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Nitrogen Release Patterns of Mixed *Gliricidia sepium* and *Acacia auriculiformis* Leaves as Influenced by Polyphenol, Lignin and Nitrogen Contents

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Abstract: A field decomposition experiment was conducted for 120 days during the rainy season. Mixed leaf proportions of *Gliricidia sepium* (high quality litter i.e., high N, low lignin, low polyphenol) and *Acacia auriculiformis* (low quality litter i.e., high N, high lignin, high polyphenol) were placed in polyvinyl litter bags at 100 g bag⁻¹. This was to evaluate the sensitivity of the quality of legume litter as predictor of the rate of decomposition and N release. The legume polyphenol concentration was significantly correlated with N release at the early stage of decomposition (8 days) ($R^2 = 0.619$; $p < 0.05^*$) and at 120 days of decomposition ($R^2 = 0.880$; $p < 0.05^*$). The C-to-N ratio of legume was significantly correlated with N release at 64 days ($R^2 = 0.617$; $p < 0.05^*$) and 120 days ($R^2 = 0.6611$; $p < 0.05^*$). The polyphenol-to-N ratio was only significantly correlated with N mineralization after 120 days ($R^2 = 0.881$; $p < 0.05^*$). The polyphenol concentration appeared to be a good chemical indicator for N release during the early stage of decomposition while the polyphenol-to-N ratio appeared to be the best predictor of N mineralization at all stages. Also, N mineralization was only significantly correlated with the initial N concentration ($R^2 = 0.766$; $p < 0.05^*$), polyphenol concentration ($R^2 = 0.643$; $p < 0.05^*$) and polyphenol-to-N ratio ($R^2 = 0.664$; $p < 0.05^*$) of legumes at 16 days of decomposition. In spite of the changes anticipated in the factors controlling decomposition over a prolonged period, the predictive value of the initial composition of legume litter for N release appears largely valid. However the initial N concentration of legume litter was the best chemical index of N released when relying on simple measurements of initial legume constituents.

Key words: Leaf decomposition, nitrogen release, polyphenols, lignin, *Gliricidia sepium*, *Acacia auriculiformis*

INTRODUCTION

Agroforestry systems, based on biomass transfer from perennial legumes (transfer of prunings from tree legumes as mulch for arable crops on another site) have been identified as a potentially important management practice in the tropics (Yamoah *et al.*, 1986; Swarup, 1987). The influence of the quality of litter on its subsequent rate of decomposition and its influence on soil fertility have been recognized since the early stages of agriculture. However, rates of decomposition and nutrient release from legume tree pruning determine the short-term benefits of the prunings to crop N uptake (Handayanto *et al.*, 1997). The mineralization of a specific type of plant residue, given favourable environmental conditions, has been shown to be largely determined by a variety of chemical and physical plant quality values (Heal *et al.*, 1997). The main thrust in this study area has been to find ways of predicting the rate of litter

decomposition and even more importantly, the rate of nutrient release from the chemical components of the resources.

Several plant quality variables and indices have been proposed for predicting decomposition and N release patterns. They include nutrient quality (total N, total P, total C), C quality (lignin, soluble C, soluble polyphenol) and physical quality (specific leaf area, toughness, waxiness) (Palm and Rowland, 1997). A variety of predictive equations have been proposed mainly using various ratios of C, N, lignin and polyphenols to mineralization (Melillo *et al.*, 1982; Fox *et al.*, 1990; Palm and Sanchez, 1991). The C-to-N ratio remains a significantly important feature of such formulae (Giller and Cadish 1997), although a number of lines of research have demonstrated important interactions with other factors. In some studies, the rate of N-mineralization from a material was dependent on the lignin-to-N ratio (Melillo *et al.*, 1989; Becker *et al.*, 1994) or the polyphenol-to-N ratio

