar 1961 a and b). In many semi-arid tropical soils, there is a flush of mineralization of organic matter in the surface soil layers at the start of the rainy season, which is referred as "Birch effect" (Birch 1960). Such accelerated mineralization of organic matter is due to drying and wetting cycles, which accelerate mineralization of the labile fraction of soil organic matter such as microbial biomass, resulting in a flush of mineral N in the top soil layer.

It is generally assumed that farmers' fields in the semi-arid tropics (SAT) would have lower mineral N concentration in soil than those observed on research stations. In order to verify this hypothesis, we analyzed several soil samples from farmers' fields in Bangladesh, India, and Vietnam, and also from the fields at ICRISAT Asia Center (IAC). In India, before sowing the postrainy season crop, the soil samples from fields at IAC contained an average of 26 μg N g^{-1} soil (range 16–42 μg N g^{-1} soil), and farmers' fields at Gwalior (Madhya Pradesh) and Hisar (Haryana) contained about 15 μg N g^{-1} soil (range 9–32 μg N g^{-1} soil) (Fig. 1). The samples from farmers'

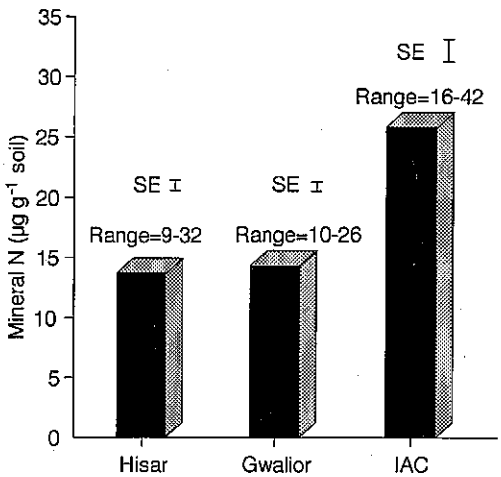

Figure 1. Mineral N concentration in soil (0–60 cm) samples from farmers' fields at Hisar (Haryana), Gwalior (Madhya Pradesh), and research fields at ICRISAT Asia Center, India (Note: All the samples were air dried before extraction in 2M KCl)

Table 1. Mineral N, total N, organic carbon, available P content, and most probable number (MPN) of rhizobia in soil samples collected from farmers' fields in Bangladesh during 1992.

Sample no.[1]	MPN of rhizobia (log_{10} g^{-1} soil) Pigeonpea	Cowpea	Chickpea	Mineral-N (mg kg^{-1} soil)	Available P (mg kg^{-1} soil)	Total N (mg kg^{-1} soil)	Organic C (%)
1	3.1	5.3	5.4	25.6	1.5	1128	0.91
2	1.7	1.7	0.3	8.5	4.0	955	0.82
3	2.7	4.2	1.7	26.1	1.5	735	0.53
4	3.1	5.1	0.7	5.4	2.2	658	0.49
5	2.7	4.0	0.3	41.2	7.0	669	0.41
6	2.4	4.2	0.3	35.8	5.2	768	0.50
7	3.7	1.7	1.3	9.6	2.0	597	0.42
8	2.5	2.3	5.3	8.5	1.5	806	0.64
9	3.7	3.2	0.6	52.3	4.2	915	0.73
10	2.3	4.3	0.4	4.5	5.0	1024	0.84

1. A total of 42 samples was analyzed; results of selected soil samples to represent range of mineral-N are given here.

fields in Bangladesh contained 4.5–52.3 μg N g^{-1} soil (Table 1), and those from north and south Vietnam contained 9.7–70.7 μg N g^{-1} soil. These results indicated that many farmers' fields contained high (>20 μg N g^{-1} soil) levels of mineral N concentrations (before sowing of a legume crop), perhaps due to the factors explained in the preceding paragraphs. These levels have been calculated to reduce nodulation and/or BNF by about half in several legumes (Rupela and Johansen 1995). All these areas with high levels of mineral N concentration in farmers' fields are from regions where legumes are grown during the postrainy season, suggesting that many farmers could be using mineral N fertilizers for nonlegume crops preceding the legumes, due to assured water availability to the crops.

Effect of Different Agricultural Practices on Soil N Fractions and BNF by Legumes

Mineral N concentration in soil is influenced by several agricultural practices and environmental factors such as soil water content and soil temperature. Mineral N in soil is in dynamic state and it varies with the soil depth.

