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# **Leguminous Cover Crop Effects on Nitrogen Mineralization Rates and Kinetics in Soils**

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With 4 tables

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### **Abstract**

Soils were collected from an experimental site (initiated in 1991) at which leguminous crops were grown as natural soil cover in the interspaces of a 19-year-old coconut plantation. Atylosia scarabaeoides, Centrosema pubescens, Calopogonium mucunoides and Pueraria phaseoloides were grown in separate plots during the rainy season and ploughed into the soil towards the end of the monsoon (in December every year). Soil samples were collected from this site at the end of the 7th year and incubated in PVC columns at  $35 \pm 1^{\circ}$ C and 0.01 MPa moisture content for 36 weeks. The soils were then leached at periodic intervals for up to 36 weeks and nitrogen (N) mineralization rates and kinetics were determined by the double exponential model. The N mineralization rates were highest during the first week and decreased with time in all soils. Soils amended (in situ ploughing) with cover crops leached 191 mg kg<sup>-1</sup> more  $NO_3^- + NO_2^-$ -N than the unamended control. The per cent organic N mineralized (total and net) and the cumulative inorganic N mineralized (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>-N) varied with the amount (biomass) and type of cover crop incorporated into the soil. In general, soils amended with cover crops had greater N mineralization potentials and rate constants than the unamended control. The kinetic parameters  $N_oS$  and  $N_o(1 - S)$  and their respective rate constants h and k also varied with the amount and type of cover crops incorporated into the soil. The results further indicated that the lignin + polyphenol: N ratio of the cover crops is extremely important in predicting the rate of decomposition and N mineralization in soils.

**Key words:** C : N ratio — leguminous cover crops — lignin + polyphenol : N ratio — lignin : N ratio — N mineralization kinetics — organic N

# Introduction

Loss of soil productivity due to excessive erosion, intensive cultivation and plant nutrient losses and

depletion is a well-documented constraint to crop production in many parts of the world. Much research has been directed towards correcting this problem using cultural and management practices such as crop rotation, nutrient recycling and soil conservation (Doran et al. 1996). Several management practices such as increasing manure application (Uhlen 1991) and cropping intensity are viable means of returning plant residues to soil (Franzluebbers et al. 1995). Growing certain crops as soil cover may increase residue return as well as shorten the length of time the soil is left fallow (Kuo et al. 1997). Cover crops may not only reduce soil erosion (Smith et al. 1987) and NO<sub>3</sub>-N leaching (McCracken et al. 1994) but also enhance nutrient status (Broughton 1977) and biological activity in soil (Dinesh et al. 1999). A mixture of cover crops (Centrosema pubescens, Calopogonium mucunoides and Pueraria phaseoloides) grown in the interspaces of a coconut plantation was estimated to contribute annually 3-14 tons of green matter, equivalent to 20–111 kg N ha<sup>-1</sup> (Hegde et al. 1993). However, cover crops must undergo decomposition before the nitrogen (N) becomes available to the main crop. Since the rate of N mineralization differs among species (Palm and Sanchez 1991) and among tissues (e.g. leaves, stems and roots) within a species (Frankenberger and Abdelmagid 1985), a close examination of the N mineralization rates and kinetics is needed to evaluate their overall effect on soil N levels and to facilitate the selection of the appropriate leguminous species for soil cover.

Though numerous studies have been performed on the N mineralization rates and kinetics in soil amended with tropical legumes (Oglesby and Dinesh et al.

Fownes 1992, Singh and Kumar 1996, Dinesh and Dubey 1998), there has been little research to document the long-term effects of leguminous cover crops on N mineralization rates and kinetics in soils. Hence soils covered and incorporated with leguminous cover crops for the past 7 years were collected from a mature coconut plantation and subjected to an aerobic incubation study (36 weeks) to determine the N mineralization rates and kinetics in these soils. Efforts were also made to derive the relationship between various N mineralization parameters and the chemical composition of the cover crops incorporated into the soil.

