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The use of nuclear techniques in the management of nitrogen fixation by trees to enhance fertility of fragile tropical soils

Results of a co-ordinated research project organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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THE USE OF NUCLEAR TECHNIQUES IN THE MANAGEMENT OF NITROGEN FIXATION BY TREES TO ENHANCE FERTILITY OF FRAGILE TROPICAL SOILS

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FOREWORD

Increasing population pressure on arable land in the tropics is leading to replacement of traditional shifting cultivation with unsustainable systems such as those with shorter-duration fallow periods, sedentary agriculture on small-scale land holdings and expansion onto marginal areas, causing soil degradation and deterioration of the environment. Some 65% of tropical soils are fragile and lose fertility rapidly under cultivation; within as little as two years, yields can fall to a small fraction of those obtained in the first season after clearing native vegetation, a trend that is particularly marked in marginal soils. Loss of soil organic matter accompanies cultivation, with concomitant decrease in desirable soil physical properties and increased erosion. Nutrient depletion through leaching and run-off exacerbates the problem.

Depletion of soil nutrients can be arrested by addition of chemical fertilizers, but financial considerations preclude this as a solution for the majority of farmers in developing countries. Therefore, it is imperative to explore alternative integrated soil and nutrient-management approaches with minimum or zero risk of environmental degradation. In this context, the use of trees for rehabilitating soils and maintaining fertility is particularly attractive. In dry areas, trees are often able to exploit soil water not available to shallow-rooted plants, and their perennial nature obviates the need for annual planting, thus reducing erosion.

In the humid tropics leguminous and actinorhizal trees have been incorporated into agroforestry systems, thereby enhancing their contributions due to fixation of atmospheric nitrogen. Depending on the type of agroforestry system and management practices, a substantial portion of this fixed nitrogen can be transferred to soil and to arable crops. Trees also provide fodder and fuel-wood, components of great importance to the subsistence farmer.

Despite broad recognition of the value of nitrogen-fixing trees in agroforestry, there are few reliable data on the magnitude of fixed nitrogen contributions or on the potential to manage and maximize nitrogen fixation by trees. There has been little effort to identify species and provenances of superior nitrogen-fixing potential. Even for the well studied *Leucaena leucocephala* there has been no critical determination of the amounts of nitrogen fixed compared with nitrogen taken up from soil, of its nutritional constraints to nitrogen fixation or, in particular, of the effects of management practices, e.g. frequency and severity of pruning, on fixation and transfer of nitrogen to soil or to associated crops via prunings. Changes in soil characteristics due to the presence of trees have also received scant attention.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a Co-ordinated Research Project in 1990 on The Use of Nuclear and Related Techniques in Management of Nitrogen Fixation by Trees for Enhancing Soil Fertility and Soil Conservation in Fragile Tropical Soils. This project was underpinned by extensive experience in the use of ^{15}N-labelled fertilizer in quantifying nitrogen fixation by food and pasture legumes; the isotope-dilution technique, recognized as the most accurate mode of quantifying fixation, was developed at the IAEA and has been used profitably in Co-ordinated Research Projects that have focused on aspects of sustainability in agriculture in developing countries in which food security is most under threat. The effort to improve understanding of the potential contributions of trees to soil fertility and to increased production of food for human consumption, which are timely in terms of current global needs, grew out of a realization at the IAEA that a comprehensive multi-disciplinary approach was needed to optimize results from Co-ordinated Research Projects. S.K.A. Danso and G. Keerthisinghe were the Project Officers during 1990–1995 and 1997, respectively. The IAEA thanks the OPEC Fund for International Development for its generous financial support. The TECDOC was edited for publication by A.R.J. Eaglesham.

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SUMMARY

The global population is expected to double within the next four decades, and much of that increase will occur in developing countries in which hunger is already a reality The need to make arable land satisfy the present demand for food is resulting in briefer fallow periods, with the commissioning of bush-fallow land that has not fully regenerated since its previous use for crop production – thus a vicious cycle is initiated that results in ever decreasing productivity per unit area When soil fertility rapidly declines, erosion often increases with far-reaching deleterious environmental consequences that may be impossible to reverse In such circumstances, crop productivity may not be significantly improved by applications of fertilizers, even if available to the subsistence farmer, because levels of organic matter that sustain fertility have reached critically low values

The pressing need to reverse these trends demands that land use systems be developed to achieve sustainable agricultural production with minimum environmental degradation Soil fertility should not be sacrificed for the short-term production of crops The recycling of renewable resources is an important factor in the maintenance of soil organic matter and hence soil fertility Furthermore, the nutrients that are removed from the system in the harvested crop must be replaced Where organic sources of nutrients are available, new systems must be designed and developed to exploit them In this context, leguminous or actinorhizal trees have a potential role in the rehabilitation of nutrient-depleted soils for food production because of their capacity to fix atmospheric nitrogen Depending on the type of cropping system involved and management practices employed, a substantial portion of this fixed nitrogen can be transferred to companion crops and to soil. Trees that grow rapidly and produce much leaf material are particularly attractive as sources of organic matter that is high in N.

Alley-cropping has been proposed as an alternative to shifting cultivation It usually involves intercropping leguminous trees or shrubs with cereals, but it may include actinorhizal species of woody plants The trees or shrubs are pruned regularly to prevent shading and reduce competition with the arable crop, the productivity of which is maintained through application of leaf prunings to the soil Thus, soil organic matter levels are maintained or even increased, and nutrients are constantly added in organic form. In addition, root residues may contribute substantially to the nutrient supply to crops A significant additional feature of alley cropping that is particularly important on hilly terrain is the reduction of soil erosion There is also the potential for the production of fuel-wood, the major source of energy for over half the world and a commodity in extremely short supply in many countries

Legume residues are known to improve crop productivity by two modes of action a direct effect on nutrient supply and an indirect effect on the soil micro-climate Legume green manure, whether composed of crop residues or prunings from shrub or tree, are low in C N, lignin and polyphenol, characteristics that result in rapid decomposition, particularly under warm, moist tropical conditions This rapid decomposition makes it difficult to synchronize the release of nutrients with crop demand To optimize the utilization of organic sources of nutrients, it is essential to develop management options that ensure that nutrient release occurs when the crop may exploit it efficiently, otherwise significant losses may occur

To evaluate the possible contribution of N_2-fixing trees (NFTs) to the improvement of soil fertility in alley-cropping and related agroforestry systems, it is important to quantify the symbiotic N_2-fixation by trees and shrubs under field conditions The study of N_2-fixation by trees is more difficult than with annual legumes. The major difference is that trees are perennial, therefore exacerbating the problem of selecting a non-fixing reference species that is valid across seasons and years Also, trees have deeply penetrating roots. On the assumption that most of those roots are capable of nutrient uptake, then it becomes critical to ascertain if substantial N uptake occurs beyond the top soil within which most of the applied ^{15}N is located, and if so, whether both the reference and nitrogen fixing tree are well matched in their pattern of nutrient utilization throughout the soil profile Given the potential importance of nitrogen fixing trees in enhancing soil fertility, decreasing erosion, and in providing fodder and fuel wood, there is an urgent need to find solutions to these methodological difficulties so that accurate measurements of fixed nitrogen may be obtained in diverse agro-ecosystems

The achievement of maximum inputs of fixed N from NFTs requires management practices and conditions that favour the functioning of the N_2-fixing system, including the presence of compatible strains of the rhizobia and/or *Frankia* particularly under stressed conditions, such as salt-affected or arid soils. Moreover, consideration must be given to the possible need for inoculation with vesicular arbuscular mycorrhiza, in view of reported positive effects on fast-growing trees. In the past, much agricultural research has been done on research stations that may not be representative of prevailing farming conditions. Where the objectives are to achieve agricultural sustainability using newly developed strategies, it is particularly important that the field research is done on degraded soils that are typical of local, or preferably regional or national, farming conditions.

Within the above context, the Co-ordinated Research Project on The Use of Nuclear and Related Techniques in Management of Nitrogen Fixation by Trees for Enhancing Soil Fertility and Soil Conservation in Fragile Tropical Soils was initiated in 1990. The research had three broad components:

- to improve the methodology for estimating nitrogen fixation by trees,
- to identify fast-growing, high-N_2-fixing tree species as plentiful sources of leaf prunings for use as green manure and as sources of fuel wood,
- to examine the patterns of decomposition of these leaf prunings and release of mineral nutrients and their effects on the yield of arable crops.

Few of the research projects included all three of these aspects. However, each project had locally relevant components.

Estimation of nitrogen fixation by trees

Several of the projects revealed difficulties with the isotope-dilution technique. Some negative estimates were obtained for N fixed, and superior growth of the non-fixing reference species was a recurring theme. In Ghana, for example, *Cassia siamea* consistently produced more dry matter than did *Leucaena leucocephala*; the total N yield of *C. siamea* was between two- and eleven- fold higher than that of *L. leucocephala*, depending on the pruning treatment. These consistently high total-N yields in *C. siamea* call for a critical evaluation of this species as a source of N. In Chile, the two reference species *Schinus polygamus* and *Fraxinus excelsior* produced more than five-fold the biomass of two *Prosopis* species. In Uganda, the estimates of %Ndfa for *L. leucocephala* differed with reference species: *Cassia spectabilis* generally gave higher values than did *C. siamea*, and some negative values were obtained with the latter. On the other hand, in Senegal only insignificant differences in the estimated proportions and amounts of N fixed by three *Acacia* species were obtained using either *Parkia biglobosa* or *Tamarindus indica* as non-fixing reference species.

Data reported from the United States of America illustrated the potential utility of ^{15}N-depleted fertilizer as a tool in the investigation of the N-nutrition of trees. Being much cheaper than its ^{15}N-enriched counterpart, it is particularly attractive for work with trees with which large experimental areas are needed to ensure adequate replication. The US data indicated that mature walnut (*Juglans regia* L.) used most of the N accumulated from soil and fertilizer for storage purposes, to be remobilized for new growth within two years, and about half of the total-N pool in a mature tree was present as non-structural compounds, available for recycling. It remains to be determined whether leguminous and actinorhizal trees use fixed N similarly.

The following points were learned with regard to the measurement of N_2 fixation by trees:

(1) Results obtained with young trees may not reflect the true potential of older trees, as N_2 fixation varies with age.

(2) Site characteristics are important when comparing results from disparate locations. Climate, physical and chemical properties of soil, etc., markedly influence fixation.

(3) Certain measures minimized methodological errors:

- The application of ^{15}N in small split-doses;
- The application of ^{15}N within lined trenches dug around the trees;
- The inter-planting of reference and N_2-fixing trees on the same plot;
- Correction for unlabelled N present in the tree before ^{15}N application.

Despite the difficulties with the techniques, the results presented by the participants demonstrated that the ^{15}N-dilution technique was useful at least for comparative purposes in identifying promising N_2-fixing tree species and provenances within species. In several countries, species of *Gliricidia, Acacia, Chamaecytisus* and *Leucaena* performed well in terms of both fixation and biomass production. For example, field studies in Sri Lanka showed that the average symbiotic contribution to the N nutrition of *Gliricidia* was about 55%.

Fast growing nitrogen fixing trees

The research in Chile under arid conditions showed that the introduction of an exotic species, Tagasaste (*Chamaecytisus proliferus* ssp. *Palmensis*) was a more viable approach to increasing inputs of legume-derived green manure than working with native *Acacia* species; the biomass production of Tagasaste was about ten times greater than that of *Acacia caven*, which is commonly grown in that region for animal fodder. Studies conducted in Sri Lanka demonstrated that *Gliricidia sepium* grown under coconut outperformed *Leucaena leucocephala* in terms of dry matter and amounts of nitrogen fixation.

Release of nutrients from legume-derived green manure and their effects on crop yields

Under humid tropical conditions, most of the legume-derived nutrients were released and available during the growth period of the crop even with surface-application as a mulch. In Uganda, the time taken for 50% loss of *C. siamea* leaf litter biomass and its macro-nutrient content was less than 20 days, corresponding to the addition of about 50 kg N ha^{-1} to the soil in the same period. In Malaysia, a rapid rate of decomposition of *Gliricidia sepium* leaf dry matter, 0.966 g/d^{-1}, occurred within the first 10 days, resulting in a 56% loss. The release of N, P and K were also rapid during the first 10 days but slower thereafter. About 60% of the N was lost within 10 days and a total of 76% of the original N content of the leaves was released within a 70-day period. Release of N and P were observed to be unrelated to that of C; initially the releases of N or P were more rapid than C, whereas between 10 and 30 days, the converse occurred.

Consistent with these observations, significant positive effects from leaf-pruning green manures were demonstrated on cereal and other crops, whether incorporated into the soil or applied as a mulch, both in Malaysia and the Democratic Republic of the Congo. In Sri Lanka, the application of prunings from the tree legumes significantly increased girth and canopy size of the coconut saplings; in general the performance of the saplings supplied with *L. leucocephala* and *G. sepium* prunings was better than when supplied with *S. siamea* prunings, which was better than that of the untreated control. The coconut saplings obtained about 36% of their N from *L. leucocephala* and 40% from *G. sepium* prunings. In the Democratic Republic of the Congo, the biomass, N concentration and grain yield of corn fertilized with a mixture of leaf prunings from *Albizia lebbeck* and *Acacia auriculiformis* were increased by 52, 54 and 67%, respectively, over the control.

Tolerance to soil salinity

In Pakistan, the mere presence of *Acacia ampliceps*, a salt-tolerant tree legume, resulted in the amelioration of saline conditions in the rhizosphere soil, showing promise for regenerating vast areas of currently unproductive saline soils. In that country alone, 5 M/ha are salt-affected with significant effects on local food production. Such salinization will be an increasing problem in arid conditions where ground-water irrigation methods are causing the accumulation of ions in topsoil. The research in Pakistan showed that infection of roots by vesicular-arbuscular mycorrhiza, and numbers of spores,

decreased with increasing salt concentration. On the other hand, the greatest mycorrhizal infections were observed in the roots of the trees that fixed the most N. The mycorrhizal spores isolated from saline soils were dark brown and thick walled compared with those from non-saline soil, indicating the possible strategy of selecting mycorrhiza for improved establishment of salt-tolerant trees in extreme conditions.

General discussion

In addition to the quantification of N_2 fixation it is of practical importance to develop ways of efficiently using the fixed N in prunings to enhance crop production and to improve and sustain soil fertility. Nutrient turnover from decomposing leaves must be considered over the long term. The accumulation of litter under N_2-fixing trees has the tendency to enrich the topsoil with available N that may, in turn, inhibit fixation. Future studies should address this issue. Data generated in Malaysia and the Democratic Republic of the Congo showed that an initial rapid release of nutrients and C was followed by much-reduced rates of decomposition, indicating the presence of recalcitrant fractions. Leaching seems to be the dominant mode of release of K, Mg and Ca. Early increases in C:N and C:P ratios were followed by decreases, with peak values at approximately 10 days, demonstrating that the processes of mineralization of these elements are not closely linked during the early period of rapid decomposition. On average, about 50% of applied leaf litter was decomposed within 10–20 days. An understanding of nutrient dynamics in decomposing plant residues is crucial to the matching of nutrient supply with crop demand. For example, studies in Malaysia with *Gliricidia sepium* in hedgerows showed that application of leaf prunings to sweet corn at 21 and 45 days after planting proved to be most beneficial to the crop, demonstrating that, in terms of nutrient supply, there is no advantage of the cut-and-carry system over permanent hedgerows.

Particularly noteworthy was the success in identifying N_2-fixing tree species capable of growing under adverse conditions. In Pakistan, *Acacia ampliceps* was found to thrive in hyper-saline soils where other crops fail; declines in electrical conductivity levels of rhizosphere soil indicated the potential to reclaim such soils. In Chile on degraded soils under arid conditions, the exotic species of *Chamaecytisus* was found to have particular utility as a source of green manure, fuel wood and animal fodder.

The data published in this TECDOC demonstrate the important potential for using N_2-fixing trees with nuclear techniques as invaluable tools in research to develop novel technologies to improve and maintain soil fertility and thus achieve sustainable crop production in countries in which food security is most at risk. The pressing need to reverse current processes by which soil productivity is being lost over vast areas, without resorting to the now-outmoded traditional bush-fallow means of regeneration, must be addressed. Alley cropping and related agro-forestry systems have the potential to renew organic inputs that contribute organic matter and plant-available nutrients, and offer, with modest inputs of fertilizers, long-term sustainability. The employment of trees as a central component for the development of affordable resources for subsistence-farmer use is not an easy solution, and perhaps the greatest utility of the data herein lies in defining some of the most pressing difficulties that must be addressed. Judicious use of trees in the rural environment can provide the ecological framework within which food, wood, and fiber production can be integrated, enhancing the quality and preservation of land systems.

MANAGEMENT AND QUANTIFICATION OF NITROGEN FIXATION IN *LEUCAENA LEUCOCEPHALA*

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Abstract

The effects of pruning and age on N_2 fixation were studied using *Leucaena leucocephala* isoline K28, with *Cassia siamea* as the non-N_2-fixing reference species, at a site of degraded soil fertility typical of much of the farming land in Ghana. The ^{15}N-dilution method was used to estimate N_2 fixation. *Cassia siamea* consistently produced higher total biomass and total N yields than did *L. leucocephala*. The mean value for the fraction of N derived from fixation (%Ndfa) was higher for pruned *L. leucocephala* (36%) than for unpruned trees (18%). There was some underestimation of N_2 fixation as a result of using *C. siamea* as the reference, and because root N-contents were not determined. Strong, significant linear correlations were observed between foliar and whole-tree (weighted average) percent ^{15}N atom excess in unpruned *L. leucocephala* and *C. siamea*, suggesting that foliar ^{15}N enrichment can be used to accurately estimate %Ndfa. The results demonstrated that the ^{15}N-enrichment methodology can provide meaningful estimates of %Ndfa and total N_2 fixed for mixed tree plantations under field conditions, when adequate spacing is provided.

1. INTRODUCTION

The possible contribution of N_2-fixing trees (NFTs) to the improvement of soil fertility, particularly in terms of N contribution, requires attention. There is a need for quantitative assessment of symbiotic N_2 fixation in such trees under field conditions, to determine their utility for agroforestry systems in tropical agriculture. The application of isotope-dilution methodology, using fertilizer enriched in ^{15}N, has been extensively reviewed and discussed [1-5].

The methodology involves the application of a small quantity of ^{15}N-enriched fertilizer to the soil. Nitrogen-fixing trees will absorb atmospheric N supplied by the root-nodule symbionts, in addition to the isotope-enriched N from the soil. Since non-fixing trees do not have access directly to atmospheric N_2, the resulting $^{15}N/^{14}N$ ratio of tissue N in any two tree species will differ in proportion with the amount of N fixed.

A major limitation of the methodology is the uncertainty in the choice of suitable non-N_2-fixing reference species. The $^{15}N/^{14}N$ absorbed by the fixing plant changes significantly with time, therefore, for a non-fixing plant to be a suitable reference, it must absorb $^{15}N/^{14}N$ with the same temporal pattern, and both should obtain their mineral N from similar soil-N pools [1,6].

The project was started in 1991 on a degraded soil, typical of most farming areas in Ghana, where there is the need for fertility restoration. The objectives were as follows.

- To measure N_2 fixation by *Leucaena leucocephala* using *Cassia siamea* as the reference species.
- To determine the effect of pruning and tree age on N_2 fixation.
- To study the availability to maize of N from *L. leucocephala* and *C. siamea* prunings using ^{15}N methodology.

2. MATERIALS AND METHODS

The experiment site was located on the campus of the University of Science and Technology, Kumasi (6° 41'N, 1° 38'W, elevation 288 m), within the moist semi-deciduous forest zone of Ghana that is characterized by two peak rainy seasons, with a mean annual total rainfall of about 1,250 mm. Temperatures are uniformly high throughout the year, with low and high monthly averages of about 25 and 28°C recorded in August and February, respectively. The mean monthly relative humidity ranges between 82 and 96% at 0600 h and 37 and 72% at 1500 h.

2.1. Soils

The experimental area was composed of two soil types, of the Akroso (Gleyic Alisol, Oxic Haplustult) and Nta series (Dystric Regosol, Typic Ustipsamment), both of which developed in situ over Cape Coast granite and have the characteristics given in Table I.

Seeds of the NFT *L. leucocephala* isoline K28 were soaked in hot water for 10 min and left to dry overnight. The rhizobial strain, TAL 1145, isolated from *L. leucocephala* in Colombia and obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria, was applied to seeds as a peat-based inoculant using 40% (v/v) gum arabic as sticker to ensure approximately 10^7 cells per seed.

The non-fixing reference species was *Cassia siamea,* the seeds of which were also soaked in pre-boiled water for 10 min and left to dry overnight. The seedlings were nursed in plastic bags.

2.2. Field layout

The project was initially designed for 5 years and consisted of 25 sub-plots [i.e. 5 years per five replicate plots (Fig. 1a)]. Lining, pegging and field planting of seedlings was done on 4 June 1991, with a uniform spacing of 4×3 m. The perimeter of each isotope sub-plots was trenched (Fig. 1b), with a plastic-film barrier installed to a depth of 0.9 m to serve the following purposes:

- to prevent lateral flow of water and fertilizer,
- to restrict root growth to within the experimental plots, and
- to prevent or reduce root exploration from outside the labelled zone/plot.

2.3. ^{15}N labelling and pruning

Three months after establishing the plantation in 1991 and annually for six years thereafter, ^{15}N-enriched ammonium sulphate (10% ^{15}N atom excess) was applied to the trenched sub-plots that were to be harvested that year (Fig. 1a), in solution at a rate of 2 g N/m^2. As an optional treatment, appropriate trees were pruned every 5 months to a height of 1 m throughout the period of the project.

TABLE I. SOIL CHARACTERISTICS

Series	Depth (cm)	pH (1:2.5 H_2O)	Organic C (%)	Total N (%)	Bray P_1 (mg/kg)	ECEC (meq/100g)	Base saturation (%)
Akroso	0-15	4.6	0.72	0.136	15.6	6.49	26
	15-30	4.5	0.72	0.042	17.6	8.33	18
	30-60	4.5	0.32	0.028	20.2	9.70	17
Nta	0-15	4.9	0.86	0.140	18.8	4.84	42
	15-30	4.8	0.32	0.044	13.8	5.63	36
	30-60	4.6	0.24	0.028	11.2	10.4	27

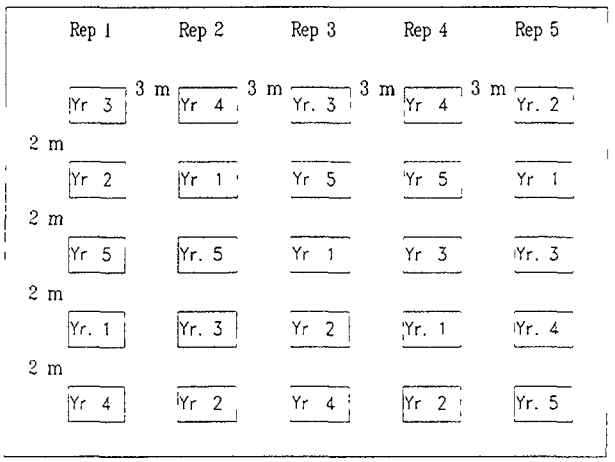

Fig 1(a) Field Layout of 5-year Project showing 5 replications and randomization of years within replicates.

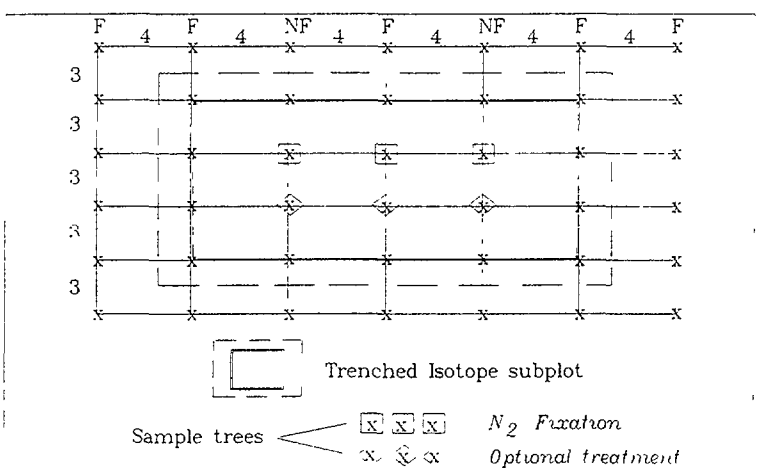

Fig 1(b). Typical plot showing spacing between trees (4m x 3m), trenched isotope subplots and sample trees

2.4. Harvesting, sample preparation and analysis

At the end of each year, selected *L. leucocephala* and *C. siamea* trees were harvested by cutting as closely as possible to soil level. Each was separated in the field into leaves, stems and branches, with each component weighed fresh. Random sub-samples of each were taken and oven-dried at 70°C. From these oven-dry weights, the total dry weight of the whole components were calculated.

The dried tree parts were milled to pass through a 1-mm sieve. Analyses for total N and ^{15}N atom excess were made on a 1500 Carlo-Erba coupled to a VG-Isogas mass spectrometer at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria.

2.5. Calculation of N_2 fixation

The isotope-dilution method [3] was used to estimate N_2 fixation, either for individual plant components or on a whole-plant basis. Component estimates of N_2 in *L. leucocephala* used the corresponding plant part of *C. siamea* as reference. Fixation by the whole plant was calculated from the weighted atom $\%^{15}N$ excess (WAE) in the fixing and non-fixing trees, using the following equation [6]:

$$WAE = \frac{AE_L \times TN_L + AE_{Br} \times TN_{Br} + AE_S \times TN_S}{TN_L + TN_{Br} + TN_S}$$

where

AE is the atom $\%^{15}N$ excess of each component [leaves (L), branches (Br) and stems (S)],
TN is the total N yield of each of L, Br and S.

The fraction of N in *L. leucocephala* derived from fixation (i.e. from the atmosphere, %Ndfa) was calculated as follows:

$$\%Ndfa = \left(1 - \frac{WAE_{Leucaena}}{WAE_{Cassia}}\right) \times 100$$

The amount of N fixed in *L. leucocephala* was calculated by the equation:

$$\text{Total N fixed (g/plant)} = \frac{\%Ndfa}{100} \times \text{Total N yield}$$

2.6. Organic-matter decomposition

As an optional study in 1994 and 1996, prunings from *L. leucocephala* and *C. siamea* were used as sources of organic matter. The aim was to examine rates of mineralization of N and assess the quantities available to a test crop (maize, 'Abeleehi') and remaining in the soil. The experiment had a randomized complete-block design with three treatments with ^{15}N-fertilizer applied:

- without prunings,
- with *L. leucocephala* prunings (3.38% N),
- with *C. siamea* prunings (2.50% N).

The prunings were incorporated into the soil, to supply N at rates equivalent to 160 kg N/ha, replicated four times. Plots were 3.2 m^2 in area and ^{15}N-labelled ammonium sulphate (10.1 atom $\%^{15}N$ excess) was applied at a rate of 2 g N/m^2. Basal application of triple superphosphate and muriate of potash were made at rates equivalent to 50 kg P_2O_5/ha and 50 kg K_2O/ha. The plots were sown to maize (50×20 cm spacing with two plants per hill, i.e. 72 plants per 3.2 m^2) and harvested for total dry matter after 50 days, i.e. at tasselling. Nitrogen in the maize derived from pruning organic matter (OM) was calculated using the following relationships.

$$\%\text{N derived from OM} = 1 - \left(\frac{^{15}\text{N a.e. in plant part}}{^{15}\text{N a.e. in control}} \right) \times 100 \qquad (1)$$

Multiplication of (1) by total N gives N derived from OM in kg/ha.

2.7. Statistical analysis

Differences between means were analyzed for statistical significance by ANOVA, using MSTAT software.

3. RESULTS AND DISCUSSION

3.1. Total dry-matter yield and distribution

A summary of total above-ground biomass accumulations over 6 years is presented in Table II. *Cassia siamea* consistently produced more dry matter than did *L. leucocephala.*

At the first harvest, leaves constituted the greatest proportion of total dry matter in both *L. leucocephala* and *C. siamea* (data not shown). However in the second, third and fourth harvests, branches formed the largest proportion. In the fifth harvest, branches (in *Cassia*) and stems (in *Leucaena*) formed the greater proportions. In the sixth harvest, branches formed the greatest proportion of unpruned trees, and stems constituted the greatest component of pruned trees.

3.2. Total N yield

Table III shows the total-N yields for 5 years. Differences between the two species in all the biomass harvests were highly significant ($P = 0.01$), with total N yield of *C. siamea* being between 2 to 11 times higher than that of *L. leucocephala*, depending on the pruning treatment. The consistently high total-N yields in *C. siamea* calls for a critical evaluation of this species as a source of N.

TABLE II. ABOVE-GROUND BIOMASS ACCUMULATIONS OVER 6 YEARS

Species/ option	Mean total dry matter (kg/plant)					
	1992	1993	1994	1995	1996	1997
L. leucoceph.						
Pruned	1.23	2.69	4.97	2.17	2.60	2.71
Unpruned	1.97	4.82	7.89	11.9	21.1	30.9
C. siamea						
Pruned	4.48	15.1	19.1	19.8	14.1	17.7
Unpruned	6.02	36.6	88.4	123	145	234
Signfce	**	**	**	**	**	**
$LSD_{0.05}$	1.70	5.80	9.07	33.4	20.6	61.7
CV(%)	39	31	24	67	35	68

TABLE III. TOTAL N YIELDS (NOT INCLUDING PRUNINGS REMOVED FROM THE PLOTS) OF *L. LEUCOCEPHALA* AND *C. SIAMEA* OVER 5 YEARS

Species	Treatment	Total N yield (g/plant)				
		1992	1993	1994	1995	1996
L. leuco.	Pruned	23.2	24.3	45.6	14.1	17.9
	Unpruned	38.5	47.4	63.2	94.1	179
C. siamea	Pruned	68.7	151	152	153	110
	Unpruned	81.2	303	740	787	1,329
Significance		**	**	**	**	**
$LSD_{0.05}$		26.3	64.3	180	210	224
CV(%)		39.0	38.4	56.4	62.7	43.0

TABLE IV. PERCENTAGE N_2 FIXED BY *L. LEUCOCEPHALA* 2 TO 5 YEARS AFTER PLANTATION ESTABLISHMENT

Treatment	%Ndfa[a]				
	1993	1994	1995	1996	Mean
Pruned	45	27	40	34	36
Unpruned	32	6.7	22	11	18

[a]Calculated using weighted atom $\%^{15}N$ excess (WAE) for the whole plant.

The total N yields among the various components of the *L. leucocephala* and *C. siamea* trees varied considerably with pruning and age. In the early stages of the establishment of the plantation, higher total-N accumulation occurred in leaves of both species, but with time relatively higher N accumulations were found in branches (data not shown).

3.3. %Ndfa

On a whole-plant basis, 2- to 5-year-old *L. leucocephala* trees, without considering the prunings removed from the plots, had higher %Ndfa values when pruned (mean of 36%) than when unpruned (18%) (Table IV). However, the differences were not significant owing to high CV values. The %Ndfa determinations were under-estimates due to the fact that *C. siamea* is a poor reference species for *L. leucocephala,* and also because the roots were notcollected.

3.4. Foliar sampling for whole-tree estimates

Throughout the study, estimates of %Ndfa and total N fixed were made using the whole-tree (weighted average) $\%^{15}N$ atom excess values, which involved a lot of work. The possibility of using foliar $\%^{15}N$ atom excess (FAE) data to estimate WAE was explored.

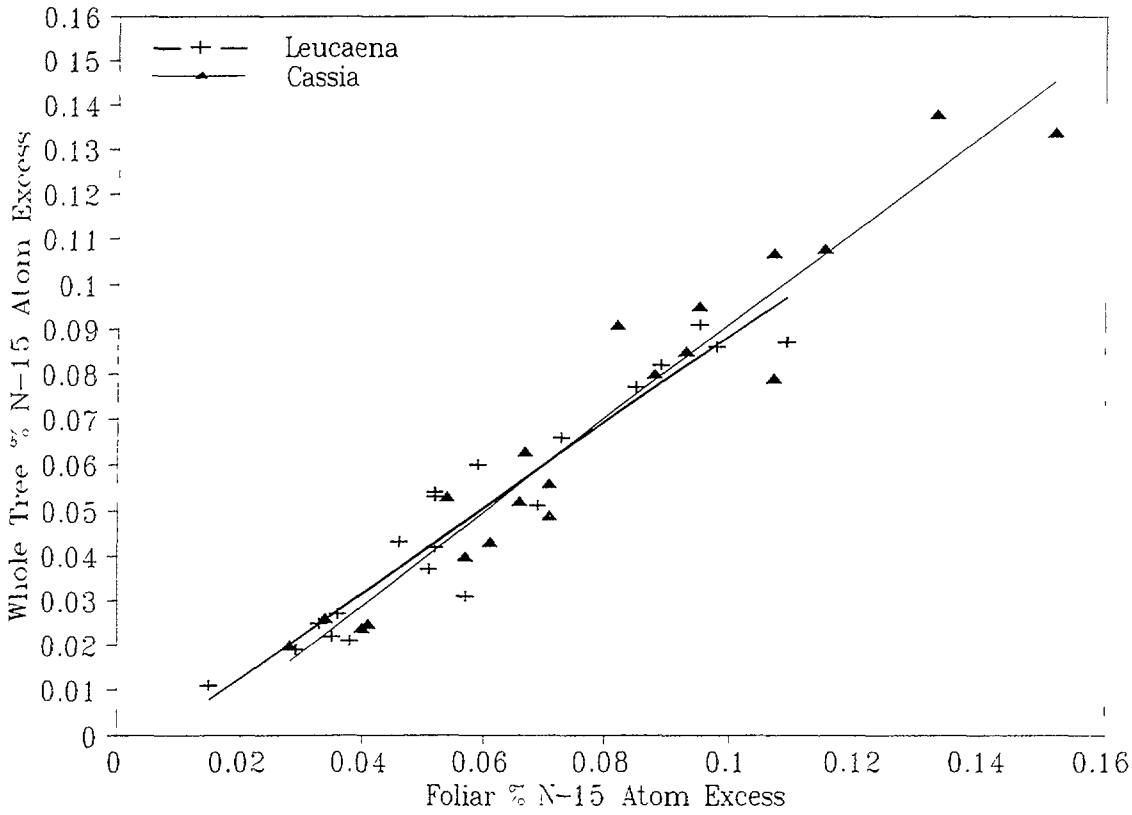

FIG. 2. Relationship between foliar and whole-tree (weighted-average) $\%^{15}N$ a.e. in unpruned L. leucocephala *and* C. siamea, *based on whole-tree harvests at 2, 3, 4, and 5 years. For* L. leucocephala: WAE = 0.948.FAE – 0.006; $r^2 = 0.92$. *For* C. siamea: WAE = 1.040.FAE – 0.013; r^2=0.93. *For both species:* WAE = 1.003.FAE – 0.010; r^2=0.93, *where WAE and FAE are $\%^{15}N$ a.e. based on whole-tree biomass sampling and foliar biomass sampling, respectively.*

There were strong, significant linear relationships between FAE and WAE for pruned and unpruned *L. leucocephala* and *C. siamea* (Fig. 2), supporting previous indications [7] that FAE can be used to accurately estimate whole-tree %Ndfa.

3.5. Organic matter decomposition

There were no significant differences between the fractions of N, or the total amounts of N, derived from *L. leucocephala* and *C. siamea* prunings, in the two maize crops (Table V). The cereal derived 85 and 69 mg plant^{-1} from *L. leucocephala* and *C. siamea* prunings, respectively, in 1994, and 164 and 142 mg, respectively, in 1996. These contributions of N from the green manures resulted in increases, albeit statistically insignificant due to high CV values, in maize dry weight and total-N accumulation, particularly in 1996. In future, heavier rates of residue should be applied and followed for longer periods of time.

4. CONCLUSIONS

The results from the mixed 6-year plantation indicate that the ^{15}N-enrichment methodology provided meaningful estimates of %Ndfa and of total N fixed by *L. leucocephala*, using *C. siamea* as the non-fixing check (however, see below).

The following conclusions are drawn from experience gained and from the data.

- In establishing a mixed plantation for assessing N_2 fixation, due consideration should be given to spacing. With the uniform spacing of 4 x 3 m, *C. siamea* outgrew and appeared to suppress the *L. leucocephala*, hence *C. siamea* was an inappropriate choice as the non-fixing reference species.
- The consistently higher total biomass and total N yields of *C. siamea* calls for its critical evaluation as a source of N.
- The N yields of the components of *L. leucocephala* and *C. siamea* varied considerably with tree age.
- Strong significant linear relationships between foliar and whole-tree (weighted average) $\%^{15}N$ atom excess values in trenched unpruned *L. leucocephala* and *C. siamea* indicate that foliar values alone can be used to accurately estimate %Ndfa.
- Organic-matter decomposition data showed similar values for the release of N from *L. leucocephala* and *C. siamea* prunings. Similar future research should include heavier rates of residue application over longer periods of time.

TABLE V. EFFECTS OF PRUNINGS FROM *LEUCAENA LEUCOCEPHALA* AND *CASSIA SIAMEA* INCORPORATED INTO SOIL ON GROWTH AND N NUTRITION OF MAIZE, 1994 AND 1996

	Total dry wt.		Total N		^{15}N enrichment[a]		N from OM		Total N from OM	
	1994	1996	1994	1996	1994	1996	1994	1996	1994	1996
	(g/plant)		(g/plant)		(%)		(%)		(mg/plant)	
No pruning	22.7	24.4	0.21	0.25	1.249	0.995				
+ *L. l.*	33.4	38.8	0.28	0.47	0.804	0.676	33	32	85.0	164
+ *C. s.*	26.6	41.5	0.23	0.43	0.806	0.586	32	42	68.6	142
Sig.	NS	NS	NS	NS	**	*	NS	NS	NS	NS
$LSD_{0\,05}$					0.245	0.221				
CV(%)	27	40	32	56	21	23	30	63	19	68

[a]From labelled fertilizer.

ACKNOWLEDGEMENTS

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NITROGEN FIXATION IMPROVEMENT IN *FAIDHERBIA ALBIDA*

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Abstract

A greenhouse experiment investigated growth, N accumulation and N_2 fixation (using the ^{15}N-dilution method) by *Faidherbia albida* in comparison with three species of *Acacia*, with *Parkia biglobosa* and *Tamarindus indica* as non-fixing reference plants. *Faidherbia albida* was mediocre in comparison with *A. seyel*, therefore seven provenances of the former were examined in a second pot experiment to investigate within-species variability for the same performance components; a provenance from Kabrousse, Senegal, showed particular promise in terms of dry weight and N accumulation, and fixation of N. This promise was confirmed with a 15-month field experiment, but revealed that there is opportunity for further improvement in N_2-fixing ability. *Faidherbia albida* is a slow-growing tree, therefore further field experiments with provenance Kabrousse should be longer term in scope. The data indicate that trenching of the ^{15}N-labelled area may not be necessary.

1. INTRODUCTION

Nitrogen fixing trees (NFTs) have an important role as sources of N and organic matter needed to rehabilitate degraded soils and to sustain fertility. However, some NFTs are potentially more useful than others, for example, analysis of soil under *Faidherbia albida* (synonym *Acacia albida*) in Senegal [1] indicated remarkable fertility: protein yields of millet near trees were three to four-fold higher than in distant plants. Although the usefulness of *F. albida* has been recognized, the few estimates that have been reported indicate that it is a poor N_2 fixer, but with genetic variability among provenances [2]. However, this evidence is based mainly on plant growth and nodulation.

This paper focuses on the N_2-fixing potential of *F. albida* in comparison with Sahelian *Acacia* species, namely *A. raddiana, A. senegal* and *A. seyal,* with emphasis on a provenance from Kabrousse, Senegal.

2. MATERIALS AND METHODS

2.1. Greenhouse experiments

The first experiment was carried out at the Bel-Air Experimental Station, Dakar, using a local soil of 93% sand that contains approximately 10^2 g^{-1} native *Bradyrhizobium* sp. *Albida,* and has a pH of 7.0, 1.9% C and 0.025 %N. The soil was sieved (<1 mm), mixed and weighed into 30-cm diameter pots (20 kg pot^{-1}). To each pot 1g K_2HPO_4 was added. Surface-sterilized seeds of the tree species were germinated in Petri dishes containing 0.8% agar. Treatments in H_2S0_4 were as follows: *A. raddiana* 120 min, *A. senegal* 14, *A. seyal,* 60, *F. albida* 30, *Parkia biglobosa* 60, *Tamarindus indica* 30. Two days after germination, the seedlings were transplanted singly into the pots. There were six treatments with four replicates: the two non-fixing reference species, *P. biglobosa* and *T. indica* and the four N_2-fixing species, *A. raddiana* and *A. senegal* (inoculated with *Rhizobium* strain ORS 1016), *A. seyal* (*Rhizobium* strain ORS 1088) and *F. albida* (*Bradyrhizobium* strain ORS 136). The rhizobial inoculants, containing 10^9 cells mL^{-1}, were applied in liquid form (10 mL pot^{-1}). At transplanting, ^{15}N-labelled fertilizer was applied at the rate of 0.2 g N pot^{-1} as a solution of $(NH_4)_2S0_4$ enriched in ^{15}N at 10.01 atom % excess. The pots were arranged randomly and watered so that soil moisture was kept close to field capacity. Five months after transplanting, plants were harvested.

A second greenhouse experiment was carried out at the same location. There were eight treatments with five replicates: the non N_2-fixing *P. biglobosa* was the reference species and provenances of *F. albida* from Senegal (Merina, Dangalma, Ndiongolor, Pire and Kabrousse) and

Burkina Faso (Gomblora and Dem) inoculated with *Bradyrhizobium* strain ORS 136, 10^9 cells mL^{-1} applied as liquid inoculum at 10 mL pot^{-1}. To all pots, N fertilizer was then applied at 0.2 g N pot^{-1} as a solution of $(NH_4)_2SO_4$ enriched at 9.60 ^{15}N atom % excess. The pots were arranged randomly and watered as in the first experiment. At 6 months after transplantation, plants were harvested.

2.2. Field experiments

The first field experiment was carried out at Mbao Forestry Station on a sandy soil, pH 7.6, 0.10% C and 0.010% N. Surface-sterilized seeds of *P. biglobosa* and *F. albida* from Kabrousse were germinated for 2 days and then transplanted into plastic pouches (one seedling per pouch). At transplantation, *F. albida* was supplied with the *Bradyrhizobium* strain ISRA 232 as liquid inoculum, 10^9 cells mL^{-1} and 10 mL per pouch. After 2 months of growth in the nursery, all plants were transplanted into the field in a randomized complete-block design with four replicates. Each plot was 3×2 m and the planting holes were 1-m spaced. Within each plot, a 2×1 m sub-plot was demarcated for ^{15}N-fertilizer application at the rate of 20 kg N ha^{-1} as a solution of $(NH_4)_2SO_4$ enriched at 9.60 ^{15}N atom % excess. Unlabelled ammonium sulphate was applied at the same rate to the remaining trees outside the sub-plot. A basal fertilizer was then applied to each planting hole at the rate of 100 kg ha^{-1} of triple superphosphate and 80 kg KCl ha^{-1}. The subplots were harvested at 3, 6 and 15 months after application of ^{15}N.

Nioro Experimental Station was selected for the second field experiment, on a sandy soil with a pH of 5.1, 0.31% C and 0.032% N. Scarified seeds of *F. albida* and *Parkinsonia aculeata* were pregerminated in Petri dishes containing 0.8% agar. After 2 days they were transplanted into plastic pouches (one seedling per pouch), each immediately inoculated with a 10-mL suspension of *Bradyrhizobium* strain ISRA 232 containing 10^9 cells mL^{-1}. After 2 months of growth in the nursery, the saplings were transplanted into the field in a factorial randomized complete-block design with four replicates. The main plot was time with two levels as Year 1 and Year 2. (The data for Year 2 will be published elsewhere.) The sub-plot was for *F. albida* as cultivated alone or mixed with the reference tree within a plot. The sub-sub-plot was the labelled area treatment as trenched or untrenched. The size of each plot was 12.5×5 m with a 2.5-m spacing between planting holes. Within each plot, 2.5×2.5 m and 5×2.5 m sub-plots for *F. albida* mono-cultured and associated with the reference tree, respectively, were demarcated for applying $(NH_4)_2SO_4$, enriched at 2 atom %^{15}N excess, at the rate of 20 kg N ha^{-1}. Unlabelled $(NH_4)_2SO_4$ was applied at the same rate to the remaining trees outside the sub-plot. A basal fertilizer was then applied to each planting hole at the rate of 100 kg ha^{-1} of triple superphosphate and 42 kg ha^{-1} of KCl. Plants were harvested at 12 months after transplantation,.

2.3. Plant analyses

In all experiments, dry weights of plant parts were recorded. Nitrogen concentration and ^{15}N atom % excess were determined at the FAO/IAEA Soil Science Unit at Seibersdorf, Austria. Nitrogen was estimated using an automated analyzer coupled to a mass spectrometer. Nitrogen fixation was calculated using the isotope-dilution equation [3]. Data were statistically analyzed using Newman and Keul's test.

3. RESULTS AND DISCUSSION

3.1. Nitrogen-fixing potential of *F. albida* in comparison with Sahelian *Acacia* species

The mean dry weight of nodules on *F. albida* was similar to that on *A. raddiana* and *A. senegal* (average 0.35 g $plant^{-1}$) and much lower than that on *A. seyal* (6.58 g $plant^{-1}$) (data not shown). The ^{15}N enrichments in the four NFTs were lower than those of the reference species, indicating significant N_2 fixation (Table I). *Acacia seyal* and *A. raddiana* had similar ^{15}N-enrichment values that were lower than those of *A. senegal* and *F. albida.*

The differences in the proportions and amounts of N fixed using *P. biglobosa* in comparison with *T. indica* (Table II) were not significant. Although the best estimates are obtained using the averaged atom $\%^{15}N$ excess for both reference species [4,5], subsequent decisions to use only *P. biglobosa* ensured that the N_2-fixation estimates were conservative.

TABLE I. DRY WEIGHT, TOTAL N, AND ATOM $\%^{15}N$ EXCESS IN COMPONENTS OF THREE *ACACIA* SPECIES AND *F. ALBIDA*, AND *P. BIGLOBOSA* OR *T. INDICA* AS REFERENCE SPECIES, CULTIVATED IN POTS AT THE BEL AIR EXPERIMENTAL STATION

Plant part	Species	Dry weight (g plant^{-1})	Total N (g plant^{-1})	$\%^{15}N$ a.e.
Leaf	*P. biglobosa*	5.7^{cd}	0.2^{c}	0.31^{b}
	T. indica	1.3^{d}	0.1^{c}	0.42^{a}
	A. raddiana	13.0^{b}	0.4^{b}	0.13^{c}
	A. senegal	11.9^{bc}	0.4^{b}	0.22^{bc}
	A. seyal	48.5^{a}	1.2^{a}	0.17^{c}
	F. albida	17.2^{b}	0.5^{b}	0.23^{bc}
	CV(%)	26	35	27
Stem	*P. biglobosa*	4.1^{d}	0.1^{c}	0.32^{b}
	T. indica	1.5^{d}	0.1^{c}	0.42^{a}
	A. raddiana	23.4^{bc}	0.3^{b}	0.14^{cd}
	A. senegal	37.3^{b}	0.6^{a}	0.25^{b}
	A. seyal	80.9^{a}	0.8^{a}	0.13^{d}
	F. albida	21.0^{c}	0.3^{b}	0.24^{bc}
	CV(%)	36	38	26
Root	*P. biglobosa*	3.4^{c}	0.1^{bc}	0.32^{b}
	T. indica	2.1^{c}	0.1^{c}	0.42^{a}
	A. raddiana	10.7^{bc}	0.2^{bc}	0.11^{d}
	A. senegal	14.4^{bc}	0.3^{bc}	0.23^{c}
	A. seyal	55.0^{a}	0.8^{a}	0.10^{d}
	F. albida	22.0^{b}	0.4^{b}	0.20^{c}
	CV(%)	62	66	20
Total	*P. biglobosa*	13.1^{cd}	0.3^{cd}	0.32^{b}
	T. indica	4.9^{d}	0.1^{d}	0.42^{a}
	A. raddiana	47.2^{bc}	0.9^{bc}	0.13^{d}
	A. senegal	63.6^{b}	1.3^{b}	0.23^{c}
	A. seyal	184.4^{a}	2.8^{a}	0.13^{d}
	F. albida	60.2^{b}	1.2^{b}	0.22^{c}
	CV(%)	31	32	22

Numbers within plant part in the same column followed by the same letter are not significantly different ($P \leq 0.05$)

TABLE II. PERCENTAGE AND AMOUNT OF N DERIVED FROM FIXATION IN PLANT PARTS OF THREE *ACACIA* SPECIES AND *F. ALBIDA* USING EITHER *P. BIGLOBOSA* OR *T. INDICA* AS THE NON-FIXING REFERENCE, CULTIVATED IN POTS AT BEL AIR EXPERIMENTAL STATION.

