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Full Length Research Paper

Optimization of Nitrogen released and immobilization from soil-applied prunings of *Sesbania sesban* and maize stover

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A glasshouse experiment was conducted using the double-pot technique involving mixtures of low and high quality N sources with the objectives of determining 1) N mineralization and substitution value of high quality organic materials, 2) N immobilization by low quality maize stover, and the subsequent remineralization of immobilized N, and 3) N mineralization from or immobilization by mixtures of high and low quality organic materials. The experiment was designed in a 6 x 3 factorial combinations of sources of easily available N (inorganic fertilizer and *Sesbania sesban*) with a source of low level of available N (maize stover). The six levels of easily available N were 0, 50, 100 and 150 mg N per pot in the form of NH_4NO_3-N, and about 34 and 136 mg organic N per pot with *S. sesban* prunings. The levels of maize stover were 0, 2.5 and 5.0 g per pot. Application of 2.5 and 5 g maize reduced maize biomass yield by 4 and 16%, and N immobilization by 18-24% of added equivalent fertilizer N at 28 days after sowing (DAS), and 22% at 49 DAS. The decline of immobilized N at 42 and 49 DAS for the 2.5 g stover was ascribed to remineralization of N immobilized earlier. From the results of N uptake, the substitution value recovered was between 0.23 and 0.41 for *S. sesban*. Application of low quality organic materials (maize stover) in combination with high quality organic materials has shown initial immobilization of inorganic N that has been mineralized by the high quality organic materials and is slowly remineralized later.

Key words: Residues quality, N-immobilization, remineralization, substitution value

INTRODUCTION

Leguminous tree prunings are capable of releasing substantial amounts of nitrogen (N) that can support crop growth. The use of N-rich tree prunings as a substitute to inorganic fertilizers has proven to be a viable alternative source of soil fertility replenishment in low input smallholder farming systems where N deficient soils are the major limitation to production (Akinnifesi et al., 1997, 2007; Ikerra et al., 1999; Kang and Shanon, 2001). Decomposition studies of various leguminous tree prunings have shown that the rates of N release from various organic materials is dependent on the quality of the material (Mafongoya et al., 1998; Seneviratne et al., 1998). This has led to a distinction between high quality materials with low C:N ratio, and low polyphenol and lignin contents, and low quality materials with high C:N ratio, and high polyphenol and lignin contents. High quality organic materials decompose very fast releasing N initially in excess of plant needs (Mafongoya et al., 1998; Handayanto et al., 1994).

In a field experiment conducted in the sub-humid highlands of Kenya, high quality leguminous tree prunings released up to 107 kg N ha^{-1} in excess of the plant demand within 4 weeks after application (Mugendi et al., 1999). The excess N was lost through leaching and other

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other natural processes, resulting in N deficits a few weeks after peak mineralization. Therefore, reduction of such losses would greatly improve N use efficiency by the crops. Synchrony between N release from the organic materials with the crop's N demand can be achieved by optimizing timing of the application of organic materials, and by combining high and low quality organic materials (Swift, 1987). Mulongoy et al. (1993) and Mafongoya et al. (1997) demonstrated that application of prunings at the time of planting result in significantly higher maize N uptake than application four weeks before or after planting. However, correct timing of application of organic materials relative to the crop planting is difficult in the tropics because of unpredictable rainfall patterns. Therefore, mixing of high and low quality organic materials becomes the next option for optimization of N uptake and crop yield in agroforestry systems. The synchrony of N release and demand by the crop is based on the hypothesis that low quality organic material will initially immobilize part of the N mineralized from the easily decomposable materials and is later slowly remineralized.

In this study, high quality leguminous tree prunings, low quality maize stover, and their combinations were compared. When organic materials are compared via the uptake by plants, precautions must be taken that the supplies of nutrients other than N are non-limiting and equal for all materials. For organic materials this is difficult, because it is not known beforehand which fractions of their nutrients will become available to the plant during the uptake period. Such problems can be overcome with the so-called double-pot technique (Janssen, 1990).

