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Estimating Dinitrogen Fixation of Hedgerow Vegetation Using the Nitrogen-15 Natural Abundance Method

J. K. Ladha,* M. B. Peoples, D. P. Garrity, V. T. Capuno, and P. J. Dart

ABSTRACT

Leguminous trees play a major role in alley farming or hedgerow by providing or recycling N and organic matter to annual crops. Little is known, however, about their capacity to fix N_2 under field conditions because of methodological difficulties. This study evaluated whether the ^{15}N natural abundance ($\delta^{15}N$) method could be applied successfully to an alley-cropping system to estimate N_2 fixation by *Gliricidia sepium* (Jacq.) Walp. The study also assessed the suitability of the non-nodulating legume *Cassia spectabilis* (L.) DC [syn. *Senna* spectabilis (DC.) Irwin and Barneby] as a suitable reference for investigations with N_2-fixing trees. The hedgerow species were planted in double rows, 3 m long with 5-m-wide alleyways, at an acid upland site in the Philippines. The $\delta^{15}N$ of total N of Cassia and Gliricidia prunings was determined from six samplings between January 1990 (17 mo after establishment) and July 1991. The $\delta^{15}N$ of the total N of samples from nonfixing Cassia ranged from 4.47 to 7.28‰ with an average and standard error of 6.16 ± 0.41‰. These values were similar to those of extractable N of soil from different soil depths, ranging from 4.66 to 7.33‰, suggesting that Cassia is a suitable nonfixing reference species. The changes in $\delta^{15}N$ of total N of prunings were similar in both tree species; therefore, the observed variation was considered not to have interferred with estimation of N_2 fixation (%Ndfa). At four of the six sampling times, Gliricidia had an Ndfa close to 50%, whereas at other two sampling dates the Ndfa dropped to 30 and 35%. This study also provides the first quantitative data demonstrating that Cassia is a non-N_2-fixing legume.

MANY TROPICAL SOILS are low in both total and plant-available N. Crop yields are often limited by N supply, and animal productivity and fecundity are restricted by poor-quality forage. Apart from high-return cash crops, however, N fertilizers are used only to a limited extent because of low per-capita incomes, limited credit facilities to most farmers, and lack of effective infrastructures for fertilizer production and distribution (Peoples and Herridge, 1990).

The capacity for tree and shrub legumes to accumulate N and organic matter can be substantial (Peoples and Craswell, 1992). Increasingly, woody perennial legumes are being incorporated into tropical farming systems for forage or green manure, or are being used to reclaim degraded wastelands, retard erosion, or provide shade, fuel, and timber (Giller and Wilson, 1991). The potential for the integrated use of these species is well illustrated in alley farming systems. Alley cropping involves growing arable crops in the interspaces (alleys) between rows of planted trees that are trimmed periodically and maintained as hedgerows. The leaf and twig prunings are added to the soil as green manure or mulch during cropping, or hedgerows can be left to produce firewood or used to provide animal fodder during noncropping periods (Kang et al., 1990).

Although tree legumes appear to have considerable potential for improving the N fertility of soils in the tropics, and increasing the quality and level of protein in the diets of both animals and humans, relatively little is known about their capacity to fix N_2 under field conditions (Peoples and Herridge, 1990; Peoples and Craswell, 1992) because of methodological and technical difficulties (Peoples et al., 1988; Danso et al., 1991).

Commonly, the N_2-fixing status of woody species has been based on measures of nodule mass, changes in tree or soil N, tree size or dry matter production, or derived from acetylene reduction assays on detached nodules (Peoples et al., 1988; Danso et al., 1992). The reliability and accuracy of such estimates is questionable. More recently, ^{15}N techniques using ^{15}N-enriched fertilizers have been applied in studies on different N_2-fixing trees and shrubs (Danso et al., 1991, 1992). With this methodology, it is essential that a non-N_2-fixing reference plant be included in the experimental design to indicate the level of ^{15}N in the pool of plant-available soil N. For accurate estimates of N_2 fixation, it is necessary that the reference and N_2-fixing legume use soil N of identical ^{15}N enrichment. It is difficult to choose an appropriate reference plant to satisfy this requirement for long-term studies with perennial tree species, particularly when ^{15}N-enriched materials are applied to the surface soil and the level of ^{15}N in the enriched zone is likely to change with both time and soil depth (Danso et al., 1991; Giller and Wilson, 1991; Danso et al., 1992).