Plant part	Species	%Ndfa with		Ndfa with	
		P. biglobosa	*T. indica*	*P. biglobosa*	*T. indica*
				(g plant^{-1})	
Leaf	*A. raddiana*	57^{a}	67^{a}	0.2^{b}	0.3^{b}
	A. senegal	29^{b}	44^{b}	0.1^{b}	0.2^{b}
	A. seyal	46^{ab}	58^{ab}	0.6^{a}	0.7^{a}
	F. albida	27^{b}	42^{b}	0.2^{b}	0.2^{b}
	CV(%)	40	28	70	54
Stem	*A. raddiana*	53^{a}	61^{a}	0.2^{b}	0.2^{b}
	A. senegal	24^{b}	36^{b}	0.1^{b}	0.2^{b}
	A. seyal	59^{a}	67^{a}	0.5^{a}	0.5^{a}
	F. albida	28^{b}	39^{b}	0.1^{b}	0.1^{b}
	CV(%)	30	28	55	53
Root	*A. raddiana*	64^{a}	72^{a}	0.1^{b}	0.1^{b}
	A. senegal	29^{b}	45^{b}	0.1^{b}	0.1^{b}
	A. seyal	69^{a}	75^{a}	0.6^{a}	0.6^{a}
	F. albida	37^{b}	51^{b}	0.2^{b}	0.2^{b}
	CV(%)	24	14	65	64
Total	*A. raddiana*	58^{a}	67^{a}	0.5^{b}	0.6^{b}
	A. senegal	27^{b}	42^{b}	0.4^{b}	0.5^{b}
	A. seyal	60^{a}	67^{a}	1.6^{a}	1.9^{a}
	F. albida	30^{b}	44^{b}	0.4^{b}	0.5^{b}
	CV(%)	29	21	48	47

For each plant part, values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

Our data indicated significant differences among the four NFTs in proportions and amounts of N fixed, in spite of high coefficients of variation (Table II) due probably to variable nodulation: whichever reference tree was used, *A. seyal* and *A. raddiana* had the highest %Ndfa values (averages 63% and 62%, respectively). Due to a high N-content (Table I), *A. seyal* accumulated more fixed N than did any other NFT. The %Ndfa values for leaves, stems and roots were higher in *A. raddiana* and *A. seyal* than in *A. senegal* and *F. albida* (Table II).

These results confirm the low N_2-fixing ability of *F. albida* and *A. senegal* reported by others [2,6,7]. In agreement with Dommergues [6], we classify *F. albida* with *A. senegal* as having low N_2-fixing potential (NFP), and categorize *A. seyal* as having a high NFP. As for *A. raddiana*, which had relatively high %Ndfa values but was low in the agronomically important total N fixed, we retain it in the high-NFP category.

TABLE III. $\%^{15}N$ ATOM EXCESS, DRY WEIGHT, N CONTENT AND TOTAL N IN PLANT PARTS OF *P. BIGLOBOSA* AND PROVENANCES of *F. ALBIDA*, CULTIVATED IN POTS AT BEL AIR EXPERIMENTAL STATION

Plant part	Species	Provenance	$\%^{15}N$ a.e.	D. wt. (g plant^{-1})	%N	Total N (g plant^{-1})	Ndfa (%)	Ndfa (g plnt^{-1})
Leaf	*P. biglobosa*		0.69^{ab}	13.4^{a}	2.71^{b}	0.36^{ab}	0.0	0.00
	F. albida	Merina (S)	0.78^{a}	7.4^{b}	2.87^{b}	0.23^{b}	0.0	0.00
		Dangalma (S)	0.75^{a}	8.4^{b}	3.06^{b}	0.26^{ab}	0.0	0.00
		Ndiongolor (S)	0.51^{bc}	12.2^{a}	2.75^{b}	0.33^{ab}	26^{ab}	0.09^{b}
		Pire (S)	0.52^{bc}	10.0^{ab}	3.08^{b}	0.31^{ab}	24^{ab}	0.07^{b}
		Kabrousse (S)	0.42^{c}	12.4^{a}	3.11^{b}	0.38^{a}	40^{a}	0.16^{a}
		Gomblora (BF)	0.60^{b}	6.7^{b}	3.59^{a}	0.23^{b}	14^{b}	003^{b}
		Dem (BF)	0.53^{bc}	7.8^{b}	3.58^{a}	0.28^{ab}	23^{ab}	0.06^{b}
		CV(%)	15	24	8.7	25	41	55
Stem	*P. biglobosa*		0.65^{bc}	12.0^{bc}	1.02^{c}	0.12^{b}	0.0	0.00
	F. albida	Merina	0.85^{a}	7.8^{c}	1.12^{bc}	0.09^{b}	0.0	0.00
		Dangalma	0.73^{ab}	9.1^{bc}	1.18^{abc}	0.11^{b}	0.0	0.00
		Ndiongolor	0.50^{c}	13.1^{abc}	1.06^{bc}	0.14^{ab}	23^{a}	0.03^{ab}
		Pire	0.50^{c}	14.5^{ab}	1.13^{bc}	0.16^{ab}	24^{a}	0.04^{ab}
		Kabrousse	0.39^{c}	18.4^{a}	1.07^{bc}	0.20^{a}	39^{a}	0.08^{a}
		Gomblora	0.58^{bc}	7.5^{c}	1.37^{a}	0.10^{b}	10^{a}	0.01^{b}
		Dem	0.54^{c}	9.8^{bc}	1.28^{ab}	0.13^{ab}	16^{a}	0.02^{b}
		CV(%)	19	31	11	32	65	82
Root	*P. biglobosa*		0.62^{ab}	27.4^{a}	1.28^{c}	0.35^{a}	0.0	0.00
	F. albida	Merina	0.73^{a}	20.5^{a}	1.73^{b}	0.36^{a}	0.0	0.00
		Dangalma	0.61^{ab}	21.0^{a}	1.76^{ab}	0.37^{a}	0.0	0.00
		Ndiongolor	0.45^{c}	27.6^{a}	1.87^{ab}	0.52^{a}	28^{a}	0.16^{a}
		Pire	0.45^{c}	24.7^{a}	1.66^{b}	0.40^{a}	28^{a}	0.12^{a}
		Kabrousse	0.40^{c}	27.9^{a}	1.84^{ab}	0.50^{a}	35^{a}	0.20^{a}
		Gomblora	0.53^{b}	20.4^{a}	2.06^{a}	0.40^{a}	15^{a}	0.06^{a}
		Dem	0.51^{b}	19.5^{a}	1.90^{ab}	0.36^{a}	18^{a}	0.08^{a}
		CV(%)	21	36	9.6	36	67	84
Total	*P. biglobosa*		0.66^{ab}	52.7^{a}	1.67^{c}	0.83^{a}	0.0	0.00
	F. albida	Merina	0.78^{a}	35.6^{a}	1.91^{b}	0.67^{a}	0.0	0.00
		Dangalma	0.70^{a}	38.5^{a}	2.00^{b}	0.73^{a}	0.0	0.00
		Ndiongolor	0.49^{b}	52.9^{a}	1.90^{b}	0.99^{a}	26^{ab}	0.26^{ab}
		Pire	0.49^{b}	49.9^{a}	1.96^{b}	0.87^{a}	25^{ab}	0.24^{ab}
		Kabrousse	0.41^{b}	58.7^{a}	2.01^{b}	1.08^{a}	38^{a}	0.44^{a}
		Gomblora	0.57^{b}	34.5^{a}	2.34^{a}	0.74^{a}	13^{b}	0.10^{bc}
		Dem	0.53^{b}	37.1^{a}	2.26^{a}	0.77^{a}	19^{ab}	0.16^{bc}
		CV(%)	16	30	6.6	30	50	70

(S): Senegal, (BF): Burkina Faso.
For each plant part, values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

The root is often not harvested in BNF studies, especially in grain legumes, on the assumption that it contributes little to the fixed-N total for the whole plant [8,9]. On the other hand, in trees, although ignoring roots may have little effect on %Ndfa estimates, our data (Table II) indicate that it may result in significant underestimation of N fixed, as suggested by Sanginga et al. [10]. In this study, the roots contained 26 to 38% of the fixed N.

3.2. Genetic variability in N_2 fixation among *F. albida* provenances

There were significant differences among the provenances of *F. albida* in terms of ^{15}N enrichment, dry matter and total-N yield (Table III). For each plant part, the ^{15}N atom % excess values for the Merina and Dangalma provenances were highest, including the reference species, whereas the provenance from Kabrousse was lowest. The latter yielded most in terms of dry weight and total N of leaf and stem, whereas there were no significant differences among the provenances in terms of root or whole plant. Kabrousse had the highest values for the proportion (%Ndfa) and amount (Ndfa) of N derived from fixation, although its superiority was not always statistically significant (Table III). The coefficients of variation were high resulting in negative Ndfa values for Merina and Dangalma, indicating zero N_2 fixation. In general, fixation of N_2 was low, with a maximum of 38 for %Ndfa, i.e. 440 mg N $plant^{-1}$.

Although *F. albida* has a low N_2-fixing potential, it presents agronomic characteristics that are of greater utility than do the *Acacia* species. Therefore, enhancing its ability to fix N_2 would increase its ability to improve soil N fertility. Provenance selection is needed to reach this goal. Sanginga et al. [2] recommended that N_2-fixation improvement of *F. albida* should include low capacity for soil-N uptake. Our determinations of amounts of N derived from soil indicated no significant differences among the seven provenances (data not shown); appraisal of this component should be included in future provenance-selection of *F. albida*

3.2. Nitrogen fixed in *F. albida*

The amounts of N fixed by the provenance from Kabrousse were estimated 3, 6 and 15 months after the transplantation into the field at Mbao, as 540, 790 and 1,410 mg N $plant^{-1}$ (Table IV), corresponding to 54, 79 and 141 g N ha^{-1}, respectively, at the adopted plant density of 100 plants ha^{-1}.

At Nioro, nodule-like swellings were found on the lateral roots of *F. albida* only. Dry-weight and total-N yields were higher in *F. albida* cultivated alone in untrenched treatments. The ^{15}N enrichment was lower in *F. albida* than in the reference tree, indicating N_2 fixation. The enrichment was lower in mixture with the reference tree within the untrenched treatments, indicating that *F. albida* fixed more N in such conditions and that it was not necessary to trench the ^{15}N-labelled area. The N_2-fixation estimates, at up to 25 g N $plant^{-1}$ (Table V), were likely overestimated with *P. aculeata* as the reference tree. Previous studies of *F. albida* have indicated less ability to fix N_2 in comparison with *A. holosericea* [11], *Gliricidia sepium* [12], 1994) and *Leucaena leucocephala* [2], confirming data reported by Ndoye et al. [13] and Gueye et al. [14].

4. CONCLUSION

Although the *F. albida* provenance from Kabrousse fixed the highest amounts of N, its contributions to the restoration and the maintenance of soil fertility through N_2 fixation were meagre, therefore the status of the species remains unchanged as a poor fixer. However, it should be borne in mind that *F. albida* is a slow-growing NFT; long-term field studies are need with the provenance from Kabrousse to determine its true potential to fix N_2. One year after transplantation of *F. albida* into the field, the fixed N measured in the mono-cultured *F. albida* plots was significantly higher than that of mixed plants, indicating no need for trenching the ^{15}N-labelled area for accurate measurement of fixed N, due probably to its root structure.

TABLE IV. NITROGEN DERIVED FROM FIXATION IN PLANT PARTS OF 3-, 6- AND 15-MONTH-OLD FIELD-GROWN *F. ALBIDA* FROM KABROUSSE, SENEGAL, CULTIVATED AT MBAO EXPERIMENTAL STATION USING *P. BIGLOBOSA* AS REFERENCE

Age (months)	Plant part	%^{15}N a.e.	%Ndfa	Ndfa (g plant^{-1})
3	Leaf	0.89^{a}	60^{a}	0.25^{a}
	Stem	0.87^{a}	48^{a}	0.10^{a}
	Root	0.74^{a}	54^{a}	0.17^{a}
	Total	0.83^{a}	55^{a}	0.54^{a}
	CV(%)	48.3	40	81.9
6	Leaf	1.22^{a}	56^{a}	0.17^{a}
	Stem	1.07^{a}	59^{a}	0.15^{b}
	Root	1.21^{a}	60^{a}	0.38^{b}
	Total	1.18^{a}	58^{a}	0.79^{a}
	CV(%)	61.5	45	63.6
15	Leaf	1.01^{a}	56^{a}	0.21^{b}
	Stem	1.08^{a}	54^{a}	0.34^{b}
	Root	1.08^{a}	51^{a}	0.77^{ab}
	Total	0.83^{a}	55^{a}	1.41^{a}
	CV(%)	40.8	35	70.9

At each age, values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE V. DRY WEIGHT, TOTAL N, PROPORTION (%NDFA) AND AMOUNT (NDFA) OF FIXED N IN 12-MONTH-OLD FIELD-GROWN *F. ALBIDA* FROM KABROUSSE, SENEGAL, USING *P. ACULEATA* AS REFERENCE

Treatment	Dry weight (g plant^{-1})	Total N (g plant^{-1})	%^{15}N a.e.	%Ndfa	Ndfa (g plant^{-1})
Alone	1,277^{b}	29.0^{b}	0.153^{a}	46^{ab}	16.5^{ab}
Trenched	2,128^{a}	46.4^{a}	0.108^{ab}	26^{b}	10.0^{b}
Untrenched					
Mixed	1,577^{ab}	34.9^{ab}	0.155^{a}	46^{ab}	16.1^{ab}
Trenched	1,469^{ab}	32.7^{ab}	0.092^{b}	69^{a}	24.7^{a}
Untrenched					

Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

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NITROGEN FIXATION IN FOUR DRYLAND TREE SPECIES IN CENTRAL CHILE

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Abstract

Results are presented from a 5-year experiment using ^{15}N-enriched fertilizer to determine N_2 fixation in four tree species on degraded soils in a Mediterranean-climate region of central Chile in which there are 5 months of drought. Species tested included three slow-growing but long-lived savannah trees native to southern South America, (*Acacia caven, Prosopis alba and P. chilensis*; *Mimosoideae*), and Tagasaste (*Chamaecytisus proliferus* ssp. *palmensis*; *Papilionoideae*), a fast-growing but medium-lived tree from the Canary Islands. Tagasaste produced four- to twenty-fold more biomass than the other species, but showed declining N_2 fixation and biomass accumulation during the 5th year, corresponding to the juvenile-to-adult developmental transition. Nitrogen content was significantly higher in Tagasaste and *Acacia caven* than in the other species. The data revealed inter-specific differences in resource allocation and phenology of N_2 fixation rarely detailed for woody plants in dryland regions.

1. INTRODUCTION

Although studies have been conducted on biological N_2 fixation (BNF) by trees of temperate and moist-tropical regions [1,2,3, other chapters in this volume], little is known of the factors that affect BNF by shrubs and trees in arid and semiarid areas [4,5]. Given the water deficits, the low levels of soil N and P, and variability in micro-symbiont rhizobial population density and composition, large variability in BNF and growth patterns in general is to be expected in N_2-fixing trees (NFTs). This variability needs to be better understood if NFTs are to be used for managing ecosystems more efficiently and effectively, and to aid the design of agroforestry systems [6-9].

An anthropogenic savanna-like formation, locally known as "espinales", occupies over 2 M ha of the Mediterranean climate region of central Chile [10]. Yields in this region are low, both in dryland farming systems and livestock husbandry. We have postulated that an invasive woody weed, also a NFT, has "parasitized" the espinales [10-12]. This species is *Acacia caven* (Mol.) Mol., the "espino" after which, along with several *Prosopis* species, the area is named. In the interior dry-lands of central Chile, where espinales dominate, approximately 300,000 people farm, graze and cut firewood intensively. These farmers generally lack the financial resources required to improve or even maintain agricultural productivity. Furthermore, there is increasing pressure on land-owners to sell their farmland for commercial purposes, such as paper-pulp production. Thus, there is an urgent need to improve these agroforestry systems with appropriate trees, pastures and crops [10,12-18].

We conducted a 5-year study with the objective of comparing BNF in three economically important *Mimosoideae* species from South America, and an unusually fast-growing *Papilionoideae*

species from the Canary Islands, *Chamaecytisus proliferus*, that has potential for agroforestry systems in central Chile [17].

The data presented here cover a) annual and cumulative biomass in both above-and below-ground organs, and, for the NFTs, b) root nodulation and BNF, extending a report of the first 2 years of the experiment [14]. Trends apparent after 5 years' growth are discussed in terms of both ecological and applied aspects, including varying resource-uptake and -utilization strategies related to ontological strategy and biogeographic origin, as well as the niches these species could occupy in the development of agroforestry systems.

Genetic variability within species was not taken into account, even though such variation in BNF is known to occur [19]. Instead, a long-term in-situ approach was adopted in order to reveal year-to-year variations in the components studied.

2. MATERIALS AND METHODS

The experimental site was located in the sub-humid Mediterranean-climate zone of central Chile, in a field representative of local conditions [18] at the Instituto de Investigaciones Agropecuarias (INIA) Experimental Center in Cauquenes, in the sub-humid portion of the Mediterranean-climate zone of Chile (35°58'S; 72°17'W) with mean annual precipitation of 695 mm, and a summer drought period of 5-6 months. Soils are loamy and of granitic origin (Maule series in Chilean terminology). Prior to planting, six randomly spaced soil samples were analyzed, yielding the following baseline data for the 0-30 cm layer (mean $\pm$ SD): organic matter 1.45 $\pm$ 0.15%; pH 6.03 $\pm$ 0.35; total N 0.06 $\pm$ 0.0%; available N (ppm) 5.67 $\pm$ 4.82; available P (ppm) 2.5 $\pm$ 0.5; and available K (ppm) 95.8 $\pm$ 49.3. Total rainfall at the site for 1992 to 1996 was 975, 655, 502, 509 and 499 mm, respectively (Fig. 1).

2.1. Plant material

The following NFTs were evaluated: *Acacia caven, Prosopis alba, Prosopis chilensis* and *Chamaecytisus proliferus* ssp. *palmensis* ("Tagasaste"). The *Prosopis* species have a taller, more widely-spreading growth habit than the locally dominant *Acacia caven*, and thus potentially provide greater shade and canopy cover while bearing large quantities of palatable, nutritious pods [7,20]. Like *A. caven*, these *Prosopis* species are slow growing but long-lived desert or savanna trees of the *Mimosoideae* sub-family, and native to southern South America. Tagasaste, a fast-growing but short-lived *Papilionoideae* species tree endemic to La Palma, Canary Islands, is a valuable source of animal fodder in the Mediterranean-climate zones of Australia, Spain and elsewhere [21], and is well adapted to central Chile [14,22]. The two non-fixing reference species used for isotope-dilution calculations were the native *Schinus polygamus* (*Anacardiaceae*), and the introduced, but widely naturalized, *Fraxinus excelsior* (*Oleaceae*).

2.2. Rhizobial strains and nursery propagation

From preliminary examination (S. Dhillion, personal communication) it appears that Chilean espinal soils are particularly deficient in microbial biomass, including rhizobia. Therefore, inoculation of the legume trees was deemed advisable. Inoculum for the three native *Mimosoideae* was prepared from nodules collected in the field from tree roots, and then transported to the laboratory in Venoject tubes charged with a desiccant. Rhizobial strains were isolated on yeast-extract mannitol agar and conserved on YEM slants at 5°C. Separate strains for *Acacia caven* and the two *Prosopis* species were then multiplied in YEM broths incubated for 5-7 days at 25°C. A specific rhizobial strain for Tagasaste was obtained from the Phoenix Company, Tasmania. Fresh seeds of all species were scarified with rough sandpaper and pre-germinated (48 h) in an incubator at 20°C. Pre-germinated seeds of the NFTs were surface sterilized, and then inoculated with broth containing the appropriate rhizobial isolate for each species, immediately prior to planting in a shadehouse. Plastic sleeves (10×50 cm) were used, each containing 1.5 kg of a potting medium that favours root

development. Seedlings were irrigated daily until well established, and held in the nursery for 7 months prior to field planting.

2.3. Experimental site and layout

The experiment had a randomized block design, with four replications per species, and included plots with and without ^{15}N that were established simultaneously. Labelled plots were used for non-destructive plant sampling for N_2-fixation measurements. Reference plants and N_2-fixing plants were planted together randomly with a square spacing of 2 m to minimize root-grafting between adjacent trees. The perimeter of each area amended with ^{15}N, and which contained four plants of each tree species, was trenched to a depth of 80 cm and lined with 0.8-mm plastic film.

Unlabelled plots were established to determine growth rates (aerial and below ground), nodule number and weight, and time courses of biomass accumulation, and N content of all plant parts through destructive, whole-plant sampling. The five plots were divided into four sub-plots comprising three plants of each NFT (or control). Each plot was used every year to provide sample plants.

Following planting, Type I plots were labelled by applying 18 kg N ha^{-1} as a solution of ammonium sulphate enriched in ^{15}N at 10 atom % excess. The soil was then cultivated with a walk-behind rototiller. The area of each labelled plot was 96 m^2 and thus 384 m^2 were labelled in total. Type II plots and the borders of Type I plots were fertilized with the same rate of unlabelled ammonium sulphate. In view of the known macro- and micro-nutrient deficiencies affecting the soil of the site, additional fertilizer was supplied in the planting holes of all trees: 100 g normal superphosphate, 15 g mixed K_2SO_4 and $MgSO_4$, and 0.5 g boron calcite.

The entire experimental site (0.4 ha) was fenced off to prevent damage by rabbits. The field was kept clear of weeds by herbicide application plus a twice-yearly hoeing around each tree.

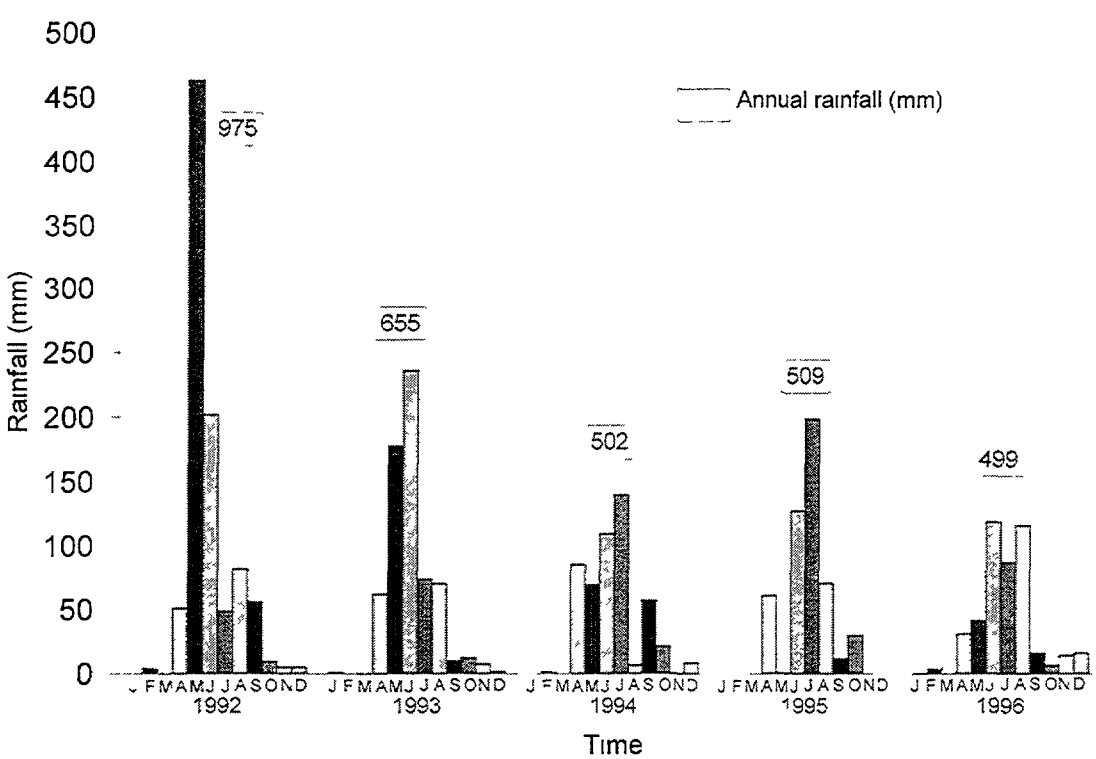

FIG 1. Annual rainfall during the 5-year experimental period

During the dry summer months of the first year, supplementary irrigation was applied each month at 15 L tree^{-1} to ensure good stand establishment. No further irrigation was applied.

2.4. Data collection and analysis

2.4.1. Type I plots

Growth rates were evaluated by measurement of tree height, trunk diameter and crown width following each season of active growth. Sampling for total N and isotope enrichment was carried out twice yearly: at the height of the most active growth (November) and at the end of the growing season (March). From each of the four trees on a plot, random samples of leaves were collected by hand and analyzed by mass-spectrometry at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria.

The isotope-dilution method, for measuring the amount of N fixed, involves the application of a small amount of ^{15}N-enriched fertilizer to the soil. The NFT assimilates non-enriched atmospheric N_2 supplied by the root nodules [3, 23-25], in addition to the fertilizer-enriched mineral N in the soil. Accordingly, a comparison of the $^{15}N/^{14}N$ ratios of tissue N in the NFT and a non-fixing species allows an assessment of the relative contribution of BNF. The proportions and amounts of N derived from fixation (i.e. from the atmosphere, Ndfa) were obtained using the isotope-dilution equation [23]; average values for the two non-fixing control species are presented.

2.4.2. Type II plots

At the end of the active growing season of each year, three trees of each NFT were harvested from randomly selected sub-plots. Plant parts were separated into leaves, young twigs, lignified twigs and branches, nodules and roots, and then weighed following oven-drying at 60°C for 72 h. Root samples were divided into two groups: 0-40 cm and >40 cm. Statistical comparisons of mean values were made using Duncan's multiple-range test.

3. RESULTS

The data reveal trends and inter-specific differences in resource allocation and BNF rarely documented in similar detail for woody plants, whether in a Mediterranean-climate region, or elsewhere.

3.1. Plant growth

Plant-growth patterns in all six tree species are reported in Table I. Tagasaste grew faster than the other three NFTs and the two non-fixing reference species, as shown in height, trunk diameter, and root length. After 5 years, Tagasaste had attained an average height of 3.2 m, 8.6 cm in trunk diameter, and 2.1 m in rooting depth.

3.2. Biomass accumulation

The relatively poor performance of the two *Prosopis* species in terms of biomass production (Table II, Fig. 2) was probably due to poor adaptation to local conditions, in particular a sensitivity to the slightly acid soil pH. In contrast, total biomass accumulation by Tagasaste was consistently and significantly greater (P<0.01) than by the other NFTs – indeed, its total biomass production was nearly five times that of *A. caven*, and twenty times that of either Prosopis species. Plant height did not increase significantly in Tagasaste after the third year (Table I); instead, biomass was added in the form of a denser, and wider crown. The two reference species produced more than five-fold the biomass of either *Prosopis* species, slightly more than *A. caven*, but only one-quarter as much as Tagasaste (Table II).

TABLE I. GROWTH OF FOUR NFTs AND TWO REFERENCE SPECIES OVER 5 YEARS

Year	*P. chilensis*	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
	Plant height (m)					
1992	0.91bc[a]	0.92bc	1.11b	1.87a	0.62d	0.88c
1993	1.36b	1.12b	1.38b	3.15a	1.12b	1.27b
1994	0.99c	1.08c	1.65b	3.21a	1.55b	1.56b
1995	1.02d	1.17d	1.71c	3.49a	2.20b	1.77c
1996	1.19d	1.43d	2.37b	3.21a	2.94a	1.90c
	Trunk diameter (cm)					
1992	1.40c	1.10d	1.60c	2.50a	2.20b	1.70c
1993	1.85d	1.86d	2.34c	5.44a	3.21b	2.97b
1994	2.65c	1.93d	3.04c	6.18a	3.82b	3.82b
1995	2.83d	2.60d	4.08c	9.18a	5.20b	5.19b
1996	3.20d	2.90d	4.90c	8.60a	6.60b	6.20b
	Root length (m)					
1992	1.39a	1.22ab	1.38a	1.20b	0.75c	0.82c
1993	1.62bc	1.56c	2.17a	1.89ab	1.55c	1.76bc
1994	1.82b	1.72b	2.31a	1.86b	1.74b	1.82b
1995	2.46a	1.99b	2.51a	2.39a	2.27ab	2.20ab
1996	1.93ab	1.79b	2.05a	2.08a	2.03a	1.93ab

[a]Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

3.3. Nodulation

In each of the 5 years of growth, the weight of nodules on the roots of Tagasaste was at least four-fold that on any of the other NFTs (Table III). At the end of the fifth year (1996), Tagasaste's nodule weight averaged 113 g plant^{-1}, as compared to 2-5 g plant^{-1} on the other NFTs.

3.4. Nitrogen content of major plant components

The %N values for leaves, woody material and roots was significantly greater in the NFTs than in the two non-fixing species (Table IV). Moreover, Tagasaste showed significantly greater leaf-N concentration than did the other NFTs; its 2.75% N value for Tagasaste foliage appears to be exceptionally high, considering that leaf-N for other Mediterranean trees and shrubs is reported to be in the 1-1.5% range [26,27].

3.5. Total Nitrogen accumulation and %Ndfa

Tagasaste accumulated some five to thirty-fold more N per plant than did any of the other five species (Table V). Furthermore, both species of *Prosopis* accumulated significantly less total N than did the two reference species. By contrast, and despite its somewhat slower overall growth rate,

A. caven consistently accumulated more N per plant than did the two reference species, and its %Ndfa was consistently high. The %Ndfa values for Tagasaste were also consistently high (ca. 80%) (Fig. 3). In contrast, following second-year peaks of 85%, 70%, and 52%, in *A. caven*, *P. chilensis* and *P. alba*, respectively, their %Ndfa values declined over the following 3 years.

3.6. Field scale estimates of BNF

To estimate fixed N on a per unit area basis (Table VI), we assumed a planting density of 1,666 trees ha^{-1} for all species, i.e. a spacing of 1.5 m between trees and 4 m between rows. This corresponds to accepted Tagasaste planting densities practised in areas with 650 mm of mean annual rainfall in both Chile and Australia. As reported previously [14], Tagasaste fixed approximately 8 and 74 kg N ha^{-1} in the first and second years, respectively, representing an order of magnitude greater than shown by the three mimosoid NFTs. In subsequent years, the gap only widened, with Tagasaste fixing a total of 367 kg ha^{-1}, some ten-fold higher than that fixed by *A. caven* and at least ninety-fold more than either of the *Prosopis* species.

TABLE II. BIOMASS ACCUMULATION IN FOUR NFTs AND TWO REFERENCE SPECIES OVER 5 YEARS

Year	*P. chilensis*	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
	Aerial dry weight (g $plant^{-1}$)					
1992	48b[a]	44b	80b	343a	42b	65b
1993	109b	162b	311b	4,141a	174b	341b
1994	228b	153b	637b	6,042a	349b	757b
1995	319c	336c	1,103b	10,251a	976c	1,240b
1996	340c	458c	2,205b	13,505a	2,210b	2,664b
	Root dry weight (g $plant^{-1}$)					
1992	41c	35c	68c	108a	111b	43c
1993	107c	86c	203c	1,158a	417b	246c
1994	237c	149c	494bc	2,617a	858b	500bc
1995	320c	322c	665c	5,235a	1,587b	819c
1996	454c	398c	1,174bc	6,011a	2,493b	1,667bc
	Total-plant dry weight (g $plant^{-1}$)					
1992	89b	78b	148b	451a	153b	108b
1993	216b	248b	514b	5,299a	591b	587b
1994	465b	302b	494bc	8,659a	1,207b	1,257b
1995	639c	657c	1,795b	15,486a	2,563b	2,059b
1996	794b	856b	3,379b	19,517a	4,703b	4,331b

[a]Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

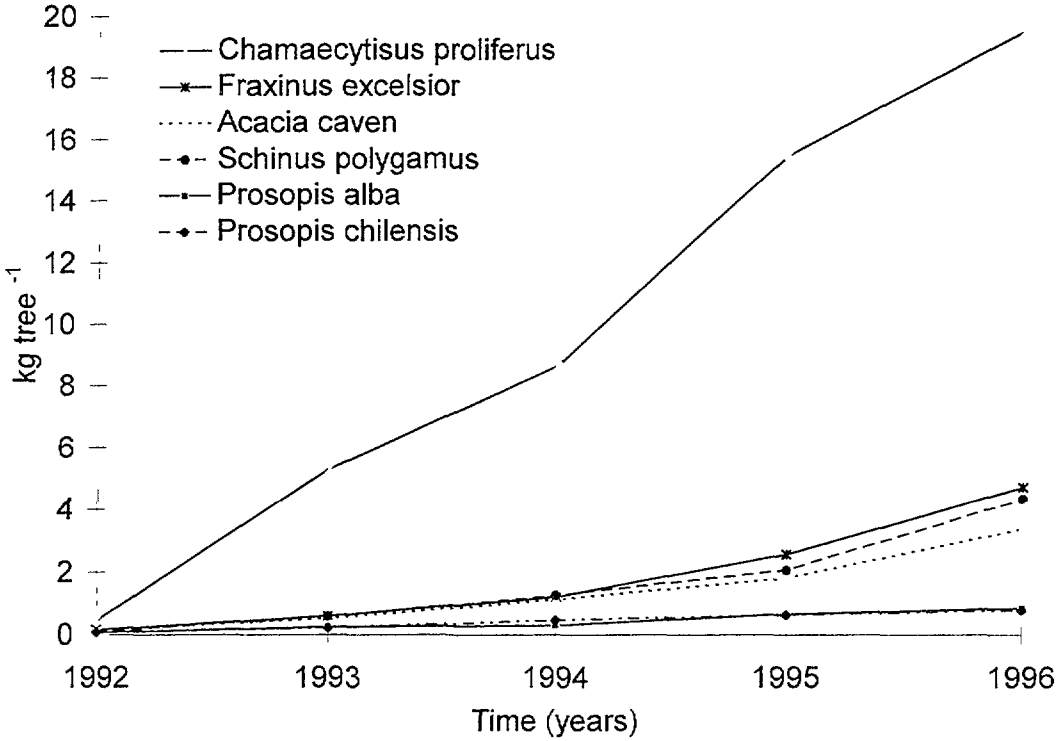

FIG 2. Total biomass accumulation in four legume and two reference trees over 5 years.

TABLE III. NODULE WEIGHT IN TWO SOIL DEPTHS ON FOUR NFTs OVER 5 YEARS

Year	0-40 cm (g plant^{-1})			
	P. chilensis	*P. alba*	*A. cavan*	*C. proliferus*
1993	1.5b[a]	3.6b	0.4b	18.5a
1994	0.38b	0.94b	0.36b	50.7a
1995	1.66b	3.41b	1.64b	59.3a
1996	3.28b	4.32b	2.01b	99.1a
	Deeper than 40 cm (g plant^{-1})			
1993	0.28b	0.35b	0.61b	9.11a
1994	0.15b	0.57b	0.47b	17.2a
1995	1.45b	2.74b	0.46b	12.5a
1996	0.08b	0.98b	0.12b	14.2a
	Total nodule dry weight (g plant^{-1})			
1992	1.2b	1.0b	1.5b	15.5a
1993	1.78b	3.95b	9.61b	18.6a
1994	0.53b	1.51b	0.83b	67.9a
1995	3.11b	6.15b	2.10b	71.8a
1996	3.36b	5.30b	2.13b	113a

[a]Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

TABLE IV. NITROGEN CONCENTRATION IN THE COMPONENTS OF FOUR NFTs AND TWO REFERENCE SPECIES

Component	N concentration (%)					
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
Leaf	1.71bc	1.93b	2.07	2.77a	1.38c	1.42c
Wood	1.04a	1.16a	1.03a	1.14a	0.72b	0.59b
Root	1.02b	1.09b	1.80a	1.92a	0.65c	0.54c

[a]Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

TABLE V. CUMULATIVE TOTAL N AND BNF ESTIMATES FOR FOUR NFTs AND TWO REFERENCE SPECIES (n =12)

Year	Total N (g plant^{-1})					
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
1992	0.97	0.95	2.22	5.53	1.27	0.83
1993	1.70	2.10	6.37	58.3	2.73	2.73
1994	3.46	2.56	13.7	91.5	5.31	5.90
1995	7.27	8.23	24.9	231	16.6	13.8
1996	8.38	9.61	49.2	270	38.6	35.3
	Fixed N (g plant^{-1})					
1992	0.30	0.24	0.31	4.81		
1993	1.19	1.10	5.45	49.1		
1994	0.96	0.48	10.1	76.2		
1995	1.15	0.64	12.8	192		
1996	2.18	1.35	23.7	220		

4. DISCUSSION

As compared to the other projects included in this Co-ordinated Research Project, the Chilean work is unusual in that it took place in a Mediterranean-climate region with 5 months of drought (Table I). Thus, the growth rates and BNF of the species tested were in general lower than those obtained in the other projects. Our relatively high year-to-year and intra-specific variability, in the destructive measurements of plants on the Type II plots, were in part due to genetic differences among individuals. This represents a source of error that can be eliminated only by using clonal material of selected tree species. However, a more critical step for the immediate future is to study these NFTs and others at the agro-ecosystem level. In other words, what happens to the N that is fixed by a given species in a research-site or on-farm study? For example, the fifth-year decline in

Tagasaste BNF was due apparently to the absence of biomass harvesting. Under normal conditions, this species would be directly grazed by livestock or pruned keeping it in a perpetual state of juvenility, therefore fixing N_2 at elevated rates. Tagasaste's significant production of N, ca. 73 kg ha^{-1} yr^{-1}, be managed in various ways, depending on the land-holder's objectives.

The next stage of our research will be aimed at developing optimum-use strategies for Tagasaste and other leading NFT candidates, in conjunction with annual legumes and grazing livestock. The isotope-dilution technique, and the stimulating interaction of an international network would certainly be of advantage for such endeavours.

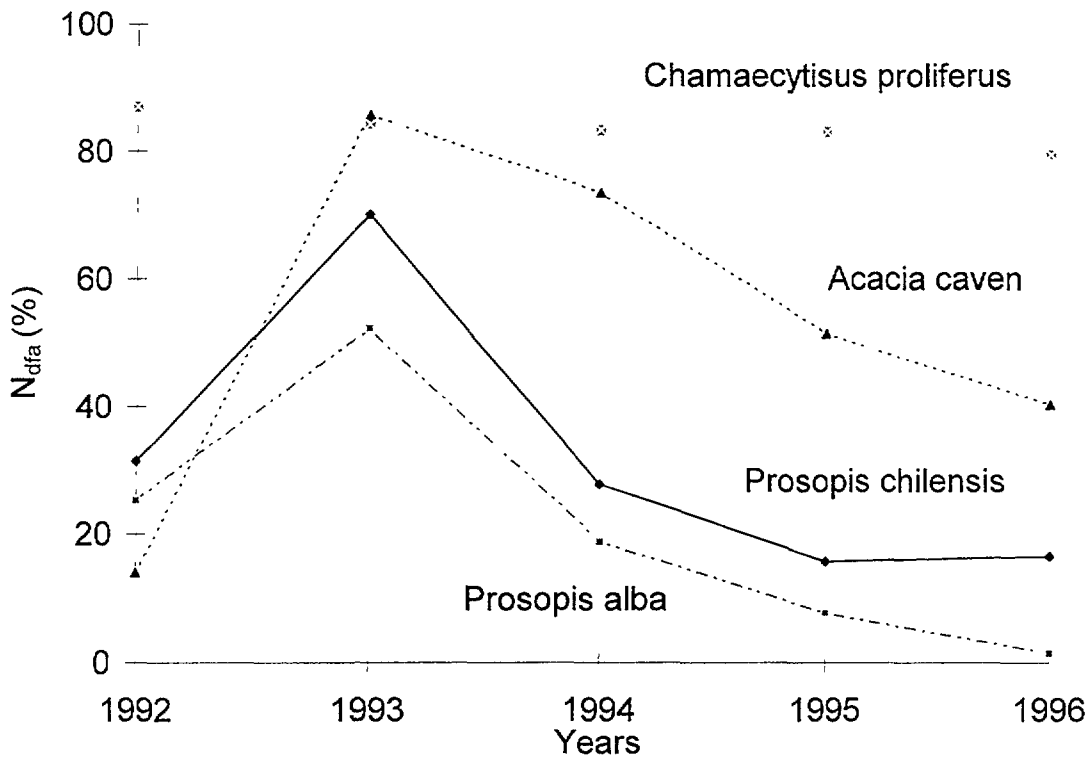

FIG. 3. %Ndfa in four NFTs over five years.

TABLE VI. ESTIMATES OF FIXED N FOR FOUR NFTs OVER 5 YEARS

Year	Fixed N (kg ha^{-1})			
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*
1992	0.50	0.40	0.52	8.01
1993	1.48	1.43	8.56	73.8
1994	0.00	0.00	7.70	45.1
1995	0.32	0.35	4.56	193
1996	1.72	1.19	18.1	46.5
Total	4.02	3.37	39.4	366

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NITROGEN FIXATION IN *LEUCAENA LEUCOCEPHALA* AND EFFECTS OF PRUNINGS ON CEREAL YIELDS

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Abstract

Leucaena leucocephala was interplanted with reference tree species, *Cassia siamea* and *Cassia spectabilis*, and estimates of percent N derived from N_2 fixation (%Ndfa) were made, by the isotope-dilution method, at 4, 6, 14, 20 and 30 months after transplanting. The %Ndfa values were low and variable throughout the growth period, except after thinning at 14 months when there was a five-fold increase. The two non-fixing reference species outperformed the N_2-fixing *Leucaena* in above-ground vegetative production, and provided different fixed-N estimates. Prunings from the *L. leucocephala* and *C. siamea* trees were applied separately to soil as green manure. Maize was planted to test the effects of the *Leucaena* green manure on soil fertility, and millet was the test crop for the *Cassia*. Whether surface-applied or incorporated, the prunings significantly improved yields, which were generally similar among rates and methods of application. The proportions of cereal N obtained from prunings ranged from 8 to 33%, with no cereal-yield correlation. The data indicate that multipurpose tree prunings are of potential use to farmers as organic sources of nutrients, even at relatively low application rates, without need for incorporation into the soil.

1. INTRODUCTION

Fertilizer use in Uganda is very low, estimated at 2,000 t for 1994 [1]. Only about 4% of farmers have used fertilizers at one time or another due to lack of the financial wherewithal and market incentives that would otherwise enable replacement of crop nutrients removed from their fields at harvest. There is widespread decline in productivity, for example to less than 10 kg per bunch of the staple banana [2].

Some tree species have the potential to produce herbage in sufficient quantities as a significant source of nutrients when applied to the soil as green manure. Many leguminous trees obtain a significant proportion of their N needs through symbiotic fixation of atmospheric N_2, which makes them of particular interest. But methods for quantification of N_2 fixation in trees have been developed using saplings because of logistical difficulties in working with mature trees. Over the past 10 years, *Leucaena* species have been introduced to Uganda as a possible means of meeting some of the N requirements of small farm-holders. In order to recommend further use of *Leucaena*, as a source of N, it is necessary to establish how much of its N requirement is met through fixation during its growth cycle.

The effectiveness of organic sources of N, such as tree prunings, on subsequent crop growth, depends upon the quality of the material, which affects decomposition and nutrient-release characteristics [3]. According to Palm and Sanchez [4] and Tian et al. [5], plant materials of low C:N ratio, of low lignin and polyphenol content, e.g. the leaves of many leguminous trees, decompose faster and may be a direct source of nutrients for crop uptake. But little is known of how best to synchronize the release of such nutrients with the growing crop's needs. Thus, residues have been used more to contribute organic matter than as direct sources of nutrients [6], although total soil organic matter frequently does not relate directly to crop yields. Nevertheless, direct yield responses and nutrient-uptake enhancement can result from addition of agroforestry tree prunings to soil [5].

In this study, we varied the placement, the time of application and the amount of leaf prunings in an attempt to influence the efficiency of nutrient transfer to growing cereals.

2. MATERIALS AND METHODS

The experiments were established on an aeric tropaquept soil [7], at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), Uganda (0° 28'N, 32° 36'E). Soil characteristics at the site are given in Table I.

TABLE I. CHARACTERISTICS OF THE SOIL

	Profile depth (cm)			
	0-15	15-36	36-52	52-97
Horizon	AP-1	AP-2	BT-1	BT-2
Clay (%)	31	18	30	48
Silt (%)	9.6	24	16	9.6
pH (H_2O)	6.1	5.1	5.1	5.0
Organic C (%)	2.4	1.4	0.9	0.7
N (%)	0.14	0.11	0.07	0.04
Truog P (ppm)	0.06	0.05	0.06	0.06
Exhangeable Ca	12	7.8	5.0	4.2
(meq/100g) Mg	2.2	1.6	0.9	1.2
K	1.2	0.1	0.1	0.0

2.1. Determination of %Ndfa

Three seedlings each of *Leucaena leucocephala*, inoculated with rhizobial strain TAL 1145, and reference species *Cassia spectabilis* and *Cassia siamea* were transplanted into 4×4 m "mixed-species" plots, each demarcated by a plastic-film barrier inserted into the soil to a depth of 0.8 m. The plants had a 1×1m spacing. There were five replicates. Ammonium sulphate, enriched in ^{15}N at 11% atom excess, was applied in solution at a rate of 13.3 kg N ha^{-1}, two weeks after transplanting. The labelled fertilizer was also applied annually as plots were harvested, to trees that had previously received non-labelled fertilizer at 13.3 kg N ha^{-1}. Single super phosphate was broadcast annually at 20 kg P ha^{-1}.

Trees were sampled at 4, 8, 14, 20 and 30 months after transplanting; leaves were randomly taken from different parts of the plants, oven dried at 70°C for 24 h, milled and sent to the FAO/IAEA Soil Science Unit at Seibersdorf in Austria, for the determination of %N and %^{15}N enrichment. Percent nitrogen derived from fixation (i.e. from the atmosphere, %Ndfa) was estimated using the isotope-dilution equation [8].

On some plots, trees were thinned after 14 months of growth to leave two plants each of *L. leucocephala*, *C. spectabilis* and *C. siamea* at a 2×1 m spacing. Labelled fertilizer was applied as before, and leaves were sampled 6 and 16 months later for ^{15}N-enrichment determination. The above-ground biomass of the thinned plants was determined, first by separating leaves and branches, and recording the fresh weights, which were converted to oven-dry weights using data obtained from dried sub-samples (70°C for 96 h).

2.2. Effects of prunings on cereal yields

The effects on soil productivity of applying *L. leucocephala* leaves and twigs to soil were determined using maize as a test crop, and the effects of *C. siamea* prunings were examined using millet.

The *Leucaena* prunings were applied at a rate equivalent to 177 kg N ha^{-1}, and treatments included application at different time points and comparison of surface-application versus incorporation (see Table IV). The *Cassia* prunings (2.63% N) were applied at various rates as surface mulch and by incorporation (see Table V). Each treatment had four replicates, and the experiment was laid out with a randomized complete- block design. The plot sizes were 3×3 m, with an area of 3×1.5 m treated with labelled fertilizer, to determine organic-N availability to the cereals using the indirect labelling method:

$$\%N \text{ derived from prunings} = 1 - \left(\frac{\%^{15}N \text{ a.e. in treated plant}}{\%^{15}N \text{ a.e. in control}} \right) \times 100$$

Maize (*Zea mays* var. Longe 1) was planted at a spacing of 75×25 cm and millet (*Eleusine coracana* var. Engenyi) at 30×5 cm. Ammonium sulphate fertilizer, enriched in ^{15}N at 10% atom excess, was applied in solution at 10 kg N ha^{-1} at 1 wk after planting. The plots were manually kept weed-free.

Maize ear-leaves, assumed to be representative of the plants as a whole, were sampled at flowering for %N and $\%^{15}N$ atom excess analyses from the inner nine plants within each ^{15}N-labelled micro-plot. For millet, nine randomly selected plants were cut at 10 cm from the soil surface and processed and analyzed as described above.

