NITROGEN FIXATION IN LEGUME TREES : MEASUREMENT BASED ON ^{15}N TECHNIQUES

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ABSTRACT

NITROGEN FIXATION IN LEGUME TREES MEASUREMENT BASED ON ^{15}N TECHNIQUES. A field experiment has been conducted to measure the N_2-fixation in six legume trees, namely *Gliricidia sepium* (F1), *Sesbania sesban* (F2), *Caliandra tetragona* (F3), *Flemengia conges-7ta* (F4), *Acacia mangium* (F5), and *Leucena leucocephala* (F6), using ^{15}N techniques, e.g. the isotope dilution method. For this technique a reference tree, that is a non N_2-fixing tree has to be used. In this experiment three reference trees were planted, but only one was used, which above ground growth was equal to the legume trees. The reference tree chosen was *Eucalyptus alba* (R1). Data obtained from this experiment show that in general the legume trees have growth then the reference trees expreesed, in dray weight of various plant parts and plants and total-N uptake (TN). At harvest some of the legume and reference tree have reached a 2.5m height. The percentage of N_2-fixation (%-Fix) ranged from 50 to 70%. The highest %N-Fix was shown by *Leucena leucocephala* (F6) (70%N-Fix). Hight %N-Fix does not necessarily mean high N-Fix uptake (gn/tree) too. The N-Fix appears to be determined by the TN (gn/tree). The highest N-Fix was contributed by the leaves, which also has the highest percentage of total –N (%TN) compared to the orther plant parts, i.e. roots, stem, and branches.

INTRODUCTION

In tropical crop production the approach to correct soil fertility problems was in the past emphasized on changing the soil condition to the plant needs. Meaning that soil fertility factor such as, pH and nutrient avaibility, were conditioned to meet optimum levels for a certain crop [1].
They [1] further stated that this high-input approach while successful in many temperate regions by obtaining high yields, has created difficulties even failures in tropical and sub-tropical regions. Such regions are usually characterized by soil of adverse chemical condition needing high costs improve [2]. For such areas including areas in Indonesia, a need to change to a more low-input technology, where plants are chosen to be adaptable to marginal soil conditions especiality in upland soils has been recognized [3,4,5].

Although most trees can fulfil the above function, nitrogen fixing trees especially legume appears to be the most suitable. Why? This is due to their ability to fix N_2 from the atmosphere. With this ability legume trees are capable to grow on infertile soils. To obtain maximum benefit of fixing trees, it is essential to select plant genotypes which have superior N_2-fixing power

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and to determine enviromental conditions which are needed to enhance nitrogen fixation. To reach these objectives suitable methodologies for assessing N_2-fixation are needed.

The strength and weakness of various methods to measure N_2-fixation have been discussed in many reviews [6,7,8,9]. Although none the methods is fully satisfactory under all conditions, the ^{15}N technique appears to be widely accepted and have the greatest potential for measuring N_2-fixation, where there would be no complications due to uptake of N from the soil [10]. Contrary to the grain and pasture legumes, the ^{15}N technique has been rarely used for legume trees. This is due to several factors. Including limited knowledges of plant parts to be sampled, problems faced by the massive size of trees, their parennial growth habit, which make the selections of reference (non N_2-fixing) trees difficult. The most important factor in the use of ^{15}N technique for assessing N_2-fixation of legume trees is the selection of a proper reference tree or the so called standard tree. This reference tree has to be a non N_2-fixing tree and has to have nearly the same rooting depth as the N_2-fixing trees. Another requirement is that of the above ground growth, which has to be nearly equal. But when the N_2-fixation capability of the legume trees are high, the criteria needed for the reference trees could be less rigid [11]. Where only N_2-fixation rate of several trees are to be determined, no reference tree is needed [10].

This paper reported the use of ^{15}N-labelled ammonium sulphate (AS) to assess the fixing ability of six legume trees in the field using one reference tree.

Obtaining data of N_2-fixation capability of legume trees, could be used for choosing a suitable legume tree as an N-Source.

Location

The experiment was conducted at the field station of the Centre for the Application of Isotopes and Radiation, Pasar Jumat, Jakarta, which soil is a red latosol type.

