Nitrogen mineralization of leguminous crops in soils

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Abstract

Organic-N production by legumes is a key benefit of growing cover crops and green manures. A soil sample was mixed with legume residue commonly used as green manure in Kenya at a rate of 500 mg N (kg soil)$^{-1}$. Silica sand equal to the weight of the soils was added and mixed thoroughly. The mixture was packed in a leaching tube and leached with 100 mL of 5 mM of $CaCl_2 \cdot 2H_2O$ and incubated at 30°C. The leaching was repeated every 2 weeks for a total of 16 weeks and analyzed for N as NH_4^+, NO_3^-, and NO_2^-. Five legume residues and five different soils were used in this study. Nitrogen mineralization of the legume residues conformed to an exponential model. Application of a two-components exponential model showed two phases of N mineralization. The relationship between the organic N remaining after each incubation period and time of incubation was controlled by two first-order reactions. The initial fast rate (k_1) changed to a slow rate (k_2) at incubation times ranging from 2 to 8 weeks, depending on the legume residue and the soil used. The percentage of N in each phase varied among the legume residue and soils. Linear regression analyses showed that net cumulative amounts of N mineralized from individual legume residues was significantly correlated with the total polyphenols and polyphenol-to-N ratios for two soils. Nitrogen mineralization of dolichos and field bean was significantly and negatively correlated with clay and sand, respectively; of field bean and alfalfa was significantly correlated with C_{mic}; and of dolichos significantly but negatively correlated with the total N and organic N in soils. Linear regression analysis of the pooled data showed that net cumulative amounts of N mineralized and percentage N mineralized were significantly correlated with C : N ratios of the residues ($r = 0.44$ and 0.48 at $p < 0.05$, respectively), and that net cumulative N mineralized was significantly correlated with (lignin + polyphenols)-to-N ratios ($r = 0.41$ at $p < 0.05$) and with lignin contents ($r = 0.61$ at $p < 0.001$).

Key words: amino acids / polyphenols / lignin / leguminous crops / nitrogen mineralization / soil properties

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1 Introduction

Decline in soil fertility and the high cost of fertilizers are the key factors limiting food production in many developing countries. Grain legumes can play a major role in nutrient capture and cycling as well as provide alternatives to shifting cultivation in replenishing soil fertility. With increased population and the demand on land use, long fallow periods are not feasible. However, short fallow periods with high-yielding legume cover crops, which result in green manure, can provide mineral N for subsequent crops in rotation systems. Biomass production in different legumes range from 1.3 to 24 t dry matter (d.m.) ha^{-1}, with largest values in the food legumes. Nitrogen accumulation ranges from 25 to 530 kg ha^{-1} and depends on the legume species, age of the crop, and environmental conditions. The history, production, N-fertilizer equivalent, effect on soil properties, and erosion control of legume winter cover crops are covered in a comprehensive review by *Smith* et al. (1987). Nutrient transformations in soils amended with green manures have been thoroughly reviewed in a second book chapter by *Singh* et al. (1992). It is evident from those review articles that a number of questions remained unanswered.

Studies demonstrated that the decomposition and nutrient-release rates are influenced by legume quality parameters such as polyphenols, lignin, and the concentration of N (*Fox* et al., 1990; *Constantinides* and *Fownes*, 1994a; *Tian* et al., 1995; *Mafongoya* et al., 1997; *Palm* et al., 1997). Further, some ratios, such as lignin-to-N, polyphenol-to-N, and (lignin + polyphenol)-to-N ratios have also been used as indexes of residue-N release (*Constantinides* and *Fownes,* 1994a; *Handayanto* et al., 1994; *Palm* and *Sanchez*, 1991; *Singh* and *Kumar*, 1996). It has not been clearly established, however, which of those variables correlate the best with N mineralization of green manures. A review article by *Hattenschwiler* and *Vitousek* (2000) on the role of polyphenols in nutrient cycling in terrestrial ecosystems concluded that several lines of evidence suggest that polyphenols influence the pools and fluxes of inorganic and organic soil nutrients.

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The significance of polyphenols for nutrient cycling and plant productivity is still uncertain, but it could provide an alternative or complementary explanation for the variability in polyphenol production by plants.

Studies by *Melillo* et al. (1982) indicated that the percentage of lignin or lignin-to-N ratio is an effective index of N release. However, where plant materials contain high concentrations of lignin or polyphenols, there may be little mineralization of plant N in spite of N being considerably greater than the critical level (*Muller* et al., 1988). Increasing lignin concentration reduces the decomposition rate of plant residues (*Tian* et al., 1992). High lignin content of plant residues could also enhance nutrient immobilization, especially of N (*Melillo* et al., 1982). Lignin contents greater than 150 g kg^{-1} slow N release considerably and polyphenols contents >30–40 g kg^{-1} can result in net immobilization of N (*Palm*, 1995). *Haynes* (1986) stated that lignin and polyphenols reduce N-mineralization rate because lignin degrades to phenolic compounds and these compounds as well as the polyphenols already present combine with plant proteins and amino acids to form humic polymers that resist decay.

The C : N ratio of incorporated plant material has been one of the most considered quality criteria of the plant material (*Heal* et al., 1997). However, this simple criterion appeared sometimes inadequate for predicting the decomposition kinetics. The C : N ratios of aboveground portions of green-manure crops range from 8.5 to 29, depending on the species (*Singh* et al., 1992). This ratio does not consider the availability of these nutrients for microbial growth. Consequently, the C : N ratio has failed to be a reliable predictor of plant-material decomposition or N mineralization (*Mckenney* et al., 1995).

