

# Subsoil Nitrogen Capture in Mixed Legume Stands as Assessed by Deep Nitrogen-15 Placement

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## ABSTRACT

The rotation of crops with planted N<sub>2</sub>-fixing legumes (improved fallows) is a promising agroforestry innovation for replenishing soil fertility in the tropics. We postulated that woody and herbaceous legumes with different rooting and growth patterns could be mixed in improved fallows to maximize utilization of belowground growth resources. We used a method of injecting a solution of <sup>15</sup>N-labeled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> into soil at 0.15- and 1.0-m depths to measure soil mineral N acquisition by sesbania [*Sesbania sesban* (L) Merr], crotalaria [*Crotalaria grahamiana* Wight and Arn.], and the understory herbaceous legume siratro [*Macroptilium atropurpureum* (DC.) Urb.] grown in mixed stands on a Kandiuudalfic Eutrudox soil in western Kenya. Crotalaria had the highest root length in the topsoil. Sesbania on the other hand had nearly half its total root length below 0.3 m at 0.3- to 1.5-m depth; sesbania took up more added <sup>15</sup>N than crotalaria and siratro from the 1.0-m depth. Mixed sesbania and crotalaria stands, as compared with growing species in monocultures, increased root length at the 0.3- to 1.2-m depth. Sesbania mixed with siratro was more effective than sesbania mixed with crotalaria in uptake of <sup>15</sup>N at 1.0-m depth but not at 0.15-m depth. At 2 mo after injection, the <sup>15</sup>N was concentrated immediately below the injection point with little lateral movement. This confirmed the utility of the methodology in determining temporal N uptake for species in mixed stands. Our results suggest that opportunities exist for enhanced subsoil N retrieval through the mixing of leguminous species, which can influence root distribution and increase rooting in the subsoil.

**A** PRIMARY OBJECTIVE for establishing fast-growing legumes, as a replacement for traditional natural fallows in tropical crop-fallow rotations is to restore fertility of nutrient-depleted soils within a short duration (Prinz, 1986). Leguminous species achieve this, not only by sourcing N from the atmosphere through N<sub>2</sub>-fixation, but also through their ability to explore subsoil nutrient pools and to capture available nutrients through their extensive root systems (van Noordwijk et al., 1996; Buresh and Tian, 1997; Gathumbi et al., 2002b). Appreciable amounts of N in the form of nitrates can accumulate in agricultural soils, especially when N inputs from fertilizer application and mineralization of soil organic matter in the topsoil layers exceed crop N demand and uptake (Guillard et al., 1995). Since NO<sub>3</sub>-N is highly mobile in soils, unutilized NO<sub>3</sub>-N is subject to rapid leaching (Suprayogo et al., 2002). Additionally, appreciable amounts of N can apparently be mineralized in

lower soil layers in deep and clay-rich soils. It has been hypothesized that active tree and shrub roots can intercept leaching nutrients and also capture leached nutrients or nutrients from deep horizons resulting from weathering (van Noordwijk et al., 1996; Rowe et al., 1999). These two concepts have been referred to in the recent literature as “the safety net hypothesis” and the “nutrient pump hypothesis,” respectively. Recent studies conducted in an oxisol in western Kenya have shown that sesbania fallows can reduce the subsoil nitrate bulge (accumulated nitrates) through plant uptake (Mekonnen et al., 1997) and also through a reduction of leaching (Hartemink et al., 1996).

The safety net and nutrient pump hypotheses can effectively be tested by use of <sup>15</sup>N tracer techniques (IAEA, 1975). Rowe et al. (1999) successfully used <sup>15</sup>N-labeling methods to test the safety net role and root activity of two species with different rooting patterns in a hedgerow intercropping agroforestry system in Indonesia. Nitrogen-15 uptake by tree roots varied with time after application and between species, but the <sup>15</sup>N signatures were well above the background natural <sup>15</sup>N abundance. Atkinson et al. (1978) reported similar variation of <sup>15</sup>N uptake while using deep placement techniques in studies on root distribution and N uptake by fruit trees. Deep placement of <sup>15</sup>N was also used in studies on subsoil N uptake by pearl millet [*Pennisetum glaucum* (L.) R. Br.] on a Kandiuudult soil of the southeastern USA (Menezes et al., 1997). Similarly, Huang et al. (1996) used this technique during an investigation of the relative recoveries of deep-point injected soil <sup>15</sup>N by switchgrass (*Panicum virgatum* L.), alfalfa (*Medicago sativa* L.), and maize (*Zea mays* L.).

The results of the above studies indicated applicability of these <sup>15</sup>N-tracer methods in monitoring rooting systems and utilization of soil N by crops and trees in agroforestry systems. However, the main limitation of this technique is the high variability in plant root distribution relative to the <sup>15</sup>N application points. This results in varying signatures of <sup>15</sup>N uptake, which is a major source of experimental variability.

All earlier <sup>15</sup>N injection experiments had examined single species stands. In this study we mixed species and tested the hypothesis that when legume species are grown in mixtures, soil N uptake is maximized at different soil depths through their different rooting patterns and distribution within the soil horizons. We also assessed lateral and vertical movement of the <sup>15</sup>N-tracer in the field to evaluate the validity of the <sup>15</sup>N placement methodology. The objectives were to assess rooting pat-

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**Abbreviations:** DM, dry matter; Lrv, root-length density; PVC, polyvinyl chloride; SE, standard error of the means; SRL, specific root length.

tern and distribution of fallow legume species in sole and mixed fallow systems and to assess uptake and recovery of  $^{15}\text{N}$ -enriched fertilizer in mixed species stands.

## MATERIALS AND METHODS

This study was conducted in a farmer's field in western Kenya ( $0^{\circ}06'\text{N}$  lat.,  $34^{\circ}34'\text{E}$  long., 1330 m above sea level). Rainfall in the study area is distributed in two crop-growing seasons per year with an annual mean of 1800 mm. The long rainy cropping season extends from March to August and the short rainy cropping season extends from September to January. Daily rainfall pattern recorded during the course of the  $^{15}\text{N}$  injection experiment is shown in Fig. 1. Soils are highly weathered and are generally classified as very fine, kaolinitic, isohyperthermic Kandiodalfic Eutrudox. The initial soil physical and chemical characteristics at a depth of 0 to 0.15 m were: pH = 5.6 (in a 1:2.5 soil/water suspension); organic C = 14.0 g  $\text{kg}^{-1}$ , by wet oxidation with heated acidified dichromate followed by colorimetric determination of  $\text{Cr}^{3+}$  (Anderson and Ingram, 1993); extractable P = 1.3 mg  $\text{kg}^{-1}$  and exchangeable K = 0.3 cmol $\cdot\text{kg}^{-1}$  (by extraction with 0.5 M  $\text{NaHCO}_3$  + 0.01 M ethylenediaminetetraacetic acid, pH 8.5); and exchangeable Ca = 5.4 cmol $\cdot\text{kg}^{-1}$ , Mg = 1.70 cmol $\cdot\text{kg}^{-1}$ , and exchangeable acidity = 0.5 cmol $\cdot\text{kg}^{-1}$  (by 1 M KCl extraction); sand = 27%; clay = 52%; silt = 21%; and bulk density = 1.3 g  $\text{cm}^{-3}$ .

