Quantification of biological N_2 fixation of hedgerow trees in Northern Lampung

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Abstract

The contribution of the leguminous hedgerow trees *Flemingia congesta* and mixed *Gliricidia sepium – Peltophorum dasyrrachis* established on an Ultisol (Grossarenic Kandiudult) in Northern Lampung, Sumatra to the N economy of the hedgerow intercropping system was assessed. N_2 fixation estimates of hedgerow trees over a two year period after establishment were obtained by the ^{15}N dilution method using *Peltophorum dasyrrachis* as the non-fixing reference plant.

Average yearly tree pruning dry matter and total N yields of the two hedgerow systems were not significantly different although seasonal differences occurred. *Gliricidia* obtained a larger (average 51%) proportion of its N from N_2 fixation than *Flemingia* (average 25%) the effect being consistent over the two years. Seasonal variation in the % N derived from N_2 fixation was associated with changes in soil mineral N availability. The amount of N_2 fixed was not significantly different between the two N fixing trees (26 vs. 35 kg N ha^{-1} yr^{-1} respectively). This was due to the higher total N yield of *Flemingia* compared to *Gliricidia* compensating for the smaller proportion of N derived from N_2 fixation. The amount of tree soil N uptake was similar for the two hedgerow systems suggesting that the higher proportion of N derived from N_2 fixation in *Gliricidia* was probably due to competition for soil mineral N by the associated non-fixing *Peltophorum*. Rapid temporal and spatial decline in plant available ^{15}N occurred suggesting that matching of fixing and non-fixing reference plants is important. Compared with estimates of N_2 fixation obtained by the natural abundance method the ^{15}N dilution method overestimated N_2 fixation by the tree legumes (an average of up to 18%), however the difference between the two fixing legume trees was maintained. N balance estimates suggested that N_2 fixation inputs of alley trees were sufficient to sustain moderate crop yields but for higher food crop yields, N_2 fixing leguminous crops would have to be included to balance N off-take.

Keywords: Flemingia congesta, Gliricidia sepium, hedgerows, biological N_2 fixation, natural ^{15}N abundance, ^{15}N dilution

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Introduction

The legume tree-*Rhizobium* symbiosis provides potentially an alternative to N fertilizers to balance N losses of agricultural systems through its ability to fix atmospheric N_2. Hedgerow trees whose prunings are returned to the soil can theoretically make a larger contribution to the systems N balance than grain legumes as their N harvest index is zero (Giller & Cadisch, 1995). Catchpoole & Blair (1990) reported N yields of up to 700 kg ha^{-1} in 14 month old stands of *Gliricidia sepium* in the humid tropics of Indonesia. Estimates of the proportion of such N derived from N_2 fixation in agroforestry trees vary widely (0–100%) depending on species, system, management and environmental conditions (Giller *et al.*, 1994). Estimates under field conditions suggest that up to 274 kg N ha^{-1} may be fixed in half a year by *Leucaena leucocephala* in dense stands in the humid tropics using the N difference method (Sanginga *et al.*, 1986). However, the amounts of N_2 fixed by hedgerow trees are likely to be much less than this due to the sparser tree density in this type of cropping system.

Adapted young trees established in degraded soils will nodulate profusely and fix N_2 actively due to the limited N supply from the soil. However, in mature tree based systems N_2 fixation often declines due to the recycling of fixed nitrogen in litterfall and prunings (Van Kessel *et al.*, 1994). The extent to which such autoregulation of N_2 fixation occurs depends on how much crop competition for N and N losses occur which limit the supply of soil N available to the tree.

Despite the large interest in nutrient cycling in hedgerow intercropping systems few reliable estimates of N_2 fixation of the tree component are available. Yield independent estimates of the different N sources in plants compared to the N difference method can be obtained by using the ^{15}N dilution method with the application of a small dose of ^{15}N enriched fertilizer. While the ^{15}N dilution method has found widespread acceptability for annual crops and herbaceous forage legumes the use of this method is more problematic with trees. This is due to the large variation in rooting depth of trees and the non-uniform vertical distribution of applied ^{15}N, which makes it more difficult to find matching non-fixing reference plants. Choice of the reference plant appears less critical when the natural ^{15}N enrichment of the soil is used instead as variations in the natural ^{15}N abundance with depth are often smaller (Cadisch *et al.*, 2000) but not necessarily so (Ledgard *et al.*, 1984). A further complication for assessment of N_2 fixation in perennial plants is the remobilization of N from roots and trunks at different phases of growth (cf. Ledgard *et al.* (1991) hence fixation rates measured in prunings may not always reflect current N_2 fixation activity. The use of several methods has been suggested to check for potential errors of individual approaches (Viera-Vargas *et al.*, 1995). Monitoring the xylem ureide content is potentially an alternative method to estimate N_2 fixation in trees. However, the method is restricted to ureide producers, mainly of the tribes *Phaseoleae* and *Desmodieae* (Giller & Wilson, 1991), and appears thus not to be suitable for *Gliricidia sepium* and *Flemingia congesta* used in this study (Herridge *et al.*, 1996). While the natural ^{15}N abundance method maybe be better suited to account for differences in rooting depth between the fixing and non-fixing reference plant it is potentially more susceptible to errors associated with remobilization and recycling of

fixed N as well as isotopic discrimination processes during N transformations particularly in established ecosystems (Handley et al., 1994; Shearer & Kohl, 1986). We used the ^{15}N enrichment method to estimate N_2 fixation of established alley cropping trees Gliricidia sepium and Flemingia congesta in Northern Lampung, Indonesia and included comparisions with the natural ^{15}N abundance method to estimate the degree of potential methodological errors.