Fertilizer N Applied to the Preceding Crop

At Solapur, Maharastra, India, an experiment being conducted since 1990–91 on a Vertisol showed that cropping history and application of 25 kg N ha^{-1} to a postrainy-season sorghum affected mineral N concentration in soil samples collected before sowing the following rainy-season crop. A maximum mean mineral N concentration of 16.2 μg N g^{-1} soil was observed in case of soybean+sorghum system. In the plots of blackgram+sorghum and cowpea+sorghum systems, mineral N concentration in soil was similar to that from fallow+sorghum plots. Application of 25 kg N ha^{-1} to postrainy-season sorghum increased mineral N concentration in soil by 2.6 times over the mineral N concentration of treatments that did not receive any N for postrainy-season sorghum (13.6 vs 5.2 μg N g^{-1} soil). In this experiment, nodulation and nitrogenase activity of all the legumes (blackgram, soybean, and cowpea) were lower in case of plots where 25 kg N ha^{-1} was applied, than that of plots where zero-N was applied (27.5 vs 50 μ mol C_2H_4 m^{-1} row h^{-1}, Patil et al. 1996).

Organic Matter Amendment

In a pot experiment using Vertisol at IAC, the effect of 0.5% farmyard manure (FYM) and 0.5% Bioearth® (a commercial organic product prepared from agricultural wastes and distillery effluents using microbial inoculants) was studied on soil N fractions, nodulation, and nitrogenase activity of pigeonpea. Pots were prepared as per the treatments (Table 2) 1 week before sowing of pigeonpea, and watered to 80% water-holding capacity (WHC). Soil samples were collected at sowing of pigeonpea. Soil mineral N concentration increased from 8.4 μg N g^{-1} soil in the control (no amendments) to 10.4 μg N g^{-1} in the case of 0.5% FYM and 16.6 μg N g^{-1} soil in the case of 0.5% Bioearth® treatments (Table 2). There was N immobilization in the no amendment and 0.5% Bioearth® amended pots at the time of sowing. Pigeonpea plants were grown up to flowering (100 days after sowing, DAS), and assayed for nodulation and nitrogenase activity, and soil samples were collected to study soil N fractions. At flowering stage, net N mineralization and microbial biomass N (flush N/0.57) varied significantly, but soil mineral N concentration did not vary due to the amendment of Vertisol with 0.5% each of FYM or Bioearth® (Table 2). Total dry matter at flowering stage (100 DAS) was significantly ($P \leq 0.01$) higher in case of 0.5% Bioearth®-amended treatment than the no-amendment or 0.5% FYM treatments (Table 3). In pigeonpea, nodule number per plant and nitrogenase activity per plant were not influenced significantly by FYM or Bioearth® amendments. However, specific nitrogenase activity (μmole C_2H_4 mg^{-1} of dry nodules h^{-1}) was substantially increased to 41.6 in the 0.5% FYM treatment, and decreased to 22.1 in the 0.5% Bioearth® treatment (Table 3). A maximum

Table 2. Effect of organic matter amendments on mineral N, net N mineralization, and microbial biomass N in pigeonpea grown in Vertisol in pots, ICRISAT Asia Center, India, 1993.

	Treatments			
	Control	0.5% FYM[1]	0.5% Bioearth®	SE[2]
At sowing				
Mineral N (μg N g^{-1} soil)	8.4	10.4	16.6	±0.92**
Net N mineralization (μg N g^{-1} soil $10d^{-1}$)	–0.4	0.4	–1.9	±0.65^{ns}
94 days after emergence				
Mineral N (μg N g^{-1} soil)	8.8	8.1	9.2	±0.502^{ns}
Net N mineralization (μg N g^{-1} soil $10d^{-1}$)	–0.86	3.3	1.9	±0.654**
Microbial biomass N (μg N g^{-1} soil)	28.1	27.5	32.4	±0.814**

1. FYM = farmyard manure
2. ** = $P \leq 0.01$; ns = not significant.

Table 3. Total plant dry mass, nodule number, and nitrogenase activity of pigeonpea cv ICP 1–6, grown on a Vertisol amended with farmyard manure (FYM) and Bioearth®, 100 days after sowing , ICRISAT Asia Center, India.

