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Biological nitrogen fixation in trees in agro-ecosystems

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Key words: A-value, acetylene reduction assay, agroforestry, isotope dilution, ^{15}N, nitrogen fixation, nodules, trees, ureide technique

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Abstract

The integration of trees, especially nitrogen fixing trees (NFTs), into agroforestry and silvo-pastoral systems can make a major contribution to sustainable agriculture by restoring and maintaining soil fertility, and in combating erosion and desertification as well as providing fuelwood. The particular advantage of NFTs is their biological nitrogen fixation (BNF), their ability to establish in nitrogen-deficient soils and the benefits of the nitrogen fixed (and extra organic matter) to succeeding or associated crops.

The importance of NFTs leads to the question of how we can maximise or optimize their effects and how we can manage BNF and the transfer of nitrogen to associated or succeeding plantings. To be able to achieve these goals, suitable methods of measuring BNF in trees are necessary. The total nitrogen difference (TND) method is simple, but is better suited for low than high soil N conditions. The acetylene reduction assay (ARA), although sensitive and simple, has many technical limitations especially for NFTs, and the estimates of BNF have generally been very low, compared to other methods. For NFTs, the ^{15}N techniques are still under development, but have already given some promising results (e.g., has been used to measure large genetic variability in BNF within different NFTs).

Various factors affect BNF in trees. They include the age of trees, the microbial component, soil moisture, temperature, salinity, pH, soil N level and plant nutrient deficiencies. Some of the factors, e.g. temperature, affect the symbiosis more than plant growth, and differences in the effects of these factors on BNF in different NFT genotypes have been reported. These factors and research needs for improving BNF in trees are discussed.

Introduction

The use of trees, especially nitrogen fixing trees (NFTs), in agroforestry and silvopastoral systems, is receiving considerable attention for several reasons. Population increases are outstripping food production in many parts of the world and more forest land is being brought into agricultural production – several million hectares of forest are being lost every year. Most tropical soils are extremely fragile and give very poor crop yields after only a few years of cultivation without expensive fertilizer inputs. The decline in soil fertility is followed in many cases by soil erosion and further decline in soil nutrients. The use of nitrogen-fixing trees in agroforestry systems in the humid/sub-humid tropics is therefore attractive as a source of N and organic matter needed in the rehabilitation of damaged soils or sustainability in undamaged soils, as protection against erosion, and for fuelwood – the major source of energy for over half the world and a commodity in extremely short supply in many countries. Sanginga et al. (1986) found that the additions of prunings of 6-month-old inoculated *Leucaena leucocephala* increased subsequent maize yield from 2.2 t ha^{-1} to 4.6 t ha^{-1}. Lal (1989) reported that *L. leucocephala* planted at 2-m intervals reduced erosion from interrow maize plantings from 4.3 t ha^{-1} (plow-till) to 0.1 t ha^{-1}, i.e. the same as no-till. In a different context, Ladha et al. (1989) found that green manure from the nitrogen-fixing *Sesbania rostrata* (a stem- and root-nodulating legume) was equivalent to 60 to 120 kg N ha^{-1} as urea in a rice ecosystem.

Similar problems as in the humid/sub-humid areas also occur in the vast semi-arid/arid areas

of the world. Here, wind erosion and desertification also become major problems and NFTs – preferably deep rooted ones with access to groundwater – will probably have an important role. Analysis of soils from under *Acacia albida* (syn. *Faidherbia albida*) in Senegal (Charreau and Vidal, 1965) indicated a remarkable fertility gradient from bare soil to soil under the foliage; protein yields of millet near trees were increased 3- to 4-fold. Leguminous trees such as *Acacia cyanophylla* are commencing to have an important agroforestry role in areas of North Africa with 250–400 mm rainfall. Significant accumulations of organic C, N, Ca, Mg, P and K have been measured in the surface soil beneath *Prosopis* canopies in natural ecosystems (Virginia, 1986), due partly to transport of these elements from lower depths and to BNF. *Casuarina equisetifolia* (symbiotic with the actinomycete *Frankia*) has found extensive use to consolidate sandy coastal soils.

While there is recognition of the importance of NFTs in maintaining fertility, in soil conservation and in providing wood, there have been few studies on the nitrogen-fixing symbioses, their potential and constraints, and management strategies to enhance their effects. In this paper we address the problem of measuring biological nitrogen fixation in trees, indicate some of the variables affecting fixation and some of the opportunities for management. We have focussed particularly on leguminous trees, with some reference to the actinorrhizal trees, especially casuarinas. While casuarinas have roles in soil conservation and reclamation of saline sites, they have not yet found as wide an acceptance in agroforestry as the leguminous trees.

Measurement of BNF in trees

The problems associated with BNF measurements are much more complex with NTFs than with annuals due largely to the size of trees and their perennial nature – hence, only few studies have been conducted on BNF in trees. The practical difficulties in harvesting whole trees or of realistic non-destructive sampling, and in obtaining suitable controls valid over seasons or years, are not easy to overcome, and for this reason many researchers have estimated BNF in young trees over short periods. However, questions such as – For how long do trees continue to fix N_2? What are the effects of root and litter turnover on N_2 fixation and soil fertility? – cannot be resolved through studies on N_2 fixation in young trees. Another problem is the number of trees to be used per study consistent with the larger area required by tree experiments, and with available resources. For a given isoline or provenance the variation in a given trait can be large. Sanginga et al. (1989a) observed very high variability in nodulation by pot-grown *Allocasuarina* spp.; although all plants were inoculated, some plants in the same pot nodulated, but others did not. For practical reasons, economic reasons and also to reduce the degree of soil variability in studies with trees, single or few tree-plots have however had to be used.