(Palm and Sanchez, 1991; Oglesby and Fownes, 1992) or the (lignin+polyphenol)-to-N ratio (Mafongaya *et al.*, 1998). It is believed, that large part of the differences between these various results stems from the different ranges of material used by different researchers. Thus, when one group (Becker *et al.* 1994) used materials with a wide range of lignin and N but a narrow range of polyphenol concentration, lignin-to-N was the best predictor. When another group (Palm and Sanchez, 1991) used materials with a wide range of polyphenol and N-concentration but narrow range of lignin, then polyphenol-to-N was the best predictor. There is therefore the need for further studies in which a wide range of all the three components is involved. This could be achieved by manipulating litter quality through mixing prunings of high quality litter (e.g., *Gliricidia*) and low quality litter (e.g., *Acacia*) in varying proportions. The use of few species with closely related properties (Jama and Nair, 1996) and the use of initial litter quality (Oglesby and Fownes, 1992) as index of decomposition and N release limits the ability of some studies to establish the relative importance of variables that regulate the rates of decomposition during different phases. The consequence of changes in the composition of a litter during decomposition is that correlation between the rate of mass or nutrient loss and simple expressions of the initial composition of the litter will have limitations. Indices of decomposition based on initial litter quality provide a rough guide of decomposition pattern. This is because of changes in the factors controlling decomposition over prolonged periods and the progressive effects of extrinsic factors. Consequently, the limitation in the predictive value of simple measures of initial composition must be recognized.

The objectives of the present study were: (1) to evaluate the sensitivity of the quality of legume litter as a predictor of the rate of decomposition and N release after placement in the field in litter bags and (2) to compare the effects of the initial polyphenol, lignin and N concentrations of legumes and the changes in chemical constituents of litter during decomposition on nutrient release.

MATERIALS AND METHODS

Site description: The field site for the experiment is a fallow forest of *Tectona grandis*. The study was conducted from June to August 2000 at the Federal University of Technology, Akure (lat. 7° 17'N, long. 5° 10'E). During this period, the mean temperature was 24.5°C; mean relative humidity (84%) and mean rainfall (76 mm). The soil is classified as ferruginous tropical soil (Alfisol) on crystalline rock of basement complex. The soil

belongs to the Egbeda series (Smyth and Montgomery, 1962). They are derived from fine grained biotite genesis and schist parent materials. The soil profile shows some medium to light textured materials near the surface, followed by sandy clay subsoil and a layer of sub angular quartz below.

Decomposition study: Green leaves of high quality litter (i.e., high N, low lignin, low polyphenol) of *Gliricidia sepium* were mixed with low quality litter (i.e., high N, high lignin, high polyphenol) of *Acacia auriculiformis* with varying proportions of *Gliricidia* and *Acacia*, i.e., 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100. Samples of each proportion were sealed in polyvinyl litterbags measuring 10×20 cm with 7 mm mesh at 100 g bag⁻¹ and placed randomly in the field under fallow forest of *Tectona grandis* in a completely randomized design at three replicates per treatment. After 4, 8, 16, 32, 64, 96 and 120 days, three bags of each treatment were retrieved from the experimental field by carefully lifting them to avoid losses of particulate materials. The bags were brushed to remove soil debris while the litter remaining in the bag was sieved through 200 mm mesh to separate soil particles from the litter.

Determination of litter quality values: The litter samples retrieved at each sampling were dried at 60°C for 72 h, ground and analysed for C, N, lignin and polyphenol contents. Ash free dry weight of litter was obtained following combustion in a furnace at 550°C for 3 h (Cochran, 1991). Total C was determined from its ash free dry weight (TSBF, 1993) and total N was determined by Kjeldahl method (AOAC, 1990). The lignin content was determined from acid-free detergent fibre described by Williams *et al.* (1986). After initial depigmentation with diethyl ether containing 1% acetic acid, the polyphenol content of the litter was extracted in 70% aqueous acetone. The total polyphenol (as tannic acid equivalent) in the extract was thereafter determined using Folin Ciocalteu reagent as described by Makkar and Goodchild (1996). Initial properties of the mixed legume leaves used are shown in Table 1

Estimation of rates of decomposition and N release: At each sampling, the materials recovered from litter bags were air dried and weighed. Sub-samples were then taken for oven dry weight correction. The litter decomposition over time was determined as percent of litter remaining while the N release was determined as dry weight N concentration in the litter. The rate constants of decomposition (kD) and N-mineralized (kN) were

estimated using a single exponent equation $Y = Y_0 \cdot \exp(-kt)$, where Y = the percentage of remaining weight of constituents (drymatter or N) at time t . Correlation analyses were also carried out (Steel and Torrie, 1980) to establish the relationship between litter quality parameters (C, lignin, polyphenol) and N-mineralization.