### Experimental Design

The experiment was superimposed within a larger legume screening experiment, consisting of single and mixed species stands, established in October 1997 (short rainy season) (Gathumbi et al., 2002b). Two fallow mixture types were selected for the  $^{15}\text{N}$  injection study based on their contrasting growth and above and potential belowground resource acquisition patterns. They were sesbania + crotalaria (50% sesbania and 50% crotalaria planted in alternate rows) and sesbania (100%) + siratro (as an understory plant) (Gathumbi et al., 2002b). Plant spacing was 0.75 by 0.75 m for sesbania + crotalaria, 0.75 by 0.75 m for sesbania in sesbania + siratro, and 0.375 by 0.75 m

for undersown siratro (Fig. 2). Two subplots for  $^{15}\text{N}$  injection each measuring 0.75 by 0.75 m were systematically laid out at diagonal corners of the main plot, which measured 6 by 5.25 m. To reduce risk of cross-contamination two replicates of the same application depth were located on the same main fallow plot. The experimental layout hence consisted of 16  $^{15}\text{N}$  placement subplots, eight of each 0.15- and 1.0-m application depths. Each  $^{15}\text{N}$  placement depth was tested in two species stands and replicated four times.

### Nitrogen-15 Depth Placement Procedures

Four months after fallow establishment ( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$  with an enrichment of 30.0 atom%  $^{15}\text{N}$ , was injected in equal aliquots through five polyvinyl chloride (PVC) tubes with a 2-cm i.d. previously installed in subplots at 0.15- or 1-m depths (Fig. 2). For each tube the  $^{15}\text{N}$  solution was applied through a funnel with an attached tube reaching to the bottom of the PVC tube at a rate of 25 mL (containing 0.4 g of N ( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$ ) and then flushed with an extra 25 mL of deionized water.

### Plant Sampling and Nitrogen-15 Recovery Calculations

To assess the time course of  $^{15}\text{N}$  uptake, the first four fully developed leaflets from the shoot tips from the center plants of the application subplot were sampled at 7, 14, 21, 28, and 35 d after  $^{15}\text{N}$  application. Final harvesting of the plants in the  $^{15}\text{N}$  application sites was performed on 24 March 1998, about 5 wk after the  $^{15}\text{N}$  application. Recovery of the applied  $^{15}\text{N}$  was measured only for the aboveground plant components. Center plants of woody species ( $n = 4$ ) were harvested individually while border plants ( $n = 12$ ) were bulked to give composite samples. For siratro center plants ( $n = 3$ ) and borders ( $n = 18$ ), all vines for individual plants were tracked down and harvested. All plant samples were separated into plant components (main stem, branches, and foliage) and total fresh weight determined. The stems and branches were then chopped into small pieces. All plant materials were thoroughly mixed before taking representative subsamples of each plant component using the quartering procedure. Subsamples weighing approximately 500 g were taken for oven-dry weight determination.

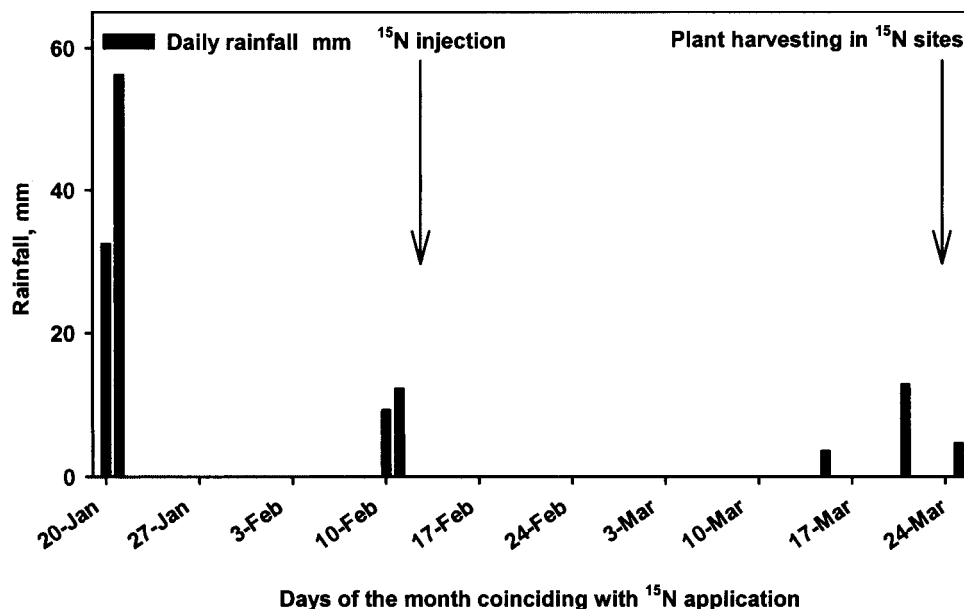


Fig. 1. Daily rainfall received from January through March 1998 during the period the  $^{15}\text{N}$  injection experiment was conducted in western Kenya.