The commonly used methods for measuring BNF are: total nitrogen difference (TND), acetylene reduction assay (ARA), the natural ^{15}N abundance method, and procedures with ^{15}N fertilizer addition to soil (isotope dilution (ID) and A-value (Av), all of which have been extensively reviewed (Danso, 1985; Knowles, 1980; Rennie and Rennie, 1983; Shearer and Kohl, 1986).

The total nitrogen difference (TND) method

The TND method is simple, and has provided several estimates of BNF in trees, with examples in Table 1. The TND method is based on estimating how much of the total (N_T) in the fixing plant was accumulated from soil (N_S); the remainder is then attributed to fixed N. The total N in a control non-N_2-fixing plant is assumed to reflect the N_S in the N_2-fixer.

Unlike some grain legumes, non-nodulated NFT isolines are not yet available, and uninoculated NFT controls have been used in soils without indigenous rhizobia (Ndoye and Dreyfus, 1988; Sougoufara et al., 1990), while completely different species have also served as controls (Pareek et al., 1990). Serious errors in the estimates of N_S will therefore affect the accuracy of the BNF estimates (Danso, 1985; Rennie and Rennie, 1983). The chances for errors are small

Table 1. Estimates of BNF in nitrogen-fixing trees by the total nitrogen difference (TND) method

Species	Estimated BNF		Reference
	%	kg N ha^{-1} yr^{-1}	
Leucaena leucocephala	52–64	448–548	Sanginga et al. (1985)
L. leucocephala	65	304	Liya et al. (1990)
L. leucocephala	45–63		Sanginga et al. (1990d)
Casuarina equisetifolia	49	53	Duhoux and Dommergues (1985)
C. equisetifolia	55–76		Sougoufara et al. (1990)
C. equisetifolia	39–55	43–60	Gauthier et al. (1985)
Sesbania rostrata	35–45	505–581	Ndoye and Dreyfus (1988)
S. sesban	11–18	43–102	Ndoye and Dreyfus (1988)
Gliricidia sepium	72	108	Liya et al. (1990)
Albizia lebbeck	60	94	Liya et al. (1990)

in low-N soils because of the small contribution from N_S relative to fixed N_2 (which would be the main source of N_T). With increasing soil N, N_S may contribute significantly to plant growth and errors in estimating N_S may then have a significant impact on the estimate of BNF. The simple TND method is therefore most recommended for measuring BNF in sandy or low-N soils and then shows little difference from other methods (e.g. Gauthier et al., 1985; Ndoye and Dreyfus, 1988). Even so, the gradual build-up of N under NFTs should be considered for long-term studies.

For trees more than a year old, distinguishing the N reabsorbed from the decomposition of fallen leaves and senesced roots and nodules from native soil N and fixed N_2 is an additional problem. These factors might account for several cases of poor agreement between TND estimates of BNF and other techniques (Pareek et al., 1990; Sanginga et al., 1990a, d; Stewart and Pearson, 1967).

The acetylene-reduction assay (ARA)

The ARA technique is simple, inexpensive, and the most sensitive method for detecting BNF, and accounts for most of the estimates of BNF in trees. The amount of ethylene produced by incubating excised nodules, decapitated roots or whole plants in an atmosphere containing acetylene is converted into total N_2 fixed by multiplying with a conversion factor which, on theoretical grounds, was fixed at 3 (Hardy et al., 1968).

Several limitations of the ARA technique have been reported. The two major ones are (i) it is an instantaneous assay and may not truly reflect BNF over long durations (Fried et al., 1983) and (ii) the conversion ratio of 3 does not apply in all cases (Rennie et al., 1978; Hansen et al., 1987). The accurate estimation of BNF in woody perennials would therefore require a burdensome number of measurements and calibrations. Even then, (iii) large errors are likely to arise because it is very difficult to recover all nodules, under most circumstances. Nodules located at great depths, up to 10 m, for example (Jenkins et al., 1988), can be difficult to recover.

Some reported ARA estimates of BNF in NFTs are presented in Table 2. The values are very low, including even the 110 kg N ha^{-1} yr^{-1} reported for *Leucaena leucocephala* when compared to values obtained using other methods. (e.g. Sanginga et al., 1985; Zaharah et al., 1986). Where detached nodules were used, the values are most likely to be underestimates, given the drastic declines in ARA that commonly result from detaching nodules from plants (Langkamp et al., 1979). We know of no in situ ARA determinations on field-grown NFTs.