			Nitrogenase activity	
Treatment	Total plant dry matter (g pot^{-1})	Nodule no. $plant^{-1}$	μmol C_2H_4 $plant^{-1}$ h^{-1}	μmol C_2H_4 mg^{-1} nodule
Control	20.7	111	10.6	30.4
0.5% FYM	21.0	105	15.6	41.6
0.5% Bioearth®	24.0	122	10.6	22.1
SE[1]	±0.71**	±13.1^{ns}	±1.84^{ns}	±4.9**

1. ** = $P \leq 0.01$.ns = not significant.

specific nitrogenase activity (41.6 μmol C_2H_4 mg^{-1} nodules h^{-1}) in case of 0.5% FYM could be due to other nutritional/growth-promoting factors from the FYM over the control. In case of the 0.5% Bioearth® treatment, specific nitrogenase activity was least, and this could be due to the highest mineral N concentration in soil at sowing, or perhaps other inhibitory substances that could be present in it as it includes distillery effluents.

A long-term fertility management experiment on a well-drained Vertic Inceptisol of 60-cm depth has been conducted since 1987 at Akola, Maharashtra, India. Surface soil (0–30 cm) samples collected during the 1995 rainy season showed that treatments that received half (25 kg ha^{-1}) or full (50 kg ha^{-1}) N through *Leucaena* loppings or FYM contained significantly higher ($P \leq 0.01$) mineral N concentration (4.9–6.9 μg N g^{-1} soil) than the treatments that received zero N (3.2 μg N g^{-1} soil) or 25 and 50 kg N ha^{-1} (3.7–4.4 μg N g^{-1} soil) through chemical fertilizers (Deshmukh et al. 1996). In the same experiment, nitrogenase activity of mung bean grown as an intercrop with cotton was adversely affected due to the application of chemical fertilizer (50 kg N ha^{-1}) alone, or in combination with organic sources (9–14 μmol C_2H_4 m^{-1} row length h^{-1}), while 50 kg N ha^{-1} through *Leucaena* loppings did not decrease nitrogenase activity (28 μmol C_2H_4 m^{-1} row length h^{-1}), compared with the zero N applied treatment (28 μmol C_2H_4 m^{-1} row length h^{-1}, Deshmukh et al. 1996).

Cropping History

Growing legumes in rotation increases mineral N content in soil, compared with the cultivation of nonlegume crops. At IAC, a long-term rotation experiment is being conducted on a Vertisol since 1983, using 2-year crop rotation treatments. The surface soil (0–20 cm) samples collected after the harvest of the 9th season crop showed in general, higher amounts of mineral N concentration in the soil from the legume-based cropping system than from the nonlegume-based cropping system (Fig. 2). Inclusion of mung bean in the cropping sequence increased available N in the soil at harvest to the extent of 12.6% in the nonfertilized control plot (Rao and Singh 1991). A five-times higher mineral N concentration from the soil under an 8-year rotation using fababeans as green manure was observed, compared with that from the soil under continuous barley treatment fertilized with 90 kg N ha^{-1} y^{-1} (Wani et al. 1991).

Effect of Fertilizer N levels on Soil N Fractions and on BNF by Legumes

Application of N fertilizer results in increased mineral N concentration in soil, and the increase is directly related to the rate of fertilizer N application. At IAC, two contrasting soil mineral N regimes to represent those on farmers' fields were created