An alternative to ^{15}N-enrichment procedures has been evaluated in agricultural and natural ecosystems, whereby the very small differences in the natural abundance of ^{15}N between air (0.3663 atom % ^{15}N) and soil (0.3680–0.3730 atom % ^{15}N) are used for the measurement of N_2 fixation (Shearer and Kohl, 1986). The ^{15}N natural abundance method has a number of advantages over the ^{15}N-enrichment studies. Investigation can be undertaken without the use of N fertilizers, and, in some soils, the level of ^{15}N in plant-available N can be reasonably uniform with depth and remain relatively stable for long periods of time (Högberg, 1990; Peoples et al., 1992) so that estimates of N_2 fixation may not be greatly affected by the choice of the nonfixing reference (Ledgard et al., 1985; Bergersen et al., 1989; Peoples et al., 1991). Although natural levels of ^{15}N in soil under trees tend to be

J.K. Ladha and D.P. Garrity, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines; M.B. Peoples, CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, ACT, 2601 Australia; V.T. Capuno, Philippine National Institute of Biotechnology, Univ. of the Philippines at Los Baños, Philippines; and P.J. Dart, Dep. of Agriculture, Univ. of Queensland, Qld 4072, Australia. This study is part of a collaborative program between IRRI and the Australian Centre for International Agricultural Research (ACIAR) under the project no. 8800 and 8731. The work was supported in part by the Australian International Development Assistance Bureau (AIDAB). Received 15 Apr. 1992.
*Corresponding author.

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Abbreviations: % Ndfa, N_2 fixation; CEC, cation-exchange capacity; SE, standard error of mean; tNdfa, total amount of N fixed.

lower than those in agricultural systems (Peoples et al., 1991), and can be variable in some forest and woodland ecosystems (Hansen and Pate, 1987), the natural abundance method has been applied to many different woody species growing in diverse environments (e.g., Shearer et al., 1983; Domenach et al., 1989, Yoneyama et al., 1990; Peoples et al., 1991; Schulze et al., 1991; Mariotti et al., 1992). Despite the potential errors in using the technique (Shearer and Kohl, 1986, 1991), the ^{15}N natural abundance approach has given similar estimates of N_2 fixation with precision similar to other measurement techniques in a variety of different cropping (Kohl et al., 1980; Evans et al., 1987; Ofori et al., 1987; Bremer and van Kessel, 1990; Herridge et al., 1990), pasture (Ledgard et al., 1985), or forest plantation systems (Domenach et al., 1989); Mariotti et al., 1992).

Our study was initiated to ascertain whether the natural abundance ($\delta^{15}N$) method could be applied successfully to an alley-cropping system to estimate N_2 fixation by Gliricidia, a multipurpose tree used commonly in the tropics, and to assess whether the vigorous, nonnodulating legume Cassia is a suitable nonfixing reference plant for investigations with N_2-fixing trees. Taxonomic revision has now placed Cassia within the genus *Senna*. For the sake of familiarity, however, we use the name Cassia.

MATERIALS AND METHODS

Site Description

The study was conducted at the International Rice Research Institute acid soil upland research site at Claveria, Misamis Oriental, Northern Mindanao, Philippines (8°38′N, 124°55′E, elevation 390 m). The climate of the region is warm; average January and July temperatures are 23.7 and 25.4 °C, respectively. Based on rainfall data from the past 5 yr, the annual precipitation averages 2500 mm. The soil is an acid clay Ultic Haplorthox with air-dried pH (1:1 [w/v] soil/water) = 4.8, organic C = 1.93%, total N = 0.175%, CEC = 11.9 $cmol_c$ kg^{-1}, and exchangeable Al = 1.85 $cmol_c$ kg^{-1}. The slope of the experimental field was ≈15%.

