PN-ABK-215

Soil Biol. Biochem. Vol. 23, No. 1, pp. 83–88, 1991
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0038-0717/91 $3.00 + 0.00
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NITROGEN RELEASE FROM THE LEAVES OF SOME TROPICAL LEGUMES AS AFFECTED BY THEIR LIGNIN AND POLYPHENOLIC CONTENTS

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(*Accepted* 30 *June* 1990)

Summary—Leguminous plant materials used as mulches, green manures and cover crops are generally assumed to provide a readily-available source of N to crops. However, little is known about the chemical composition and N release patterns of the variety of legumes being used in tropical agroecosystems. N release patterns from the leaflets of 10 tropical legumes and rice straw were determined in a laboratory experiment. Ground leaf material was allowed to decompose in an acid soil (pH 4.5) for 8 weeks and the soil was analyzed periodically for extractable NH_4^+-N and NO_3^--N. N release in the soil plus plant material were compared to that of the soil without plant material added and related to the N, lignin and polyphenolic concentrations of the leaflets. Three patterns of net N mineralization emerged during the 8-weeks. One pattern exhibited by the control soil, rice straw and leaves of two of the leguminous plants was a low, positive net mineralization. Another pattern showed much higher rates of mineralization than the control soil and the third pattern showed initial net immobilization followed by low but positive net mineralization rates. The amount of N mineralized during the 8 weeks as compared to the control soil ranged from +46 to −20% of the N added in plant material. Net mineralization was not correlated to % N or % lignin in the leaf material but was found to be negatively correlated to the polyphenolic concentration, $r = -0.63$, or the polyphenolic-to-N ratio, $r = -0.75$. Mineralization in excess of the control soil was found only for materials with a polyphenolic-to-N ratio <0.5. Mechanisms to explain the low mineralization by materials high in polyphenolics include the formation of stable polymers between polyphenolics and amino groups, and nitrosation, a chemical reaction of nitrite (NO_2^-) with polyphenolics. Our results show that leguminous plant material with a high polyphenolic content or polyphenolic-to-N ratio may not be a readily-available source of N.

INTRODUCTION

The use of leguminous plants as a source of N for crops is particularly important in many parts of the humid tropics where N fertilizers are not often economically feasible due to poor market and infrastructure development. In areas where use of N fertilizers is possible, large losses of applied N due to leaching from heavy and frequent rainstorms result in reduced yield and economic return. Leguminous plant material used as mulches, green manures and cover crops provides an economical source of N (Broughton, 1977; Kang *et al.*, 1981; Wade and Sanchez, 1983). The efficiency of N use might also be improved by selecting and mixing plant materials that decompose and release N in synchrony with plant demand (Vallis and Jones, 1973; Swift, 1985).

The importance of the quality of organic additions to N dynamics in natural and managed ecosystems has long been recognized (Harmsen and van Schreven, 1955; Swift *et al.*, 1979; Melillo *et al.*, 1982; Vitousek and Matson, 1985). There is general consensus that net N mineralization occurs if the N concentration is above 2% and immobilization occurs below that concentration. It has also been shown that it is the form of N (Iritani and Arnold, 1960; Berg and Staaf, 1981; Sivapalan *et al.*, 1985) and C (Tenney and Waksman, 1929) and the C-to-N ratio present in the material that is important. The % lignin or lignin-to-N ratio is often an effective index for N release patterns (Melillo *et al.*, 1982; Melillo and Aber, 1984).

With high N content, leguminous plant material should release N quickly. Work by Crowther and Mirchandani (1931), Weeraratna (1979) and Ladd *et al.* (1981) confirm this; however, Vallis and Jones (1973) found this did not hold for *Desmodium intortum* and suggested that polyphenolic content may affect N release patterns. In general, polyphenolics are reactive compounds and can form stable polymers with many forms of N (Martin and Haider, 1980; Stevenson, 1986).