The physical and chemical properties of the soil are as follows

Sand	0.7 %
Silt	30.3 %
Clay	69.0 %
PH (H2O)	5.4
(KCl)	4.3
Cation/100g	14.2 me
K	0.3 me
Ca	10.1 me
Mg	3.4 me
Na	0.4 me

CEC/100g	27 me
P2O5 (Olsen)	9 ppm
K2O	11 ppm
Organic matter	
C	1.25 %
N	0.14 %
C/N	9 %

Plant matterial

The plants used in this experiment are as follows,

Legume trees (N$_2$-fixing)	Scientific name	Local name
F1	*Gliricia sepium*	Gliricidia
F2	*Sesbania sesban*	Sesbania
F3	*Caliandra tetragona*	Kaliandra
F4	*Flemengia congesta*	Flemengia
F5	*Acacia Mangium*	Akasia
F6	*Leucena leucocephala*	Lamtoro gung
Reference trees (non N$_2$-fixing)		
R1	*Eucalyptus alba*	Kayu putih
R2	*Swietenia mahagoni*	Mahoni
R3	*Carica papaya*	Papaya

After evaluation the above ground growth of the reference trees, R1 (*Eucalyptus alba*) was chosen to be used as a reference tree. The two other reference trees were too small, especially in height compared to the legume trees. All the trees used in this experiment were grown from seeds. The seeds were planted in PVC containers filled with cleand sand. About one month later the seedlings were transplanted to polyethelene bags which were filled with 1 kg soil derived from the experiment site and this was mixed with 100g manure. Three monthslater the seedlings were transplanted into field. Rhyzobium for trees was not applied because it was not available, although attemps have been made to get it from abroad and locally. Inspite of this the seedlings have quitte a high number of nodules when transplanted as shown in Table 1.

Experimental plots

The design used in this experiment was a randomized block design (RDB) with four replications.

Each experimental plot has a size of 10m x 10m planted at random with a total 100 trees of legume and reference tress, using a planting distance of 1m x 1m. In the middle of the plot a plot with a size of 6m x 6m used to be applied with ^{15}N-labelled ammonium sulphate (AS) with 9.634% atom excess (%a.c). This plot is called an isotope plot. Surrounding the isotopes plot a trench was dug to a depth of 2m. Into the trench a polyethelene sheet was inserted, so that the isotope plot was surrounded by the sheet. The purpose to surround the isotope plot with a polyethelene sheet was to prevent the roots of the trees in the isotope plots to cross out side the plots and vice versa. In the isotope plots 18 legume and 18 reference trees were planted, as shown in Fig. 1.

Application of fertilizer

^{15}N-labelled AS was used in the isotope plots, while for the rest of the trees non-labelled AS was applied. AS was applied by broadcasting around cach tree. P and K were applied at a rate of 0.1 kg TSP and 0.1 kg KCl per tree respectively, and were broadcasted around the tree. The rate of ^{15}N-labelled AS and non-labelled AS applied was 12g AS/tree, which was about 25 kg N/ha or 0.25 kg N/100m^2.

This amount was applied in three splits. The first N application was three months after transplanting, the second and third application were done at a one month interval after the first application. The dates of planting and fertilizers application were as follow,

12 June 1995	Seeds planted in PVC containers filled with clean sand
16 July 1995	Seedlings transplanted to polyethelene bags filled with 1 kg soil from the experimental site and mixed with 100 g mahure
16 August 1995	Plants from polyethelene bags transplanted the field
27 December 1995	First application of ^{15}N-labelled and non-labelled AS (3.5 g N/tree), P and K fertilizers
30 January 1996	Second application of ^{15}N-labelled and non-labelled AS (3.5g N/tree)
4 March 1996	Third application of ^{15}N-labelled and non labelled AS (5g N/tree)
8 April 1996	Havest

Tree harvest

The whole plant including the roots of the legume and reference trees were harvest. Each plant part, e.g. roots, stem, branches, and leaves were analyzed separately. Measurement of percentage of total –N (%TN) was done by the Kyeldahl method. The percentage of atom excess (%a.e) of each plant part was determined by an emission spectrophotometer, YASCO N-151. The calculation of percentage of N-derived from fertilizer (%NF) and percentage of N-derived fixation (%NFix) is presented in the attachment.