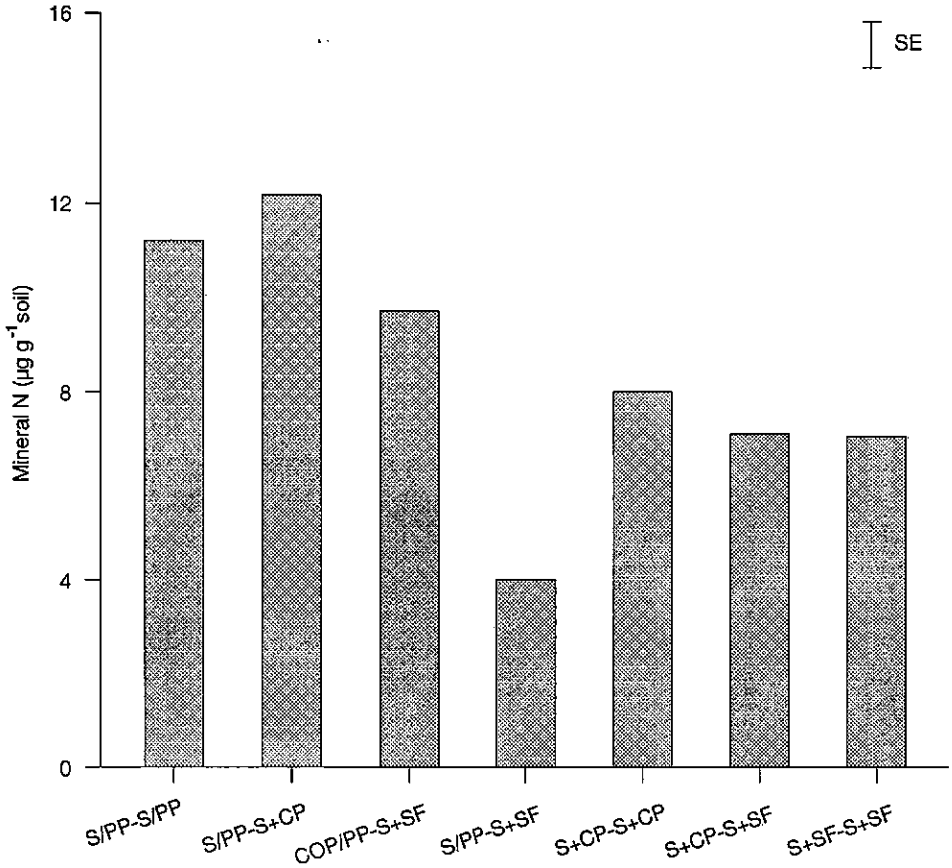

Figure 2. Mineral N concentration in soil samples (0–20 cm) from plots under different cropping systems since the last 9 years (1983–1991). (S = sorghum, PP = pigeonpea, SF = safflower, CP = chickpea, COP = cowpea, / = intercrop, + = sole crop grown during the postrainy season). Source : Wani et al. 1995.

on a Vertisol (previously depleted of mineral N by growing cover crops), through application of 20 and 100 kg N ha^{-1} in big plots replicated four times. The field received about 150 mm rain after N fertilizer was applied. On analysis of the soil samples, it was observed that mineral N concentration increased to 31 µg g^{-1} soil (in plots receiving 100 kg N) and 7.3 µg g^{-1} soil (in plots receiving 20 kg N) compared with 5 µg g^{-1} soil in the top 15-cm layer before fertilizer application (Fig. 3). Increased mineral N concentration to 90 cm depth due to 100 kg N ha^{-1} application was observed. However, such an increase was proportionatly lower than the increased mineral N concentration observed for the top 15-cm layer (Fig. 3). Nodulation, proportion of N_2-fixed, and the amount of N_2-fixed by the four chickpea lines sown 24 days after N application were adversely affected due to the high mineral N concentration in soil (Table 4). Mean (of the four chickpea lines with