The ^{15}N isotope methodology

The major strengths of BNF measurements using either added ^{15}N fertilizers or the natural ^{15}N abundance in soil are: they can estimate the separate contributions of N from soil, fertilizer and BNF, and measure the integrated amounts and proportions of N_2 fixed over desired periods. They are therefore useful for assessing BNF differences among genotypes belonging to the

Table 2. Some estimates of BNF in nitrogen-fixing trees by the acetylene reduction assay (ARA)

Species	Estimated BNF		Reference
	%	kg N ha^{-1} yr^{-1}	
Acacia pulchella	13.0		Hansen and Pate (1987b)
A. pulchella		0.49	Hansen et al. (1987)
A. alata	3.7		Hansen and Pate (1987b)
A. alata		1.6	Hansen et al. (1987)
A. pellita		12.0	Langkamp et al. (1979)
A. pennatula		34.0	Roskoski et al. (1982)
A. extensa		0.1	Hansen et al. (1987)
Brassiaea aquilifolim		0.19	Hansen et al. (1987)
Gliricidia sepium		13.0	Roskoski et al. (1982)
Inga jinicuil		35.0	Roskoski (1981)
Leucaena leucocephala		110.0	Högberg and Kvarnström (1982)
Purshia tridenta		0.06	Dalton and Zobel (1977)

same species or different species (Sanginga et al., 1990a,b) and effects such as soil N on BNF (Sanginga et al., 1987). In pot studies Sanginga et al. (1990b) found that % Ndfa varied from 37 to 72% (mean, 65%) with *L. leucocephala* isolines and 6 to 36% (mean, 20%) with *A. albida* provenances. Ndoye and Dreyfus (1988) found that stem-nodulated *Sesbania rostrata* fixed much more N_2 in 60 days (83–109 kg N ha^{-1}) than the root-nodulated *S. sesban* (7–18 kg N ha^{-1}). With the ^{15}N techniques, it should be possible to identify NFT genotypes in which BNF is less affected by soil N.

The ^{15}N methods are however better developed for herbaceous legume systems than for woody perennials. Although most measurements of BNF in trees so far are on greenhouse-grown plants there appears to be much promise in the use of the ^{15}N techniques to measure BNF in woody perennials (e.g. large genetic variability in BNF within isolines and provenances of NFTs were reported by Sanginga et al., 1990a, b). The ^{15}N soil approaches are the ^{15}N natural abundance, the ^{15}N isotope dilution (ID) (Fried and Middelboe, 1977; McAuliffe et al., 1958) and the A-value (Av) approaches (Fried and Broeshart, 1975).

The natural ^{15}N abundance or $\delta^{15}N$ method

Many soils have N of slightly higher ^{15}N abundance than that of atmospheric N_2, and thus relative to atmospheric N_2, most soils are slightly enriched in ^{15}N. The extent to which the ^{15}N (or $\delta^{15}N$) accumulated from soil is diluted by N_2 fixed in the fixing plant is then used to estimate BNF (Delwiche and Steyn, 1970; Mariotti, 1983; Shearer et al., 1974).

The advantages of the $\delta^{15}N$ methodology include: (i) A fairly stable $\delta^{15}N$ with time, resulting in less errors in the determination of BNF; (ii) Does not require addition of costly ^{15}N fertilizers. For trees, this could involve substantial savings; (iii) the $\delta^{15}N$ method does not involve any disturbance of the soil as occurs with incorporation of ^{15}N fertilizer and therefore is more appropriate to natural ecosystems and established plantations.

Shearer et al. (1983) observed significantly lower $\delta^{15}N$ in *Prosopis*, the dominant tree species in a Sonoran desert ecosystem, than in either soil N or presumed non-N_2-fixing plants deriving their N entirely from soil. Shearer and Kohl (1986) estimated that *P. glandulosa* derived between 43 and 65% of its N from BNF, and also measured differences in BNF in *P. glandulosa* at different sites. With Alnus, depending on age, the percentage of N derived from fixation (% Ndfa) in *A. incana* was shown by the $\delta^{15}N$ method to be between 85 and 100%, while % Ndfa differed between 40 and 80% in seven *A. glutinosa* genotypes of different origin (Domenach et al., 1989). The method was able to detect legumes that did not fix N_2 (Shearer et al., 1983), and shows much promise for exploratory studies in natural ecosystems for potential NFTs.

A major drawback with the $\delta^{15}N$ method is

that unlike the ^{15}N enrichment methods, small isotope fractionations or variability (site or among plant parts) can cause significant errors (Shearer and Kohl, 1986). Also, the ^{15}N natural abundance method may be unsuitable for BNF studies in some NFTs in natural communities, because the $\delta^{15}N$ in these soils is usually lower than in agricultural soils (Peoples et al., 1991). Although some reports have suggested high variability across sites (Broadbent et al., 1980; Selles et al., 1986), Shearer et al. (1978) suggested that the spatial variability of $\delta^{15}N$ in many soils may well be within acceptable limits.

Because of their big sizes and difficulty in whole-tree sampling, the idea of sampling representative plant parts to estimate BNF for whole trees is appealing. However, large differences in $\delta^{15}N$ have been observed among different plant parts (Shearer et al., 1983), thus questioning the validity of using the $\delta^{15}N$ in a particular organ to estimate BNF on a whole-tree basis. More research is needed.