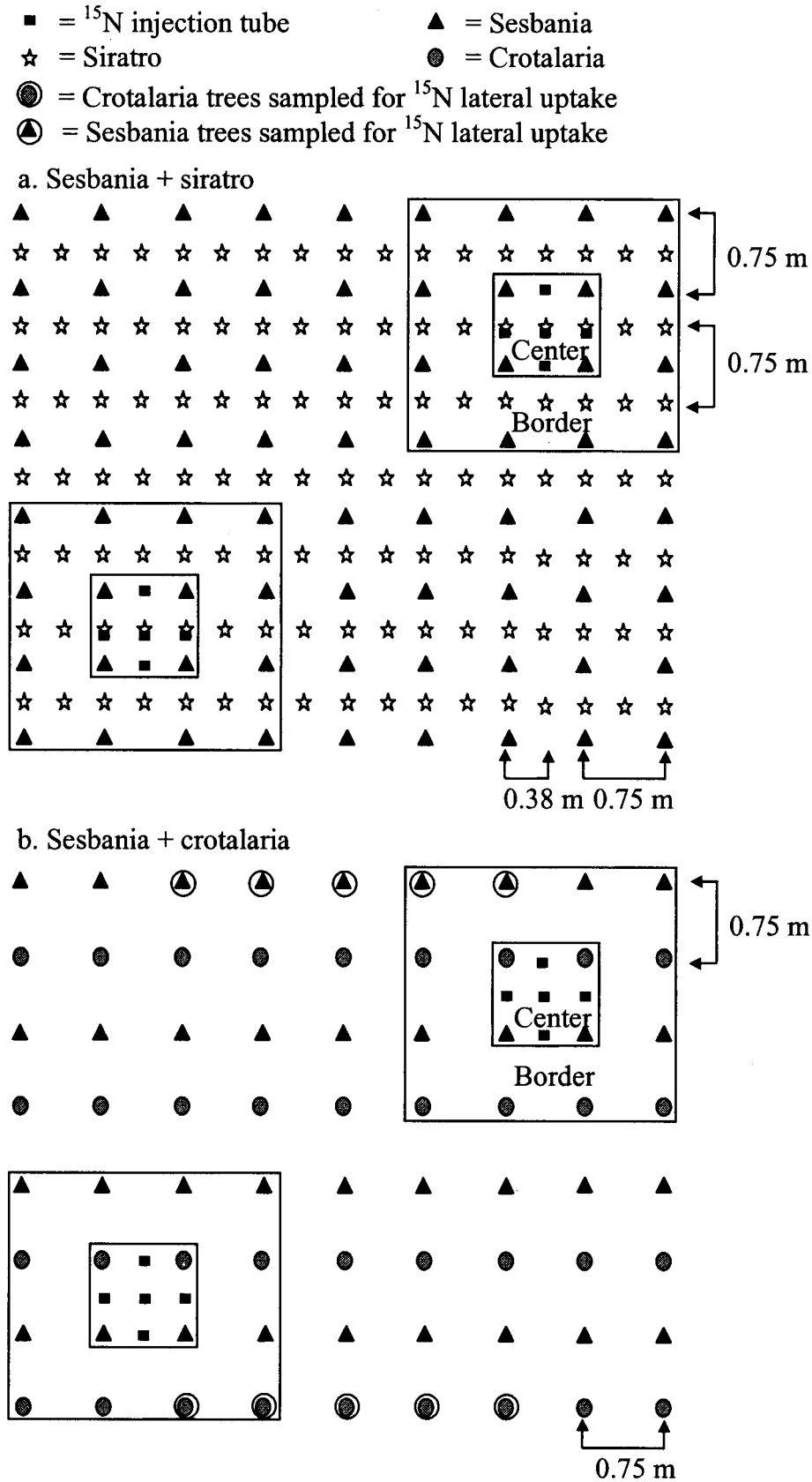


Fig. 2. Field layout of  $^{15}\text{N}$ -application subplots in two improved fallow systems in western Kenya.

Additional young fully developed leaf samples were taken from plants located at 0.75, 1.06, 1.68, 2.37, and 3.09 m from the injection point to generate data on uptake of labeled N by lateral roots and the minimum spatial distance required to separate  $^{15}\text{N}$  placement sites and avoid interference between plots (Fig. 2). The minimum distance between the sampled plant in the outer row and the nearest corner of the  $^{15}\text{N}$  site was calculated using the formula:

$$d = \sqrt{(x^2 + y^2)} \quad [1]$$

where  $d$  is the minimum distance between sampled plant and the nearest corner of  $^{15}\text{N}$  injection site,  $x$  is the distance between the corner of  $^{15}\text{N}$  injection site and the nearest plant in the outer row (0.75 m), and  $y$  is the distance between the nearest plant and the sampled plant.

All plant samples were oven dried (50°C, 72 h) and ground in order of expected enrichment to avoid cross contamination using a micro hammer mill. The percentage of N and the  $^{15}\text{N}$  enrichment of the samples were then determined using a 20-20 stable isotope mass spectrometer (PDZ formerly Europa Scientific, Crewe, UK) coupled to an automated CN analyzer (Crewe, UK).

The  $^{15}\text{N}$  enrichment of all materials (fertilizer and plant samples) was expressed as atom %  $^{15}\text{N}$  excess, which was determined from the natural  $^{15}\text{N}$  abundance of the soil (0.3668) or the respective plant N pool (Rowe and Cadisch, 2002) measured before application of  $^{15}\text{N}$  enriched fertilizer. The amount of  $^{15}\text{N}$  applied was calculated as  $^{15}\text{N}$  excess over the background abundance of  $^{15}\text{N}$  in soil, since it is the soil N pool to which enriched fertilizer was applied. The proportion of the total applied labeled N recovered by target plants was calculated using the formula:

$$\% \text{ N recovery} = \left[ \frac{\sum_i^n \text{Dry weight} \times \% \text{ N}/100 \times \text{atom \% } ^{15}\text{N excess}_{\text{sample}}}{\text{N}_{\text{fertilizer}} \times \text{atom \% } ^{15}\text{N excess}_{\text{fertilizer}}} \right] \times 100 \quad [2]$$

where  $i$  = plant components ( $n = 3$ ) of center ( $n = 4$ ) or border ( $n = 1$  composite) plants.

### Soil Sampling at Nitrogen-15 Application Sites

Soil samples were taken from under the  $^{15}\text{N}$  injection points on 9 Apr. 1998 (2 mo after application), to generate information on the possible vertical and lateral movement of the labeled fertilizer. Sampling was performed so that the auger hole was directly over the injection point. For the 0.15-m placement depth, vertical sampling was done from 0.15 to 1.15 m in 0.1-m intervals, and lateral sampling was done at 0.1-m intervals up to a 0.5-m distance. Lateral sampling was done at perpendicular distances away from the  $^{15}\text{N}$  application site with reference to one outer  $^{15}\text{N}$  injection tube at the 0.15-m application depth. For the 1.0-m placement depth, the same sampling intervals were used but starting at 1.0 m deep. The soil samples were air dried and finely ground and analyzed for percentage of N and  $^{15}\text{N}$ .