The main assumption in the study was that addition of maize stover would increase soluble organic compounds in the mixtures and by that stimulates the immobilization of N from chemical fertilizers or released from decomposing high quality legume tree prunings. The objectives of the study were to assess (1) N mineralization from high quality organic materials (2) N immobilization by low quality maize stover and the subsequent remineralization of immobilized N, and (3) determine interactive effects on N mineralization or immobilization when high and low quality organic materials are applied in mixtures.

MATERIALS AND METHODS

Experimental design of the greenhouse study

A greenhouse experiment was conducted in June 2000 at the Sub-department of Soil Quality of Wageningen University and Research Center in The Netherlands. Six levels of high-quality N sources were combined with three levels of a low-quality nutrient source. The high-quality sources were NH_4NO_3 and *Sesbania sesban (L)* from Zalewa provenance, Malawi. The low-quality material was maize stover, for which the hybrid maize, LG 11, was used.

The six levels of high-quality N sources were 0 (control), 50, 100, and 150 mg N per pot applied with NH_4NO_3, and 34 and 136 mg N applied with 1.0 and 4.0 grams of sesbania prunings (Table 1). The three levels of the low-quality nutrient source were 0, 2.5 and 5.0 grams of maize stover, corresponding to 0, 12 and 24 mg N per pot. The treatments with chemical fertilizer were chosen because NH_4NO_3 acted as reference with which the other N sources were compared. The treatments were arranged in a completely randomized design with 3 replications.

The experimental crop was maize, hybrid LG 11. Initially four harvest dates had been foreseen, each with two replicates. In view of the poor maize growth after 40 days, it was decided to reduce the number of harvest dates to three and to increase the number of replicates to three for the second and third harvest dates.

To obtain the required quantities of the organic materials, sesbania and maize were raised in pots in the greenhouse during a preceding period of 50 days. The maize stover was cut into two parts, *i.e.* a lower part below the ear leaf and an upper part above the ear leaf. Samples of the organic materials were dried in an oven at 75 °C for 48 h and were finely ground for total N, P, K and C analyses, prior to the start of the main experiment (Table 2). The N content of the high quality sesbania prunings was about 6 times higher and the C:N ratio about 6 times lower than that of the low quality maize stover. The upper part of the maize stover had a C:N ratio of 38, about 3 times higher than the C:N ratio in high quality sesbania prunings. The lower part of maize, with the lower N content and highest C:N ratio, was used in this experiment.

The double-pot technique

The double-pot technique is a method where plants can take up nutrients simultaneously from two compartments (Janssen, 1990). An upper pot contains the substrate to be tested and a container under-neath is filled with nutrient solution. The roots of the plants growing in the upper pot pass through a mesh that forms the bottom of the pot and reach the nutrient solution in the container. When a nutrient is omitted from the solution, plants can take it up only from the substrate in the upper pot. In this way, the nutrients are supplied to the plants without mixing them with the soil, and the effect of the missing nutrient can be tested. Raijmakers and Janssen (1993) have modified the method for the study of organic substrates. Instead of individual lower pots, a 150 L tank is used for the nutrient solution. It is covered with a board with holes in which the upper pots fit and in such away that their bases do not touch the solution in the tank below. A perforated bucket is under each pot to keep the roots separated.

Details of the greenhouse study

The organic materials were chopped and mixed thoroughly with 2000 g of quartz sand. The maize biomass was dried at 40 °C for 72 h before application in the pots whereas for gliricidia and sesbania fresh materials were applied. The organic materials-sand mixtures were pot-ted in 2.5 l pots with bottom gauze, and moistened to field capacity with distilled water. Field capacity was 300 ml per 1000 g sand. Only 60% of the water needed to bring the soil to field capacity was applied before potting and the remaining 40% was applied after sowing. For the inoculation of the pots with soil microorganisms, to each pot 10 ml were added of an extract obtained by shaking fresh forest soil in demineralized water. Ammonium nitrate (NH_4-NO_3) was used as a source of inorganic N fertilizer. The 60% of the water field capacity applied at potting consisted of a mixture of (where required) an appro-priate aliquot of inorganic N (NH_4-NO_3) fertilizer solution, 10 ml of the inoculating solution from the forest soil extract and demineralized water was added to make up the balance. The pots were covered with a polyethylene sheet and left to stand for five days prior to planting. Four maize seeds were sown in each pot. After planting, the pots were watered with the remaining 40% to bring the moisture level to field capacity. The pots were then placed on the 150 l tank with a