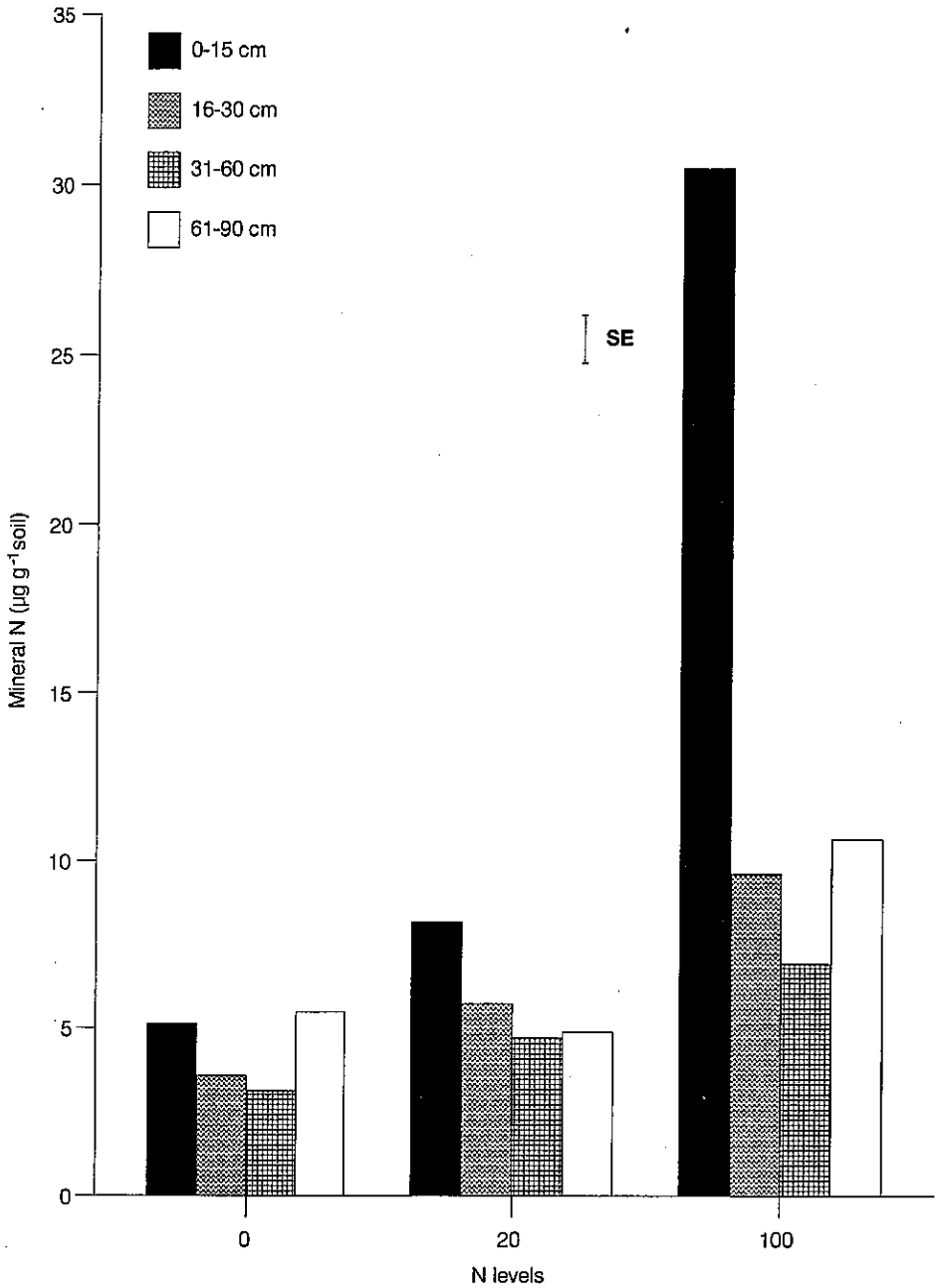

Figure 3. Mineral N concentration in a Vertisol at ICRISAT Asia Center, as influenced by the application of nitrogen as urea. About 150 mm rain fell on 8 days between application of fertilizer on 12 Oct 1993, and soil sampling on 28 Oct 1993.

Table 4. Nodulation, N_2-fixation, yield and N-yield of different chickpea selections as affected by soil N levels, ICRISAT Asia Center, India[1], postrainy season 1993/94.

N-Level (surface 15 cm soil)	Nodule no. m^{-2}	Nodule mass ($g\ m^{-2}$)	% N derived f rom air (Pfix)	Amount ($kg\ ha^{-1}$) of N_2-fixed (^{15}N dilution method)	Amount ($kg\ ha^{-1}$) of N_2-fixed (Difference method)	N-yield through grains ($kg\ ha^{-1}$)	Grain yield ($t\ ha^{-1}$)
N1 (7.3 $\mu g\ g^{-1}$ soil)[2]	736	1.22	52.4	35	35	66	2.13
N2 (31.2 $\mu g\ g^{-1}$ soil)	635	0.86	19.2	14	6	73	2.02
SE[3]	$\pm 47.2^{ns}$	$\pm 0.132^{ns}$	$\pm 7.66^{*}$	$\pm 5.0^{*}$	$\pm 2.5^{**}$	$\pm 2.5^{ns}$	$\pm 0.107^{ns}$

1. The experiment had 12 cropping systems, of which five were on chickpea-sorghum involving five different types of chickpea: mineral N tolerant symbiotic chickpea, high nodulating, low nodulating, advanced breeding lines, and nonnodulating chickpea (the latter two included as controls). All these treatments were grown as subplots on the two main plots (N1 = 20 kg N ha^{-1}, N2 = 100 kg N ha^{-1} as urea) of the split-plot design with four replications. Nitrogen was applied 24 days before sowing chickpea. About 150 mm rain fell between N application on 12 Oct 1993 and sowing on 6 Nov 1993. Data presented here are the means of the four nodulated chickpea types. Nonnodulating chickpea line was used as reference to quantify N_2-fixation by the ^{15}N dilution method, and the difference method.