Nitrogen-15 fertilizer enrichment methods

The addition of ^{15}N-enriched fertilizers to soil significantly increases the differences in the ^{15}N enrichments between soil N and atmospheric N_2, and increases the ease of ^{15}N detection in plants and the chances of obtaining more precise and possibly more accurate estimates of BNF. Duhoux and Dommergues (1985) suggested that the ^{15}N enrichment techniques are probably the most reliable for measuring BNF in trees. Despite its advantages, ^{15}N enrichment of soil has not been used routinely for measuring BNF in trees, and may relate to the difficulty of adapting the techniques to the massive sizes of trees, and their long duration of growth.

Selection of reference plant

The $^{15}N/^{14}N$ ratio in the available soil N needs to be accurately determined to obtain accurate estimates of BNF. The reference plant used for assessing the integrated ^{15}N enrichment of the N absorbed by the NFT from soil is therefore very critical for the ^{15}N methods. Sanginga et al. (1990c) and Pareek et al. (1990) are among the few to have examined the selection of reference plants for NFTs and clearly showed that highly erroneous values of BNF can be obtained with unsuitable reference plants.

Errors due to the use of unsuitable reference plants are however less critical when BNF is high (Hardarson et al., 1988); Sanginga et al. (1990c) showed that the errors in the estimates of BNF were far greater in the poorer fixer *A. albida* than in *L. leucocephala*. Danso et al. (1986) and Hardarson et al. (1988) suggested that errors in BNF estimates due to the reference plant are quite insignificant when % Ndfa is in the 80% range, but the % Ndfa in many NFTs fall significantly below 80% (e.g. Gauthier et al., 1985; Hansen and Pate, 1987a; Sanginga et al., 1990b). Also, when the ^{15}N enrichment in soil does not decline rapidly, the chances for accurate BNF estimates are greatly improved (Witty and Ritz, 1984). However, there are no reported studies of BNF in NFTs using slow-release ^{15}N formulations or ^{15}N-labelled organic matter. The practical difficulty in incorporating the ^{15}N-labelled organic matter into soil in an established tree stand, limits the applicability of this labelling method in NFTs. Many studies don't however need to quantify the N_2 fixed accurately while for some, there may be little need for a reference plant. For example, the ranking of Rhizobium strains or NFT genotypes for BNF capacity in a soil enriched with ^{15}N should be the same with or without a reference plant. Under such circumstances, not much advantage is gained by including a reference plant.

The criteria for selecting a reference crop for grain legumes (Fried et al., 1983) apply to NFTs as well. The reference plant should be a non-N_2-fixer, with similar growth and time course of N uptake as the NFT, unless the $^{15}N/^{14}N$ ratio of soil is stable, and both plants should obtain their N from a similar soil N pool.

Absence of nitrogenase activity by ARA can be used to ascertain if a putative reference plant fixes N_2 (Fried et al., 1983). Non-nodulating isolines have not yet been identified for NFTs. A closer examination of some of the poorly or irregularly nodulated NFTs, e.g. *Allocasuarina* spp. (Reddell et al., 1986b; Sanginga et al., 1989a) might reveal some non-nodulating isolines. *Allocasuarina*, which is poorly nodulated in nature, may be a good reference plant for naturally nodulating *Casuarina* spp. as they may

have similar rooting habits. Uninoculated NFTs have sometimes been used as references plants (Sanginga et al., 1990e; Gauthier et al., 1985; Ndoye and Dreyfus, 1988), with the advantage that BNF can be estimated by two independent methods, TND and ID. In practice, however, it has often been difficult to avoid cross-contamination of the uninoculated reference plant, particularly in the field (e.g. Gauthier et al., 1985; Sanginga et al., 1988).

The selection of fixing and non-N_2-fixing plants with similar growth and N uptake patterns (Witty, 1983) is easier in annuals than in trees in which growth progresses through several, often contrasting seasons which could affect growth of the reference and N_2-fixing plants differently. However, because the decline in ^{15}N enrichment in soil into which a ^{15}N fertilizer has been added, decreases with time (Pareek et al., 1990), the subsequent greater stabilization of ^{15}N enrichment should minimize potential errors in the long-term estimations of BNF.

The requirement for the fixing and non-N_2-fixing plants to obtain their N from the same N pool does not necessarily mean that plants should have equal rooting depths. In the hypothetical case in Figure 1, the reference plant No. 2 is for all purposes ideal, and perhaps as good a reference as is plant No. 4, which although has shallower roots, but is absorbing its N from the same zone A, within which the ^{15}N was incorporated. This therefore allows for some flexibility in selecting for reference crops, even if their rooting habits differ slightly from those of the putative N_2-fixer (Fried et al., 1983). For NFTs, this is very important. Many trees may be absorbing their nutrients predominantly from the topsoil even though roots may penetrate far deeper (Anonymous, 1975). Furthermore, plant-available N in most soils is mainly in the topsoil, in which case any dilution of ^{15}N by unlabelled N from the subsoil (e.g. plant No. 3 in Fig. 1) could be small. Is it then justified to use non-tree species, such as grasses and cereals, with different root systems as reference crops for trees? Pareek et al. (1990), found the ^{15}N enrichment of soil extract was similar to that assessed by four non-fixing weeds and cereal crops, and BNF estimates were also similar using these as references. Unfortunately, no trees were used as references. Also, because BNF was very high, and the influence of reference crop errors is minimal under such situations, it is difficult to make a generalization from this study.