RESULTS

Leaf chemistry: Total C, total N, lignin and polyphenol contents in the legume leaf mixture ranged from 47.2, 4.8, 9.4 and 2.3% respectively in pure *Gliricidia* to 49.6, 3.9, 19.6 and 7.7% respectively in pure *Acacia*. The N concentration of legume leaf mixtures (Table 1) decreased with increased proportion of *Acacia* to *Gliricidia*, whereas, the concentration of lignin, polyphenol-to-N ratio and lignin-to-N ratio increased.

Pattern of mixed litter decomposition and N-mineralization: All mixed leaf proportions had an initial phase of rapid decomposition (0-4 days) followed by a

second phase of comparatively lower decomposition rate (4-120 days) (Fig. 1). The decomposition rate constant (k) days^{-1} was highest in 100:0 (pure *Gliricidia*) proportion (-0.1376). The rate of decomposition of legumes leaves mixtures decreased with increased proportion of *Acacia* (Fig. 1). At 96 days, the percent decomposition were 99.5, 88.1, 89.2, 84.2, 70.8, 70.1 and 73.3% for (*Gliricidia*: *Acacia* mixture) 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100, respectively. After 96 days, pure *Gliricidia* had decomposed completely. The percent of original N-mineralized at 96 days ranged from 25.7% for the 50:50 proportion to 53.5% for the 100:0 (pure *Gliricidia*) proportion and the shapes of the N release curves differed among proportions of *Gliricidia* : *Acacia* mixture (Fig. 2). With the exception of 0:100 (pure *Acacia*) proportions, all mixtures followed an initial rapid N-release (0-4 days) followed by N immobilization between 4 and 8 days but the 50:50 proportions immobilized N up to day 16.

The mineralization rate constant (k) days^{-1} was highest for the 100:0 (pure *Gliricidia*) proportion (0.2085) and lower for the 50:50 proportion (0.0131). In between

Table 1: Chemical characteristic of mixed legume leaves used for the experiment (means and standard errors) (n = 3)

Mixed legume used <i>Gliricidia</i> : <i>Acacia</i> (%)	Total N	Lignin	Polyphenol (TAE)	Total Carbon (%)	C/N	L/N	PP/N	(L+PP)/N
100:0	4.8±1.0	9.4±0.0	2.3±0.3	47.2±0.2	9.8	1.9	0.4	2.4
80:20	4.6±0.4	9.8±1.1	2.6±0.4	46.9±1.1	10.1	2.1	0.5	2.6
60:40	4.4±0.0	10.2±0.1	3.2±0.0	48.4±1.0	11.0	2.5	0.7	3.0
50:50	4.3±0.8	12.6±0.2	3.6±1.1	46.8±0.8	10.8	2.9	0.8	3.7
40:60	4.2±0.1	15.2±0.2	4.8±0.5	48.7±0.0	11.5	3.6	1.1	4.7
20:80	4.0±0.2	17.8±0.8	5.1±0.0	47.9±0.4	11.9	4.4	1.2	5.7
0:100	3.9±0.3	19.6±0.4	7.7±0.8	49.6±0.1	12.7	5.0	1.9	7.0

TAE = Tannic Acid Equivalent; PP = Polyphenol; L+PP = Lignin + Polyphenol; L = Lignin; C = Carbon; N = Nitrogen; n = Sample size

Table 2: Coefficients of determination (R^2) for linear regressions between decomposing legume leaf constituents and (%) N remaining in the litter at each stage of decomposition

Time (days)	Chemical components					
	PP	Lignin	Lignin/N ratio	PP/N ratio	C/N ratio	(Lignin+PP)/N ratio
4	0.157	0.068	0.0157	0.027	0.0001	0.010
8	0.619*	0.010	0.066	0.568	0.004	0.059
16	0.041	0.223	0.070	0.001	0.453	0.069
32	0.043	0.297	0.015	0.012	0.244	0.015
64	0.0006	0.001	0.046	0.0007	0.617*	0.047
96	0.048	0.182	0.011	0.070	0.156	0.007
120	0.880*	0.524	0.554	0.881*	0.61*	0.56