# **Materials and Methods**

The study site (Port Blair, Andaman Islands, India) is a humid tropical region with a mean annual temperature of 24-31°C and annual rainfall of ~3000 mm, the rainfall period extending from May to November. Severe soil erosion and associated nutrient losses are common problems in this region. A study dealing with the ecological sustainability of plantation-based land use was initiated in 1991 in a 19-year-old coconut plantation at the Central Agricultural Research Institute, Port Blair. The study consists of growing certain leguminous crops as natural soil cover in separate plots demarcated by living boundaries of lemon grass (Cymbopogon flexuosus). Cover crops such as Atylosia scarabaeoides, Centrosema pubescens, Calopogonium mucunoides and Pueraria phaseoloides were grown in separate plots during the rainy season and incorporated into the soil by repeated in situ ploughing to a depth of 30 cm towards the end of the monsoon (in December every year). The study also comprised appropriate controls consisting of only coconut trees with neither cover crops nor living boundaries. The experiment had four replications laid out in a randomized block design. The soil of the experimental site was a sandy clay loam, isohyperthermic, Fluventic sulfaquent. The relevant properties of the soil were: pH (1 : 5  $H_2O$ ): 5.4; organic C: 4.4 g kg<sup>-1</sup> and total N: 0.49 g kg<sup>-1</sup>. The soil texture was determined by the pipette method (Day 1965), total N by the Kjeldahl method (Bremner and Mulvaney 1982) and organic C by the Walkley-Black method (Nelson and Sommers 1982).

The biomass was harvested in the maximum growth period (before plough-down) in the inner 1-m<sup>2</sup> area of each plot and the yield estimated. Mature plants (leaves, stem and roots) of the respective cover crop were washed thoroughly, oven-dried (60°C) and ground to pass a 100-mm 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 cover crops is given in Table 1.

# **Incubation procedure**

At the end of the 7th year, 1 week after incorporation of cover crops into the soil (January 1998), about 15 soil samples (0-30 cm) were drawn randomly from the inner two-thirds of each plot using a soil core (6 cm diameter) and pooled into a composite sample. About 80 g of the respective soil (<2 mm) and 80 g of a silica-sand mixture were thoroughly mixed and transferred to PVC leaching tubes (40 cm length  $\times$  3.5 cm diameter). The soil-sand mixture was supported in the tube on a glass wool pad above a one-hole stopper fitted with a glass drainage 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 and K, respectively; its pH was approximately 7. The tubes were stoppered, the moisture potential was brought to 0.01 MPa by overnight equilibration on a suction manifold apparatus, and the tubes were incubated at 35  $\pm$  1°C.

Mineralized N (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>) 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), mineralized 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><sup>+</sup>-N from total N. Unaccounted N, enhanced N loss, total N mineralized and net N mineralized (Table 1) were estimated according to Lindemann et al. (1988).

Table 1: Biomass	yield and chemical	composition of the co	over crops incorporated	into the soil

Cover crop	Biomass yield <sup>1</sup> (t ha <sup>-1</sup> )	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> )
Atylosia scarabaeoides	28.3	498	28.6	17.4	67.2	26.4
Pueraria phaseoloides	32.9	528	32.4	16.3	61.5	22.4
Centrosema pubescens	16.0	467	25.1	18.6	74.2	30.7
Calopogonium mucunoides	14.0	453	22.1	20.5	79.3	35.4
LSD (0.05)	1.8	4	0.5	0.6	0.3	5.8

<sup>&</sup>lt;sup>1</sup>Sum of 7 years (includes above-ground and below-ground yields).

# **Determination of kinetic parameters**

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

$$N_t = N_o S(1-e^{-ht}) + N_o (1-S)(1-e^{-kt}), \label{eq:Nt}$$

where N<sub>t</sub> represents the cumulative amount of inorganic N mineralized at various times (t), No represents the potential mineralizable N and S and 1 - S represent the labile and recalcitrant (slowly decomposable) organic N fractions decomposing at specific rates h and k, respectively. The potential mineralizable N (N<sub>o</sub>) was determined by non-linear least-square fitting of data on inorganic N leached at t = 1, 2, 4, 8, 10, 12, 14, 20, 24, 32 and 36 weeks.

All values reported are means of four replications on a dry weight g<sup>-1</sup> oven-dried (110°C) weight of soil basis.

The data were analysed with the ANOVA procedure (Gomez and Gomez 1984) using an F-test to determine if there were any significant differences (0.05 level). Mean separations were performed using the least significant difference (LSD) test at the 0.05 level.