The tropics house a diversity of legumes, from ground covers to trees, that could be used as green manures or mulch. Little is known about the secondary compounds of tropical legumes but it is quite possible that those growing on acid soils have high polyphenolic contents. N dynamics could therefore be affected if polyphenolics do, in fact, alter N release. Criteria that predict the release of N from leguminous plant material based on their chemical composition would facilitate the selection and management of such materials.

Our purpose was to determine the range of N, lignin and polyphenolic concentrations in the leaves of several tropical legumes and to determine the relationship between these factors and N release. A laboratory experiment using the leaflets of tropical legumes commonly used in agricultural production systems was designed to address these objectives.

MATERIALS AND METHODS

Analysis of leaf material

Plant material was analyzed for % N, % lignin and % soluble polyphenolics. Total N was determined by microKjeldahl, lignin by the acid detergent fiber method (Van Soest and Wine, 1968), and soluble phenolics by a revised Folin–Denis method (King and Heath, 1967). Polyphenolics were extracted from the leaves in 50% aqueous methanol for 1 h in a water bath (80°C) before the extract was analyzed. Total soluble polyphenolics are expressed as % tannic acid equivalents.

Laboratory experiment

Leaflets from ten leguminous trees and shrubs and mature rice straw (*Oryza sativa*) were used as treatments in the laboratory incubation (Table 1). The plant materials were collected from agroforestry trials at the Yurimaguas Experiment Station in the Peruvian Amazon. All plants, except *Leuceana leucocephala* (Lamb) de Witt, were growing on acid, upland soils (Typic Paleudults, pH 4.5); *L. leucocephala* was growing on a more fertile alluvial soil (Tropofluvent, pH 6.0). The acid, upland soils are representative of *ca* 30% of the soils found in the lowland humid tropics (Sanchez, 1989). All of the species are used in agroforestry systems in the humid tropics, four species are native to South or Central America and the others are exotics (Table 1). A mixture of young and mature leaves were hand picked from several trees of each species, only leaflets were used in the experiment to avoid confounding N dynamics by including lignified rachises. The fresh leaflets were air dried and then ground (< 1 mm).

Soil used for the experiment was taken from the top 10 cm of the Typic Paleudult. The soil had a loamy sand texture, 1% organic matter content, pH value of 4.5, and effective cation exchange capacity of 3.3 cmol dm^{-3}. Fresh soil was sieved (< 2 mm) to remove roots and organic debris. Extractable NH_4^+-N and NO_3^--N at the beginning of the incubation were 4.6 and 15.9 μg N g^{-1} soil, respectively.

For each treatment, 88 mg of ground leaves were mixed with fresh soil equivalent to 500 g oven dry weight, to approximate a mulch rate of 3.3 t dried plant material ha^{-1}. Deionized water was added to attain a moisture content of 110 mg g^{-1}, field capacity for the soil being *ca* 150 mg g^{-1}. Soil without added plant material served as a control and soil with mature rice straw as a non-leguminous comparison. The soil and plant mixtures were sealed in plastic bags and kept in the dark at room temperature of *ca* 26°C. Each treatment was replicated three times.

Subsamples (10 g) were taken from each bag and analyzed for exchangeable NH_4^+-N and NO_3^--N immediately after addition of plant material and then again at 1, 2, 3, 4 and 8 weeks. Soil was extracted by shaking for 30 min in 30 ml of 0.5 M K_2SO_4, centrifuged, and aliquots analyzed for NH_4^+ and NO_3^-. Ammonium was analyzed by the indophenol blue method (Dorich and Nelson, 1982) and nitrate by the method of Cataldo *et al.* (1975). Net mineralization was considered the difference in exchangeable NH_4^+ and NO_3^- between two samplings.