Although the literature on green manure is voluminous, the studies involving nutrient transformations have been carried out more often in the past three decades. Many of those studies involved adding a constant rate of legume residues to soils, even though the residues varied in their N contents, or the studies involved one legume residue or one soil (*Frankenberger* and *Abdelmagid*, 1985; *Handayanto* et al., 1992; *Singh* et al., 1992; *Singh* and *Kumar*, 1996; *Mafongoya* et al., 1998). To utilize green-manure N to the fullest extent, a more comprehensive understanding of N-mineralization process is required, and the factors affecting this process are essential for synchronizing the release of N from green manure with uptake by subsequent crops. Therefore, information on the N-mineralization rates of legume residues added on an equal N rate to well-characterized soils is needed. The objectives of this work, therefore, were: (1) to study the N-mineralization kinetics of different legume plants, (2) to assess the relationships among their N mineralization and selected organic constituents, including organic N and C, lignin, hemicellulose, cellulose, and amino acid composition, and (3) to assess the relationships between net legume-residue N mineralization and the soil properties. For this purpose, we studied the N mineralization of five leguminous crops added on an equal N rate (500 mg N [kg soil]$^{-1}$) to each of five surface soils differing in their chemical, biochemical, and physical properties.

2 Materials and methods

2.1 Legume crops

The crop materials used consisted of field bean (*Phaseolus vulgaris* L.), dolichos (*Lablab purpureus* L. Sweet), soybean (*Glycine max* L.), alfalfa (*Medicago sativa* L.), and garden pea (*Pisum sativum* L.). The plants were grown in a greenhouse for a period of 6 weeks on Nicollet surface soil (fine-loamy, mixed, superactive, mesic Aquic Hapludoll; pH = 6.4, C_{org} = 3.7%, clay = 21%, sand = 40%) after treating the seeds with specific *Rhizobium* inoculants supplied by Nitragin Inc. (Milwaukee, WI, USA) by using a succrose solution (200 g in 900 mL deionized water) to aid the adhesion of the inoculants to the seeds and promote N_2 fixation. The legume tops were harvested, dried at 65°C for 3 d, ground by hand to pass through a 1 mm plastic sieve; a portion of which was ground to pass an 80-mesh sieve (<180 μm). In the analyses reported in Tab. 1, total C and N were determined by a LECO Model 600 C and N analyzer (LECO corporation, St Joseph, MI, USA), NH_4^+-N and NO_3^--N by steam distillation, organic N by the difference between the total-N and inorganic-N values, and polyphenols by the method of Folin-Ciocalteau (*Contantinides* and *Fownes,* 1994b). The above analyses were done on the portion ground to pass a 180 μm sieve. Lignin, cellulose, and acid-detergent lignin-ash contents were determined on the sample ground to pass a 1 mm sieve as described by

Table 1: Chemical characteristics of legume residues used.

Plant[a]	Total N	Inorganic N as		Organic N	Crude protein	Total C	Total polyphenols	Lignin	Hemicellulose	Cellulose	ADL ash[b]
		NH_4^+	NO_3^-								
	g kg^{-1}										
Field bean	27.0	0.23	1.69	25.08	157	420	6.3	34.0	791	167	7.4
Dolichos	27.4	0.35	0.49	26.56	166	429	12.4	36.0	793	166	4.7
Soybean	41.6	0	3.22	38.40	240	427	9.6	60.7	763	170	6.0
Alfalfa	43.5	0	1.88	41.62	260	423	8.0	44.5	807	143	4.3
Garden pea	62.7	0.19	3.49	59.21	370	434	10.1	46.2	786	164	3.5

[a] Field bean (*Phaseolus vulgaris L.*); dolichos (*Lablab purpureus* (L.) Sweet); soybean (*Glycine max* L.); alfalfa (*Medicago sativa* L.); garden pea (*Pisum sativum* L.)
[b] ADL ash, acid-detergent lignin ash

Vogel et al. (1999) by using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Faiport, NY). All analyses were done in duplicates and expressed on a moisture-free basis (65°C for 48 h).

2.2 Amino acid analysis

The determination of the amino acid composition of the legume residues (Tab. 2) was performed on the <180 μm sample in accordance with published protocols (*Gehrke* et al., 1987; *AOAC*, 2000). Formic and performic acids hydrolysis reactions were conducted on each of the samples. Eluted fractions from samples were analyzed using an amino acid analyzer utilizing a Na-citrate buffer system. Amino acids were separated using ion-exchange chromatography. Sulfur-containing amino acids were extracted as methionine sulfone and cysteic acid by a performic acid protocol (*Gehrke* et al., 1987). From the percentage of N in each amino acid chemical formula, the amount of N associated with each quantity of amino acid was calculated.

2.3 Soils and their properties

Five Iowa surface (0–15 cm) soils were selected to include a range of chemical, biochemical, and physical properties. The soils were sampled from unfertilized fields, mixed, and sieved to pass a 2 mm mesh sieve and stored at 4°C. A portion of the field-moist soil was air-dried (22°C), and a portion of this was ground to pass through an 80-mesh (180 μm) sieve.

In the analyses reported in Tab. 2, pH was determined by a combination electrode on ratio of soil to water or 0.01 M $CaCl_2$ of 1:2.5, total N by a LECO Model 600 C and N analyzer, NH_4^+-N and NO_3^--N by steam distillation (*Keeney* and *Nelson*, 1982), organic N by subtracting inorganic N from the total-N values, total C by a LECO 600 C and N analyzer, and particle-size distribution by a pipette method (*Kilmer* and *Alexander,* 1949). Microbial biomass C (C_{mic}) was determined by the chloroform fumigation-extraction method (*Vance* et al., 1987) and microbial biomass N (N_{mic}) by the chloroform fumigation-incubation method (*Horwath* and *Paul*, 1994) on the freshly sampled soils. Tests showed that none of the soil samples contained soluble, extractable polyphenols. Total C and N were determined on the <180 μm samples, all other analyses were performed on the <2 mm–mesh samples. All results reported are averages of duplicates and expressed on a moisture-free basis, moisture being determined from weight loss after drying at 105°C for 24 h.

