

Biological N₂ fixing capacity of *Gliricidia sepium* and *Calliandra calothyrsus* under different agroclimatic conditions

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ABSTRACT

Biological nitrogen fixing capacity of *Gliricidia sepium* and *Calliandra calothyrsus* was investigated under field conditions in two contrasting locations in the Mid- country Intermediate and Mid-country Wet Zones of Sri Lanka at Nillambe and Matale respectively using the ¹⁵N isotope dilution method. Two non nodulating tree legume species *Senna siamea* and *Senna spectabilis* were used as reference crops. Under Matale conditions, all the species performed well. The highest above ground biomass and total N content were found in *S. spectabilis* (5622g and 126.97gN plant⁻¹). *C. calothyrsus* produced a higher biomass and contained more N than *G. sepium*. However, under Nillambe conditions, total biomass production was extremely low in comparison to Matale and the highest biomass recorded, by *C. calothyrsus* was only 197g plant⁻¹. Low biomass production under these conditions is attributed to the moisture stress and strong windy conditions prevailed during the growth period. Under Matale conditions no significant (P=0.01) difference was found between the two reference crops and estimated average Pfix values for *G. sepium* and *C. calothyrsus* were 56 and 42%, respectively. Total amount of N₂ fixed by above ground parts of *C. calothyrsus* (33g N plant⁻¹) was greater than *G. sepium* (26g plant⁻¹). The values are equivalent to 110 and 88kg N ha⁻¹ (3333 plants ha⁻¹). Under Nillambe conditions Pfix values for both fixing species were higher than at Matale but total N fixed were limited to the maximum of 6.0 and 9.2 kg N ha⁻¹ yr⁻¹ for *G. sepium* and *C. calothyrsus*, respectively. Performance of two reference species was also significantly (p=0.01) different at Nillambe. Overall results suggest that the performance of N₂ fixing trees varies with the agroclimatic conditions and *S. siamea* as well as *S. spectabilis* appear to be equally suitable as reference plants for estimation of BNF potential of *G. sepium* and *C. calothyrsus* under the conditions of this study.

Keywords: multipurpose trees, isotope dilution technique, reference crops, Sri Lanka, dry matter yield.

INTRODUCTION

Low productivity associated with degradation of soil physical, chemical and biological properties resulting from the adoption of unprotected cultivation practices is one of the major problems in mid country of Sri Lanka. Unpredictable rainfall patterns have further aggravated the problem. With the increase in cost of fertilizer and relatively low prices received for the agricultural products, most of the small scale farmers have deviated from the practice of manuring their crops. This situation has compelled to find alternatives for inputs and conservation of the resources. In respect to Nitrogen fertilizers, use of biological N₂ fixation process associated with living organisms has a great potential (Peoples and Craswell 1992; Liyanage *et al.* 1994; Mc Donagh *et al.* 1995). The terrestrial flux of N from of biological N₂ fixation has been estimated to range from 129-170x10⁶ tN/yr (Burns and Hardy

1975; Paul 1988) and this is several fold higher than input of N from N fertilizer (65x10⁶ t N/yr; Paul 1988).

Multipurpose trees are used for purposes such as timber, fuel wood shade and support for other crops, to reduce water table, to prevent leaching losses, recycle nutrients from depth, or reclaim and ameliorate the soil through litter fall, root turnover or incorporation of prunings (Garrity 1995). Perennial crop based agro forestry systems are common in Sri Lanka and widely accepted due to low productivity of the land. Black pepper, coffee and cocoa based agro forestry systems are common, especially in the mid country region in Sri Lanka and *Gliricidia sepium* (*Gliricidia*) is the most common leguminous tree species found in these systems. It is widely used as a shade tree for cocoa, coffee, tea and many other crops and as a support and shade tree for black pepper. Peoples *et al.* (1996) highlighted the importance of looking for alternative tree species for agro forestry systems, taking soil acidity and pest

susceptibility of *Leucaena leucocephala* as an example. It is essential to evaluate and identify other alternative species for *Gliricidia*, though it has the ability to produce high biomass and good quality fodder as well as green manure. Several workers have reported *G. sepium* as a high N₂-fixer (Awonaikie *et al.* 1992; Sanginga 1992; Liyanage *et al.* 1994) and recent work has proved equally good performances of *Calliandra calothyrsus* (Herridge *et al.* 1996; Peoples *et al.* 1996). Biomass production of *Calliandra* is higher than *Gliricidia* and leaf to stem ratio (0.89) also is significantly higher than that of *Gliricidia* grown under mid country conditions of Sri Lanka (Gunarathne and Heenkenda 1995).