C = Carbon; PP = Polyphenol; * = Significant at 0.05 level; N = Nitrogen

Table 3: Coefficients of determination (R^2) for linear regression of (%) N remaining in decomposing legume litter at each stage of decomposition versus initial green leaves properties

Time (days)	Legend green leaf constituents					
	N	Lignin	Polyphenol	Lignin/N ratio	PP/N ratio	(Lignin+PP)/N ratio
4	0.02	0.071	0.032	0.04	0.034	0.020
8	0.144	0.26	0.09	0.231	0.085	0.211
16	0.766*	0.409	0.643*	0.510	0.664*	0.0546
32	0.535	0.260	0.510	0.340	0.521	0.374
64	0.468	0.365	0.410	0.397	0.416	0.412
96	0.490	0.443	0.180	0.430	0.186	0.398
120	0.006	0.001	0.033	0.001	0.027	0.001

PP = Polyphenol; * = Significant at 0.05 level; N = Nitrogen

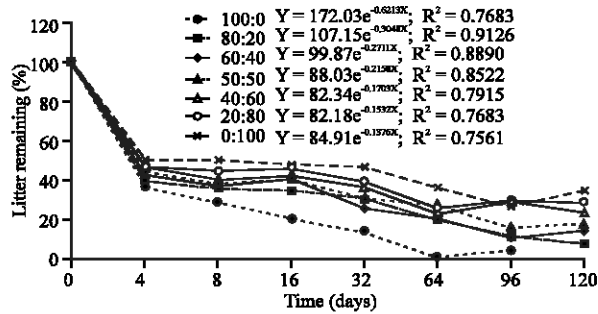


Fig. 1: Decomposition patterns of leaf mixtures of *Gliricidia sepium: Acacia auriculiformis* (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100) under humid tropical conditions ($p < 0.05$)

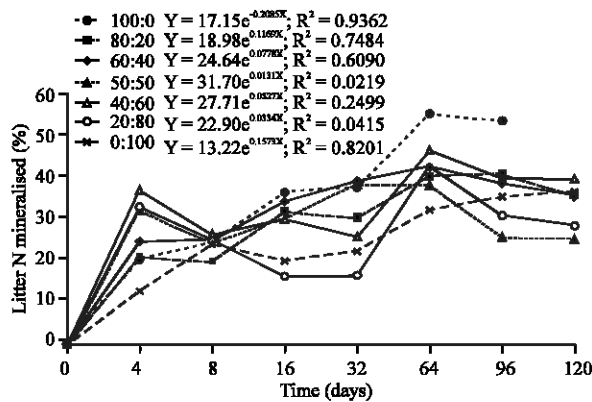


Fig. 2: Percent of original N mineralized from leaf mixtures of *Gliricidia sepium: Acacia auriculiformis* (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100) under humid tropical condition. (Equations were derived from data from day 4-120)

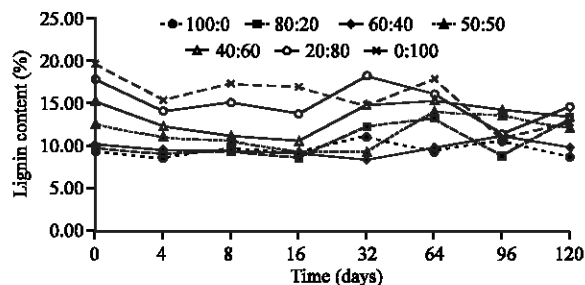


Fig. 3: Pattern of changes in lignin content of leaf mixtures of *Gliricidia sepium: Acacia auriculiformis* (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100) under humid tropical condition

these two extremes, the N release constants varied with varying proportions of *Gliricidia:Acacia* mixtures with no definite pattern.

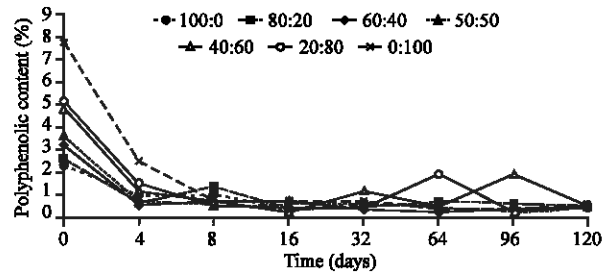


Fig. 4: Changes in polyphenolic content of leaf mixtures of *Gliricidia sepium: Acacia auriculiformis* (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100) during decomposition under humid tropical conditions

