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## Nitrogen Mineralization Rates and Kinetics in Soils Freshly Amended with Green Manures

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With 4 tables

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### Abstract

Long term incubation studies to determine the nitrogen (N) mineralization rates and kinetics in soils freshly amended with some commonly used green manures such as *Sesbania rostrata*, *Gliricidia maculata*, *Leucaena leucocephala* and *Azolla pinnata* are scarce. A long term aerobic study was, therefore, conducted by incubating soils freshly amended with the above-mentioned green manures in PVC columns at  $35 \pm 1^\circ\text{C}$  and with 0.01 Mpa moisture content. The soils were then leached at periodic intervals for up to 36 weeks.

The N-mineralization rates were greatest during the first week and decreased with time in all soils. The green manure amended soils leached  $247\text{ mg kg}^{-1}$  more  $\text{NO}_3 + \text{NO}_2 - \text{N}$  than the unamended control. In general, the total N mineralized (mean 61%) was almost twice that of net N mineralized (mean 30%) in the amended soils. The percent N mineralized (total and net), however, varied with the nature of green manure incorporated into the soil. It was greatest in the soil amended with sesbania and lowest in the soil amended with azolla. The kinetic parameters derived using the double exponential model indicated that green manure amended soils possessed significantly higher N-mineralization potentials and rate constants compared to the unamended control. The kinetic parameters also varied with the nature of green manure incorporated into the soil. Among the various parameters lignin content, lignin to N ratio and lignin + polyphenol to N ratio of the green manures were the key factors governing the rate of decomposition and subsequent N mineralization from the amended soils.

**Key words:** Green manures — organic N — N-mineralization kinetics — C:N ratio — lignin + polyphenol:N ratio — lignin:N ratio

term soil fertility in sustainable crop production. Incorporation of green manures into soils has, therefore, become a common practice in several parts of the world (Singh et al. 1992). However, green manures must undergo decomposition before their nitrogen (N) becomes available to the crop. The suitability of green manures, therefore, depends to a great extent on their N-mineralization rates in soil in relation to crop demand.

The N-mineralization rates and kinetics have been determined in soils amended with sewage sludge (Lindemann et al. 1988), poultry manure and fermentation residues (Griffin and Laine 1983) and leguminous crop residues (Frankenberger and Abdelmagid 1985, Wilson and Hargrove 1986, Singh and Vinod Kumar 1996). However, most of the studies concerning N-mineralization rates in soils amended with green manures (see review of Singh et al. 1992) have either been short term incubation studies (8 to 16 weeks) or have employed simple first order equations which give a poor fit to the N-mineralization data (Molina et al. 1980, Broadbent 1986). We, therefore, conducted a long term aerobic incubation study with the primary objective of determining the N-mineralization rates in soils freshly amended with green manures like sesbania, gliricidia, leucaena, and azolla. A secondary objective was to investigate the relationship between various N-mineralization parameters and chemical composition of the green manures incorporated into the soil.

### Introduction

Green manures are viable and ecologically sound alternatives which serve the dual purpose of increasing crop productivity as well as improving long-

### Materials and Methods

The soil used in the study was a fine, sandy loam, *Typic Sulfaquent* and had no history of being applied with either chemical fertilizers or green manures. Relevant properties

Table 1: Important characteristics of the organic manures used in the study

Organic amendment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	C : N ratio	Lignin (g kg <sup>-1</sup> )	Polyphenol (g kg <sup>-1</sup> )
Sesbania	482.4	41.0	11.8	90.0	24.6
Gliricidia	361.9	26.6	13.6	112.0	30.9
Leucaena	391.7	31.6	12.4	104.0	26.2
Azolla	213.5	12.2	17.5	204.0	38.4

of the soil are: pH = 6.7; organic carbon = 2.3 g kg<sup>-1</sup>; total N = 0.18 g kg<sup>-1</sup>.

Soil pH was determined in a 1 : 5 soil water suspension using a glass electrode, texture was determined by the pipette method (Day 1965), total N by the kjeldahl method (Bremner and Mulvaney 1982) and organic carbon by the Modified Mebius method (Nelson and Sommers 1982).

Whole plants (leaves, stem and roots) of *Sesbania rostrata*, *Gliricidia maculata*, *Leucaena leucocephala* and fronds of *Azolla pinnata* were the green manures used for the study. The green manures were harvested at full maturity, nodules removed and the roots thoroughly washed. *A. pinnata* fronds were harvested 30 days after inoculation of *A. pinnata* culture in N-free nutrient medium (Watanabe et al. 1977). The green manures were then oven dried (60 °C) and ground to pass a 100 µm sieve before analysis for total N (Bremner and Mulvaney 1982), organic carbon (Nelson and Sommers 1982), lignin (Goering and van Soest 1970) and polyphenols (Anderson and Ingram 1989). The chemical composition of the green manures are given in Table 1.