Use of isotope dilution technique has a wide acceptance in N₂ fixation studies but several methodological issues are to be solved for tree crops. Selection of a suitable reference crop is of prime importance since the values estimated primarily depend on the root activity and growth pattern of the reference crop (Danso and Kumarasinghe 1990; Parrotta *et al.* 1994; Peoples *et al.* 1996). Use of more than one reference crop may increase the precision of the results over a single reference crop, due to inherent variation of the root distribution patterns, growth rates of the reference crops and influences on the mineralization of organic N (Boddey *et al.* 1990; Witty 1983; Witty and Giller 1991). Present study was undertaken to estimate the biological N₂-fixing capacity of *Gliricidia sepium* and *Calliandra calothyrsus* under different agro ecological conditions in Sri Lanka. Two non-nodulating tree legumes, *Senna siamea* and *Senna spectabilis* were used as non N₂ fixing reference species.

MATERIALS AND METHODS

Seeds of *Gliricidia sepium* (Jacq.) Walp. (*Gliricidia*), *Calliandra calothyrsus* Meissn. (*calliandra*) and non-N₂-fixing shrub legumes *Senna siamea* (*Siamea*) and *Senna spectabilis* (*Spectabilis*) were grown in 15x21 cm polyethylene bags filled with

equal parts of top soil, coir dust and sand. A slurry of the crushed nodules (5g fresh nodules in 100ml of water) obtained from field grown *Gliricidia* and *Calliandra* was applied to bags containing N₂-fixing plant at the rate of 5ml per bag. *Calliandra* and *Spectabilis* seeds were scarified before planting with concentrated H₂SO₄ for 8 and 15 minutes, respectively, and thoroughly washed in running water. Potted plants were kept inside a nursery shed providing adequate water until planting out in the field.

Two field sites were selected for the experiment from the mid country region of Sri Lanka, with contrasting agro ecological conditions. The first site was at the main research station of the Department of Export Agriculture at Matale (07° 30'N., 80° 5'E., 357m amsl) and the second site was located at one of the sub-stations at Nillambe (07° 10'N., 80° 40'E., 540m amsl). Annual rainfall during the study period for Matale was 2857mm and 2418mm for Nillambe. Soil properties of the sites are given in Table 1. Matale site is flat and has been used for growing cocoa for a long period before it was abandoned for the last 20 years. Nillambe site is sloping and has been used for tea cultivation and subsequently for growing annual crops for about 15 years with frequent ploughing.

Five weeks old tree seedlings were field planted in weed free plots in rows 2.0m apart and 1.5m between plants in 6x6m plots during the rainy season in early June 1993. Each plot consisted of 12 plants and replicated three times in a randomized complete block design. Within each plot, 3x4m isotope sub-plot was demarcated to cover two center plants. To limit lateral migration of labeled N and to prevent the horizontal spread of roots, 50 cm deep trenches were dug and lined with two layers of thick polythene sheet. One month after the field planting, 100 and 85 kg ha⁻¹ of K₂O and P₂O₅ respectively were applied as muriate of potash and triple super phosphate to all the plots and incorporated in to the soil.