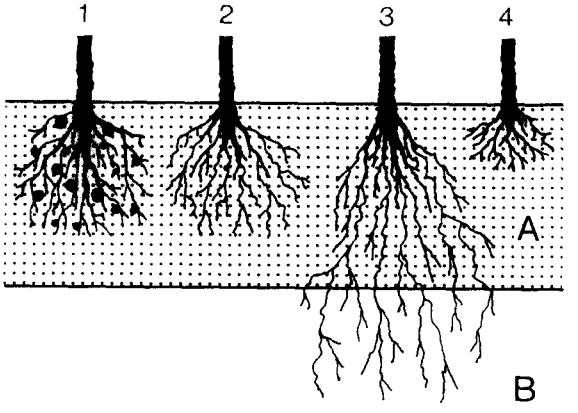

Fig. 1. The effect of root depth of reference plants on $^{15}N/^{14}N$ uptake. The fixing plant is represented by 1, while 2, 3 and 4 represent potential reference plants. **A** and **B** represent soil horizons.

A special problem with trees could be the horizontal spread of roots; they are likely to absorb N from well outside the zones within which ^{15}N was incorporated, particularly if ^{15}N was applied as a band. For this reason, Baker et al. (1990) recommend the trenching of the perimeter of each replication block and installing a multi-layered plastic film barrier to a depth of 1.5 m to contain the roots within the zone in which ^{15}N was applied.

Rate, formulation, time and frequency of ^{15}N fertilizer addition

To enrich a soil with ^{15}N for BNF measurement, the atom % ^{15}N excess, rate, frequency and method of application of the ^{15}N fertilizer will all influence the precision and the accuracy of the estimate.

Essentially, the amount of N applied should be small enough not to suppress BNF significantly, while that for the reference plant should be large enough to allow good but not necessarily maximum growth. Where the native soil N would adequately support the growth of the reference non-N_2-fixing plant, the ID method is preferred,

because of the greater simplicity of the ID procedure (only one N rate is used) and calculations. The AV method is preferred where soil N is low, and where it may be necessary to eliminate traces of N_2 fixation in the reference plant, or where nodules from cross-contamination are likely to form on the reference crop at a lower N rate. Sanginga et al. (1990c) used both the ID and Av approaches, to measure BNF in *L. leucocephala* and *A. albida*, with the corresponding uninoculated species included with two other non-NFTs as reference plants. They found that at the 10 mg N kg^{-1} soil rate (ID method), the uninoculated legumes were nodulated, and large differences in the estimates of BNF by the different reference trees were obtained, including some negative values for the poorer fixer *A. albida*. No nodules were, however, found when the N applied to the reference plants was raised to 100 mg N kg $soil^{-1}$ (AV method), and the estimates of BNF with all reference plants were quite close for each NFT. Sanginga et al. (1990c) observed that the pattern of N uptake by the reference and fixing plant was closer (a requirement for a satisfactory reference plant) when 100 rather than 10 mg N kg $soil^{-1}$ was applied.

For perennial pastures, the frequent, small additions of ^{15}N to soil is a suitable approach for measuring BNF (Vallis et al. 1967; 1977) as the application of N as small doses prevents the suppressive effects of applied N on BNF, and also results in a more stable $^{15}N/^{14}N$ ratio in soil (Hardarson et al., 1988; Labandera et al., 1988). For trees, the use of multiple ^{15}N fertilizer applications (2–4 a year) probably before periods of high root activity appears to be an attractive method, but has been little examined. Preliminary study by N. Sanginga, F. Zapata, S.K.A. Danso and G.D. Bowen (unpublished) compared the effect of different amounts of ^{15}N-labelled fertilizer and the frequency of application on estimates of N_2 fixed in *L. leucocephala* and *G. sepium* over 9 months. They found that a single application of 20 mg kg $soil^{-1}$ decreased BNF, and that applying this amount in three splits gave different results, depending on whether it was repeatedly applied to the same plot or each split application was on a previously unlabelled plot.

Sampling of plants

The practical difficulty in harvesting whole trees increases with age, and it is difficult and labour-intensive to attempt to recover all roots. A representative organ would be ideal for BNF estimation, provided the ^{15}N enrichment is representative for the whole tree. The ^{15}N enrichments in different organs of trees can however vary widely (Sanginga et al., 1990e), and raises the question as to which plant part is most representative. Aboveground organs might be preferred for ease of sampling, and the leaves which contain most of the aboveground N seem the most appropriate choice. Even where only leaves are to be sampled, it could be laborious to sample all leaves. For this reason, Baker et al. (1990) tested different sampling strategies, and suggested that the simple procedure of collecting small numbers of leaves (20 to 60) at random from the trees was sufficient to estimate % Ndfa in *Leucaena leucocephala*. However, measuring the total BNF should be more problematic

While the % Ndfa in the leaves of soybean was found to be close to that of the whole plant, total N fixed differed (Danso and Kumarasinghe, 1990). Studies by Sanginga et al. (1990e) showed that *L. leucocephala* roots alone contained as much as 60% of the N fixed in the whole plant. This could still well be an underestimate because of fine-root turnover which can be extremely large. Thus, any estimates of BNF in trees that exclude roots should be regarded as underestimates. Allometric relations, worked out with forest trees (Felker et al., 1982), may be of some use in estimating total biomass, but even these are only approximate. The costs and practicalities of doing tree experiments dictate that the sampling problem needs urgent attention, especially if one is trying to quantify both above-ground and below-ground nitrogen which will influence the N available to associated or succeeding plants.