Yield data were taken from the non-labelled area of each plot. Maize plants were harvested at tasseling and chopped into small pieces, dried in the oven at 70°C for 48 h and weighed. Maize grain yields could not be determined due to theft. Millet was harvested at maturity, air dried, threshed and the grain oven-dried at 65°C for 24 h before weighing. The significance of differences between mean values was determined by ANOVA procedures.

Included in the *Cassia* study was the determination of rates of decay of surface-applied and incorporated leaves, using the litter-bag method. Litter bags of 5-mm mesh had a box configuration (30×30×3 cm) that held the equivalent of 4 t leaf ha^{-1}. At planting, thirty-two bags were placed in the field, eight per plot. Four replicate bags were recovered every fourteen days. The residues were washed with deionized water to remove adhering soil particles, dried at 72°C, weighed and milled to pass through a 2-mm sieve. One-gram sub-samples were combusted in porcelain crucibles at 550°C for 2 h and the residue mass expressed on an ash-free basis. The mass of litter (and nutrients N, P and K) was fitted to a first-order exponential decline function:

$$\frac{\text{Leaf remaining}}{\text{Initial leaf}} = \exp^{-k.\text{time}}$$

which was used to calculate the time to 50% decomposition (t_{50}).

3. RESULTS AND DISCUSSION

3.1. Determination of %Ndfa

The %Ndfa values remained low throughout the study (Table II), a circumstance that provides imprecise data [9]; indeed, standard deviation values were relatively high. There was no trend with growth, possibly due to variable soil fertility across the experiment site. The estimates of %Ndfa differed with reference species: *C. spectabilis* generally gave higher values than did *C. siamea*, and some negative values were obtained with the latter. Negative estimates of %Ndfa have been reported for *A. albida* using *C. siamea* as the reference, in a pot experiment [10]. It would appear, therefore, that *C. siamea* fails to meet the requirements of a satisfactory reference crop described by Fried et al. [11].

Thinning at 14 months increased %Ndfa, mostly over the subsequent first 6 months, indicating that increased N_2-fixation activity resulted from increased sunlight penetration. By 14 months, the non-fixing trees were larger (Table III) and shaded the *L. leucocephala*. By 16 months after thinning, the shading effect was again evident in terms of decreased %Ndfa values (Table II). It appears that, in this soil, the 2×1 m spacing was too close for optimal growth of, and N_2 fixation by, *L. leucocephala* when mixed with *C. spectabilis* and *C. siamea* as reference trees. Under these conditions, the non-fixing reference trees were superior, producing 1.5 (*C. spectabilis*) to 2.3 (*C. siamea*) times more wood and green-manure material (Table III), and would be preferable to *L. leucocephala* if it could be established that they obtain their nutrition from soil horizons and/or areas not exploited by interplanted crops.

TABLE II. TIME COURSE FOR %NDFA VALUES IN *L. LEUCOCEPHALA* WITH *C. SPECTABILIS* AND *C. SIAMEA* AS REFERENCE PLANTS

Time of harvest	Not thinned		Thinned	
	C. spectabilis	*C. siamea*	*C. spectabilis*	*C. siamea*
(months)	(%Ndfa)			
4	5.8 (15)[a]	-7.3 (16)	ND[b]	ND
6	12 (12)	-19 (23)	ND	ND
14	6.0 (6.5)	-16 (8.7)	ND	ND
20	9.3 (10)	10 (11)	59 (11)	37 (18)
30	8.4 (11)	7.8 (8.4)	15 (16)	12 (15)

[a](Standard deviation).
[b]Not determined.

TABLE III. ABOVE-GROUND BIOMASS AND FOLIAR N OF *L. LEUCOCEPHALA*, *C. SPECTABILIS* AND *C. SIAMEA* AFTER 14 MONTHS OF GROWTH

Component	*L. leucocephala*	*C. spectabilis*	*C. siamea*
		(g/plant)	
Leaves	134 (72.6)[a]	478 (126)	1,105 (259)
Leaves + stems	1,264 (578)	1,882 (784)	2,945 (622)
Foliar N	5.20	15.6	30.1

[a](Standard deviation).

TABLE IV. PROPORTION OF MAIZE N DERIVED FROM *L. LEUCOCEPHALA* PRUNINGS (APPLIED AT 177 kg N/ha), AND EFFECTS ON MAIZE DRY WEIGHT

Application of prunings		N from prunings	Dry weight
Method	Time	(%)	(g/plant)
Control			888 (39.3)[a]
Incorporated	4 weeks pre-planting	28 (3.9)	995 (10.8)
Surface applied	4 weeks pre-planting	15 (5.3)	1,026 (11.4)
Surface applied	At planting	13 (15)	954 (62.0)
Surface applied	4 weeks post-planting	16 (12)	1,258 (25.0)

[a](Standard deviation).

3.2. Effects of prunings on cereal yields

The largest uptake of organic N by subsequently planted maize resulted from incorporation into the soil (Table IV): 28% of cereal N was derived from the *L. leucocephala* prunings. Ploughing in organic materials, as opposed to surface mulching, has been shown to accelerate the release of nutrients [12]. Surface application of prunings, before, at, or after planting resulted in only 13 to 16% of the maize N originating from the legume (Table IV). However, it is noteworthy that higher %N derived from the prunings did not correlate with higher dry-matter accumulation. The highest dry-matter yield was obtained when mulch was added at 4 weeks after planting. These difficult-to-interpret data raise a methodological question: is it acceptable to apply labelled fertilizer at planting when prunings are applied at other times?

With millet, the values for %N derived from prunings were similar to those obtained with maize (Table V) and also did not correlate with cereal grain yield. Similar data for N uptake from tree litter have been obtained using other methodologies [13].

All of the *C. simea* amendments resulted in significantly higher yields than from the control plants, except where residue was incorporated at 1 t/ha. The millet grain yields were low, ranging from 266 (control) to 402 kg/ha (6.6 t/ha incorporated), the result of low soil moisture after flowering. There were no significant grain-yield differences between the two placement methods, suggesting that, even if incorporation were to have enhanced decomposition [12], the low-moisture conditions precluded benefit to the millet.

The decomposition characteristics given in Fig. 1 show that even with surface application, most of the nutrients were released and available during the crop's growth period. The times to 50% (t_{50}) of mass loss of *C. siamea* leaf litter and its macro-nutrients were less than 20 days, corresponding to the addition of about 50 kg N/ha to the soil in the same period. This is more than the 17 kg N/ha total uptake that corresponded to the 15% of millet N that was derived from the surface application of 4 t/ha of prunings (Table V), indicating poor synchrony between nutrient release and crop uptake. Although there were no significant millet-yield differences among the various levels of residue application (Table V). However, greater yield returns per unit input were realized at lower rates of litter application (Fig. 2); the most efficient rates were 1,000 kg/ha for surface application, and 2,000 kg/ha for incorporation.

TABLE V. PROPORTION OF MILLET N DERIVED FROM *C. SIAMEA* PRUNINGS, AND EFFECTS ON GRAIN YIELD

Application of prunings			N from	
Method	Rate (t/ha)	N content (kg/ha)	prunings (%)	Grain yield (kg/ha)
Control				266d[a]
Incorporated	1	26.3	16 (20)[b]	301cd
Incorporated	2	52.6	17 (32)	364ab
Incorporated	4	105	10 (22)	376ab
Incorporated	6.6	174	33 (13)	402a
Surface	1	26.3	8.0 (14)	314bcd
Surface	2	52.6	17 (0.1)	334bc
Surface	4	105	15 (9.2)	350abc

[a] Numbers followed by the same letter are not significantly different ($P \leq 0.05$.

[b] (Standard deviation).

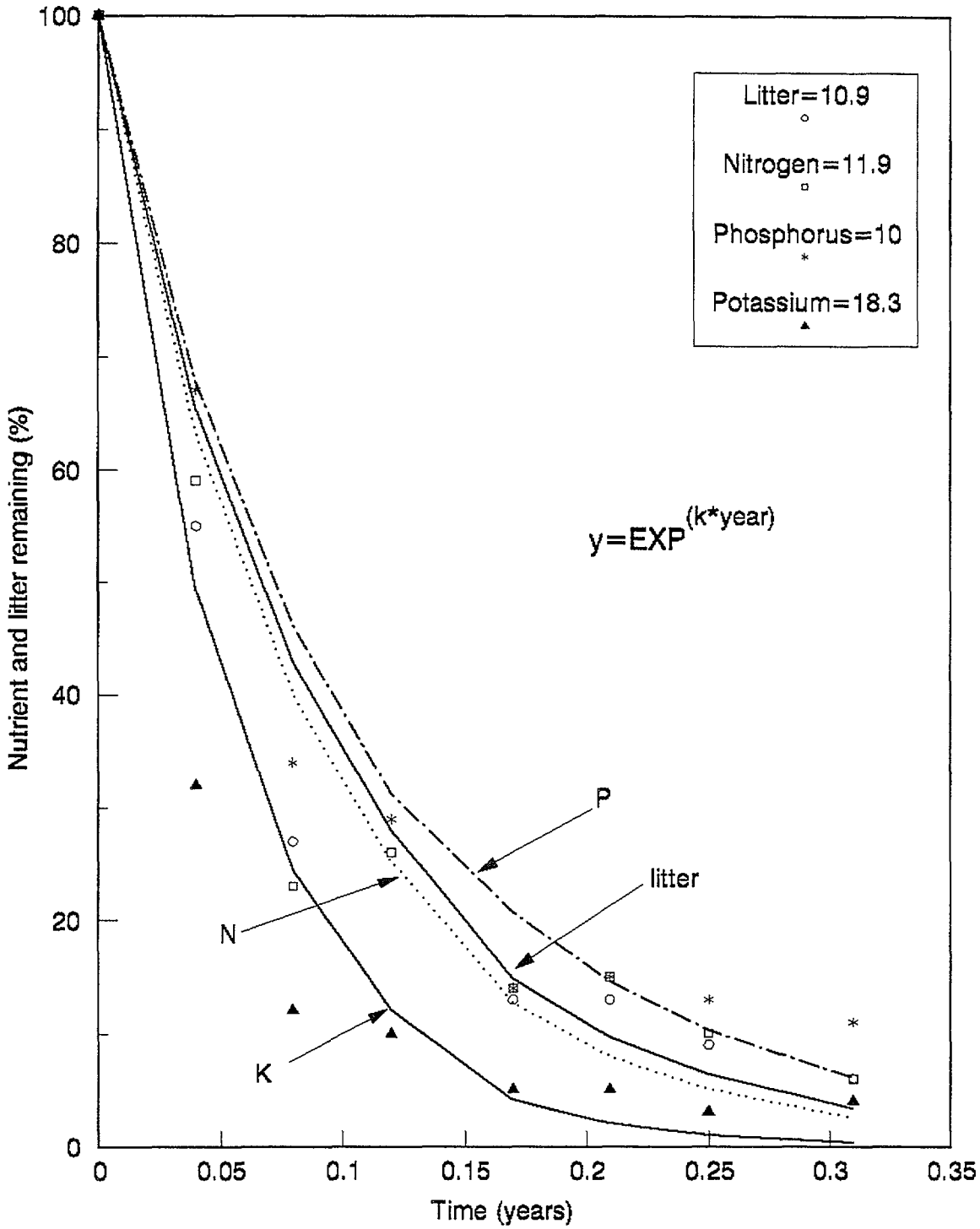

FIG 1. Decay patterns for surface-applied C. siamea *prunings.*

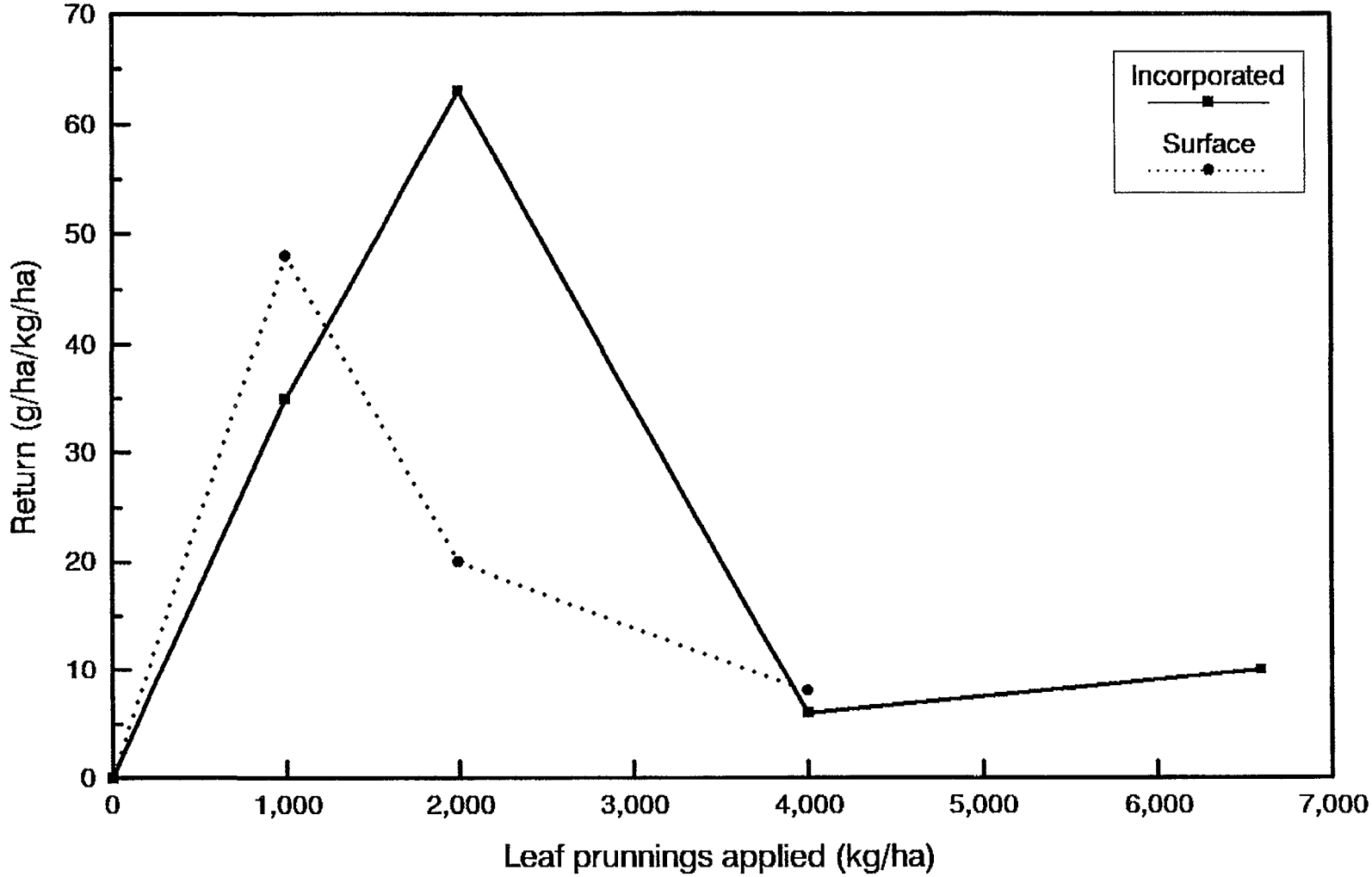

FIG 2. The effect of C. siamea *leaf placement and quantity on efficiency of use by millet.*

The results of this study offer insights into better management of multipurpose tree residues; application at high rates and incorporation of the residues appears not to be beneficial in terms of yield nutrient-use efficiency. However, this needs to be confirmed with a detailed economic analysis of the treatment effects.

4. CONCLUSIONS

- Different reference species may give differing estimates of fixed N estimates. *C. siamea* was an unsuitable non-fixing check for *L. leucocephala.*
- Spacing, and consequently shading, affected N_2 fixation; the closer the spacing, the less the fixation.
- The isotope-dilution methodology in determining N uptake from tree-pruning mulches gave results in the range predicted using other methodologies elsewhere.
- The values for %N derived from prunings did not differ for N_2-fixing and non-fixing trees.
- Neither incorporation of prunings nor increased rates of application affected cereal yields or N-use efficiency in the short term, possibly because of limiting factors unrelated to N nutrition, e.g. drought. Under higher-yielding conditions, correlations between cereal yields and rate and method of application of prunings may be obtained.

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NITROGEN FIXATION AND EFFECTS OF PRUNING ON *GLIRICIDIA SEPIUM* AND *LEUCAENA LEUCOCEPHALA*

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Abstract

This 7-year study examined genetic variability in N_2 fixation by *Gliricidia sepium* and the N_2-fixing capacity in *G sepium and Leucaena leucocephala* as influenced by frequency of pruning, age, and shade from coconut. The ^{15}N-dilution method was used with the non-nodulating tree legume *Senna siamea* as the non-fixing reference. There were significant differences in total dry matter, N yield and N_2-fixation capacity among four *G sepium* provenances. *Gliricidia* had higher values than *Leucaena* for dry matter, N yield, and amount of N fixed; %Ndfa was comparable in both species (47-55%). A substantial amount (18%) of fixed N_2 was present in the roots of both species. In a long-term study aimed at comparing the effect of pruning practices and age of trees, *G. sepium* grown under coconut outperformed *L leucocephala* in terms of dry matter, N yield and amounts of N_2 fixation. Coconut saplings supplied with *G. sepium* and *L leucocephala* prunings as green manure grew better than those supplied with *S siamea*, the fraction of coconut-sapling N obtained from *Gliricidia* and *Leucaena* was 40 and 36%, respectively. These results suggest that *G sepium*, which demonstrated a high potential for biomass production and N_2 fixation, is appropriate for interplanting with coconut palms. Also, *S siamea* was found to be a suitable reference species

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a major plantation crop, playing a vital role in Sri Lanka's economy and contributing to the food security of its 18 million people. It has been reported that the majority of coconut-growing soils are inherently low in N, K and Mg, the major reason for the crop's low productivity – 7,000 nuts ha^{-1} yr^{-1} in many parts of the country. Declining fertility is due mainly to inadequate attention to the return of organic residues to plantation soils.

In Sri Lanka, numerous agroforestry systems associated with coconut, including crop and livestock components have been established during the past few decades, with a view to increasing productivity and maximizing income for coconut farmers. The interplanting of coconut with N_2-fixing trees (NFTs) has been successfully introduced to the coconut ecosystem. Such species are considered to be one of the most appropriate components in agroforestry systems because of their inherent ability to utilize atmospheric N_2 and grow well in soil low in mineral N. Among them, *Gliricidia sepium* (Jacq.) Walp. and *Leucaena leucocephala* (Lam) de Wit are recognized as having potential for production of green manure, animal fodder and for maintaining soil fertility [1,2]. By far, they are the most common species grown in association with coconut. The growth and biomass production of these two species and their effect on coconut production have been documented [3].

In view of increasing cost of N fertilizer, attention is now being focused on biological N_2 fixation (BNF) in tree legume species such as *G. sepium* and *L. leucocephala* in agroforestry systems. Estimates of BNF by these species have been reported under both glass-house and field conditions in young trees [2,4-6]. It has been shown that fixation in tree legumes is strongly influenced by genotype within species, by management practice [2,5] and tree age. However, these aspects are not well documented for older trees. This research was undertaken to strengthen our knowledge and understanding on the N_2-fixing potential in *G. sepium* and *L. leucocephala*, as influenced by genotype, management and age.

The objectives were as follows.

- To study genotypic variation in BNF.
- To quantify BNF capacity of tree-legume species under coconut canopy.
- To study the effect of tree management on BNF under coconut canopy.
- To study changes in BNF with tree age.
- To study N contribution from prunings of tree legumes to an associate crop.

2. MATERIALS AND METHODS

2.1. Experiment 1. N_2-fixation in four *Gliricidia sepium* genotypes

The experiment was conducted at the Coconut Research Institute (CRI) of Sri Lanka (08° 02'N, 79° 50'E, 2 m) from December 1990 to November 1991. The average annual rainfall at the site is 1,850 mm, with maximum and minimum air temperatures of 31 and 24°C, and relative humidity of 70 to 80%. The rainfall data for the experimental period are given in Table I. The soil was a sandy loam (Xanthic Ferralsols) and its chemical characteristics are given in Table II.

The treatments consisted of four *G. sepium* genotypes viz. provenances 14/84, 17/84 (Guatemala), 12/86 (Costa Rica) obtained from the Oxford Forestry Institute, UK, and the local land-race (designated LL). The non N_2-fixing legume tree species *Senna siamea* (syn. *Cassia siamea*) was used as the reference for the ^{15}N-dilution method [7]. *Senna* seeds were obtained from Tree Seed Centre, Thailand. The experiment had a randomized complete-block design with four replicates.

TABLE I. ANNUAL RAINFALL AND ITS DISTRIBUTION

Year	Total rainfall (mm)	Distribution (No. of rainy days)
1991 (Site 1)[a]	1,676	129
1992 (Site 2)[b]	1,868	86
1993	1,764	110
1994	1,718	115
1995	1,630	86
1996	1,199	95

[a](Experiment 1).
[b](Experiments 2 and 3).

TABLE II. SOIL CHARACTERISTICS (EXPT. 1)

Depth (cm)	pH[a]	Total N (%)	Organic C (%)	Bray P (mg kg^{-1})	Exchangeable cations (meq 100^{-1} g) K	Ca	Mg
0-20	5.41	0.096	0.89	8.43	0.326	0.40	< 0.001
20-40	5.28	0.097	0.85	7.29	0.215	0.30	< 0.001

[a]1:5, soil:H_2O.

Four-week-old inoculated seedlings (inoculum supplied by the BNF Resource Centre, Bangkok, Thailand), raised in polythene bags, were transplanted into holes (30×30×30 cm) made at a spacing of 2×1 m (equivalent to 5,000 plants ha^{-1})). After planting, the holes were refilled with top soil containing a basal fertilizer mixture (kg ha^{-1}): triple superphosphate 100, KCl 50, S 10, Cu 5.4, B 2.7, Zn 5.0 and Mo 0.5.

Each replicated block (plot) measuring 8×5 m consisted of four *G. sepium* provenances in the centre row and two rows of *S. siamea* on either side with the 2×1 m spacing, surrounded by a border row of *S. siamea.* Within each plot, an isotope sub-plot measuring 6×4 m was demarcated and the area under each tree within the sub-plot was contained by trenching and installation of galvanized sheets to a 45-cm depth. Three months after planting, a solution containing urea enriched with 5 atom % ^{15}N excess was sprayed (200 mL m^{-2}) onto the isotope sub-plot, at a rate equivalent to 20 kg N ha^{-1}. Unlabelled urea was applied at the same rate to the border trees outside the sub-plot.

The trees within the isotope sub-plot were harvested 11 months after transplanting, in November 1991. After removing the above-ground plant parts, the root system of each tree was exposed by washing the soil within the enclosed area and the whole plant was taken for sampling. Plants were separated into leaves, branches, stems and roots, chopped into 5-cm pieces and then weighed fresh. A 300-g sub-sample was taken for drying at 70°C until constant weight in a forced-draft oven, then milled to a fine powder.

Total-N content and ^{15}N enrichment in each plant part were determined [8] on an automatic N analyzer (1500 Carlo-Erba) coupled to a VG-Isogas mass spectrometer at the FAO/IAEA Soil Science Unit, Austrian Research Centre, Seibersdorf. The isotope-dilution equation [7] was used to estimate the percent N derived from fixation (i.e. from the atmosphere, %Ndfa) for individual plant parts. Subsequently, BNF in the whole plant was calculated from the weighted atom % ^{15}N excess (WAE) in the fixing and reference plants [9] as follows:

$$WAE = \frac{[(AE_L \times TN_L) + (AE_S \times TN_S) + (AE_B \times TN_B) + (AE_R \times TN_R)]}{[TN_{(L+S+B+R)}]}$$

Where

AE is atom % ^{15}N excess,
TN is total N,
L is leaves,
S is stems,
B is branches,
R is roots.

The proportion of total N derived from atmospheric N_2 (%Ndfa) was then calculated as follows:

$$\%Ndfa = \left(1 - \frac{\text{WAE in } N_2 \text{ fixer}}{\text{WAE in } S.\ siamea}\right) \times 100$$

And the amount of N_2 fixed (NF) was calculated as follows:

$$NF = \frac{\%Ndfa}{100} \times \text{Total N (g plant}^{-1}\text{)}$$

The significance of differences between mean values was determined by analysis of variance by the General Linear Model of SAS [10].

TABLE III. SOIL CHARACTERISTICS (EXPTS. 2, 3 AND 4)

Depth (cm)	pH[a]	Total N (%)	Organic C (%)	Bray P (mg kg^{-1})	Exchangeable cations K Ca Mg (meq 100^{-1} g)		
0-20	5.24	0.082	0.85	12.8	0.104	0.849	<0.339
20-40	5.52	0.057	0.78	11.4	0.052	0.818	<0.285

[a]1:5, soil:H_2O.

2.2. Experiment 2. N_2 fixation by *G. sepium* and *L. leucocephala* under coconut

This study was conducted in a 50-year-old plantation (planted 8.5×8.5 m) at CRI, for a period of 12 months, commencing 15 May, 1992.

The photosynthetically active radiation (PAR) transmitted to the under-storey on a sunny day was 70%. The average annual rainfall at the site is 1,600 mm and the maximum and minimum air temperatures were 31 and 23°C, respectively. Rainfall data during the experimental period are in Table I. The soil was a lateritic gravel (ferric acrisols) and its chemical characteristics are given in Table III.

The treatments consisted of the NFTs *G. sepium* (provenance 12/86)and *L. leucocephala* (K 636) and the non-fixing reference legume *S. siamea*, arranged in a randomized complete-block design with four replicates. Within each replicate, a 6×2 m sub-plot was enclosed by cutting a trench and placing galvanized sheets to a 45-cm depth on all sides and between trees.

The seedlings were raised in polythene bags for 8 weeks, the NFTs inoculated with appropriate rhizobium cultures. They were transplanted to the field in planting holes (30×30×30 cm) in single rows, 2 m apart, between the rows of coconut palms (640 trees ha^{-1}). Prior to transplanting, a fertilizer mixture consisting of triple superphosphate (equivalent to 100 kg ha^{-1}), KCl (50 kg ha^{-1}), B (2.7), Zn (5.0) and Mo (0.5), was applied to each planting hole and mixed with top soil.

Three months after establishment, a solution containing ammonium sulphate enriched at 5 atom % ^{15}N excess was applied to the isotope sub-plots at the rate of 20 kg N ha^{-1} as 200 mL m^{-2} followed by thorough watering to ensure uniform distribution. Unlabelled ammonium sulphate was applied to border rows at the same rate. The plots were weeded thrice and the weeds left to decompose on the surface.

Twelve months after planting, four trees of each species were harvested. The above-ground parts were sampled first by cutting the main stem 10 cm above ground level and separating branches, leaves and stems. When uprooting trees, the soil within each enclosed area was excavated to a depth of 90 cm and most of the root biomass was recovered by hand-sorting. Each component plant part was chopped into 5-cm pieces and weighed fresh. A 300-g sub-sample of each was dried in a forced-draft oven at 70°C to constant weight for determination of dry weight. All dried plant samples were milled, analyzed for N content and ^{15}N enrichment, and determinations of %Ndfa and NF made, as described above.

2.3. Experiment 3. Pruning-frequency and tree-age effects on N_2 fixation by *G. sepium* and *L. leucocephala* under coconut.

The details of the experiment site, species, date, method of planting and fertilizer applied were as described for Experiment 2. Each 18×2 m sub-plot consisting of nine legume trees, three of each species, was enclosed by cutting a trench and placing a two-layered black polythene sheet to a depth of 90 cm along the border. There were three pruning regimes (4-monthly, 6-monthly, and once per year designated "unpruned") arranged with the three species in a 3^3 factorial randomized complete-block design with four replicates.

Twelve months after establishment in the field, all trees were cut back to 1 m. Each replicate consisted of three plots and one pruning treatment was assigned per plot. Following the initial cut-back in the sub-plots and thereafter at completion of each pruning interval, a solution of ammonium sulphate enriched with 5 atom % ^{15}N excess was applied at 20 kg N ha^{-1} to the soil in two or three split doses. Trees that were pruned every 4 months were applied with fertilizer solution in three doses each year, and those pruned every 6 months and unpruned trees were applied with fertilizer solution twice per year. The border trees outside the isotope sub-plot were applied with unlabelled ammonium sulphate at the same rate. All plots were weeded thrice per year.

At each harvest, the above-ground biomass of each tree was collected and separated into leaves and branches and weighed fresh. Thereafter, 300-g sub-samples were taken from the three trees of each species within a sub-plot. Each sub-sample was dried and milled for estimation of total N and ^{15}N enrichment.

The pruning treatments were imposed over four years (1993/94, 1994/95, 1995/96, 1996/97) after the initial pruning In plots with unpruned trees, sub-samples of leaves and branches were taken for estimation of %N and ^{15}N enrichment on completion of a pruning cycle at the end of each year, while a set of unpruned trees in each species was used to estimate pruning biomass after the cut back to 1 m.

2.4. Experiment 4. Recovery of N from prunings applied to coconut seedlings

This follow-up study utilized the prunings labelled with ^{15}N in Experiment 3.

Six-month-old coconut seedlings (var. Tall × Tall) were established in the field on 15 May, 1995, by planting them in holes (45×45×45 cm) at a spacing of 1 m in the area between coconut-palm rows, which was divided into two main blocks, each of four plots of equal size (4×3 m) consisting of six coconut seedlings. The seedlings in three plots in one block were supplied with ^{15}N-labelled prunings from either *G. sepium, L. leucocephala* or *S. siamea,* at the rates of 108, 86 and 159 kg per plot, respectively, during the experimental period. In the other block, three similar plots were mulched with the same quantities of unlabelled prunings from three species. One plot in each block served as the control without mulching.

At the end of 12 months growth, the third leaf of each coconut seedling was sampled, dried and milled for determination of %N and ^{15}N enrichment. Subsequently, fresh and dry weights of the third frond were recorded. The growth of each coconut seedling within a plot was determined by measuring plant height, basal girth and canopy size. For statistical analysis, individual coconut seedlings in each plot within a block were considered as replicates. The experiment had a fully randomized design with four replicates. The N obtained by the coconut seedlings from the prunings was calculated as follows:

$$\%N \text{ derived from prunings} = \frac{\%^{15}N \text{ a.e. in coconut with prunings} - \%^{15}N \text{ a.e. in coconut control}}{\%^{15}N \text{ in prunings.}} \times 100$$

The ^{15}N enrichments in the prunings were, *Leucaena* 0.189%, *Gliricidia* 0.162% and *Senna* 0.346%.

3. RESULTS

3.1. Experiment 1

3.1.1. Dry matter and N yields

There were significant differences ($P \leq 0.001$) among the *G. sepium* provenances (Tables IV and V). Provenance OFI 14/84 produced the highest dry matter (DM) and N yields followed by the local land-race (LL). Although the non-N_2-fixing reference *S. siamea* accumulated the highest total DM, its N yield was lower than that of provenance 14/84 but, almost double that of other two provenances 17/84 and 12/86. The above-ground plant parts accumulated 80-90% of the total DM and N yields; the roots had 10-20% irrespective of species and provenance (Tables IV and V). In terms of N-use efficiency (NUE), the amount of dry matter produced by the plant per unit of taken up, *S. siamea* was significantly ($P \leq 0.001$) more efficient than *G. sepium* provenance OFI 14/84 and the local land-race (Table V).

TABLE IV. DRY MATTER AND ITS DISTRIBUTION IN PLANT COMPONENTS OF FOUR *GLIRICIDIA SEPIUM* GENOTYPES AND *SENNA SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaves	Branches	Stem	Roots	Total
			(kg plant^{-1})		
OFI 14/84	0.80(16)[a]	0.60(12)	2.90(58)	0.72(14)	5.02
OFI 17/84	0.31(10)	0.57(18)	1.66(53)	0.61(19)	3.15
OFI 12/86	0.42(15)	0.46(16)	1.49(51)	0.53(18)	2.90
LL	0.97(22)	0.58(13)	1.99(44)	0.94(21)	4.48
Senna	0.88(15)	0.56(26)	2.65(44)	0.97(16)	6.06
Significance	*	**	*	NS	**
$LSD_{0.05}$	0.39	0.84	0.98	-	1.56
CV(%)	37	73	29	39	23

[a](%).

TABLE V. TOTAL N YIELD, ITS DISTRIBUTION IN PLANT COMPONENTS AND N-USE EFFICIENCY (NUE) OF FOUR *G. SEPIUM* GENOTYPES AND *S. SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem	Root	Total	NUE
			(g N plant^{-1})			(g DM g^{-1} N)
OFI 14/84	21.3(22)[a]	3.4(4)	65.2(68)	6.3(7)	96.1	53
OFI 17/84	3.0(8)	3.5(9)	26.4(69)	5.5(14)	38.3	85
OFI 12/86	5.5(14)	2.2(6)	26.7(69)	4.2(11)	38.5	78
LL	36.1(46)	4.9(6)	29.8(38)	7.6(10)	78.4	59
Senna	25.2(38)	6.1(9)	30.5(46)	4.3(7)	66.2	101
Significance	***	NS	*	NS	**	**
$LSD_{0.05}$	12.7	-	24.1	-	33.1	21
CV(%)	45	63	44	45	34	18

[a](%).

TABLE VI. DISTRIBUTION OF ATOM % ^{15}N EXCESS IN PLANT COMPONENTS AND THE MEAN WEIGHTED ^{15}N ATOM EXCESS (WAE) OF FOUR *G. SEPIUM* GENOTYPES AND *S. SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem	Root	WAE (whole plant)
	(atom % ^{15}N excess)				
OFI 14/84	0.094	0.137	0.135	0.151	0.120
OFI 17/84	0.137	0.168	0.177	0.152	0.160
OFI 12/86	0.150	0.219	0.166	0.167	0.163
LL	0.138	0.123	0.191	0.177	0.159
Senna	0.313	0.358	0.346	0.363	0.337
Significance	**	***	***	***	***
$LSD_{0.05}$	0.089	0.070	0.076	0.077	0.071
CV(%)	35	23	34	25	24

3.1.2. Atom %^{15}N excess

The ^{15}N enrichment in plant parts and weighted average for the whole plant in *S. siamea* was significantly higher ($P \leq 0.001$) than those of the *G. sepium* genotypes (Table VI). However, differences in atom %^{15}N excess among *G. sepium* provenances were not significant. Enrichment values in the leaves of *G. sepium* genotypes (mean 0.130), were lower than in the stems branches and roots (0.162-0.167). There was a similar trend with *S. siamea.*

3.1.3. Nitrogen fixation

On a whole-plant basis, provenance OFI 14/84 had the highest %Ndfa (64%), however owing to the high coefficient of variation, this was not significantly greater than for the other *G. sepium* genotypes (Table VII). In contrast, the amount of N fixed by OFI 14/84 (61.7 g. N plant^{-1}) was significantly higher than by OFI 1784 and 12/86, but not the local land-race (40 g N plant^{-1}). It is noteworthy that, except in a few instances, %Ndfa for any individual plant part was similar to that estimated for the whole plant (Table VII). Of the total N fixed in the whole plant, the amounts in leaves, branches, stems and roots represented 29, 5.8, 57 and 9.0%, irrespective of *G. sepium* provenance.

3.2. Experiment 2

3.2.1. Dry Matter and its distribution

Among the three tree-legume species, total DM accumulations by *G. sepium* and the reference *S. siamea* were comparable at around 2.8 kg plant^{-1} with *L. leucocephala* producing only 1.7 kg plant^{-1}. However, the differences between species were not significant, owing to a high CV (Table VIII). Of the total DM, a high proportion (70-80%) was distributed in the above-ground plant parts, the accumulation of DM in roots accounting for 20-30% in all three species. Furthermore, the distribution of DM in above ground plant parts in *G. sepium* and *S. siamea* was similar, in contrast to *L. leucocephala* in which the trunk accumulated the highest proportion of the DM.

TABLE VII. ESTIMATES OF PERCENTAGE (%NDFA) AND AMOUNT OF N_2 FIXATION (IN PARENTHESIS) BY FOUR *G. SEPIUM* GENOTYPES, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem	Root	Total
	%Ndfa (g N plant^{-1})				
OFI 14/84	70 ± 7[a] (14.9 ± 5)	61 ± 9 (2.1 ± 1)	62 ± 4 (40.2 ± 12)	57 ± 11 (3.6 ± 1)	64 (61.7)
OFI 17/84	56 ± 12 (1.7 ± 1)	51 ± 15 (1.8 ± 1)	45 ± 19 (11.3 ± 6)	56 ± 14 (3.1 ± 1)	53 (18.4)
OFI 12/86	52 ± 2 (2.9 ± 1)	36 ± 19 (0.8 ± 1)	48 ± 18 (11.8 ± 2)	52 ± 11 (2.2 ± 1)	52 (17.8)
LL	56 ± 9 (20.2 ± 4)	63 ± 18 (3.3 ± 2)	42 ± 24 (15.4 ± 6)	47 ± 24 (3.6 ± 2)	53 (40.4)
Significance					NS (*)
$LSD_{0\,05}$					25.7

[a] Mean ± SE.

TABLE VIII. DRY MATTER AND ITS DISTRIBUTION IN PLANT COMPONENTS OF *G. SEPIUM, L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2)

Species	Leaf	Branch	Trunk	Root	Total	% DM in root
			(g plant^{-1})			
L. leuco.	287	148	835	478	1,747	27
G. sepia	486	736	675	905	2,801	32
S. siamea	687	871	661	603	2,822	22
Significance	*	*	NS	NS	NS	NS
$LSD_{0\,05}$	323	536	-	-	-	-
CV(%)	40	57	45	45	40	19

3.2.2. N yield and its distribution

A trend similar to that for dry matter occurred in total-N yield, *G. sepium* being the highest with 36 g N plant^{-1}, followed by the reference *S. siamea*, at 27 g N plant^{-1} (Table IX), and *L. leucocephala* accumulated the least N. However, these values were not significantly different due to the high CV. Among the above-ground plant parts, leaves accounted for the highest proportion of the N in all three

species. The NFTs had relatively more N in the root than did the reference plants. Also, the NUE in the reference plant *S. siamea* was significantly ($P \leq 0.001$) higher than those of the NFT species, which had similar values (Table IX).

3.2.3. Atom $\%^{15}N$ excess

The ^{15}N enrichments within individual plant parts and their whole-plant weighted averages differed significantly among the three legumes. As expected, the concentration of ^{15}N in the component parts of the reference species, *S. siamea* was higher ($P \leq 0.001$) than in the two N_2 fixing species, *G. sepium* and *L. leucocephala* (Table X) indicating that significant fixation had occurred. Whereas the ^{15}N enrichments in the leaves of the NFTs tended to be lower than in the branches, stems and roots, in *S. siamea* the enrichments were more uniform across organs.

TABLE IX. TOTAL N YIELD, ITS DISTRIBUTION IN DIFFERENT PLANT COMPONENTS AND N-USE EFFICIENCY (NUE) OF *G. SEPIUM*, *L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2).

Species	Leaf	Branch	Trunk	Root	Total	% of total in root	NUE
			(g N plant^{-1})				(g DM g^{-1} N)
L. leuco.	9.8	1.0	6.1	5.6	22.6	25	78
G. sepium	15.3	5.0	4.1	11.8	36.2	32	75
S. siamea	15.6	4.1	2.5	4.7	26.9	17	107
Significance	NS	*	NS	*	NS	**	**
$LSD_{0.05}$	-	2.83	-	5.45	-	7.9	17
CV(%)	40	51	48	46	40	20	12

TABLE X. ATOM % ^{15}N EXCESS IN PLANT COMPONENTS AND THE WEIGHTED ATOM % ^{15}N EXCESS (WAE) FOR THE WHOLE PLANT IN *G. SEPIUM*, *L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2)

Species	Leaves	Branches	Trunk	Roots	WAE
			(Atom $\%^{15}N$ excess)		
L. leuco.	0.083	0.089	0.108	0.133	0.102
G. sepium	0.088	0.114	0.135	0.152	0.118
S. siamea	0.223	0.246	0.219	0.227	0.224
Significance	**	**	**	*	**
$LSD_{0.05}$	0.07	0.05	0.05	0.07	0.06
CV(%)	32	20	22	25	23

TABLE XI. ESTIMATES OF %N DERIVED FROM FIXATION (%NDFA), AMOUNTS OF FIXED N, AND PERCENT FIXED N IN THE ROOT OF *G. SEPIUM* AND *L. LEUCOCEPHALA* IN THE ESTABLISHMENT YEAR , 1992/93 (EXPT. 2)

Species	Lf.	Brnch.	Trnk	Root	Whole plant	Lf.	Brnch.	Trnk.	Root	Whole plant	N in root (%)
	(%Ndfa)					(Ndfa g N plant^{-1})					
L. leuco.	61	64	52	41	55	5.8	0.7	3.1	2.0	11.3	19
G. sepium	59	54	38	32	47	9.5	2.7	1.6	3.1	17.4	22
Significance	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
$LSD_{0\,05}$	-	-	-	-	-	-	1.5	-	-	-	-
CV(%)	25	20	30	35	27	26	50	56	31	42	52

3.2.4. N_2 fixation

On a whole-plant basis, %Ndfa values in *G. sepium* and *L. leucocephala* were not significantly different, at 47 and 55%, respectively (Table XI). Furthermore, %Ndfa values in individual plant parts were similar to that of the whole plant. This contrasted with the total amount of N fixed which was higher in *G. sepium* (17 g plant^{-1}) than in *L. leucocephala* (11 g plant^{-1}), although not significantly so due to the high CV. Among above-ground plant parts, leaves had the highest proportion of the fixed N (50-55%), and a substantial amount (18-22%) was present in roots.

3.3. Experiment 3

3.3.1. Total dry matter of prunings

The total DM of prunings (leaves and branches) as affected by pruning practice, tree age and species are shown in Table XII. Total pruning DM was higher ($P \leq 0.01$) in *G. sepium* and *S. siamea* than in *L. leucocephala.* Also, except in the first year (1993/94) after initial pruning, total DM values for *G. sepium* and *S. siamea* were comparable and significantly higher than for *L. leucocephala.* In all three species, there was a progressive increase in total DM with age to the second year (1994/95), but the rates of DM accumulation in pruned material was higher in *G. sepium* and *S. siamea* than in *L. leucocephala.* Although pruned and unpruned trees produced similar biomass yields in the first year, pruned trees produced more in the third year (1995/96). High biomass in unpruned trees in the second year after initial pruning was followed by a marked drop in the third year. Except in the second year, the 4-monthly pruning was superior to the 6-month interval in terms of total biomass yield (Table XII). The DM yields from prunings increased progressively with tree age. Among above-ground plant parts, the distribution of DM in branches was higher ($P \leq 0.01$) than that in leaves in both NFT species but, the opposite trend occurred with *S. siamea* (Table XII).

3.3.2. Total N yield and NUE

The total-N yields in the prunings were higher ($P \leq 0.01$) in *G. sepium* (58 g N plant^{-1}) followed by *L. leucocephala* then *S. siamea* (33 g N plant^{-1}) in the first year after initial pruning (Table XIII). But, in the second and third years the non-N_2 fixing *S. siamea* accumulated more ($P \leq 0.01$) N in prunings (104 and 95 g N plant^{-1}, respectively) than did *L. leucocephala* (46 and 41 g N plant^{-1}, respectively). Also, the rates of N accumulation with age were higher in *G. sepium* and *S. siamea* compared to *L. leucocephala.*

TABLE XII. DRY MATTER PRODUCTION OF *L. LEUCOCEPHALA*, *G. SEPIUM*, AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(kg plant^{-1})											
Leaves												
L. leuco.	1.21	0.94	0.60	0.92	0.63	0.73	1.25	0.87	0.94	0.91	0.08	0.65
G. sepium	1.71	1.13	1.09	1.31	1.62	2.45	3.17	2.41	2.14	2.04	0.44	1.54
S. siamia	1.29	1.49	0.68	1.15	3.06	3.58	2.89	3.18	5.95	3.37	0.26	3.19
Mean	1.41	1.19	0.79		1.77	2.25	2.44		3.01	2.10	0.26	
$LSD_{0\,05}$[a]		0.12				0.15				0.14		
Branches												
L. leuco.	1.44	1.53	1.76	1.58	0.80	1.30	3.35	1.82	2.62	2.70	0.61	1.98
G. sepium	1.34	1.51	1.90	1.58	1.06	3.38	5.67	3.37	3.98	4.16	0.98	3.04
S. siamia	0.78	0.94	1.34	1.02	1.55	2.80	3.49	2.61	2.64	3.74	0.15	2.17
Mean	1.19	1.33	1.67		1.14	2.49	4.17		3.08	3.53	0.58	
$LSD_{0\,05}$[a]		0.22				0.18				0.49		
Whole pruning												
L. leuco.	2.65	2.47	2.37	2.50	1.43	2.03	4.60	2.69	3.56	3.61	0.70	2.62
G. sepium	3.05	2.64	2.99	2.90	2.68	5.82	8.84	5.78	6.12	6.20	1.42	4.58
S. siamea	2.08	2.43	2.02	2.18	4.60	6.39	6.38	5.79	8.60	7.10	0.41	5.37
Mean	2.59	2.51	2.46		2.91	4.75	6.61		6.09	5.00	0.84	
$LSD_{0\,05}$[a]		0.33				0.32				1.57		

[a]For comparing means of species or pruning practices.

TABLE XIII. NITROGEN YIELDS OF *L. LEUCOCEPHALA* , *G. SEPIUM*, AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(g N plant^{-1})											
Leaves												
L. leuco.	44	29	22	32	23	26	42	30	33	34	3	23
G. sepium	59	39	38	45	53	88	102	81	69	73	15	52
S. siamea	33	34	14	27	76	96	81	84	152	91	7	83
Mean	45	34	24		51	70	75		85	66	8	
$LSD_{0.05}$ [a]		5.2				6.1				35		
Branches												
L. leuco.	11	12	15	13	6	13	27	15	22	25	6	17
G. sepium	14	12	14	13	10	37	50	32	32	35	11	26
S. siamea	6	5	7	6	12	27	19	19	15	19	0.8	12
Mean	10	10	12		9	26	32		23	26	5.9	
$LSD_{0.05}$ [a]		2.3				2.8				9.1		
Whole pruning												
L. leuco.	56	41	36	44	29	39	69	46	55	58	9	41
G. sepium	73	51	51	58	64	125	152	113	101	108	25	78
S. siamea	39	39	21	33	88	123	100	104	168	110	8	95
Mean	56	44	36		60	96	107		108	92	14	
$LSD_{0.05}$ [a]		7.0				9.0				36		

[a]For comparing means of species or pruning practices.

The effects of pruning on total-N yields of all three species followed trends similar to those of DM production, i.e. pruned trees accumulated more N than did unpruned trees, except in the second year after initial pruning. The 4-monthly interval was superior to the 6-monthly, except in the second year. Irrespective of species and pruning regime, accumulation of N in leaves was higher ($P \leq 0.01$) than in branches, with *G. sepium* and *S. siamea* accumulating more N than did *L. leucocephala*. Furthermore, among pruning treatments, leaf-N accumulation over the 3-year period on trees pruned at 4 monthly intervals (100-182 g N plant^{-1} was higher or comparable to these harvested twice yearly (89-200 g N plant^{-1}), whereas unpruned trees accumulated less leaf N (66-154 g N plant^{-1}).

Non-fixing *S. siamea* more efficiently utilized soil mineral N for biomass production than did the NFTs in years 1 and 2 (Table XIV). However, the NUE of *L. leucocephala* was significantly higher than that of *S. siamea* in year 3. Generally, NUE remained fairly constant with tree age in all three species, with generally higher values ($P \leq 0.01$) in unpruned trees at least in the first and second years, but with no marked effect of pruning frequency.

3.3.3. Atom %^{15}N excess

Senna siamea had the highest ^{15}N enrichments in its prunings (0.279-0.351%), followed by *L. leucocephala*, whereas ^{15}N enrichment was significantly lower ($P \leq 0.01$) in *G. sepium* (Table XV). The unpruned trees in all three species had the lowest ^{15}N enrichments in leaves and branches and, except in the second year, the enrichments of 6-monthly prunings were less than those cut every 4 months. Irrespective of the species and pruning treatment, enrichment declined with age, except in a few instances.

3.3.4. N_2 fixation

The prunings from *G. sepium* contained a higher ($P \leq 0.01$) proportion of fixed N (%Ndfa 48-65) than *L. leucocephala* (41-60) except in year 2 (Table XVI). A similar trend existed in leaves and branches although the differences in %Ndfa were not significant. In neither species was there marked change in %Ndfa with age except in the second year. Unpruned trees derived a higher proportion of total N from fixation (53-76%) except in the second year. The %Ndfa was not influenced by pruning interval.