Hedgerow Establishment and Pruning

Stem cuttings of Gliricidia and Napier grass (*Pennisetum purpureum* Schum.) and the seedlings of Cassia were planted in double rows during the rainy season of 1988. The experiment was laid out in a split-plot design with four replicates of each hedgerow species. No fertilizer or rhizobial inoculum was applied. The stem cuttings or seedlings were established along the contour line with 25-cm spacing between plants in two rows spaced 50 cm apart on a contour bund 25 cm high. Each experimental subplot was 3 m long with a 5-m-wide alleyway, giving a plot area of 16.5 m^2. In 1989, a rainfed upland rice (*Oryza sativa* L.)–maize (*Zea mays* L.) crop sequence was established in the alleys, and the pruning of the hedgerow species was initiated. Gliricidia and Cassia were pruned to ≈50 cm above the ground surface four times per year (Fig. 1) (January, May or June, July or August, and December in each of the 3 yr of 1989, 1990, and 1991). The Napier grass was cut to about ≈5 cm above the ground surface every month. The prunings from the tree hedgerows were applied to the alley crops as green manure in the respective plots, while the grass clippings were removed from the field for animal fodder. This study describes analyses and estimates of N_2 fixation for hedgerow material sampled in 1990 and 1991 (Fig. 1).

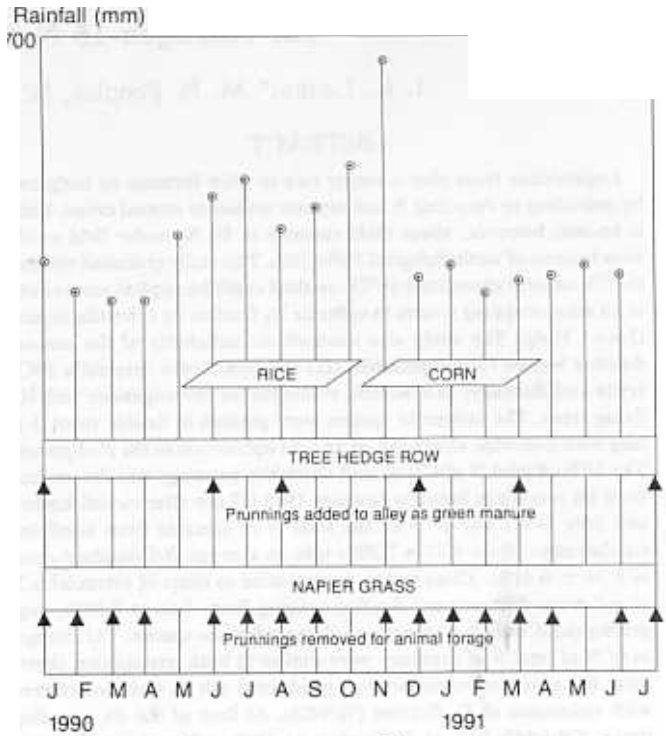

Fig. 1. The pruning management of the tree legume and grass hedgerows species in relation to the rainfall pattern and the annual crop sequence in the alleyways.

Plant Sampling and Preparation for Biomass, Nitrogen and Nitrogen-15 Natural Abundance Determination

The pruned regrowth collected from the middle 2 m of both the upper and lower hedgerows was combined and weighed. For $\delta^{15}N$ determination, the leaves and stems were separated from one-half of the total sample. Each component (leaves, stems, and leaves + stems) was cut into pieces of 2 to 3 cm, subsampled (≈500 g of fresh tissue), washed in tap water followed by rinsing in distilled deionized water, oven dried at 65 °C for 3 d, and weighed. The sampling of May 1991 also included the trunk portion (without root) of the plant; one tree from each replicate was sampled and the component parts (leaves, stems, and trunks) were processed. Each plant sample was milled to a fine powder and stored in a desiccator until analyzed.