Effect of remobilized N

Perennial plants go through periods of N mobilization and remobilization. Domenach and Kurdali (1989) estimated that retranslocated N is important for regrowth, with 10% of the N in *A. glutinosa* at the end of the growing period being

traced to retranslocated N. Retranslocation of N, however, introduces methodological limitations because the ^{15}N enrichment of the translocated N in the NFT may be significantly different (lower) from that of the reference plant. These differences will therefore be compounded in the differences in the final ^{15}N enrichments in the fixing and reference plant, and unless corrected for, will result in erroneous BNF estimates. The following equation is used to correct for retranslocated N and also for differences in amounts of stored N retranslocated from a preceding harvest (e.g. in sequentially harvested NFTs where ^{15}N is applied always to a previously unlabelled tree) on the ^{15}N enrichment at final harvest:

$$\text{Corrected atom \% } ^{15}\text{N excess (CAE) in plant} = \frac{^{AE}(T_2) \times {}^{N}(T_2)}{{}^{N}(T_2) - {}^{N}(T_1)} \quad \ldots\ldots \quad (1)$$

Where T_2 is the time of the final (or later) harvest and T_1 is the harvest immediately preceding regrowth and ^{15}N application, and N is the total N in plant at the indicated times.

The relative contribution or importance of retranslocated N, however, diminishes with time (Domenach and Kurdali, 1989) and therefore the potential error in BNF estimates is reduced with time. Much of the errors will also depend on the frequency and severity of pruning. Domenach and Kurdali (1989) suggested that harvesting the most recently formed leaves at the end of the growing season significantly reduced the influence of nitrogenous reserves on measurements of BNF. Also, the frequent addition of ^{15}N fertilizers compared to reliance on residual ^{15}N in soil seems to have the advantage, that plants (both fixing and non-N_2-fixing) would be absorbing higher enriched ^{15}N than already present in the plants, resulting quickly in a narrowing of the existing ^{15}N enrichment differences in the plants (Danso et al., 1988).

Effect of litter turnover

Nutrient cycling (largely from litter turnover) is a major source of nutrients in tree growth (Mahendrappa et al., 1986), and also influences the ^{15}N enrichment in the soil. The ID approach assumes that both fixing and non-N_2-fixing plants are growing on soil of the same $^{15}N/^{14}N$ ratio. However, with the litter of the fixing plant being lower in ^{15}N enrichment than that of the non-N_2-fixing plant, the soil under a NTF may have a lower ^{15}N enrichment than that under the non-N_2-fixing plant. Thus, a crucial assumption of the methodology, that both the fixing and non-N_2-fixing plants are sampling N of the same ^{15}N enrichment is invalidated. Again, this is where frequent addition of ^{15}N has special advantages. By superimposing fresh ^{15}N fertilizer on an already low level of ^{15}N enrichment, it should be possible to override such $^{15}N/^{14}N$ differences in the two root zones (Danso et al., 1988).

Ureides as a measure of BNF

Another method which has shown much promise in some grain legumes is the measurement of BNF based on the composition of nitrogen compounds in the xylem sap. Where ureides are uniquely produced by BNF, their relative abundance in the total nitrogen exported from the nodules in the xylem stream to the leaves serves as an indication of the proportion of N fixed (Herridge, 1982; McClure et al., 1980). This approach therefore overcomes problems of quantifying N uptake from soil separately. However, of 35 NFTs studied by van Kessel et al. (1988), only two showed a high abundance of ureides in the xylem sap. Sanginga et al. (1988) could not find significant correlation between ureide production and BNF in *Leucaena*, while the study by Hansen and Pate (1987a) indicated that xylem sap analysis was not suitable for measuring BNF in three *Acacia* species. Results to date do not therefore indicate that this method has much potential for measuring BNF in trees.

Nitrogen fixation potential of trees

Dommergues (1987) defined two parameters:

(i) The *nitrogen fixing potential* (NFP) of a species, i.e. the nitrogen fixed with all environmental constraints removed, including the possible inhibitory effect of soil nitrogen.

However, almost without exception the field data reported are subject to *some* environmental constraints and the concept of NFP is a qualified one, and

(ii) the *actual nitrogen fixed* (ANF), which is the resultant of NFP, modified by environmental constraints.

Dommergues (1987) identified high and low NFP species. The former included such species as *L. leucocephala* and *A. mangium* for which records occur of 100 to 300 (sometimes 500) kg N fixed ha^{-1} yr^{-1}; the latter include such species as *A. albida*, *A. senegal*, and *A. pellita* (Langkamp et al., 1979) with which fixation has been reported as less than 20 kg N ha^{-1} yr^{-1}. It is likely that when more species are studied there will be more of a continuum. The major factor in high NFP, a high percentage of nitrogen derived from the atmosphere (% Ndfa), generally appear to be less affected by environmental conditions than total N fixed (Danso, 1986; Materon and Danso, 1991).