Parameter observed

Parameter observed in this experiment are,

- dry weight of roots, stem, branch, leaves, and plants (roots + stem + branches + leaves)
- percentage of total-N (%TN), percentage of atom excess (%a.e), percentage of N derived from fertilizer (%NF), and percentage derived from N_2-fixation (%NFix) were determined for each plant part.
- Total N uptake (TN), N-uptake from fertilizer (NF), and N-uptake from N_2-fixation (NFix) of the various plant parts and plants, the %NFix and NFix are expressed only for the whole plant (roots + stem + branches + leaves)

RESULTS AND DISCUSSION

At harvest most of the trees were about 2m to 2.5m heigh. Data in Table 1 show the plant condition at transplanting of seedling into the fields. For legume tree it was shown, that nodules were formed on the roots although Rhyzobium innoculation was not carried out. Apparently the soil has indigenous rhizobia which were able to infect the root of the legume trees. For the reference trees no nodules were found at all on the roots. At transplanting the reference trees R2 and R3 have better growth expressed in dry weigh of various plant parts and plants compared to R1 (Table 1) but in the field R1's growth surpassed R2 and R3 (Table 2). Due to this R1 was choosen to be used as a reference trece to assess the N_2-fixation of the legume trees by the ^{15}N method.

High dry weight of the legume trees were found in F1, F2, F3, and F6, while F4 and F5 showed the lowest dry weight (Table 2). In general it could be mention that the legume trees have better growth than the reference trees if expressed in dry weight of various plant parts and dry weight of the plants (Table 2) Looking at the dry weight of the various plant parts, it appears that for the legume and reference trees the roots have the lowest dry weight. At harvest when digging out the roots, it was observed that the roots were mostly concentrated at the 0-10 cm soil depth. This could be due the heavy rains received at Pasar Jumat area since tansplanting. It could be that these heavy rains caused heigh water tables and this was able to restrict the root growth to a certain depth. Further it appears that root growth restriction caused low dry weight, and this is obviously the reason why the weight of roots were less than the other plant parts.

An other facts to be mentioned is that the fresh and dry weight of the legume and reference trees showed high CV (Coefficient of Variation). This appear to be due the different plant species used as mentioned by SANGINGA et al [12]. Apparently in this experiment the high CV obtained due to the different species in the same block (replication) as well as the differences between blocks.

For the legume trees the highest percentage of total-N (%TN) was found in the leaves, followed by the %TN of branches, roots, and stem respectively (Table 3). Like in legume trees, the highest %TN for reference trees were in the leaves followed by the roots and lowest was for the stem (Table 3). While for the branches there was only one value that was for R1, R2 and R3 were considered to have no branches. Having higher dry weight (Table 2) and higher %TN resulted in higher total-N uptake (TN) of the legume trees compared to that of the reference trees, especially for F1, F2, F3, and F6 (Table 4). Field observations and results of fresh and dry weight showed that F4 and F5 are slow growers compared to the other legume trees even compared to R1.

The most important data to be used to measure percentage N-derived from fixation (%NFix) is the percentage of atom exess (%a.e) of the verious plant parts. It was obtained in this experiment that the reference trees have higher % a.e in their various plant parts than the legume trees (Table 5). If this is due to better growth of the legume trees expressed in higher dry weight and TN causing higher dilution of the N-fertilizer, Making the %NF of the legume trees lower than the reference trees (Tables 2, 4, and 5). But for legume trees with the low dry weight and TN this is not the case, they still have low %NF theoriti cally they should have high %TN due to lower dilution of N-fertilizer (Tables 2 and 4 : F4, F5 vs R1, R2, R3). Based on these data it could be suggested that the legume trees has another N source beside N from soil and N form fertilizer which dilute the N-fertilizer, resulting in lower %NF in legume trees than in the reference trees. This source is the N_2-fixed from the atmosphere.

To assess the ability of N_2-fixation (%N-Fix) of the legume trees, the %N-Fix is expressed for the whole plant and not for the various plant parts. The calculation of this is presented in the attachment. Based on this calculation the N-Fix of the legume trees range from 50-70% (Table 6). Such a range of various legume trees has been obtained by other research workers as quoted by [12] where the values range from 33 to 78% using the same isotope dilution technique as employed in this experiment. Table 6 furher shows that the tree with the highest %N-Fix does not necessary resulted in the highest N-Fixation uptake (Table 6, F3 vs F6).

It appears that high N-fixation uptake values is determined by the TN of the tree, which is a total of TN of each various plant parts (Table 4) and here it show that the highest TN for F3 was found in the leaves (Table 4, F3 vs F6). From this experiment it looks that the highest TN is derived from the leaves for all the legume trees (Table 4). This high TN in leaves is derived from high dry weight and %TN (Tables 2 and 3). In short it could be suggested that when choosing a tree legume to be used a nitrogen source for other crops several parameters have to be taken into consideration. The %N-fixation alone would not be enough to be the base for choosing a legume tree as a source of N. Other parameters such as %TN. Dry weight of various plant parts have to be considered too. As well known any tree which would be used as an N-source need to have plenty biomass and if possible have also a high %TN. In this experiment it appears that dry weight and %TN of the leaves are the tree`s part which could be the highest N source.