Soil Sampling and Nitrogen-15 Natural Abundance Determination of Plant-Extractable Soil Nitrogen

The soil was sampled (January 1990) with an auger at 0 to 20-, 20- to 40-, 40- to 60- and 60- to 100-cm depths. Four sites, two each from the upper and lower hedgerows, were sampled to collect a composite sample from each replicate in the Gliricidia and Cassia treatments. The soil from each layer was passed through a 1-mm sieve, mixed, and placed in a 250-mL beaker. The moisture content of the soil was adjusted to 75% of field capacity and maintained near this level by daily watering with deionized distilled water. Rice (cv. 0S4) seeds were sown in each beaker, thinned to five plants per pot after emergence, and grown in a greenhouse. Seven weeks after sowing, the rice plants (including roots) were harvested, washed, dried, and total plant N analyzed for $\delta^{15}N$ using the methods described below.

Nitrogen and Nitrogen-15 Natural Abundance Analysis

Plant subsamples (300 mg) were analyzed for total N content by the micro-Kjeldahl technique, modified to include NO_3 and

Table 1. Variation in ^{15}N of plant-extractable N derived from soil taken from different depths.

Depth	$\delta^{15}N$†
cm	
0–15	
15–30	
30–60	
60–100	
Standard error of mean	

† Determined by ^{15}N analysis of total N accumulated by rice seedings grown for 7 wk in soil recovered from different depths.

NO_2 with the salicyclic acid treatment, as described by Peoples et al. (1989). Special precautions were taken during analysis to avoid isotopic discrimination and to ensure complete recovery of N (Bergersen et al., 1990). The digestion was carried out in a block digestor at 280 °C for ≈16 h. Steam distillation was performed in a complete glass distillation unit with 250-mL sample flasks. The distillates (80 mL) were acidified with one or two drops of 1% H_2SO_4 (v/v), and the volume reduced to ≈1 to 2 mL on a hot plate. Complete drying of the sample was avoided. The ^{15}N analyses were carried out on a dual inlet, triple collector isotope ratio mass spectrometer (VG Model 903 VG Isogas, Middlewich, England; Bergersen et al., 1989) and results were expressed as $\delta^{15}N$ (‰) with reference to air N_2, where:

$$\delta^{15}N = 1000\ (R_{sample} - R_{air\ N_2})/R_{air\ N_2}.$$

$$R = \text{mass } 29/\text{mass } 28 = {}^{15}N\ {}^{14}N/{}^{14}N_2.$$

Calculations and Statistical Evaluation

Treatment means and the SE were calculated for all sampling times and parameters. Data of $\delta^{15}N$ and total plant N of all samplings of Cassia and Gliricidia were also analyzed with standard analysis of variance techniques. The proportion of plant N derived from N_2 fixation (% Ndfa) was calculated after Shearer and Kohl (1986) as:

$$\%\ \text{Ndfa} = \frac{\delta^{15}N\ (\text{soil-derived N}) - \delta^{15}N\ (N_2\text{-fixing plant})}{\delta^{15}N\ (\text{soil-derived N}) - \delta^{15}N\ (\text{fixed N})} \times 100$$

In this formula, $\delta^{15}N$ (soil-derived N) is the $\delta^{15}N$ of the non-N_2-fixing reference plant and $\delta^{15}N$ (N_2-fixing plant) is the $\delta^{15}N$ of the N_2-fixing plant. The value of $\delta^{15}N$ (fixed N) was determined experimentally as -1.45 ± 0.02 ‰, by analysis of shoot N of Gliricidia inoculated with *Rhizobium* spp. strain CB3090 grown in N-free media in a temperature-controlled (30 °C day–25 °C night) glasshouse. The standard error of % Ndfa was calculated as described by Shearer and Kohl (1986).

RESULTS

Nitrogen-15 Natural Abundance of Plant-Extractable Nitrogen at Different Soil Depths

The variation in $\delta^{15}N$ of plant-available soil N was examined by growing rice in soil taken from Gliricidia and Cassia hedgerow treatments. Since $\delta^{15}N$ values of rice plants from Gliricidia and Cassia soils did not differ significantly, the values were combined for presentation in Table 1. Although the mean $\delta^{15}N$ of rice grown in soil recovered from different depths ranged from 4.66 to 7.33, there were no statistically significant differences between depths.