2. Data in parentheses are soil mineral N concentration at sowing of chickpea.

3. ns = not significant; * = $P<0.05$, ** = $P<0.01$.

large differences in nodulation capacities) nodule number decreased by 14%, nodule mass by 30%, and proportion of N derived from air (Pfix) by 63% due to the high N in the plots (31.2 μg N g^{-1} soil in top 15 cm), compared with the low N plots (7.3 μg N g^{-1} soil in top 15 cm). The chickpea grains produced at the low N plots derived 35 kg N ha^{-1} from BNF, while those from high N plots derived only 6–14 kg N ha^{-1} from BNF, although they recorded a 11% higher N yield (Table 4).

In a pot experiment (replicated four times), a range of soil N concentration was simulated through the application of five levels (0, 20, 50, 100, 200 kg ha^{-1} equivalent) of N through urea to an Alfisol. Phosphorus was applied basally to all the pots at the rate of 20 kg P ha^{-1} equivalent as single superphosphate. Twelve days later, at sowing of five different legumes, the five fertilizer levels were 23, 31, 43, 66, and 92 μg N g^{-1} soil. The five legumes were grown up to flowering stage (43–82 DAS), and observed for nodulation, plant growth, and nitrogenase activity. Soil samples were collected to estimate soil N fractions. At the flowering stage (43 DAS for mung bean, 55 DAS for groundnut, 58 DAS for soybean and cowpea, and 82 DAS for pigeonpea), mean mineral N concentration in soil varied significantly ($P \leq 0.01$) among the legumes, 9.3 μg N g^{-1} soil for pigeonpea to 36.8 μg N g^{-1} soil for mung bean (Table 5). The highest mean soil mineral N concentration (45.1 μg Ng^{-1} soil) was observed when mineral N content at sowing was greatest, and the least mineral N concentration of 7.9 μg N g^{-1} when the mineral N was lowest at sowing (Table 5). In the case of the zero N applied treatment (23 μg N g^{-1} soil at sowing), maximum mineral N concentration of 17.8 μg N g^{-1} soil was observed in case of mung bean, and least (0.3 μg N g^{-1} soil) in case of cowpea. A significant ($P \leq 0.10$) interaction for soil mineral N concentration (at flowering) between crops and the mineral N at sowing was observed (Table 5). These results indicated differential N uptake by the legumes, resulting in varied soil mineral N concentrations at flowering stage, which in turn, could affect nodulation and nitrogenase activity in the legumes.

The different concentrations of mineral N in soil at sowing also affected net N mineralization in soil at flowering stage of the different legumes. At 23 and 31 μg N g^{-1} soil concentration, immobilization of N was observed, resulting in negative net N mineralization (Table 5). In case of 43 and 92 μg N g^{-1} soil amount of net N mineralized during 10 days laboratory incubation increased from 0.9 to 6.9 μg N g^{-1} soil 10 d^{-1} (Table 5). Similarly, mean net N mineralization was influenced by the crops, and there was a significant ($P \leq 0.01$) interaction between crops and the mineral N concentration at sowing for the net N mineralization in soil at flowering stage. It was noticed that with the increasing mineral N concentration in the soil, the amount of net N mineralization also increased at flowering (Table 5). However, no such relationship was observed between mean soil mineral N concentration for the legumes (Table 5) and net N mineralization (Table 5) at flowering. For example, mean soil mineral N concentration at flowering was maximum in case of mung

Table 5. Mineral N[1], net N mineralization[2] in soil, and nitrogenase activity at flowering stage of different legumes grown in Alfisol in pots, ICRISAT Asia Center, India, 1994.