Data from this experiment show that there were differences in the ability of N_2-fixation by different legume trees. Here it was found that the legume tree with the highest N_2-fixation ability expressed in %N-Fix is F6 (*leucena leucocephala*), followed in order by F3 (*Caliandra tetragona*), F2 (*Sesbania sesban*), F1 (*Gliricidia sepium*), F5 (*Acacia mangium*), and F4 (*Flemengia congesta*) (Table 6).But as mentioned before, the %N-Fix alone is not enough to consider a legume tree to be a good source of nitrogen which could be used for other crops, but other parameters needed to be taken into consideration, such as high weight and high %TN of biomass

CONCLUSIONS

Several conclusions could be taken from the experiment carried out. The conclusions are as described below,

1. Ammonium sulphate labelled with 10% 15N could be used to obtain detectable percentage of atom excess (%a.e) in various tree parts.
2. In general it appears that legume trees have better growth than non legume trees. This might be due to the ability of the legume trees to fix N_2 from the air, resulting in higher N available for growth compared to the non legume trees (standard trees). In this experiment growth is expressed in dry weight of several plant parts and total dry weight of plants.
3. Using the ^{15}N technique it was found that the highest N_2-fixation (%N-Fix) was obtain by F1 (*Leucena leucocephala*), followed by F3 (*Caliandra tetragona*), F2 (*Sesbania sesban*), F1 (*Gliricidia sepium*), F5 (*Acacia mangium*), and F4 (*Flemengia congesta*).
4. The ability of a tree legume to fix N_2 alone could not be the base for choosing a legume tree to be used as a nitrogen source for other crops, other parameters should be taken into consideration like the dry weight and percentage of total N (%TN) of the various plant parts.

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Table 1. Dry weight, %total-N Uptake, of roots, system + leaves, plants, and number of nodules at transplanting.

		Roots	Leaves +Stem	Plant	Number Of nodules
F1	a. dw (g)	0.373	2.818	3.191	33
	b. %to-n	1.969	2.654	--	--
	c. To-N Uptake (mg N-Plant)	7.3	74.8	82.1	--
F2	a.	0.479	3.172	3.651	50
	b.	1.824	2.792	--	--
	c.	8.7	88.6	97.3	--
F3	a.	0.523	2.414	2.667	100
	b.	1.646	2.483	--	--
	c.	4.2	59.9	64.1	--
F4	a.	0.107	0.878	0.986	121
	b.	1.624	2.246	--	--
	c.	1.7	19.7	21.4	--
F5	a.	0.081	0.705	0.786	15
	b.	1.948	2.346	--	--
	c.	1.6	16.5	18.1	--
F6	a.	0.609	4.045	4.654	43
	b.	1.527	2.477	--	--
	c.	9.3	100.2	109.5	--
R1	a.	0.031	0.304	0.335	--
	b.	1.210	2.287	--	--
	c.	0.4	7.0	7.4	--
R2	a.	0.164	1.487	1.651	--
	b.	1.210	2.287	--	--
	c.	2.0	34.0	36.0	--
R3	a.	0.217	2.010	2.227	--
	b.	1.867	2.531	--	--
	c.	4.1	50.9	55.0	--

Table 2. Fresh Weight (FW), and Dry Weight (DW) of plant parts and plants of various legume and reference trees at harvest.

Trees	Roots		Stem		Branches		Leaves		Plant	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
	……………………………….. g/tree …………………………………………………									
F1	1021	339	1575	557	947	247	2011	438	5548	1581
F2	704	257	2713	1149	1793	624	1511	3807	6720	2338
F3	356	156	1925	769	1448	417	2051	664	5780	2106
F4	116	45	613	258	384	107	448	86	1560	496
F5	64	21	400	155	279	78	821	175	1564	430
F6	477	205	2263	879	1255	425	1493	371	5150	1881
R1	158	65	1075	403	528	224	766	241	2528	934
R2	16	6	63	28	--	--	112	36	200	70
R3	429	43	1750	115	--	--	1131	149	3310	307
LSD										
5%	322	112	880	350	442	161	594	156	2032	656
1%	436	152	1192	474	606	221	805	212	2754	889
CV (%)	59.6	61.0	43.5	50.0	31.4	34.2	35.4	39.0	38.7	39.8

Notes : each values is an average of 4 replicates
FW : Fresh Weight
W : Dry Weight
Height of the tree range from 0.8 – 2.5 m

Table 3. Total-N percentage (%to-N) of plant parts base on dry of various legume and reference trees at harvest.