Irrespective of age, *G. sepium* had higher amounts of fixed N in prunings, than did *L. leucocephala* (Table XVII). Nitrogen derived from fixation was appreciably higher in leaves than in branches. Also, BNF capacity in both species increased up to the second year and declined thereafter. Pruned trees in both species produced significantly higher amounts of fixed N, and there was no consistent pattern in the amounts of fixed N in unpruned trees except in year 2. There was no consistent trend in terms of superiority of any one pruning regime.

3.4. Experiment 4

3.4.1. Growth of coconut

The application of prunings from the tree legumes significantly increased girth and canopy size of the coconut saplings (Table XVIII). A similar trend was observed also in plant height, although the differences were significant only with the *Leucaena* prunings. In general the performance of the coconut saplings supplied with *L. leucocephala* and *G. sepium* prunings was better than when applied with *S. simea* prunings, which was better than that of the untreated control, albeit not necessarily significantly.

TABLE XIV. NITROGEN USE EFFICIENCY OF *L. LEUCOCEPHALA, G. SEPIUM* AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(g DM g^{-1} plant N)											
L. leuco.	48	60	66	58	46	52	67	55	64	61	75	67
G. sepium	43	52	59	50	44	47	59	50	60	60	56	59
S. siamea	54	62	104	73	54	53	64	57	56	66	58	60
Mean	48	58	76		48	51	63		60	62	63	
$LSD_{0.05}$ [a]		10.2				2.4				3.7		

[a]For comparing means of species or pruning practice.

TABLE XV. ATOM % ^{15}N EXCESS IN HARVESTED PLANT MATERIALS OF *L. LEUCOCEPHALA, G. SEPIUM*, AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(% ^{15}N a.e.)											
Leaves												
L. leuco.	0.223	0.211	0.127	0.190	0.145	0.168	0.080	0.131	0.207	0.160	0.025	0.130
G. sepium	0.214	0.200	0.130	0.181	0.108	0.166	0.133	0.136	0.151	0.171	0.024	0.277
S. siamea	0.394	0.334	0.316	0.351	0.364	0.444	0.312	0.374	0.387	0.330	0.114	0.277
Mean	0.278	0.245	0.191		0.206	0.259	0.174		0.245	0.221	0.054	
$LSD_{0.05}$ [a]		0.027				0.045				0.020		
Branches												
L. leuco.	0.239	0.244	0.221	0.235	0.153	0.171	0.196	0.173	0.214	0.156	0.041	0.137
G. sepium	0.187	0.208	0.148	0.182	0.100	0.156	0.148	0.134	0.134	0.141	0.031	0.101
S. siamea	0.360	0.327	0.363	0.350	0.358	0.364	0.363	0.362	0.362	0.337	0.138	0.279
Mean	0.262	0.260	0.244		0.205	0.230	0.235		0.237	0.211	0.071	
$LSD_{0.05}$ [a]		0.037				0.041				0.020		
Whole pruning materials												
L. leuco.	0.226	0.222	0.165	0.204	0.148	0.167	0.126	0.147	0.211	0.161	0.032	0.134
G. sepium	0.210	0.203	0.134	0.182	0.107	0.163	0.136	0.136	0.147	0.167	0.024	0.112
S. siamea	0.389	0.333	0.330	0.351	0.365	0.429	0.322	0.373	0.383	0.333	0.120	0.279
Mean	0.275	0.253	0.210		0.207	0.253	0.194		0.247	0.220	0.059	
$LSD_{0.05}$ [a]		0.027				0.038				0.016		

[a]For comparing means of species or pruning practices.

TABLE XVI. PERCENT N DERIVED FROM FIXATION IN HARVESTED PLANT MATERIALS OF *L. LEUCOCEPHALA* AND *G. SEPIUM* AS AFFECTED BY PRUNING PRACTICE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(%Ndfa))											
Leaves												
L. leuco.	43	36	59	46	59	62	73	65	47	53	77	59
G. sepium	45	41	58	48	70	62	54	62	61	49	81	64
Mean	44	38	58		64	62	64		56	51	79	
$LSD_{0.05}$[a]				NS				NS				NS
[b]	7.7				NS				5.1			
Branches												
L. leuco.	32	25	34	31	56	53	41	50	40	54	65	53
G. sepium	47	37	56	47	71	57	56	61	63	58	79	67
Mean	40	31	45		64	55	48		52	56	72	
$LSD_{0.05}$[a]				NS				NS				11
[b]	NS				NS				14			
Whole pruning materials												
L. leuco.	42	32	49	41	59	61	59	60	45	53	73	57
G. sepium	46	40	58	48	70	61	55	62	62	51	80	65
Mean	44	36	53		65	61	57		53	52	76	
$LSD_{0.05}$[a]				6.8				NS				4.1
[b]	8.3				NS				5.0			

[a]For comparing means of species. [b]For comparing means of pruning practices.

TABLE XVII. AMOUNT OF N FIXED BY *L. LEUCOCEPHALA* AND *G. SEPIUM* AS AFFECTED BY PRUNING PRACTICE (EXPT.3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(g N plant^{-1})											
Leaves												
L. leuco.	19.8	10.5	12.8	14.4	13.5	16.0	29.9	24.7	15.2	17.6	2.3	11.7
G. sepium	27.7	16.0	21.4	21.7	37.6	54.3	54.7	55.4	42.5	34.1	12.1	29.5
Mean	23.7	13.2	17.1		25.6	35.2	42.3		28.8	25.8	7.2	
$LSD_{0.05}$[a]				4.5				10.9				4.8
[b]	3.7				NS				5.9			
Branches												
L. leuco.	3.9	2.7	5.4	4.0	3.4	7.2	16.2	8.9	8.5	14.1	4.3	9.0
G. sepium	7.0	4.8	7.8	6.5	7.2	20.9	26.9	18.3	19.5	20.7	8.5	16.2
Mean	5.4	3.7	6.6		5.3	14.1	21.6		14.0	17.4	6.4	
$LSD_{0.05}$[a]				2.6				5.9				3.2
[b]	3.2				4.8				3.9			
Whole pruning materials												
L. leuco.	24.0	13.1	17.8	18.6	17.0	23.8	46.1	28.9	24.4	31.3	6.7	20.8
G. sepium	34.6	20.8	29.4	28.3	44.9	76.3	81.6	67.6	62.4	54.4	20.5	45.8
Mean	29.3	17.0	23.6		31.0	50.0	63.9		43.4	42.9	13.6	
$LSD_{0\,05}$[a]				5.5				14.5				6.8
[b]	6.8				17.8				8.4			

[a]For comparing means of species
[b]For comparing means of pruning practices.

3.4.2. Availability of N from prunings

The coconut saplings obtained about 36% of their N from *L. leucocephala* and 40% from *G. sepium* prunings (Table XVIII). The fraction of N taken up from *Senna* green manure was significantly lower.

4. CONCLUSIONS

The selection of a suitable reference species is a critical consideration in estimating BNF by trees, when using the ^{15}N-dilution method. In this work, biomass production of the non-nodulating tree legume *S. siamea* was similar to those of the NFTs, and had higher ^{15}N enrichments In the light of these observations, *S. siamea* appeared to be a good choice as a reference for estimating BNF in *G. sepium* and *L. leucocephala* for Sri Lankan conditions.

4.2. Experiment 1

In view of the widespread use of *G. sepium* as a component of agroforestry systems in many ecological niches, it is important to select genotypes/provenances with superior BNF ability. An important finding arising from this study is the significant variation in BNF ability among *G. sepium* genotypes, provenance OFI 14/84 from Guatemala being superior to others. Also, it is noteworthy that the local land-race demonstrated considerable capacity for fixing atmospheric N_2. The lower ^{15}N enrichment in plant tissues of provenance 14/84 indicates superior ability to fix N_2. This study shows that the selection of superior genotypes with high biomass yield and N_2-fixation ability is a valid strategy for increasing the N contribution from fixation in agroforestry systems. Although %Ndfa values for the whole plant did not differ appreciably among *G. sepium* genotypes, (52-64%), the amounts of fixed N varied significantly (18-62 g N plant^{-1}) as a result of differences in biomass production, suggesting that BNF depends heavily on the supply of photosynthates. Furthermore, results of this study suggest that exclusion of roots in calculating BNF would not seriously underestimate (a mean of 9%) the amounts of N_2 fixed by *G. sepium*, at least during the first year.

TABLE XVIII. LEAF %N, ^{15}N ENRICHMENTS AND GROWTH COMPONENTS OF COCONUT SAPLINGS AND THE RECOVERY OF N FROM PRUNINGS (EXPT. 4)

Pruning source	Leaf N (%)	^{15}N (% excess)	Height (cm)	Girth (cm)	Canopy (cm)	%N from prunings
L. leucocephala	1.74	0.086	143	82.5	205	36
G. sepium	1.66	0.083	121	79.1	184	40
S. siamea	1.74	0.070	114	71.2	151	15
Control	1.63	0.019	91.8	52.4	124	
Significance	NS	*	*	*	***	*
$LSD_{0.05}$	-	0.04	34.3	10.1	40.1	17
CV (%)	12	41	19	9	16	36

4.2. Experiment 2

The comparison of *G. sepium* (12/86) and *L. leucocephala* (K 636) under coconut over a 1-year period revealed the former to be superior in terms of total DM production, N yield and amount of N fixed. A notable feature was the high NUE achieved by *Gliricidia* and *Leucaena* (75-78 g DM g^{-1} N). In these species, %Ndfa values did not differ markedly, ranging between 47 and 55% ,whereas fixed N in *G. sepium* (17 g N $plant^{-1}$) was higher than in *L. leucocephala* (11 g), although not significantly so due to a high CV. In Experiment 1 the same provenance of *G. sepium* grown in full sunlight produced similar amounts of fixed N_2, showing that BNF capacity of *G. sepium* was not adversely affected by the 30% shade under the coconut-tree canopy. In this study, roots accounted for substantial proportions of total DM (27-32%), total N (25-32%) and fixed N (19-22 g $plant^{-1}$) in both NFT species, suggesting that exclusion of roots would lead to gross underestimation of BNF in *G. sepium* and *L. leucocephala* grown under such conditions.

4.3. Experiment 3

Currently, there is a serious lack of information and understanding of the BNF capacity of NFT species, as influenced by tree management and age. This issue was addressed by estimating the BNF potential of *G. sepium* and *L. leucocephala* grown under coconut and subjected to three pruning frequencies. *Gliricidia sepium* was found to be the superior N_2 fixer, as shown by higher amounts of fixed N in prunings (28-68 g N $plant^{-1}$), irrespective of tree age. Also, amounts of fixed in both species increased up to the second year after initial pruning, which resulted in declines in ^{15}N enrichment. The total DM in its prunings was significantly higher in *G. sepium* than in *L. leucocephala,* which correlates with its high N_2-fixing capacity. Pruning increased biomass production and N yield except in the second year after initial pruning, 4-monthly pruning being superior to 6-monthly. In contrast, unpruned trees produced higher total DM and N yields in the second year of growth after initial pruning. The amount of fixation by NFTs with frequent pruning may have been due to less soil N being available as a result of greater demand for N for regrowth. In contrast, there was a remarkable drop in the N_2 fixation by trees not subjected to pruning, particularly in the third year, probably as a result of more soil N being made available via root and nodule senescence and due to absence of regrowth. These results suggest that pruning would have a positive effect on BNF capacity in trees in the long term.

4.4. Experiment 4

Increased growth of coconut saplings by the application of *G. sepium* and *L. leucocephala* as a mulch is a reflection of rapid decomposition and availability of nutrients; 35-40% of sapling N was obtained from the green manure. In the light of these results, it would appear that *G. sepium* could be recommended as a component in agroforestry systems associated with coconut. However, the amounts of fixation and contribution of atmospheric N by regularly pruned *Gliricidia* and its transfer to coconut palms have to be investigated in longer-term studies. Also, long-term effects of *G. sepium* on maintaining and restoring soil fertility of marginal soils under coconut, its impact on the soil-plant environment and implications on socio-economic considerations also warrant study, in order to promote its widespread use in the development of sustainable farming systems in the tropics.

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EVALUATION OF *FRANKIA* AND RHIZOBIAL STRAINS AS INOCULA FOR NITROGEN-FIXING TREES IN SALINE CONDITIONS

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Abstract

Frankia strains isolated from various *Casuarina* species were screened for nodulation and N_2-fixing ability on *C. glauca* and *C. obesa* under controlled-environment conditions. Five out of thirteen strains induced effective root nodules on *C. glauca*, but none did so on *C. obesa*; two strains were selected. Similarly, various rhizobial strains were screened for nodulation and N_2 fixation on four *Acacia* species and finally three were selected for compatibility with *A. ampliceps*. The two *Frankia* strains (CcOl and CcI3) and three *Rhizobium* strains (Abal, Ar2-1 and PMA63/1) were checked for NaCl-tolerance in vitro, and were used as inocula to estimate N_2 fixation in fast-growing trees under highly saline field conditions. The isotope-dilution method was used to estimate the proportion and amount of N_2-fixed by *A. ampliceps* and *C. glauca* with *Eucalyptus camaldulensis* as the non-fixing check. After a year, *A. ampliceps* plants formed a few root nodules at low Ec_e levels, but during the second and third years profuse nodulation was observed. In 1-year-old plants the fraction of N derived from fixation (Ndfa) ranged from 7 to 55% (average 31%) in *A. ampliceps* and from 7 to 24% (average 15%) in *C. glauca*, and after two years %Ndfa for *A. ampliceps* increased markedly, with values up to 86%. On the other hand, increases in %Ndfa for *C. glauca* were insignificant, possibly due to the use of *E. camaldulensis* as the non-fixing reference plant. Infection of tree roots by vesicular arbuscular mycorrhiza (VAM), scored after 3 years, showed a negative relationship with soil electric conductivity, as did VAM spore number. The spores isolated from saline soils had thicker walls than those from a fertile soil. Decreases in the soil salinity levels were observed at the end of the 3-year experiment.

1. INTRODUCTION

Soil salinity is a serious environmental and agricultural problem causing extensive areas of arable land to be excluded from cultivation. In Pakistan, 13 M acres are salt-affected with significant effects of food production. These soils have high concentrations of NaCl, high pH, low content of available N and P, and are very low in organic matter. Various approaches are being attempted to ameliorate these conditions and restore economic and biological viability to these soils.

An effective strategy is to initiate plant successions using salinity-tolerant species, even those of limited economic utility. Agroforestry approaches are receiving attention, involving the planting of N_2-fixing trees (NFTs) that, by the applications of prunings as green manure, can contribute N as well as organic matter, combat erosion and also provide fuel wood [1,2]. Many *Casuarina* and *Acacia* species are reportedly tolerant of high soil temperatures and salinity [3,4,5]. Some have been introduced in Pakistan, but early field observations revealed poor root nodulation [6,7], possibly due to the absence or non-compatibility of endemic strains of the necessary microsymbionts, *Frankia* and rhizobia.

The achievement of maximum inputs of fixed N from NFTs into agro-ecosystems requires management practices and conditions that favour the functioning of the N_2-fixing system [3], including the presence of compatible salt-tolerant strains of the rhizobia and/or *Frankia* [7-10]. Moreover, consideration must be giver to the actinorhizal symbiosis and the possible need for inoculation with vesicular arbuscular mycorrhiza, in view of their positive effects on fast-growing trees [11-13].

The long-term evaluation of N_2 fixation by trees poses problems associated with their perennial nature, massive size and difficulty in obtaining representative samples [14]. With modifications, the ^{15}N-dilution technique has been recommended for estimating the proportion and amount of N fixed by trees [15,16].

Our objectives were to find the most compatible strains of *Frankia* and rhizobia for their specific hosts for improved N_2-fixing ability, and to use them with *Acacia ampliceps* and *Casuarina glauca* for field measurements of N_2 fixation and to investigate possible ameliorative effects on a salt-affected soil.

2. MATERIALS AND METHODS

2.1. Evaluation of N_2-fixing microsymbionts

2.1.1. Isolation of endophytes

Root nodules were collected from *Casuarina* species growing at the Punjab Forest Research Institute (PFRI), Faisalabad, the Bio-Saline Research Station (BSRS), Lahore, and at Quaid-I-Azam University (QAU), Islamabad. Nodules were obtained also from *Acacia* species growing in a highly saline area at BSRS.

Frankia strains were isolated from *Casuarina glauca* by two methods. (1) After surface-sterilization with 3% OsO_4 and rinsing with sterile distilled water, the nodules were crushed in a drop of 0.1 *M* phosphate buffer under aseptic conditions and used to inoculate Qmod medium [17]. The inoculated medium was incubated at 28 to 30°C and growth of the actinomycete was monitored. (2) The suspension from a surface-sterilized nodule in 0.1 *M* phosphate buffer was poured on top of a sucrose gradient of 1.0, 1.6 and 2.5 *M* in a teflon centrifuge tube [18] and centrifuged at 5,000×g in a swing-out rotor, at room temperature for 20 min. The fraction at the interface between 1.6 and 2.5 *M* was collected and mixed with basal propionate (BAP) agar medium [19] while still warm (35-40°C). The plates were incubated at 28 to 30°C, for 25 to 30 days and typical star-shaped hyphal colonies of *Frankia* were aseptically picked singly and added to fresh BAP broth. The strains were sub-cultured every two months in BAP broth supplemented with Na and K phosphate, Fe-EDTA and trace elements, and incubated at 28°C. Fully developed *Frankia* colonies were repeatedly passed through an 18-gauge syringe needle to separate and homogenize them before sub-culturing or use as inocula

Rhizobia were isolated from *Acacia ampliceps, A. aneura* and *A. senegal*. The nodules were surface-sterilized with 0.2% $HgCl_2$, rinsed with sterile distilled water and aseptically crushed in a drop of 0.1 *M* phosphate buffer and added to yeast extract mannitol agar (YEMA) plates [20] which were then incubated at 28 to 30°C until colony appearance. Single colonies were sub-cultured on YEMA supplemented with congo red or bromothymol blue (BTB) to monitor time to colony appearance and acid or alkali production. The strains were authenticated by their ability to form nodules on the host of derivation in growth pouches containing N-free nutrient solution.

2.1.2. Nodulation studies

Seeds of *C. glauca* and *C. obesa*, obtained from the CSIRO Australian Tree Seed Centre, Canberra, Australia, were surface sterilized in 0.2% $HgCl_2$, rinsed with sterile distilled water, and sown in sterile sand. The seedlings were irrigated with N-free nutrient solution. Cultures of *Frankia*, isolated from *Casuarina* species, were obtained from culture collections in various parts of the world.

The roots of 1-month-old seedlings of *C. glauca* and *C. obesa* were inoculated by being held in suspensions of *Frankia* strains for 18 to 24 h then sown two per Leonard-jar assembly containing sterile sand. There were three replicates for each treatment, with uninoculated controls. The assemblies were watered with N-free nutrient solution and the plants were grown under controlled-environment conditions, with a 14-h photoperiod of 4,750 lux at 28±2°C and relative humidity of 50 to 70%. Plants were harvested after two months and examined for nodulation.

Cross-inoculating ability of locally isolated and exotic strains of rhizobia was checked on *Acacia ampliceps, A. nilotica, A. aneura, A. saligna* and *A. sterophylla*, in Leonard jars and growth pouches. Seeds were surface-scarified with concentrated H_2SO_4 for 10 min, rinsed in sterile water and allowed to imbibe in the final rinse for 2 h, then planted in fours and thinned to one after establishment. There were four replicates per strain. One mL of culture broth of each strain (10^9 cells mL^{-1}) was applied per plant. The experiment was conducted under controlled-environment conditions with a 16-h photoperiod of 4,750 lux, day/night temperatures of 28/22°C, and 50 to 70% relative humidity. Plants were harvested at six weeks, and nodule numbers and fresh weight determined.

2.1.3. Nitrogenase activity

Acetylene-reduction assays [21] were run on fresh root nodules from *C. glauca*. The excised nodules were placed in 13-mL vacutainer tubes fitted with air-tight rubber seals, from which 0.1 atm. was replaced with acetylene using a pressure-lock gas syringe (Precision Sampling Corporation). Control tubes were (a) without nodules in acetylene and (b) with nodules and no acetylene, and incubation was for 1 h at room temperature. Gas samples (100 µL) were analyzed for ethylene production by gas chromatography.

2.1.4. Endophyte salt tolerance in vitro

The two most effective *Frankia* strains, CcO1 and CcI3, isolated from *C. cunninghamiana,* were selected for in-vitro NaCl-tolerance studies. A cell-packed volume of 0.5 mL of each strain was added to 10 mL of BAP broth in screw-capped tubes containing NaCl at concentrations of 0, 100 m*M* (approximately 9 dS m^{-1}, 300 m*M* (28 dS m^{-1}), 500 m*M* (47), 700 m*M* (66) and 900 m*M* (84). The amount of soluble protein present at the time of inoculation was estimated by Lowry's method [22]. The cultures were incubated at 28°C for 45 days without shaking, and protein content and percent growth recorded. Similarly, the selected rhizobial strains, i.e. Aba1, Ar2-1 and PMA63/1 were checked for in-vitro NaCl tolerance. The rhizobial cells (10^6 mL^{-1}) were inoculated in triplicate into 100-mL YEM broths supplemented with NaCl concentrations of 0 to 500 m*M*. The cultures were grown at 28°C with continuous stirring for 3 to 5 days, and viable-cell counts made.

2.1.5. Effects of salinity on native rhizobia

Two soils of different salinity level (5 to 7 and 15 dS m^{-1}) were counted for indigenous rhizobia able to nodulate *A. ampliceps* and for *Frankia* nodulating *C. glauca,* by plant-infectivity tests and the most-probable-number (MPN) method.

Also examined were the effects of salinity, 0.8 to 35 dS m^{-1}, on nodulation, dry weight, nitrogenase activity and rhizosphere populations of rhizobial strains Aba1, Ar2-1 PMA 63/1, with *A. ampliceps* under controlled-environment conditions in Leonard-jar assemblies. The initial population was 2.6×10^6 cells mL^{-1}. There were four replicates, with negative controls as described above; data were recorded at 15, 30 and 45 days.

2.1.6. Sporulation studies

Frankia strains CcC 1 and CcI3 were used to study sporulation using modified BAP medium [23]. The experiment was carried out using 25-mL screw-capped glass tubes and 100-mL conical flasks containing 10 or 50 mL of medium. The tubes were incubated without shaking at 28°C, whereas the flasks were shaken at 100 rpm. Sets of test-tube samples were oven-dried at 65 to 70°C at 8-day intervals up to 32 days. Three replicates were kept for each reading. Strains grown in the flasks were used for morphological studies, as well as to compare rates of growth between shaken and unshaken cultures.

2.2. Evaluation of NFTs

2.2.1. Field experiment

The area selected was highly saline (Table I), dry and unsuitable for most plant cultivation. *Acacia ampliceps*, and *C. glauca*, were selected as potential N_2-fixers tolerant of high salinity. *Eucalyptus camaldulensis,* also a fast grower, was chosen as the non-fixing reference. The experiment was designed to last three years. The total area treated with ^{15}N was 462 m^2, with twelve plots of 38.5 m^2 (Figs. 1 and 2). Each ^{15}N plot was contained by digging 1-m deep trenches on all sides and placing

a plastic sheet as a shield to prevent soil and nutrient mixing. There were four replicates and the experiment had a randomized complete-block design.

Soil samples were collected from each plot at a depth of 10 to 12 inches. In excess of 212 saplings of *A. ampliceps*, *C. glauca* and *E. camaldulensis* were raised in a nursery and transplanted into the field. Ammonium sulphate was applied at 20 kg N ha^{-1} enriched in ^{15}N at 10% atom excess to twelve plots, with P_2O_5 at the rate of 80 kg ha^{-1}. Similar amounts of P and unlabelled N were applied to adjoining areas. The N fertilizer was dissolved in 5-L aliquots of canal water and sprayed onto the surface.

2.2.2. *Inoculation with* Frankia *and rhizobia*

Acacia ampliceps seedlings were inoculated with a mixture containing two local rhizobial strains, Aba1 and Ar2-1, and the exotic PMA 63/1, that had been previously evaluated for salt tolerance and N_2-fixing compatibility with the host under controlled-environment conditions. Similarly, *C. glauca* seedlings were inoculated with a mixture of *Frankia* strains CcO1 and Cc13 that were known to be salt tolerant and compatible with the host. Plants were and irrigated with canal water and inoculated again after three months.

2.2.3. *First-year harvest*

Acacia ampliceps and *E. camaldulensis* plants were selected from four of the twelve ^{15}N plots at one year from transplanting. Whole plants were harvested and nodulation data recorded for *A. ampliceps*. Leaves, stems and branches, and roots were separated, chopped into small pieces and air dried in a sun room. Fresh and dry weights were recorded. Sub-samples were milled, sieved and sent for analysis of ^{15}N enrichment at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria, using a mass spectrometer [24]. The N concentrations of shoot and grain were estimated byKjeldahl's method [25].

TABLE I. SOIL ELECTRIC CONDUCTIVITY AND pH OF 12 PLOTS AT THE INITIAL AND FINAL STAGES OF THE EXPERIMENT

Plot no.	Initial data[a]		Final data[b]	
	pH	Ec_e (dS m^{-1})	pH	Ec_e (dS m^{-1})
1	9.2[c]	7.8	8.4	2.2
2	8.9	6.0	8.3	2.4
3	9.0	5.9	8.4	3.8
4	9.0	7.3	8.7	4.3
5	8.8	5.7	8.3	4.4
6	8.8	4.8	8.5	2.6
7	8.9	10	8.4	10
8	8.8	5.8	8.5	4.8
9	8.7	11	8.4	8.4
10	7.9	13	7.2	7.6
11	7.9	15	7.0	10
12	8.0	13	7.9	9.1

[a]Before transplanting. [b]After third-year plant-sample collection. [c]Averages of five replicates.

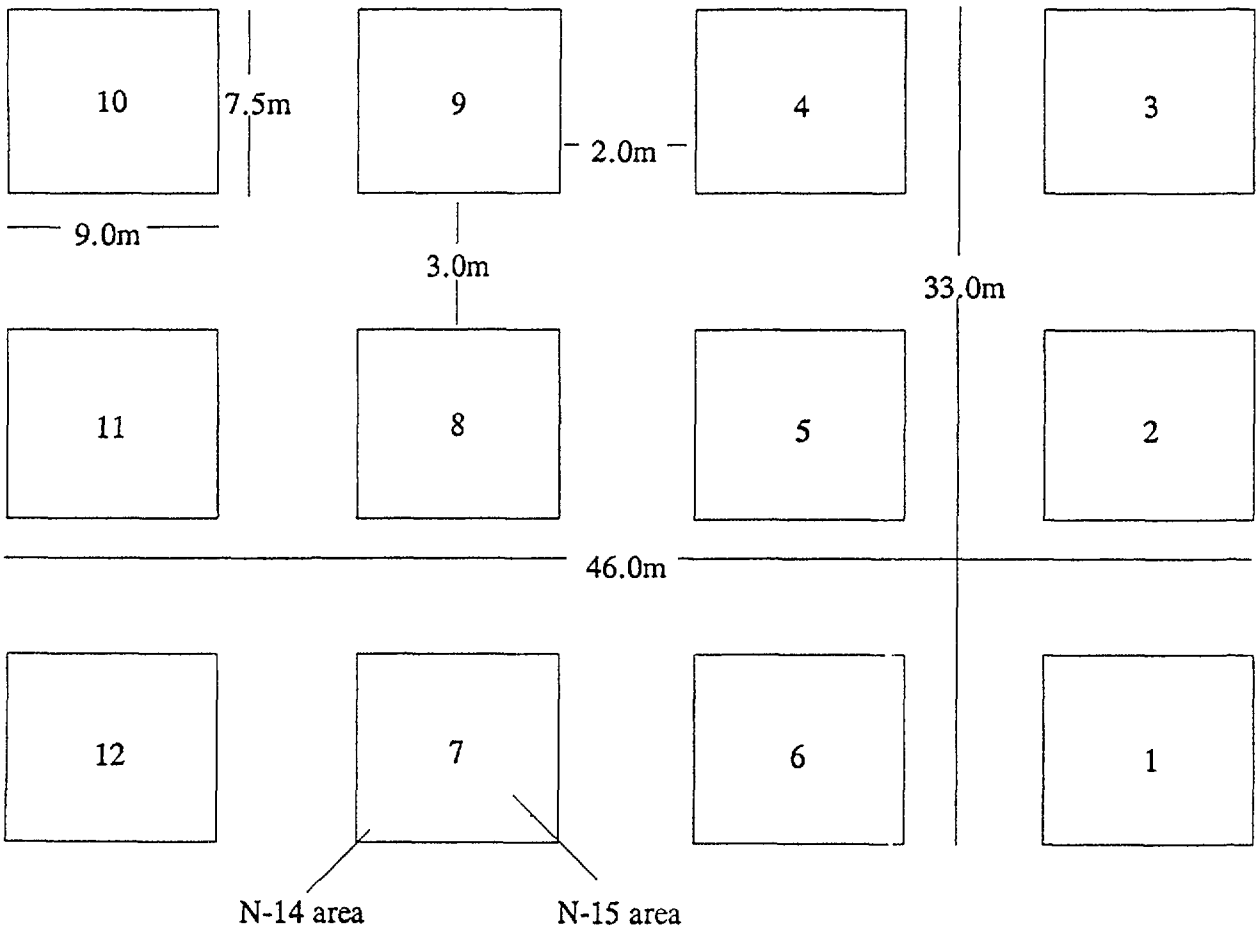
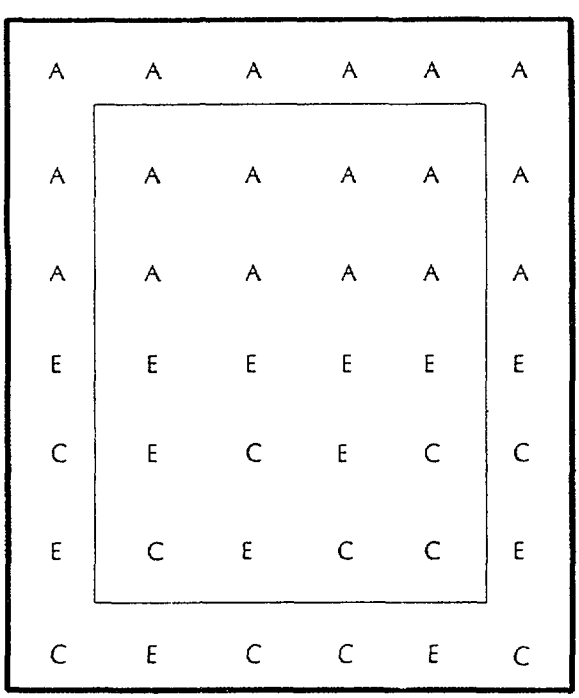


FIG 1 Layout of the experiment



A=*Acacia ampliceps*

C=*Casuarina glauca*

E=*Eucalyptus camaldulnesis*

Plant to plant distance = 1.5m

N-15 area

N-14 area

FIG 2 Arrangement of N_2-fixing and non-fixing trees in a plot

Milled samples were digested with concentrated nitric acid, filtered and analyzed on a flame photometer for K and Na content [26].

2.2.4. Second- and third-year harvests

Due to large size, the trees were sub-sampled, then chopped, dried and milled in preparation for ^{15}N analysis.

Rhizosphere-soil samples were taken at the first- and second-year harvests, and from all twelve plots at 10-12 inches depth at the third-year harvest, for determinations of pH and Ec_e.

2.2.5. Mycorrhizal infection

Roots and rhizosphere soil were collected from *C. glauca, A. ampliceps* and *E. camaldulensis* on the twelve ^{15}N plots, and used for mycorrhizal studies. In addition, root samples were collected from a non-saline soil (Ec_e<1 dS m^{-1}). The roots were cleared and stained with trypan blue in lactic acid [27] and a slide technique was employed to assess mycorrhizal infection [28]. Spores were extracted from the rhizosphere soil [27] and counted, and different types were picked up and mounted in lactic acid for detailed observation under the microscope.

3. RESULTS AND DISCUSSION

3.1. **Evaluation of N_2-fixing microsymbionts**

3.1.1. Frankia-Casuarina *symbiosis*

Nodules were observed on the roots of a few plants of *C. glauca* and *C. obesa* at the Pakistan Forest Research Institute, and only a few *C. glauca* plants growing in saline soil at BSRS had root nodules. In contrast, *C. glauca* plants at QAU had enormous numbers of root nodules.

The isolates were identified at *Frankia* on the basis of their growth on specific Qmod media: star-shaped colonies with thinseptate hyphae, numerous vesicles and pear-shaped sporangia [29-31].

Strain CGQU, isolated from a well nodulated *C. glauca* at QAU, formed effective N_2-fixing nodules on seedlings of its host of derivation. Four out of eleven exotic *Frankia* strains, isolated from various *Casuarina* species, also formed nodules on *C. glauca*, thus showing cross-infectivity. The local isolate CGQU formed the highest number of nodules, but had low acetylene-reduction activity as compared with exotic strains CcO1 and CcI3 (Table II). None of the isolates formed nodules on *C. obesa.*

The exotic strain CcO1, showed high salt tolerance in vitro, with growth at up to 500 m*M* NaCl (approximately 47 dS m^{-1}) whereas strain CcI3 was sensitive to even 100 m*M* (9 dS m^{-1}). The local strain CGQU did not survive even under very low NaCl concentrations. Miettinen [32] reported that a *Frankia* strain from *C. equisetifolia* was tolerant of 16 dS m^{-1} under free-living conditions.

Frankia stain CcO1 was grown on modified basal propionate medium, in an effort to enhance sporulation (Fig. 3), resulting in an increased total biomass yield within a given period of time. High nitrogenase activity and superior growth in vitro at high salt concentrations, indicated that CcO1 was a promising endophyte for *C. glauca.*

TABLE II. SCREENING OF *FRANKIA* STRAINS FOR NODULATION ON *C. GLAUCA* AND ACETYLENE REDUCTION ACTIVITY

Strain	Nodule number (per plant)	Nodule fresh wt. (mg)	C_2H_2 reduction ($\mu mol\ g^{-1}h^{-1}$)
Local			
CGQAU	13	40	1.7
CGBSRS	0	0	0
Exotic			
CM21	0	0	0
CM22	0	0	0
CcI017	0	0	0
AlII01	0	0	0
CcO1	9	106	36
CcI3	11	110	4.1
Ce24	0	0	0
53024	0	0	0
Ino03593	0	0	0
JCT-287	4	30	ND[a]
F-49	6	37	ND

[a]Not determined.

3.1.2. *Rhizobia*-Acacia *symbiosis*

Rhizobial endophytes were isolated from the root nodules of three *Acacia* species and were characterized on the basis of growth rate and acid or alkaline production. The strains isolated from *A. ampliceps* Aba1, Aba2 and Ar2-1 showed maximum growth within 72 h of incubation, produced large amounts of exopolysaccharide and turned the BTB medium yellow due to acid production. Therefore, these strains were grouped as *Rhizobium* strains similar to those isolated by Milnitsky *et al.*, [33] (1977) and Zou *et al.*, [10] from *A. ampliceps*. Isolates from *A. senegal*, AS1, AS2 and As3, and from *A. aneura*, Aa2, reached maximum growth after 120 h, i.e. were slow-growing, and produced less exopolysaccharide and turned the medium blue indicating alkali-production; these were *Bradyrhizobium* strains. All the exotic strains listed in Tables II and III had characteristics of the genus *Bradyrhizobium*, except PMA 63/1.

Infectivity tests on two *Acacia* species by locally isolated and exotic strains showed nodule induction on *A. ampliceps* and *A. nilotica* by most (Table III). No nodulation was observed on *A. aneura* except by the local isolate Aa2 (Table IV). Two exotic and one local isolate formed nodules on *A. saligna*. Cross-inoculation among a wide range of strains suggests that they can be effectively utilized as inoculum for a range of *Acacia* species in the field.

Strains Aba1, Ar2-1 and PMA63/1 showed tolerance of up to 300 m*M* NaCl (data not shown).

The indigenous population of rhizobia was found to be only two cells per g for *A. ampliceps* in a soil with an Ec_e 5-7 dS m^{-1}. A negative correlation of native rhizobial numbers with increasing salinity, indicates the need for inoculation of legumes in highly saline conditions.

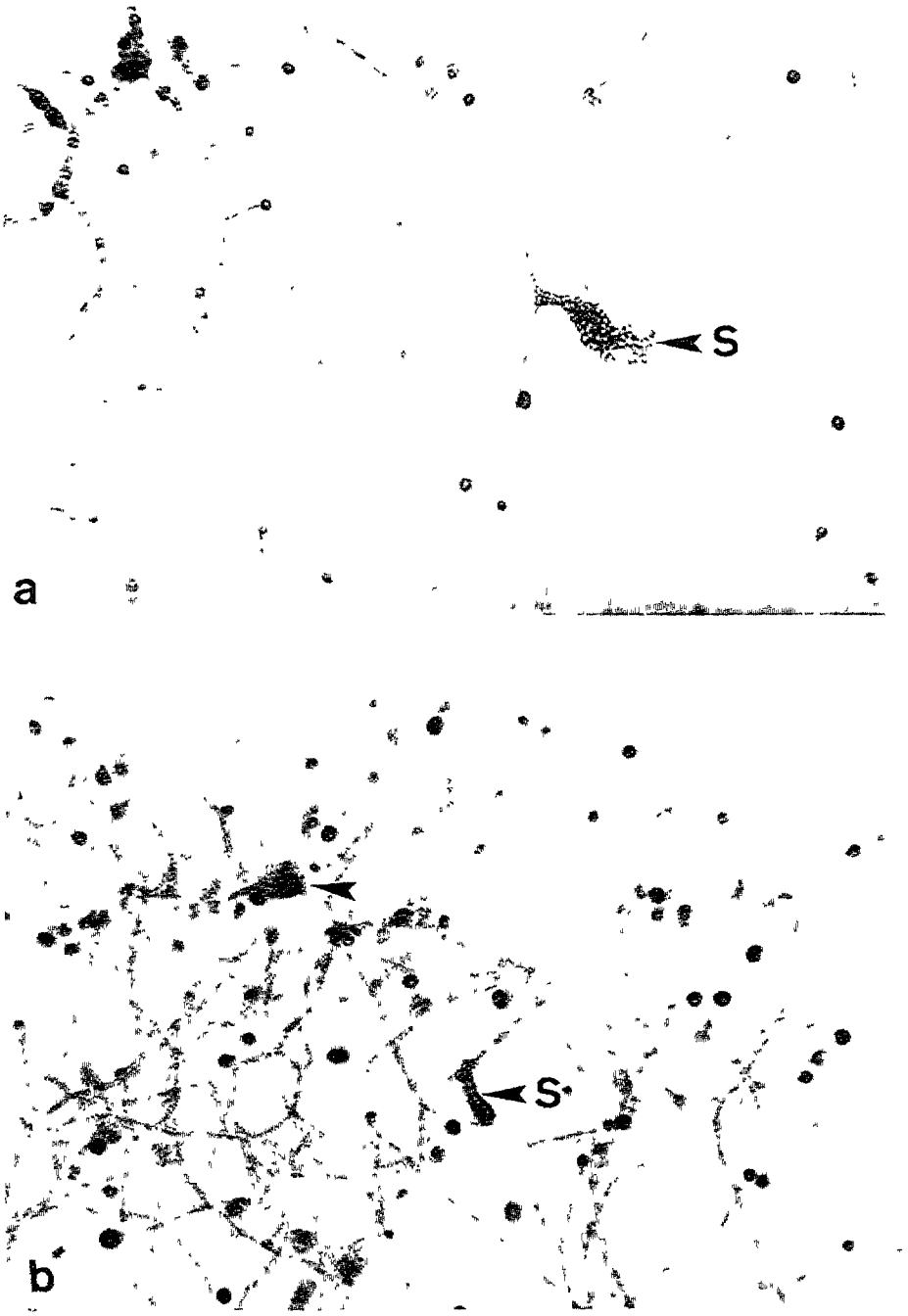


FIG. 3. Photomicrographs of Frankia *strain CCO1 colonies, showing (a) a typical mature sporangium, and (b) many young sporangia induced by the modified medium* [23]. ×1800.

In another experiment, the initial observations after 15 days showed higher cell counts of all strains at salinity levels of 25 and 30 dS m^{-1} in sand, whereas the numbers decreased after 45 days (data not shown). Local strain Aba1 nodulated at 10 dS m^{-1}, and local Ar2-1 and exotic PMA63/1 formed effective nodules up to 15 dS m^{-1}. The nodule numbers decreased towards 25 dS m^{-1} and none were observed above 30 dS m^{-1}. These three rhizobial strains were selected for later field experiments. *Rhizobium* strains, in general, may be more tolerant of salinity than *Bradyrhizobium* [10].

The most compatible rhizobia and *Frankia* strains were tested in the field on their respective host plants in saline soils.

3.2. Field evaluation of NFTs in saline conditions

Soil samples showed a pH range from 7.9 to 9.2, and Ec_e levels of 4.8 to 15 dS m^{-1} (Table I), indicating a wide range of salt concentrations, rendering it unsuitable for cultivation of most species. Although the salinity levels were generally higher in plots 7-12, plant growth was inferior on plots 1-6 due to them being more calcareous and less permeable to irrigation water.

3.2.1. First-year harvest

A year's growth had ameliorative effects on the electric conductivity of rhizosphere soil (Table V). Nodules were found on some of the *A. ampliceps* plants growing in plots at the lower levels of salinity, whereas there was no nodulation in plots with Ec_e levels of 10 to 13 dS m^{-1} (Table VI). The *C. glauca* plants showed a complete absence of nodules. Only nodule-like thickenings were observed on one plant.

However, the ^{15}N data indicate that fixation contributed significant N not only to *A. ampliceps* but also to *C. glauca.* (Tables VII and VIII), indicating that nodules had been present on the latter, but not observed. Although plot 10 had higher Ec_e values (Table V), plants growing there had the highest values for %Ndfa (Tables VII and VIII).

Sodium and K content analyses of *A. ampliceps* showed generally higher K contents in leaves and shoots than in roots (Tables IX and X). Some leaf samples showed very high accumulations of Na, up to 27-fold higher than in the corresponding shoot sample, and, in most cases, Na concentrations were higher in root than in the corresponding shoot. Similar trends were present in*E. camaldulensis.*

3.2.2. Second- and third-year harvests

After two years of growth there was further evidence of ameliorative effects on Ec_e of rhizosphere soil (Table XI). *A. ampliceps* had nodules on all plants with profuse nodulation on some with rhizosphere Ec_e levels of 7.4 and 9.2 dS m^{-1} and pH of 8.6 and 7.7, respectively (data not shown). Again *C. glauca* showed a complete absence of nodules, in spite of the fact that it had been inoculated again with *Frankia*, with crushed root nodules, as well as with *C. glauca* rhizosphere soil. The weights of plants in the plots initially of the highest salinity were comparable with those of plants in plots of lower salinity levels (data not shown).

TABLE III. SCREENING OF LOCALLY ISOLATED AND EXOTIC RHIZOBIAL STRAINS FOR NODULATION ON TWO *ACACIA* SPECIES

Strain	*A. ampliceps*	*A. nilotica*	Strain	*A. ampliceps*	*A. nilotica*
Local			Exotic		
Aba1	+[a]	–	TAL1521	+	+
Aba2	+	–	TAL1446	+	+
AS1	+	+	PMA63/1	+	+
AS2	+	+	PMA311/1	+	–
AS3	–	+	14631.1	+	+
Ar2-1	+	+	19361	+	–
			N.A2538	–	+
			Amm1	+	+
			TAL1436	+	+

[a]Nodules present.

TABLE IV. SCREENING OF RHIZOBIAL STRAINS FOR NODULE NUMBER PER PLANT AND NODULE FRESH WEIGHT ON *ACACIA* SPECIES

Strain	*A. aneura*		*A. saligna*		*A. ampliceps*		*A. sterophylla*	
	No.	Wt. (mg)	No.	Wt. (mg)	No.	Wt. (mg)	No.	Wt. (mg)
Local								
Aa2	10	20	0	0	0	0	20	20
Aba1	0	0	0	0	14	30	32	30
AS1	0	0	0	0	10	30	2	10
AS2	0	0	0	0	3	1	10	50
AS3	0	0	6	2	0	0	0	0
Ar2-1	ND[a]	ND	ND	ND	20	36	ND	ND
Exotic								
14631.1	0	0	0	0	8	20	14	20
19631	0	0	13	10	15	30	9	10
NA2538	0	0	24	40	0	0	20	130

[a]Not determined.

TABLE V. RHIZOSPHERE SOIL pH AND CHANGES IN ELECTRIC CONDUCTIVITY AFTER ONE YEAR OF *A. AMPLICEPS* AND *E. CAMALDULENSIS*

Plot no.	Soil sample	pH	EC_e (dS m^{-1})	Initial EC_e
10	A1[a]	8.7[b]	12	13
	A2	8.4	7.3	
	E1	N.D[c]	N.D	
	E2	8.5	5.2	
3	A1	8.9	2.0	5.9
	A2	8.9	2.4	
	E1	8.8	N.D	
	E2	N.D	N.D	
5	A1	8.9	2.4	5.7
	A2	8.8	3.0	
	E1	8.6	2.8	
	E2	8.7	5.4	
7	A1	9.1	8.0	10
	A2	9.0	7.4	
	E1	9.0	9.0	
	E2	N.D	N.D	

[a]*Acacia* plant 1 and 2; *Eucalyptus* plant 1 and 2. [b]Averages of five replicates. [c]Not determined.

TABLE VI. NODULATION DATA OF *A. AMPLICEPS* AT THE FIRST-YEAR HARVEST

Plot No.	Plant	Nodule no.	Fresh wt. (mg)	Dry wt. (mg)
10	A1[a]	5	828	221
"	A2	2	132	27
3	A1, A2	0	0	0
5	A1	5	93	8
"	A2	10	1,105	350
7	A1, A2	0	0	0

[a] *Acacia* plant 1, etc.

TABLE VII. *ACACIA AMPLICEPS* AND *E. CAMALDULENSIS* GROWTH, TOTAL N AND SOURCES OF N AT THE ONE-YEAR HARVEST

Species/ Plot no.	D. Wt. (g plant^{-1})	Total N (g plant^{-1})	%Ndff[a]		%Ndfa[b]		WAE[c]
			Leaf	Shoot	Leaf	Shoot	(%Ndfa)
Acacia							
10	2,802a[d]	34.0a	0.6a	1.0a	62a	33a	55a
3	531b	6.0b	0.8a	0.8a	12b	30ab	16b
5	412b	5.0b	0.7a	1.0a	8.0b	7.4b	7.8b
7	383b	5.0b	0.2b	0.5b	50b	29ab	46a
Eucalyptus							
10	584a	7.2a	1.6a	1.5 a			
3	457b	4.3b	0.9b	1.1ab			
5	210bc	2.1bc	0.8b	1.0ab			
7	29.0c	0.4c	0.3c	0.7b			

[a] %N derived from fertilizer. [b] %N derived from fixation. [c] Weighted average.
[d] Averages of two values; within species, if followed by the same letter, numbers are not significantly different ($P \leq 0.05$).

The ^{15}N data for *A. ampliceps* gave %Ndfa values of 11 to 83% for leaves, 4 to 82% for pods and 8 to 86% for shoots, on average, almost double the values obtained after one year's growth (data not shown). On the other hand, *E. camaldulensis* plant parts showed greater ^{15}N dilution than in *C. glauca*, possibly due to:

- Complete absence of root nodules on *C. glauca* (as discussed above).
- *E. camaldulensis* had two to three fold the biomass of *C. glauca*.
- Deeper roots of *E. camaldulensis* than of *C. glauca* exploiting non-enriched sources of N.

TABLE VIII. *CASUARINA GLAUCA* AND *E. CAMALDULENSIS* GROWTH, TOTAL N AND SOURCES OF N AT THE ONE-YEAR HARVEST

Species/ Plot no.	Dry Wt. (g plant^{-1})	Total N (g plant^{-1})	%Ndff[a]		%Ndfa[b]		WAE[c] (%Ndfa)
			Leaf	Shoot	Leaf	Shoot	
Casuarina							
10	55.0ab[d]	0.6ab	0.6a	0.5ab	24a	23a	24a
3	69.0a	0.9a	0.4b	0.6a	10a	6.0a	9.3a
5	40.0bc	0.5bc	0.5ab	0.5b	18a	23a	20a
7	16.0c	0.3c	0.3c	0.3c	6.1a	13a	7.3a
Eucalyptus							
10	132a	1.3a	0.8a	0.7a			
3	92.0b	0.8b	0.5bc	0.7a			
5	77.0b	0.7b	0.6ab	0.6a			
7	13.0c	0.2c	0.4c	0.3b			

[a]%N derived from fertilizer. [b]%N derived from fixation. [c]Weighted average.
[d]Averages of two values; within species, if followed by the same letter, numbers are not significantly different ($P \leq 0.05$).