Table 1. Mass fractions of C, N, P and K and C:N ratios of the organic materials applied in the pot experiment.

Organic material	N (mg/g)	P (mg/kg)	K (mg/kg)	C (mg/g)	C:N ratio
Sesbania	34	1.5	11	431	13
Maize stover, upper part	11	0.8	10	416	38
Maize stover, lower part	5	0.8	7	403	80

Table 2. Quantities of N (mg/pot) applied with the various combinations of high and low quality N sources in the upper pot. The number of pots was doubled for the treatments with one asterisk in one replicate, and for the treatments with two asterisks in the other replicate (see text).

High quality N source		Low quality N source (maize stover), (g per pot)		
Name	Rate (g per pot)			
		0	2.5	5.0
Control	0	0*	12	24**
NH_4NO_3	0.143	50*	62	74**
	0.286	100*	112	124**
	0.429	150*	162	174**
Sesbania prunings	1.0	34	46	57
	4.0	136	147	159

Table 3. Composition of the minus N nutrient solution and amounts of the stock solutions added to each 150 L tank in a double pot technique. The solution was refreshed after every two weeks

Stock solution	Concentration of stock Solution	Volume of stock solution added in a 150 L tank (ml)
Macronutrients		
$MgSO_4.7H_2O$	2 N	112.5
KH_2PO_4	1 N	450
K_2SO_4	1 N	150
$CaCl_2.6H_2O$	1 N	450
Micronutrients*		A mixture of micronutrients 150
H_3BO_3	2.86 g/L	
$MnCl_2.4H_2O$	1.81 g/L	
$ZnSO_4$	0.22 g/L	
$CuSO_4$	0.16 g/L	
$(NH_4)_6MO_7O_{24}.4H_2O$	0.04 g/L	
Fe(Fe-EDTA)	35.0 g/L	

*The micronutrients were dissolved in one bottle to make a mixture of micronutrients stock solution, except for the Fe(Fe-EDTA) was prepared in a separate container

nutrient solution containing all the nutrients except N; the composition is given in Table 3. The pots were covered again with the polyethylene sheet until all the maize seeds had germinated. Seven days after sowing (DAS) the maize plants were thinned to three per pot. After thinning the surface of the pots were covered with 100 g gravel (0.5 - 1.0 mm in diameter) to reduce evaporation. The pots were watered daily with distilled water and weighed every other day to determine the amount of water required to maintain the pots at field capacity. The nutrient solution (minus N) in the lower tank was changed every 2 weeks.

During the experiment, the greenhouse daily temperatures varied between 27 and 32°C. Water was pumped at 30 min intervals into the glasshouse chamber floor to maintain the chamber humidity at 60%.

The maize was harvested at 28, 42 and 49 DAS. At harvest, the maize shoots were cut just above the gravel surface, and the roots were washed and rinsed with distilled water. Roots were subdivided into roots growing in the soil and roots growing in the solution for the second and third harvests; after the first harvest the roots growing in the soil and solution were combined. Shoots and roots were dried in an oven at 75°C for 48 h and dry-matter weight was determined. The dried biomass was finely ground and analyzed

Table 4. Maize shoot yield (g DM/pot) harvested at 28, 42 and 49 days after sowing.