Trees	Roots	Stems	Branches	Leaves
	………………………… %to-N …………………………			
F1	1.180	0.620	1.212	2.868
F2	0.838	0.428	1.022	3.924
F3	1.016	0.563	1.163	3.217
F4	1.200	0.730	1.262	3.040
F5	0.944	0.599	0.946	3.231
F6	1.139	0.592	1.004	3.806
R1	0.664	0.616	0.544	2.039
R2	0.617	0.499	--	1.621
R3	1.223	1.246	--	3.802
LSD 5%	0.201	0.178	0.248	0.576
1%	0.271	0.241	0.339	0.781
CV (%)	14.0	18.6	16.3	12.9

Table 4. Total-N uptake of plant parts and plants of various legume and reference trees at harvest.

Trees	Roots	Stems	Branches	Leaves	
	…………………………….....	gN/tree	…………………………		
F1	3.900	3.428	2.453	12.377	22.658
F2	2.134	4.933	6.510	12.102	25.679
F3	1.603	4.214	6.038	21.293	33.147
F4	0.559	1.854	1.307	2.604	6.324
F5	0.198	0.930	0.720	5.681	7.529
F6	2..414	5.359	4.284	14.261	25.203
R1	0.358	2.494	1.136	4.586	8.575
R2	0.039	0.139	--	0.561	0.739
R3	1.527	1.114	--	5.724	7.365
LSD 5%	1.094	2.128	1.883	4.951	7.909
1%	1.482	2.884	2.580	6.710	10.717
CV (%)	57.5	53.6	39.5	38.6	35.5

Table 5. Percentage of ^{15}N atom excess (%a.c) and percentage devired from fertilizer (%NF) of various plants of legume trees at harvest.

Trees	Roots		Stems		Branches		Leaves	
	%a.e	%NF	%a.e	%NF	%a.e	%NF	%a.e	%NF
F1	0.906	9.29	0.703	7.28	0.866	8.87	0.934	9.37
F2	0.877	8.98	0.544	5.57	0.719	7.37	0.862	8.84
F3	0.870	8.92	0.582	5.96	0.672	6.89	0.716	7.49
F4	1.037	10.64	0.855	8.77	1.000	10.27	0.893	9.15
F5	1.015	10.49	0.612	6.28	0.831	8.55	1.108	9.59
F6	0.734	7.60	0.447	4.58	0.536	5.49	0.501	5.14
R1	1.638	16.89	1.645	15.67	1.840	18.69	2.836	20.51
R2	2.180	22.35	2.613	23.02	--	--	3.045	31.12
R3	2.289	23.47	1.742	19.07	--	--	2.000	24.64
LSD 5%		16.89		7.71		6.99		6.36
1%		22.35		10.49		9.59		8.65
CV (%)		23.47		49.06		35.27		30.85

$$\%NF = \frac{\%a.e\ of\ \ sample}{\%a.e\ of\ \ fertilizer} x100$$

Table 6. Dry weight (DW), total N-uptake (TN), N-fertilizer uptake (NF), N2-fixation (%N-Fix) of various legume and reference trees at harvest.

Legume trees	Plant DW (g)	TN (gN/tree)	N-Fert (gN/tree)	N-Fix (gN/Tree)	%N-Fix
F1	1581	22.658	1.996	11.640	52.44
F2	2338	25.679	2.016	14.823	57.48
F3	2106	33.149	2.455	19.926	59.48
F4	496	6.324	0.576	3.223	51.05
F5	430	7.259	0.719	3.710	52.05
F6	1881	25.203	1.456	17.361	70.03
R1	934	8.575	1.335	--	--
R2	70	0.739	0.211	--	--
R3	307	7.365	1.721	--	--
LSD 5%	656	7.909	0.869	--	--
1%	889	10.717	0.911	--	--
CV (%)	39.8	35.5	34.40	--	--

Attachment
Calculation of %N-Fix and N-Fix uptake using the 15N technique for legume and reference trees :

$$\%\text{N-derived from fertilizer (\%NF)} = \frac{\%\ atom\ \ excess\ of\ plant\ sample}{\%\ atom\ \ excess\ \ of\ \ fertilizer}\ x\ 100\%$$