Commencing from the third month after

Table 1. Soil characteristics of experimental sites

Depth	Texture	pH (1.5 soil: water)	Total N (%)	Organic C (%)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	CEC (me q/ 100g)
Matale							
0-30cm	sandy clay loam	5.28	0.263	2.82	51	55	38.91
30-45cm	sandy clay loam	5.06	0.211	1.86	14	34	28.66
Nillambe							
0-30cm	sandy loam	6.10	0.141	1.23	30	32	22.10
30-45cm	sandy clay loam	5.82	0.096	0.89	24	29	23.42

planting (map), 10.39 atom % ^{15}N ammonium sulfate was applied as a solution in water in three equal splits (3,6 & 9 map), to the isotope sub-plots using an atomizer at the rate of 20kg N ha⁻¹. Split applications of isotope was practiced rather than a single large applications to minimize errors in determination of N₂ fixation associated with rapid decline in $^{15}\text{N}/^{14}\text{N}$ ratio in plant available soil N (Witty 1983). After each application, plots were watered thoroughly using a watering can. Plots were kept weeds free by hand weeding and weeds removed from the isotope sub plots were allowed to decompose within the plot.

At the end of 12 months after field planting, above ground parts of the fixing and no fixing reference trees were carefully harvested at 3cm from the base and separated in to leaves and stem. Wet weight of them were recorded and twigs and trunk were cut into small pieces (<3cm) and mixed thoroughly. Sub samples from leaves and stems were dried at 70°C to a constant weight, and subsequently used for the determination of N content and ^{15}N a.e. Total N content was determined by micro Kjeldahl method and ^{15}N a.e. was determined on VG-Isogas mass spectrometer. Nitrogen fixation for individual plant parts (leaves and stems) as well as for above ground parts were calculated using the isotope dilution equation (Freed and Middelboe 1977). Weighted atom % ^{15}N excess (WAE) was used for the calculation of the N₂ fixation by whole above ground parts (Fried *et al.* 1983).

RESULTS

Dry matter yield

Total dry matter production of both N₂- fixing and

reference crop species were significantly higher under Matale conditions over Nillambe. Poor growth at Nillambe may be attributed to strong wind experienced from June to August and January to March period (Table2). Although the total rainfall during the growth period was not a limiting factor, strong wind and low soil water retention capacity in Nillambe have limited the amount of available water for the plants during short dry spells. Moreover, ploughpan developed for over a period of 10 years may have limited the root penetration to the lower horizons. Non fixing reference crop *S. spectabilis* showed a profuse growth at Matale and produced significantly (P=0.01) higher leaf and stem biomass over both fixing trees and the other reference crop, Siamea. Liyanage *et al.* (1994) also observed a similar trend with *S. siamea* over *G. sepium*. However, at Nilambe the growth of Calliandra was superior to the other crops though leaf biomass did not show any significant difference among the species, but stem biomass of Calliandra was significantly (P=0.05) higher than in Spectabilis.

N yield

Leaf and stem N% of Gliricidia and Calliandra were significantly (P=0.01) higher than the reference species under Matale conditions but under Nillambe conditions only Gliricidia indicated a significantly (P=0.01) higher leaf N% over the others and the highest stem N% was found in Spectabilis. The highest total leaf and stem N yields were found in Spectabilis but the value for Calliandra leaves did not differ significantly (P=0.05) from that of Matale condition (Table 2). In contrast to the results at Matale, total N yields are very low despite similar N% found in plants. Calliandra recorded the highest

Table 2. Above ground dry matter, N% and N yield and their distribution in leaves and stem

	Dry weight (g plant ⁻¹)			N%		N Yield(g plant ⁻¹)		
	Leaves	Stems	Total	Leaves	Stems	Leaves	Stems	Total
Matale								
<i>G. Sepium</i>	832.1	928.7	1760.9	3.3	2.2	27.4	20.5	47.8
<i>C. Calothyrsus</i>	1465.1	1458.9	2924.0	3.2	2.2	46.5	32.2	78.7
<i>S. siamea</i>	932.9	1038.8	1971.7	2.3	1.9	21.3	14.8	36.1
<i>S. Spectabilis</i>	2871.2	2750.8	5622.0	2.6	1.4	75.8	51.1	127.0
Significance	***	***	ND	***	***	**	***	ND
LSD(p=0.05)	904.5	836.7		0.3	0.2	29.7	17.0	
CV%	29.7	34.7		4.4	4.3	34.7	28.6	
Nillambe								
<i>G. Sepium</i>	55.6	63.1	118.7	3.4	1.3	1.9	0.8	2.7
<i>C. calothyrsus</i>	113.5	83.7	197.2	2.7	1.1	2.9	0.9	3.8
<i>S. Siamea</i>	103.7	60.7	164.4	2.4	1.1	2.4	0.7	3.1
<i>S. Spectabilis</i>	93.1	47.7	140.8	2.6	1.8	2.4	0.9	3.3
Significance	NS	*	ND	***	***	NS	NS	ND
LSD(p=0.05)		34.6		0.4	0.2			
CV%		26.6		9.3	35.2			