Table 2. Total N content in regrowth prunings of Cassia and Gliricidia.

Pruning time after establishment	Time	Total N	
		Cassia	Gliricidia
mo		kg/ha	
17	Jan. 1990	na†	na
22	June 1990	129	42
24	Aug. 1990	54	51
28	Dec. 1990	85	74
33	May 1991	70	70
35	July 1991	145	135
Analysis of variance		df	*F*
Sampling time (T)		4	24.71**
C. spectabilis vs. G. sepium (SP)		1	5.02*
T vs. SP		4	2.95

*, ** Significant at the 0.05 and 0.01 probability levels, respectively
† na = not available.

Trends in Nitrogen and Nitrogen-15 Natural Abundance in Dinitrogen-fixing and Reference Trees

The two species accumulated similar amounts of total N in four of five sample dates (Table 2). In the June 1990 sampling, Cassia prunings contained significantly more N than Gliricidia, but differences in N accumulation were not significant in the subsequent harvests. Total N in regrowth ranged from 3.7 to 10.0 g N $tree^{-1}$ (or 54 to 145 kg N ha^{-1}) for Cassia and 3.7 to 12.1 g N $tree^{-1}$ (or 42 to 135 kg N ha^{-1}) for Gliricidia. Gliricidia, due to lower survival, had fewer trees (11 250 ha^{-1}) than Cassia (14 500 ha^{-1}). The largest N accumulations occurred in the June 1990 and July 1991 (early wet season) cuttings (Fig. 1).

Figure 2 shows $\delta^{15}N$ of the total N of Cassia and Gliricidia in all samplings between January 1990 and July 1991. The overall mean and standard error of $\delta^{15}N$ for the six samplings of Cassia was 6.16 ± 0.41‰, while for Gliricidia it was 2.87 ± 0.44‰. Levels of $\delta^{15}N$ declined significantly ($P < 0.05$) during the 11-mo period from the first to the fourth sampling, and in-

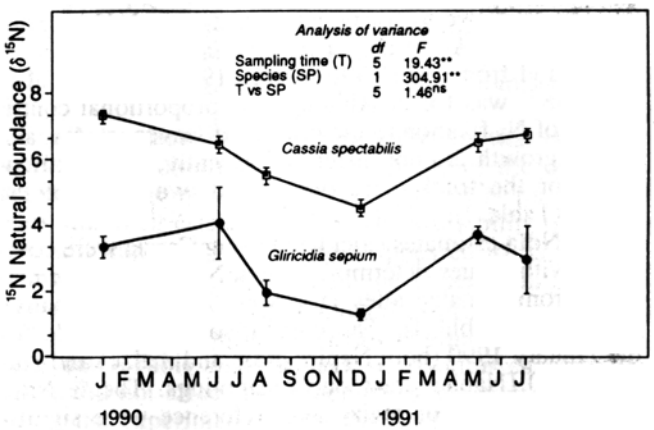

Fig. 2. The time course of changes in the $\delta^{15}N$ (‰) of the total N of regrowth of *Cassia spectabilis* and *Gliricidia sepium* in the hedgerow intercropping system. Vertical bars indicate ± standard error. ** Significant at 0.01 probability level.

Table 3. Distribution of ^{15}N natural abundance ($\delta^{15}N$) in Cassia and Gliricidia and estimation of the proportion of Gliricidia N derived from N_2 fixation (%Ndfa) (May 1991).

	$\delta^{15}N$		Gliricidia Ndfa
	Cassia	Gliricidia	
	‰		
Total regrowth	6.51	3.72	
Leaves	6.77	4.04	
Stems	6.22	3.14	
Trunk	5.06	3.89	
Standard error of mean	0.68	0.60	

creased in subsequent harvests. The difference of $\delta^{15}N$ between the two species ranged from 2.4 to 3.9‰. The values of $\delta^{15}N$ of Gliricidia in June 1990 and July 1991 were highly variable, and were consequently not significantly different from the $\delta^{15}N$ of Cassia at those dates. The $\delta^{15}N$ of the two genera, however, differed significantly ($P < 0.01$) in the other four samplings.