Table 1. Characteristics of organic additions, changes in ($NH_4^+ + NO_3^-$)-N, 8-week cumulative mineral N and percent of N mineralized

Treatment	N	Lignin (%)	Phenolic	($NH_4^+ + NO_3^-$)-N Week[a] (μg N g^{-1} soil) 1	2	3	4	8	8-Week Total	% Of added N as NH_4^+ and NO_3^-
Control				0.47	2.93	8.70	4.17	7.74	24.01	
Gliricidia sepium	3.74	7.8	1.02	12.12*	15.64*	9.30	3.72	13.23	54.00a*	45.57
Erythrina sp.	3.52	9.7	1.04	8.67*	10.86*	11.58	2.06	15.10*	48.25b*	39.53
Albizia saman	3.20	6.3	1.48	1.84	9.05*	10.86	0.70	18.23*	40.68c*	29.60
Inga edulis	3.18	16.3	3.43	1.47	0.92	6.07	4.27	8.89	21.52ef	−4.27
O. sativa	1.13	6.2	0.69	0.33	3.04	6.75	0.85	12.33	23.31de	−3.50
Inga sp.	2.97	17.9	1.61	−2.17	3.50	6.32	2.55	12.01	22.21e	−3.44
Leucaena leucocephala	3.94	5.2	2.94	−4.59	4.79	7.54	9.22	8.23	25.19d	1.71
Cassia reticulata	2.50	10.8	2.20	−9.44*	6.85	8.64	−0.58	18.06*	23.52de	−1.11
Cajanus cajan[b]	3.48	10.2	3.34	−5.38*	4.71	4.57	4.18	10.67	18.74fg*	−8.60
Desmodium gyroides[b]	2.99	11.7	1.91	−4.40*	4.75	3.22*	6.59	7.38	17.53g*	−12.31
Desmodium ovalifolium[b]	2.37	9.1	3.61	−7.00*	4.13	3.01*	3.78	11.99	15.91g*	−19.41

[a]Week 1 indicates change in mineral N during the first week. Week 2 is for the period between week 1 and week 2, etc.
[b]Exotics to Central and South America.
Values within each column followed by * are significantly different from the control, Dunnet's LSD, $P = 0.05$. Values followed by the same letter are not different, LSD $P = 0.05$.

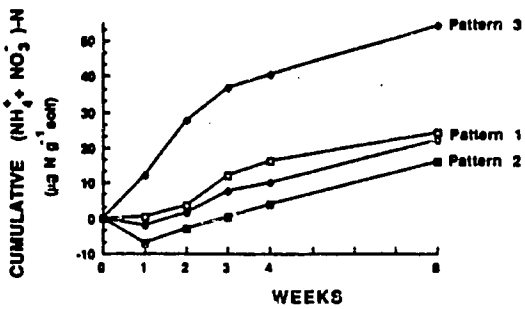

Fig. 1. Examples of the three N release patterns shown by the plant materials as compared to the control (□), soil with no amendment: *Inga* sp. (◇) showed no difference from the control throughout the incubation, *Desmodium ovalifolium* (■) showed net immobilization during the first week followed by low but positive net mineralization, and *G. sepium* (◆) showed immediate net mineralization higher than the control soil.

RESULTS

Characteristics of plant material

N concentrations for the legumes ranged from a high of 3.94% in *L. leucocephala* to a low of 2.37% in *Desmodium ovalifolium* Wall, compared to 1.13% N in rice straw (Table 1). Lignin contents ranged from 17.9 to 5.2% in *Inga* sp. and *L. leucocephala*, respectively. Rice straw was at the lower end of the range. Soluble polyphenolics ranged from 3.61 to 1.02% for the legumes compared to a low value of 0.59% for rice straw. There were no significant correlations among % N, % lignin or % polyphenolics in the leaves.

Patterns of net N mineralization

Net mineralization in the soil without amendment was low throughout the 8 weeks but was always positive (Pattern 1). The plant materials showed three patterns of net N mineralization (Table 1, Fig. 1). Mineralization from *Inga edulis* Mart., *Inga* sp., and rice, never differed significantly from the control at any period during the 8-week incubation. Pattern 2, exhibited by *Cassia reticulata* Willd., *Cajanus cajan* (L.) Millsp., *L. leucocephala*, and the two *Desmodium* species, showed negative net mineralization (immobilization) during the first week, followed by low but positive net mineralization during the remainder of the experiment. In these species net mineralization generally occurred at rates equal to or below that of the control. Pattern 3 is that shown by *Gliricidia sepium* (Jacq.) Walp., *Erythrina* sp., and *Albizia saman* (Jacq.) F.V.M. (syn. *Samanea saman*), in which net mineralization was higher than the control during the first 2 weeks and then again at 4–8 weeks.