Materials and methods

Site

The experiment was carried out at the BMSF (Biological Management of Soil Fertility) project site of Brawijaya University at North Lampung, Sumatra, Indonesia (4°30'S, 104°98'E). The soil at the study site is a Grossarenic Kandiudult (Van der Heide et al., 1992) with 65% sand, 17% silt and 18% clay. Soil fertility characteristics (0–15 cm) were: pH (H_2O) 5.4; 2.2 % organic C (Walkley-Black); 0.13% total N (Kjeldahl), 11 mg kg^{-1} P (Bray II), cation exchange capacity 5.02 $cmol_{(+)}$ kg^{-1}; 0.34, 0.16, 2.29 and 1.10 $cmol_{(+)}$ kg^{-1} of Na^+, K^+, Ca^{2+} and Mg^{2+}, respectively in ammonium acetate pH 7 (Hairiah et al., 2000). Mineral N analysis were done by distillation of 1 M KCl soil extracts. The area has an average annual temperature of 26.3°C, humidity of 96% and rainfall of 2580 mm.

Hedgerow management

Hedgerows were established in a comparative multi-species rotation system experiment in November 1994 described in detail by Hairiah et al. (2000). Hegederow systems included: i) mixed Gliricidia sepium-Peltophorum dasyrrachis alley cropping with mixed rice+maize followed by groundnut and cowpea and ii) single species Flemingia congesta alley cropping with mixed rice+maize followed by groundnut and cowpea. Plot size was 12 × 13 m^2 with four blocks. Gliricidia and Peltophorum were planted alternately within single rows, using a plant distance of 4 × 0.5 m. Flemingia was planted with a plant distance of 4 × 1 m. Thus the individual tree populations became 2500 per ha. A basal fertilizer rate of 17 kg P ha^{-1} and 50 kg K ha^{-1} was applied to the rice+maize and groundnut crops. Trees were first pruned in April 1995 (establishment phase, not included in this study) and thereafter regularly pruned at 75 cm for two years until March 1997. The pruning biomass was weighed freshly, subsampled and the remainder returned and spread evenly onto the plot. Biomass subsamples were separated into leaves and stems, dried, ground and analyzed.

N_2 fixation

^{15}N dilution method
In April 1995 ^{15}N was applied as ammonium sulphate at a rate of 10 kg N ha^{-1} (10.2 atom %^{15}N) in solution mixed with glucose at a C:N ratio of 10:1 in order to immo-

bilize the ^{15}N more rapidly into the soil microbial biomass (Giller & Witty, 1987). The ^{15}N application area was 5 × 3 m in the hedgerow system covering a 3 m wide strip across the alley. ^{15}N was applied in three replicates. For ^{15}N enrichment measurements the three central trees were harvested at the time of pruning the unlabelled main plot. Prunings were separated into leaves and stems and analyzed for ^{15}N enrichment using an automated CN analyzer (Roboprep) coupled to an isotope ratio mass-spectrometer (Model 20–20, Europa Scientific, Crewe).

Based on the assumption that the non-fixing reference plant takes up a similar proportion of soil-N:fertilizer-^{15}N as the fixing plant the proportion of N derived from the atmosphere can be calculated as (McAuliffe *et al.*, 1958):

$$\% \, N_2 \, fixation = [1 - \frac{atom \, \%^{15}N \, excess \, fixing \, legume}{atom \, \%^{15}N \, excess \, non \, fixing \, reference}] \times 100 \qquad 1$$

where atom % ^{15}N excess = atom % ^{15}N – 0.3663 (natural ^{15}N abundance of atmospheric N$_2$). Pruning atom % ^{15}N excess was calculated as a weighted mean of the enrichments of leaves and stems. *Peltophorum dasyrrachis* was used as the non-fixing reference plant (Rowe *et al.*, 1999).

Natural ^{15}N abundance method:
Due to potential problems with ^{15}N contamination only trees in replicate four which received no fertilizer ^{15}N were sampled in July and October 1995 and March and October 1996. Harvesting procedures were as above however, and harvested material was handled separately from enriched samples to avoid contamination. Calculations for N$_2$ fixation for the natural abundance method were as follows (Amarger *et al.*, 1979):

$$\% \, N_2 \, fixation = [1 - \frac{\delta^{15}N \, reference - \delta^{15}N \, fixing \, legume}{\delta^{15}N \, non \, fixing \, reference - B}] \times 100 \qquad 2$$

where δ^{15}N ‰ = [(^{15}N/^{14}N sample / ^{15}N/^{14}N standard) – 1] × 1000 and where standard is atmospheric N$_2$. *B* is the δ^{15}N value of the same N$_2$ fixing plant when grown with N$_2$ as the sole source of N. B values of –1.11 and –1.32 for *Gliricidia sepium* and *Flemingia congesta* respectively were used (unpublished data).