NS- not significant ND- not determined

leaf and stem N at Nillambe though the values are statistically not significant.

Atom% ¹⁵N

Atom% ¹⁵N enrichment in leaves and stems as well as calculated weighted average of both the fixing tree species *G. sepium* and *C. calothyrsus*, were significantly lower than the reference species confirming that fixing trees do not rely on soil and applied nitrogen at both locations (Table 3). However, leaf atom% ¹⁵N excess in *S. siamea* was significantly ($P=0.01$) higher than in *S. spectabilis* under Nillambe conditions, indicating that the pattern of soil N absorption differs between two reference species. Lower atom % ¹⁵N distribution could be found in leaves than in stems at both locations expect in *S. siamea* at Nillambe, which is the highest value recorded. Under Matale conditions, *G. sepium* gave lower enrichment values than *C. calothyrsus* but the reverse was true under Nillambe conditions.

N₂ Fixation

Percentage of N derived through fixation (Pfix) by *G. sepium* and *C. Calothyrsus* in above ground parts, calculated on the basis of two reference crops are given in Table 4. Estimated values for leaves and stems based on atom% ¹⁵N excess in individual parts and for whole above ground parts based on weighted atom excess(WAE) are given separately. Under Matale conditions, *G. sepium* and *C. calothyrsus* have derived 52.8-58.3% and 38.4-45.6% of N, respectively, in above ground plant parts from fixation. Comparatively higher percentage of leaf N than stem N has been derived from fixation. Based on

Pfix values, *G. sepium* has a significantly ($P=0.01$) higher ability to obtain its N requirement through fixation than *C. calothyrsus*. Although *S. siamea* estimated a higher value of Pfix than *S. spectabilis*, no significant difference was observed between them for the cumulative Pfix values either for Gliricidia or Calliandra. Pfix values based on *S. siamea* for leaves of both the fixers and for stems of Calliandra were significantly ($P=0.05$) higher. Total amount of N²- fixed by Calliandra is higher than Gliricidia due to high biomass and total N content but the difference is not statistically significant. During the first year of growth, Calliandra fixed 30.32-35.72g N plant⁻¹ (100-119kg ha⁻¹) while Gliricidia fixed 24.81-27.77g plant⁻¹ (83-93kg ha⁻¹). In both the cases 58-61% of the fixed N of above ground parts were found in leaves.

Situation at Nillambe was quite different from Matale and Pfix was higher for both Gliricidia and Calliandra ranging from 51.1-70.2% and 36.1-72.2%, respectively. Pfix values were highly significant for the reference crops but no difference could be found between two N₂- fixers either for whole above ground parts or individual plant parts with respect to a single reference crop. Despite high Pfix values, the amount of N₂ fixed was extremely low for both fixing species due to poor biomass production. Total amount of N₂ was also highly significant between two fixers as well for the values based on two reference crops. Highly variable N-fix values observed for Gliricidia (0.95-1.78g N plant⁻¹ or 3.17-5.96kg N ha⁻¹) and for Calliandra (1.80-2.76g N plant⁻¹ or 6.00-9.21kg ha⁻¹) suggest the uncertainty of the values obtained under the conditions, where plant growth is arrested due to

Table 3. ¹⁵N atom % excess in different plant parts and mean weighted ¹⁵N atom % excess (WAE) for the whole plant