2.4 Nitrogen mineralization

Nitrogen mineralization was studied by the incubation method of *Stanford* and *Smith* (1972) as described in details by *Chae* and *Tabatabai* (1986). Briefly, the method involves incubating at 30°C, after mixing thoroughly, a mixture of 20 g of soil sample (<2 mm mesh, on an oven-dried basis) of field-moist soil and an equal weight of acid-washed silica sand. The mixture was treated with (or without for control) legume residue (1 mm) containing 10 mg of total N (equivalent to 500 mg N per kg of soil). The mixture was placed in a leaching tube and retained by a thin glass-wool pad. A thin pad of glass wool was placed at the top of the mixture in the tube to prevent disturbance of the soil surface upon leaching. The soil–sand–legume residue mixture in the column was leached with 100 mL of 5 mM $CaCl_2$ in five increments to remove the initial mineral N. A suction of 6 kPa (60 cm Hg) was applied to remove the excess solution. The volume of the leachate thus obtained was adjusted to 100 mL with deionized water. The columns were covered with Saran Wrap with a small hole (∅ 0.5 cm) for aeration. The leaching procedure was repeated every 2 weeks for a total of 16 weeks. Controls for each soil were included by using soil-sand mixtures without treatment with legume residue. All treatments were done in duplicates.

The leachates were analyzed for NO_3^--N and NO_2^--N by steam distillation (*Keeney* and *Nelson*, 1982). The leachates obtained after the first 2 weeks of incubation were also analyzed for NH_4^+-N by steam distillation and for NO_2^--N by the modified Griess-Ilosvay colorimetric method (*Keeney* and *Nelson,* 1982). The leachates obtained thereafter were analyzed for NO_3^--N only, as no NH_4^+-N or NO_2^--N could be detected.

2.5 Statistical analysis

The nonlinear regression approach described by *Smith* et al. (1980) for N mineralization was used to solve the following

Table 2: Selected chemical and physical properties of the soils used.

Soil[a]	pH		Total N	Inorganic N as		Organic N	Total C	C_{mic}[b]	N_{mic}[b]	Clay	Sand	Moisture
	H_2O	$CaCl_2$		NH_4^+	NO_3-							
			g kg^{-1}	— mg kg^{-1} —		—— g kg^{-1} ——		— mg kg^{-1} —		—— g kg^{-1} —		g kg^{-1}
Storden	7.8	7.4	2.2	2.5	14.4	2.18	28.9	167	22.0	133	480	164
Webster	8.0	7.4	3.5	1.4	8.4	3.49	32.3	553	41.2	232	370	253
Clarion	5.0	4.6	3.7	4.6	9.7	3.69	21.1	335	13.2	235	356	198
Canisteo	7.5	7.4	3.8	1.9	7.1	3.79	37.7	527	40.3	275	318	235
Harps	7.9	7.6	4.3	1.4	6.8	4.29	53.9	273	37.8	281	335	247

[a] Storden, fine-loamy, mixed, superactive, mesic Typic Eutrudept; Webster, fine-loamy, mixed, superactive, mesic Typic Haplaquoll; Clarion, fine-loamy, mixed, supeactive, mesic Typic Hapludoll; Canisteo, fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquoll; Harps, fine-loamy, mixed, superactive, mesic Typic Calciaquoll

[b] C_{mic} and N_{mic}, microbial biomass C and N, respectively

equation for estimating the readily mineralizable organic-N pool in the legume residue (N_o) and the first-order rate constant (k):

$$N_m = N_o [1 - \exp(-kt)], \quad (1)$$

where N_m is the amount of N mineralized in legume residue–treated soil minus that mineralized in the control soil at a specific time (t). The statistical analysis–system computer language was used to calculate N_o and k (*Barr* et al., 1976).

To estimate the decomposition rates (k_i) of the various organic-N pools in each legume residue, k_i values were calculated from slopes of linear segments of curves obtained from plotting the natural log of organic N remaining after each incubation period against time of incubation as described by *Gilmour* et al. (1977) and *Ajwa* and *Tabatabai* (1994). The segment with the steeper slope (higher k_i value) was considered to represent the more readily decomposable organic-N fraction. The segment with the flatter slope (smaller k_i value) was considered to represent the less decomposable (resistant) fraction of organic N. The k_i value of the resistant fraction was used in calculating the half-life ($t_{1/2}$) of the resistant N fraction in the legume residue:

$$t_{1/2} = 0.693/k_i . \quad (2)$$

3 Results and discussion

3.1 Chemical composition of legume residue

Selected chemical constituents of the legume residues are summarized in Tab. 1. The total N contents of the legumes ranged from 2.70% to 6.27%, with garden pea having the highest concentration. The total organic-C contents were uniform, at about 426 g kg^{-1}. The concentration of crude protein was estimated from organic N multiplied by 6.25; the values ranged from 157 g kg^{-1} in field bean to 370 g kg^{-1} for garden pea. Dolichos contained the highest concentration of polyphenols (12.4 g kg^{-1}), while field bean contained the lowest concentration (6.3 g kg^{-1}). Field bean contained also the lowest lignin concentration (34.0 g kg^{-1}), while soybean contained the highest (60.7 g kg^{-1}). The percentages of cell soluble, hemicellulose, and cellulose did not differ markedly among the legume residues; but the concentrations of the acid-detergent lignin ash ranged from 3.5 g kg^{-1} in garden pea to 7.4 g kg^{-1} in field bean.

The amino acid composition (Tab. 3) of the legume residues varied considerably, with aspartic acid and glutamic acid being the most predominant, and alanine, arginine, glycine, isoleucine, leucine, phenylalanine, serine, and valine being second group as dominant. The concentrations of histidine, hydroxyproline, methionine, ornithine, taurine, and tryptophan were the least. The total amounts of 22 amino acids determined in the residues ranged from 141.3 g kg^{-1} for dolichos to 272.7 g kg^{-1} for garden pea. Expressed as percentages of crude protein in legume residues, the total amino acids ranged from 73.7 for garden pea to 92.2 for field bean (avg. = 83.3%). The total amounts of amino acid N associated with the 22 amino acids ranged from 20.8 to 37.6 g (kg residue)$^{-1}$. Expressed as percentages of N in the total amino acids (Tab. 3), the values ranged from 13.7% in alfalfa to 14.4% in field bean (avg. = 13.9%); almost constant.