Fast growth rate alone is not the sole indicator of high nitrogen fixation. Some species like the non-fixing legume *Cassia siamea* achieve extremely high growth rates because of an extensive root system which, at least in the first year, explores a considerable volume of soil (S. Hauser, pers. comm.). Rapid growth rates may also be achieved in poorly fixing species by efficient retranslocation of absorbed or fixed N. There are few studies of genotype differences in the *physiological efficiency of use* of nitrogen in legumes, whether it be absorbed from soil or fixed from the atmosphere.

The occurrence of nodulation

Although the vast majority of the Leguminosae form nodules, a number do not. In the subfamily Caesalpinioideae, nodulation is largely restricted to the tribe Caesalpinieae and the genus *Chamaecrista* from the Cassieae. In the Mimosoideae, nodulation is general, except for 4 groups within the tribe Mimoseae, and a few species of *Acacia*. The only tribe from the Papilionoideae which appears not to nodulate is the Dipterygeae. A number of genera in the Swartzieae do not nodulate (de Faria et al., 1989).

Cassia siamea, often used in agroforestry systems, does not nodulate and its vigorous growth is due to a highly effective rooting system. Other prominent woody legumes lacking nodulation are the carob (*Ceratonia siliqua*) *Bauhinia* spp. (Sprent et al., 1988), *Parkia biglobosa* (Dommergues, 1987) and *Gleditsia* spp. (Allen and Allen, 1981).

Attention is drawn to stem nodulation of legumes – usually shrubs occurring in wet (or swampy) sites – and their potential for green manure in rice ecosystems. Stem nodulation, by *Azorhizobium* and some strains of *Rhizobium* (Alazard et al., 1988) occur on 17 species of *Aeschynomene*, 3 species of *Sesbania* and 1 species of *Neptunia* (Becker et al., 1988).

The nodulation phenomenon in actinorrhizal genera (symbiotic with *Frankia* spp.) needs more examination. For example, in the Casuarinaeae, less than half of the 70–80 species have been examined. In the genus *Casuarina*, nodulation appears to be high and nodules at a site are usually well developed and uniform. By contrast, for many species of the large genus *Allocasuarina*, nodules are often absent in the field (or extremely difficult to find), and frequently only a few of the individual plants are nodulated (Reddell et al., 1986b).

Genotypic variation in BNF

Pot studies, and limited field studies, have shown large differences between genotypes within a species in their nitrogen fixation. Pot studies give indications of differences in nitrogen-fixing potential, but have their shortcomings. Figure 2 indicates not only significant differences in nitrogen fixation between *Rhizobium* strains, but also large plant genotype differences within *L. leucocephala* which disappeared after some 24 weeks. Herein lies a problem with pot studies: are the marked genotype differences for the first 24 weeks only a reflection of speed of infection/response or is the equality of growth in the third period merely a reflection of root/plant growth constraints imposed by pot studies? While screening for genotypic differences by pot/glasshouse studies might be useful, the major findings must be field-tested. The marked genotypic differences (2.5-fold over 7 months) observed in

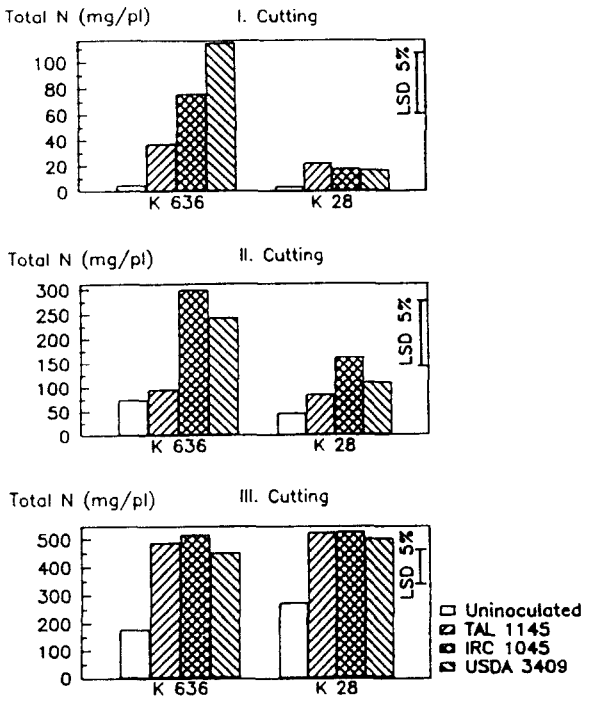

Fig. 2. Total nitrogen (mg plant $^{-1}$) of *Leucaena leucocephala* genotypes K636 and K28 inoculated with different Rhizobium strains during successive cuttings at 12-week intervals. From Sanginga et al. (1990d).

nitrogen fixed by *C. equisetifolia* in pot studies by Sougoufara et al. (1987) were also substantiated in the field (Dreyfus et al., 1988).