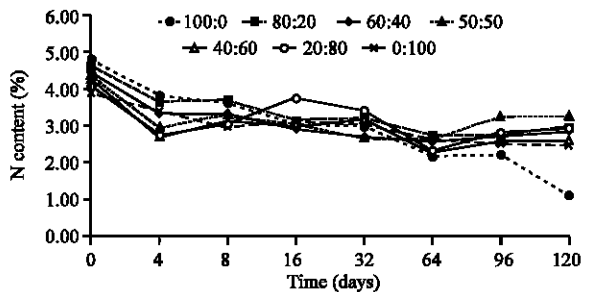


Fig. 5: Pattern of changes in nitrogen content of leaf mixtures of *Gliricidia sepium: Acacia auriculiformis* (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100) during decomposition under humid tropical conditions

Relationship between legume constituents and N-mineralization:

The pattern of changes in lignin, polyphenol and N contents of *Gliricidia: Acacia* leaf mixtures during decomposition is shown in (Fig. 3-5), respectively. The lignin and the N contents of the mixed *Gliricidia* and *Acacia* litter during decomposition from 0 to days 120 followed an undefined pattern of increases and decreases while that of polyphenol content showed a rather rapid reduction in polyphenol content (Fig. 4) between 0 and 4 days of decomposition before assuming the undefined pattern as observed for lignin and N content (Fig. 3-5). Legume polyphenol concentration (Table 2) was significantly correlated with N-mineralization at the early stage of decomposition (8 days) and at 120 days of decomposition. The C-to-N ratio of the legume leaves was significantly correlated with N release at 64 days and at 120 days. The polyphenol-to-N ratio was only significantly correlated with N-mineralization after 120 days. Legume lignin, lignin-to-N ratio and (lignin+polyphenol)-to-N ratio were each not significantly

correlated with N-mineralization at any time during the decomposition. Similarly N-mineralization was only significantly correlated (Table 3) with initial N concentration and polyphenol-to-N ratio of legumes at 16 days of decomposition.

DISCUSSION

Litter quality rating of legume leaves: The data on the properties of the mixed green legume rated *G. sepium* as a high quality legume while *A. auriculiformis* was shown to be low quality legume with respect to the chemical constituents that regulate decomposition and N-mineralization of their respective litters. For example, in *Gliricidia* the N (4.8%) polyphenol (2.3%) and lignin (9.4%) and for *Acacia* the N (3.9%) polyphenol (7.7%) and lignin (19.6%) contents in their fresh leaves and their subsequent rate of decomposition and N-mineralization compared favourably with the reports of Fox *et al.* (1990), Oglesby and Fownes (1992) and Handayanto *et al.* (1997) for high and low quality litter, respectively. Similarly, the decrease in N concentration with increased proportions of *Acacia* in the *Gliricidia:Acacia* leaf mixtures used for our experiment which resulted in increased lignin and polyphenol concentrations was similar to that reported by Handayanto *et al.* (1997).

Rate of litter decomposition and N-mineralization: The rate of decomposition and N-mineralization (96 days) of high quality litter such as *Gliricidia* (99.5, 53.5%), respectively, was higher than low quality litter like *Acacia* (70, 35.3%), respectively. These rates compared favourably with those reported by Oglesby and Fownes (1992). The decreased rate of decomposition and subsequent N-mineralization with an increased proportion of *Acacia* in the *Gliricidia:Acacia* leaf mixtures found in the present study confirmed the findings of Handayanto *et al.* (1997) who reported a similar effect on N-mineralization when prunings of *Peltophorum dasyrrachis* (low quality litter) was mixed with *Gliricidia sepium* (high quality litter). The altered litter quality slowed down the decomposition and N-mineralization rate of the high quality litter (*Gliricidia*). These findings suggest that the decomposability of litters is, in part, a function of their chemical composition. The decreased N-mineralization with increased proportion of *Acacia* leaves in *Gliricidia: Acacia* mixtures, indicated that N-mineralization of leaf prunings can be manipulated by mixing materials of different quality although this may be at the expense of reducing the total amount of N released at least in the short term. By mixing with slow N release legume leaves like *Acacia*, losses of N-mineralized

from the *Gliricidia* leaves through leaching may be minimized while *Acacia* leaves can contribute to the maintenance of soil organic matter and hence increase the long-term residual benefits of the leaf prunings.