3.2 Nitrogen mineralization

Nitrogen-mineralization patterns were similar for all legume residue–treated soils; all showed parabolic curves with time

Table 3: Amino acid composition of the legume residues used (figures in parentheses are amounts of N).

Amino Acid	Field bean	Dolichos	Soybean	Alfalfa	Garden pea
			g kg^{-1}		
Alanine	9.3 (1.46)	9.1 (1.43)	12.3 (1.93)	15.2 (2.39)	18.0 (2.83)
Arginine	8.1 (2.59)	8.3 (2.66)	11.8 (3.78)	12.5 (4.00)	14.5 (4.64)
Aspartic acid	17.8 (1.86)	16.4 (1.74)	24.7 (2.62)	26.3 (2.79)	49.8 (5.28)
Cysteine	1.6 (0.19)	1.5 (0.17)	2.1 (0.24)	2.9 (0.15)	2.8 (0.32)
Glutamic acid	7.6 (1.69)	15.1 (1.45)	20.9 (2.01)	23.5 (2.26)	26.5 (2.54)
Glycine	8.2 (1.53)	7.8 (1.46)	10.6 (1.98)	11.7 (2.19)	13.9 (2.60)
Histidine	3.8 (1.21)	3.8 (1.21)	4.8 (1.53)	5.4 (1.72)	6.4 (2.04)
Hydroxyproline	1.1 (0.12)	1.5 (0.18)	1.7 (0.20)	1.5 (0.18)	2.4 (0.28)
Isoleucine	7.4 (0.79)	7.3 (0.78)	9.5 (1.02)	10.8 (1.15)	11.9 (1.27)
Lathionine	0 (0)	0 (0)	0.5 (0.07)	0.1 (0.01)	0 (0)
Leucine	13.6 (1.46)	13.8 (1.48)	18.5 (1.98)	19.7 (2.11)	21.6 (2.31)
Lysine	8.4 (1.60)	8.7 (1.65)	12.5 (2.38)	14.2 (2.70)	16.3 (3.10)
Methionine	2.2 (0.21)	2.3 (0.22)	3.9 (0.37)	4.2 (0.39)	4.3 (0.41)
Ornithine	0 (0)	0.40 (0.08)	0.5 (0.11)	0.6 (0.13)	6.8 (0.144)
Phenylalanine	8.6 (0.73)	8.6 (0.73)	11.8 (1.00)	13.1 (1.11)	14.4 (1.22)
Proline	7.6 (0.93)	7.4 (0.90)	9.6 (1.17)	11.2 (1.37)	11.3 (1.38)
Serine	6.5 (0.86)	6.2 (0.82)	8.3 (1.10)	9.3 (1.24)	11.0 (1.46)
Taurine	1.4 (0.16)	1.4 (0.16)	1.0 (0.11)	0.3 (0.03)	0.7 (0.08)
Threonine	7.1 (0.84)	6.8 (0.80)	9.6 (1.13)	10.9 (1.29)	11.7 (1.38)
Tyrosine	4.6 (0.35)	5.0 (0.39)	7.1 (0.55)	8.0 (0.62)	9.0 (0.69)
Tryptophan	1.0 (0.14)	1.0 (0.14)	2.1 (0.29)	3.2 (0.44)	3.6 (0.49)
Valine	8.9 (1.06)	8.9 (1.06)	12.2 (1.45)	13.6 (1.62)	15.8 (1.88)
Total	**144.8 (20.8)**	**141.3 (19.5)**	**196.0 (27.0)**	**218.2 (29.9)**	**272.7 (37.6)**
Percentage N	**14.4**	**13.8**	**13.8**	**13.7**	**13.8**

of incubation. Plots of the amounts of N mineralized in the untreated soils (controls) *vs.* incubation time were linear as shown in Figs. 1 and 2 for Canisteo and Webster soils, respectively. The amounts of N mineralized within successive incubation times for the other three soils used are shown in Tab. 4. In general, the amount of N mineralized in legume residue–treated soils increased at a decreasing rate. This was seen as rapid increase during the initial stages of incubation, followed by a slower, nearly linear increase. The mineralization rate of the native organic N in soils in the presence of legume residue (priming effect) assumed to be the same for the legume residues used. This assumption is based on early finding that suggested a similar priming effect in soils whether different crop residues or residues from various parts of the same plant were applied (*Pinck* et al., 1950; *Hallam* and *Bartholomew*, 1953; *Broadbent* and *Nakashima,* 1974). Other

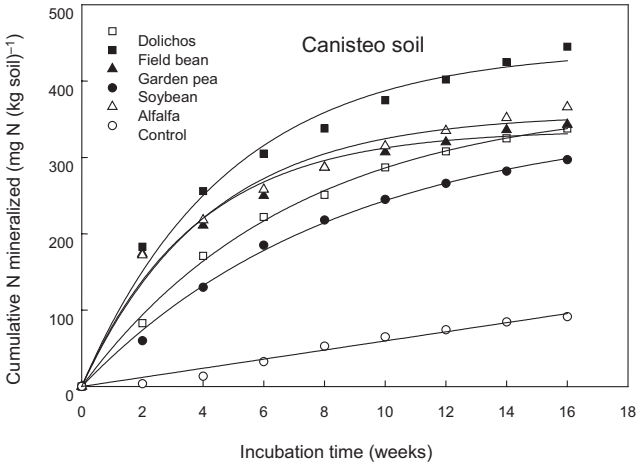

Figure 1: Relationship between cumulative amounts of N mineralized in Canisteo soil treated with (or without) different leguminous crops and incubation time. Least significant difference of cumulative N mineralized at 16 weeks of incubation = 64.