TABLE IX. THE Na AND K CONTENTS OF PARTS OF *A. AMPLICEPS* AND *E. CAMALDULENSIS* PLANTS AT THE ONE-YEAR HARVEST

Plot	Plant	Leaf		Shoot		Root	
		K	Na	K	Na	K	Na
		(meq)					
10	A1[a]	335[b]	650	225	68	125	93
	A2	230	1,250	255	45	140	325
3	A1	290	375	230	70	120	53
	A2	255	300	240	62	143	253
5	A1	205	950	275	65	142	74
	A2	162	50	240	62	135	275
7	A1	120	950	335	58	135	55
	A2	170	100	270	65	112	80
10	E1	285	75	305	40	115	68
	E2	430	1,100	135	75	140	55
3	E1	142	750	135	43	162	74
	E2	205	75	230	75	110	70
5	E1	210	725	150	62	115	80
	E2	170	1,150	195	74	100	85
7	E1	255	525	152	30	120	62
	E2	90	50	260	45	120	325

[a]*Acacia* plant 1 and 2; *Eucalyptus* plant 1 and 2. [b]Average of three replicates.

It appears that *Eucalyptus camaldulensis* was a poor choice as the reference species for estimating N_2 fixation in *C. glauca* using the ^{15}N-dilution technique. The requirements for a suitable reference species have been discussed [14,34,35].

At the third-year harvest, heavy nodulation was observed on all of the *A. ampliceps* plants, but on none of the *C. glauca* (data not shown).

3.2.3. Vesicular-arbuscular mycorrhiza

Percent infection of roots by vesicular-arbuscular mycorrhiza, and numbers of spores, decreased with increasing salt concentration in the 12 plots (Table XII). The greatest mycorrhizal infection was observed in the roots of *A. ampliceps* with generally less in *C. glauca* and *E. camaldulensis* (Table XII, Fig. 4) indicating, some correlation with N_2 fixation. Mycorrhiza-rhizobia-infected plants have shown increased growth, N uptake and nitrogenase activity [11-13]. Most of the spores from these treatments were identified as *Glomus* spp., with a few *Sclerocystis* spp. The mycorrhizal spores isolated from saline soils were dark brown and thick walled as compared to those from non-saline soil (Fig. 5).

TABLE X. THE Na AND K CONTENTS OF DIFFERENT PARTS OF *C. GLAUCA* AND *E. CAMALDULENSIS* PLANTS AFTER THE FIRST YEAR

Plot	Plant	Leaf		Shoot		Root	
		K	Na	K	Na	K	Na
		(meq)					
10	C1[a]	180[b]	330	90	155	100	260
	C2	150	325	90	125	85	200
3	C1	160	320	100	110	70	105
	C2	210	330	105	75	90	125
5	C1	140	90	120	80	80	120
	C2	150	320	85	90	80	140
7	C1	220	350	100	90	70	135
	C2	150	290	85	105	70	105
10	E1	255	80	130	60	100	95
	E2	320	30	140	30	110	70
3	E1	220	120	120	45	95	60
	E2	220	50	110	125	110	95
5	E1	220	70	130	100	110	80
	E2	180	60	130	40	105	75
7	E1	230	40	105	40	100	90
	E2	210	50	90	70	90	80

[a] *Casuarina* plant 1 and 2; *Eucalyptus* plant 1 and 2.

[b] Average of three replicates.

TABLE XI. RHIZOSPHERE-SOIL pH AND CHANGES IN ELECTRIC CONDUCTIVITY AFTER TWO YEARS OF *A. AMPLICEPS* AND *E. CAMALDULENSIS*

Plot no.	Soil sample	pH	Ece (dS m^{-1})	Initial Ece
2	A1[a]	8.6[b]	8.4	6.0
	A2	8.6	7.4	
	E1	9.0	1.8	
	E2	8.7	2.1	
6	A1	8.6	2.2	4.8
	A2	8.3	1.8	
	E1	8.0	6.5	
	E2	8.2	1.8	
9	A1	7.8	6.2	11
	A2	8.2	4.4	
	E1	8.3	7.6	
	E2	8.5	10.0	
12	A1	7.8	10.8	13
	A2	7.7	9.2	
	E1	7.8	11.8	
	E2	7.8	9.8	

[a]From *Acacia* plant 1 and 2 and from *Eucalyptus* plant 1 and 2. [b]Averages of five replicates.

TABLE XII. INFECTION OF ROOTS BY VESICULAR-ARBUSCULAR MYCORRHIZA AND RHIZOSPHERE SPORE POPULATIONS AFTER 3 YEARS

Soil type/ Plot no.	*A. ampliceps*		*C. glauca*		*E. camaldulensis*	
	Infection (%)	Spore no. (per 100 g soil)	Infection (%)	Spore no. (per 100 g soil)	Infection (%)	Spore no. (per 100 g soil)
Saline[a]						
1	95	541	100	321	100	349
2	100	542	95	333	95	320
3	100	560	90	340	90	340
4	95	496	95	315	90	345
5	90	398	75	307	80	202
6	90	392	65	308	80	185
7	85	410	70	295	75	160
8	85	388	65	285	75	165
9	75	395	60	270	60	180
10	80	406	60	280	65	120
11	65	225	45	240	65	64
12	65	220	30	219	55	60
Non-saline	100	1,210	100	645	100	533

[a]Ec_e levels are in Table I.

In most plots there were marked reductions in the salinity levels after 3 years as indicated by decreases in electrical conductivities after final harvest (Table I) confirming other data [2] that planting of N_2-fixing trees and their symbiotic interactions with rhizobia, *Frankia* and vesicular-arbuscular mycorrhiza may strongly contribute to the restoration of fertility of degraded soils, even those heavily affected by salinity.

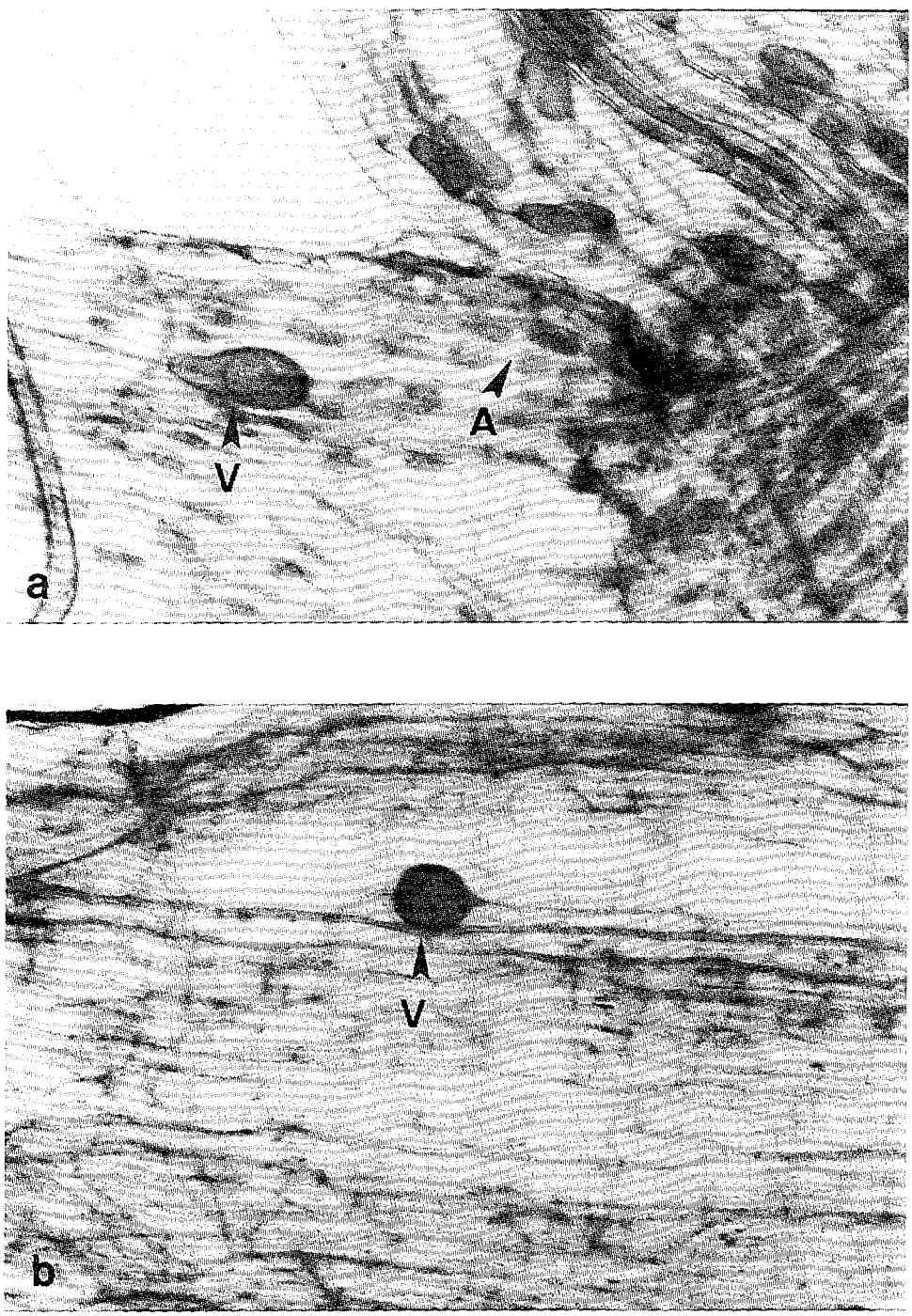


FIG 4. Vesicular-arbuscular mycorrhizal infection showing arbuscules (A) and vesicles (V) in the roots of (a) A. ampliceps *and (b)* G. glauca. ×200.

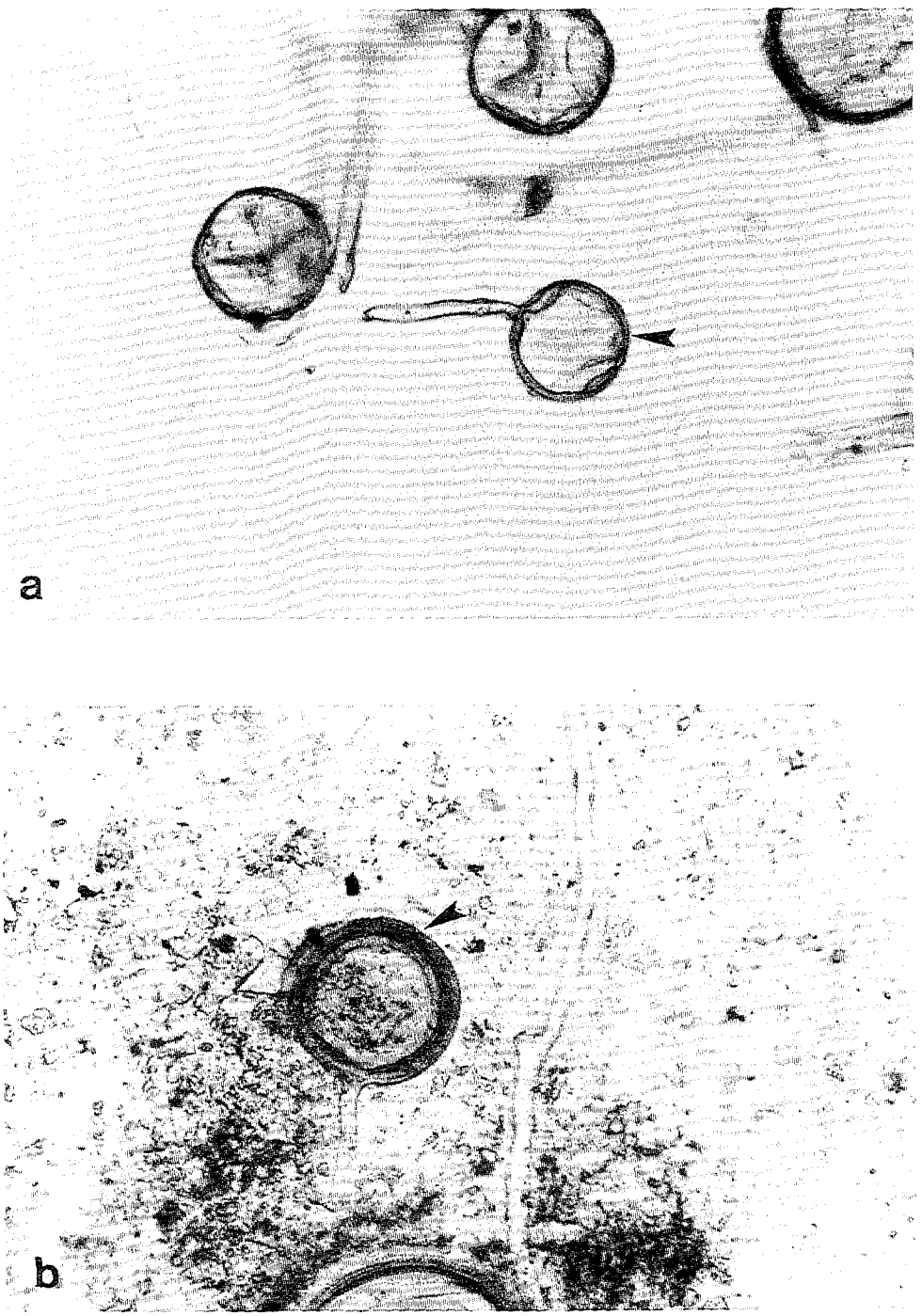


FIG. 5 Photomicrographs showing spores of Glomus *sp. (a) from non-saline soil, and (b) a dark brown, thick-walled spore from saline soil.*×200.

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NITROGEN FIXATION IN *ACACIA AURICULIFORMIS* AND *ALBIZIA LEBBECK* AND THEIR CONTRIBUTIONS TO CROP-PRODUCTIVITY IMPROVEMENT

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Abstract

Pot and field experiments assessed N_2 fixation by *Albizia lebbeck* and *Acacia auriculiformis* and contributions from prunings to yields of corn and hibiscus. Nitrogen fixation in these tree legumes was poor, with less than 50% N derived from fixation (%Ndfa) when grown in pots, but higher (>70%) in field conditions, after inoculation with compatible *Bradyrhizobium* strains. Prunings from *A. lebbeck,* as green manure improved growth of maize and hibiscus, inducing greater corn-kernel yields than did urea. *Acacia auriculiformis* prunings were similarly beneficial when mixed with leaves of *A. lebbeck* or *L. leucocephala.* Application of slow- and fast-nutrient-releasing leaves is required to maximize their contributions to crop productivity.

1. INTRODUCTION

The potentially beneficial effects of N_2-fixing tree legumes (NFTs) on the fertility of the fragile and often impoverished soils of the tropics have recently deserved attention from soil scientists [1-6].

Tropical forests are subject to increasing human pressures resulting from rapid population growth, scarcity of fertile agricultural land, energy demands and other needs. Over-exploited soils are losing fertility and crop productivity is declining.

As in many other countries of the tropics, the Democratic Republic of the Congo (ex-Zaïre) has, since 1975, adopted tree replantation strategies throughout the country to combat the serious environmental consequences of deforestation. In this programme, NFTs, *Acacia auriculiformis, A. mangium, Albizia lebbeck, A. chinensis, Leucaena leucocephala* etc., are being planted in locations that have been dramatically affected by erosion and soil-fertility loss. Kinzono, one of the sites selected in this challenging enterprise, is a rural locality on the Bateke Plateau, 150 km north of Kinshasa, at which *A. auriculiformis* and other NFTs have been planted on some 10,000 ha.

The overall objectives of this programme are to provide urban areas, such as Kinshasa, with fuel wood, and for soil conservation and restoration of fertility in rural areas. Scientists at the the Congolese High Commission for Atomic Energy (CGEA) participate in the programme, undertaking research on rhizobia/tree-legume symbioses and assessing contributions of NFTs to soil-fertility restoration and soil conservation.

This paper summarizes research on N_2 fixation in *Albizia lebbeck* and *Acacia auriculiformis,* and their impact on the fertility of Kinshasa's sandy soil.

2. MATERIAL AND METHODS

Pot and field trials were carried out at the Regional Centre for Nuclear Studies of Kinshasa (CREN-K) at Mont-Amba (4°30'S, 15°18'E, altitude 430 m). Climatic data recorded during the experiment are shown in Table I. The soil is an arenoferral orthotype with the physico-chemical characteristics described in Table II.

TABLE I. CLIMATIC DATA FOR THE EXPERIMENTAL SITE

Component	Value
Precipitation	1,600 mm yr^{-1}
Mean temperature	26°C
Relative humidity	82%
Daily evaporation	1.8 mm

TABLE II. SOME SOIL PHYSICO-CHEMICAL CHARACTERISTICS (0-30 cm)

Component	Value
pH_{water}	5.5
pH_{KCl}	4.2
Clay (%)	3.9
Silt (%)	2.9
Sand (50 to 100 m) (%)	16
Sand (100 to 250 m) (%)	40
Sand (250 to 500 m) (%)	35
Sand (500 to 1000 m) (%)	1.2
Organic C (%)	1.4
Total N (%)	0.08
C:N	17
Bray-II P (ppm)	4.1
Exchangeable cations (mmole kg^{-1})	
Ca	12
Mg	3.2

2.1. Experiment 1. N_2 fixation in *Acacia auriculiformis* and *Albizia lebbeck*

2.1.1. Pot experiment

Sieved (2 mm) soil was autoclaved (120°C/1 h) and placed in black polyethylene bags, 2 kg soil per bag. Three seeds of *A. auriculiformis* and *A. lebbeck* were sown per bag. Seedlings were thinned to one at 2 weeks after sowing and inoculated with approximately 1.8×10^8 cells of the following local *Bradyrhizobium* strains, from the CREN-K collection: Aa5, Aa6, Aa19, Aa25, Aa29, Aa30 and Aa41. These rhizobia were previously isolated from nodules of *A. auriculiformis* growing at Kinzono.

Ammonium sulphate was applied at 20 mg N kg^{-1} soil, enriched at 9.73% ^{15}N atom excess to the NFTs. The non-fixing reference species, *Eucalyptus camaldulensis, Oryza sativa* and *Leucaena leucocephala*, which is not nodulated by the strains listed above [7], received 80 mg N kg^{-1} soil enriched at 2% ^{15}N atom excess. A basal application of 30 mg P kg^{-1} soil was made to all bags 5 days after sowing. A completely randomized design was used with four replications.

Plants were harvested 3 months after sowing. Roots were carefully washed with tap water to remove soil, and nodules were detached and counted. Plants were then oven-dried (75°C/3 days) and ground. Percent N was assayed using the Kjeldahl method and $^{15}N/^{14}N$ ratio determined using an emission spectrometer (NOI6-PC). Nitrogen fixation, N derived from fertilizer and from soil were quantified using formulae described by Zapata [8] and by Hardarson and Danso [9].

2.1.2. Field experiment

Seedlings of *A. auriculiformis* and *A. lebbeck* were grown in plastic bags and inoculated with Aa5 and Aa29 *Bradyrhizobium* strains (1.5×10^8 cells per bag) in nursery conditions. Three months later, they were transplanted in the field at 2×1 m on four plots, 6×6 m each and spaced at 2 m. There were two treatments plus and uninoculated control for both NFTs: (1) inoculated with Aa5, and (2) inoculated with Aa29. The experiment had a randomized complete-block design with four replicates.

One and 9 months after transplanting, the central 12 m^2 of each plot were labelled with 20 kg N ha^{-1} as ammonium sulfate enriched at 10.2% ^{15}N atom excess. *Senna siamea* was the non-fixing reference species and was planted on the same plots as the NFTs.

Plant height and basal stem diameter were measured monthly. At the harvest time (11 months after planting), leaves were collected, sub-sampled, oven-dried (65°C/4 days), milled, then analyzed for %N and $^{15}N/^{14}N$ ratio as before.

2.2. Experiment 2. Contribution of leaves to crop growth and yield

2.2.1. Pot experiment

Fifteen kg of sieved (2 mm) soil were transferred to black polyethylene bags (40×40 cm). Leaves of *A. lebbeck* (3% N), *L. leucocephala* (2.8% N) and *A. auriculiformis* (2% N) were incorporated into the soil in each bag, either homogeneously or in combinations (1:1), equivalent to 100 kg N ha^{-1}.

The indirect ^{15}N-labelling technique, see below, was used to determine the availability of leaf N; ammonium sulfate, enriched at 5.4% ^{15}N atom excess, was applied at 20 mg N kg^{-1} soil. To alleviate soil-P deficiency, a basal 45 mg P kg^{-1} soil, was applied as triple superphosphate to all bags 37 days before leaf amendment.

The test-crops were *Zea mays* var. Kasaï (from SENASEM/Congo-Kinshasa) and *Hibiscus sabdariffa* var. sabdariffa (from a local market), sown in the bags at 2 and 15 days after leaf application, respectively.

The following treatments, with a control, were applied as a randomized complete-block design of four replicates: leaves of (1) *A. lebbeck* (Al), (2) *L. leucocephala* (Ll), (3) *A. auriculiformis* (Aa), (4) Al+Aa, (5) Al+Ll, (6) Aa+Ll.

Two corn or hibiscus plants were maintained per pot after thinning, and were grown outside with tap water applied when rain was insufficient.

Shoots were sampled at 35, 60 and 90 days after sowing (DAS), oven-dried (65°C/4 days) and analyzed for dry matter, %N as done previously. The $^{15}N/^{14}N$ ratios were determined only for the 90-day harvest.

Nitrogen derived from leaves was calculated using the following formula.

$$\text{N derived from leaves} = \left(1 - \frac{\%^{15}\text{N a.e. in treated plants}}{\%^{15}\text{N a.e. in control plants}}\right) \times 100$$

2.2.2. Field experiment

The crop used in this experiment was *Zea mays* var. Kasaï. Plant spacing was 75×25 cm and the field was weeded twice with a hoe.

Green leaves of *A. lebbeck* (4.5% N), *L. leucocephala* (3.2% N) and *A. auriculiformis* (2.1% N), grown at CREN-K, were incorporated into the soil (0-20 cm deep) between the rows 3 days after emergence of the corn at a rate equivalent to 120 kg N ha^{-1}.

The treatments were as described for the pot experiment above, with the addition of three rates of urea: 40, 80 and 160 kg N ha^{-1}. A randomized complete-block design was used with three replicates. Distance between plots and blocks were, respectively, 1 m and 1.50 m. Each plot measured 2.5×2.5 m.

The soil was labelled with 10.1% ^{15}N atom excess with 20 kg N ha^{-1} urea on the central 1 m^2 of each plot, the rest of which received unenriched urea at the same rate of application.

Corn samples harvested 12 weeks after sowing (WAS) were oven-dried (65°C/4 days). Dry matter, N and grain yields were extrapolated to kg ha^{-1} [9]. Nitrogen analysis and determinations of N derived from soil, fertilizer and leaf were as described above.

Analyses of variance (ANOVA) were computed using MSTATC software (Michigan State University, 1989-1991) and the least significant difference test was used to compare treatment means.

3. RESULTS

3.1. Experiment 1

3.1.1. Pot experiment

3.1.1.1 Nodulation and total N

The rhizobial strains induced nodules on *A. auriculiformis* and *A. lebbeck* roots. Means of 16 and 20 nodules $plant^{-1}$ were obtained for *A. auriculiformis* and *A. lebbeck*, respectively (Table III), with average accumulations of 19 mg N pot^{-1} in the former and 32 in the latter.

3.1.1.2. Nitrogen fixation

Results indicated little N_2 fixation in *A. lebbeck* and *A. auriculiformis*, with %Ndfa estimates of between 29 and 49% in the former and between 25 and 45% in the latter, after 3 months of growth (Fig. 1). The %Ndfa values obtained with *O. sativa* as the reference crop were at least 50% higher than those obtained with *L. leucocephala* or *E. camaldulensis*.

TABLE III. *ALBIZIA LEBBECK* AND *ACACIA AURICULIFORMIS* NODULATION, TOTAL N

	Nodule number		Total N	
	A. auriculiformis	*A. lebbeck*	*A. auriculiformis*	*A. lebbeck*
	(per plant)		(mg/pot)	
Not inoculated	0.0	0.0	10	19
Aa5	6.5	19.8	17	24
Aa6	5.5	10.5	23	35
Aa19	19.3	29.0	23	40
Aa25	17.8	11.5	15	26
Aa29	21.0	34.8	17	41
Aa30	2.5	6.3	18	29
Aa41	9.5	28.5	22	31
Inoc'd Mean	16.1	20.1	19.3	32.3

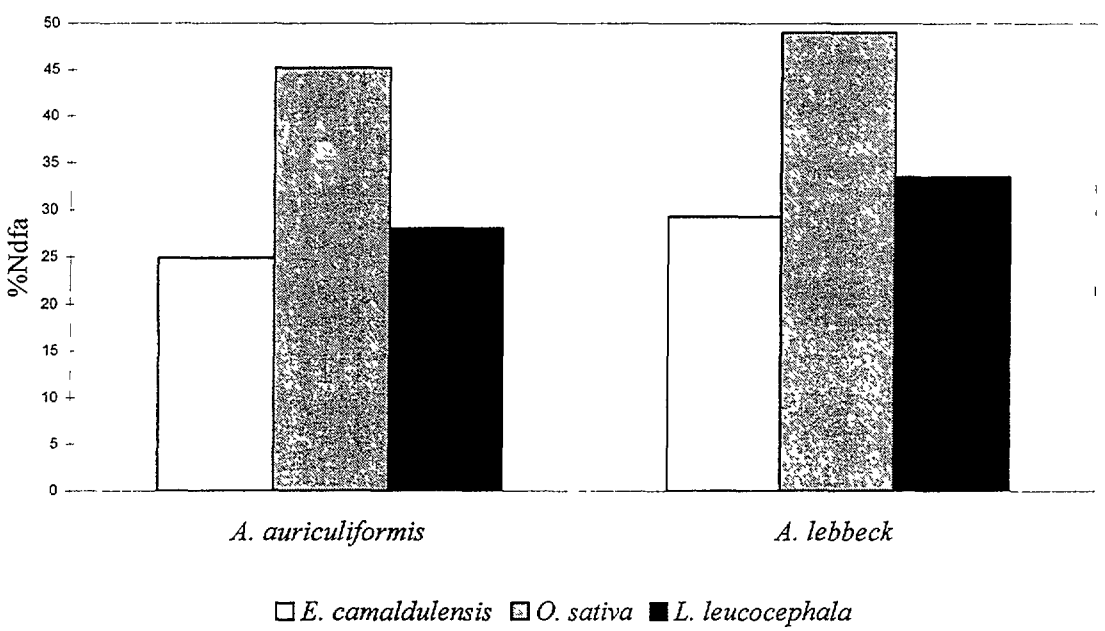


FIG. 1. Percent N derived from fixation in Albizia lebbeck *and* Acacia auriculiformis *as estimated using* Eucalyptus camaldulensis, Oryza sativa *and un-nodulated* Leucaena leucocephala *as reference crop (Expt. 1, pot).*

3.1.2. Field experiment

3.1.2.1. Growth and total N

Acacia auriculiformis grew faster than did *A. lebbeck* (Fig. 2); the height of the former increased from 39 to 125 cm, whereas the latter recorded 18 to 62 cm in the same period. The same trend was observed on the basal stem diameter data: values ranged from 5 to 20 mm for *A. auriculiformis* and from 4 to 10 mm for *A. lebbeck*. Later, *A. auriculiformis* and *Senna siamea* shaded *A. lebbeck* in the field, negatively affected its growth and its leaves accumulated N to concentrations in excess of 3% (data no shown). The highest N concentration in *A. auriculiformis*, 2.8%, was recorded in plants inoculated with Aa29 (data not shown).

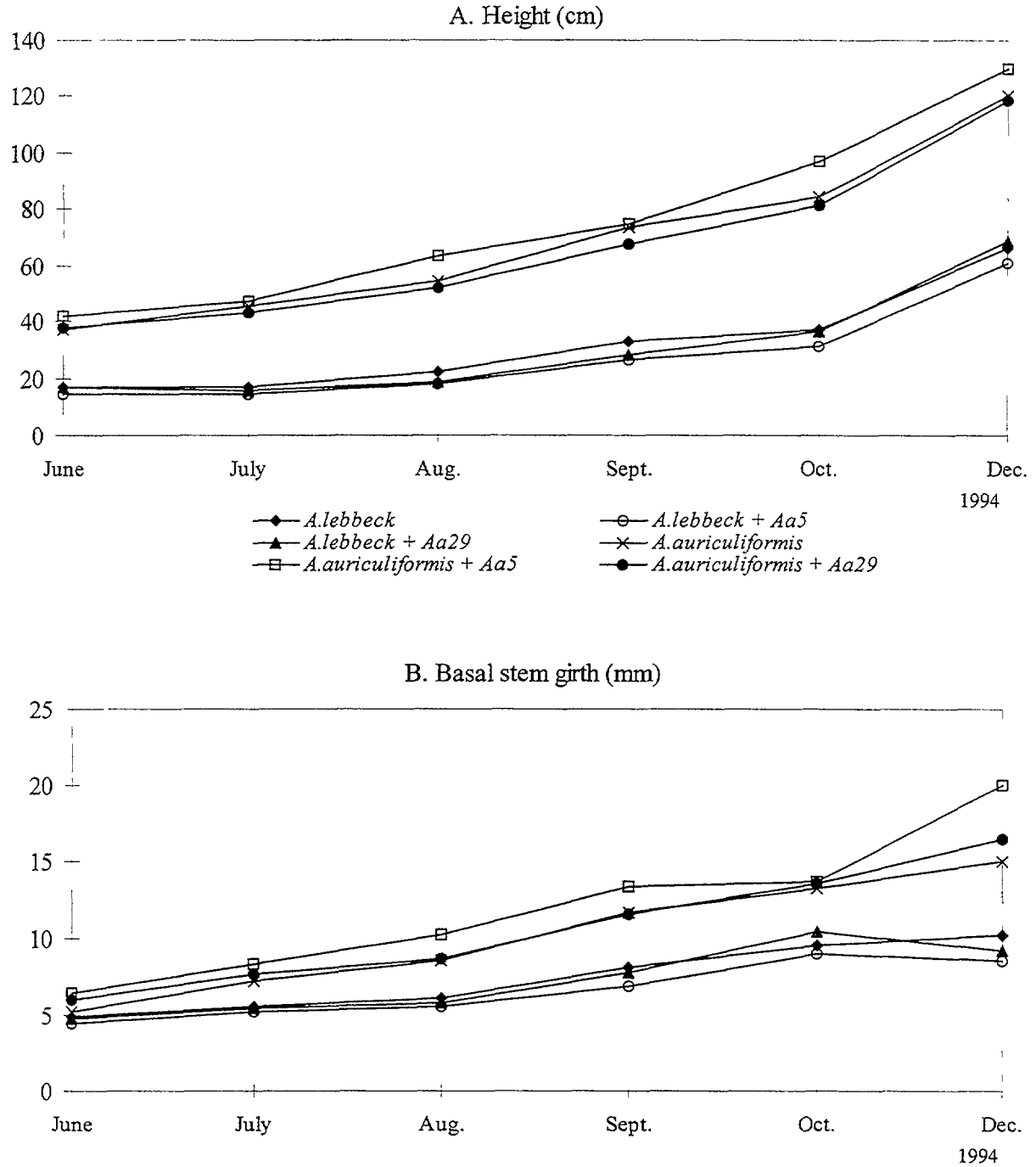


FIG. 2. Growth of Albizia lebbeck *and* Acacia auriculiformis *in field conditions (Expt. 1, field).*

3.1.2.2. N_2 fixation

The largest portion of N in *A. auriculiformis* and *A. lebbeck*, in excess of 70%, was from fixation in plants inoculated with Aa29 (Fig. 3).

Uninoculated *A. lebbeck* and *A. auriculiformis* inoculated with Aa5 accumulated more N from soil (approximately 70 and 60%, respectively) than from fixation or fertilizer. Contribution of fertilizer to N uptake by the tree legumes was low and did not reach 10%.

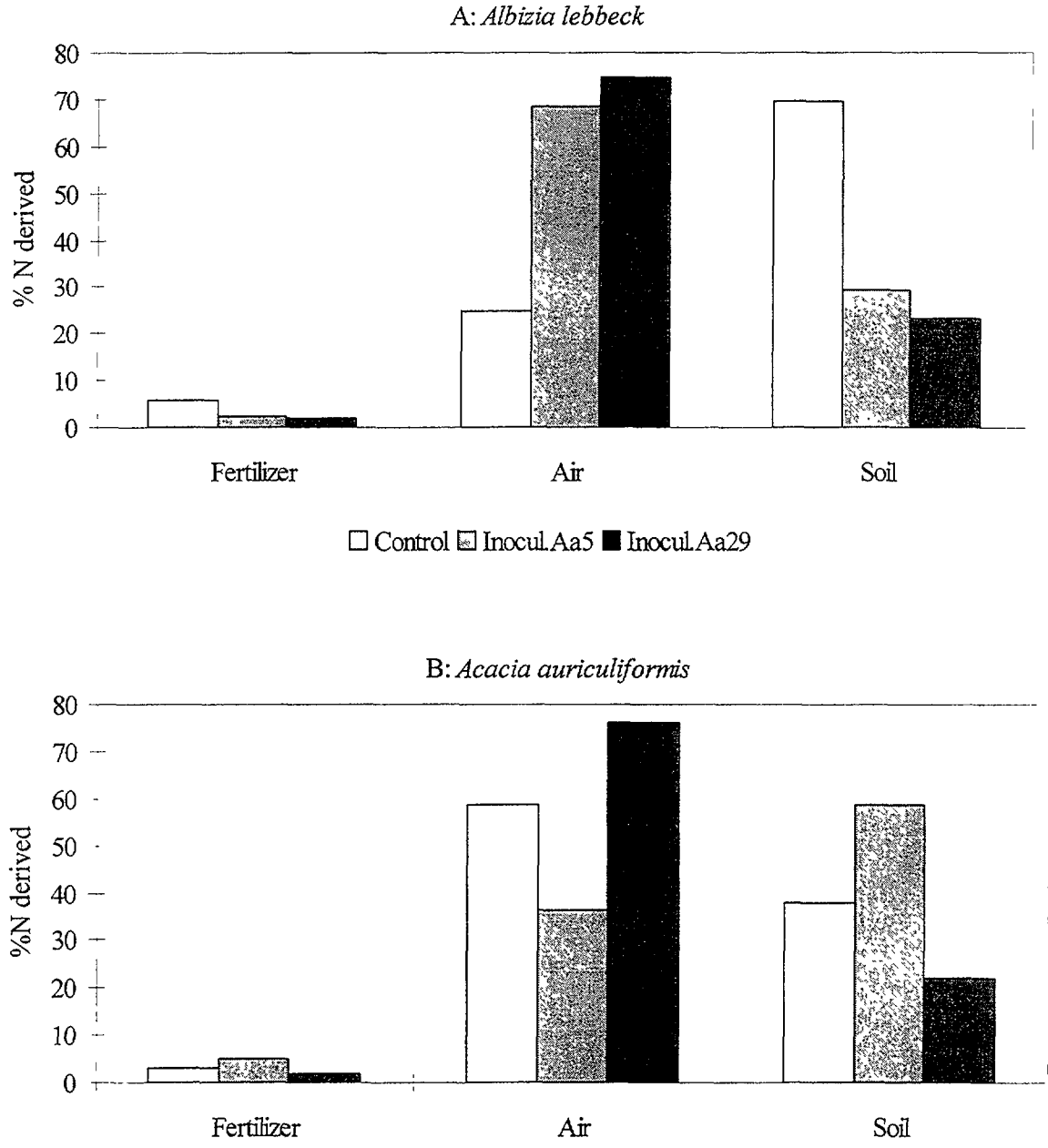


FIG. 3. Sources of N in leaves of Acacia auriculiformis *and* Albizia lebbeck *as inoculated with native* Bradyrhizobium *strains in Kinshasa's sandy soil (Expt. 1, field).*

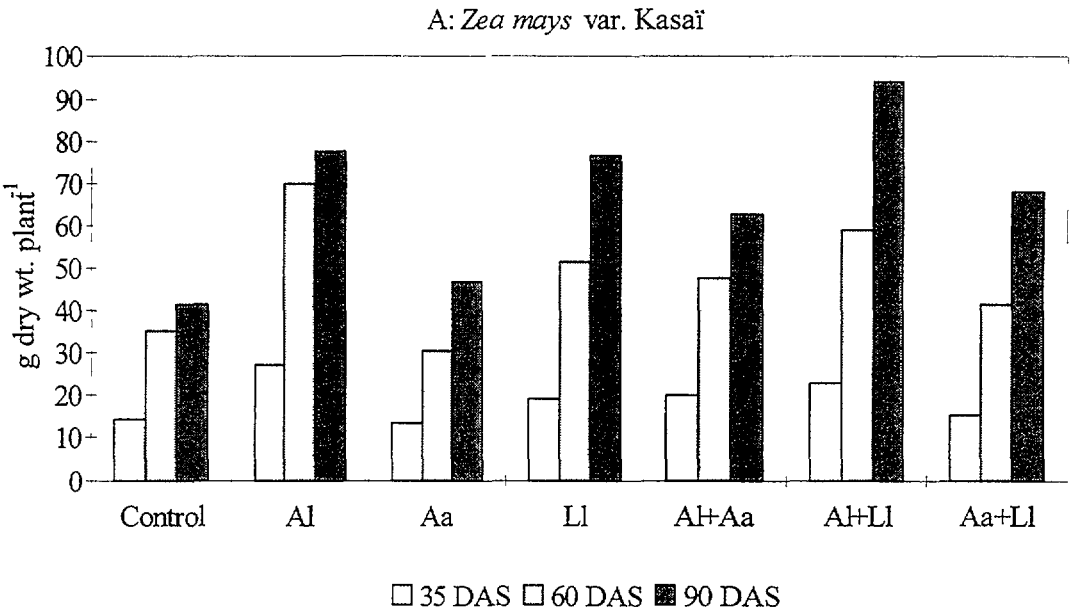
3.2. Experiment 2

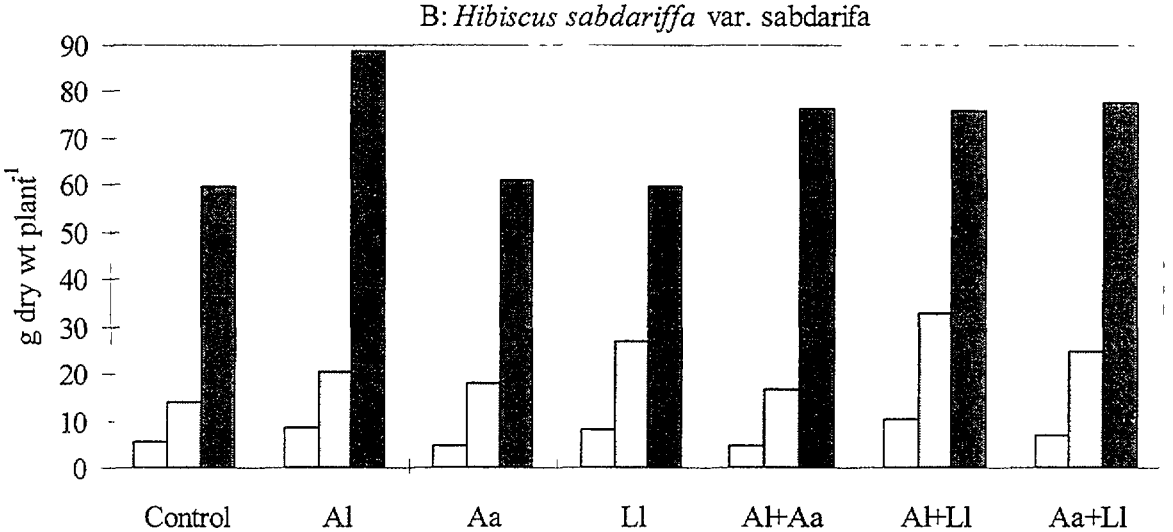
3.2.1. Pot experiment

3.2.1.1. Dry matter

At 35 and at 60 DAS, *Zea mays* produced most biomass, 27 and 69 g plant^{-1}, respectively, when treated with *A. lebbeck* leaves and at 90 DAS when treated with the Al+Ll mixture(94 g plant^{-1})

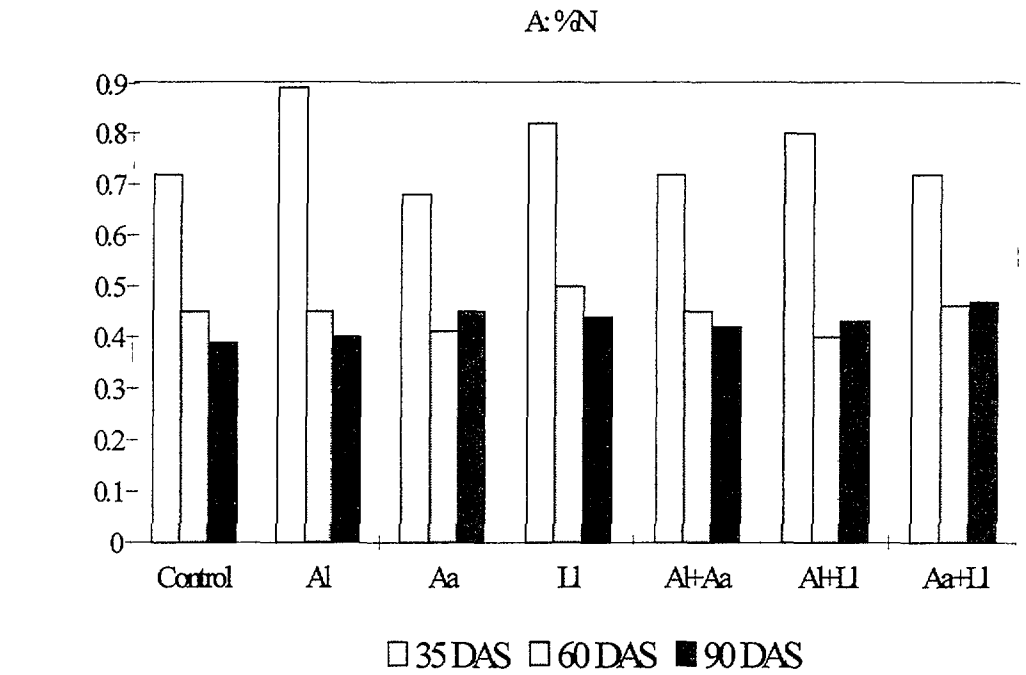
(Fig. 4A). In *H. sabdariffa*, the highest biomass at 35 DAS was recorded with *A. lebbeck* leaves (8.5 g plant^{-1}), *L. leucocephala* leaves (8.1 g plant^{-1}) and the combination Al+Ll (10.4 g plant^{-1}) (Fig. 4B); these values were not significantly different.

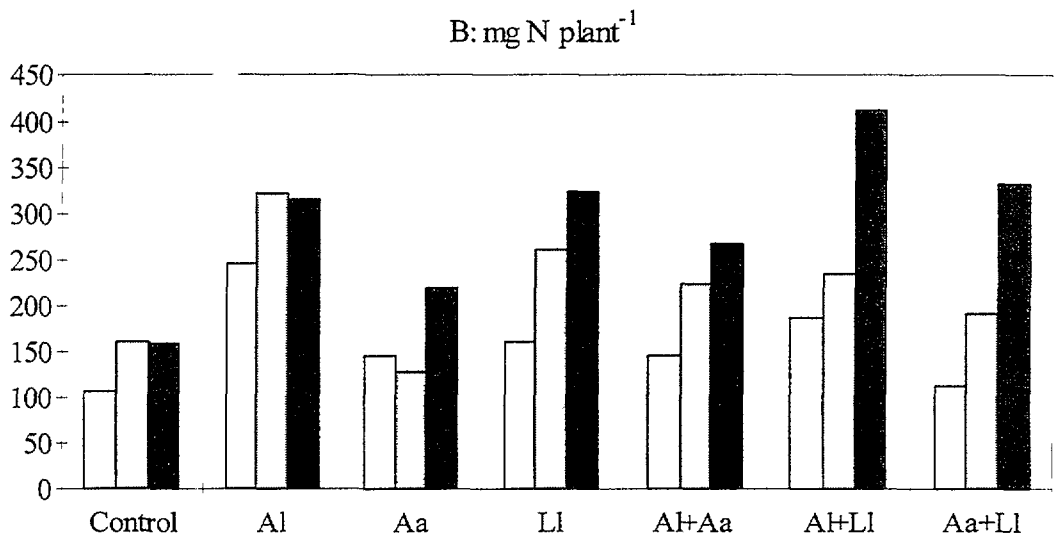




		35DAS	60DAS	90DAS
$LSD_{0.05}$	A	5.5	9.8	14
	B	2.9	12	49

FIG. 4. Dry weight of Zea mays *var. Kasaï and* Hibiscus sabdariffa *var. sabdariffa as affected by tree-legume leaves (Expt. 2, pot).*





		35DAS	60DAS	90DAS
$LSD_{0.05}$	A	0.12	NS	0.07
	B	94	83	95

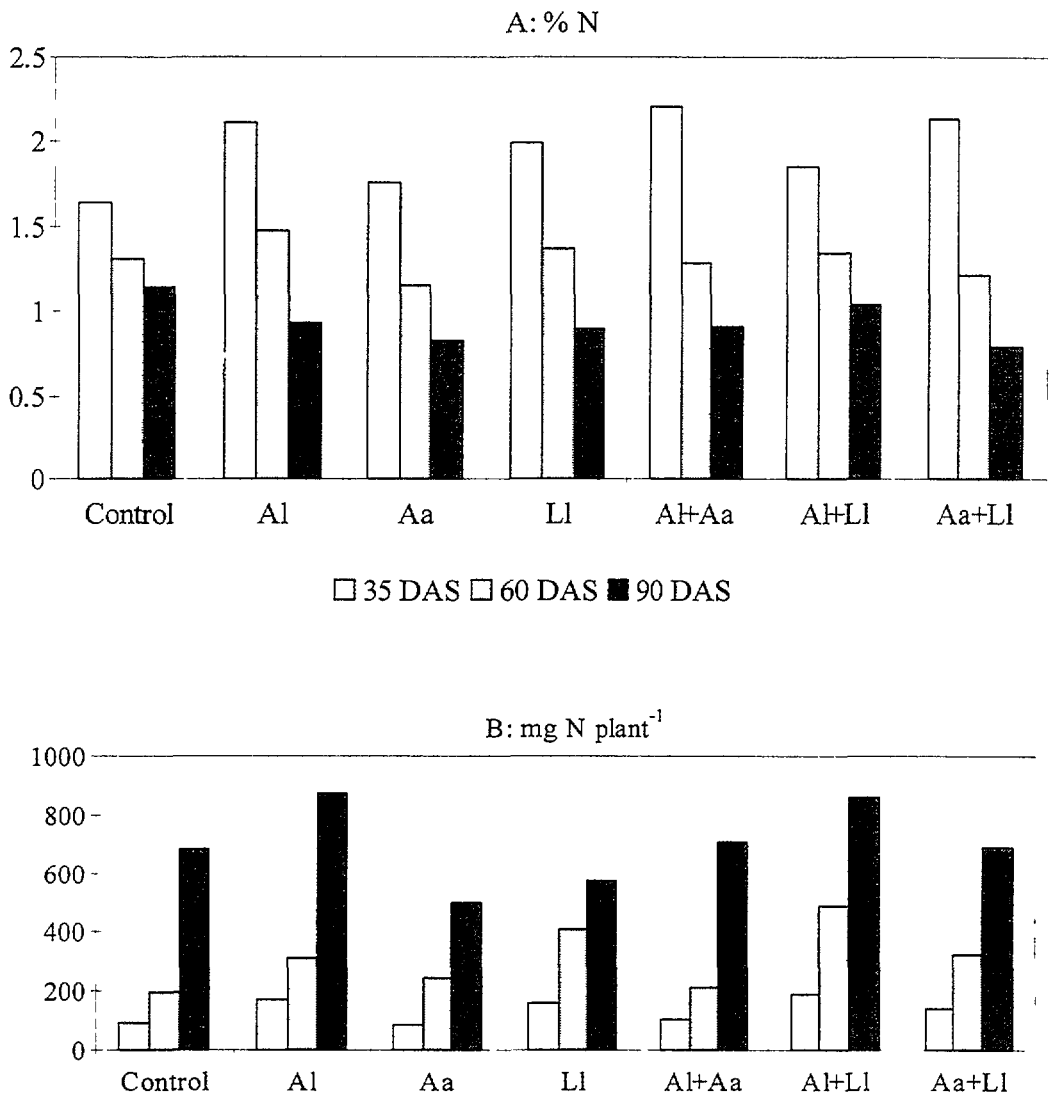
FIG. 5 N uptake by Zea mays *var. Kasai as affected by tree-legume leaves, at 35, 60 and 90 days after sowing (Expt. 2, pot).*

3.2.1.2. Total N

The highest corn-N concentration (0.89%) at 35 days was obtained in plants amended with *A. lebbeck* (Fig. 5A). At 60 and 90 DAS, corn %N was not affected by any treatment., The N accumulation by *Z. mays* treated with *A. lebbeck* leaves was significantly higher than with other

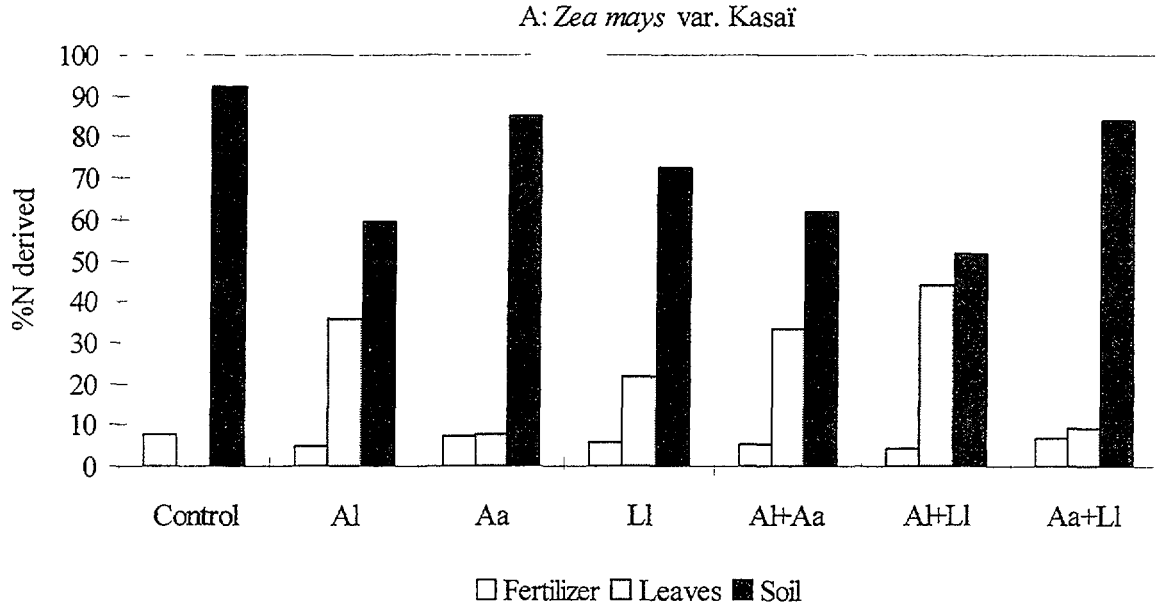
treatments at 35 and 60 DAS (244 and 320 mg N plant^{-1}, respectively) (Fig. 5B). However, at 90DAS the mixture of *L. leucocephala* and *A. lebbeck* leaves resulted in significantly more N accumulated than with *A. lebbeck* alone.