Time of harvesting		**28 days after planting**			**42 days after planting**			**49 days after planting**		
Maize stover rate (g/pot)		0	2.5	5.0	0	2.5	5.0	0	2.5	5.0
N-Source	N-rate									
NH_4NO_3	0	2.20	2.35	2.01	3.79	3.70	3.21	3.62	3.51	3.41
	50	3.41	3.68	2.66	7.39	6.62	5.40	7.94	6.83	6.36
	100	3.97	4.39	4.10	11.11	10.75	8.80	12.86	12.54	11.04
	150	5.82	5.33	5.46	16.37	15.53	14.24	18.95	17.44	15.37
Sesbania	34	2.64	2.40	2.41	5.63	4.46	4.29	5.37	5.38	4.92
	136	3.84	3.40	2.89	7.64	6.99	6.00	8.54	7.25	6.05
N-Source		0.355***			0.551***			0.541***		
Maize stover		0.233**			0.361***			0.354***		
-Source x Maize stover		NS			NS			0.936***		

The significant levels **, *** represent $p < 0.01$ and $p < 0.001$ respectively and NS = Not significant.

for N using the methods described by Teminghoff et al. (2000). Nitrogen contents in shoots and roots were calculated as the product of N mass fraction and dry-matter yield, and total N uptake was calculated as the sum of the N contents in the shoot and roots.

Substitution value of organic N sources

The response of a crop to an organic N source may be compared to the response to a reference N source, either in a vertical way (at common nutrient rates) or in a horizontal way (at common levels of yield or nutrient uptake). We used the horizontal comparison and N uptake. The substitution value (SV) is then the ratio of the recovery fractions (RF) of the organic N source (ONS) and the reference N source (RNS), being NH_4NO_3 in the present study. Recovery fraction (RF) was calculated as:

$$\mathbf{RF = \delta NU / N_A} \text{ -- } 1$$

Where δNU is the difference between N uptake in the treatment and N uptake in the control and N_A is applied N.

And the substitution value (SV) is given by:

$$\mathbf{SV = RF_{ONS}/RF_{RNS}} \text{ -- } \mathbf{2}$$

SV has to be split into an organic and an inorganic part:

$$\mathbf{SV = SV_O * F_O + SV_I * F_I} \text{ -- } \mathbf{3}$$

where F stands for fraction and the subscripts O and I for organic and inorganic.

In case F_I is negligible, as in the present study, equation 2 can be used and SV equals the fraction of organic N that is mineralized. Because this fraction increases over time, also the value of SV increases over time.

For the derivation of SV from our experimental results we plotted the increases in N uptake above the control (henceforth denoted by δN uptake) to the rate of N application. We then calculated the recovery fractions as the slopes of the regression lines, and the SV of the particular ONS as the ratio of the slopes of an ONS and RNS. The SV's obtained were then used to calculate the rate of equivalent N fertilizer of the organic materials:

$$\mathbf{EF_{ONS} = SV * N_{A,\ ONS}} \text{ --- } \mathbf{4}$$

Where, EF_{ONS} is the equivalent fertilizer rate of the organic N source and $N_{A,\ ONS}$ is the rate of N applied with the organic source.

Data analysis

Data was analyzed using two way ANOVA using GENSTAT version 5. Linear, power and exponential regressions were done to relate ΔN uptake and equivalent fertilizer N and derive substitution rates and fraction of N immobilized. Means were separated using the least significant difference (LSD) at 0.05 probability level.

RESULTS

Maize biomass yield

Inorganic N fertilizer significantly (P>0.001) increased maize biomass yield (Table 4) up to the highest application rate. Maize growth was faster in the pots with inorganic N fertilizer than in the pots with organic materials. Although 4 g sesbania prunings is equivalent to 136 mg organic N, maize biomass yield was equivalent to 50 mg fertilizer N only.

Application of 2.5 and 5 g maize stover reduced average maize shoot yield at 28 DAS by 4 and 16%, respectively (Figure 1), but the pattern for the various N sources was rather irregular. The maize biomass yield reductions due to application of 2.5 and 5 g maize stover were 9 and 17% at 42 DAS and 7 and 16% at 49 DAS.