Results and discussion

The average dry matter yield of prunings was similar for both hedgerow systems over the 2 years (Table 1). In the mixed hedgerow, *Gliricidia* performed poorly during the first year after establishment but showed better pruning yields than the associated *Peltophorum* towards the end of the evaluation period (Figure 1). There was little production during the dry period between April and September 1996.

Yearly average total N yield was similar between the two hedgerow systems (Table 1). *Peltophorum* accumulated more N than *Gliricidia* in the mixed hedgerow as an
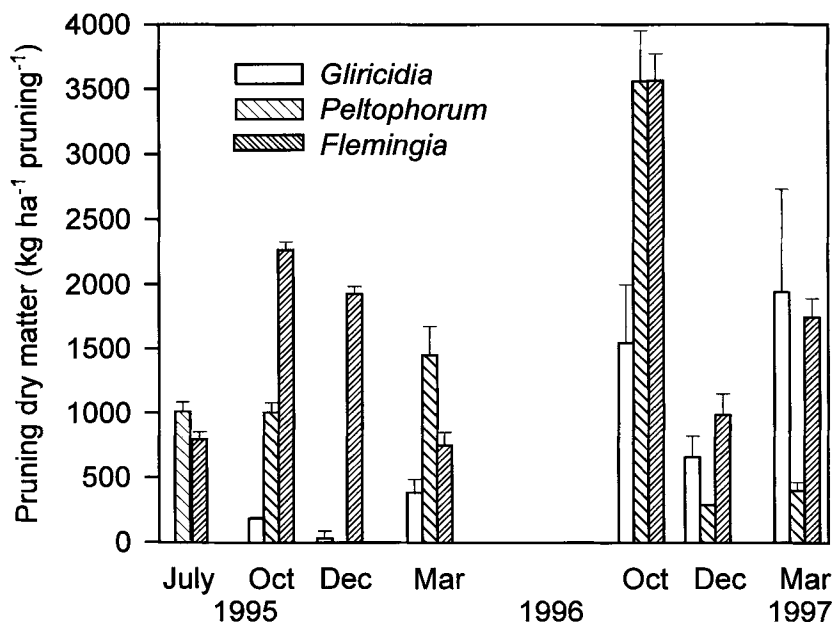
Figure 1. Dry weight of prunings of *Flemingia congesta* and mixed *Gliricidia sepium-Peltophorum dasyrrachis* hedgerows. Vertical bars are standard errors of means.

average of the two years but this was mainly due to its better performance in the first year. Pruning N yields in this study were slightly smaller compared to previous observations in a nearby long-term experiment where Van Noordwijk *et al.* (1997) observed N yields of 145 and 168 kg N ha^{-1} $year^{-1}$ for single species alleys of *Gliricidia* and *Flemingia* respectively.

Table 1. Yearly tree pruning growth, N_2 fixation (^{15}N dilution method) and soil-N uptake of established mixed *Peltophorum dasyrrachis – Gliricidia sepium* and sole *Flemingia congesta* hedgerows in Northern Lampung, Sumatra (average of 2 years).

	Dry weight	Total N yield	Avg. % N derived form N_2 fixation	Amount of N_2 fixed[2]	Soil-N uptake
	(kg ha^{-1} yr^{-1})		%	(kg ha^{-1} yr^{-1})	
Peltophorum	3848	70	0	0	70
Gliricidia	2064	52	51	26	26
Total	5912	122		26	96
Flemingia	6014	136	25	35	101
SED[1]	164[ns]	9.1[ns]	5.1*	8.7[ns]	5.2[ns]

[1] for comparisons of total systems performance; ns = not significant; * = P<0.05.
[2] for calculations of N_2 fixed at harvest March 97 % N_2 fixation values of March 96 were used