### Incubation procedure

Exactly 80 g of soil, 80 g of silica-sand mixture and 2 g (on an oven dry basis) of the specific green manure were thoroughly mixed and transferred to PVC leaching tubes (40 cm length × 3.5 cm dia.). The soil-sand-green manure mixture was supported in the tube on a glass wool pad above a one-hole stopper fitted with a glass drainage tube. A thin glass wool pad was placed on top of the soil to avoid soil dispersion when the leaching solution is poured into the tube. The mineral N initially present was leached from the system using 100 ml of 0.01 M CaCl<sub>2</sub> in small increments (10 ml at a time) followed by 25 ml of N-minus nutrient solution prepared with KH<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub> and CaSO<sub>4</sub> containing 100, 24, 113, 0.5 and 4 mg l<sup>-1</sup> of Ca, Mg, S, P, K respectively; its pH was approximately 7. The tubes were stoppered, moisture potential brought to 0.01 Mpa by overnight equilibration on a suction manifold apparatus and incubated at 35 ± 1 °C.

Mineralized N (NH<sub>4</sub>, NO<sub>2</sub> and NO<sub>3</sub>) and organic N were determined following 1, 2, 4, 8, 10, 12, 14, 20, 24, 32 and 36 weeks of incubation. After each extraction, the moisture potential was brought to 0.01 Mpa and the

tubes returned to the incubator. Total N in the soil and leachate was determined by the regular kjeldahl method (Bremner and Mulvaney 1982), inorganic N (NH<sub>4</sub>, NO<sub>3</sub> + NO<sub>2</sub>) - N by steam distillation (Keeney and Nelson 1982) and nitrite by the modified Griess-Ilosvay method (Keeney and Nelson 1982). Organic N was calculated by subtraction of NH<sub>4</sub>-N from total N. Unaccounted N, enhanced N loss, total N mineralized and net N mineralized (Table 2) were estimated according to Lindemann et al. (1988). Soil-sand-silica mixture without any green manure formed the control.

### Determination of kinetic parameters

The N-mineralization potentials and rate constants were determined using the double exponential model (Molina et al. 1980):

$$N_t = N_o S(1 - e^{-ht}) + N_o(1 - S)(1 - e^{-kt})$$

where, N<sub>t</sub> represents the cumulative amount of inorganic N mineralized at various times (t). N<sub>o</sub> represents the potential mineralizable N and S and 1 - S represent the labile and recalcitrant organic N fractions decomposing at specific rates h and k respectively.

All values reported are means of three replications expressed on a moisture-free basis.

### Results

Leachates were analysed for NO<sub>2</sub>, NH<sub>4</sub> and total N from the initial leaching date through the second week of incubation; thereafter levels of NO<sub>2</sub>, NH<sub>4</sub> and total N were below detection limits. Ammonium N was detected only in the initial leachate and averaged 1.1 mg kg<sup>-1</sup> for the unamended soil and 26 mg kg<sup>-1</sup> for soils amended with green manures. Nitrite levels were below detection limits, except for the initial leaching (0.3 mg kg<sup>-1</sup> for all treatments). Much of the organic N loss was unaccounted for in the leachates and was reflected in the low net organic N-mineralization rates (Table 2). The inorganic N data (Table 2) reveals that soils amended with green manures leached higher NO<sub>3</sub> + NO<sub>2</sub> - N than the unamended soil. Green manure amended soils

Table 2: Organic N mineralized from the soils amended with green manures

Amendment incorporated	mgN kg <sup>-1</sup> soil							Organic N mineralization (%)	
	Initial organic N	Final organic N	Organic N loss	Initial leached N	0-36 week leached N	Unaccounted N <sup>a</sup>	Enhanced N loss <sup>b</sup>	Total <sup>c</sup>	Net <sup>d</sup>
None	521	188	333	4.2	110	223	—	63.9	21.1
Sesbania	1434	509	925	33.1	478	447	368	64.5	33.3
Leucaena	1262	461	801	21.0	408	393	298	63.5	32.3
Gliricidia	1098	429	669	19.2	334	335	224	61.0	30.4
Azolla	823	360	463	28.1	207	256	97	56.3	25.2
Isd 0.05	121	56	32	4.6	23	24			

<sup>a</sup> Organic N loss-leached N; <sup>b</sup> leached N from amended soil-leached N from control soil; <sup>c</sup> organic N loss/initial organic N  $\times 100$ ; <sup>d</sup> 0-36 week leached N/initial organic N  $\times 100$ .

leached 247 mg kg<sup>-1</sup> (average of soils amended with green manures) more NO<sub>3</sub>+NO<sub>2</sub>-N than the unamended soil (Table 2; see enhanced N loss). Although net organic N mineralization was higher in amended soils, organic N loss and unaccounted N were greater than in the control soil.