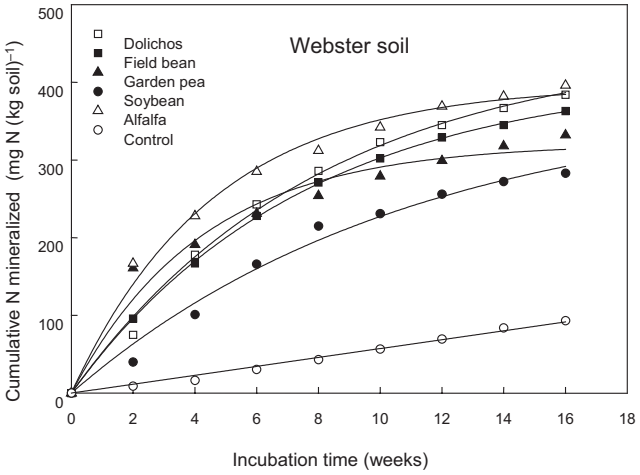

Figure 2: Relationship between cumulative amounts of N mineralized in Webster soil treated with (or without) different leguminous crops and incubation time. Least significant difference of cumulative N mineralized at 16 weeks of incubation = 57.

Table 4: Amounts of N mineralized within successive incubation periods in soils treated with legume residues.

Soil	Legume	N mineralized within successive incubation periods (weeks) specified							
		0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	Total
		mg (kg soil)$^{-1}$								
Storden	None	12	12	16	9	21	11	6	5	92
	Field bean	136	53	31	8	21	26	27	10	312
	Dolichos	143	102	53	37	34	26	27	15	437
	Soybean	57	86	38	32	15	29	25	25	307
	Alfalfa	171	22	24	19	10	14	21	7	288
	Garden pea	149	33	32	16	11	17	17	16	291
	LSD $p < 0.05$[a]									42
Clarion	None	7	6	5	1	4	8	4	2	37
	Field bean	64	121	58	19	14	17	15	14	322
	Dolichos	37	103	51	23	16	13	13	9	265
	Soybean	28	61	65	23	18	14	14	9	232
	Alfalfa	87	76	32	14	11	10	8	8	246
	Garden pea	79	96	43	14	14	12	12	8	278
	LSD $p < 0.05$									59
Harps	None	5	3	8	8	9	12	8	6	59
	Field bean	91	73	37	31	28	25	24	24	333
	Dolichos	66	77	33	33	29	25	26	26	315
	Soybean	26	51	53	34	15	26	25	22	252
	Alfalfa	141	39	30	17	19	19	24	20	309
	Garden pea	139	43	25	18	18	21	21	19	304
	LSD $p < 0.05$									38

[a] LSD, Least significant difference

studies showed that the priming effect caused by adding plant materials high in solubles was as effective as adding insoluble materials if they were added to soils at the same rate (*Bingeman* et al., 1953).

Statistical analysis showed that net N mineralization of the legume residues in the five soils studied were not significantly correlated with the total amino acids of the residues. This is not surprising because the proportion of each amino acid varied among the five residues and a constant rate of N was added to the soils (Tab. 3).

The total amounts of net N mineralized from the legume residues varied considerably; they ranged from 170 mg (kg soil)$^{-1}$ in Storden soil treated with alfalfa residue to 353 mg N (kg soil)$^{-1}$ in Canisteo soil treated with field bean residue. The captions of Figs. 1 and 2 show the least significant difference values (LSD) obtained for the Canisteo and Webster soils, and Tab. 4 shows the amounts of total N mineralized within successive incubation periods and the LSD values of the other three soils treated with legume residues. The variation in net N mineralization (not shown) is expected, because the chemical composition varied among the legume residues (Tab. 1) and the soils (Tab. 2). Expressed as percentages of legume N added to soils, the net cumulative amounts of N mineralized in 16 weeks varied among the soils and the legume residues studied; they ranged from 38.0% in Webster soil treated with soybean residue to 70.6% in Canisteo soil treated with field bean residue (Tab. 5). In contrast, the amounts of N mineralized in the control soils ranged from 36 mg N in Clarion soil to 93 mg N in Webster soil. Expressed as percentages of organic N in soils, the values ranged from 1% in Clarion soil to 4.2% in Storden soil. The results clearly show that the efficiency of the legume residues in providing mineral N to crops is dependent on the type of legume and the soil involved.

Analysis of the leachates obtained after the first 2 weeks of incubation contains some NO_2^--N, the values ranged from 0.06 mg N (kg soil)$^{-1}$ for the Canisteo soil treated with soybean or alfalfa to 1.78 and 3.31 mg N (kg soil)$^{-1}$ for the Harps soil treated with garden pea and field bean, respectively; all other values were <1 mg (kg soil)$^{-1}$. None of the leachates contained NH_4^+-N. The reported amounts of N mineralized include the trace amounts of NO_2^--N, because the steam-distillation method used for determination of inorganic N in the leachates includes NO_2^--N (*Keeney* and *Nelson* 1982).

3.3 Nitrogen mineralization models

A variety of models has been proposed for calculating plant residue–decomposition rates and soil organic-matter levels. These include models with decomposition rates that are constants in time, models with decomposition constants that are variable in time, and models with different fractions of organic matter, each having a different decomposition rate (*Bouwman,* 1990). All these models involve equations with exponential functions. Because the N-mineralization patterns were similar, but the rates of mineralization of the organic N of the legume residues were markedly different and unpredictable, we used an exponential equation developed for describing N mineralization in soils to calculate the decomposition rate constants and the readily mineralizable organic N in the legume residues. All results of net legume-residue N mineralized in soils conformed well to the exponential model developed for N mineralization (*Smith* et al., 1980). Convergence of the estimates occurred with <11 iterations. The first-order rate constants (k) and potentially mineralizable–organic N values (N_o) for the legume residues are presented in Tab. 6. The N_o values varied from 182 mg kg^{-1} of Storden soil treated with garden pea to 352 mg kg^{-1} of Storden soil treated with dolichos (values well below the added 500 mg N [kg soil]$^{-1}$), and the k values ranged from 0.07 week^{-1} for Canisteo soil treated with soybean to 0.63 week^{-1} for Storden soil treated with garden pea.