The proportion of N derived from N_2 fixation estimated using the ^{15}N dilution method varied during the season (Figure 2a) but was apparently not associated with fluctuations in dry matter production. With the onset of rains the % N_2 fixation declined until December but recovered towards the end of the rainy season. This decline in %N_2 fixation after the dry season may be associated with a flush of mineral-
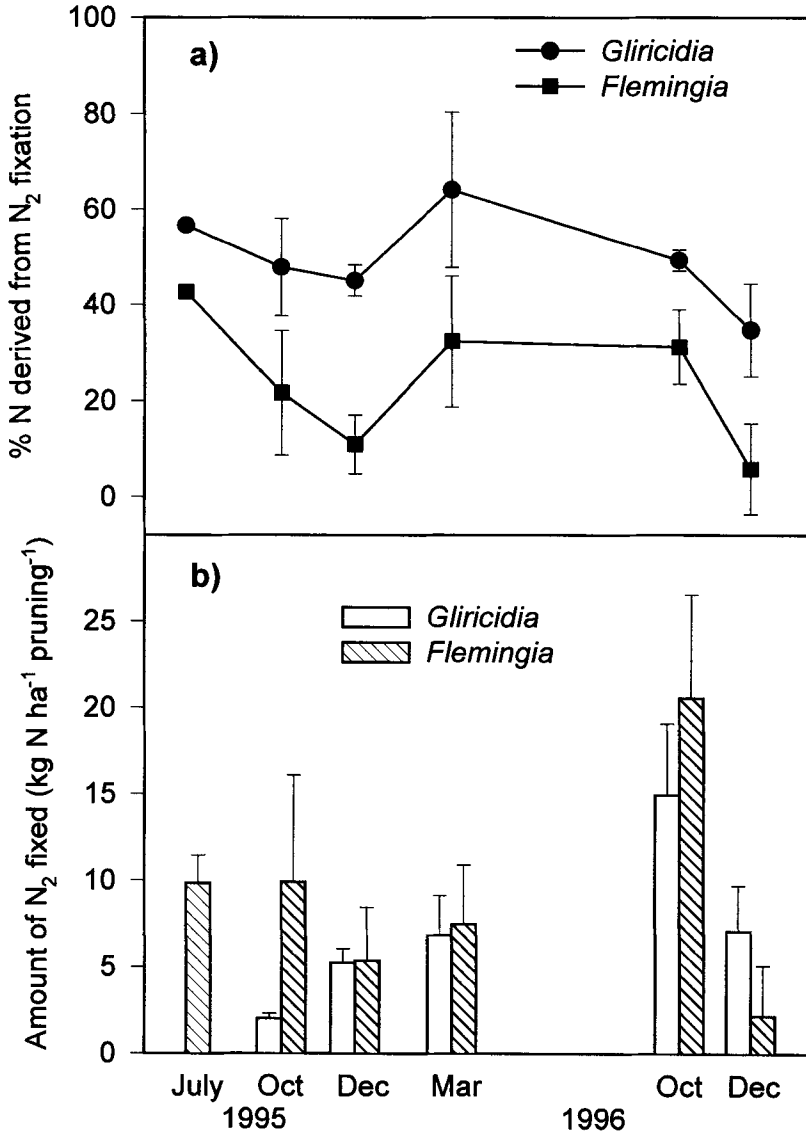

Figure 2. Seasonal variation in a) percent N derived from N_2 fixation (^{15}N dilution method) and b) the amount of N_2 fixed of *Flemingia congesta* and mixed *Gliricidia sepium-Peltophorum dasyrrachis* hedgerows.

ization after re-wetting of the soil (Birch, 1958). Indeed, mineral N measurements in 1996 confirmed a large increase in ammonium-N during the dry season followed by a rapid nitrification upon the onset of the rains (Figure 3). Mineral N concentrations decreased thereafter with the development of the rice-maize crop and presumably leaching and/or gaseous losses. Increased mineral N availability is known to reduce
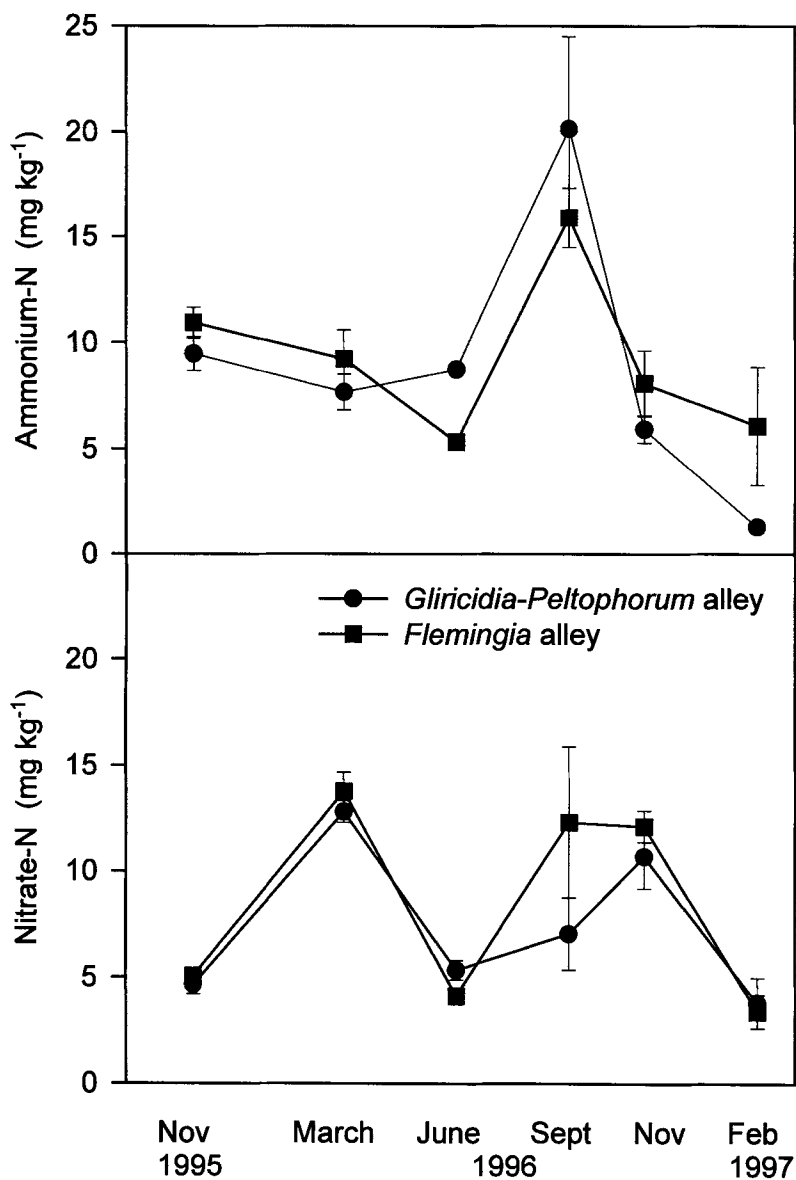