Maize N uptake

The N uptake by maize increased over time, except for the controls (Table 5). The control plants could derive N only from the seeds (5.4 mg N per seed) and from the forest soil extract (2 mg N), and apparently the seeds had already been emptied for N at the first harvest date.

Table 5. Total N uptake (mg/pot) by maize grown in a glasshouse using a double pot technique sampled at 28, 42 and 49 days after sowing. The data for the highest rate of gliricidia treatment (139 mg N/pot) has not been included.

Time of harvesting		**28 days after planting**			**42 days after planting**			**49 days after planting**		
Maize stover rate (g/pot)		0	2.5	5.0	0	2.5	5.0	0	2.5	5.0
N-Source	N-rate									
NH_4NO_3	0	24.45	25.34	21.95	23.93	22.46	20.40	21.45	21.34	19.67
	50	45.74	43.88	33.43	53.12	45.16	38.14	53.85	43.20	39.64
	100	72.77	64.96	63.66	81.86	76.64	61.45	89.46	84.69	72.90
	150	120.50	84.46	91.04	120.52	108.71	99.73	131.51	115.72	97.68
Sesbania	34	30.25	27.37	25.45	34.95	28.31	25.92	34.46	36.89	33.59
	136	48.42	44.20	33.99	49.52	45.76	39.79	60.30	50.96	42.42
N-Source		6.71***			5.52***			4.96***		
Maize stover		4.93***			3.62***			3.24***		
N-Source x Maize stover		11.62**			NS			8.58***		

The significant levels **, *** represent $p < 0.01$ and 0.001 respectively and NS = Not significant.

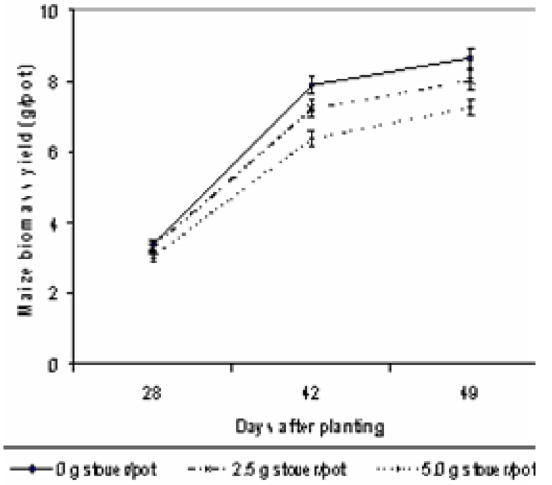

Figure 1. The effect of application of maize stover on maize biomass yield.

A clear response was found to the inorganic as well as to the organic N sources.

Application of maize stover on average reduced maize N uptake at 28 DAS, but not for control. The average reductions for the treatments except for the control were 17 and 22% in the case of 2.5 and 5 g maize stover, respectively. At 42 DAS, these figures were 12% for 2.5 g stover and 23% for 5 g maize stover, and at 49 DAS the N uptake reductions were 10% for 2.5 g stover and 21% for 5 g stover.

Substitution values

The data on δN uptake was regressed against N applied (through NH_4NO_3) and the organic N sources (the uptake for the controls were averaged over the three stover treatments). Only the treatments without maize stover were included in the regression. Again only the lower application level of gliricidia was considered. Regression lines were forced through the origin because by definition δN is zero when no N is applied. Linear functions ($y = bx$), power functions ($y = ax^b$) and exponential functions ($y = ae^{bx}$), regressions were explored because the res-ponse to NH_4NO_3 seemed to increase with increasing application rates. Although the exponential function gave the highest R^2 values for the regression graphs, however, the regression lines did not comply with our definition of δN = 0, when x = 0, whereas the power function gave lower R^2 values than the linear regression. Hence linear regression was opted for. Table 6 presents the regression coefficients and the substitution values (SV) derived from the linear function.