In general, mineralization rates (mg NO<sub>3</sub>+NO<sub>2</sub>-N kg<sup>-1</sup> soil wk<sup>-1</sup>) were greatest during the first week and decreased with time in all soils (data not given). Among the amended soils, total and net N mineralization were highest in the soil amended with sesbania, while they were least in the soil amended with azolla (Table 2).

### Kinetics

The kinetic parameters estimated using the double exponential model (Table 3) indicated that soils amended with green manures possessed greater N-mineralization potential than the unamended control. Among the amended soils, the soil amended with sesbania had the greatest N-mineralization

potentials and rate constants, while the soil amended with azolla had the least values.

### Discussion

Data in Table 2 indicates lower net organic mineralization in all soils. The unaccounted N is presumed to have been denitrified or volatilized. Either gaseous loss of N occurred resulting in much of the organic N loss being unaccounted for in the leachates or the moisture of 0.01 MPa may have encouraged denitrification (Lindemann et al. 1988). However, other researchers have found the optimum moisture potential for N mineralization to be 0.01 to 0.03 MPa (Stanford and Epstein 1974, Terry et al. 1981). The flush of (NO<sub>3</sub>+NO<sub>2</sub>)-N and corresponding high mineralization rates during the first week of incubation were attributed to the decomposition of very labile organic N. These results are in good agreement with the common knowledge that as the N mineralization proceeds, the mineralizable

Table 3: Nitrogen mineralization rates and kinetic parameters of soils amended with green manures

Amendment incorporated	N mineralization rate (mg N kg <sup>-1</sup> wk <sup>-1</sup> )	N mineralization potential (mg N kg <sup>-1</sup> )		Rate constants (wk <sup>-1</sup> )	
		NoS	No (1-S)	h	k
None	3.1	204	1.2	0.213	0.020
Sesbania	13.3	745	16.3	1.423	0.073
Leucaena	11.3	655	15.2	1.102	0.069
Gliricidia	9.3	545	13.2	0.889	0.058
Azolla	8.0	352	10.2	0.521	0.033
Isd 0.05	0.2	21	0.9	0.030	0.010

N resistance becomes greater and the mineralization rate becomes smaller (Sierra 1990).

Estimates of the percent organic N mineralized are given in Table 2. While the first estimate (total organic N mineralized) reflects true N mineralization by definition and is a better estimate of plant available N during a growing season, the second estimate (net organic N mineralization) is the recoverable organic N and gives a true picture of how much organic N remains for decomposition the following growing season. Most investigators report net N mineralization (Lindemann et al. 1988).

Variations in percent organic N-mineralized (total and net) among the amended soils is apparently due to variations in the chemical composition of the green manures incorporated into the soil. Incorporation of green manures with higher lignin and polyphenol contents and wider C to N ratio led to relatively lower organic N-mineralization rates (Table 2). However, among the various parameters lignin content, lignin to N ratio and lignin + polyphenol to N ratio accounted for maximum variation in the total and net N-mineralized from the soils (Table 4). Polyphenol and lignin reduce the rate of N mineralization from decomposing plant materials by complexing with protein (see review of Singh et al. 1992). Lower organic N mineralization in soils amended with azolla would, therefore, be due to its higher lignin and polyphenol content. However, lignin + polyphenol to N ratio has been found to be a better parameter in predicting N mineralization in soils from added organic materials as compared to lignin to N ratio and to polyphenol to N ratio (Handayanto et al. 1994).

### Kinetics

The double exponential model gives a relatively better fit to the experimental data by considering two mineralizable N pools of different resistance and

thus two rate constants (Broadbent 1986). In this study, estimated values of  $N_0$  were always larger than the cumulative values obtained in the experiment (Tables 2 and 3) indicating that some organic N remained in the amended soils even after 36 weeks.