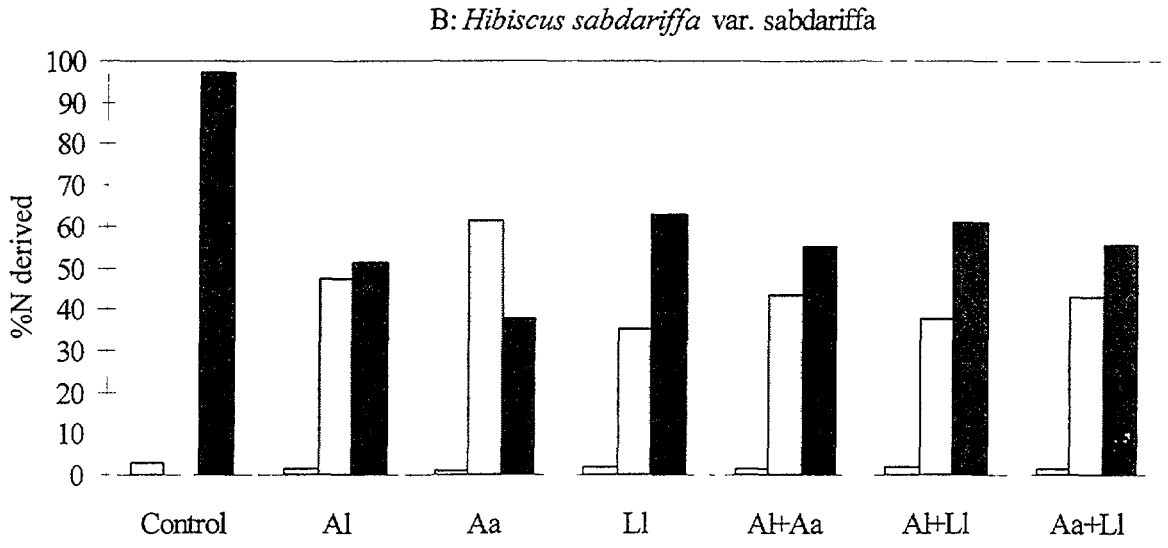
In *H sabdariffa*, organic fertilization with *A. lebbeck* leaves alone and the mixtures Al+Aa and Aa+Ll significantly increased N concentration to 2.1 to 2.2% from the control level of 1.6% at 35 DAS (Fig. 5B). Broadly, *H. sabdcriffa* tissue N was more abundant in plants receiving the combination of *A. lebbeck* and *L. leucocephala* leaves, inducing accumulations of 188, 487 and 862 mg N plant^{-1}, respectively, at 35, 60 and 90 DAS (Fig. 6B). *A. auriculiformis* leaves did not affect the N nutrition of *Z. mays* or of *H. sabdariffa* at any harvest.



		35DAS	60DAS	90DAS
$LSD_{0.05}$	A	0 44	0 29	0 25
	B	45	248	NS

Fig 6. Nitrogen uptake by Hibiscus sabda. fa *var sabdariffa as affected by tree-legume leaves, at 35, 60 and 90 days after sowing (Expt. 2, pot).*





		Fert	Leaves	Soil
$LSD_{0.05}$	A	1.1	9.2	7.1
	B	NS	NS	14

Fig 7 Assessment of %N derived from fertilizer, tree-legume leaves and soil in Zea mays *var. Kasai and* Hibiscus sabdariffa *var. sabdariffa at 90 days (Expt. 2, pot).*

3.2.1.3. N derived from leaves

Albizia lebbeck leaves alone and in Al+Aa and Al+Ll mixtures furnished more N to *Z. mays* tissue than did the other treatments, contributing 36, 33 and 44% of the corn N, respectively (Fig. 7A). On the other hand, there were no significant differences in the leaf-N treatment effects on *H sabdariffa* (Fig. 7B). The soil provided most of the N, i.e. 51 to 84% in *Z. mays* and 51 to 63% in *H. sabdariffa*; the fertilizer contributed a maximum of only 7%.

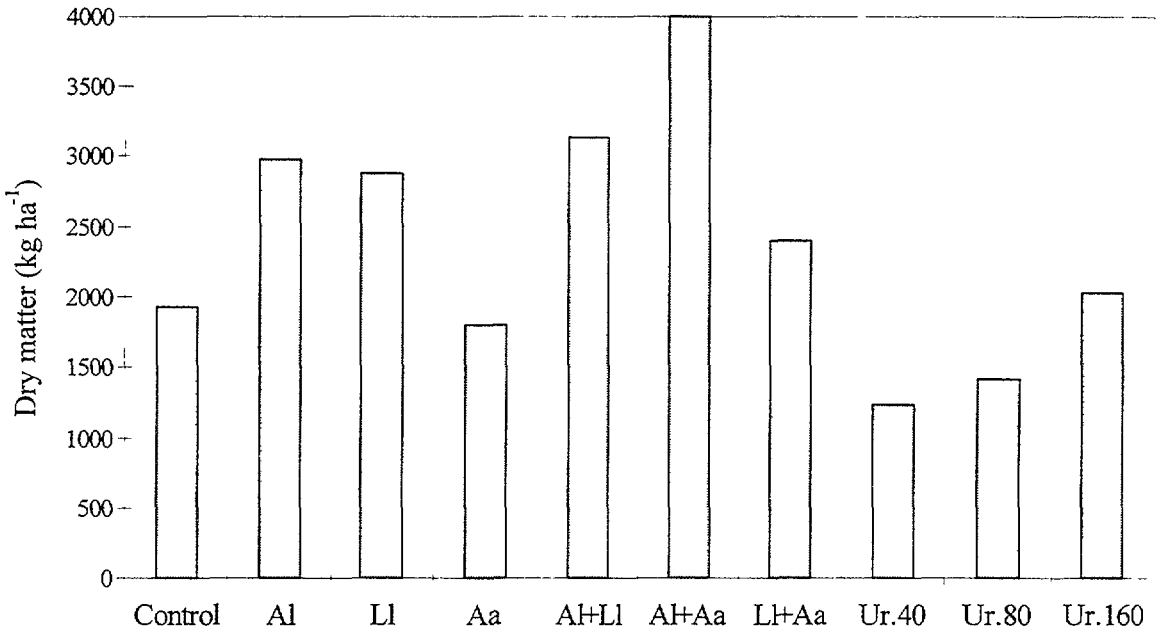


FIG. 8. Corn dry-matter production as affected by tree-legume leaves and urea (Expt. 2, field). $LSD_{0\,05} = 1205$.

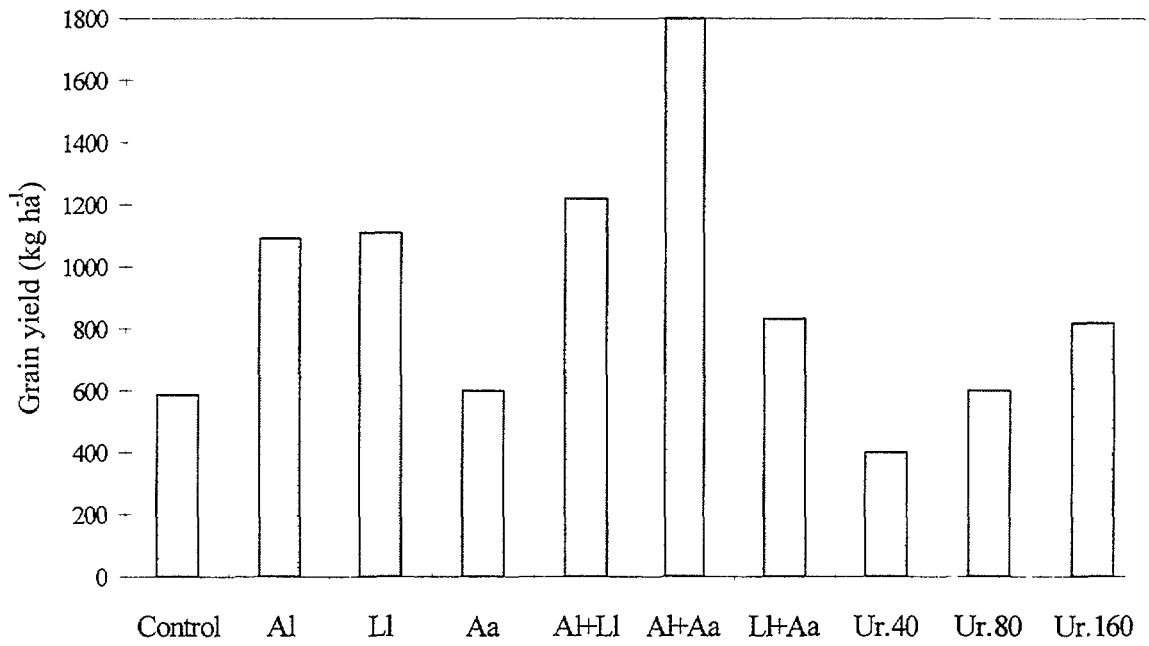


FIG. 9. Corn grain yield as affected by tree-legume leaves and urea (Expt. 2, field). $LSD_{0\,05} = 608$.

3 2.2. Field experiment

3.2.2.1. Dry-matter yields

In contrast with data obtained in the pot experiment in which higher corn biomass yields were obtained with *A. lebbeck* or *L. leucocephala* leaves, in the field the highest corn yield was obtained with the mixture Al+Aa (3,987 kg ha^{-1}) (Fig. 8). None of the three doses of urea (40, 80 and 160 kg N ha^{-1}) positively affected biomass accumulation.

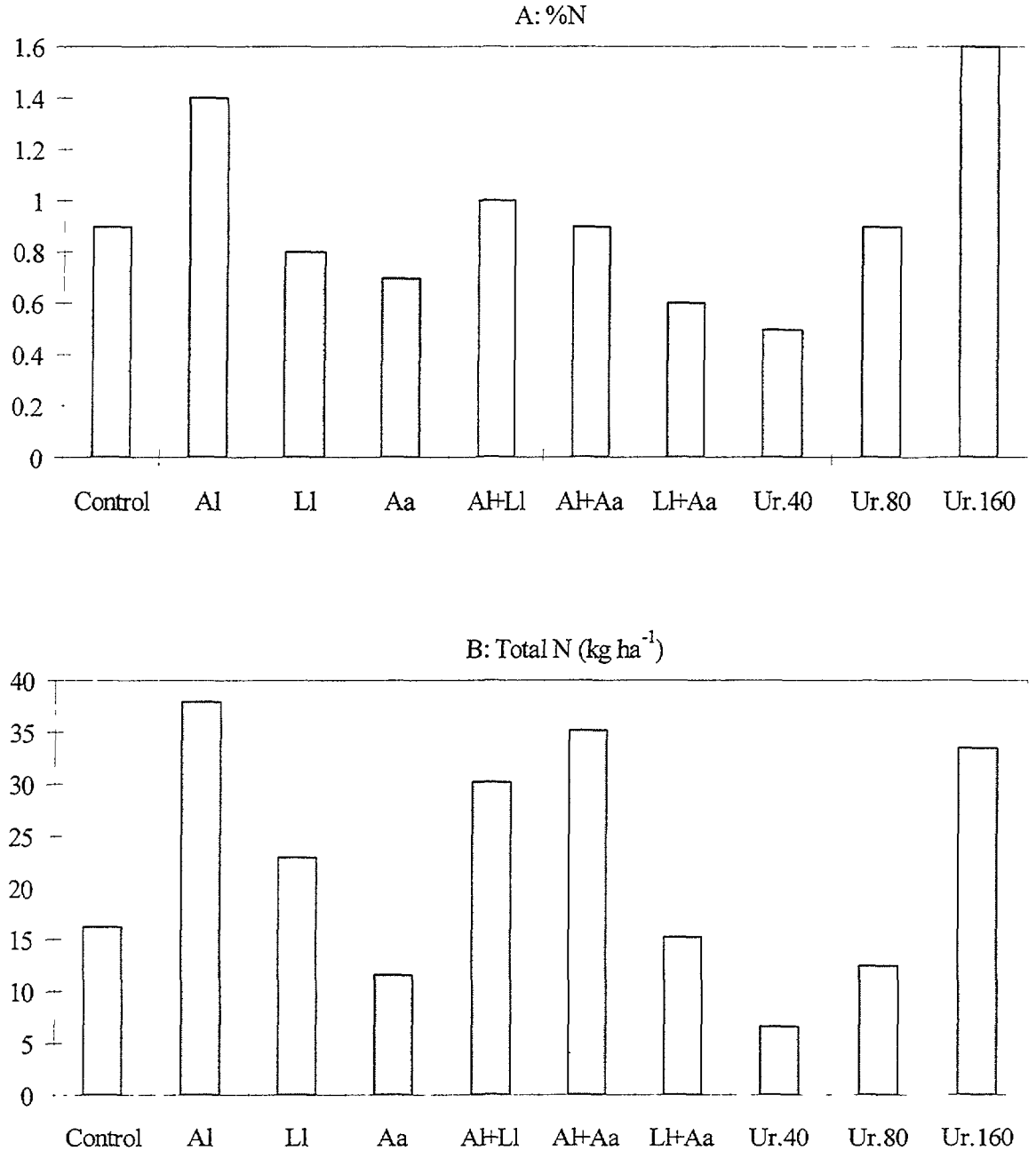
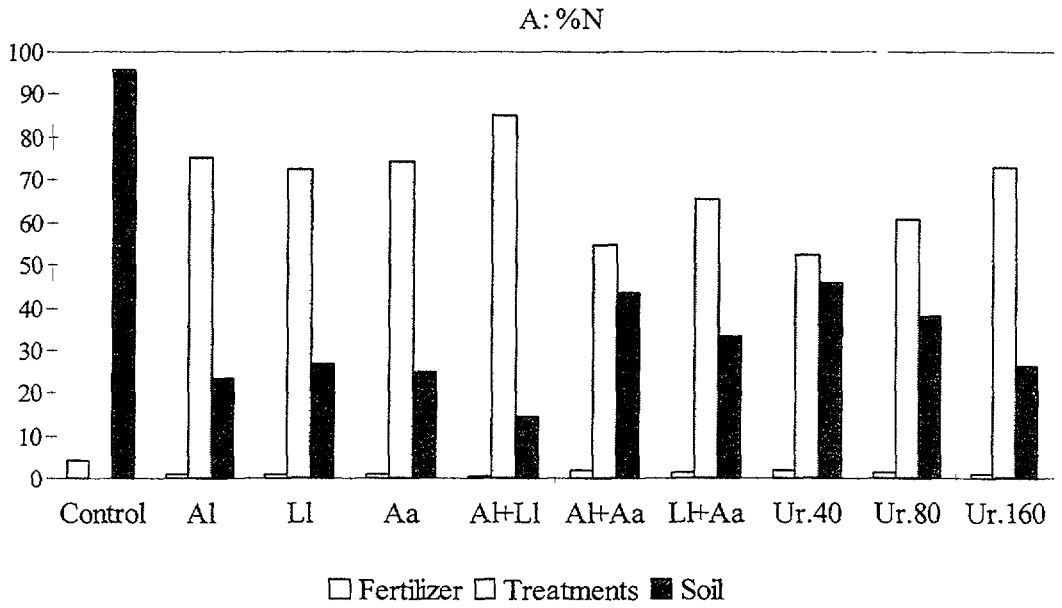
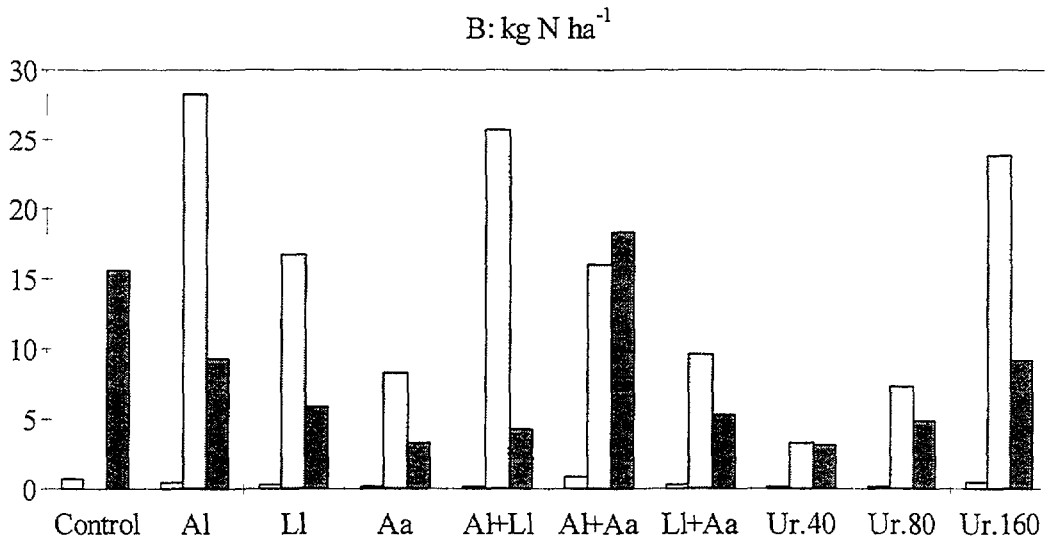


FIG. 10. Corn N-nutrition as affected by tree-legume leaves and urea (Expt. 2, field). A $LSD_{0\,05} = 0.4$; B $LSD_{0\,05} = 14$.

3.2.2.2. Grain yield

Most of the leaf treatments increased *Z. mays* grain yields over the control, particularly the Al+Aa mixture (1,797 kg ha^{-1}), followed by Al+Ll (1,220 kg ha^{-1}) and Al and Ll alone (approximately 1,200 kg ha^{-1}) (Fig. 9). Grain production with Aa leaves (600 kg ha^{-1}) was similar to those obtained with 40 kg N ha^{-1} of urea (603 kg ha^{-1}) and the control (587 kg ha^{-1}).





		Fert	Leaves	Soil
$LSD_{0.05}$	A	NS	NS	38
	B	NS	14	NS

FIG.11. N derived from basal fertilizer, tree-legume leaves or urea, and soil in Z. mays *var. Kasai.*

3.2.2.3. Total N

The N nutrition of *Z. mays* (Fig. 10) was significantly improved by leaves from *A. lebbeck* (1.35%, 38 kg N ha^{-1}), followed by the Al + Aa (0.87%, 35 kg N ha^{-1}) and the Al+Aa (0.97%, 30 kg N ha^{-1}) mixtures, compared with untreated plants and those amended with 40 and 80 kg N ha^{-1} as urea.

3.2.2.4. N derived from treatments

The fractions of N derived from the leaf amendments and urea were in the range 52 to 85% (Fig. 11A). The soil supplied between 15 and 46%, whereas less than 5% of N came from the basal urea fertilizer (see Control). *Albizia lebbeck* leaves produced the best N yield in *Z. mays*, 28 kg N ha^{-1}, albeit not significantly different from that obtained with the Al+Ll mixture (26 kg N ha^{-1}) or the highest dose of urea (24 kg N ha^{-1}). The contributions from Aa and Ll+Aa and from 40 and 80 kg N ha^{-1} as urea were less than 10 kg N ha^{-1}.

4. DISCUSSION

Kinzono's soil contains native strains of *Bradyrhizobium* that effectively nodulate *A. auriculiformis* [10]. These strains are also infective on *A. lebbeck* roots, but not on *L. leucocephala* [7,11]. *Albizia lebbeck* belongs to a group of tree legumes with broad infectivity, whereas *L. leucocephala* is more specific [12,13,14]

Experimentation in pots allows greater control than in the field, of factors that affect plant growth and hence indirectly affect root nodulation, N_2 fixation and N accumulation. On the other hand, restricted root development in pots may affect uptake of nutrients that are important to physiological development. The low values for nodulation (15, 17 nodules $plant^{-1}$), total N (19, 32 mg $plant^{-1}$) and %Ndfa (<50%) recorded with *A. auriculiformis* and *A. lebbeck* in our pot experiment may be due to such limitation. Awonaike et al. [15] found that dry matter, N content and total amount of N fixed by *L. leucocephala* were affected by decreasing rooting volume whereas the reverse was observed for %Ndfa. They concluded that rooting volume, rather than cropping pattern, influences the proportion and amount of fixed N.

In the field, *A. auriculiformis* grew better than *A. lebbeck* in terms of height and basal stem girth (Fig. 2). *Acacia auriculiformis* is known for fast growth and rapid biomass accumulation, and is reported to accommodate somewhat unfavorablepedo-climatic conditions [11,16-18].

Acacia auriculiformis and *A. lebbeck* accumulated 70% of their N from fixation (Fig. 3) when inoculated with a compatible *Bradyrhizobium* strain, confirming why they are regarded as having utility in agroforestry systems for soil-fertility restoration [6,19].

The incorporation of green manures of the above legumes and of *L. leucocephala* into Kinshasa's sandy soil increased corn and hibiscus growth and yield. Up to 60 DAS, the pot experiment revealed increases of dry matter and N accumulation when the soil was amended with pure or mixed *A. lebbeck* and *L. leucocephala* leaves. Later, stimulation was obtained in mixtures with leaves from *A. auriculiformis*. This delayed, but complementary, effect of *A. auriculiformis* green manure on maize productivity was more evident in field conditions. Biomass, N concentration and grain yield of corn fertilized with the Al+Aa mixture were increased by 52, 54 and 67%, respectively, over the control.

In our pot and field trials, the leaves of *A. lebbeck* and *L. leucocephala* could not be physically traced after 90 days in the soil, whereas *A. auriculiformis* materials remained only partially decomposed. This was probably because of higher N content (>4%) in the former two species and the size of their leaflets [6,20-22]. Green manures of high C:N ratio and thicker leaves, such as *A. auriculiformis*, decompose more slowly.

It has been demonstrated that chemical composition, application time and decomposition rate of green manures greatly influence their effectiveness on crop productivity improvement [23]. Synchrony of nutrient release with crop needs may be lost in the absence of ingenious management practices. The increase of yield noted at 90 DAS on crops treated with combinations including *A. auriculiformis* leaves could be due to delayed nutrient release. Giashuddin et al [22] showed that N liberation from *A. auriculiformis* leaves reached 40% after 90 days and exceeded 60% only after 160

days in the soil, whereas *Gliricidia sepium* released more than 90% of its leaf N by 90 days. *Acacia auriculiformis* leaves should be utilized in green-manure mixtures for crops with a growing season longer than 3 months.

Our results are in keeping with the findings of several others on the superiority of green manures over synthetic fertilizers in terms of biomass productivity, grain yield and tissue-N accumulation [24-29]. The green manure's effectiveness is attributed not only to nutrient release during the decomposition, but also to improvements in structure and physico-chemical properties of the soil [30,31].

In addition, the efficiency of use of green manure by a crop greatly depends on the technique adopted at the time of application to the soil. Practices that favor synchronization of nutrient release with the period of greatest need in the crop will bring the greatest benefit to small-holder farmers of developing countries. To minimize nutrient losses that may occur through volatilization, immobilization, leaching, fixation, denitrification, etc., farmers must be cognizant of influencing factors.

- Application mode: green manure incorporation into soil versus surface-placement [32,33].
- Application time: this should be such that nutrient release will occur most rapidly during the period of greatest need by the crop.
- Chemical quality: plant materials with high C:N ratio are slower in nutrient-release, whereas lower C:N ratio material decomposes more rapidly [34,35].

In our field conditions, green manures supplied between 54 and 84% of maize's total N, whereas urea contributed between 52 and 72%. Rathert and Werasopon [26] found that NFT foliar biomass is an excellent source of N, P and K. The high-N content in *A. lebbeck* and *L. leucocephala* leaves significantly influenced N uptake by maize; some 84% of its N content came from the mixture Al+Ll.

5. CONCLUSION

The potential importance of NFTs in fertility restoration and conservation of marginal tropical soils is undeniable. However, selection of productive NFT provenances should be done in concert with selection of compatible rhizobial strains. Inoculation of *A. lebbeck* and *A. auriculiformis* with *Bradyrhizobium* increased N_2 fixation (>70%) compared with uninoculated plants.

Also, incorporation into soil of *A. lebbeck*, *A. auriculiformis* and *L. leucocephala* green leaves significantly influenced N nutrition as well as crop productivity. Studies should be conducted to better evaluate the benefits that result from managing inputs of slow- and fast-decomposing green manures, in order to synchronize nutrient release with crop needs. In addition to improvinging soil structure, the humus of slowly decaying leaves may allow retention of released nutrients from rapidly decomposing materials within the rooting zone, thus fostering greater efficiency of uptake.

ACKNOWLEDGEMENTS

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NITROGEN FIXATION BY *GLIRICIDIA SEPIUM:* DECOMPOSITION OF ITS LEAVES IN SOIL AND EFFECTS ON SWEET-CORN YIELDS

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Abstract

Nitrogen fixation by *Gliricidia sepium* subjected to three pruning regimes (one, two or four cuts per year) was measured using the ^{15}N-dilution technique with *Cassia siamea* as the reference species Over a 4-year period, estimates of the fraction of N derived from fixation, generally <50%, indicated that field-grown *G sepium* is a low N_2 fixer *Gliricidia sepium* leaves were placed in litter-bags, buried in an ultisol and sampled at intervals over 70 days The half-life for dry matter was 17 days, and about 60% of the N was lost within 10 days, K and Ca were the most rapidly released nutrients, with half-lives of only 1 and 3 days, respectively The N contributions from *G sepium* leaves and roots to alley-cropped sweet corn were quantified by the ^{15}N-dilution technique over three growing seasons The application of leaves with roots resulted in increased N uptake and dry matter yield in corn Below-ground competition between hedgerow and corn, assessed using ^{32}P with the third crop, occurred under conditions of low nutrient-availability The data imply that there is no advantage of the cut-and-carry system over permanent hedgerows, provided that prunings are applied at the time of nutrient demand in the crop

1 INTRODUCTION

The traditional land-use system of shifting cultivation has proved to be unsustainable in the tropics. The fallow period is becoming increasingly short due to demographic pressure and land-tenure systems, resulting in rapid declines in soil fertility and productivity, with deleterious environmental consequences, mainly soil erosion [1,2]. The decline in availability of nutrients, especially N, is not mitigated by the use of chemical fertilizers where available [3,4], due to low organic-matter inputs and high losses of N [5,6].

Alley-cropping has been proposed as an alternative to shifting cultivation [7]. This usually involves intercropping leguminous trees or shrubs with cereals. The former are pruned regularly to prevent shading and reduce competition with the crop, the productivity of which is maintained through application of the prunings to the soil. In addition, root residues are suspected of contributing substantially to crop-nutrient availability. This system has been reported to improve soil fertility and nutrient uptake, and to reduce soil erosion [8,9].

Legume residues are generally known to improve crop productivity by enhancing the availability of soil N in addition to supplying plant-available N [10]. Depending on the nature of the materials, two modes of action are distinguished, namely a direct effect on nutrient supply and an indirect effect on the soil micro-climate. Plant materials of low C:N, lignin and polyphenol decompose rapidly and are readily available sources of nutrients for crops. There is need to develop management options to ensure that nutrient release from green manure matches crop needs while minimizing losses [11-13]. Poor-quality residues (high C:N, lignin and polyphenol) also improve crop performance, through positive effects on the soil microclimate, even when applied as mulch [9,14,15]. The long-term build-up of soil organic matter has been established to be more important than the immediate supply of nutrients [16].

Although the potential for alley-cropping is widely acknowledged, information on competition for nutrients between the hedgerows and crops is still lacking. There have been reports of substantial proportions of the residue N being recovered in hedgerow above-ground biomass [17,18]. This phenomenon and the immobilization of N in soil organic matter are believed to have contributed to low utilization of residue N in first maize crops in *Leucaena* alley-cropping systems [19]. However, this N becomes available to subsequent crops through pruning of hedgerow regrowth, application to the soil, and mineralization.

Nitrogen-fixing trees can play a special role in alley-cropping systems through the ability to thrive in N-deficient soils [20]. *Gliricidia* species in particular have potential due to their fast-growing characteristic, however, N_2-fixing ability, as measured by acetylene reduction, was found to be poor [21]. On the other hand, research using the ^{15}N-dilution technique indicated that *G. sepium* was comparable to *Leucaena leucocephala,* which is regarded as a high N_2 fixer [21]. Danso et al. [20] found that *G. sepium* derived 72% of its N from fixation (i.e. from the atmosphere, Ndfa), and Liya et al. reported 85% Ndfa when *G. sepium* was grown in concrete cylinders sunk 1 m into the ground [22].

The potential importance of nutrient-rich green manures in building and sustaining soil fertility and crop productivity in low-input agricultural systems is now widely acknowledged [8,10,23]. These organic amendments not only contribute plant-available nutrients during decomposition by soil micro-organisms, but also improve soil physico-chemical and biological properties [8,24]. These processes are dependent on the quality of the material, on climatic factors, and on the nature of the soil flora and fauna [11,25-27].

A direct relationship exists between decomposition rate and initial N or lignin and soluble polyphenols in the green manure. High initial N, low C:N, (lignin+polyphenol):N and polyphenol:N ratios favour high rates of decomposition, fresh leguminous leaves for example [12,28-30]. Thus, nutrient releases differ according to the quality of the organic material, in extent and pattern. Previous investigations have been directed at the dynamics of release and availability of N during green-manure decomposition. More information on release patterns and interactions with other factors is required for the development of effective nutrient-management practices for low-input cropping systems.

This research had several objectives.

- To evaluate the effects of successive cutting on N_2 fixation by field-grown *Gliricidia sepium* over four years, using the ^{15}N-dilution technique with *Cassia siamea* as the reference crop.
- The elucidation of decomposition and nutrient-release patterns of fresh leaves of *G. sepium*.
- The quantification of *G. sepium* leaf N used by alley-cropped sweet corn using the ^{15}N dilution technique.
- To assess below-ground competition for P between *G. sepium* hedgerows and alley-cropped sweet corn.

2. MATERIALS AND METHODS

2.1. Nitrogen fixation by *G. sepium*

A field experiment was conducted at the Puchong experimental site of the Department of Soil Science, Universiti Putra, Malaysia, where the climate is humid tropical with a mean annual rainfall of 2,100 mm. The soil at the trial site is classified as a kandic tropudult with the following characteristics: pH(H_2O) 5.2; total N 0.16%, Bray-2 extractable P 29 mg kg^{-1}, ammonium acetate exchangeable K, Ca and Mg of 120, 376 and 90 mg kg^{-1}, respectively. Plots of 5×5 m were marked out, with 0.5 m between, and basal fertilizers of 100 kg P ha^{-1} and 100 kg K ha^{-1} were applied.

Three-month-old *Gliricidia sepium* seedlings were transplanted from the greenhouse at 1×1 m. *Cassia siamea* plants, as the reference, were similarly transplanted. The centre 3×3 m area in each plot was trenched to a depth of 1 m and lined with plastic sheet, and the original soil replaced. This area was evenly applied with 3 g N as ammonium sulphate enriched with ^{15}N at 10% atom excess. The same rate of N as unenriched ammonium sulphate was applied around the trenched area. Trees in the trenched area were divided into one row to be pruned at 1 m above the ground level every 3 months, one row to be pruned every 6 months, and the remaining row to be cut on a yearly basis (designated "unpruned"). The same treatments were applied to the *C. siamea*. The plots were established for trees to be harvested at 1, 2, 3 and 4 years after transplanting. The ^{15}N for the plots to be harvested at the end of the second, third and fourth years was applied at the beginning of the respective year, as described above. There were five replications, randomly arranged.

At the end of each year, individual trees from each cutting-regime from two replications were dug out, separated into leaves, stem and root, weighed and chopped using a garden shredder, and sub-sampled. The trunks of large trees were cut into small pieces using an electric saw, and the saw dust used as the sub-sample. The sub-samples were weighed and dried in a forced-air oven at 65°C until constant weight was achieved. The samples were finely ground and mixed again. Total N and N-isotope ratios were determined at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria, with a 1500 Carlo Erba Automatic Nitrogen Analyzer coupled to a SIRA mass spectrometer. The isotope-dilution equation [31] was used to calculate the fraction of N derived from fixation, with *C. siamea* as the non-fixing reference. The significance ($P \leq 0.05$) of differences between mean values was determined by analysis of variance using a SAS-PC package [32].

2.2. *Gliricidia sepium* leaf decomposition

This experiment was carried out also at the Puchong experimental site of Universiti Putra. The temperature ranged between 19 and 36°C with average relative humidity of 97% during the experimental period. The rainfall pattern is shown in Fig. 1. The soil used is of the Bungor series (typic paleudult) in an undulating land-form with the following properties at 0-15 cm depth: pH (water, 1:2.5) 4.6, pH (KCl, 1:2.5) 3.6, 4.4 mg kg^{-1} Bray-1 P, 0.013% N, 1.4% organic C, cation exchange capacity (CEC) of 5.7 cmol(+)kg^{-1}, and exchangeable K, Ca and Mg values of 0.16, 0.07 and 0.08 cmol(+)kg^{-1}, respectively.

Two-hundred g of fresh *G. sepium* leaves were placed in plastic litter-bags, 30×40 cm, with a mesh size of 5 mm, and buried 10 cm in the soil. Bags were randomly excavated in fours at 5, 10, 20, 30, 40, 50, 60 and 70 days. Soil was carefully removed by rinsing in water for 1 min, after which the residue was oven-dried at 70°C, milled and then analyzed for C by the Walkley-Black method, for total N by steam-distillation and titration after digestion with salicylic acid-H_2SO_4, and for other nutrients after ashing at 550°C (4 h): P by the molybdate-blue method, and K, Ca and Mg by atomic-absorption spectrometry. The leaf samples were analyzed initially also for lignin and polyphenol content [13].

Data means were computed and compared by analysis, as described above. The following exponential model was fitted:

$$M_t = M_0.e^{-k.t}$$

where

M_0 is the original amount of dry material or nutrient,
M_t is the proportion of dry matter or nutrient remaining after a period of time t, in days,
k is a constant,
t is time.

A plot of time against logarithm of this first-order exponential model was made for each component. These plots revealed that a single negative exponential model did not give best-fit curves for dry matter and nutrient loss. Therefore, non-linear curves were made, the slopes of which give the k values for each component. Half-lives for dry-matter loss and nutrient release were calculated from the best-fit equations.

2.3 Sweet corn alley-cropped with *Gliricidia sepium*

This research was carried out also at the Puchong experimental site. The relative humidity was between 88 and 93% during the experimental period with maximum and minimum temperatures of 36°C and 20°C, respectively. Rainfall averaged around 2,100 mm. The soil was of the Bungor series (typic paleudult), pH(H_2O) 4.6, pH(KCl) of 3.9, organic C (Walkley-Black) of 1.48%, Bray-1 P of 4.39 mg kg^{-1}, 0.06% N, cation exchange capacity (1 *N* ammonium acetate, pH 7.0) of 8.3 cmol(+) kg^{-1}, exchangeable Ca, K and Mg of 0.32, 0.17 and 0.18 cmol(+)kg^{-1} soil, respectively.

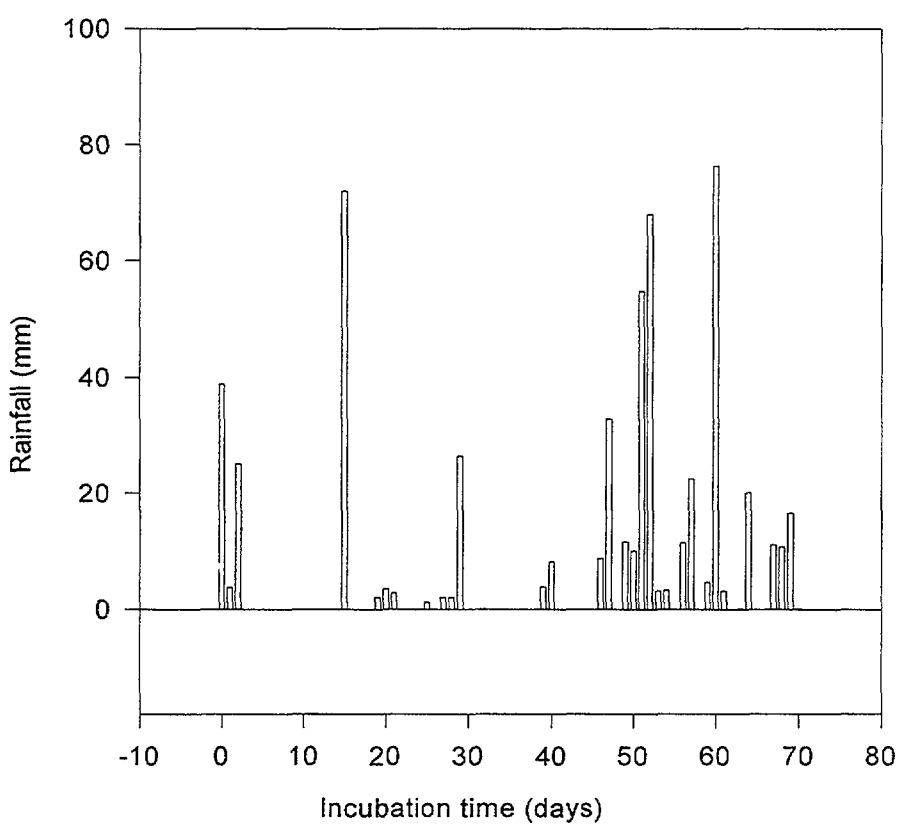


FIG. 1. Rainfall distribution during the period of study.

The following treatments were arranged in a randomized complete-block design with four replicates:

(1) Control without hedgerows.
(2) Control with hedgerows.
(3) Leaf prunings with hedgerows.
(4) Leaf prunings without hedgerows.
(5) Roots without hedgerows.
(6) Roots with hedgerows.
(7) Leaf prunings + roots without hedgerows.
(8) Leaf prunings + roots with hedgerows.

Experimental plots measuring 5×3 m were made and *G. sepium* was planted in May 1992 with a 1×1 m spacing. For the hedgerow plots, *G. sepium* was planted on the plot edges. Where root incorporation was to be made, *G. sepium* seedlings were planted over the plot at the specified distance, i.e. a total of 20 plants plot^{-1}. Before planting the first crop of sweet corn (*Zea mays* L.) in November 1993 on the root-incorporation plots, the trees were cut below ground level and the stumps smeared with the herbicide Garlon 250 to prevent regrowth. The second corn crop was established in October 1994 and the third in January 1996 on these plots with leaf prunings added.

Trees in hedgerow plots were pruned at a height of 1 m. The leaf material had an average composition of 4.2% N, 12% lignin, 2.3% polyphenol, 39% C, 0.88% Ca, 2.6% K and 0.16% P. Lime was applied at 2 t ha^{-1} at about 4 weeks prior to planting the second crop. Sweet corn was then planted at intra- and inter-row spacings of 0.25 and 0.75 m, respectively, giving five rows of twenty-five plants (125 plants plot^{-1}). Applications of 100 kg ha^{-1} as triple superphosphate and 120 kg ha^{-1} KCl, were made with the first pruning application. Then prunings, equivalent to 120 kg N ha^{-1}, were incorporated between the maize rows at 0 and 30 days after planting (DAP) for the first crop. Prunings were applied to the second crop at 5 and 30 DAP and to the third crop at 160 kg N ha^{-1} at 21 and 45 DAP. Nitrogen, P and K fertilizers were added as ammonium sulphate, TSP and KCl at equivalent rates to control plots. Supplementary P and K was added to all pruning treatments as TSP and KCl, respectively. The total amounts of major nutrients supplied by the prunings to each crop are shown in Table I.

In the central 1.5×1.5 m area of each plot, (seven plants) ^{15}N micro-plots were demarcated and 40 kg N ha^{-1} was applied as ammonium sulphate enriched in ^{15}N at 10% atom excess with the other fertilizers. For the third crop, additional 1×1 m micro-plots in each centre row were set up in hedgerow plots, to assess the *G. sepium*'s access to ^{32}P applied to the corn. To each micro-plot, 29.6 MBq ^{32}P was added with 5 mg P as carrier (KH_2PO_4) in 200 mL of water. Hedgerow plants, at 1.7, 2.2, 3.2, and 4.2 m from the centre of each micro-plot, i.e. from the designated "point of application," were harvested individually at 4 and 6 weeks after radioisotope application.

TABLE I. QUANTITIES OF NUTRIENTS ADDED AS *G. SEPIUM* PRUNINGS

Nutrient	Quantity added to		
	Crop 1[a]	Crop 2[b]	Crop 3[c]
	(kg ha^{-1})		
Nitrogen	120	120	160
Phosphorus	4.6	4.6	6.1
Potassium	75.5	75.5	101

[a]Added at 0 and 30 days after planting. [b]Added at 5 and 30 days after planting.
[c]Added at 21 and 45 days after planting.

Weeding and irrigation were carried out manually when necessary. The corn plants were harvested at the milk stage of kernel development, approximately 65 days. Three plants from the centre of the ^{15}N micro-plots were cut at ground level, separated into stover and cobs and total fresh weights recorded. Sub-sample fresh weights were noted before drying at 70°C until constant weight was achieved. Yields were obtained by harvesting the three centre rows. Grain yields for crop 1 were insignificant, therefore they were combined with stovers for determination of total dry matter.

Leaf samples of the hedgerow regrowth were collected at 4 and 6 weeks after radioisotope application for Cerenkov counting of ^{32}P activity after ashing at 550°C for 4 h, and dissolving the cooled ash in 2 *M* HCl.

Samples from the corn plants were milled and total N determined by micro-Kjeldahl distillation and titration after salicylic acid-H_2SO_4 digestion. The ^{15}N enrichments in the samples were determined with a NOI-6 analyzer [33] for crop 1, and by mass spectrometry for crops 2 and 3.

Soil samples were collected for analysis from the plots prior to the experiment and before the third crop.

Nitrogen derived from a treatment (NdfT) was calculated using the indirect isotope-dilution method, as follows.

$$\%NdfT = 1 - \left(\frac{^{15}N \text{ a.e. in plant part}}{^{15}N \text{ a.e. in control}}\right) \times 100 \quad (1)$$

Multiplication of (1) by total N gives NdfT in kg ha^{-1}.

Results were subjected to analysis of variance to evaluate treatment effects as above. Differences between means were tested for significance by Duncan Multiple Range Test ($P \leq 0.05$).

3. RESULTS

3.1. Nitrogen fixation by *G. sepium*

3.1.1. Plant growth and total N

The total dry matter produced by *G. sepium* pruned four times per year was 2,305 g $tree^{-1}$ in the first year and 1,397, 1,771, 1,443 g $tree^{-1}$ in years 2 to 4, respectively. The re-growth was hindered latterly probably due to shading from surrounding trees.

At the end of the first year, the stem contributed 42% of the total dry weight, while 26% was from roots and 32% from leaves (Table II). For trees pruned twice per year, the stem contributed 57% of the total dry matter, and the leaves and roots consisted of 22% and 21% respectively. Similarly, trees cut only at the end of the first year consisted of 57%, 21% and 21% as stem, leaves and roots, respectively.

In year 2, for *G. sepium* pruned four times per year, total dry matter was 1,397 g $tree^{-1}$ with 764 g (54%) as leaves, 454 g (33%) as stem and 179 g (13%) as roots, whereas trees pruned twice per year had a total dry-mass production of only 505 g consisting of 29% leaves, 34% stem and 37% roots (Table III). Unpruned tree dry weight was 449 g, with much higher root development (75%) than stem (15%) or leaves (10%).