	Soil mineral N levels (μg Ng^{-1} soil) at sowing					
Crop	23	31	43	66	92	Mean
Mineral (NH_4+NO_3) N (μg g^{-1} soil)						
Mung bean	17.8	19.5	18.6	56.8	71.2	36.8
Groundnut	6.9	7.3	17.1	33.7	80.3	29.1
Cowpea	0.3	1.7	3.2	8.1	33.9	9.4
Soybean	7.9	13.0	13.5	25.3	20.8	16.1
Pigeonpea	6.5	5.8	7.3	7.5	19.5	9.3
SE			±0.82**[3]			±0.36**
Mean	7.9	9.5	11.9	26.3	45.1	
SE			±0.37**			
Net N mineralization (μg g^{-1} soil $10d^{-1}$)						
Mung bean	-10.7	-9.0	1.6	-5.8	0.2	-4.6
Groundnut	0.6	0.9	0.3	1.2	0.0	0.6
Cowpea	3.5	5.7	4.5	7.4	2.1	4.6
Soybean	-3.1	-3.1	4.9	12.3	27.6	7.7
Pigeonpea	-5.4	-4.5	-6.7	-5.3	4.5	-3.5
SE			±0.83**			±0.39*[3]
Mean	-2.9	-2.0	0.9	2.0	6.9	
SE			±0.30**			
Nitrogenase activity (μmol C_2H_4 $plant^{-1}$ h^{-1})						
Mung bean	0.11	0.05	0.04	0.03	0.04	0.09 0
Groundnut	9.34	7.94	5.61	2.22	1.75	5.37
Cowpea	1.31	2.71	4.44	1.04	0.65	2.02
Soybean	1.00	0.40	0	0	0	0.26
Pigeonpea	7.02	14.14	8.08	6.76	0.14	7.23
SE			±1.19**			±0.53**
Mean	3.76	5.04	3.62	2.05	0.51	
SE			±0.53**			

1. Mineral N content in soil was estimated using moist soil samples extracted in 2 M KCl.
2. Net N mineralized = Mineral N content in soil at t_{10} - Mineral N content in soil at t_0.
3. * = $P \leq 0.05$; ** = $P \leq 0.01$.

bean, and least in case of pigeonpea (Table 5), but in both the cases immobilization of N in soil was observed, while in all other crops, net N mineralization was observed (Table 5). These results suggest that internal N cycle in the soil is influenced by the crop N uptake and mineral N fertilizer applied, and in turn, rate of mineral N fertilizer applied would have varying effect on nitrogenase activity of different crops.

Flush N in the soil was calculated as the difference between mineral N content in soil with and without chloroform-fumigated soil, incubated for 10 days. It is a direct index of N released from the killed microorganisms due to chloroform-fumigation in the soil, and is an estimate of microbial biomass N. Highest mean flush N (5.5 μg N g^{-1} soil) was observed in case of soybean, and lowest in case of pigeonpea (3.3 μg N g^{-1} soil). As observed earlier for net N mineralization, there was no apparent relationship between flush N values for the different legumes and different mineral N levels at sowing (results not shown).

Mean nodule number decreased significantly ($P \leq 0.01$) from 61 per plant in case of 23 μg N g^{-1} soil to 36 per plant in case of 92 μg N g^{-1} soil. Similarly, mean nodule mass also decreased significantly ($P \leq 0.01$) from 0.22 mg $plant^{-1}$ in case of 23 μg N g^{-1} soil to 0.044 mg $plant^{-1}$ in case of 92 μg N g^{-1} soil (detailed results not shown). Nitrogenase activity (C_2H_4 production) was measured at flowering stage of the five different legumes. A significant interaction for nitrogenase activity between legumes and soil mineral N levels at sowing was observed. In case of pigeonpea and cowpea, nitrogenase activity at 31 μg N g^{-1} soil (at sowing) increased twofold over the nitrogenase activity at 23 μg N g^{-1} soil, whereas in all the other legumes, increased mineral N concentration greater than 23 μg N g^{-1} soil at sowing reduced nitrogenase activity (Table 5). Mean nitrogenase activity, however, increased from 3.8 μmol C_2H_4 $plant^{-1}$ in case of 23 μg N g^{-1} soil to 5.0 μmol C_2H_4 $plant^{-1}$ h^{-1} in case of 31 μg N g^{-1} soil, and then decreased with the increasing soil mineral N concentration (Table 5). Among the different legumes, highest nitrogenase activity was observed in the case of pigeonpea followed by ≥ groundnut ≥ cowpea ≥ soybean and mung bean (Table 5).