Variation of Nitrogen and Nitrogen-15 Natural Abundance within Plants

The N and $\delta^{15}N$ present in different plant parts (total regrowth, leaves, stems, and trunk) of Cassia and Gliricidia was examined in the May 1991 sampling. The N in the total regrowth and leaves of Cassia and Gliricidia (1.75–1.94 and 1.74–2.38% N in Cassia and Gliricidia, respectively) were significantly higher than in their stem (0.64 and 1.00% N) and trunk (0.45 and 0.64% N). The $\delta^{15}N$ of the regrowth of the leaves, stems, and trunks, however, did not differ significantly within each species (Table 3). Gliricidia had a significantly lower $\delta^{15}N$ than Cassia in all plant parts.

Estimation of Plant Nitrogen Derived from Dinitrogen and Amounts of Dinitrogen Fixed by Gliricidia

The proportion of plant N derived from N_2 fixation (% Ndfa) in Gliricidia was estimated for each of the six samplings between January 1990 and July 1991 using Cassia as a nonfixing reference. The % Ndfa ranged from 30% in June 1990 to 55% in December 1990, with an average of 43% during the entire study period (Fig. 3). The estimated total amount of N_2 fixed (tNdfa) ranged from 13 to 66 kg N ha^{-1} per sampling. During the 13-mo period from June 1990 to July 1991, a total of 170 kg N ha^{-1} was fixed. Although the proportional contribution of N_2 fixation to the growth of leaves, stems, and total regrowth did not differ significantly, values calculated for the trunk were only about two-thirds of the others (Table 3).

The Ndfa estimates calculated using Cassia were compared with values determined when Napier grass or material from a maize alley crop were used as alternative references (Table 4). The comparisons were made for the January 1990 (both Napier grass and maize) and August 1990 (Napier grass only) samplings. The % Ndfa based on Cassia or maize as a reference were significantly greater than those determined using Napier grass in the January 1990 sampling, while in August 1990 the % Ndfa values were similar regardless of which reference material was used.

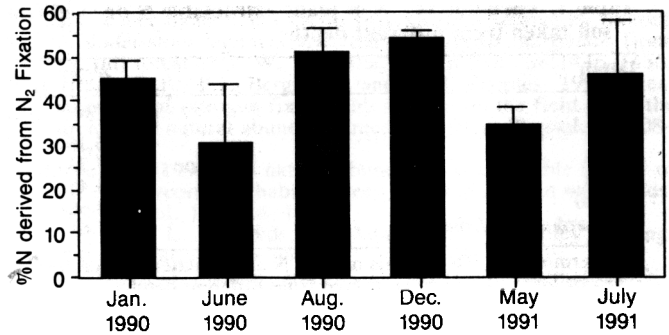

Fig. 3. The contribution of biological N_2 fixation (%Ndfa) to the N content of regrowth prunings at different sampling times of the *Gliricidia sepium* hedgerow intercropping system. Vertical bars indicate ± standard error.

DISCUSSION

There are a number of basic requirements that are necessary if accurate estimates of N_2 fixation are to be obtained using $\delta^{15}N$ methods (Shearer and Kohl, 1986; Peoples et al., 1989):

1. An appropriate non-N_2-fixing reference plant is available to provide a measure of the $\delta^{15}N$ of soil plant-available N.
2. The plant-available soil N contains an adequate level of $\delta^{15}N$ (preferably >5‰), that does not change greatly with depth or rapidly with time, and that is relatively uniform across the experimental site.
3. Plant parts sampled are representative of the whole plant. These three criteria were met in this study.