TABLE II. DRY-MATTER YIELDS, N CONCENTRATION AND TOTAL-N YIELDS OF PLANT COMPONENT PARTS. YEAR 1

Treatment / Species / Component	Dry matter Harvest no. 1	2	3	4	Total	N concentration Harvest no. 1	2	3	4	Mean	Total N Harvest no. 1	2	3	4	Total
	(g tree^{-1})					(%N)					(g tree^{-1})				
4 cuts per yr															
G. sepium															
Leaf	113	261	102	258	734	4.5	3.6	3.0	3.3	3.6	5.04	9.26	3.04	8.46	25.8
Stem	116	144	316	396	972	1.4	1.0	1.1	0.7	0.95	1.63	1.45	3.48	2.69	9.25
Root				601	601				1.4	1.4				8.41	8.41
			(Total)		(2,307)								(Total)		(43.5)
C. siamea															
Leaf	141	293	83	186	703	3.5	3.2	3.3	2.4	3.0	4.89	9.32	2.75	4.43	21.4
Stem	126	332	305	379	1,142	1.1	0.8	0.9	0.4	0.7	1.35	2.69	2.72	1.52	8.28
Root				344	344				0.6	0.6				1.89	1.89
			(Total)		(2,189)								(Total)		(31.5)
$LSD_{0\,05}$	n.s	n.s	102	n.s							1.03	3.15	2.35	5.91	9.92
2 cuts per yr															
G. sepium															
Leaf		326		142	468		3.6		3.2	3.5		11.8		4.50	16.3
Stem		418		784	1,202		0.8		0.8	0.8		3.34		6.35	9.69
Root				443	443				1.2	1.2				5.45	5.45
				(Total)	(2,113)									(Total)	(31.5)
C. siamea															
Leaf		282		230	512		2.6		2.3	2.5		7.28		5.22	12.5
Stem		332		387	719		0.7		0.5	0.6		2.29		1.82	4.11
Root				406	406				0.6	0.6				2.39	2.39
				(Total)	(1,637)									(Total)	(19.0)
$LSD_{0\,05}$		224		441								4.43		1.02	3.35
1 cut per yr															
G. sepium															
Leaf				213	213				3.1	3.1				6.54	6.54
Stem				576	576				0.9	0.9				5.41	5.41
Root				213	213				1.2	1.2				2.58	2.58
				(Total)	(1,002)									(Total)	(14.5)
C. siamea															
Leaf				1,070	1,070				2.1	2.1				22.3	22.3
Stem				783	783				0.4	0.4				3.29	3.29
Root				799	799				0.4	0.4				3.28	3.28
				(Total)	(2,653)									(Total)	(28.8)

TABLE III. DRY-MATTER YIELDS, N CONCENTRATION AND TOTAL-N YIELDS OF PLANT COMPONENT PARTS. YEAR 2

Treatment	Dry matter					N concentration					Total N				
Species	Harvest no.					Harvest no.					Harvest no.				
Component	1	2	3	4	Total	1	2	3	4	Mean	1	2	3	4	Total
	(g tree^{-1})					(%)					(g tree^{-1})				
4 cuts per yr															
G. sepium															
Leaf	299	316	122	27	764	3.6	3.6	3.6	3.3	3.6	10.8	11.3	4.4	0.9	27.4
Stem	144	216	68	6	454	1.3	1.6	1.2	1.5	1.4	1.8	3.5	0.8	0.4	6.5
Root				179	179				1.5	1.5				2.7	2.7
		(Total)			(1,397)							(Total)			36.6
C. siamea															
Leaf	164	182	389	55	790	2.6	2.4	2.5	3.7	2.6	4.2	4.4	9.6	2.1	20.3
Stem	90	155	175	25	445	0.6	0.9	0.8	1.4	0.8	0.6	1.4	1.4	0.4	3.8
Root				112	112				0.6	0.6				0.7	0.7
		(Total)			(1,347)							(Total)			(24.8)
2 cuts per yr															
G. sepium															
Leaf		102		42	144		3.8		3.5	3.7		3.9		1.5	5.4
Stem		78		95	173		1.3		1.5	1.4		1.0		1.3	3.3
Root				188	188				1.6	1.6				3.1	3.1
		(Total)			(505)							(Total)			(11.7)
C. siamea															
Leaf		96		27	123		2.6		3.6	2.8		2.5		1.0	3.5
Stem		74		42	115		0.7		1.0	0.9		0.5		1.5	2.0
Root				87	87				0.7	0.7				6.0	6.0
		(Total)			(325)							(Total)			(11.5)
1 cut per yr															
G. sepium															
Leaf				45	45				3.5	3.5				1.6	1.6
Stem				69	69				1.1	1.1				0.8	0.8
Root				335	335				1.7	1.7				5.9	5.9
		(Total)			(449)							(Total)			(8.3)
C. siamea															
Leaf				64	64				3.2	3.2				2.0	2.0
Stem				77	77				0.7	0.7				5.4	5.4
Root				143	143				0.8	0.8				1.1	1.1
		(Total)			(284)							(Total)			(8.5)

TABLE IV. DRY-MATTER YIELDS, N CONCENTRATION AND TOTAL-N YIELDS OF PLANT COMPONENT PARTS. YEAR 3

Treatment	Dry matter					N concentration					Total N				
Species	Harvest no.					Harvest no.					Harvest no.				
Component	1	2	3	4	Total	1	2	3	4	Mean	1	2	3	4	Total
	(g tree^{-1})					(%)					(g tree^{-1})				
4 cuts per yr															
G. sepium															
Leaf	176	162	14	136	620	2.7	3.4	3.8	3.6	3.4	4.78	5.60	5.59	4.94	20.9
Stem	131	137	117	137	522	1.4	1.4	1.3	1.4	1.3	1.86	1.90	1.54	1.90	7.20
Root				629	629				1.1	1.1				7.17	7.17
		(Total)		(1,771)								(Total)		(35.3)	
C. siamea															
Leaf	155	144	126	123	548	2.7	2.5	3.1	3.3	2.8	4.12	3.96	4.03	4.04	15.8
Stem	125	125	108	119	477	0.90	0.85	0.91	1.2	0.90	1.13	1.06	0.98	1.38	4.55
Root				1200	1200				0.56	0.56				6.72	6.72
		(Total)		(2,225)								(Total)		(27.0)	
2 cuts per yr															
G. sepium															
Leaf		136		643	779		3.5		4.1	3.7		4.80		26.7	31.5
Stem		225		171	396		1.1		1.5	1.3		2.48		2.51	4.99
Root				1679	1679				1.8	1.8				29.6	29.6
		(Total)		(2,854)								(Total)		(66.0)	
C. siamea															
Leaf		225		173	398		3.5		2.9	3.2		7.9		5.02	13.0
Stem		108		124	232		0.73		0.91	0.81		0.79		1.13	1.92
Root				1323	1323				0.65	0.65				8.60	8.60
		(Total)		(1,953)								(Total)		(23.5)	
1 cut per yr															
G. sepium															
Leaf				336	336				3.6	3.6				12.2	12.2
Stem				1660	1660				1.2	1.2				20.7	20.7
Root				1200	1200				1.4	1.4				16.8	16.8
		(Total)		(3,196)								(Total)		(49.7)	
C. siamea															
Leaf				6106	6106				2.6	2.6				158	158
Stem				19085	19085				0.55	0.55				105	105
Root				6530	6530				0.75	0.75				49.0	49.0
		(Total)		(31,721)								(Total)		(312)	

In year 3, total dry matter produced by *G. sepium* trees pruned four times annually increased to 1,771 g tree^{-1}, whereas trees pruned twice per year weighed 2,854 g tree^{-1} and those unpruned had a mean dry weight of 3,196 g (Table IV). In the fourth year, the unpruned *Gliricidia* produced 6,986 g tree^{-1}, comprised mainly of roots and leaves (Table V).

TABLE V. DRY-MATTER YIELDS, N CONCENTRATION AND TOTAL-N YIELDS OF PLANT COMPONENT PARTS. YEAR 4

Treatments / Species / Component	Dry matter Harvest no. 1	2	3	4	Total	N concentration Harvest no. 1	2	3	4	Mean	Total N Harvest no. 1	2	3	4	Total
	(g tree^{-1})					(%)					(g tree^{-1})				
4 cuts per yr															
G. sepium															
Leaf	130	103	54	41	328	4.1	4.2	4.1	4.7	4.2	5.36	4.32	2.23	1.91	13.8
Stem	201		29	21	251	1.2		1.3	2.1	1.3	2.70		0. 36	0.37	3.45
Root				864	864				1.5	1.5				13.2	13.2
		(Total)			(1,443)							(Total)			(30.5)
C. siamea															
Leaf	666	64	105	21	856	3.0	3.1	3.5	3.1	3.1	19.8	2.00	3.64	0.66	26.1
Stem	2004	56	13		2073	0.67		1.2	1.7	1.21	13.4		0.70	0.22	14.3
Root				2325	2325				0.55	0.55				12.8	12.8
		(Total)			(5,254)							(Total)			(53.2)
2 cuts per yr															
G. sepium															
Leaf		557	107		664		3.8	4.2		4.0		21.3	4.51		25.8
Stem		904	111		1015		1.2	1.4		1.3		11.0	1.58		12.6
Root			1018		1018			1.8		1.8			18.0		18.0
		(Total)			(2,697)							(Total)			(56.5)
C. siamea															
Leaf		1119	160		1279		3.2	2.9		3.1		36.3	4.64		40.9
Stem		4379	157		4536		0.77	0.98		0.78		33.7	1.54		35.3
Root			3121		3121			0.76		0.76			23.7		23.7
		(Total)			(8,936)							(Total)			(99.9)
1 cut per yr															
G. sepium															
Leaf			2997		2997			4.0		4.0			119		119
Stem			673		673			1.2		1.2			8.0		8.0
Root			3316		3316			1.9		1.9			64.3		64.3
		(Total)			(6,986)							(Total)			(191)
C. siamea															
Leaf			11298		11298			2.8		2.8			317		317
Stem			85929		85929			0.74		0.74			636		636
Root			19989		19989			0.75		0.75			150		150
		(Total)			(117,216)							(Total)			(1,103)

In year 1, *Cassia siamea* trees pruned four times showed almost the same dry weight as *G. sepium*, whereas unpruned trees produced much higher yields than *Gliricidia* (Table II). *Cassia* trees in year 2 also showed no increase in growth due to shading (Table III). In the third year, after the tall border-row trees had been felled, growth improved, with the highest increase occurring in the fourth year, with the stem constituting the bulk of the dry matter (Tables IV and V).

TABLE VI. ENRICHMENT IN ^{15}N OF PLANT COMPONENT PARTS AND N DERIVED FROM FIXATION. YEAR 1

Treatment Species Component	^{15}N enrichment Harvest no. 1	2	3	4	Ndfa Harvest no. 1	2	3	4	N fixed Harvest no. 1	2	3	4	Total
	(atom % excess)				(%)				(g tree^{-1})				
4 cuts per yr													
G. sepium													
Leaf	1.028	0.425	0.195	0.112	nf[a]	nf	14	39			0.44	3.34	3.78
Stem	1.141	0.425	0.206	0.345	nf	nf	17	23			0.58	0.63	1.21
Root				0.491				6.1				0.52	0.52
											(Total)		(5.51)
C. siamea													
Leaf	0.685	0.347	0.228	0.185									
Stem	0.751	0.406	0.247	0.451									
Root				0.523									
$LSD_{0.05}$	0.496	0.120	0.061	0.123									
2 cuts per yr													
G. sepium													
Leaf		0.519		0.133		1.4		38	0.17		1.71		1.88
Stem		0.674		0.351		nf		11			0.68		0.68
Root				0.604				nf					
										(Total)			(2.56)
C. siamea													
Leaf		0.430		0.215									
Root		0.503		0.393									
Stem				0.364									
$LSD_{0.05}$		0.114		0.116									
1 cut per yr													
G. sepium													
Leaf				0.132				nf					
Stem				0.254				nf					
Root				0.709				nf					
C. siamea													
Leaf				0.101									
Stem				0.156									
Root				0.204									

[a]No fixation, *Cassia* enrichment < *Gliricidia*.

The N concentrations of the leaves of *Gliricidia* were consistently higher than those of the stem or root, and higher than the leaves, stem and root of *Cassia* for all treatments and years of growth (Tables II-V).

Most of the N accumulated in *Gliricidia* and *Cassia* at the end of the first year was in the leaves, for all treatments (Table II). At the end of year 2, all treatments resulted in decreases in N assimilation (Tables II and III), whereas, in year 3, all treatments, except pruned four times, showed increases in total N from year 2 (Tables III and IV). Between years 3 and 4, *G. sepium* pruned twice and four times resulted in decreases in N accumulation (Table IV and V).

3.1.2. ^{15}N enrichment and N_2 fixation

In year 1, the ^{15}N enrichments in *G. sepium* pruned four times per year were higher, in the first 2 cuts, than those of the reference trees, therefore no fixation of N was measurable (Table VI). The third and fourth cuts showed lower ^{15}N enrichment levels compared to *C. siamea*. A total of 5.5 g N tree^{-1}, 13% of total N accumulated, was calculated to be fixed by *Gliricidia* pruned four times, at the end of the first year. The trees pruned twice fixed 2.6 g N tree^{-1}, which was equivalent to 8.1% of the total N accumulated in the first year. No fixation was detectable in unpruned *Gliricidia* trees in year 1.

In year 2, *G. sepium* in all treatments had lower ^{15}N enrichments than *C. siamea*, indicating that fixation contributed significant N (Table VII). Trees pruned four times per year had fixation of 2.9 g N tree^{-1} (26% Ndfa). In unpruned *Gliricidia*, only the roots showed N increases from year 1 and of this, 50% was calculated to come from N_2 fixation, equivalent to 1.7 g N tree^{-1}. The Ndfa values in kg ha^{-1} were determined on the basis of N accumulation for each year. Therefore in year 2, since no increases in total N were observed for unpruned trees or those pruned twice per year, fixation could not be calculated.

At the end of year 3, most treatments showed N increases from year 2, with N_2 fixation contributing up to 50% of total N (Table VIII). In year 4, %Ndfa was even up to 60% in trees pruned four times per year (Table IX).

3.2. *Gliricidia sepium* leaf decomposition

The original nutrient concentrations of the fresh leaves were: 4.2% N, 0.16% P, 2.6% K, 0.88% Ca, 0.31% Mg, 39% C, 2.3% polyphenol and 12% lignin. Therefore, the leaves had a C:N ratio of 9.3, and polyphenol+lignin:N ratio of 3.4.

3.2.1. Dry matter and C loss

A rapid rate of decomposition of leaf dry matter, 0.966 g day^{-1}, occurred within the first 10 days, resulting in a 56% loss (Fig. 2). The rate of decay became more gradual up to 40 days (>60% lost) and little change occurred thereafter.

A similar pattern was observed for loss of C (Fig. 3). In this case, the rate was faster than for dry matter. Nearly 40% of C was lost during the first 5 days and much of it, 75%, was released by the thirtieth day. Rainfall appeared to have little influence on rate of loss of dry matter or of C from this material.

3.2.2. Nutrient release

3.2.2.1. N, P and K

The releases of N, P and K were rapid during the first 10 days and slower thereafter (Figs. 4, 5 and 6). About 60% of the N was lost within 10 days (Fig. 4) and a total of 76% of the original N

content of the leaves was released within the 70-day period. Releases of N and P were observed to be unrelated to that of C between (Figs. 7 and 8); initially the release of C was more rapid than of N or P, whereas between 10 and 30 days, the converse occurred.

TABLE VII. ENRICHMENT IN ^{15}N AND N DERIVED FROM FIXATION OF PLANT COMPONENT PARTS. YEAR 2

Treatment	^{15}N enrichment				Ndfa				N increase yr 2				Ndfa				
Species	Harvest no.				Harvest no.				Harvest no.				Harvest no.				
Component	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	Total
	(atom % excess)				(%)				(g tree^{-1})				(g tree^{-1})				
4 cuts per yr																	
G. sepium																	
Leaf	0.414	0.239	0.140	0.077	31	15	9.1	nf[a]	5.8	2.0	1.0	1.4	1.8	0.3	0.12		2.2
Stem	0.388	0.236	0.147	0.015	20	33	5.8	nf	0.17	2.0	0	0	0.03	0.7	nc[b]		0.71
Root				0.111				nf				0					
															(Total)		(2.91)
C. siamea																	
Leaf	0.601	0.280	0.154	0.073													
Stem	0.487	0.352	0.156	0.085													
Root				0.093													
2 cuts per yr																	
G. sepium																	
Leaf		0.378		0.065		2.1		54		0		0		nc		nc	
Stem		0.292		0.073		42		53		0		0		nc		nc	
Root				0.116				45		0		0				nc	
C. siamea																	
Leaf		0.473		0.142													
Stem		0.501		0.157													
Root				0.210													
1 cut per yr																	
G. sepium																	
Leaf				0.081				50				0				nc	
Stem				0.218				25				0				nc	
Root				0.136				50				3.38				1.67	1.67
																(Total)	(1.67)
C. siamea																	
Leaf				0.163													
Stem				0.289													
Root				0.274													

[a]No fixation, *Cassia* enrichment < *Gliricidia*.

[b]Fixation non-calculable because N did not increase.

TABLE VIII. ENRICHMENT IN ^{15}N, N DERIVED FROM FIXATION, AND N INCREASE OF PLANT COMPONENT PARTS. YEAR 3

Treatments Species Component	^{15}N enrichment Harvest no. 1 2 3 4	Ndfa Harvest no. 1 2 3 4	N increase yr 3 Harvest no. 1 2 3 4 Total	Ndfa Harvest no. 1 2 3 4 Total
	(atom % excess)	(%)	(g tree^{-1})	(g tree^{-1})
4 cuts per yr				
G. sepium				
Leaf	0.414 0.150 0.102 0.086	31 30 28 22	1.2 4.0 5.2	0. 34 0.90 1.24
Stem	0.388 0.213 0.127 0.105	2 18 26 nf[a]	0.06 0.7 1.5 2.3	0.01 0.19 0.20
Root	0.066	13	4.5 4.5	0.59 0.59
				(Total) (2.03)
C. siamea				
Leaf	0.601 0.213 0.142 0.110			
Stem	0.487 0.259 0.170 0.099			
Root	0.191			
2 cuts per yr				
G. sepium				
Leaf	0.105 0.087	50 nf	0.9 25.8 26.6	0.45 0.45
Stem	0.182 0.091	43 13	1.5 1.0 2.5	0.64 0.13 0.77
Root	0.176	43	0 0	nc[b]
				(Total) (1.22)
C. siamea				
Leaf	0.212 0.083			
Stem	0.319 0.105			
Root	0.308			
1 cut per yr				
G. sepium				
Leaf	0.049	50	10.6 10.6	5.31 5.31
Stem	0.066	25	19.9 19.9	4.90 4.90
Root	0.128	nf	10.9 10.9	
				(Total) (10.2)
C. siamea				
Leaf	0.056			
Stem	0.100			
Root	0.123			

[a] No fixation, *Cassia* enrichment < *Gliricidia*.

[b] Fixation non-calculable because N did not increase.

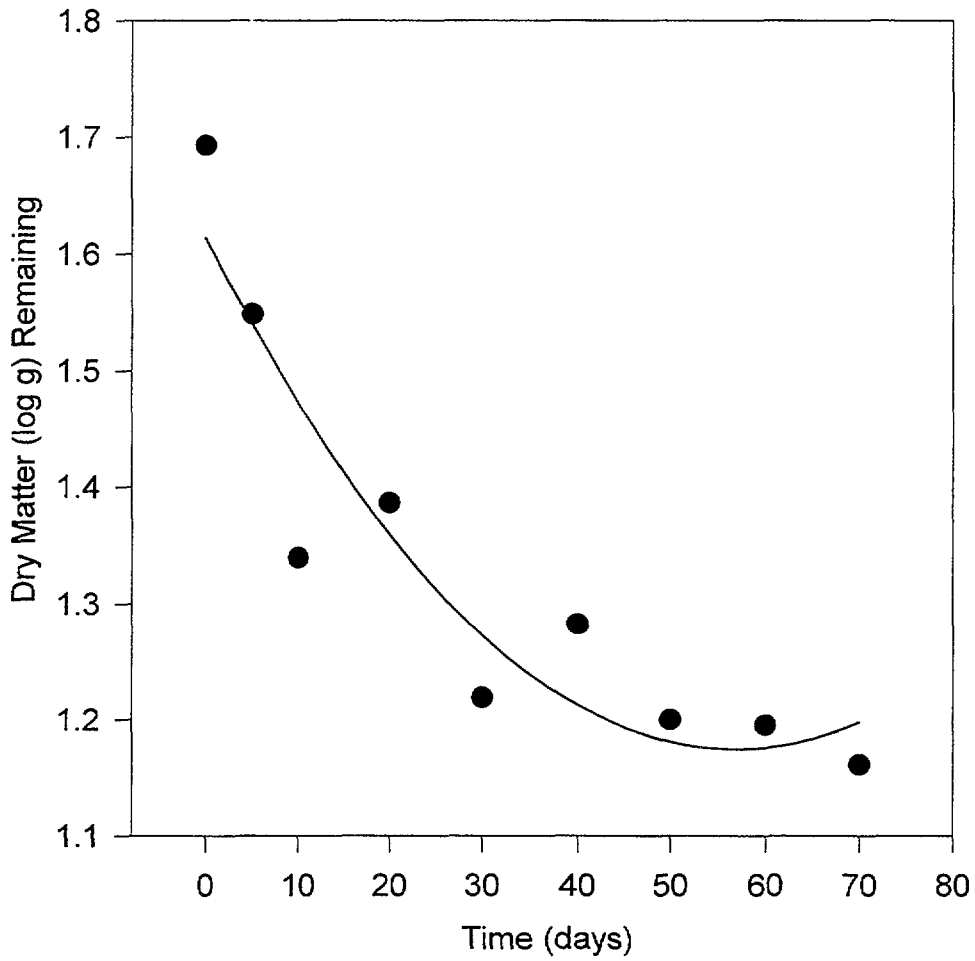
TABLE IX. ENRICHMENT IN ^{15}N, N DERIVED FROM FIXATION, AND N INCREASE OF PLANT COMPONENT PARTS. YEAR 4

Treatment Species Cmpnt.	^{15}N enrichment Harvest no. 1	2	3	4	Ndfa Harvest no. 1	2	3	4	N increase yr 4 Harvest no. 1	2	3	4	Total	Ndfa Harvest no. 1	2	3	4	Total
	(atom % excess)				(%)				(g tree^{-1})					(kg ha^{-1})				
4 cuts/year																		
G. sepium																		
Leaf	0.381	0.226	0.077	0.070	20	29	61	62	0.58	0	0	0	0.58	0.11	nc[a]	nc	nc	0.11
Stem	0.260		0.123	0.087	43		16	0	0.51	0	0	0	0.51	0.22		nc		0.22
Root				0.159				21				6.05	6.05				1.29	1.29
															(Total)			(1.62)
C. siamea																		
Leaf	0.475	0.317	0.197	0.185														
Stem	0.459		0.147	0.087														
Root				0.202														
2 cuts/year																		
G. sepium																		
Leaf		0.265		0.085		9		14		16.5		0	16.5		1.53		nc	1.53
Stem		0.284		0.131		11		nf[b]		8.55		2.13	10.7		0.96			0.96
Root				0.260				nf				6.05	6.05					
															(Total)			(2.65)
C. siamea																		
Leaf		0.292		0.099														
Stem		0.320		0.110														
Root				0.161														
1 cut/year																		
G. sepium																		
Leaf				0.065				nf				0	0					
Stem				0.093				nf				0	0					
Root				0.212				nf				0	0					
C. siamea																		
Leaf				0.058														
Stem				0.074														
Root				0.135														

[a]Fixation non-calculable because N did not increase.

[b]No fixation, *Cassia* enrichment < *Gliricidia*.

Phosphorus showed a pattern of loss similar to that of N, albeit at a much reduced rate, with a half-life of 18 days (Fig. 5). About 35% was lost within the first 10 days and a total of 73% by 70 days (Fig. 5). Figure 8 shows a no-P-release phase between 10 and 40 days.



$y = 1.61 - 0.015x + 0.00014x^2$ ($r^2 = 0.96$)

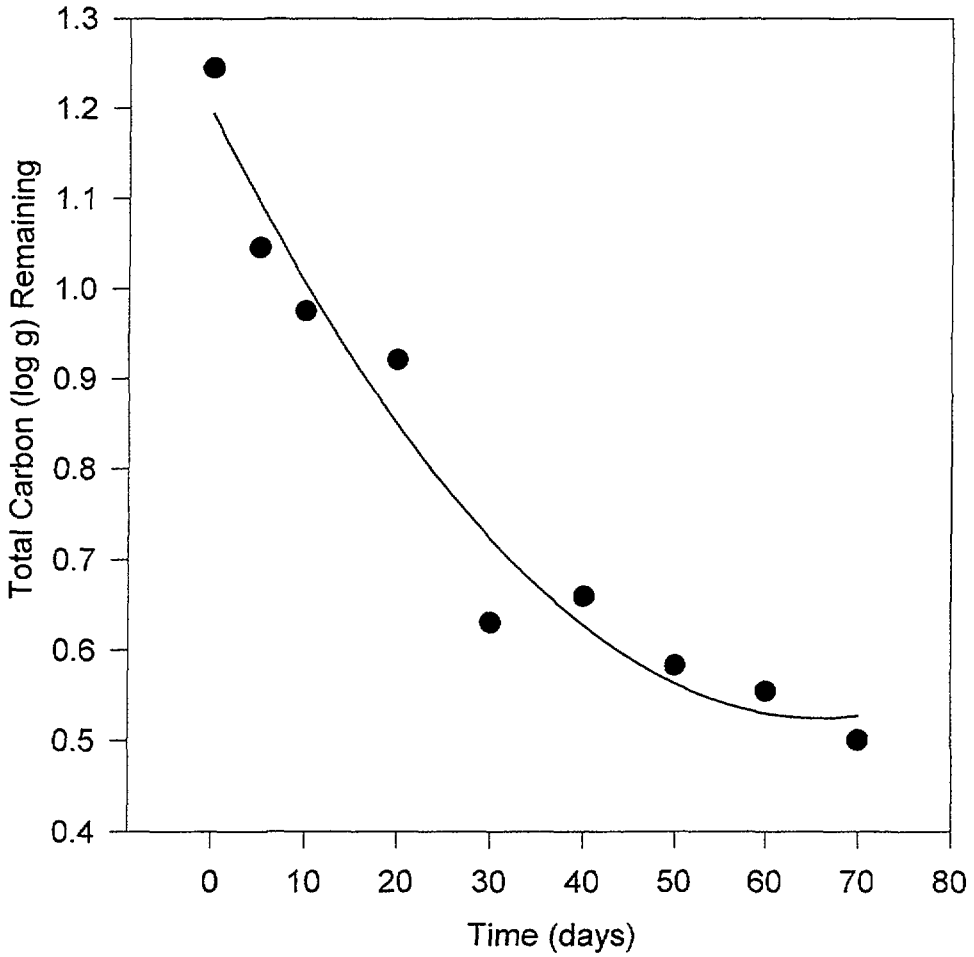
FIG. 2. Gliricidia sepium *leaf decomposition: dry matter loss.*

The rate of release of K was highest, with almost 90% gone within 10 days and 98% released by the thirtieth day. Little release occurred after 20 days of incubation. The half-life for K was 3 days (Fig. 6).

3.2.2.2. Ca and Mg

The pattern of release of Ca from the *G. sepia* leaves was similar to that of K, with about 85% lost within the first 5 days (Fig. 9). The half-life was calculated to be 1.1 days.

Loss of Mg closely followed that of C, with about 46% released within 5 days (Fig. 10). This high rate continued on to day 30 with a total of 86% being lost.



$y = 1.194 - 0.020x + 0.00019x^2$ ($r^2 = 0.96$)

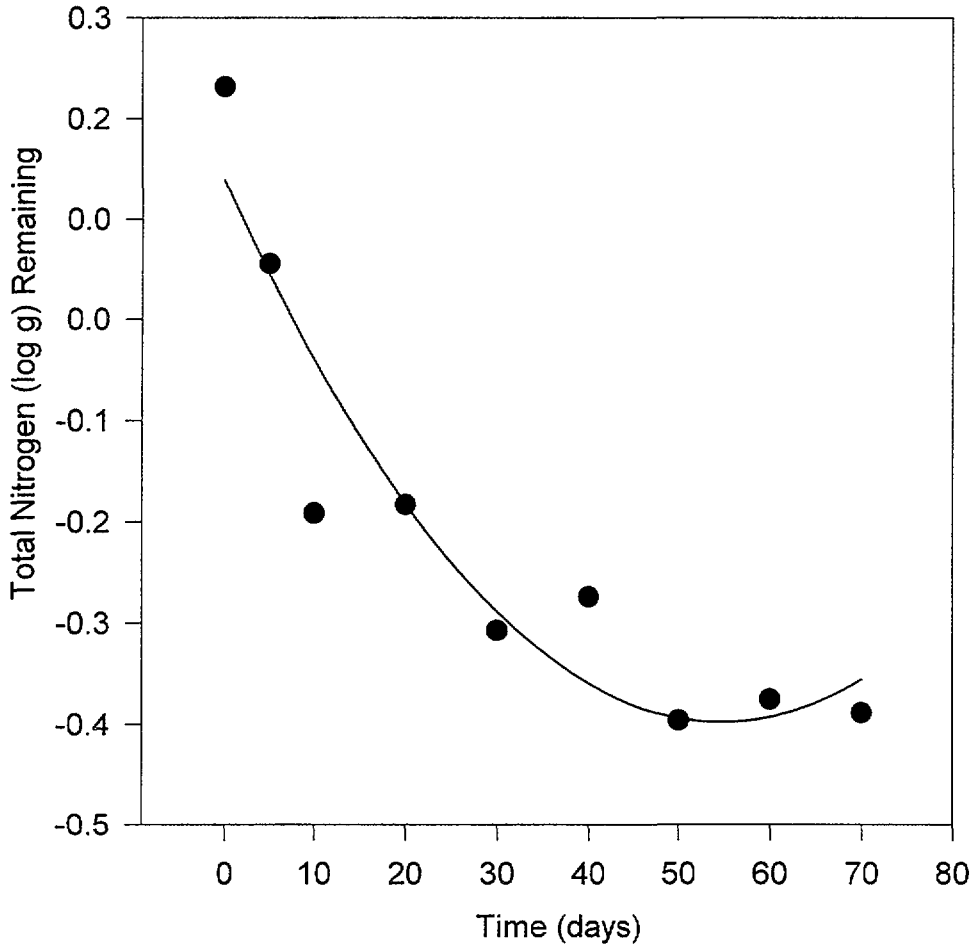
FIG. 3. Gliricidia sepium *leaf decomposition: C loss.*

3.3. Sweet corn alley-cropped with *Gliricidia sepium*

The treatments had significant effects on corn dry-matter yields and N uptake.

3.3.1. Dry-matter yields

In general, corn-stover yields increased from crop 1 to crop 3 except for the "root alone" and "root + hedgerow" treatments (Table X). The highest dry matter in crop 1 occurred in the "leaf + root" treatment, which was significantly higher than that of the control. However, no significant differences were observed between "root alone" and "leaf + root," irrespective of the presence or absence of hedgerow. Leaf prunings alone resulted in yields comparable to the control. There were similar trends in the second crop except that "leaf alone," and most of the other treatments resulted in dry-matter yields significantly higher than that of the control. The control plots of crop 3 produced plants of higher dry weight; and "control + hedgerow" gave a yield that was significantly higher than all others. The "root alone" and "root + hedgerow" treatments resulted in relatively low stover yields.



$$y = 0.139 - 0.0196x + 0.00018x^2 \quad (r^2 = 0.89)$$

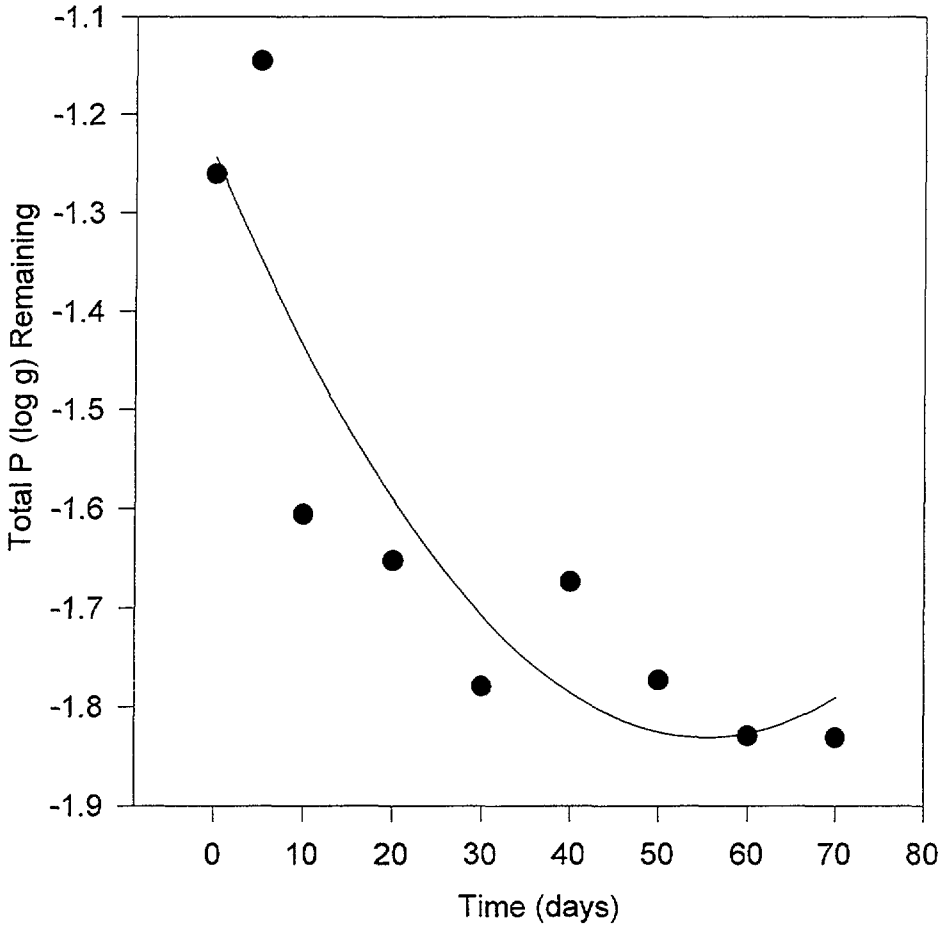
FIG. 4. Gliricidia sepium *leaf decomposition: N loss.*

Although no significant differences in ear yield were discerned among treatments, the trends in crop 2 corresponded with those for stover (Table XI). More-pronounced differences occurred in crop 3, again with trends similar to those for stover.

3.3.2. N concentration

In crop 1, the highest N concentration, obtained in corn stovers in the "leaf alone" treatment, was significantly greater than those produced by the other treatments (Table X). By contrast, in crop 2, the "leaf + root" combination with and without hedgerow resulted in the highest stover %N values; but, as with crop 1, the control and "root alone" treatment still resulted in relatively low N concentrations. Treatment effects in crop 3 were similar to those in crop 2 with values generally lower than those in crop 1.

The %N values for ears (Table XI) were generally higher than for stovers (Table X) for all treatments, with similar trends.



$$y = -1.23 - 0.021x + 0.0019x^2 \quad (r^2 = 0.81)$$

FIG. 5. Gliricidia sepium *leaf decomposition: P loss.*

3.3.3. N uptake

In crop 1, "leaf + root" produced the highest accumulation of N in sweet-corn stover (Table X), although not significantly greater than with four other treatments; the lowest uptake value was obtained with the control treatment. The trends in crop 2 were similar, with "leaf + root + hedgerow" and "leaf + root" showing the highest values. In crop 3, leaf prunings alone and in combination with root or hedgerow resulted in significantly higher N uptake values than the control. The lowest N uptake was by "root alone" and "root + hedgerow." Nitrogen uptake values in plants generally increased from crop 1 to crop 3; however, "root alone" and "root + hedgerow" showed declining trends.

The N contents of the ears (Table XI) were generally lower than those of the corresponding stovers (Table X), but with similar trends of responses to the treatments for crops 2 and 3.

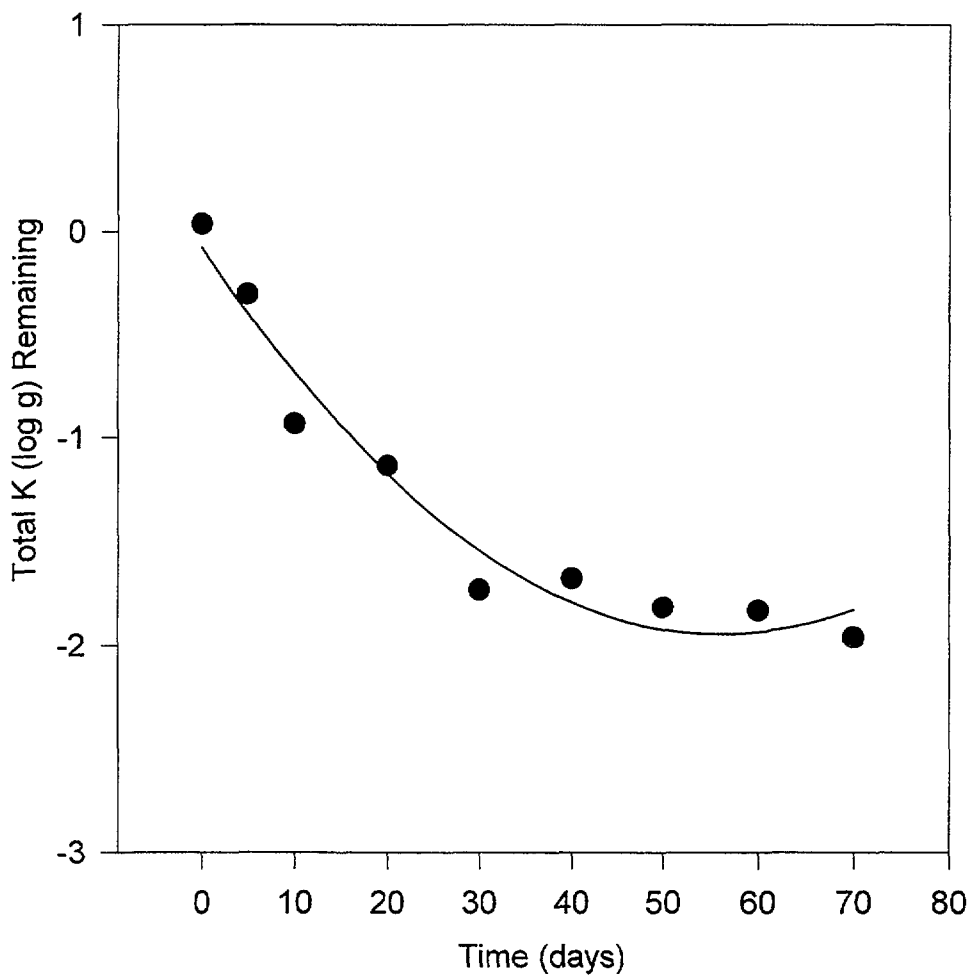
3.3.4. N derived from treatments

The highest contributions to stover N of corn crop 1, 63 and 57%, came from the "leaf alone" and "leaf + hedgerow" treatments, respectively." Trends in crop 2 differed slightly in that "leaf + root + hedgerow" made the highest contribution to N uptake, 39%, with "leaf + hedgerow," "leaf + root" and

"leaf alone" treatments giving insignificantly different %NdfT values. As in crop 1, the lowest contributions were from the "root alone," "root + hedgerow" and "control + hedgerow" treatments. In crop 3, leaf prunings alone or in combination with roots or hedgerow made the highest contributions to N uptake (25-33%). Again, little N originated from roots. Trends in ear-N accumulation were generally similar to those for stovers in crops 2 and 3 although the values were lower (Table XIII). On the whole, the N derived from the prunings alone, or when combined with roots, in the presence or absence of hedgerow increased from crop 1 to 3. The contribution of the roots declined in crop 3.

3.3.5. Competition between hedgerow and crop

No significant differences in ^{32}P activity in hedgerow plants were observed from the point of application up to 4.2 m, with the "leaf + root" treatment or the control plots at 4 weeks after fertilizer application (WAF) (Table XIV). Where prunings were applied, highest activity was observed at 1.7 m from the ^{32}P micro-plot, insignificantly different from regrowths on trees at 2.2 m, but significantly higher than those at 3.2 and 4.2 m. However, there were no differences between the activities of regrowths at 2.2, 3.2 and 4.2 m from the point of application for all treatments. The situation remained unchanged at 6 WAF except that significant differences were observed in the "leaf + root" plot.



$$y = -0.077 - 0.067x + 0.00595x^2 \quad (r^2 = 0.96)$$

FIG. 6. Gliricidia sepium *leaf decomposition: K loss.*

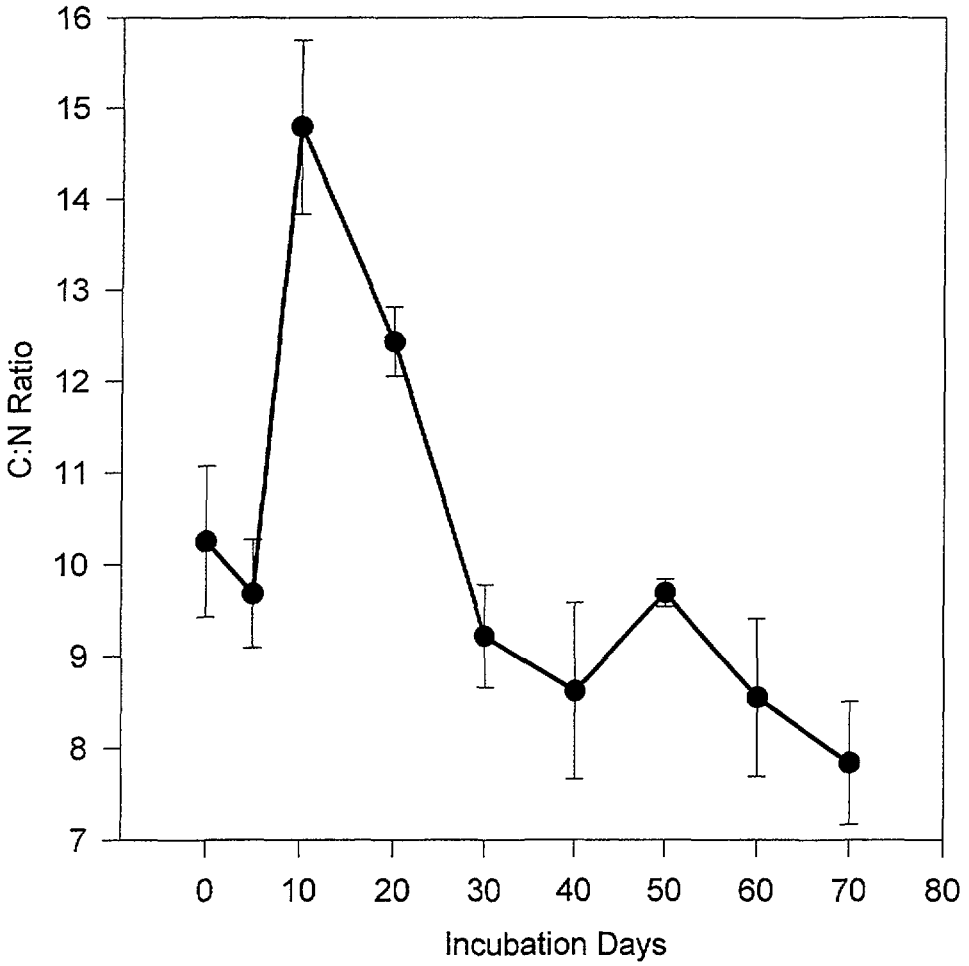


FIG. 7. Gliricidia sepium *leaf decomposition: C:N ratio.*

4. DISCUSSION

4.1. Nitrogen fixation by *G. sepium*

The ^{15}N enrichments of all plant parts showed descending trends from year to year, probably due to declines in the ^{15}N:^{14}N ratio in the soil [34]. The %Ndfa values ranged from 13 to 26 for trees pruned four times per year, 0 to 29 for trees pruned twice per year, and 0 to 50 for unpruned trees, indicating that field-grown *G. sepium* trees are not high N_2 fixers, even though others working with plants in pots and in concrete cylinders have reported otherwise [21,22]. Unpruned trees showed variable %Ndfa values, possibly due to growth-rates differences between fixing and reference trees. The roots of the reference trees may have explored more deeply than did those of *G. sepium*, resulting in lower ^{15}N-enrichment values.

Anomalous aspects of the data indicate difficulties in working with trees, e.g. %Ndfa values in excess of 50% were obtained with plants that apparently did not accumulate N, precluding the calculation of fixed N (Table VII). Large tree-to-tree variation in vigour is contributory.

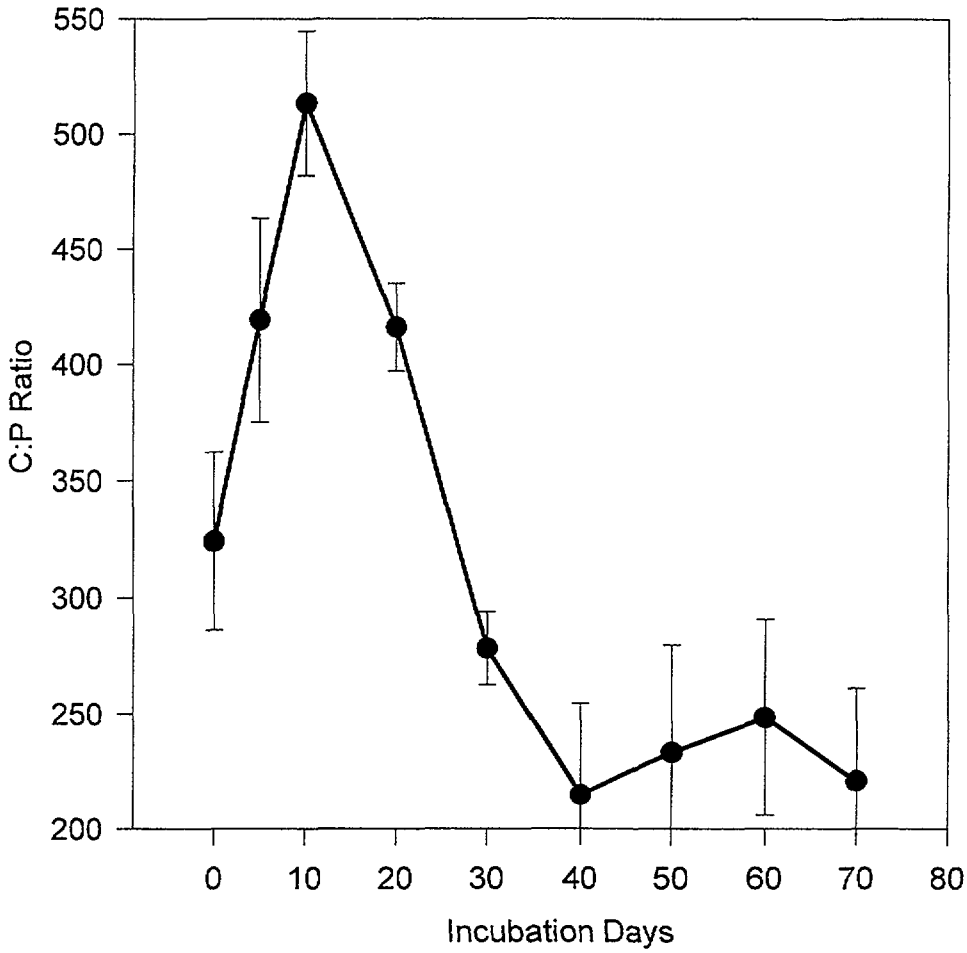
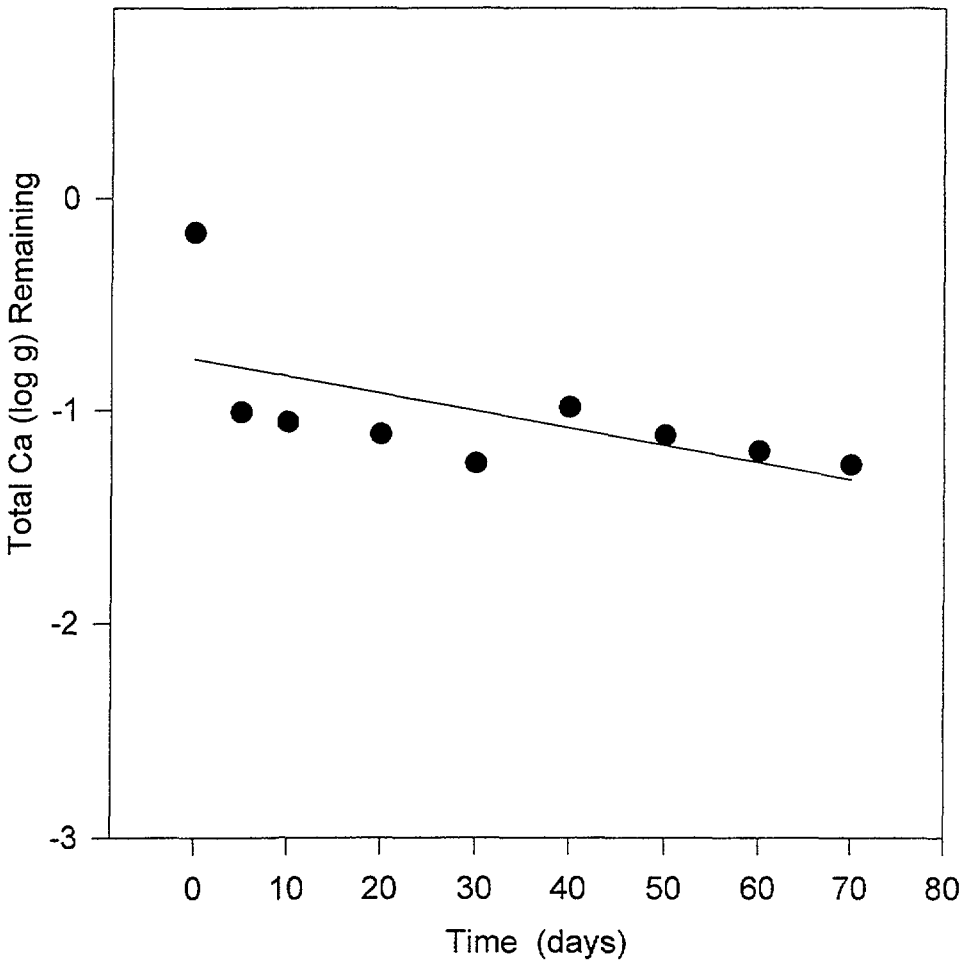


FIG. 8. Gliricidia sepium *leaf decomposition: C:P ratio.*

4.2. *Gliricidia sepium* leaf decomposition

Nutrient concentration, quality, soil biotic and environmental factors influence decomposition rates and patterns of nutrient release from plant materials [14,17,28,35]. The fast decomposition rate of the *G. sepium* leaves is attributable to relatively higher nutrient concentrations (see 3.2.) [35].