Total net materialization after 8-weeks differed significantly from the 24.0 μg N g^{-1} of the control soil in 6 treatments (Table 1): *G. sepium*, *Erythrina* sp. and *A. saman* with higher concentrations and the two *Desmodium* species and *C. cajan* with lower concentrations. *G. sepium* had the highest net mineralization, 54.0 μg N g^{-1} soil, and *D. ovalifolium* the lowest, 15.9 μg N g^{-1} soil.

The net immobilization or mineralization during the first week is reflected in the 8-week cumulative net mineralization. This relationship is shown by a correlation coefficient of 0.81 ($P = 0.0001$) between the amount mineralized in the first week and the net cumulative mineralization for the 8 weeks. The correlation increases to 0.95 ($P = 0.0001$) when the amount mineralized during the first 2 weeks is compared to the 8-week total. The correlation does not improve after that time, suggesting a 1 or 2 week incubation may be long enough to provide information on the relative availability of N from the leaves.

Relation of net mineralization to chemical characteristics of leaves

The amount of N mineralized, as compared to the control, ranged from 46% of the N added in plant material, in the case for *G. sepium*, to −20% for *D. ovalifolium* (Table 1). N mineralized during the first week and after 8 weeks was not significantly correlated to the N concentration of the leaves (Table 2), or to their lignin concentration. The highest correlation for N mineralized was with % polyphenolic content, $r = -0.59$ and $r = -0.63$ for the first week and 8-week totals, respectively. The lignin-to-N ratio was only slightly correlated to the 8-week total while the polyphenolic-to-N ratio was significantly correlated for the two periods, $r = -0.69$ and $r = -0.75$, respectively.

The relationship between N mineralized and polyphenolic-to-N ratio is best described by a log function, $MIN = -21.78 \cdot \ln(PP/N) + 18.77$ ($r^2 = 0.76$), where MIN is the cumulative 8-week mineralization and PP/N is the polyphenolic-to-N ratio (Fig. 2). Net mineralization in excess of the control was found only for materials with a polyphenolic-to-N ratio <0.5. For those materials N mineralization was linearly related to the N content, $r = 0.997$; for the remaining materials the correlation between net mineralization and N content was only 0.03.

Table 2. Correlation coefficients relating cumulative amounts of ($NH_4^+ + NO_3^-$)-N to chemical characteristics of plant material

Cumulative $NH_4^+ + NO_3^-$	% N	% Lignin	% Phenolics	% Lignin / % N	% Phenolic / % N
Week 1	0.33	−0.11	−0.59	−0.28	−0.69
	(0.32)	(0.74)	(0.06)	(0.41)	(0.02)
Week 8	0.42	−0.34	−0.63	−0.53	−0.75
	(0.20)	(0.31)	(0.04)	(0.09)	(0.01)

Numbers in parentheses are the probability level of the correlation.

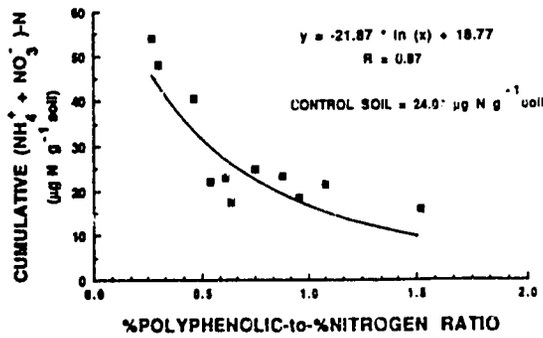

Fig. 2. Relationship between (NH_4^+ + NO_3^-)-N levels at the 8-week incubation period and the polyphenolic-to-N ratio of the plant material.