As the maximum N uptake with the organic N sources was equal to the N uptake obtained with application of 50 mg N in the form of NH_4NO_3 (Table 5), the ratio of the recovery fraction of the organic N source and the recovery fraction of NH_4NO_3-N obtained at the rate of 50 mg N was also calculated using equation 2, to serve as a practical solution for SV (PSV in Table 6). Substitution values of sesbania given by ESV and PSV (Table 6) increased as mineralization progressed with time. The SV values obtained with the various methods increased from 0.23 to 0.44 for sesbania.

Using the PSV values, the application rates of 1.0 g sesbania can be translated into 7.8 mg N at 28 DAS, 12.6 mg N at 42 DAS and 13.9 mg N at 49 DAS whereas the rate of 4.0 g sesbania can be translated into 31.3 mg N at 28 DAS, 50.3 mg N at 42 Das and 55.8 mg N at 49 DAS.

Nitrogen immobilization by maize stover

The fraction N immobilized was calculated from the regression lines as the difference between the slopes of the regression lines without maize stover and with maize stover. The δN uptake versus N application, for gliricidia

Table 6. Values of the regression coefficient a and of R-square of the linear regression equations (y = ax) relating δN uptake at harvest of 28, 42 and 49 DAS to applied N, and substitution values for sesbania. ESV stands for 'equation' SV and PSV for 'practical' SV.

DAS	N source	Constanta	R square	ESV	PSV
28	NH_4NO_3	0.5847	0.9753	0.23	0.30
	Sesbania	0.1345	0.9256		
42	NH_4NO_3	0.6726	0.9364	0.37	0.39
	Sesbania	0.2461	0.8787		
49	NH_4NO_3	0.7177	0.9567	0.41	0.44
	Sesbania	0.2968	0.9456		

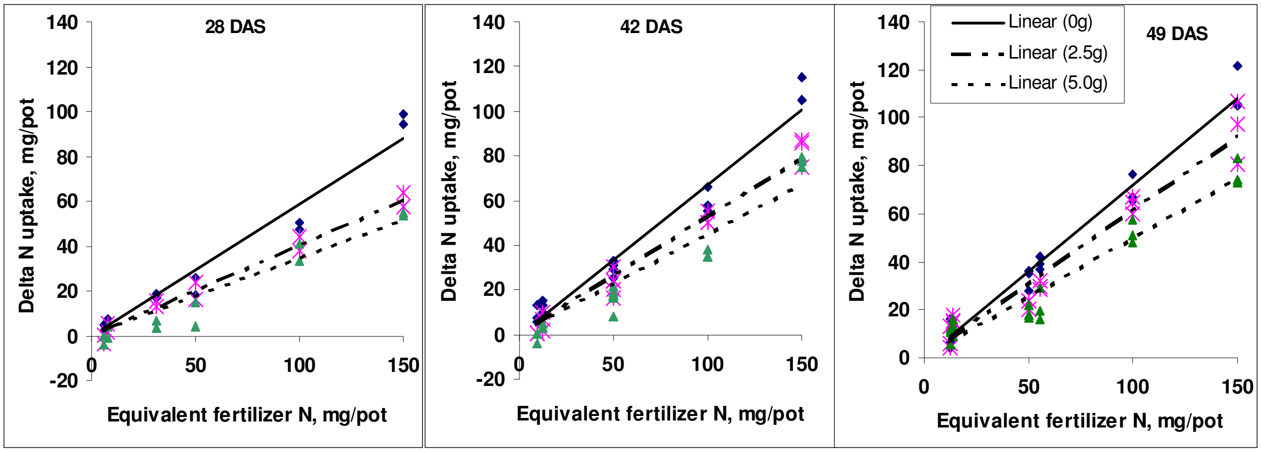

Figure 2. The relations between Delta N uptake (δN) and applied equivalent fertilizer N for the harvests at 28, 42 and 49 DAS, derivation of fraction N immobilized.

Table 7. Values of the regression coefficient a and R-square of the relations between δN uptake and applied equivalent fertilizer N.