Figure 3. Soil mineral N pattern (0–15 cm) under two hedgerows.

the proportion of N derived from fixation (Streeter, 1988; Senaratne et al., 1987). Van Kessel et al. (1994) showed N_2 fixation decreased with time in Leucaena leuco-cephala hedgerows as a result of recycling of fixed N_2. However, no consistent decline in the proportion of N derived from N_2 fixation with time was found in the current experiment. This could have been because the experiment was only two years old and hence recycling of fixed N was not significant compared with the N mineralization from native soil organic matter. Additionally, unlike in the study of van Kessel et al. (1994) substantial amounts of N were removed in harvestable products (Hairiah et al., 2000). Hence, the overall cropping systems N balance was only slightly positive not considering leaching or other losses (Table 2). In this situation it is likely that N_2 fixation continues with little change.

Gliricidia obtained a larger proportion of its N from N_2 fixation compared with Flemingia throughout the 2 years (Figure 2a). On average, this resulted in Flemingia obtaining 25% less of its N from N_2 fixation compared to Gliricidia (Table 1). Average percent N derived from N_2 fixation by Gliricidia in this study was similar to the 44–58% reported by Rowe et al. (1999) using the natural ^{15}N abundance method at a nearby site. Similarly active N_2 fixation of 31–54% in periodically-pruned Gliricidia has been measured by Ladha et al. (1993) in an hedgerow intercropping association in the Philippines. Peoples et al. (1996) found higher %N_2 fixation in protein banks of Gliricidia (56–89%) probably because of the continuous complete removal of prunings. Sisworo et al. (1998) found the proportion of N derived from N_2 fixation to be the same for Gliricidia sepium (51%) as for Flemingia congesta (52%) in Java. Both legumes appeared to be equally adapted to the prevailing environment, hence the increased proportion of N derived from N_2 fixation by Gliricidia compared to Flemingia in this study was probably due to the competition by Peltophorum for soil mineral N as soil N uptake by the trees was similar in both systems (Table 1). This

Table 2: N balance of 2 and 3 (in brackets) year old hedgerow or crop only rotation systems.

	Hedgerow system		Crop rotation
	Peltophorum-Gliricidia	Flemingia (kg N ha^{-1} yr^{-1})	Groundnut-Cowpea
N export:			
Total system harvest N off-take[1]	34 (70)	37 (82)	77 (110)
N imports:			
N_2 fixation by trees	38	35	0
N_2 fixation groundnut[2]	20	16	34
N_2 fixation cowpea[3]	3	8	18
Systems N balance[3]	27 (–9)	22 (–19)	–25 (–53)

[1] Hairiah et al. (2000); crop yields were higher in year 3 (values in brackets) hence leading to greater N off-takes
[2] Cadisch et al. (2000)
[3] for year 3 assuming same fixation rates as year 2

suggests that N_2 fixation in these trees was largely regulated by the availability of soil mineral N e.g. significant N_2 fixation only occurred when N demand was not met by accessible mineral N resources. This may explain why some non-fixing trees such as *Senna* and *Peltophorum* appear to be equally suitable agroforestry trees, at least in the short term, as fixing trees as both sequester similar amounts of soil mineral N. Analysis of the chemical properties of tree materials by Hairiah *et al.* (2000) revealed that the concentration of phosphorus in leaves of N_2 fixing trees was >0.4 % P. It is known that P deficiency can reduce N_2 fixation in legumes (Cadisch *et al.*, 1993) but concentrations above 0.3 % P are often considered to be sufficient and hence P appeared not to have been limiting in this experiment. The uptake of up to 101 kg N ha^{-1} year^{-1} of soil N is potentially a large source of competition with associated crops. In the current experiment N appeared not to have been limiting crop production as no significant N fertilizer effect was observed probably due to other constraints (temporary drought stress, Hairiah *et al.*, 2000). Competition for mineral N however, may be crucial in more N limited systems and requires appropriate management (pruning regime). Estimated N uptake from soil in this study agrees with the finding of Rowe *et al.* (1999) in a similar soil in the same area. Soil N uptake by trees did not vary between systems. This suggests that despite *Peltphorum* having a better safety-net root system (Rowe *et al.*, 1999) soil-N recycling apparently was not substantially improved in the mixed hedgerow in the short-term.