Relationship Between Nitrogenase Activity and Soil N Fractions

In the same pot experiment described above, the effect of different fertilizer N levels on such soil N fractions as mineral N, net N mineralized, and flush N was determined at sowing and flowering stages. Using stepwise regression, the relationship between nitrogenase activity of the five legumes and the different soil N pools at sowing and at flowering stages was studied. It was observed that in all the legumes except mung bean, there was a significant ($P \leq 0.01$) relationship (R^2 = 0.56–0.80) between nitrogenase activity and different soil N frac-

Table 6. Equation to estimate nitrogenase activity of legumes using regression coefficient for different soil N fractions.

Legume	Regression equation[1]	Regression coefficient (R^2) [2]
Mung bean	$\mu mol\ C_2H_4\ Plant^{-1}\ h^{-1} = 212 - 3.4x_1 - 7.7x_2 + 17.3x_4 - 22.3x_6$	31^{ns}
Groundnut	$\mu mol\ C_2H_4\ Plant^{-1}\ h^{-1} = 25920 + 314x_1 + 995x_2 - 2287x_3 - 3366x_4 - 1160x_6$	65**
Cowpea	$\mu mol\ C_2H_4\ Plant^{-1}\ h^{-1} = 5956 - 42.2x_1 - 278x_2 - 734x_5$	56**
Soybean	$\mu mol\ C_2H_4\ Plant^{-1}\ h^{-1} = 779 - 23.6x_1 - 57.2x_2 + 263x_4 - 205x_5$	80**
Pigeonpea	$\mu mol\ C_2H_4\ Plant^{-1}\ h^{-1} = 22571 - 749x_2 - 1250x_5 - 3726x_6$	68**

1. x_1 = Mineral N in soil at sowing ($\mu g\ g^{-1}$), x_2 = net N mineralization in soil at sowing ($\mu g\ g^{-1}$ soil 10 d^{-1}), x_3 = flush N in soil at sowing (μg N g^{-1} soil), x_4 = mineral N in soil at flowering, x_5 = net N mineralized at flowering (μg N g^{-1} soil 10 d^{-1}), x_6 = flush N in soil (μg N g^{-1} soil) at flowering.

2. Percentage of variance accounted; ** = $P<0.01$, ns = not significant, degrees of freedom = 14.

tions (Table 6). These results suggested that nitrogenase activity in the legumes was adversely affected by different soil N fractions such as mineral N, net N mineralized, and flush N observed at sowing or at flowering stages.

Conclusion

In the tropics, during dry periods, upward movement of NO_3 takes place due to capillary movement of water, resulting in increased mineral N concentrations in the top soil layer before the sowing of the rainy-season crop (Nye and Greenland 1960, Wetselaar 1961 a and b). Frequent drying and wetting of soil is a common feature in the tropics, and results in a flush of mineral N in soil due to accelerated mineralization of labile organic matter fraction (Birch 1960). In addition to these two natural processes, human interventions such as fertilizer application to the preceding crop, or to intercropped nonlegumes; organic matter amendments such as FYM or compost, inclusion of legumes in crop rotations or grown as intercrops, change mineral N concentration in soil. As a result of all such factors, farmers' fields can have >20 μg N g^{-1} soil. Such levels of mineral N (>20 μg N g^{-1} soil) reduced BNF in most tropical legumes almost by half. It therefore seems that the general recommendation of applying starter dose of 20–30 kg N ha^{-1} to legumes will not be valid for all the situations. The situations that may need starter N doses for legumes in the tropics could be few, such as when sole legumes are grown following a high-yielding cereal crop fertilized moderately (less N applied than what is harvested). Even in such cases, and also when legumes are grown as intercrops, use of such organic sources of N as FYM, compost, or *Glyricidia* loppings where

available, should be preferred. Addition of organic sources of N alone, up to 50 kg N ha^{-1} did not adversely affect BNF by legumes and yield of cotton (Deshmukh et al. 1996). In a Vertic Inseptisol watershed experiment when soybean fixed 230 kg N ha^{-1} (80% of its N requirement), soil N budget could be adjusted by applying loppings of *Glyricidia* grown on contour bunds without affecting crop productivity adversely (Wani et al. unpublished). Based on the available data, we hypothezise that most tropical grain legumes can be grown without a starter N dose. Any excess removal of N by sole crop of legume can be compensated by applying N though organic sources, to ensure high productivity of the system without affecting BNF by legumes.

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