### Root Sampling and Analysis

Root studies were performed between 27 Feb. and 1 Mar. 1998 on replicated soil profiles for four selected treatments within the same main experiment (Gathumbi et al., 2002a), which included sesbania + crotalaria and sesbania + siratro among the mixed species fallows. In addition, root samples

were also taken from sesbania and crotalaria sole species fallow plots to offer additional data on rooting patterns of the same species when grown in monoculture arrangement. Root sampling was conducted on a profile wall exposed from a pit dug in one of the corners of each plot. The pits were dug 0.10 m from the base of the target plants and extended to 1.8-m depth. The orientation of the profile wall was such that it was diagonal to the line of trees and shrubs to be sampled. This was to ensure that the sampled area was representative of the unit area and soil covered by each plant following the suggestions of Rao and Coe (1991). The profile wall was smoothed using a machete.

Soil-root sampling was done using a monolith metal box sampler with measurements of 15 by 15 by 10 cm, giving a volume of 2250 cm<sup>3</sup>. The root-sampling depths were 0 to 0.15, 0.15 to 0.30, 0.30 to 0.45, 0.45 to 0.60, 0.60 to 0.90, 0.90 to 1.20, and 1.20 to 1.50 m. Soil-root samples were soaked in water for at least 12 h following the procedures of Bohm (1979). The stirred soil-root suspension was then passed through a 0.5-mm sieve. Roots and organic debris retained on the sieve were stored at 5°C in plastic bags containing 17% (v/v) acetic acid solution. The roots were separated from organic debris. In mixed fallow plots, roots of the respective species could not be recognized with certainty and hence the calculations were based on the composite root sample for each depth. The separated roots were stained with methyl violet solution (0.1% in 10% [v/v] ethanol), spread on a glass tray, and scanned with Delta-T Scan system (Delta-T Devices, Ltd., Cambridge, UK). Root images were obtained with a Hewlett Packard scanner (Hewlett Packard Analytical Group, Palo Alto, CA) and Aldus Photostyler image analysis software (Aldus Corp., Seattle, WA) at a resolution of 95 dots per centimeter. Root-length density (Lrv) was calculated by the formula of Newman (1966) when the tray contained nonoverlapping roots and by the formula of Harris and Campbell (1989) when the tray contained overlapping roots. Immediately after scanning, the root samples were oven dried (70°C, 48 h) and weighed.

### Statistical Data Analysis

Root data were tested for normality by plotting residuals against fitted values. They were not normally distributed and hence a square root transformation was performed for the percentage data; all other data sets were log transformed (logarithm to base 10) before analysis (Gomez and Gomez, 1984). Analysis of variance (ANOVA) was then performed using the general linear model procedure of the SAS program (SAS Institute, 1990). All data, however, are reported on an untransformed scale for comparison of treatment means.

## RESULTS

### Nitrogen-15 Uptake Patterns

The  $^{15}\text{N}$  enrichment of young leaves from the 0.15-m  $^{15}\text{N}$  placement depth was highest for sesbania followed by crotalaria and siratro (Fig. 3a). Even after only 1 wk, significant  $^{15}\text{N}$  enrichment was detected showing highly active plant roots at that soil depth. The  $^{15}\text{N}$  enrichments of young leaves increased progressively with time, but increments decreased in sesbania after 21 d. At all sampling times the  $^{15}\text{N}$  leaf enrichment was highest for sesbania and lowest for siratro.

At 1-m placement depth, the  $^{15}\text{N}$  leaf enrichment remained low for the first 14 d after  $^{15}\text{N}$  placement for all species tested, but thereafter started to increase signifi-

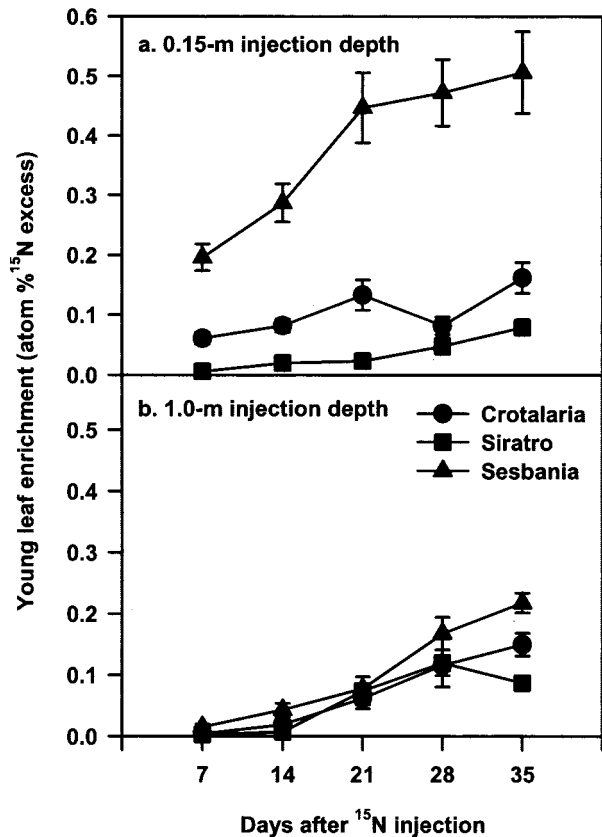


Fig. 3. Nitrogen-15 concentration in young leaves after application of  $^{15}\text{N}$  at two soil depths in western Kenya.

cantly, except for siratro for which  $^{15}\text{N}$  enrichment declined after 28 d (Fig. 3b). The leaf  $^{15}\text{N}$  enrichment of sesbania was less with 1-m placement than with 0.15-m placement, while enrichments were unaffected by placement depth with crotalaria and siratro at 35 d. The highest  $^{15}\text{N}$  enrichment was again obtained from sesbania at 35 d after  $^{15}\text{N}$  placement at 1-m.

#### Nitrogen-15 Uptake at Lateral Distances From the Application Site

Young leaf  $^{15}\text{N}$  enrichment for sesbania and crotalaria plants sampled at varying lateral distances from  $^{15}\text{N}$  application sites showed a decreasing enrichment with increasing distance (Fig. 4). At any one distance, the  $^{15}\text{N}$  concentration was higher for sesbania than crotalaria. The  $^{15}\text{N}$  enrichment for the leaf materials sampled at 0.75 m from the nearest sampling point was 0.01 and 0.07 atom%  $^{15}\text{N}$  excess for crotalaria and sesbania, respectively. No significant  $^{15}\text{N}$  enrichment was detected in plant samples taken >1.7 m (crotalaria) and >2.4 m (sesbania) away from the injection point when compared with the background  $^{15}\text{N}$  enrichment of plant materials grown in unenriched soil.