Harvest date, DAS	Stover rate, g/pot	Regressioncoefficient a	R-square	Fractionimmobilized
28	0	0.5847	0.9593	
	2.5	0.4061	0.9778	0.18
	5.0	0.3461	0.9332	0.24
Difference (5.0-2.5)				0.06
42	0	0.6691	0.9600	
	2.5	0.5281	0.9711	0.14
	5.0	0.4477	0.9212	0.22
Difference (5.0-2.5)				0.08
49	0	0.7210	0.9776	
	2.5	0.6174	0.9473	0.10
	5.0	0.4987	0.9419	0.22
Difference (5.0-2.5)				0.12

and sesbania expressed in the above-calculated fertilizer N equivalents were plotted (Figure 2). The resulting regression coefficients and R^2 are presented in Table 7. At 28 DAS, 2.5 g maize stover immobilized 18% and 5 g maize stover immobilized 24% of the added fertilizer N. For the pots that received 2.5g maize stover immobilization decreased over time indicating that remineralization had taken place. For the 5.0 g stover treatment, the fraction immobilized N stabilized at 22% at 42 and 49 days, hardly lower than initial 24%. The difference between 2.5 and 5.0 g maize stover in fractions N immobilized increased over time.

DISCUSSION

The recovery fractions of 13% (at 28 days), 24% (at 42 days), and 29% (at 49 days) of N applied with sesbania are within the range of N recoveries of agroforestry prunings reported by other scientists. Akinnifesi et al. (1997) reported a 10-16% recovery in maize plants from the application of ^{15}N labeled *Leucaena leucocephala* (Lam.) de Wit. In a laboratory experiment Handayanto et al. (1994) found 14-44% N recovery by maize from different mixtures of *Gliricidia sepium* and *Peltophorum pterocarpa* prunings. Cobo et al. (2002) in a glasshouse experiment found N recoveries by rice ranging from 13.1 to 54.6% after applying leaves of nine different green manures.

The amount of N immobilized was consistently lower in the mixtures for 2.5 g stover than in 5.0 g stover. Increasing the amount of stover in the mixture increased N immobilization, and hence reduction in N uptake by the maize resulting in lower maize biomass. The reduction of the fraction of immobilized N from 18% at 28 DAS to 10% at 49 DAS in the treatment with mixtures of 2.5 g stover is likely the result of re-mineralization of the earlier immobilized N. This result agrees with the hypo-thesis that mineral N immobilized initially by the maize stover is slowly remineralized.

The reductions in maize dry-matter yield induced by addition of maize stover are comparable to the results obtained in Kenya where maize grain yield was reduced by 3 - 30% after incorporating maize stover (Qureshi, 1987; Nandwa, 1995). Apparently both the uptake by plants and the uptake by microorganisms were constant portions of the available N, irrespective of the amounts. Intuitively, one would expect that the portion taken up would be higher at low than at high quantities of available N. The opposite seemed to have been found, as δN uptake gave the impression of increasing with increasing quantities of NH_4NO_3-N, independent of the presence of maize stover. Alternatively, there might be some N immobilized due to soluble C released by roots and maize stover.

The calculated fractions of immobilized N by maize stover were similar for fertilizer N and N released from sesbania. The effects of the low and high quality organic materials seemed just additional without any special interaction between the organic sources.

Conclusion

Although the mixing of high quality organic materials is a viable option for optimization of N uptake and crop yield in agroforestry systems, the short-term effect is note-worthy. The substitution values (SV) of sesbania increa-sed over time. Maize N uptake was lower in the treat-ments with maize stover than in the treatments without. Apparently, maize stover immobilized mineral N. The immobilized N was later partially released and was taken up by the maize crop (especially in 2.5 g stover). Althou-gh, the experiment did not run long enough to allow for complete decomposition of the maize stover, the results clearly demonstrate that a combination of high and low quality organic materials the low quality organic material will initially immobilize part of the N mineralized from the easily decomposable materials and slowly remineralizes it later. Hence our hypothesis is correct, however, there is need to find out the optimum mixture ratio of the high and low quality organic materials that will give the best synchrony between N release from the mixture and demand by the crop.

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