Because the results obtained indicate that initially there was a rapid mineralization of easily decomposable compounds (*e.g.*, sugars and proteins) and the remaining material being more resistant, we also used a two-components model to describe the N-mineralization patterns of N in the legume residues. To identify the various phases involved in mineralization of organic N of the legume residues added to soils and to estimate the decomposition rate (k_i) of the various organic pools in each legume residue, we constructed graphs by plotting the natural log of N remaining *vs.* time (weeks) for each set of data collected. For illustration, the results obtained with the Canisteo soil treated with field bean or soybean residue are shown in Fig. 3. Transformation of the N-mineralization data revealed that the decomposition of organic N in all the legume residues used occurred in two phases. The rate constants of phase I were much larger than those of phase II. The amount of organic N involved in phase I (presented as a percentage of legume N added, 500 mg (kg soil)$^{-1}$, D_1) is the easily decomposable fraction of organic N added. Phase II (presented by D_2) of decomposition represents the slowly decomposing compounds and resistant fraction (Tab. 6). The time involved in mineralizing phase I ranged from 2 to 8

Table 5: Net cumulative N mineralized in 16 weeks in soils treated with legume residues expressed as percentages of total N added to soils.

Legume residue	Percentage of N mineralized in soil specified				
	Storden	Webster	Clarion	Canisteo	Harps
Field bean	44.0	54.0	57.2	70.6	54.8
Dolichos	69.0	58.2	45.6	49.2	51.2
Soybean	43.0	38.0	39.0	41.0	38.6
Alfalfa	39.2	60.6	41.8	54.8	50.0
Garden pea	39.8	47.8	48.2	41.0	49.0
Mean	47.0	51.7	46.4	51.3	48.7

Table 6: First-order rate constants for mineralization of organic N in soils treated with legume residues.

Soil or legume	Rate constant (week^{-1})[a]			N_o (mg kg^{-1})	Percentage of N Min. at each phase		Half-life of N remaining (weeks)
	k	k_1	k_2		D_1	D_2	
Storden soil							
Field bean	0.50	0.10	0.01	197	33.0	7.6	53.3
Dolichos	0.23	0.15	0.05	352	44.2	17.4	13.9
Soybean	0.17	0.07	0.02	207	24.0	14.4	31.5
Alfalfa	0.22	0.21	0.01	186	34.2	5.4	99.0
Garden pea	0.63	0.16	0.01	182	27.4	8.2	77.0
Webster soil							
Field bean	0.17	0.08	0.02	324	39.4	8.2	33.0
Dolichos	0.19	0.09	0.03	284	42.4	9.4	27.7
Soybean	0.14	0.05	0.01	223	27.0	3.6	86.6
Alfalfa	0.33	0.14	0.02	299	42.2	39.4	31.5
Garden pea	0.44	0.18	0.02	227	30.4	12.8	38.5
Clarion soil							
Field bean	0.19	0.07	0.02	298	45.0	8.6	30.1
Dolichos	0.16	0.07	0.02	252	34.6	6.8	46.2
Soybean	0.12	0.05	0.01	237	27.4	7.4	49.5
Alfalfa	0.29	0.09	0.01	210	30.0	6.6	63.0
Garden pea	0.25	0.10	0.01	241	32.4	8.2	49.5
Canisteo soil							
Field bean	0.31	0.17	0.04	336	48.4	16.2	15.8
Dolichos	0.22	0.09	0.02	251	31.6	11.4	34.7
Soybean	0.07	0.07	0.02	205	23.4	10.6	43.3
Alfalfa	0.19	0.20	0.01	215	32.6	8.0	69.3
Garden pea	0.54	0.20	0.02	235	33.0	14.2	36.5
Harps soil							
Field bean	0.19	0.09	0.03	265	58.6	17.8	21.0
Dolichos	0.15	0.08	0.03	273	27.0	19.2	21.0
Soybean	0.09	0.04	0.02	255	23.0	10.6	34.7
Alfalfa	0.26	0.16	0.02	225	27.2	15.8	30.1
Garden pea	0.37	0.16	0.02	227	26.8	14.4	33.0

[a] k was calculated from net cumulative N mineralized during 16 weeks of incubation at 30°C by using the exponential equation, $N_m = N_0(1–\exp[–kt])$, where N_m is organic N mineralized at time t; k_1 and k_2 were calculated from graphs prepared by plotting organic N remaining after each incubation time *vs.* time; N_0 is mineralizable organic-N pool.

weeks, depending on the legume residue and soil used. During phase I, a large fraction of organic N of the legume residues was mineralized. The values ranged from 23% of N in soybean residue to 58.6% of N in field bean residue added to Harps soil. A relatively small fraction of organic N was mineralized from the legume N added in phase II during the 16 weeks of incubation.

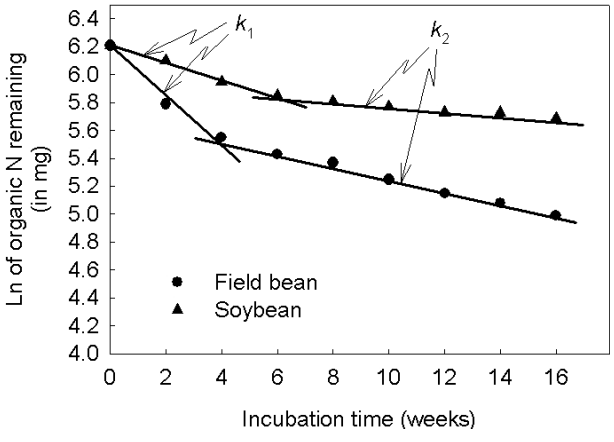

Figure 3: Natural log of organic N remaining in Canisteo soil treated with field bean or soybean residue as a function of incubation time.

The half-lives of organic N associated with phase II varied considerably, depending on the soil and legume residue used. They ranged from 13.9 weeks for dolichos to 99 weeks for alfalfa in the Storden sandy soil (Tab. 6), indicating the difference in potential efficiency of the legume residues in providing mineral N to subsequent crops in rotation systems.