Initially rapid release of nutrients and C followed by a much-reduced rates is consistent with an initial leaching phase in the decomposition process [12,36,37]. The relatively slower rate of release of nutrients after the leaching phase could be due to relative increases in recalcitrant fractions, indicating dominance of soil fauna [27]. After the initial leaching phase for N, there was an accumulation or no-release stage followed by another release phase. Increases in C:N and C:P ratios (Figs. 7 and 8) indicate higher rates of release of N and P relative to C, possibly due to high proportions of N and P in soluble fractions.

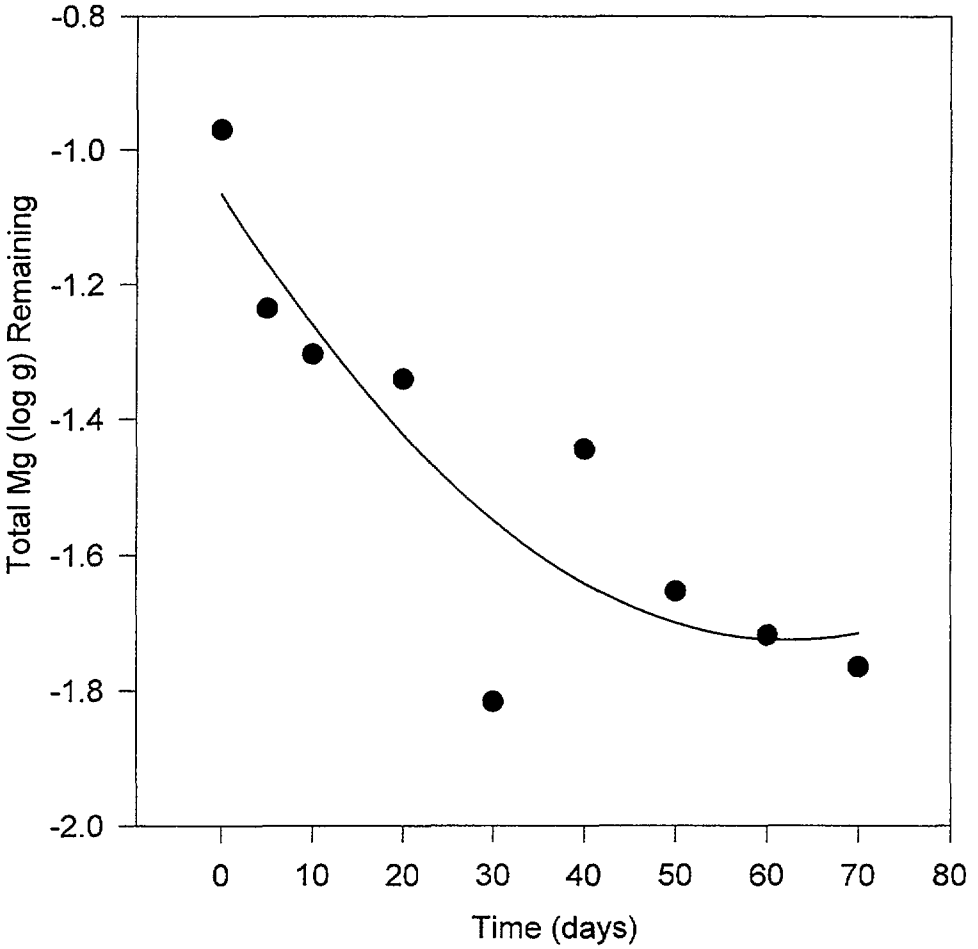


$$y = -0.758 - 0.0082x \quad (r^2 = 0.37)$$

FIG. 9. Gliricidia sepium *leaf decomposition: Ca loss.*

The rapid early loss of K indicates that leaching is the dominant mode of its release. Similar observations have been reported before [12,14,35], attributable to high mobility of K and Ca. The rapid leaching rates of Ca and Mg are also consistent with previous observations [11,38]. Apparently, leaching proceeds until a critical minimum concentration is reached. Our data have important implications for synchronizing nutrient release with crop-nutrient needs in low-input cropping systems. As such, split applications of fresh organic inputs may be the most efficient management option.

The fact that no pronounced changes occurred in the rate of nutrient release with precipitation (see Fig. 1) suggests that the influence of the latter is minimal above a threshold level that affects fauna activity [17]. Frequency of rainfall should be considered when establishing suitable nutrient-management systems in the humid tropics to ensure that necessary minimum soil-moisture content is achieved.



$$y = -1.066 - 0.021x + 0.00017x^2 \quad (r^2 = 0.79)$$

FIG 10. Gliricidia sepium *leaf decomposition: Mg loss.*

4.3. Sweet corn alley-cropped with *Gliricidia sepium*

The importance of leguminous green manures in maintaining soil fertility and crop productivity has been widely reported [8,16,19,39,40]. In view of the rapid decomposition of *G sepium* leaves, increases in growth and yields of the corn were attributable to the release of nutrients from the decomposing leaf prunings. In contrast, roots are generally believed to decompose at a slower rate. Although the results from our root-litter decomposition studies are contradictory, the longer response times may be due to high lignin content. In addition to this, the onset of the process itself could be delayed as a result of slower root-cell desiccation and mortality in the relatively moist environment of the sub-soil. The mixture of roots and leaves is expected to have a decomposition rate between the two extremes due to initial immobilization of nutrients released by the leaves.

The situation in crop 1 was such that the nutrients from prunings could not have been available at the time of crop demand. Most of the N could have been lost before optimum corn-root development occurred. Also, nutrient availability and uptake could have been severely limited by soil acidity and aluminium toxicity, factors to which corn is sensitive, hence the application of lime for the second crop.

Nonetheless, initial N immobilization and subsequent release in the "leaf + root" treatment probably reduced the losses resulting in the relatively high dry-matter yield in this treatment in crop 1. Other workers reported low residue-N recovery by the first maize crop when alley-cropped with *Leucaena* [18,19], attributed to immobilization of N in the soil organic matter and uptake by the hedgerows [11,41,42].

The pronounced effect of the prunings in crop 3 may have been due to the nutrients being available in better synchrony with crop demand [12,13], considering that the period of greatest need for N in maize is between 30 and 60 days after planting [16,43]. Similarly, since the control plants were supplied with readily available nutrients in the form of synthetic fertilizers, they would be expected to perform better than with the treatments. The relative decline in yields in the presence of hedgerows is attributable to competition effects [44].

The leaf treatments resulted in higher soil mineral N levels (Table XV). Competition with hedgerow could have been masked by the high nutrient availability resulting from decomposition of leaves and roots [45]. It is apparent that the competition effect of the hedgerows became more pronounced in conditions of relatively low nutrient availability and with proximity (Tables XIV). For instance, a substantial competition effect was observed with "root + hedgerow." Leaf prunings, on the other hand, provide more nutrients at the time of crop demand thereby masking the effect of the hedgerows. The passages created by decaying roots could also improve soil structure for root development and so enhance the exploitation of soil moisture and nutrient resources.

In summary, our data show that although N_2 fixation is not high in *G. sepium*, its leaf prunings improved N uptake and yields in sweet corn much more than did the residual roots. Application of leaf prunings at 21 and 45 days after planting proved to be most beneficial to the crop – the importance of applying organic materials to provide nutrients at the time of crop demand was thus confirmed. There was evidence of competition from hedgerow plants for P applied to alley-cropped corn; further research is needed for quantification. The practical implication of the data as a whole is that there is no advantage of the cut-and-carry system over permanent hedgerows, provided that prunings are applied at the time of crop demand.

TABLE X. DRY-MATTER YIELDS, N CONCENTRATION AND TOTAL-N UPTAKE BY CORN STOVER, CROPS 1, 2 AND 3

Treatment	Dry matter			N concentration			Total N		
	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3
	(kg ha^{-1})			(%)			(kg ha^{-1})		
Control	898d[a]	1751b	3284b	1.8bc	1.2bcd	1.1bc	15.8c	21.7c	36.4b
Leaf alone	1082cd	2783a	3049b	2.4a	1.3abc	1.5a	26.2bc	36.2bc	45.1a
Root alone	2175a-d	2628a	1849d	1.5c	1.2cd	0.94cd	31.8abc	31.8cd	17.4c
Leaf + Root	3158a	2745a	3288b	1.8bc	1.5a	1.5a	56.2a	40.1ab	48.7a
Cntrl+Hdgrw	1628cd	2401ab	4278a	1.8bc	1.1d	1.1b	28.5bc	26.9de	49.2a
Leaf+Hdgrw	2686ab	2713a	3410b	1.6bc	1.3abc	1.5a	43.2ab	35.5bc	52.5a
Root+Hdgrw	2421abc	2453ab	2413c	1.5c	1.4ab	0.93cd	35.3ab	35.5bc	22.4c
Lf+Rt+Hgrw	1836a-d	3154a	3586b	1.9b	1.5a	1.4a	35.4ab	46.4a	51.6a

[a]Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE XI. DRY MATTER, %N AND TOTAL N OF CORN EARS, CROPS 2 AND 3

Treatment	Dry matter		N concentration		Total N	
	Crop 2	Crop3	Crop 2	Crop 3	Crop 2	Crop 3
	(kg ha^{-1})		(%)		(kg ha^{-1})	
Control	990a[a]	1084ab	1.7ab	1.8bc	16.8ab	19.19ab
Leaf alone	1092a	1234ab	1.8ab	2.1a	19.8ab	26.3a
Root alone	1085a	575c	1.8ab	2.0abc	19.2ab	11.3b
Leaf + Root	1278a	1193ab	1.6ab	2.0ab	21.1ab	24.5a
Cntrl+Hdgrw	993a	1546a	1.5b	1.8bc	15.3b	27.5a
Leaf+Hdgrw	1443a	1084ab	1.7 ab	1.8abc	25.0a	19.6ab
Root+Hdgrw	1237a	808bc	1.9a	1.7c	23.6a	13.4b
Lf+Rt+Hgrw	1034a	1488a	1.9a	1.9abc	19.7ab	27.8a

[a]Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE XII. NITROGEN IN STOVERS DERIVED FROM THE TREATMENTS, CROPS 1, 2, 3

Treatment	NdfT			NdfT		
	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3
	(%)			(kg ha^{-1})		
Control	0.0c[a]	0.0d	0.0b	0.00c	0.00d	0.00b
Leaf alone	63a	23ab	32a	6.06b	9.16abc	14.0a
Root alone	0.0c	26abc	6.3b	0.00c	7.76bcd	1.12b
Leaf + Root	26b	31abc	33a	6.30b	12.7ab	16.3a
Cntrl+Hedgerow	0.0c	10cd	10b	0.00c	2.52cd	4.66b
Leaf+Hedgerow	57a	34ab	25a	11.0a	11.4abc	13.3a
Root+Hedgerow	7.6bc	14cd	0.0b	1.37c	5.78bcd	0.00b
Lf+Rt+Hdgerow	24b	39a	30a	2.84 c	18.0a	60.7a

[a]Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE XIII. NITROGEN IN EARS DERIVED FROM THE TREATMENTS, CROPS 2 AND 3

Treatment	NdfT		NdfT	
	Crop 2	Crop 3	Crop 2	Crop 3
	(%)		(kg ha^{-1})	
Control	0.0c[a]	0.0d	0.00c	0.00d
Leaf alone	24abc	36a	4.95abc	9.38a
Root alone	27ab	9.0cd	4.27abc	0.72cd
Leaf + Root	38a	27ab	8.55a	7.27ab
Cntrl+Hedgerow	10c	18bc	1.62bc	4.82bc
Leaf+Hedgerow	37a	23abc	8.71a	4.57bcd
Root+Hedgerow	15bc	0.92d	4.27abc	0.17d
Lf+Rt+Hdgerow	37a	37a	6.96ab	10.26 a

[a]Means in the same column fc ed by the same letter are not significantly different at ($P \leq 0.05$).

TABLE XIV. ^{32}P ACTIVITY IN *GLIRICIDIA SEPIUM* REGROWTH WITH DISTANCE FROM POINT OF APPLICATION AT 4 AND 6 WEEKS AFTER ^{32}P APPLICATION

Time / Treatment	^{32}P activity at distance from point of application			
	1.7 m	2.2 m	3.2 m	4.2 m
	($\log_{10}$ ^{32}P activity)			
4 weeks				
Control + Hedgerow	2.886abc[a]	2.957abc	2.798abc	2.688abc
Leaf + Hedgerow	3.065a	2.766abc	2.521c	2.529bc
Root + Hedgerow	3.039ab	2.879abc	2.60abc	2.503c
Lf + Rt + Hedgerow	2.947abc	2.740abc	2.531bc	2.517c
6 weeks				
Control + Hedgerow	2.679abcd	2.537bcd	2.425d	2.571abcd
Leaf + Hedgerow	2.791ab	2.468d	2.463d	2.475cd
Root + Hedgerow	2.765abc	2.610abcd	2.477cd	2.484cd
Lf + Rt + Hedgerow	2.832a	2.509bcd	2.510bcd	2.502bcd

[a]Numbers followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE XV. MINERAL-N STATUS OF TREATMENT PLOTS PRIOR TO ESTABLISHMENT OF CROP 3

Treatment	Mineral N (mg kg^{-1})
Control	8.91c[a]
Leaf alone	24.6abc
Root alone	16.1bc
Leaf + Root	14.3bc
Control + Hedgerow	11.5c
Leaf + Hedgerow	31.3ab
Root + Hedgerow	14.0bc
Leaf + Root + Hedgerow	34.8a

[a]Numbers followed by the same letter are not significantly different ($P \leq 0.05$).

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QUANTITATIVE ESTIMATES OF UPTAKE AND INTERNAL CYCLING OF ^{15}N-DEPLETED FERTILIZER IN MATURE WALNUT TREES

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Abstract

In mature fruit trees, internal recycling is an important source of N for the growth of new wood, leaves and fruits. Using ^{15}N-depleted fertilizer, i.e. ^{14}N-enriched, N-uptake efficiency and the magnitude of internal N cycling were studied in mature walnut trees. Two kg of ^{14}N-labelled ammonium sulfate N were applied per tree, and compartmentation of N was followed over a period of 6 years by analyzing catkins, pistillate flowers, leaves and fruits each year for total N content and isotopic composition. Subsequently, two of the six labelled trees were excavated and analyzed for labelled-N content. The data indicate that mature walnut uses most of the N accumulated from soil and fertilizer for storage purposes, to be remobilized for new growth within 2 years, and about half of the total-N pool in a mature tree is present as non-structural compounds, available for recycling.

1. INTRODUCTION

Increasing societal concern with nitrate contamination of ground water has brought orchard fertilization practices under increasing scrutiny [1]. Horticultural studies that typically monitor site-specific tree responses to various fertilizer application rates [2,3] may have local relevance, but do not necessarily promote more efficient usage of fertilizer N. That is, a quantitative relationship is seldom established between the level of fertilizer N applied and actual N usage by the tree. That fertilizer-N recovery and annual-N usage by mature trees has received scant attention may be associated with the difficulties inherent in excavation and analysis of their large woody biomass and/or the expense associated with the use of stable isotope methodology. The possibility of meaningful extrapolation to mature trees from study of immature trees, even of the same species, appears remote because the magnitude and patterns of tree N demand and the availability of N from internal cycling vary with age [4]. Also, unlike immature trees, the N contents and biomass of the "perennial" parts of mature trees remain essentially constant from year-to-year. Consequently, in mature trees, net annual N uptake may reflect the quantity of N lost in deciduous and transient organs including the harvested fruit, leaf litter, pruning wood, abortive immature reproductive organs and root turnover [5,6].

Besides the year-to-year constancy of the "perennial" biomass in mature trees, the current study was founded on the following assumptions:

- accumulation of labelled N is essentially complete within one year of application [7,8] and, perhaps within 2 to 3 months of application [9],
- uptake of soil N during dormancy, i.e. between autumn leaf fall and spring growth resumption, is minimal [10,11].

Our objectives were to a) determine annual N uptake by mature walnut trees, b) determine the magnitude of internal cycling, i.e. size of the N-storage pool in "perennial" tree parts. (Other methods, such as whole-tree N budgets are often in error because root systems are not fully recovered, leaf-N resorption prior to senescence is not quantified adequately, etc. [11], c) assess the efficiency of fertilizer-N recovery by mature, field-grown trees under commercial conditions, and d) estimate the magnitude of N demand relative to total annual-N usage.

2. MATERIALS AND METHODS

2.1. Plant material

Mature (9-year-old) walnut trees (*Juglans regia* L. cv. Hartley) were growing in a Tajunga loamy sand (mixed thermic typic xeropsamment) in a commercial orchard at Oakdale, California (37°41'N, 120°50'W). 'Hartley' was selected because it is the most widely grown walnut cultivar in California.

2.2. Isotopic labelling

Six individual tree replicates were pulsed with a 40% solution (w/v) of ^{15}N-depleted $(NH_4)_2SO_4$, at 1.5 kg N $tree^{-1}$ equivalent to 180 kg N ha^{-1}, during February (year zero) as described by Deng et al. [13]. Labelled N was "chased" with annual applications of 2 kg $tree^{-1}$ of non-labelled $(NH_4)_2SO_4$ through year 5.

2.3. Sampling

Leaves and fruit were sampled periodically between March and November throughout each of the 6 years of experimentation. At each sampling date, five representative fruiting spurs between 2 and 4 m from the ground and within 0.6 m of the tree's periphery were excised and transported back to the laboratory packed in ice. Also, twenty catkins and twenty pistillate flowers per tree were sampled each year in March and April, respectively.

2.4. Total N and isotope analysis

The total-N contents of tissue sub-samples were determined by a modified macro-Kjeldahl method [14]. Nitrate N was included in the total-N measurement by the pretreatment of samples with salicylic acid. Analysis of labelled N was performed by Isotope Services, Los Alamos, NM. Samples were oxidized to N_2 gas, and isotopic composition determined by mass spectrometry. The results are expressed as ^{14}N atom % excess and N derived from labelled fertilizer, on the basis of standard conversions [15,16]. The fraction derived from labelled fertilizer in each organ or tree part was then multiplied by its N content to determine recovery of labelled N.

2.5. Tree excavation

Two of the six labelled trees were excavated during dormancy (February) exactly 6 years after labelled-N application. Each tree was separated into three fractions above the graft union: new wood, i.e. wood produced during the previous growing season, canopy branches, and trunk, i.e. the part remaining above the graft union after removal of new wood and canopy branches. Two fractions were below the graft union: the rootstock stump and the root, which were excavated using a back-hoe. Soil from a pit with radius equivalent to half the distance to the next tree in the row and 2 m deep was screened using pitchforks, and all visible roots were collected. An unknown quantity of fine roots, presumably a small fraction, escaped detection and, therefore, was not analyzed.

Fresh weights of the various "perennial" fractions were obtained using a load-cell scale. "perennial" organs were passed through a mechanical chipper, fresh weights of sub-samples determined. All samples were transported back to Davis and dried to a constant weight at 60°C. Determination of fresh-to-dry weight ratios permitted subsequent calculation of whole-tree N contents. Samples were ground in a Wiley mill to pass a 30-mesh screen and analyzed for total and labelled N as described above.

2.6. N removal in fruit and abscised leaves

The amounts of N loss per tree in senescent leaflets (leaf litter) and harvested crop were determined annually. Twenty mature fruits were collected randomly each year from the four quadrants of each tree just prior to harvest, separated into seed, shell and hull, and oven-dried, weighed and analyzed for total N and isotope composition as described previously.

Each tree was harvested individually, and the crop was weighed. Total N and isotopic N removal in the harvested crop were determined annually for each tree as the product of total dry matter yield, percent N derived from labelled fertilizer and N concentration.

Nitrogen losses in senescent leaves were estimated using the previously established ratio of 4.29 leaves per fruit (unpublished data). Thus, at harvest, total fruit weight per tree was determined. The average number of fruit in a 2 kg sub-sample was counted to permit extrapolation to total number of fruits per tree. The product of fruit number per tree and 4.29 provides a realistic estimate of leaf number. The product of leaf number, average leaf dry matter, and N concentration at leaf abscission represents the amount of N carried to the orchard floor in leaf litter from each tree.

2.7. Calculations

The percent annual depletion (PAD) calculated as follows.

$$PAD\ (\%) = \left(\frac{NdfF_n - NdfF_{n+1}}{NdfF_n}\right) \times 100 \qquad (1)$$

where

$\%NdfF_n$ is N derived from labelled fertilizer in year n,
$\%NdfF_{n+1}$ is N derived from labelled fertilizer in the same organ type and the same developmental stage in the following year.

PAD is an indication of the magnitude by which the N in the tree is diluted with soil N [17]. The validity of this interpretation and use is limited to fully-grown trees of which biomass and N content remain stable from year-to-year.

TheNdfF is calculated as follows.

$$\%NdfF = \left(\frac{\text{Atom \% } {}^{14}\text{N excess}_{(plant)}}{\text{Atom \% } {}^{14}\text{N excess}_{(labelled\ fertilizer)}}\right) \times 100 \qquad (2)$$

Percent fertilizer use efficiency (%FUE) is the fraction of the total amount of the labelled fertilizer-N applied that is recovered by the tree and is calculated as:

$$\%FUE = \left(\frac{g\ NdfF_{(component)}}{g\ {}^{14}N\ applied}\right) \times 100 \qquad (3)$$

$$\text{where } g\ NdfF = \frac{\%NdfF \times \text{total } N_{(component)}}{100} \qquad (4)$$

TABLE I. ^{14}N-ENRICHMENT VALUES OF TREE COMPONENTS

Component/ Month	Years after isotope application 0	1	2	3	4	5
	(atom %^{14}N excess)					
Catkins/ March	0.0000[a] ±0.0010	0.0689 ±0.0089	0.0353 ±0.0041	0.0288 ±0.0046	0.0143 ±0.0008	0.0105 ±0.0007
Flower/ April	0.0040` ±0.0013	0.0467 ±0.0041	0.0352 ±0.0039	0.0214 ±0.0020	0.0151 ±0.0005	0.0106 ±0.0005
Leaves/ April	0.0055 ±0.0019	0.0466 ±0.0043	0.0324 ±0.0010	0.0154 ±0.0013	0.0139 ±0.0008	0.0088 ±0.0010
Fruit/ June	0.0449 ±0.0066	0.0481 ±0.0037	0.0345 ±0.0020	0.0149 ±0.0010	0.0138 ±0.0008	0.0085 ±0.0008
Leaves/ June	0.0233 ±0.0032	0.0466 ±0.0043	0.0335 ±0.0020	0.0154 ±0.0020	0.0139 ±0.0008	0.0088 ±0.0010
Fruits/ October	0.0651 ±0.0061	0.0463 ±0.0024	0.0294 ±0.0019	0.0130 ±0.0007	0.0124 ±0.000 2	0.0090 ±0.0008
Leaves/ October	0.0507 ±0.0040	0.0453 ±0.0027	0.0279 ±0.0016	0.0131 ±0.0006	0.0122 ±0.0003	0.0085 ±0.0006
Leaves/ November	0.0359 ±0.0024	0.0401 ±0.0034	0.0225 ±0.0015	0.0140 ±0.0009	0.0116 ±0.0008	0.0080 ±0.0007

[a]Mean ± standard error (n= 6).

The turnover of ^{14}N stored within the tree followed an exponential decay function:

$$N_t = N_o(e^{-k.t}) \quad (5)$$

where

N_o is the atom %^{14}N excess value at time = 0,
N_t is the atom %^{14}N excess value at t years,
k is the decline constant,
t is the time since the application of ^{14}N-enriched fertilizer.

3. RESULTS

3.1. Atom %^{14}N values and PAD

The atom %^{14}N excess of all the tree components declined to approximately 0.01 at 6 years after the pulse of labelled fertilizer N, but remained significantly different from natural abundance, 99.6315 atom %^{14}N (Tables I and II). One year after the application of the ^{14}N-enriched fertilizer, the enrichment values for leaves and fruits became similar and remained so for the balance of the study.

TABLE II. ^{14}N ENRICHMENT IN TREE COMPONENTS 6 YEARS AFTER THE APPLICATION OF LABELLED FERTILIZER

Tree component	Atom %^{14}N excess
New wood	0.0084±0.0009[a]
Canopy	0.0124±0.0014
Trunk	0.0118±0.0011
Root stock	0.0124±0.0014
Root	0.0118±0.0027

[a]Mean±standard error (n=2).

TABLE III. PERCENT ANNUAL DEPLETION OF LABELLED FERTILIZER IN TREE COMPONENTS, 2 AND 3 YEARS AFTER ITS APPLICATION

Tree component/ Month	Years after labelled N application	
	2	3
	(PAD %)	
Catkins/April	48±3	25±8
Flowers/April	28±9	37±9
Leaves/April	28±7	53±3
Fruit/June	26±7	57±2
Leaves/June	26±8	54±2
Fruits/October	36±4	55±3
Leaves/October	38±3	53±2
Leaves/November	43±3	36±6
Average	34 ±2	46±2

[a]Mean ± standard error (n= 6).

In the first year (i.e. the year of isotope application), no labelled fertilizer was detected in the early-spring catkins (Fig. 1, Table I). Similarly, in the first year, leaves and flowers collected in the spring, showed lower atom %^{14}N excess values than did fruits. In subsequent years, the ^{14}N enrichment values for flowers were generally higher than for leaves and fruits (Table I).

At the time of tree excavation, 6 years after the pulse of labelled fertilizer was applied, the ^{14}N enrichment in new wood was significantly lower than in the trunk, root and canopy (Table II).

The NdfF values varied significantly among annual organs during the year of application, i.e. year zero (Fig. 1). The atom %^{14}N excess of annual organs increased with time during the season (Table I). Thus, catkins, the earliest maturing organ, relied entirely on unlabelled storage N, whereas organs developing later exhibited progressively greater labelling with the influx of ^{14}N-enriched N. The only exception to that generality was senescing leaves at the November sampling in which the labelling declined relative to that of leaves sampled in October. In subsequent years, 1 through 5, during which time the within-tree storage pool was labelled, the atom %^{14}N excess in annual organs declined between March and October with the progressive influx of unenriched N (Table I).

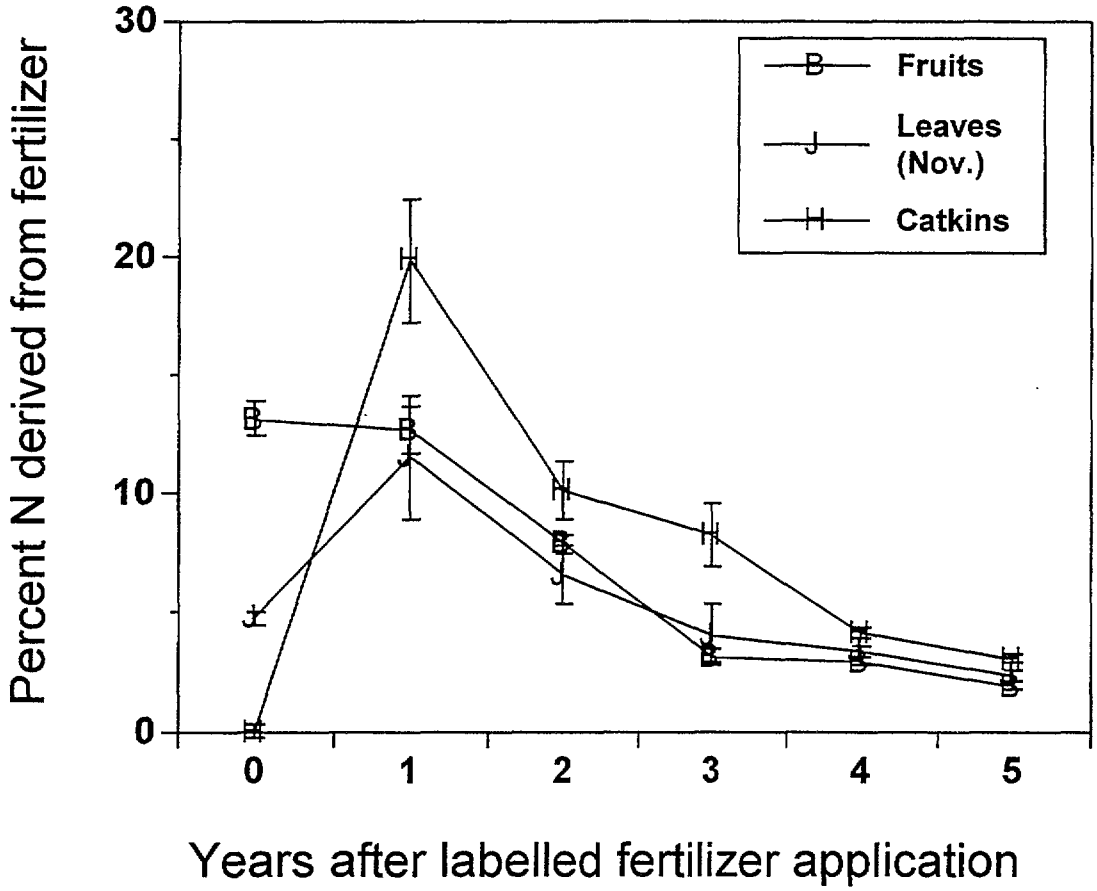

FIG. 1. Percentage of N derived from ^{14}N-enriched fertilizer in fruits, catkins and leaves of mature walnut trees over a 5- year period.

The PAD values for various components were followed throughout the second and third growing seasons (Table III). As it is based on the NdfF (Equation 1), it is possible to calculate its value for the entire experimental period. However, the differences in NdfF values from season to season became too small towards the end of the experiment to support reliable estimates (data not shown). Two years after the application of the ^{14}N-enriched fertilizer, the atom $\%^{14}N$ excess values between two consecutive years still differed by >0.01 and the PAD values ranged from a high of 48% for the catkins to a low of 26% for fruits and leaves in June (Table III). In the following year, the reverse occurred, with the lowest PAD value of 25% for catkins whereas the highest value, 57%, was observed for fruits, collected in June. The PAD values averaged across all tree components and throughout the growing season was 46% in the third year and 34% in the second. We have used the average of years 2 and 3 (PAD = 40%) to provide an annual estimate of internal N cycling.

3.2. Total-N accumulation and fertilizer recovery

The 5-year average of the N content of fruits and senesced leaves (i.e. sampled just prior to abscission) was estimated at 815 and 90 g tree^{-1}, respectively (Table IV). Fruit N content, however, fluctuated annually with the yield. A complete N budget for the entire tree was made 6 years after the application of fertilizer. The canopy branches accounted for 1,366 g N tree^{-1}, 44% of the total, followed by root and the trunk. New wood contained 57 g N tree^{-1}. In total, the N content was, on average, 3,096 g N tree^{-1} of which 2,473 g, almost 80%, was above ground, and 962 g, 31%, was in components formed the previous year: fruits, leaves, and new wood.

TABLE IV. TOTAL N CONTENT AND RECOVERY OF LABELLED FERTILIZER IN FRUITS AND SENESCED LEAVES OVER THE YEARS AND "PERENNIAL" TREE PARTS AT THE TIME OF EXCAVATION

Year/ Component	Total N (g tree^{-1})		TNdfF (g tree^{-1})		FUE (%)
Year zero					
Fruits	987±69[a]		120±20		6.0±1.1
Leaf litter	84±4		4±1		0.2±0.0
Year 1					
Fruits	922±65		124±14		6.2±0.7
Leaf litter	95±6		11±1		0.6±0.1
Year 2					
Fruits	581±59		49±5		2.4±0.2
Leaf litter	76±5		5±1		0.3±0.
Year 3					
Fruits	686±37		25±2		1.3±0.1
Leaf litter	67±4		3±0		0.1±0.0
Year 4					
Fruits	760±33		27±1		1.3±0.1
Leaf litter	95±5		3±0		0.2±0.0
Year 5					
Fruits	1,043±63		27±3		1.4±0.2
Leaf litter	123±7		3±0		0.1±0.0
Years 0-5					
Fruits Average	815±26[b]	Total	372±30	Total	18.6±1.5
Leaf litter	90±4		29±3		1.4±0.1
Year 6, spring tree excavation					
New wood	57±8[c]		1±3		0.1±0.0
Canopy	1,366±59		49±7		2.4±0.4
Trunk	145±25		5±4		0.2±0.0
Root stump	139±35		5±7		0.2±0.0
Root	484±99		17±7		0.9±0.4
Total N accumulation	3,096±106		-		-
N fertilizer recovery	-		478		23

[a]Mean±standard error (n=6). [b]Average for the 6 years. [c]Mean±standard error (n=2).

The greatest accumulation of labelled N in fruit and leaf occurred in the year of application and the subsequent year (Fig. 2, Table IV). Senesced leaves, i.e. leaf litter, retained only small amounts of labelled N and remained a small sink for fertilizer N. A sharp decline in the amount of

labelled N in the second year continued into year 3 (Fig. 2). Nevertheless, fruits and leaves continued to accumulate labelled N even 5 years after its application (Table IV). The maximum amount of N derived from labelled fertilizer in fruits and senesced leaves was 135 g tree^{-1}, in year 1; a slightly lower amount was recovered in year zero.

The recovery of fertilizer N (%FUE) by fruits and leaf litter reached maximum values in the first and second years following application, but never reached 7% (Table IV). For the last three years of the experiment, the annual recovery of labelled fertilizer of fruits remained just above 1%, whereas for leaves it was between 0.1 and 0.2%. The accumulated recovery of labelled fertilizer for the entire 6-year period was 19% for fruits and 1.4% for leaves.

The total amount of labelled N recovered, in leaf litter, fruits, canopy, trunk and root when the experiment was terminated, was 478 g N tree^{-1}, corresponding to 23% of the applied fertilizer (Table IV). Six years after application, 77 g or 16% of the fertilizer-N accumulated by the tree was recovered in the "perennial" components: trunk, roots and canopy.

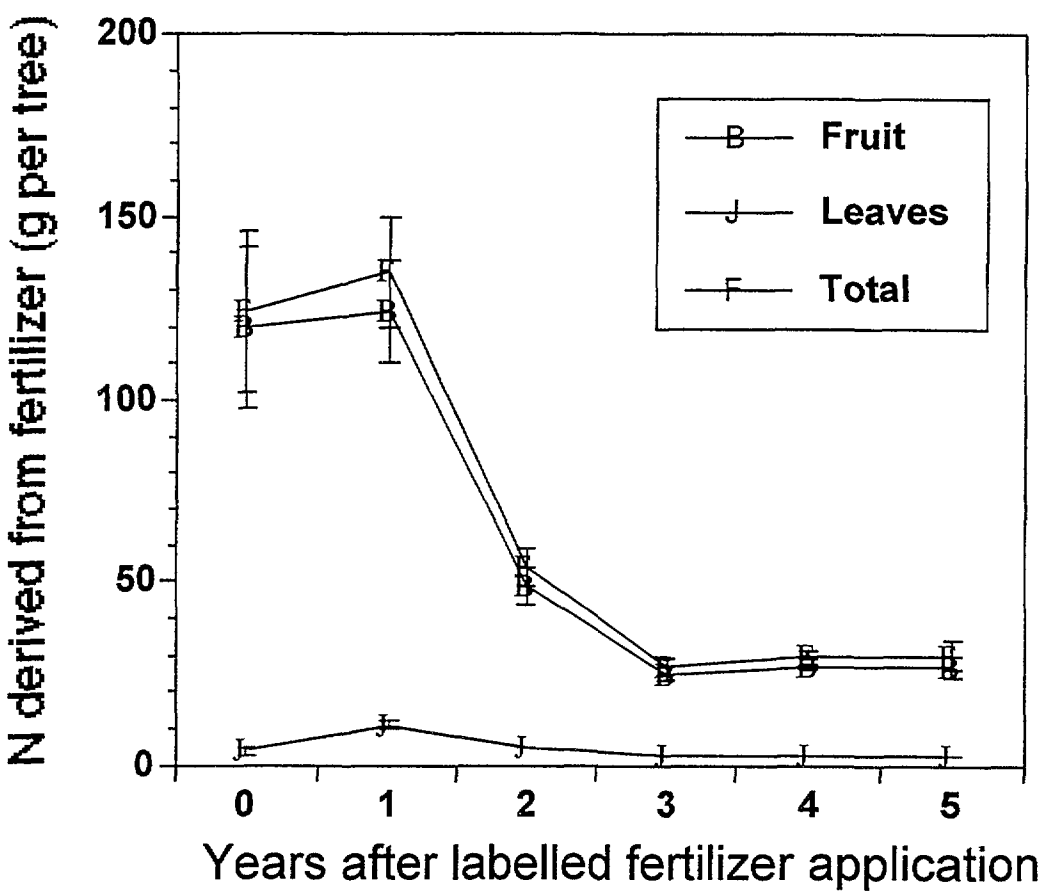

FIG. 2. Total amount of N derived from ^{14}N-enriched fertilizer in fruits, leaves and fruits + leaves of mature walnut trees over a 5-year period.

4. DISCUSSION

Despite the small difference between the natural abundance of ^{14}N in tree N, 99.6315 atom $\%^{14}N$, and the applied fertilizer, 99.9827, significant enrichments in N were observed 6 years after application. Clearly, ^{14}N-enriched, i.e. ^{15}N-depleted, fertilizer can be used successfully in long-term labelling studies to follow the fate of N in mature walnut trees as was shown for pistachio [8] and almond [17]. Whereas, conceptually, there is no difference between the use of ^{15}N-enriched or ^{15}N-depleted fertilizer, the latter costs much less, therefore, in field experiments its use results in considerable savings.

The N pool used to support growth of annual organs varies seasonally. The labelled fertilizer was applied two months prior to the maturation of, and did not become a source of N for, the catkins and was only a minor source of N for the flowers. A likely explanation is that walnut trees do not accumulate soil N in early spring, and rely completely on internal N sources for the growth and development of catkins. Similar conclusions were reached for sycamore (*Acer pseudoplatanus* L.) and Sitka spruce (*Picea sitchensis* Bong. Carr) seedlings grown under controlled conditions in sand culture [18,19] and also for immature prune [10] and mature almond trees [11].

4.1. Recovery and use efficiency of labelled N

The fruits were the dominant sink, and ultimately accounted for 78% of all the labelled fertilizer-N recovered by the trees over the 6-year period. Leaves collected just prior to abscission in the fall accounted for only 6% of the labelled N absorbed. This low value is presumably associated with N resorption in the fall. As occurs in other deciduous trees [20], about 50% of the N stored in the leaves of walnut was redistributed to "perennial" tree parts during fall senescence when proteins such as the Rubisco enzyme complex are hydrolyzed and protein content declines [21,22]. Part of the labelled ^{14}N present in the various leaf proteins is resorbed, stored and remobilized the following spring [17,23]. Because of this recycling, the importance of the leaves as a temporary sink for N would be grossly underestimated if only the labelled N in senesced leaves were considered.

After 6 years, labelled N was still present in tree components considered "perennial:" root, trunk and canopy (Tables II and IV). Labelled fertilizer-N represented <4% of the total N content in the "perennial" tree components, which corresponded to 3.7% of all the labelled fertilizer that could be accounted for in 6 years. The low recovery of the labelled fertilizer in the root, trunk and canopy is likely caused by the annual recycling to fruits, leaves and shoots. Labelled N deposited in "perennial" tree components may be stored only temporarily, with remobilization each spring and redeposition in storage in late summer/autumn. The roots of fruit trees are known to store large amounts of N during the winter months and to be an important source of N in the spring [24]. In young apple trees, ^{15}N-labelled N accumulated in the fall was stored mainly in the roots, and at least 505 of the N in the new leaves the following year were derived from root reserves [24]. A considerable concurrent decline in the concentration of root N occurred during leaf expansion.

The overall N-fertilizer use efficiency was 23% of which only 6.2%, 26% of total fertilizer-N accumulated, was recovered in fruits and leaves during the first year (Table IV). The rather low fractional recovery of labelled N in the fruits and leaves during the first year provides a strong indication that the majority of the fertilizer N was stored rather than utilized in the growth of annual organs, i.e. leaves and fruits.

Few studies have reported the percentage recovery of fertilizer-N in mature fruit trees grown under natural conditions. The recovery after 8 months of applied fertilizer of 22-year-old orange trees was dependent on the tree N status: trees that were starved for N accumulated 57% of the fertilizer, whereas trees grown under an abundance of N accumulated 40% [25]. The majority of the labelled N was found in the above-ground components with only 11% in the roots. Surprisingly, less than 5% of the label was recovered from the soil organic matter pool, therefore, N losses must have been high. Similarly, 6-year-old kiwi fruit (*Actinidia delicosa*) vines accumulated between 48 and 53% of

fertilizer N, depending on the amount applied [26]; all of the fertilizer-N uptake had occurred by the first harvest and a large portion (18-22%) was recovered in the soil where it remained largely unavailable for plant uptake during the subsequent 2 years. The removal of labelled N in harvested fruits in the first year was between 5 and 6% of the total amount applied, and the accumulated ^{15}N recovery in fruits did not exceed 85% in 3 years. Earlier work in California on mature almond trees showed that 10 to 25% of the labelled N applied was recovered in the fruits [11]. Because the soil was not analyzed for atom $\%^{15}N$, the magnitude of the fertilizer loss from the system remains unknown.

The reason for the low apparent N-fertilizer recovery by mature walnut trees is not well understood. It is possible that the absolute amount of labelled-N uptake had been larger, but that during remobilization of N in the spring and again in the fall, volatilization losses were high [27].

4.2. Internal cycling of N

Previous studies with fruit trees showed that internal cycling represents a major source of N for new growth [17,28,29]. The PAD was used to estimate the percentage of N derived from internal sources [17]. Using the ^{14}N excess values of comparable organs, i.e. leaves, fruits, etc., for two consecutive years (Equation 1), the percentage of the ^{14}N dilution in the second year reflects the of total N derived from the soil-N pool. The equation used for calculating the PAD is identical to the equation used to calculate the N derived from soil when labelled fertilizer is applied and the plant has only two sources of N: fertilizer and soil. Therefore, (100 – PAD)% is equal to the N derived from internal cycling.

In the second year, 84% of the N in mature fruits, measured in October, was derived from internal sources (PAD = 16%) which declined to approximately 50% the following year. A similar decline in the N derived from remobilization was observed for all the other components harvested throughout the growing season (Table III). A clear explanation for the yearly fluctuations of the N contribution from remobilization is not apparent. The occurrence of alternate bearing is, in some instances, plausible; however, in our situation, the yield of fruits in years 2 and 3 were similar and contained comparable total-N values (Table IV).

For mature almond trees, the average PAD over a period of 5 years for embryo, leaves and blossoms was estimated at 50% [17]. In this study, the 2-year average PAD for all the various walnut components was estimated at 40%. This would indicate that walnut is more dependent than almond on storage N as a source for new growth. Using the ^{15}N extracts of xylem sap from field-grown kiwi fruit vines, a temporal change in the N derived from remobilization was observed [30]. Whereas up to 57% of the N in xylem sap was derived from remobilization early in the growing season, this value declined to 25% as the season progressed. Using a somewhat similar approach, and analyzing the ^{15}N content of the xylem sap of mature walnut trees, Deng et al. [13] found that the ratio of storage N/uptake N, i.e. N recently absorbed from soil was 18 early in the growing season, declining to 0.9 once the leaves were fully expanded. Neilsen et al. [31] used ^{15}N-enriched fertilizer to determine the source of N in leaves of 2-year-old apple (*Malus domestica*) trees and concluded that remobilization contributed more than 50% of the total N for leaf growth. Based on the above, it is clear that soil N is of minor importance early in the growing season, with new growth dependent primarily on internal-N sources. After leaf expansion, the importance of remobilization declines and the soil becomes the major contributor of N.

The amount of remobilized N in fruits and leaves versus from direct uptake from fertilizer or soil can also be calculated by mass balance using the total-N content and the ^{14}N-labelled fertilizer budget. Of the 478 g tree^{-1} of ^{14}N-labelled fertilizer accumulated during the entire 6-year period in fruits, leaves and canopy, trunk and roots, 124 g tree^{-1} or 26% was recovered in the fruits and leaf litter in the year the labelled fertilizer was applied (Table IV). During the subsequent 5 years, a total of 276 g tree^{-1}, equivalent to 58% of all the ^{14}N-labelled fertilizer accumulated, was recovered in fruits and leaves. The ^{14}N-labelled fertilizer accumulated in the fruits and leaves during those 5 years

would have been predominately stored, assuming that the accumulation of ^{14}N-labelled fertilizer from the soil became insignificant after the first year. As trees were grown on light textured, sandy soils, the amount of ^{14}N-labelled fertilizer in the soil that remained in a plant-available form would have been small. Therefore, it can be argued that at least 58% of all the N in fruits and leaves is derived from N that was stored initially and remobilized in subsequent years. The 58% corresponds to a PAD value of 42%, which is close to the average PAD value for years 2 and 3 (Table III).

The combined, average, annual-N content in fruit and leaf litter was estimated at 905 g tree^{-1} (Table IV), of which 58% or 525 g N tree^{-1} was derived from internal sources. As 124 g tree^{-1}, equivalent to 13%, of labelled fertilizer was accumulated in the year zero, the remaining 29% (100 – 58 – 13) or 262 g tree^{-1} was derived directly from the available soil-N pool. Therefore, the N-balance approach confirms the finding obtained by the PAD approach that internal remobilization is the major source of N for fruits and leaves.

4.3. Mean Residence Time of stored N

Because catkins were not enriched in ^{14}N in the year during which labelled fertilizer was applied (Table I, Fig. 2), it is possible to calculate the Mean Residence Time (MRT) of storage N. Tree components that obtain their N from both remobilization and soil cannot be used to calculate the MRT, as no distinction can be made between soil N and unlabelled N stored during previous years. There is a possibility that the catkins accumulated soil N prior to labelled-N application. evidence suggests that most if not all of the N in almond and walnut catkins is accumulated in the spring as soil N; uptake by mature trees during the fall and winter months is minimal [13,17].

By solving for k in the exponential decline function (Equation 5) using the atom %^{14}N excess values of catkins in years 1 to 5 (Table I), k was found to be equal 0.50 yr.$^{-1}$ Therefore the MRT, equal to 1/k, was 2 years.

A MRT of 2 years indicates that, on average, N accumulated for storage is remobilized within two years. That the calculated MRT is 2 rather than 1 year indicates that this species is conservative with its N. If the MRT were 1 year and all the storage N were used in the first year, there would be inadequate reserves with which to respond via secondary growth to insect damage, diseases or other calamities.

Weinbaum et al. [17], following the decline in atom %^{14}N in almond trees over a period of 5 years, calculated that, after 4 years, the contribution of labelled fertilizer-N represented 6.2% of the storage-N pool which was equal to 3.1% of the total N in the tree, using a measured PAD value of 50%. It becomes apparent that most of the N accumulated by mature walnut trees in a particular year is stored rather than used for new growth. Subsequently, N is remobilized in support of annual and reproductive growth over a period of several years.

It is of interest to determine the size of the pools in "perennial" tree components that serve as potential sources of N for new growth. The average total-N content in mature walnut was 3,096 g tree^{-1}, with 905 g present in fruits and leaf litter (Table IV). Therefore, 2,191 g N was present in "perennial" components. As the average PAD value was 40% and the total N content in the fruits and leaves was 905 g, 543 g was derived from internal N sources. With a MRT of 2 years, the size of the internal pool that contributed N for new growth was approximately 1,100 g N tree^{-1}, about 50% of the total pool present in the "perennial" tree parts. Hence, half of the N that was present in the canopy, trunk and roots was available for internal cycling, the other half appears to have been present in a structural form that was unavailable for recycling.

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