Treatment	Atom% ¹⁵ N excess		WAE
	Leaves	Stems	
Matale			
<i>G. sepium</i>	0.0086	0.0131	0.0104
<i>C. calothyrsus</i>	0.0121	0.0154	0.0136
<i>S. Siamea</i>	0.0229	0.0284	0.0251
<i>S. spectabilis</i>	0.0194	0.0252	0.0221
Significance	***	***	***
LSD(p=0.05)	0.0028	0.0029	0.0023
CV%	8.97	7.16	6.56
Nillambe			
<i>G. sepium</i>	0.0793	0.0902	0.0821
<i>C. calothyrsus</i>	0.0603	0.0797	0.0650
<i>S. siamea</i>	0.2797	0.1299	0.1356
<i>S. spectabilis</i>	0.1240	0.1360	0.1268
Significance	***	**	**
LSD(P=0.05)	0.0823	0.0381	0.427
CV%	30.33	34.21	33.16

Table 4. Estimates of the proportion (Pfix) and amounts of N derived from N₂ fixation in leaves, stems and whole above ground parts of *G. sepium* and *C. Calothyrsus* at two locations. Estimated values based on *S. siamea* and *S. spectabilis* given against R1 and R2, respectively.

Treatment	Pfix %			Amount of N fixed (g/plant)			
	Leaves	Stems	Whole plant ^a	Leaves	Stems	Whole plant ^a	
Matale							
<i>G. sepium</i>	-R1	62.6±1.2	53.6±5.0	58.3±2.6	17.11±3.16	10.83±2.51	27.77±5.61
	-R2	47.2±0.8	45.6±3.2	52.8±3.0	15.28±2.82	9.99±2.11	24.81±4.32
<i>C. calothyrsus</i>	-R1	55.8±1.7	49.5±0.7	45.6±3.3	21.92±0.85	14.63±2.31	35.72±0.92
	-R2	37.5±2.8	38.8±1.4	38.4±2.0	17.47±1.29	12.40±1.74	30.22±2.29
Significance	***	**	***	NS	NS	NS	
LSD(p=0.05)	6.8	10.1	9.4				
CV%	6.74	10.88	9.63				
Nillambe							
<i>G. sepium</i>	-R1	70.2±5.8	52.4±15.8	67.7±4.9	1.32±0.16	0.33±0.14	1.79±0.11
	-R2	51.1±5.6	30.6±8.8	35.4±2.9	0.24±0.04	0.10±0.01	0.95±0.15
<i>C. Calothyrsus</i>	-R1	77.2±4.0	56.0±4.0	74.0±5.1	2.23±0.39	0.46±0.12	2.76±0.38
	-R2	36.1±3.8	38.1±10.8	48.5±4.7	0.37±0.09	0.12±0.01	1.80±0.02
Significance	***	*	***	***	*	***	
LSD(p=0.05)	18.5	20.0	14.0	0.572	0.299	0.412	
CV%	15.81	22.65	12.45	27.57	59.32	11.29	

^a calculation based on WAE

stress conditions.

DISCUSSION

Isotope dilution technique was used in this study for the quantification of nitrogen fixation in field based comparison of the ¹⁵N isotope enrichment of fixing and non-fixing reference plants grown on isotopically enriched soil. Calculation of N₂ fixation using this method based on the assumption that N uptake from the soil by reference plants has the same isotopic composition as the N uptake by the N₂ fixing plant, though this is not always met (Witty and Giller 1991). Discrepancies in N₂ fixing values estimated for a given crop using different reference crops often arise as a result of dissimilarities in the ¹⁵N/¹⁴N uptake from soil in which the ¹⁵N/¹⁴N ratio declines rapidly. Also errors attributable to reference crop have greater effect at lower than at higher levels of N₂ fixation (Danso and Kumarasinghe 1990; Witty and Giller 1991). In this study, fairly accurate estimates could be obtained for both fixing species under Matale conditions where environmental factors were favorable for fixing and reference crops. *Gliricidia* and *Calliandra* were found to be high potential N₂-fixers under this condition with the capacity to fix nearly 100kg of atmospheric N₂ ha⁻¹ yr⁻¹. However, under Nillambe conditions, *Gliricidia* as well as *Calliandra* did not perform well, due to the fact that biomass production was extremely restricted by stress, despite higher Pfix values observed than at Matale. Higher Pfix values may be attributed to low N status of the soil (Witty and Giller 1991; Danso *et al.* 1992) and higher ¹⁵N enrichment of both the N₂-

fixers and reference plants confirmed the accumulation of a greater proportion of applied fertilizer.