DISCUSSION

The importance of polyphenolics in decomposition and mineralization processes has been frequently debated (Swift *et al.*, 1979). The issue has been confused by the complexity and diversity of compounds referred to as polyphenolics and by the different methods used to measure them (Swain, 1979; Martin and Martin, 1982; Mole and Waterman, 1987a,b). Extraction procedures are only 30–95% efficient and the compounds extracted include hydrolyzable tannins and condensed tannins, as well as non-tannin polyphenolics (Swain, 1979).

The interaction of polyphenolics with N and the fate of the N depend on the type of polyphenolics present in the plant material. Some polyphenolics form complex structures by H-bonding with basic-N-containing groups, others form stable cross linkages with amino groups making the material resistant to decomposition. In addition, phenolics are readily oxidized to quinones either by autooxidation at neutral pH conditions or by enzymes at a range of pH values. The quinones react with other phenolics, amino acids and amino sugars to form stable polymers (Swain, 1979; Martin and Haider, 1980). The stable polymers formed between phenolics and N-containing compounds have characteristics similar to, and are considered precursors to, the fulvic and humic acids found in soil organic matter (Martin and Haider, 1980; Stevenson, 1982). Another interaction between phenolics and N that may be especially important in acid soils is referred to as nitrosation, a chemical reaction of NO_2^- with phenolics forming organic N compounds. Nelson and Bremner (1969) reported that NO_2^- was fixed by organic matter and led to gaseous losses of N in soils with neutral pH but the formation of stable organic compounds in acid soils. Although NO_2^- concentrations are generally low in soils, NO_2^- is formed during the process of nitrification, and nitrosation could account for the apparent immobilization of inorganic N (Azhar *et al.*, 1986a, b).

Our results indicate that polyphenolic content may play a more important role in influencing mineralization patterns for leguminous leaves than % N or lignin-to-N ratio. This is in contrast to mineralization patterns of non-leguminous plant material (Melillo *et al.*, 1982). The large amount of N immobilized and the extended period of net immobilization observed for material with high % N, such as *L. leucocephala* and *C. cajan* was unexpected, but both had high polyphenolic concentration. Millar *et al.* (1936) reported that net N immobilization by legumes such as alfalfa and clovers was small and the effect persisted for <2 weeks. They attributed the immobilization to an abundance of soluble organic-C relative to N. Vallis and Jones (1973) were among the first to show a significant and prolonged net immobilization of N by a legume. They suggested that the polyphenolics in the leaves bound to proteins forming complexes resistant to decomposition.

Differences in the type and amount of soluble polyphenolics present in the leaves could explain the initial net release or immobilization of N seen in our study. The patterns might also be explained by differences in relative amounts of soluble carbohydrates and soluble N in the leaves. However, the significant correlation between net mineralization and polyphenolic concentration and the lack of correlation with N and lignin demonstrate the primary importance of polyphenolics in determining the mineralization patterns obtained. Pattern 3 exhibited by *G. sepium*, *Erythrina* sp. and *A. saman*, in which there was immediate net mineralization of N, suggests the presence of both microbially-available C and N source in the leaves. These three species also shared low polyphenolic contents. The only other material used with such a low polyphenolic content was rice straw, but it had a low N content and exhibited low net mineralization.

The initial immobilization of N exhibited by *L. leucocephala*, *C. cajan*, *C. reticulata* and the two *Desmodium* species (Pattern 2), all with relatively high N and high polyphenolic but low lignin concentrations, suggests the presence of available C in the leaves that is not mateched by a sufficient amount of available N. Polyphenolics could bind to organic N (e.g., amino acids and proteins) in the leaves making the N unavailable, or could bind to the soluble forms of organic N released from the leaves, forming resistant complexes in the soil. In either case, there would be insufficient available N from the plant material to match the available C and net immobilization of soil N would be expected. Sivapalan *et al.* (1985) found lower net N mineralization from tea leaves that had high soluble N and high polyphenolic content in comparison with leaves with high soluble N content but low polyphenolics, suggesting that the polyphenolics made the soluble N unavailable. Lignin might also bind organic N making it unavailable, but the lack of correlation between mineralization and lignin content and the generally low lignin contents of these materials make this a less probable explanation.