Despite the smaller proportion of N derived from N_2 fixation observed in *Flemingia* the amount of N_2 fixed was not significantly different between the two fixing trees (Figure 4; Table 1). This was due to the larger total N yield of *Flemingia* presumably due to reduced competition as *Flemingia* was grown in single species alleys compared to the mixed species alleys in the case of *Gliricidia*.

Accurate estimates of N_2 fixation of trees under field conditions are difficult to obtain. This is because of the deep rooting of many trees and their varied growth patterns. Application of ^{15}N in solution to soil led to a rapid change in isotopic enrichment of the plant available ^{15}N pool despite the addition of carbon to stimulate a more rapid incorporation into the soil microbial biomass (Figure 5). Additionally, superficial application of ^{15}N led to a large spatial variation with depth (Cadisch *et al.*, 2000). ^{15}N methods are based on the assumption that a non-fixing reference plant takes up a similar proportion of soil-N:fertilizer-^{15}N (Ledgard *et al.*, 1985). Hence non-fixing reference plants should match spatial and temporal N uptake of fixing trees closely. The reference plant *Peltophorum dasyrrachis* used in this study however, has proportionally a greater root N uptake activity at lower soil depths than *Gliricidia* (Rowe *et al.*, 1999) and potentially *Flemingia*. Viera-Vargas *et al.* (1995) suggested the use of different ^{15}N application methods to account for the different spatial and temporal variations and to obtain a range of fixation rates. We used the elevated natural ^{15}N abundance of the soil to evaluate the validity of the ^{15}N dilution results. As the natural ^{15}N abundance varied less with depth than where ^{15}N fertilizer had been applied (Cadisch *et al.*, 2000) the former method is less prone to errors associated with different rooting depths. Comparisons of N_2 fixation estimates by the ^{15}N dilution method with those by the ^{15}N natural abundance method (Shearer & Kohl, 1986) obtained from unlabelled plants in replicate four suggest potentially an
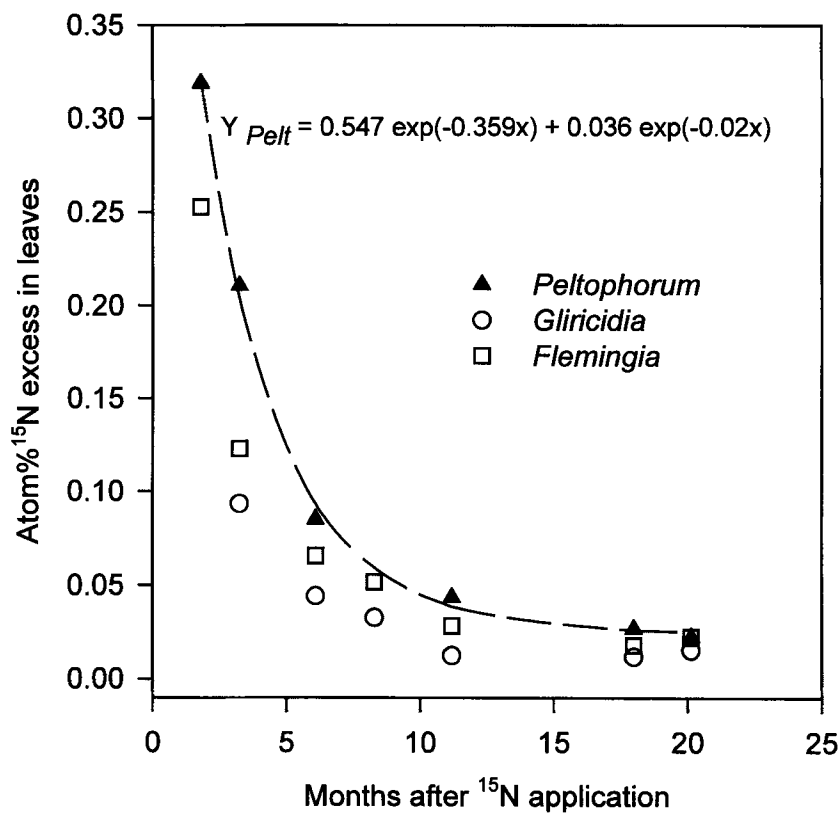
Figure 4. Temporal variation in the ¹⁵N enrichment of leaves of the reference plant (*Peltophorum dasyrrachis*).

overestimation of N_2 fixation (by 18% on average, Table 3). However, both ¹⁵N methods predict a greater proportion of N derived from N_2 fixation in *Gliricidia* than in *Flemingia*. Thus the ¹⁵N natural abundance results also support the previous observation that the % N derived from N_2 fixation is controlled by availability of soil mineral N.