#### Lateral and Vertical Residual Soil Nitrogen-15

Five weeks after  $^{15}\text{N}$ -enriched fertilizer application, most of the residual  $^{15}\text{N}$  was observed to be within 0.10-m lateral distance relative to the injection point, which averaged 0.5 atom%  $^{15}\text{N}$  excess. Soil samples

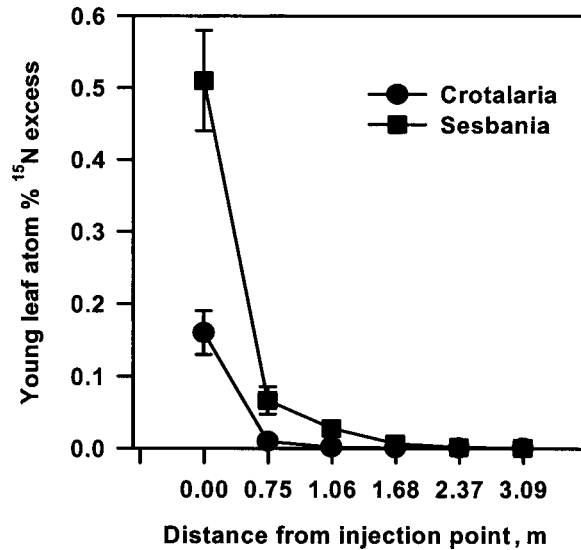


Fig. 4. Young leaf  $^{15}\text{N}$  concentration for sesbania and crotalaria plants sampled at various distances from the  $^{15}\text{N}$  injection point at 9 wk after application in the field.

taken 0.20 to 0.50 m away from the  $^{15}\text{N}$  injection point had  $^{15}\text{N}$  enrichment similar to the background soil.

Vertical atom%  $^{15}\text{N}$  excess distribution down the soil profile decreased with depth indicating some gradual downward movement (leaching) of the applied  $^{15}\text{N}$  relative to the application point (Fig. 5). Nitrogen-15 enrichment detected in soil samples taken at 0.40 m below the 0.15-m application depth and 0.80 m below the 1.0-m application depth were similar to the background enrichment of the soil (0.3668 atom%  $^{15}\text{N}$  excess).

#### Plant Root Distribution

Total root length decreased with profile depth (Table 1). The highest total root length to 1.5-m depth was observed in crotalaria + sesbania ( $6.7 \text{ km m}^{-2}$ ) and the lowest was with sesbania + siratro ( $2.2 \text{ km m}^{-2}$ ). Root data from several depths were pooled because of high variability of the data. The 0- to 0.3-m soil layer was observed to contain the highest proportion of the total root length, the highest being 76% for crotalaria. At 0.3- to 0.6-m depth a higher proportion of roots was observed for mixtures of sesbania + crotalaria (26%) compared with sole sesbania (16%) and sole crotalaria (10%) treatments. Sole crotalaria treatments had a proportionally lower root length at 0.6- to 1.5-m soil depth, accounting for only 14% of the total root length compared with 25 to 33% of the other fallow treatments. With respect to total root length, sesbania + siratro mixtures had lower ( $P < 0.05$ ) root length at 0- to 0.3-m depth compared with crotalaria. No statistical differences in root length among treatments were observed below 0.6 m.

As with root length, the Lrv ( $\text{cm cm}^{-3}$ ) decreased with depth. The highest Lrv in the 0- to 0.15-m soil layers was with crotalaria and the lowest was with sesbania + siratro (Fig. 6). The Lrv of sesbania + crotalaria was observed to be larger in the 0.30- to 0.60-m soil layer as compared with other treatments at the same depth.

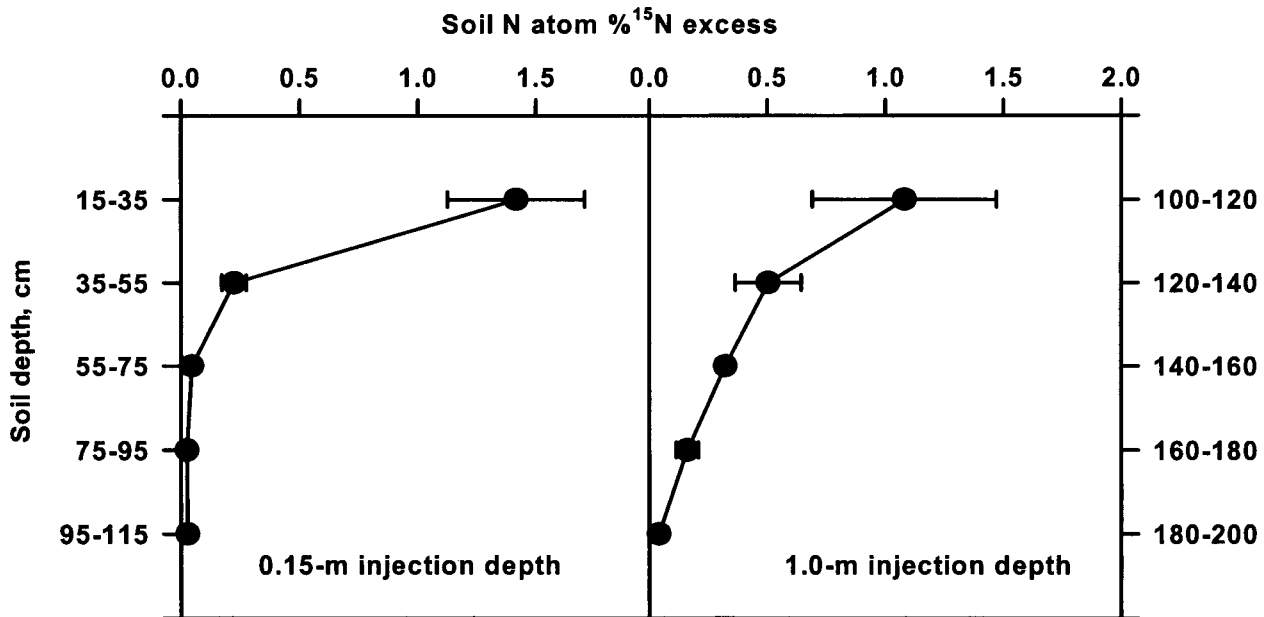


Fig. 5. Residual soil atom% <sup>15</sup>N excess at vertical distances below the 0.15- and 1.00-m depths for <sup>15</sup>N injection at 9 wk after application.