Contrary to the reports of Oglesby and Fownes (1992) and Myers *et al.* (1997), a rapid reduction in % N-mineralized was observed between 4 and 8 days of decomposition for all the treatments (leaf mixtures) particularly, for pure *Gliricidia* but not with the pure *Acacia*. This period of N-immobilization coincided with a period of very high bacterial population in the decomposing litter of *Gliricidia* than *Acacia* (Oyun, unpublsh.). As the readily-degradable forms of C are consumed, the population requires nutrients to grow. These nutrients are available in the decomposing litter and when these nutrients, particularly N, is assimilated by the microbial biomass, it could lead to a higher N content in the litter since the microbes are still attached to the litter. This probably explained the decline in the estimated percent N mineralized between 4 and 8 days of decomposition.

Correlation between litter quality indices and N-mineralization: N mineralization was most significantly correlated with polyphenol concentration after 8 days of decomposition and after 120 days of decomposition was most significantly correlated with the polyphenol-to-N ratio. Based on these observations, the polyphenol concentration would appear a good index of N release at the early stages of decomposition while the polyphenol-to-N ratio appeared to be the best predictor of N-mineralization of decomposing litter with wide range of polyphenol and lignin contents. These findings agree with those of Palm and Sanchez (1991) and Oglesby and Fownes (1992) on N mineralization from tropical legumes but contradict the observation of Melillo *et al.* (1989) who suggested that the lignin-to-N ratio predicts the early stage of decomposition; and that the lignin content alone, or the lignocellulose index, predicts the longer-term decomposition rate.

Pattern of changes in lignin, polyphenol and N content during litter decomposition: Figure 3 indicates the lignin contents of the mixed decomposing legume litter appeared to vary little from day 0 to 120 days of decomposition. This observation may be attributed to the rigid nature of lignin in plants which prevents its degradation by microorganisms. Only a limited range of organisms are known to produce the enzymes necessary for complete lignin degradation, while others attack only the side chains or cleave certain bonds to expose more easily decomposed cellulose (Paul and Clark, 1989).

Soluble phenolics can serve as a C substrate but many of them, particularly tannins, can also inhibit the growth or function of the decomposer organisms by binding to enzymes, or rendering N unavailable to organisms by chemically binding to proteins (Waterman and Mole, 1994).

Although the pattern of changes in the amounts of polyphenol (Fig. 4) in the decomposing leaves disappeared rapidly in all treatments, including pure *Acacia* 7.7% polyphenol. There was still a corresponding decrease in decomposition and N-mineralization with an increased proportion of *Acacia* in the *Gliricidia:Acacia* leaf mixture. These observations suggest a need to look beyond C quality (lignin, soluble C, soluble phenolics) as the only important intrinsic factor that influences litter decomposition and subsequent N-mineralization. Decomposition studies to a large extent have ignored the effects of physical quality. Thus, apart from C quality, attention should be drawn to certain physical variables such as toughness (Swift *et al.*, 1979), waxiness or rigidity of leaves (Grubb, 1986) that may provide additional explanations for decomposition patterns.

Although the protein binding capacity of the polyphenols was not measured, as earlier noted by Haslam (1989), high coefficient of determination (R^2) between N-mineralization and polyphenol content of the litter (Table 2) suggest that soluble polyphenols present actually interfered with net mineral-N release from *Acacia* litter as shown in Fig. 2.

The N content of the decomposing mixed litter (Fig. 5) exhibited a similar pattern to that of lignin content (Fig. 3). This pattern can be explained by the microbial activity at each stage of decomposition which may results in net N-immobilization or the amounts of polyphenols and lignin contents that may inhibit the microbial activity during N-mineralization.

CONCLUSIONS

Present results clearly indicate the relationship between N mineralization and some legume constituents during decomposition. Similarly, the results demonstrate a high potential for manipulating N release patterns for legume residues through the mixing of high quality litter (i.e., *Gliricidia*) and low quality litter (i.e., *Acacia*). However such potentials require verification through field measurements of their effect on crop production, e.g. maize, to develop credible programmes on the synchronization of soil nutrient availability and crop demand.

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