The amount of N_2 fixed (35–38 kg N ha⁻¹ yr⁻¹) by *Gliricidia* or *Flemingia* was suf-

Table 3. Comparison of % N derived from N_2 fixation estimates using different ¹⁵N methods. Average of harvests July and October 95 and March and October 96. Reference plant was *Peltophorum dasyrrachis*. Values in brackets are standard errors.

| | %N derived from N_2 fixation | |
	Gliricidia	Flemingia
¹⁵N enrichment method (n = 12)	55 (4)	32 (4)
Natural ¹⁵N abundance method (n = 4)	37 (12)	24 (11)

ficient to compensate for the whole systems harvest N off-take of approximately 34–37 kg N ha^{-1} year^{-1} during the first two years (Table 2). However, crop yields were low (<1 t ha^{-1} crop^{-1}). Considering the additional N$_2$ fixation inputs from groundnut and cowpea there was initially a positive systems N balance of between 22–27 kg N ha^{-1} yr^{-1} for the hedgerow systems. The mineral N balance (0–15cm) between November 1995 and November 1996 was +4 to +9 kg N ha^{-1} for *Gliricidia-Peltophorum* or *Flemingia* respectively (Figure 3). This could have resulted in N losses in excess of 11–22 kg N ha^{-1} during this early phase of the experiment and potentially even higher considering the 30–40 kg N ha^{-1} made available by the Birch effect (Birch, 1958) at the beginning of the rainy season. However, tree roots can act as a safety-net below the crop root zone and intercept and recycle leached N (Rowe *et al.*, 1999). When crop yields increased in the third year, and assuming the same yearly N$_2$ fixation rates, the N contributions of fixing trees were lower than N exports in harvest products. Thus, it appears that at moderate to high crop yields N$_2$ fixation by hedgerow trees may not be sufficient and hence there is a need to include a grain legume or occasional green manure into the tree-based systems to balance N exports in harvested products. N$_2$ fixation by groundnut included in the rotation amounted to 16–38 kg N ha^{-1} yr^{-1} (Cadisch *et al.*, 2000) and another 3–18 kg N ha^{-1} yr^{-1} were provided by growing cowpea on residual moisture. With these additional N inputs hedgerow systems still had slightly negative N balances on the three year basis but these were considerably less than in pure crop based systems. Some N$_2$ fixation inputs may also be obtained from tree roots. Rowe (1999) measured *Gliricidia* root biomass of 3900 kg ha^{-1} equivalent to around 70 kg N ha^{-1}. Given a similar proportion of N derived from N$_2$ fixation as in shoots (51%) the additional inputs by roots could amount to 35 kg fixed N in the hedgerow system. However, the improved N balance in hedgerow systems has to be offset against reduced yields due to lost space, shading and competition for nutrients and water and the additional labor costs which makes these systems economically less attractive than leguminous crop-green manure based systems (Whitmore *et al.*, 2000). Additionally, data from Hairiah *et al.* (2000) suggest that the benefit of N$_2$ fixation inputs by trees for crop production were delayed for more than three years at the study site.

Acknowledgements

References

Amarger, N., A. Mariotti, F. Mariotti, J.C. Durr, C. Bourguignon & B. Lagacherie, 1979. Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in ¹⁵N natural abundance. *Plant and Soil* 52: 269–280.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* 10: 9–31.

Cadisch, G., K. Hairiah & K.E. Giller, 2000. Applicability of the natural ¹⁵N abundance technique to measure N₂ fixation in *Arachis hypogaea* grown on an Ultisol. *Netherlands Journal of Agricultural Science* 48: 31–45

Cadisch G., R. Sylvester-Bradley, B.C. Boller & J. Nosberger, 1993. Effects of phosphorus and potassium on N₂ fixation (¹⁵N-dilution) of field-grown *Centrosema acutifolium* and *C. macrocarpum*. *Field Crops Research* 31: 329–340.

Catchpoole, D.W. & G.J. Blair, 1990. Forage tree legumes I: Productivity and N economy of Leucaena, Gliricidia, Calliandra and Sesbania and tree/Green Panic mixtures. *Australian Journal of Agricultural Research* 41: 521–530.

Giller, K.E. & G. Cadisch, 1995. Future benefits from biological nitrogen fixation in agriculture: An ecological approach. *Plant and Soil* 174: 255–277.

Giller, K.E., J.F. McDonagh & G. Cadisch, 1994. Can biological nitrogen fixation sustain agriculture in the tropics? In: J.K. Syers & Rimmer D.L. (Eds.), Soil Science and Sustainable Land Mangagement in the Tropics. CAB International, Wallingford, UK, pp. 173–191.

Giller, K.E. & K.J. Wilson, 1991. Nitrogen Fixation in Tropical Cropping Systems. CAB International, Wallingford, 313 pp.

Giller, K.E. & J.F. Witty, 1987. Immobilized ¹⁵N-fertilizer sources improve the accuracy of field estimates of N₂-fixation by isotope dilution. *Soil Biology and Biochemistry* 19: 459–463.

Hairiah, K., M. van Noordwijk & G. Cadisch, 2000. Crop yield, C and N balance of three types of cropping systems on an Ultisol in N. Lampung. *Netherlands Journal of Agricultural Science* 48: 3–17

Handley, L.L., D. Odee & C.M. Scrimgeour, 1994. δ¹⁵N and δ¹³C patterns in savanna vegetation: dependence on water availability and disturbance. *Functional Ecology* 8: 306–314.

Herridge, D.F., B. Palmer, D.P. Nurhayati & M.B. Peoples, 1996. Evaluation of the xylem ureide method for measuring N₂ fixation in six tree legume species. *Soil Biology and Biochemistry* 28: 281–289.

Ladha, J.K., M.B. Peoples, D.P. Garrity, V.T. Capuno & P.J. Dart, 1993. Estimating dinitrogen fixation of hedgerow vegetation in an alley-crop system using the ¹⁵N natural abundance method. *Soil Science Society of America Journal* 57: 732–737.

Ledgard, S.F., J.R. Freney & J.R. Simpson 1984. Variations in natural enrichment of ¹⁵N in the profiles of some Australian pasture soils. *Australian Journal of Soil Research* 22: 155–164.

Ledgard, S.F., R. Morton, J.R. Freney, F.J. Bergersen & J.R. Simpson, 1985. Assessment of the relative uptake of added and indigenous soil nitrogen by nodulated legumes and reference plants in the ¹⁵N dilution measurement of N₂ fixation: Derivation of method. *Soil Biology and Biochemistry* 17: 323–328.

Ledgard, S.F., G.S. Smith, J.A. Douglas, M.S. Sprosen & G.J. Brier, 1991. Utilization of fertilizer and stored nitrogen by asparagus and kiwifruit estimated using ¹⁵N. In: Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies. IAEA, Vienna, pp. 371–379.

McAuliffe C., D.S. Chamblee, H. Uribe-Arango & W. W. Woodhouse 1958. Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by ¹⁵N. *Agronomy Journal* 50: 334–347.

Peoples, M.B., B. Palmer, D.M. Lilley, L.M. Duc & D.F. Herridge, 1996. Application of ¹⁵N and xylem ureide methods for assessing N₂ fixation of three shrub legumes periodically pruned for forage. *Plant and Soil* 182: 125–137.

Rowe, E., 1999. The safety-net role of tree roots in hedgerow intercropping systems, Wye College, University of London, Wye.

Rowe, E., K. Hairiah, K.E. Giller, M. van Noordwijk & G. Cadisch, 1999. Direct testing of the 'safety-net' role of hedgerow tree roots by ¹⁵N placement experiments on an acid soil in Lampung (Indonesia). *Agroforestry Systems* 43: 81–93.

Sanginga, N., K. Mulongoy & A. Ayanaba, 1986. Inoculation of Leucaean leucocephala (Lam.) de Wit with Rhizobium and its nitrogen contribution to a subsequent maize crop. *Biological Agriculture and Horticulture* 3: 347–352.

Senaratne, R., C. Amornpimol & G. Hardarson, 1987. Effect of combined nitrogen on nitrogen fixation of soybean (*Glycine max* L. Merrill) as affected by cultivar and rhizobial strain. *Plant and Soil* 103: 45–50.

Shearer, G. & D.H. Kohl, 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. *Australian Journal of Plant Physiology* 13: 699–756.

Sisworo, E.L., H. Rasjid, W.H. Sisworo, S. Solahuddin & J. Wemay, 1998. Nitrogen fixation in legume trees: measurement based on ^{15}N techniques. Internal report. Centre for the Application of Isotopes and Radiation, Jakarta, Indonesia, 20 pp.

Streeter, J., 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. Critical *Reviews in Plant Sciences* 7: 1–23.

Van der Heide, J., S. Setijono, M.S. Syekhfani, E.N. Flach, K. Hairiah, S. Ismunandar, S.M. Sitompul & M. Van Noordwijk, 1992. Can low external input cropping systems on acid upland soils in the humid tropics be sustainable? *Agrivita* 15: 1–10.

Van Kessel, C., R.E. Farell, J.P. Roskoski & K.M. Kaene, 1994. Recycling of the naturally occuring ^{15}N in an established stand of Leucaena leucocephala. *Soil Biology and Biochemistry* 26: 757–762.

Van Noordwijk, M., K. Hairiah, B. Lusiana & G. Cadisch, 1997. Tree – Soil – Crop interactions in sequential and simultaneous agroforestry systems. In: L. Bergstrom & Kirchmann H. (Eds.) Carbon and Nutrient Dynamics in Natural and Agricultural Tropical Ecosystems. CAB International, Wallingford, pp. 175–192.

Viera-Vargas, M.S., O.C. de Oliveira, C.M. Souto, G. Cadisch, S. Urquiaga & R.M. Boddey, 1995. Use of different ^{15}N labelling techniques to quantify the contribution of biological N_2 fixation to legumes. *Soil Biology and Biochemistry* 27: 1185–1192.

Whitmore, A.P., G. Cadisch, B. Toomsan, V. Limpinuntana, M. Van Noordwijk & P. Purnomosidhi, 2000. An analysis of the economic values of novel cropping systems in N.E. Thailand and S. Sumatra. *Netherlands Journal of Agricultural Science* 48: 105–114.