The actual amount of N₂-fixed is conditioned by the plants nitrogen fixing potential and by environmental factors. This may operate in two ways, either by reducing plant growth or by direct affect on the symbiosis or both the effects (Danso *et al.* 1992). Soil moisture may affect bacterial nitrogen fixation indirectly through plant growth and directly on symbiosis. Total amount of N₂-fixed is yield dependent but % N₂ fixed is not. The stress observed on plant growth at Nillambe may have affected in two ways; moisture stress due to low water holding capacity, high evapo-transpiration due to strong wind and poor root penetration to the deeper layers due to plough pan as well as the physical stress caused by wind.

S. siamea is a suitable reference crop used in N₂-fixing studies on tree or shrub legumes in the tropics (Sanginga *et al.* 1990; Ladha *et al.* 1993; Liyanage *et al.* 1994). Accuracy of the Pfix values estimated using isotope dilution technique would largely depend on the reference crop. If the reference plant and the fixing plant differ in the time course at which they absorb N from soil, or if they sample different soil horizons, then the two crops will be utilizing pools of different ¹⁵N enrichment unless there is a constant and uniform enrichment of soil N. Although this problem can be reduced by careful choice of reference crop and method of ¹⁵N addition, it is seldom possible to achieve perfect experimental conditions using either of these two approaches (Witty and Giller 1991). Use of more than one

reference crop would increase the likelihood of obtaining accurate estimates (Boddy *et al.* 1990; Witty 1983).

S. spectabilis has been recently used as a reference crop for N₂-fixing studies in shrub legumes (Peoples *et al.* 1996) and in the present study both the reference crops performed equally well under Matale conditions but not at Nillambe. Pfix values and the total amount of N₂ fixed values estimated, based on *S. spectabilis* at Nillambe were much lower than those based in the *S. siamea*. This may be due to root penetration of *Spectabilis* beyond the isotope plots and absorbing more N from the low ¹⁵N/¹⁴N zone, in comparison to *Siamea*. According to Danso and Kumarasinghe (1990) error attributed to reference crop is higher at lower level than at higher level of N₂ fixation. However, under present situation high error was associated with higher rate of fixation (Pfix) but low total amount of fixation. Exclusion of roots from the study did not show the full N₂ fixing capacity of the plants. Danso and Kumarasinghe (1990) reported that roots of soybean may contain 10-12% of the fixed N of whole plant.

The overall results conclude that *G. sepium* and *C. calothyrses* have the potential to obtain 55% and 42%, respectively, of their N requirement from biological N₂ fixation. Total content of the fixed N in the above ground parts of the plants averaged to 88 and 110kg ha⁻¹ yr⁻¹ under favorable growing conditions. When total N input to a system is considered, total biomass production and N content are equally important as the percentage fixation. However, under moisture stress and windy climatic conditions, growth of both the species are arrested and as a result of that total N input to the system is extremely low although the percent N₂ fixation seems to be higher. The results also confirm that *S. siamea* and *S. spectabilis* are suitable reference crops for N₂ fixation studies in tree or shrub legumes. Location specific performances of *Gliricidia* and *Caliandra* suggest the importance of selecting suitable N₂ fixing species for specific conditions rather than extrapolating the results from one location to another.