Example : %a.e. plant sample = 0.670%
%a.e. fertilizer = 9.634%

$$\%\text{NF plant sample} = \frac{0.670}{9.634}\ x100\% = 20\%$$

Dry weight	(DW) (g)	%NT	NT-uptake Gn/DW	%NF	NF-uptake Gn/NT uptake
Legum tree:					
Roots	340	1.2	4.1	9.3	0.38
Branches	560	0.9	5.0	7.3	0.37
Stems	250	1.2	3.0	8.9	0.27
Leaves	440	2.9	12.8	9.6	1.23
Plants	1590	--	24.9		2.25
Reference tree:					
Roots	70	0.7	0.5	16.9	0.11
Stems	410	0.6	2.5	15.7	0.39
Branches	240	0.5	1.1	18.7	0.21
Leaves	240	2.0	4.8	20.3	0.98
plants	940	--	8.9	--	1.69

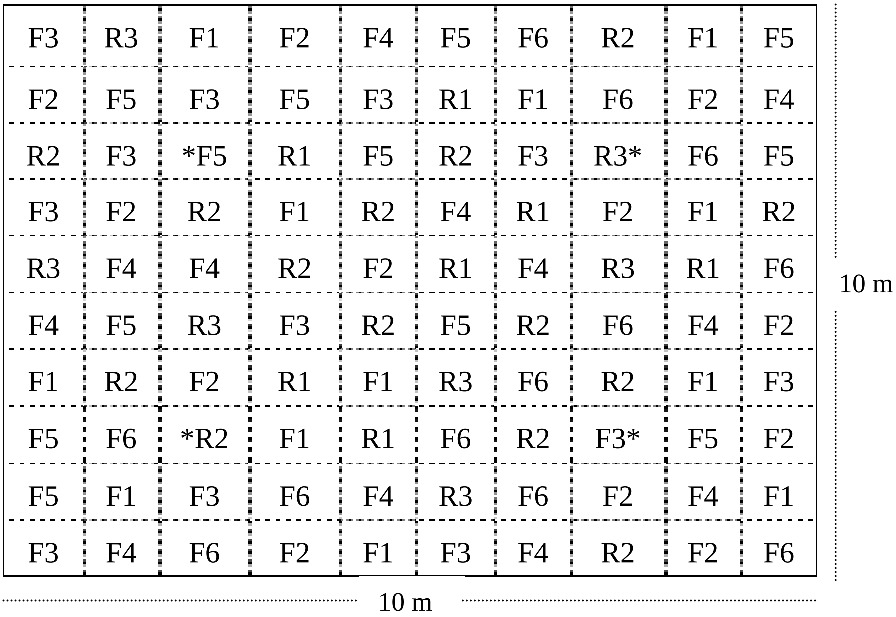
NT uptake = %N x DW ; NF uptake = %NT uptake
Legume treee %NF (plant) : 2.25/24.9 x 100% = 9.04%
Reference tree %NF (plant) : 1.69/8.9 x 100% = 18.99%
%N Soil Reference tree = 100% -18.99% = 81.01%

$$\frac{\%NF}{\%N\ \ soil}\ legume = \frac{\%NF}{\%Nsoil}\ reference$$

$$\frac{9.04}{\%N\ \ soil}\ legume = \frac{18.99}{81.01}\ reference$$

%N Soil legume = $\frac{81.01}{\%N\ \ soil}\ x\ 9.04 = 38.56$

% Fif-legume (whole plant) = 100% - 38.56% - 9.04% = 52%
%N soil   %NF



F3	R3	F1	F2	F4	F5	F6	R2	F1	F5
F2	F5	F3	F5	F3	R1	F1	F6	F2	F4
R2	F3	*F5	R1	F5	R2	F3	R3*	F6	F5
F3	F2	R2	F1	R2	F4	R1	F2	F1	R2
R3	F4	F4	R2	F2	R1	F4	R3	R1	F6
F4	F5	R3	F3	R2	F5	R2	F6	F4	F2
F1	R2	F2	R1	F1	R3	F6	R2	F1	F3
F5	F6	*R2	F1	R1	F6	R2	F3*	F5	F2
F5	F1	F3	F6	F4	R3	F6	F2	F4	F1
F3	F4	F6	F2	F1	F3	F4	R2	F2	F6

* Isotope plot : 6 m x 6 m
F : Legume tree
R. : Reference tree

Figure 1. Layout of an experimental plot (replicate I).