Although Allen and Allen (1981) reported many *Cassia* spp. to be nonnodulating, this study provides the first quantitative data confirming that Cassia, a potentially important agroforestry species (Giller and Wilson, 1991), is indeed a non-N_2-fixing legume. Cassia had $\delta^{15}N$ values similar to those of the maize alley crop and to Napier grass at one of two harvests (Table 4), and to determinations of plant-extractable soil N obtained from a glasshouse study (Table 1). Also the rooting pattern was similar to that of Gliricidia (D.P. Garrity, 1989, unpublished data). Therefore, on the basis of all these qualities, Cassia was judged to be a suitable nonfixing reference. The reasons for significantly lower $\delta^{15}N$ in Napier grass than in Cassia at the January 1990 sampling (Table 4) may be due to differences in phenology and growth habit, or to the fact that Napier grass was cut more frequently

Table 4. The $\delta^{15}N$ of various reference plants and estimates of the proportion of N derived from N_2 fixation (%Ndfa) of Gliricidia.

Sampling date	Reference		Gliricidia	
	Species	$\delta^{15}N$	$\delta^{15}N$	Ndfa
Jan. 1990				
	Napier grass	5.88 ± 0.18		35 ± 4.6
	Cassia	7.28 ± 0.21		45 ± 3.9
	Maize	7.75 ± 0.68		48 ± 5.2
Aug. 1990			1.93 ± 0.36	
	Napier grass	6.73 ± 0.36		59 ± 4.8
	Cassia	5.50 ± 0.24		51 ± 5.5

than Cassia. It may also reflect real differences occurring in fluxes through soil N pools induced by differences in handling the prunings (Cassia cuttings were incorporated on site, whereas all of the grass was removed), or to a contribution by associative N_2 fixation with the Napier grass roots during the growth period prior to the January sampling. Roots of Napier bajra, a hybrid of *P. purpureum* and *P. americanum* (L.) Leeke, have been reported to support nitrogenase (acetylene reduction) activity in field trials at ICRISAT, Hyderabad, India (Dart and Wani, 1982).

Assumptions inherent in the use of reference plants in ^{15}N investigations include (Peoples et al., 1989): (i) the ^{15}N abundance of the non-N_2-fixing reference is the same as that of soil N, and (ii) the legume and reference plant explore a soil N pool of identical ^{15}N abundance. For these assumptions to hold true it would be necessary that no differential isotopic discrimination occurs during N assimilation and metabolism by the reference plant or legume. While this has generally been held to be true for $\delta^{15}N$ studies (Shearer and Kohl, 1986), there has been a recent report of differences in ^{15}N natural abundance of plant N (1–2.5‰) between ectomycorrhizal and vesicular–arbuscular mycorrhizal tree species growing in the same soil (Hörberg, 1990). No information is available concerning the mycorrhizal status of the plants used in our study. Fairly good agreements were found, however, between the range of woody and herbaceous plants used to estimate the $\delta^{15}N$ of soil mineral N in this study (Cassia, Napier grass, and maize; Table 4), and at other tree legume field sites in the Philippines, Indonesia, and Australia (Peoples et al., 1991).

The $\delta^{15}N$ contents of the Cassia reference material were similar (6.5–7.3‰; Fig. 2) in four of the six samplings taken during the 18-mo experimental period. The two $\delta^{15}N$ values that were markedly lower than the rest were observed in August and December 1990 (5.6 and 4.5‰, respectively; Fig. 2) following the period of greatest rainfall (June–November; Fig. 1), and may have reflected moisture-induced changes in the dominant microbial transformation occurring in soil in the alley system (Shearer and Kohl, 1986, 1991). The homogeneity of soil $\delta^{15}N$ at the field site was indicated by the low standard error of mean $\delta^{15}N$ of Cassia regrowth (SE < 02.2‰ at five of six harvests; Fig. 2), and similar $\delta^{15}N$ determinations for plant-available soil N to a depth of 100 cm (Table 1).