3.4 Effect of legume and soil properties on net N mineralization

Linear regression analyses of the net N-mineralization data obtained for the individual legume residues and the legume or soil chemical properties were conducted. The results showed that, with the exception of the results reported in Figs. 4–7, none of the relationships were significant at $p < 0.05$. Therefore, the results of those findings are not reported here, but the regressions that showed significant correlations at $p < 0.05$ or less showed that net legume-N mineralization in Canisteo soil was significantly, but negatively correlated

(r = –0.99, p < 0.01) with total polyphenols of the residues (the results obtained for dolichos residue deviated from this relationship) (Fig. 4A), and in Storden soil, it was significantly correlated (r = 0.97, p < 0.01) with the polyphenols-to-N ratio of the residues (Fig. 4B). Other analyses showed that the net N of dolichos and field bean residues mineralized in the five soils in 16 weeks were significantly, but negatively correlated with clay content (r = –0.99, p < 0.001) and sand content (r = –0.94, p < 0.05); the results obtained with Clarion and Harps soils deviating from those relationships), respectively (Fig. 5). Net N mineralization in field beans and alfalfa was significantly correlated with the C_{mic} in soils, with r values of 0.996, p < 0.01 and 0.94, p < 0.05, respectively (Fig. 6). The results of the other legume residues showed similar trends, but were not significantly correlated at p < 0.05. Only net N-mineralization values of dolichos were significantly, but negatively correlated with total N and with organic N in soils, with r values of 0.91, p < 0.05 and 0.91, p < 0.05, respectively (Fig. 7). The results of the regression analyses showed that the individual legume or soil properties affect net N mineralization of the residues differently, and no general conclusions could be drawn on which of the properties could be used as an index for predicting N mineralization of any specific legume plant.

In addition to the regression analyses reported above for the net legume-N mineralization and the individual legume or soil properties, we performed statistical analyses for the pooled data. Linear regression analyses of the pooled N–mineralization data showed that net cumulative amounts of N mineralized and percentage N mineralized were significantly correlated with C : N ratio of the legume crops (r = 0.44 and 0.48 at p < 0.05, respectively), and that net cumulative N mineralized was significantly correlated with (lignin + polyphenols)-to-N ratios (r = 0.41 at p < 0.05). This finding contradicts the studies by *Handayanto* et al. (1994) on the release of mineral N from pruning of legume hedgerow trees in relation to quality of the prunings and incubation method showing that, as expected, when the residues were added at constant rate [0.5 g kg^{-1} of soil-sand mixture (1:1), even though the N contents varied significantly], the N release followed the order of N concentration of the legumes. They concluded that the (lignin + polyphenol)-to-N ratio was significantly, but negatively correlated with the N release and that this ratio was consistently among the best-quality description to predict weight losses and N release from prunings in litterbag and leaching-tube experiments but not in a pot experiment. They attributed the lack of good correlation with quality factors of the pot experiment to be due to the presence of soluble polyphenols with a greater capacity to bind protein under nonleaching conditions. Other work by *Mafongoya* et al. (1998) on N mineralization from decomposing leaves of multipurpose trees as affected by their chemical composition showed that the (lignin + polyphenols)-to-N ratio was negatively correlated (r = –0.75 at p < 0.05)

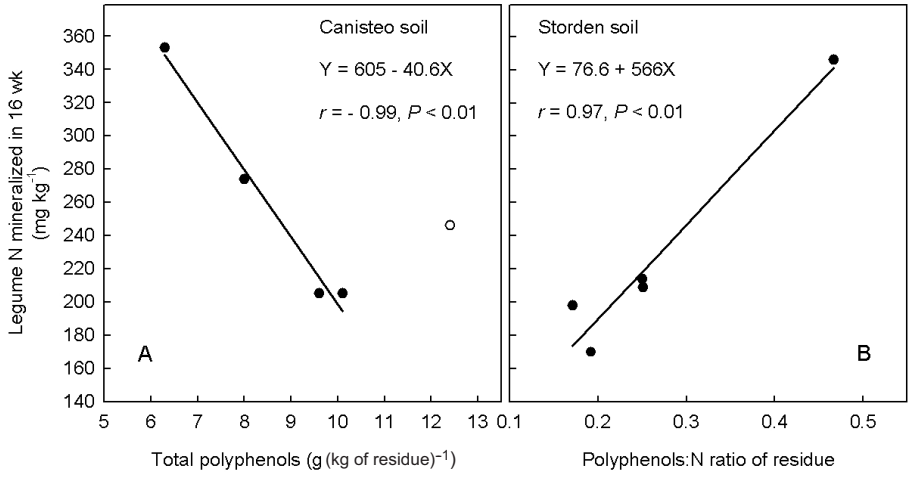

Figure 4: Relationship between net cumulative N mineralized in 16 weeks in Canisteo soil and total polyphenols in legume residue, the value of dolichos (open symbol) deviated from this relationship (A); and those mineralized in Storden soil and polyphenols-to-N ratio of the legume residue (B).

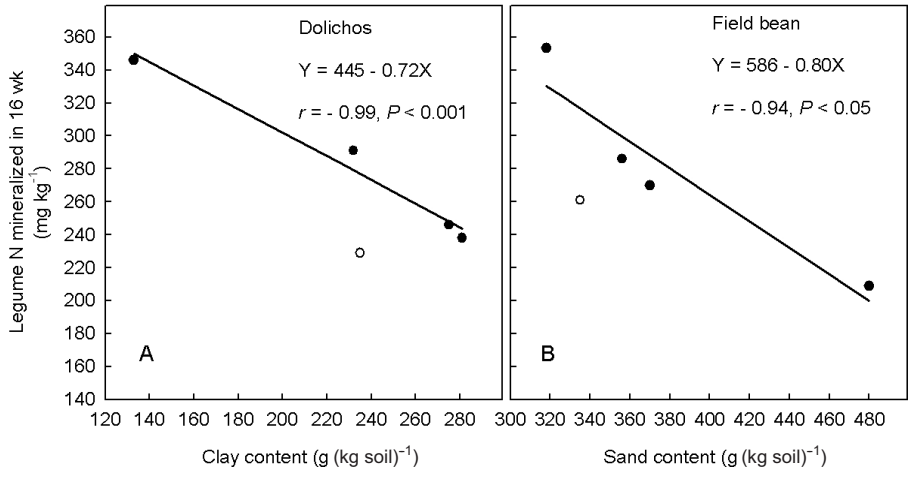

Figure 5: Relationship between net cumulative N mineralized from dolichos residue in 16 weeks and clay contents, the value of Clarion soil (open symbol) deviated from this relationship (A); and those mineralized from field bean and sand content, the value of Harps soil (open symbol) deviated from this relationship (B).