Statistical differences in specific root length (SRL, m g<sup>-1</sup>) between treatments were observed only in the top 0.3 m where SRL of sesbania was significantly less (*P* < 0.05) than that of crotalaria. With the exception of sesbania + crotalaria at the 0.3- to 0.6-m depth, the SRL of crotalaria was consistently higher in all soil depths compared with other treatments, suggesting that this species had a higher proportion of fine roots than coarse roots at all layers.

The sesbania + siratro mixed fallow was among the treatments observed to have the least root biomass at all soil depths. Total root biomass to 1.5-m soil depth was significantly higher (*P* < 0.05) for sesbania + crotalaria mixtures (1343 kg ha<sup>-1</sup>) compared with other treatments.

### Nitrogen-15 Recovery by Sesbania, Crotalaria, and Siratro

Total biomass yield determined from all plants harvested from the <sup>15</sup>N-injection subplots was not different (*P* > 0.05) between the fallow types. Total biomass for the harvested center and border plants in sesbania +

crotalaria mixture was 252 (±17 SE) g m<sup>-2</sup> (56% sesbania, 44% crotalaria) and for sesbania + siratro was 372 (±67 SE) g m<sup>-2</sup> (91% sesbania, 9% siratro). In both mixed stands sesbania trees contributed a higher proportion of the biomass than the companion species crotalaria or the understory plant siratro.

In sesbania and crotalaria mixtures, <sup>15</sup>N recovered by center trees was 64 mg <sup>15</sup>N site<sup>-1</sup> for sesbania and 78 mg <sup>15</sup>N site<sup>-1</sup> for crotalaria when labeled N was injected at the 0.15-m depth (Table 2). Crotalaria border plants recovered less (7 mg) of the applied N than sesbania border trees (13 mg). Total mean <sup>15</sup>N recovery from the 0.15-m application depth was 162 mg, representing 27% of the applied N. Sesbania and crotalaria contributed 13 and 14%, respectively (Table 3).

Sesbania and siratro in mixture recovered in total 22% of the applied N at 0.15 m with sesbania and siratro contributing 16 and 6% respectively. While sesbania center plants contributed most to the <sup>15</sup>N recovery, the stoloniferous siratro border plants were equally active in <sup>15</sup>N recovery as were sesbania border plants (Table 2). At 1.0-m application depth, sesbania grown in cro-

Table 1. Distribution of root length, specific root length, and root biomass with soil depth for different species.

Profile depth	Fallow species	Root length	Total root length†	Specific root length	Root biomass
m		km m <sup>-2</sup>	%	m g <sup>-1</sup>	kg ha <sup>-1</sup>
0-0.3	Crotalaria	4.8a	76a	80a	522ab
	Sesbania	1.5ab	54ab	35b	547ab
	Sesbania + crotalaria	3.1ab	49b	61ab	793a
	Sesbania + siratro	1.0b	46b	59ab	229b
	Crotalaria	0.6b	10b	78a	83b
0.3-0.6	Crotalaria	0.5b	16ab	56a	136b
	Sesbania	1.9a	26a	93a	214a
	Sesbania + crotalaria	0.4b	21ab	55a	85b
	Sesbania + siratro	0.9a	14b	87a	111b
	Crotalaria	0.8a	30ab	61a	136b
0.6-1.5	Sesbania + crotalaria	1.6a	25ab	85a	336a
	Sesbania + siratro	0.8a	33a	68a	125b

† Analysis of variance for total root percentage was constructed on square root transformed data. The rest of the data sets were log-transformed before conducting the ANOVA. Means in a column followed by the same letter within a profile depth are not significantly different by Tukey test (*P* = 0.05). Root data are reported on untransformed scale for comparison of treatment means.

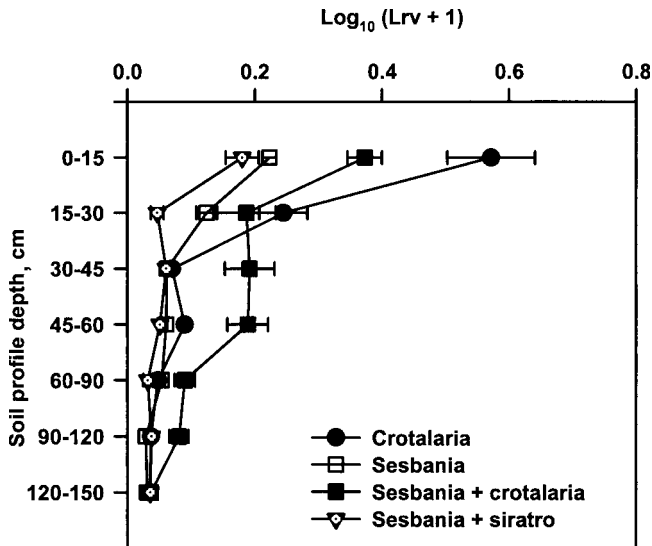


Fig. 6. Effect of soil depth on root length density ( $Lrv$ ) of pure and mixed species.

talaria + sesbania mixtures had a higher recovery of  $^{15}N$  (32 mg  $^{15}N$ ) than crotalaria (17 mg  $^{15}N$ ) for the center plants. Also sesbania border plants recovered more  $^{15}N$  than crotalaria border plants. Total mean recovery was 13% of the applied N. The sesbania + siratro mixture recovered a similar amount of  $^{15}N$  from the 1.0 m as with the 0.15-m application depth. However, most of the  $^{15}N$  was recovered by sesbania center plants (57 mg  $^{15}N$ ), compared with only 9 mg  $^{15}N$  recovered by siratro. Siratro border plants recovered a higher proportion of the applied N compared with the center plants. Total mean  $^{15}N$  recovery from 1 m was 18% (107 mg  $^{15}N$  site $^{-1}$ ) of the applied N (Table 3).

## DISCUSSION

### Use of Nitrogen-15 Placement to Differentiate Uptake in Mixed Species Stands

The recovery of applied N by plants depends on location and spatial distribution of active roots relative to the  $^{15}N$  application point. In this study there was a large variation in vertical root distribution for all the species within the profile and also across the four replications. As such the standard errors for the means in  $^{15}N$  uptake

were large as would be expected (Table 2). Large variations in root distribution were also reported for gliricidia [*Gliricidium sepium* (Jacq.) Kunth ex Walp.], peltophorum [*Peltophorum dasyrrhachis* (Miq.) Kurz.] (Rowe et al., 1999), and sesbania (Jama et al., 1998; Mekonnen et al., 1999). This variability resulted from the sporadic rooting patterns of the trees. Thus, sufficient replications must be used if small differences in  $^{15}N$  uptake are to be detected.

Residual  $^{15}N$  enrichment of the soil samples taken at various lateral and vertical distances from the  $^{15}N$  injection points revealed more downward than lateral movement of the injected  $^{15}N$  (Fig. 5). This could have been as a result of leaching, although there remained a pronounced enrichment of  $^{15}N$  close to the injection point even 9 wk after  $^{15}N$  application. To reduce  $^{15}N$  movement in soil, ( $^{15}NH_4$ ) $_2$ SO $_4$  could be applied in combination with a C source such as sucrose at a C/N ratio of 10:1 to facilitate microbial immobilization of the  $^{15}N$ , and thus restricting it more closely to the applied depth (Witty, 1983; Rowe et al., 1999). Alternatively,  $^{15}N$  can be applied in the form of resin-adsorbed  $^{15}N$  whereby weakly acidic cation adsorption resins are saturated with enriched fertilizer; however, difficulties are often encountered in achieving sufficient  $^{15}N$  saturation in resins, which leads to inefficient use of the isotope.

The vertical movement (leaching) of the placed  $^{15}N$  fertilizer leads to some of it being taken up by plant roots at soil layers below the placement point and hence the uptake should be considered to be from that soil layer rather than from a point. The application depth thus represents the minimum depth at which the labeled N is placed. The amount of  $^{15}N$  applied should provide sufficient enrichment but should not significantly increase the available soil N pool around the injection points as this could alter the uptake pattern and thereby introduce some errors (Witty, 1983). Assuming that applied N extended horizontally largely in a radius of 0.20 m (as indicated by our results), the application rate was thus equivalent to around 40 kg N ha $^{-1}$  within this area. This corresponds close to the 27 to 30 kg inorganic N (NO $_3$ -N + NH $_4$ -N) ha $^{-1}$  found at the two depths at the start of the experiment (Gathumbi et al., 2002b). Potential interferences of applied N with root activity

Table 2. Recovery of applied  $^{15}N$  from 0.15- and 1.0-m placement depths by sesbania, crotalaria, and siratro grown in mixtures as determined from "center" and "border" plants relative to the  $^{15}N$  injection point in the field in western Kenya.

Species mixture	Species	Zone	$^{15}N$ recovery, mg site $^{-1}$			
			0.15-m placement depth		1.0-m placement depth	
			Mean	SE†	Mean	SE
Sesbania + Crotalaria	Sesbania	Center	64	9.2	32	7.0
		Border	13	3.9	23	10.7
	Crotalaria	Center	78	28.8	17	6.0
		Border	7	3.3	7	4.6
<b>Total</b>		<b>162</b>	<b>28.1</b>	<b>79</b>	<b>25.3</b>	
Sesbania + siratro	Sesbania	Center	80	9.3	57	14.7
		Border	16	4.2	24	18.5
	Siratro	Center	16	6.7	9	3.8
		Border	20	8.2	17	14.4
<b>Total</b>		<b>130</b>	<b>21.3</b>	<b>107</b>	<b>29.5</b>	

† SE, Standard error of the mean.

**Table 3. Recovery of the labeled N from 0.15- and 1.0-m placement depths by sesbania, crotalaria, and siratro grown in mixtures in the field in western Kenya.**

Species mixture	Species	Plant density plants ha <sup>-1</sup>	Recovery of labeled N	
			0.15-m placement depth	1.0-m placement depth
			Mean†	Mean
Sesbania + crotalaria	Sesbania	8 890	13.0ab	9.3ab
	Crotalaria	8 890	14.3ab	4.1b
<b>Total</b>			27.3	13.4
Sesbania + siratro	Sesbania	17 780	16.0a	13.7a
	Siratro	35 560	5.9b	4.3b
<b>Total</b>			21.9	18.0

† Means in a column followed by the same letter are not significantly different by Tukey test ( $P = 0.05$ ).

patterns could be further reduced by applying less fertilizer N but at higher enrichment (e.g., 99 atom% <sup>15</sup>N).

The <sup>15</sup>N placement method was successful in quantifying and separating N uptake patterns from different depths by individual species in mixed stands. The <sup>15</sup>N recovery for the species reported here were higher than those reported for peltophorum and gliricidia (Rowe et al., 2001) or *Calliandra calothyrsus* Meissn. and *Leucaena leucocephala* (Lam.) deWit. (Mugendi et al., 1999) in hedgerow intercropping systems. In those studies the <sup>15</sup>N enriched materials were applied away from the tree rows, as opposed to this study where the <sup>15</sup>N fertilizer was applied very close to the target plants.

The low <sup>15</sup>N recovery by border tree plants at all placement depths suggested that the plant roots extended more vertically than horizontally relative to the application sites in these relatively dense stands. Plant roots in monoculture or in mixed species systems are likely to have variable horizontal growth and distribution depending on the nutrient and water availability at topsoil layers and also the degree of competition with the neighboring plants (Fitter, 1991; Caldwell, 1994; van Noordwijk and Purnomosidhi, 1995; Schroth, 1998). Trees are known to have more extended horizontal root growth than shrubby species or even annual crops and hence may source nutrients far from their growing position. As such, in studies on nutrient uptake by plant roots using <sup>15</sup>N techniques, it is important to know horizontal root activity of the specific species before setting up the experiment, to reduce the chances of plants scavenging the applied <sup>15</sup>N from the neighboring plot. In the current study, the enrichment of the leaf samples obtained at various distances from the injection point revealed that in this type of experimental setup, the minimum spatial placement distances between two <sup>15</sup>N application sites should be >4 m for crotalaria and >6 m for sesbania (Fig. 4). Rowe and Cadisch (2002) recommended a minimum interplot spacing of 6 to 8 m for short-term <sup>15</sup>N experiments with gliricidia and peltophorum hedgerow intercropping systems. Trees that are substantially larger than those studied here, however, are likely to have more extensive root systems and larger distances will be required. Tree rooting extent is also likely to be greater on sites with greater moisture deficits, more infertile soils, or shallower rooting depths (Helliwell, 1986).

### Complementarity in Soil Nitrogen Acquisition

The differences in <sup>15</sup>N uptake showed that sesbania had more root activity at lower soil depths than either siratro or crotalaria, despite sesbania and crotalaria having similar Lrvs at 1-m depth in single species stands (Fig. 6). However, mixing sesbania with crotalaria increased the amount and distribution of roots, particularly in the 0.30- to 0.60-m depth, but also at lower soil depths up to 1.2 m. This suggests that mixing sesbania with crotalaria may have increased competition for growth resources (nutrients and water) in the topsoil layer and hence this overlapping of feeding niche stimulated downward development of roots to lower soil depths. In other words, more roots needed to be established at lower layers to increase subsoil nutrient capture driven by the N demand within each species. Given the higher <sup>15</sup>N uptake of sesbania at the lower soil depths, it may be assumed that sesbania Lrv was increased in this case. Unfortunately, it was not possible to distinguish the two root systems with certainty to verify this hypothesis.

Van Noordwijk et al. (1996) noted that increased root establishment in species mixtures could enhance complementarity in resource use at subsoil horizons in an attempt to acquire an equilibrium between growth resource availability and plant-root uptake because the vertical distribution of roots is greatly influenced by the relative success of roots in taking up the most limiting resource in the upper soil profile. The higher Lrv for crotalaria than sesbania in topsoil and high <sup>15</sup>N recovery from topsoil by crotalaria supports the hypothesis that sesbania root development at lower soil depths was stimulated by competition from crotalaria. This is further corroborated by the fact that on a per plant basis sesbania took up more <sup>15</sup>N from 1-m depth when mixed with crotalaria than when mixed with siratro.

The higher topsoil recovery of the applied N by the sesbania + crotalaria mixture compared with the sesbania + siratro mixture corresponded with the higher total root length (Table 1) and Lrv (Fig. 6) in the topsoil (0–0.30 m). Because of the similar distribution of fine roots for both mixed species fallow treatments as depicted by the SRL measurements, the higher uptake of <sup>15</sup>N by sesbania + crotalaria treatments could largely be attributed to the Lrv, which is reported to greatly influence the soil nutrient accessibility to plants (van

Noordwijk and Brouwer, 1991; Schroth and Zech, 1995; Schroth, 1998).

Root data obtained for sesbania was comparable with those reported in previous studies conducted in the study area. For instance, root length recorded for the 0- to 0.3-m soil depth for 11- and 15-mo-old sesbania fallows planted at a spacing of 1.0 by 1.0 m was 1.3 km m<sup>-2</sup> (Jama et al., 1998) and 1.5 km m<sup>-2</sup> (Mekonnen et al., 1997), respectively, compared with 1.5 km m<sup>-2</sup> reported in this study. The high SRL for sesbania in this study for 0- to 0.3-m soil depth (35 m g<sup>-1</sup>) compared with 12 m g<sup>-1</sup> reported for the 11-mo-old sesbania fallow reported by Jama et al. (1998) could be attributed to the younger age and the higher plant density. Specific root length is an indication of the relationship between root biomass and the ability of the root systems of crops to penetrate a given soil (van Noordwijk and Brouwer, 1991). The values obtained for SRL measurements observed in most of the fallow tree and shrub species indicate that these species require less root biomass to produce a unit length of roots compared with other species with a higher proportion of coarse roots. In other words, more fine roots were observed for all the species, which can be attributed to their relatively young age.

### Elucidation of Temporal Changes in Nitrogen Uptake Patterns

The recovery of the labeled N per site or even for individual species did not necessarily correspond with the amount of total biomass or N yield accumulated. This could be because of the fact that <sup>15</sup>N uptake measurements corresponded only for the later growth phase of the fallows and was partly influenced by the onset of the dry season (Fig. 1). Thus <sup>15</sup>N uptake pattern are not only influenced by Lrv and <sup>15</sup>N distribution but also strongly affected by plant N demand and accessibility of water and N during this phase. The onset of the dry season could explain why a high proportion of <sup>15</sup>N was taken up from a depth of 1 m. This is further corroborated by the decline in <sup>15</sup>N uptake in the topsoil toward the end of the experiment, while the opposite occurred in the lower soil layer (Fig. 3). Thus the <sup>15</sup>N data gives additional insight into the temporal pattern of N uptake at various soil depths, which are not revealed by root mapping observations. Although <sup>15</sup>N enrichments of young leaves give useful insights into the temporal uptake patterns of labeled N of a given species, it must be taken into consideration that <sup>15</sup>N enrichment in young leaves is not an indicator of total plant <sup>15</sup>N uptake, as this depends on total plant N yield and <sup>15</sup>N distributions within the plant (Rowe et al., 1999). The index hence has to be used with caution and put in the context of other parameters, for example, in our case, the relative differences in <sup>15</sup>N uptake patterns between topsoil and subsoil (Fig. 3) correspond with the actual total <sup>15</sup>N uptake (Table 3).

A further reason why uptake of soil mineral N by legumes is not necessarily correlated to growth demand is the potential of legumes to acquire N from atmospheric N<sub>2</sub> by means of their symbiotic relationship with

*Rhizobium*. Both sesbania and crotalaria have been shown to obtain over 50% of their N demand from symbiotic N<sub>2</sub> fixation (Gathumbi et al., 2002a). In fact, crotalaria obtained over 75% of its N from N<sub>2</sub> fixation compared with around 50% by sesbania, suggesting that this species needed to acquire more soil N and this may explain the higher <sup>15</sup>N uptake activity. It may also be argued that the much faster early uptake of <sup>15</sup>N by sesbania compared with crotalaria (Fig. 3) further suggests a higher dependency of sesbania on soil mineral N.

### CONCLUSIONS

The <sup>15</sup>N placement technique for assessing subsoil N uptake by the test legume species can be used with an appreciable degree of certainty in tracing <sup>15</sup>N uptake by legume species in fallow systems and also in studies on rooting patterns. The method appears particularly useful in mixed species stands to distinguish root activity between species at a given depth and to assess root plasticity in response to competition by the companion species. In this study, the uptake of the labeled <sup>15</sup>N from different soil depths by different species in fallows corresponded with total root length and the Lrv. However, when designing such experiments, species rooting patterns and distribution should be considered because these constitute the highest source of error as has been observed in this study. Increasing the number of treatment replications and sampling points may partly reduce the experimental errors associated with plant root heterogeneity; while increasing <sup>15</sup>N enrichment of N applied will allow reducing the amount of N applied hence avoiding preferential root responses to added N.

Whereas legume species evaluated in this study could be used for subsoil N retrieval when grown in sole species fallows, the root, and <sup>15</sup>N recovery data accruing from this study suggest that there is complementarity in the system when they are grown in mixtures. For instance, when sesbania and crotalaria were mixed there was more subsoil N uptake by sesbania while crotalaria is reported to source a higher proportion of its N from N<sub>2</sub>-fixation and topsoil N uptake. Again it appears that mixing crotalaria and sesbania introduces competition of growth resources in the topsoil and thus more roots are established at lower zones, expanding the nutrient uptake zone for the two species.

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