# **Results**

Table 2 summarizes the inorganic N leachate data. In all treatments,  $NO_2^-$ ,  $NH_4^+$  and total N were present only in the initial leachate; thereafter they were below detection limits. While NH<sub>4</sub><sup>+</sup>-N in the initial leachate averaged 28 mg kg<sup>-1</sup> for soil amended with cover crops,  $NO_2$ -N averaged 0.6 mg kg<sup>-1</sup> for all treatments. Irrespective of treatment, the organic N loss was unaccounted for in the leachates, resulting in low net organic N mineralization rates (Table 2). The inorganic N data (Table 2) reveal that soils amended with cover crops leached more NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>-N than the unamended soil. In fact, cover crop amended soils leached 191 mg kg<sup>-1</sup> (average of soils amended with cover crops) more NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>-N than the unamended soil (Table 2; see enhanced N loss).

In general, mineralization rates  $NO_3^- + NO_2^- \cdot N \text{ kg}^{-1} \text{ soil week}^{-1}$ ) were highest during the first week of incubation and decreased gradually during subsequent weeks in all soils (data not given). Nevertheless, after 36 weeks the amended soils accumulated much more NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>-N than the unamended control. Among the cover crops, incorporation of *Pueraria phaseoloides* led to greater total and net N mineralization, while values were lowest in soils amended with Calopogonium mucunoides (Table 2).

Table 2: Organic N mineralized from the soils amended with cover crops

				N (mg N kg <sup>-1</sup> soil)	soil)			Organic N mineralization (9	nic N ation (%)
Cover crop incorporated	Initial organic N	Final organic N	Organic N loss	Initial leached N	0–36 week leached N	Unaccounted $N^1$	Enhanced N loss <sup>2</sup>	Total <sup>3</sup>	Net <sup>4</sup>
None	372	149	223	3.7	96	127	I	59.9	25.8
Atylosia scarabaeoides	933	337	969	16.3	305	291	209	63.9	32.7
Pueraria phaseoloides	1026	364	662	18.2	351	311	255	64.5	34.2
Centrosema pubescens	876	329	547	14.2	265	282	169	62.4	30.2
Calopogonium mucunoides	833	330	503	12.7	226	277	130	60.4	27.1
LSD(0.05)	5	5	4	0.5	4	5			
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#### **Kinetics**

Soils amended with cover crops had higher N mineralization potentials and rate constants than the control (Table 3). Among the amended soils, N mineralization potentials and rate constants were highest in soils amended with *Pueraria phaseoloides* and lowest in soils amended with *Calopogonium mucunoides*.

#### **Discussion**

The net organic N mineralization was low in all treatments, presumably due to denitrification or volatilization of the unaccounted N. Gaseous loss of N might have resulted in much of the organic N being unaccounted for in the leachates, or the moisture potential of 0.01 MPa might have encouraged denitrification (Lindemann et al. 1988). However, earlier studies indicated that a moisture potential of 0.01 MPa is optimum for N mineralization in soils (Stanford and Epstein 1974, Terry et al. 1981). The study also revealed a flush of NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>-N and corresponding high mineralization rates during the first week of incubation, especially in soils amended with cover crops (data not shown). Decomposition of very labile organic N is likely to be responsible for such high values during the initial stages of incubation. As the more labile organic N disappears, and the more recalcitrant organic N predominates in the organic N pool, the mineralization rate would be expected to slow down (Sierra 1990).

Variations in total and net organic N mineralized among the amended soils might be due to variations in the chemical composition of the cover crops incorporated into the soil. The total and net N mineralized was significantly correlated to the C: N ratio and the polyphenol and lignin contents of the cover crops. It is therefore apparent that incorporation of cover crops with

higher lignin and polyphenol contents and C: N ratio led to lower organic N mineralization rates (Table 2). While lignin reduces the rate of N mineralization from decomposing plant materials by forming lignoprotein complexes (Frankenberger and Abdelmagid 1985), the exact inhibition mechanism of polyphenols is not fully understood (Oglesby and Fownes 1992). Soluble phenols may precipitate proteins, thereby inhibiting microbial/ enzyme activities essential for N mineralization. Hence, the lower organic N mineralization in soils amended with Calopogonium mucunoides is probably due to higher lignin and polyphenol contents. However, among the various parameters, the lignin + polyphenol: N ratio accounted for maximum variation in the total and net N mineralized from the soils (Table 4). This parameter has been found to be a better index for predicting N mineralization in soils from added legumes than the lignin: N ratio or the polyphenol: N ratio (Handayanto et al. 1994).