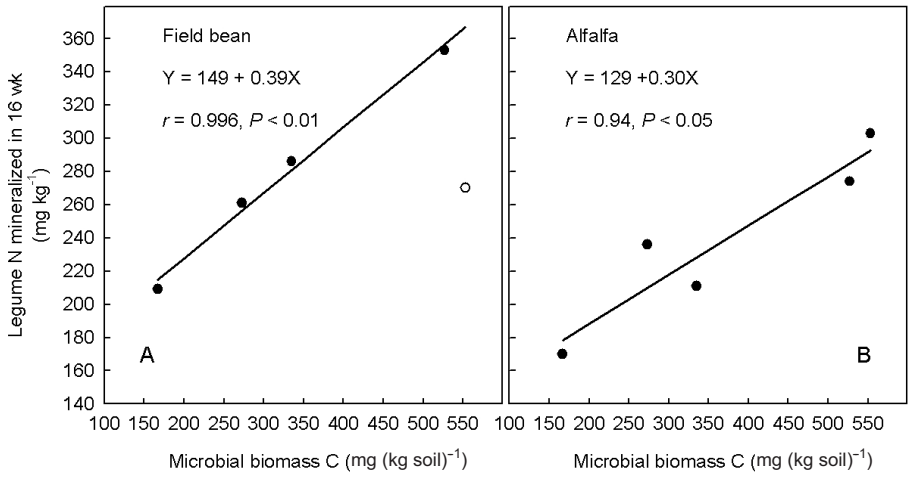

Figure 6: Relationship between net cumulative N mineralized from field bean in 16 weeks and microbial biomass C, the value of Webster soil (open symbol) deviated from this relationship (A); and those mineralized from alfalfa and microbial biomass C (C_{mic}) (B).

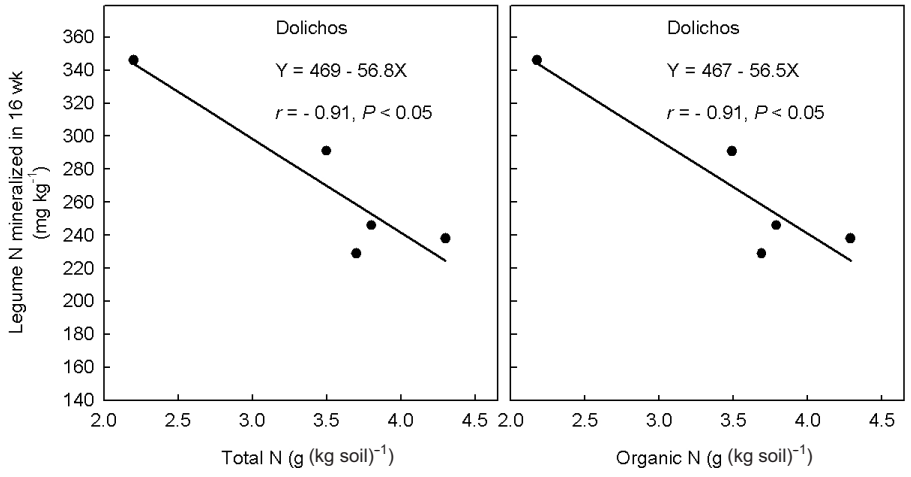

Figure 7: Relationship between net cumulative N mineralized from dolichos residue in 16 weeks and total N in soils (A); and organic N in soils (B).

with N release and that this ratio could be used to screen leguminous-tree leaves for their potential to release N in short-term experiments. They also reported that total phenol content of tree leaves was not a useful predictor of N release, but the reactivity of polyphenols as measured by their protein-binding capacity can be a useful predictor. Low–molecular weight phenolics occur universally in higher plants, some of which are common in a variety of plant species and others are species-specific. We did not test the protein-binding capacity of the polyphenols extracted from the legumes studied, because according to *Hattenschwiler* and *Vitousek* (2000) higher–molecular weight proanthocyanidins (condensed tannin) are most abundant polyphenols in woody plant, but are usually absent in herbaceous plants, such as the ones used in this work. Cumulative N mineralized was significantly correlated with lignin contents ($r = 0.61$, $p < 0.001$).

4 Conclusions

Net N mineralization of five legume residues in five soils conformed to an exponential equation, indicating that the N_o and k values of N added at a constant rate differ among the residues and soils used. Application of a two-components exponential model showed two phases (two pools) for N mineralization; a phase with rapid rates ranging from 2 to 8 weeks and a phase with slow rates. The percentage of organic N in the legume residues in each phase varied among the residues and soils. The total amounts of amino acids varied markedly among the residues. Expressed as percentages of crude protein, the total amounts of 22 amino acids ranged from 73.6% to 92.5%. The total N in the amino acids was almost constant (ranged from 13.7% to 14.4%, mean 13.9%, of the amino acids). Net N mineralization of the individual legume residues added to the five soils was significantly correlated with total polyphenols and polyphenol-to-N ratios for Canisteo and Storden soils, respectively, but not for the other three soils. The negative relationship between N mineralization of dolichos and field bean with clay and sand contents, respectively; of field bean and alfalfa with microbial biomass C; and negative relationships for dolichos with total N and organic N in soils clearly indicate that N mineralization is affected by legume species. Statistical analysis of the pooled data showed that net cumulative amounts of N mineralized in 16 weeks and percentage N mineralized were significantly correlated with C : N ratio of the legume residues and with (lignin + polyphenols)-to-N ratios. The results support the view that variability of the individual organic constituents of legume residues governs the difference in potential efficiency of green manure in providing mineral N to subsequent crop in rotation systems.

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