Variation of $\delta^{15}N$ between plant parts in annual legumes due to isotopic fractionation, or changes in the use of soil or fixed N during plant development, can complicate sampling protocols for $\delta^{15}N$ determinations and introduce error into calculations of % Ndfa (Shearer and Kohl, 1986; Peoples et al., 1991). The same might also be expected in perennial woody species. In *Prosopis glandulosa*, a N_2-fixing tree species, Shearer et al. (1980) reported a difference of >3‰ between trunk wood and the whole tree. Because the trunk wood contained only a small proprotion of the total N of the tree, however, it made little difference when data were considered on a whole-plant basis. In our study, the $\delta^{15}N$ of leaves, stems, and trunk of Gliricidia or of Cassia did not differ significantly (Table 3).

Another factor that potentially could influence the accuracy of estimates of % Ndfa and the amounts of N_2 fixed in regularly cut tree systems concerns the contribution of N remobilized from the Gliricidia roots and trunk towards foliar regrowth after cutting (Danso et al., 1992). Although tree legume roots may represent a major component of total plant N in glasshouse investigations (Sanginga et al., 1990), retranslocation of root N in N_2-fixing alder trees has been reported to contribute only 10% of regrowth N requirements in the field (Domenach and Kurdali, 1989). We have no measures of root N or $\delta^{15}N$ in our current field study to compare with incremental data for prunings. However, Gliricidia trunks contained only ≈30% of the total aboveground plant N at the time of the May 1991 sampling (data not shown) with a $\delta^{15}N$ value that was not significantly different from the $\delta^{15}N$ of prunings collected at either that same May harvest, or the following July sampling by which time regrowth had accumulated 135 kg N ha^{-1} (Tables 2 and 3; Fig. 2). Although we are unable to quantify the role of remobilization in the N-economy of the alley crop hedgerows, its impact on % Ndfa is likely to be small if root $\delta^{15}N$ followed a similar trend to that observed for the trunk, and if root N contributed as little as 10% of the regrowth N.

The reliance of a legume upon N_2 fixation for growth is dependent on a dynamic interactions between the status of soil mineral N, nodulation (number, mass per plant, and specific nitrogenase activity), and other environmental factors affecting its performance. In four of the six sampling times, Gliricidia had % Ndfa values close to 50. In two samplings, however, the % Ndfa dropped to between 30 and 35 (Fig. 3). These were the samplings occurring during the early wet season transition, when rainfall was increasing from the preceeding dry period. The relatively low % Ndfa of Gliricidia N during early wet season samplings may have been due to the inhibitory effects of more abundant soil mineral N expected after a period of dry weather followed by the onset of rains (Buresh et al., 1992). Also, nodules associated with *G. sepium*, *Acacia manguim*, and *A. auriculiformis* senesce during the dry season at other locations in the Philippines with similar climatic patterns. New nodules did not appear until the start of the rainy season in May (Almendras et al., 1987 unpublished data). Nodule seasonality has been observed in other tree legume species (Wong et al., 1989; Fownes and Anderson, 1991). The variation in estimates of % Ndfa during the early wet season may have been a consequence of differences in nodulation resulting from seasonal variation in soil moisture.

The N contents of these trees indicated that both the N_2 fixing and nonfixing tree species have the capacity to extract large quantities of soil mineral N for several years after their establishement. The availability of abundant soil mineral N to the trees appeared to have been unimpaired for the duration of the experiment. Cassia spectabilis prunings yielded quantities of N very similar to those of the N_2-fixing Gliricidia at almost every sampling date (Table 2). The lack of fixation capacity in Cassia did not affect its capacity to accumulate plant-N during the experiment (1988–1991). These results also show the difficulties likely to be encountered in measuring N_2 fixation by trees using conventional N-balance techniques. The demonstration of N_2 fixation would depend on the ability to measure small changes in the soil

N content. This would not be possible in a deep soil during a relatively short sampling period.

Also, the strong sink for mineral soil N provided by the extensive tree root systems has both positive and negative implications. Specifically, N cycling to the alley crop via the tree prunings is enhanced, but the large mineral N capture by the hedgerows may create competition for N uptake between the hedgerows and the associated annual crop species. Gliricidia and Cassia roots grow actively into the space between hedgerows during the cropping season. The soil–plant distribution and uptake of mineral and fixed N in crop-hedgerow systems composed of either fixing or nonfixing species needs further elucidation.

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