#### **Kinetics**

The soils varied markedly in their N mineralization potentials and rate constants (Table 3). This is possibly due to variation in the chemical composition and quantity of biomass incorporated into the soil. Incorporation of more biomass with higher N content, lower C: N ratio and lower lignin and polyphenol contents produced greater N mineralization potentials and rate constants. Correlations obtained between the rate constants and chemical constituents of the green manures (Table 4) indicated that the lignin + polyphenol: N ratio accounted for maximum variation in the rate of N mineralization from the cover crops incorporated into the soil. In this study, the estimated values of N<sub>o</sub> were always larger than the cumulative values obtained in the experiment (Tables 2 and 3),

Table 3: N mineralization rates and kinetic parameters of soils amended with cover crops

	N mineralization rate (mg N		ization potential g N kg <sup>-1</sup> )	Rate constants (week <sup>-1</sup> )	
Cover crop incorporated	$kg^{-1}$ week <sup>-1</sup> )	NoS	$N_o(1-S)$	h	k
None	2.8	182	1.2	0.202	0.018
Atylosia scarabaeoides	9.3	541	13.0	0.883	0.060
Pueraria phaseoloides	11.2	624	14.2	1.024	0.073
Centrosema pubescens	8.4	462	11.7	0.712	0.053
Calopogonium mucunoides	6.9	402	8.2	0.573	0.032
LSD (0.05)	0.6	2	0.8	0.002	0.005

Table 4: Simple correlation coefficients (r)<sup>1</sup> based on the correlation between chemical composition of the cover crops (x) and per cent organic N mineralized/rate constants (y)

N mineralization parameter	N content	Lignin content	Polyphenol content	C : N ratio	Lignin : N ratio	Polyphenol : N ratio	Lignin + polyphenol : N ratio
Total N mineralized Net N mineralized h k	0.80** 0.79** 0.81** 0.82**	-0.73** -0.75** -0.70** -0.74**	-0.73** $-0.73**$ $-0.74**$ $-0.73**$	-0.76** -0.76** -0.75** -0.74**	-0.77** $-0.76**$ $-0.74**$ $-0.75**$	-0.76** -0.79** -0.76** -0.75**	-0.85** -0.84** -0.80** -0.82**

 $<sup>^{1}</sup>$ n = 16: \*\*P < 0.01.

indicating that some organic N remained in the amended soils even after 36 weeks.

# Zusammenfassung

# Leguminosen als Deckfrucht und deren Wirkung auf die Stickstoffmineralisierung sowie die Boden-Kinetik

Böden wurden von einem eperimentell genutzten Standort (begonnen 1991) entnommen, der mit Leguminosen als natürlichem Bestand in den Zwischenräumen einer 19 Jahre alten Pflanzung mit mit Kokouß aufwies. Atylosia scarabaeoides, Centrosema pubescens, Calopogonium mucunoides und Oueraria phaseloides wurden in separaten Parzellen während der Regensaison angebaut und in den Boden gegen Ende des Monsuns (in jedem Jahr im Dezember) eingepflügt. Bodenproben wurden von diesem Standort am Ende des siebenten Jahrs genommen und für 36 Wochen einer Bebrütung in PVC-Röhren bei 35°C und einer Bodenfeúchte von 0,01 Mpa unterworfen. Die Böden wurden dann in periodischen Abständen ausgewaschen und N-Mineralisierung und Kinetik in einem doppelt exponentiellen Model bestimmt.Die Mineralisierungsraten waren in der ersten Woche am stärksten und nahmen dann ab. Böden, in denen die Deckfrüchte eingepflügt wurden, erreichten in den Auswaschungen 191 mg kg<sup>-1</sup> mehr an NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> als Böden ohne eingepflügte Deckfrüchte. Der Prozentsatz des mineralisierten organischen N (total und netto) variierte mit der Menge an Biomasse und der Deckfrucht, die eingepflügt wurde. Grundsätzlich wiesen Böden mit eingpflügter Deckfrucht ein größeres Mineralisierungpotential und eine höhere Rate als Böden ohne eingepflügte Deckfrucht auf. Die kinetischen Parameter,  $N_oS$  und  $N_o(1 - S)$  und ihre jeweiligen Konstanten h und k variierten mit der Menge und der Art der Deckfrucht, die eingepflügt wurde. Die Ergebnisse weisen darauf hin, daß das Lignin + Polyphenol zu N Verhältnis der Deckfrucht von wesentlicher Bedeutung in der Voraussage des Abbaues und der Mineralisierung in den Böden ist.

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