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REFERENCES

- Awonaiké KO, Hardarson G and Kumarasinghe K S 1992 Biological nitrogen fixation of *Gliricidia sepium*/ *Rhizobium* symbiosis as influenced by plant genotype, bacterial strain and their interactions. Trop. Agric. (Trinidad) 69: 381-385.
- Boddey R M, Urquiaga S, Neves MCP, Suher AR and Peres JR 1990 Quantification of the contribution of N₂ fixation to field grown grain legumes- a strategy for the practical application of the ¹⁵N isotope dilution technique. Soil Biochem. 22: 649-655.
- Burns RC and Hardy RWF 1975 Nitrogen fixation in bacteria and higher plants. Springer-Verlag. Berlin. Germany. 189p.
- Danso SKA and Kumarasinghe KS 1990 Assessment of potential sources of error in nitrogen fixation Measurements by the nitrogen-15 isotope dilution technique. Plant and Soil. 125:87-93.
- Danso SKA, Bowen GD and Sanginga N 1992 Biological nitrogen fixation in agro-ecosystems. Plant and Soil. 141: 177-196.
- Fried M, Danso SKA and Zapata F 1983 The methodology of measurement of N₂ fixation by non-legumes as inferred from field experiments with legumes. Can. J. Microbial. 29:1053-1062.
- Freed M and Middelboe V 1977 Measurement of amount of nitrogen fixed by a legume crop. Plant and Soil. 43:707-711.
- Garrity DP 1995 The fate of organic matter and nutrients in agroforestry systems. In: Soil Organic Matter Management for Sustainable Agriculture. ACIAR Monograph No.56. Eds. RDBL Efrog, GJ Blair and ET Craswell. pp 69-77. ACIAR Canberra, Australia.
- Gunaratne WDL and Heenkenda AP 1995 Biomass production and plant nutrient content of N₂-fixing and non N₂-fixing leguminous tree species. Proc. Sixth Regional Workshop on Multipurpose Trees. Kandy, Sri Lanka. 176-181.
- Herridge DF, Palmer B, Nurthayati DP and Peoples MB 1996 Evaluation of the xylem uride method for measuring N₂ fixation in six legume species. Soil Boil. Biochem. 28:281-289.
- Ladha JK, Peoples MB, Garrity DP, Capuno VT and Dart PJ 1993 Estimating nitrogen fixation of hedgerow vegetation using the

- nitrogen-15 natural abundance method. Soil Sci. Am. J.57:732-737.
- Liyange M de S, Danso SKA and Jayasundara HPS 1994 Biological nitrogen fixation in four *Gliricidia sepium* genotypes. Plant and Soil. 161:267-274.
- McDonagh JF, Toomsman B, Limpinuntana V and Giller KE 1995 Grain legumes and green manures as pre-rice crops in Northeast Thailand. Plant and Soil. 177:111-126.
- Paul EA 1998 Towards the year 2000: directions for future nitrogen research. In: Advances in Nitrogen Cycling in Agricultural Ecosystems. Ed. JR Wilson. pp.417-425. CAB International, Wallingford, UK.
- Peoples MB and Craswell ET 1992 Biological nitrogen fixation: Investments, expectations and actual contribution to agriculture. Plant and Soil. 141:13-39.
- Peoples MB, Palmer B, Lilly DM, Duc LM and Herrdgen DF 1996 Application of ^{15}N and xylem uride methods for assessing N_2 fixation of three shrub legumes periodically pruned for forage. Plant and Soil. 182: 125-137.
- Parrotta JA, Baker DD and Fried M 1994 Application of ^{15}N -enrichment methodologies to estimate nitrogen fixation in *Casurnia equisetifolia*. Can. J. For. Res. 24:201-207.
- Sanginga N, Danso SKA, Zapata F and Bowen GD 1990 Influence of reference trees on N_2 fixation estimates in *Leucaena leucocephala* and *Acacia albida* using ^{15}N -labeling techniques. Biol. Fertil. Soils. 9:341-346.
- Sanginga N 1992 Early growth and N_2 - fixation of leucaena and gliricidia at different levels of phosphorus application. Fertilizer Research. 31:165-173.
- Witty JF 1983 Estimating N_2 fixation in the field using ^{15}N - labeled fertilizer; some problems and solutions. Soil Biol. Biochem. 15:631-639.
- Witty JF and Giller KE 1991 Evaluation of errors in the measurement of biological nitrogen fixation using ^{15}N fertilizer. In: Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies. IAEA, Vienna. Pp59-72: