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 – Pultophorum dasyrrachis* established on an Ultisol (Grossarenic Kandudult) 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 *Pultophorum 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 *Pultophorum*. 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
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 N₂. 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 N₂ 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 N₂ 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 N₂ actively due to the limited N supply from the soil. However, in mature tree based systems N₂ 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 N₂ 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 N₂ 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 ¹⁵N dilution method with the application of a small dose of ¹⁵N enriched fertilizer. While the ¹³N 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 ¹⁵N, which makes it more difficult to find matching non-fixing reference plants. Choice of the reference plant appears less critical when the natural ¹⁵N enrichment of the soil is used instead as variations in the natural ¹⁵N abundance with depth are often smaller (Cadisch et al., 2000) but not necessarily so (Ledgard et al., 1984). A further complication for assessment of N₂ 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 N₂ 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 N₂ 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 ¹⁵N 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 *Glicicidia sepium* and *Flemingia congesta* in Northern Lampung, Indonesia and included comparisons 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$_2$O) 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$_c$ kg$^{-1}$; 0.34, 0.16, 2.29 and 1.10 cmol$_c$ 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). Hedgerow systems included: i) mixed *Glicicidia 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. *Glicicidia* 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 x 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):

\[ \% N_2 \text{fixation} = \left[ 1 - \frac{\text{atom} \%^{15}\text{N excess fixing legume}}{\text{atom} \%^{15}\text{N excess non fixing reference}} \right] \times 100 \]  

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 dasyrachis* 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):

\[ \% N_2 \text{fixation} = \left[ 1 - \frac{\delta^{15}\text{N reference} - \delta^{15}\text{N fixing legume}}{\delta^{15}\text{N non fixing reference} - B} \right] \times 100 \]  

where $\delta^{15}$N \%o = [(15N/14N sample / 15N/14N standard) – 1] x 1000 and where standard is atmospheric $N_2$. $B$ is the $\delta^{15}$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
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, N₂ fixation (¹⁵N 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).

<table>
<thead>
<tr>
<th></th>
<th>Dry weight (kg ha⁻¹ yr⁻¹)</th>
<th>Total N yield</th>
<th>Avg. % N derived form N₂ fixation</th>
<th>Amount of N₂ fixed (kg ha⁻¹ yr⁻¹)</th>
<th>Soil-N uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peltophorum</em></td>
<td>3848</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td><em>Gliricidia</em></td>
<td>2064</td>
<td>52</td>
<td>51</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>5912</td>
<td>122</td>
<td>25</td>
<td>26</td>
<td>96</td>
</tr>
<tr>
<td><em>Flemingia</em></td>
<td>6014</td>
<td>136</td>
<td>25</td>
<td>35</td>
<td>101</td>
</tr>
<tr>
<td>SED¹</td>
<td>164wu</td>
<td>9.1wu</td>
<td>5.1*</td>
<td>8.7ns</td>
<td>5.2wu</td>
</tr>
</tbody>
</table>

¹ for comparisons of total systems performance; ns = not significant; * = P<0.05.

² for calculations of N₂ fixed at harvest March 97 % N₂ fixation values of March 96 were used.
The proportion of N derived from N\textsubscript{2} fixation estimated using the \textsuperscript{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\textsubscript{2} fixation declined until December but recovered towards the end of the rainy season. This decline in %N\textsubscript{2} fixation after the dry season may be associated with a flush of mineral-
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

![Graph showing soil mineral N pattern (0–15 cm) under two hedgerows.](image URL)

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 N₂ fixation decreased with time in Leucaena leucocephala hedgerows as a result of recycling of fixed N₂. However, no consistent decline in the proportion of N derived from N₂ 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 N₂ fixation continues with little change.

Gliricidia obtained a larger proportion of its N from N₂ fixation compared with Flemingia throughout the 2 years (Figure 2a). On average, this resulted in Flemingia obtaining 25% less of its N from N₂ fixation compared to Gliricidia (Table 1). Average percent N derived from N₂ fixation by Gliricidia in this study was similar to the 44–58% reported by Rowe et al. (1999) using the natural ¹⁵N abundance method at a nearby site. Similarly active N₂ 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 %N₂ 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 N₂ 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 N₂ 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).

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

<table>
<thead>
<tr>
<th>Hedgerow system</th>
<th>Crop rotation</th>
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<tr>
<td>Peltrophorum-Gliricidia</td>
<td>Flemingia</td>
</tr>
<tr>
<td>(kg N ha⁻¹ yr⁻¹)</td>
<td>(kg N ha⁻¹ yr⁻¹)</td>
</tr>
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N export:
Total system harvest N off-take¹ | 34 (70) | 37 (82) | 77 (110) |

N imports:
N₂ fixation by trees | 38 | 35 | 0 |
N₂ fixation groundnut² | 20 | 16 | 34 |
N₂ fixation cowpea³ | 3 | 8 | 18 |
Systems N balance³ | 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 N₂ fixation in these trees was largely regulated by the availability of soil mineral N e.g. significant N₂ 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 N₂ fixing trees was >0.4 % P. It is known that P deficiency can reduce N₂ 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 *Peltophorum* 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 N₂ fixation observed in *Flemingia* the amount of N₂ 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 N₂ 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 ¹⁵N in solution to soil led to a rapid change in isotopic enrichment of the plant available ¹⁵N pool despite the addition of carbon to stimulate a more rapid incorporation into the soil microbial biomass (Figure 5). Additionally, superficial application of ¹⁵N led to a large spatial variation with depth (Cadisch *et al.*, 2000). ¹⁵N methods are based on the assumption that a non-fixing reference plant takes up a similar proportion of soil-N:fertilizer-¹⁵N (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 ¹⁵N application methods to account for the different spatial and temporal variations and to obtain a range of fixation rates. We used the elevated natural ¹⁵N abundance of the soil to evaluate the validity of the ¹⁵N dilution results. As the natural ¹⁵N abundance varied less with depth than where ¹⁵N fertilizer had been applied (Cadisch *et al.*, 2000) the former method is less prone to errors associated with different rooting depths. Comparisons of N₂ fixation estimates by the ¹⁵N dilution method with those by the ¹⁵N natural abundance method (Shearer & Kohl, 1986) obtained from unlabelled plants in replicate four suggest potentially an
overestimation of N₂ fixation (by 18% on average, Table 3). However, both ¹⁵N methods predict a greater proportion of N derived from N₂ fixation in *Gliricidia* than in *Flemingia*. Thus the ¹⁵N natural abundance results also support the previous observation that the % N derived from N₂ fixation is controlled by availability of soil mineral N.

The amount of N₂ fixed (35–38 kg N ha⁻¹ yr⁻¹) by *Gliricidia* or *Flemingia* was suf-

<table>
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<th>Method</th>
<th>Gliricidia</th>
<th>Flemingia</th>
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<tbody>
<tr>
<td>¹⁵N enrichment method (n = 12)</td>
<td>55 (4)</td>
<td>32 (4)</td>
</tr>
<tr>
<td>Natural ¹⁵N abundance method (n = 4)</td>
<td>37 (12)</td>
<td>24 (11)</td>
</tr>
</tbody>
</table>

Table 3. Comparison of % N derived from N₂ 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.
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.

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BIOLOGICAL N FIXATION OF HEDGEROW TREES