The initial, rapid immobilization could also be explained by nitrosation, the reaction discussed earlier. Soluble polyphenolics that leached from the leaves into the soil could bind to NO_2^- formed during nitrification and produce an apparent immobilization of mineral N. This reaction readily occurs in acid soil conditions. Nitrosation would be implicated if NH_4^+ was formed at rates similar to the control but NO_3^- was found at depleted concentrations. These relationships were not obvious for those plants with high polyphenolic content (Table 3).

Table 3. Cumulative concentrations[a] of extractable NH_4^+-N and NO_3^--N in the soil for the different treatments at 8 weeks

Treatment	NH_4^+-N	NO_3^--N
	(μg N g^{-1} soil)	
Control	6.89 (0.48)	37.64 (1.98)
G. sepium	30.10 (1.08)	44.56 (0.97)
Erythrina sp.	26.50 (1.32)	44.50 (2.26)
A. saman	18.51 (0.48)	43.26 (0.68)
I. edulis	3.97 (0.92)	38.63 (3.17)
O. sativa	4.84 (0.40)	39.20 (0.06)
Inga sp.	3.16 (0.79)	39.22 (0.55)
L. leucocephala	5.58 (0.54)	41.21 (1.49)
C. reticulata	3.70 (0.50)	41.00 (1.97)
Ca. cajan	1.89 (0.24)	37.17 (1.74)
Desmodium gyroides	0.88 (0.51)	37.14 (1.12)
D. ovalifolium	0.80 (0.11)	36.22 (0.46)

[a]Mean (SD) of three replicates.

Pattern 1 in which little net mineralization or immobilization occurred, as for both *Inga* species, could reflect their higher lignin content, and therefore supposedly lower soluble-C, of these materials. The two *Inga* species had 50% higher lignin contents than the other species. Lignin content may influence the long-term N release as is indicated by a weak correlation found for the 8-week cumulative mineralization and the lignin-to-N ratio.

The patterns of N mineralization obtained in this study are complex and involve a variety of factors. The lignin-to-N ratio, which has been shown to control decomposition and mineralization in non-leguminous leaves (Melillo *et al.*, 1982), was not a controlling factor in these leguminous leaflets, at least in the short term. The lignin-to-N ratios of the leguminous leaves used in this study, however, were below that of the plant materials used in the study by Melillo *et al.* (1982). Our study suggests that polyphenolics content could control the short-term N release and availability from legumes. Leguminous plant materials have previously been considered as readily available sources of N. This assumption may be incorrect if the plant material has a high polyphenolic content, or a polyphenolic-to-N ratio >0.5. This type of short-term incubation could be useful for screening legumes. Another experimental study using plant materials, including non-legumes, with a broader range in N, lignin and polyphenolic content would provide additional information on the overall importance of polyphenolics and their interaction with lignins in controlling N release from plant materials. Also, it must be pointed out that only leaflets were used in this study, N release patterns from the entire leaves may be different and should be investigated.

The diverse N release patterns observed from the leaflets of 10 tropical legumes and the possible role of polyphenolics on N availability have several management implications. Legumes with low polyphenolic content used as green manure or mulch may release N rapidly and provide sufficient N for plant growth, but the N may be readily lost by leaching if not released in synchrony with plant demands. Legumes with high polyphenolic content may react with organic N compounds do not provide enough N for plant growth in the short term, but they may contribute to the build-up of organic N in the soil and provide a low but continual supply of N in the long term. A combination of the two types of legumes may provide the best agroecosystem management.

Acknowledgements—We thank A. Salazar for providing the diverse plant material and W. H. Schlesinger and W. Johnson for providing facilities for polyphenolic and lignin analyses, respectively. Comments on earlier drafts by S. Buol, C. B. Davey, G. Hoyt, R. H. Miller, M. Reynolds, W. H. Schlesinger, D. Worsham, and two anonymous reviewers were helpful and much appreciated. This work was part of the North Carolina State University TROPSOILS Program, supported by US Agency for International Development (USAID).

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