Lower  $N_0$  values and rate constants in the soil amended with azolla might perhaps be due to its higher lignin and polyphenol content compared to the other green manures. Correlations obtained between the rate constants and chemical constituents of the green manures (Table 4) indicated that lignin content, lignin to N ratio and lignin + polyphenol to N ratio accounted for maximum variation in the rate of decomposition and subsequently the rate of N mineralization from green manures incorporated into the soil.

### Summary

A long-term (36 weeks) aerobic incubation experiment was conducted to assess the N-mineralization rates and kinetics in soils amended with *Sesbania rostrata*, *Gliricidia maculata*, *Leucaena leucocephala* and *Azolla pinnata*. The study revealed that the N-mineralization rates were greatest during the first week and decreased with time in all soils. The percent organic N-mineralized (total and net) and the cumulative inorganic N-mineralized ( $NO_3 + NO_2 - N$ ) varied with the type of green manure incorporated into the soil. In general, they were greatest in the soil amended with sesbania and least in the soil amended with azolla. The kinetic parameters,  $N_0S$  and  $N_0(1 - S)$  and their respective rate constants  $h$  and  $k$  were also greatest in the soil amended with sesbania followed by soils amended with leucaena, gliricidia and lastly by the soil amended with azolla. Among the various parameters, lignin content, lignin to N ratio and lignin + polyphenol to N ratio of the green manures accounted

Table 4: Simple correlation coefficient ( $r$ )<sup>a</sup> based on the correlation between chemical composition of the green manures (x) and percent organic N mineralized/rate constants (y)

N mineralization parameters	C:N ratio	Lignin content	Polyphenol content	Lignin:N ratio	Lignin + Polyphenol:N ratio
Total N mineralized	-0.89*	-0.93*	-0.83*	-0.92*	-0.92*
Net N mineralized	-0.92*	-0.95*	-0.86*	-0.95*	-0.95*
$h$	-0.85*	-0.87*	-0.82*	-0.85*	-0.85*
$k$	-0.90*	-0.94*	-0.84*	-0.94*	-0.94*

<sup>a</sup> $n = 4$ ; \*at  $P < 0.05$ .

for maximum variation in the percent organic N-mineralized and N-mineralization rates in the green manure amended soils.

## Zusammenfassung

### Stickstoffmineralisationsraten und Energieumsetzungen in Böden frisch angereichert mit Gründüngung

Untersuchungen zur Langzeitinkubation zur Bestimmung von N-Mineralisierungsraten und die Energieumsetzungen in Böden, die frisch mit übliche verwendeten Gründüngungen wie *Sesbania rostrata*, *Gliricidia maculata*, *Leucaena leucocephala* und *Azolla pinnata* versorgt wurden, sind selten. Es wurde daher eine Langzeitbelüftungsuntersuchung an inkubierten Böden, die frisch mit den genannten Gründüngungen in PVC-Säulen  $35 \pm 1^\circ\text{C}$  und 0,01 Mpa Feuchtigkeitsgehalt gehalten wurden, durchgeführt. Die Böden wurden anschließend in periodischen Intervallen bis zu 36 Wochen ausgewaschen. Die N-Mineralisierungsraten waren am größten während der ersten Woche und nahm mit der Zeit in allen Böden ab. Die mit Gründüngung versorgten Böden zeigten Auswaschungen von  $247 \text{ mg kg}^{-1}$  mehr an  $\text{NO}_3 + \text{NO}_2 - \text{N}$  als die unbehandelte Kontrolle. Grundsätzlich war die Gesamtmenge an mineralisiertem N (Mittelwert 61 %) annähernd zweimal so hoch, wie die netto N-Menge mineralisiert (im Mittel 30 %) in den angereicherten Böden. Der mineralisierte Prozentsatz an N (gesamt und netto) variierte mit der Art der Gründüngung, die in den Böden eingebracht worden war. Am stärksten war die Mineralisierung in einem Böden mit *Sesbania* und am geringsten mit *Azolla*. Die Parameter der Energieumsetzungen, die unter Verwendung eines doppelt exponentiellen Modells abgeleitet wurden, weisen darauf hin, daß Böden mit Gründüngung ein signifikant höhere N-Mineralisierungspotentiale aufweisen im Vergleich zu der unbehandelten Kontrolle. Die Energieumsetzungsparameter variierten auch mit der Art der Gründüngung, die in den Böden eingebracht wurde. Von den verschiedenen Parametern waren der Ligningehalt, das Verhältnis von Lignin zu N und das Verhältnis von Lignin + Polyphenol zu N der Gründüngungen die Schlüsselfaktoren, die die Rate der Zersetzung und die darauf folgende N-Mineralisierung in den angereicherten Böden kontrollierten.

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