Sanginga et al. (1990a,b) have shown 2–3-fold differences in nitrogen fixed between provenances/genotypes of *A. albida* and *L. leucocephala* after 12 weeks and 70% differences in pots after 36 weeks; 3–5-fold differences were found between 11 provenances of *C. equisetifolia* and of *C. cunninghamiana*. The potential application of such findings is obvious, especially as many tree species are readily propagated vegetatively. However, selection for high BNF can not be the only consideration in mixed ecosystems; for example, it is necessary to select for tolerance of stress conditions as well.

In the presence of appreciable soil nitrogen some genotypes may fix more nitrogen and absorb less soil nitrogen than others. Genotypic differences in the effect of soil N on inhibition of BNF may have important implications for the nitrogen fixed in second and subsequent seasons by different genotypes.

In a mixed cropping system like alley cropping, one may *not* always be looking for the plant genotype with the highest BNF. Such a plant may be too vigorous a competitor for associated crops. In such cases selecting for maximum BNF needs to be accompanied by selection to reduce competition, e.g. selection for particular types of root systems. The NFT needs to be considered in the context of the ecosystem, not its BNF alone.

Plant and Rhizobium factors

The age factor

To date, there are few studies on BNF by trees in the second and subsequent seasons after planting. Based on acetylene reduction activity and on nodule mass data, Hansen and Pate (1987b) concluded that % Ndfa in two species of *Acacia* in the first year were 37% and 29%, compared to only 9% and 2% in the second year. In one species, the calculated amount of N fixed in the second year was similar to that for the first year and for the second species it was 50% greater in the second year. During the first season, when plant growth is exponential, BNF will usually increase with time, e.g. data of Sanginga et al. (1990d) indicate 55 mg, 120 mg and more than 250 mg N fixed per plant of *L. leucocephala* grown in pots over successive 12-week periods.

There is a strong case for more detailed studies of nitrogen fixed in second and subsequent seasons: If as with *L. leucocephala*, some 300 kg N or more can be fixed aboveground in a year and if, as data indicate, an equal amount occurs belowground (Sanginga et al., 1990e) then soil N levels from litter fall and sloughed root material may well be sufficient to suppress BNF. Sanginga et al. (1987) showed that 40 kg N ha^{-1} of applied fertilizer reduced BNF by *L. leucocephala* by some 50%. A marked reduction in BNF in second or subsequent seasons may affect management options. Furthermore, as the tree ages, N stored in roots and stem may reduce BNF by a feedback mechanism but this needs further study also. If future studies *do* show a reduction of BNF in the second or later seasons, the two postulated mechanisms above could be discerned by pot studies compar-

ing BNF of seedlings planted to soil from under trees and adjacent soil outside their influence.

The Rhizobium component

Specificity in nodulation. Some leguminous trees appear to nodulate only with fast-growing strains of *Rhizobium*, some only with the slow-growing *Bradyrhizobium* and some with both *Rhizobium* and *Bradyrhizobium* (e.g. Dreyfus and Dommergues, 1981). However, even in each of these 3 groups considerable specificity can occur. For example, the data of Dreyfus and Dommergues (1981) show that two of five *Rhizobium* isolates, and one of five *Bradyrhizobium* isolates tested did not infect *A. sieberiana*. All five *Rhizobium* isolates infected *A. farnesiana*, but only two of the 5 *Bradyrhizobium* infected it. In studies by Habish and Khairi (1970), *A. albida* nodulated with only one isolate from *A. albida* and 1 of another 9 isolates from various *Acacia* species. The nodulation affinities of some tree legumes with potential in agroforestry are indicated in Table 3. At this stage no consistent conclusions can be made on any relationship between *Bradyrhizobium*/*Rhizobium* specificities and systematic plant classification. However, some patterns may emerge as revisions of larger genera, such as *Acacia*, are made.

Effectiveness. Table 4, from Dreyfus and Dommergues (1981), shows clearly that considerable differences can occur between rhizobia in their effectiveness on particular hosts, a finding collaborated by other workers, e.g. Olivares et al. (1988) on *A. cyanophylla*, *A. melanoxylon* and *Prosopis chilensis*. These data also indicate cases where rhizobia isolated from a species is either ineffective or poorly effective on the same species. It follows that any assumption that indigenous rhizobia are effective and thus no response will be achieved by inoculation with selected effective rhizobia for that species, is premature. Thus, *Rhizobium*/*Bradyrhizobium* strain selection is a critical part of legume tree

Table 3. Rhizobial requirements of some potentially useful leguminous trees

Species	Nodulation by B and/or R*	Reference
Acacia albida	B	Dommergues (1987)
A. constricta	R	Waldon et al. (1989)
A. holosericea	B	Dreyfus and Dommergues (1981)
A. longifolia var. sophorae	R	Lawrie (1983)
A. mearnsii	B	Lawrie (1983); Dommergues (1987)
A. melanoxylon	B	Lawrie (1983)
A. nilotica	R	Dommergues (1987)
A. raddiana	R	Dommergues (1987)
A. saligna (syn. *A. cyanophylla*)	R, B	Barnet et al. (1985) Olivares et al. 1988)
A. senegal	R	Dommergues (1987)
A. seyal	R, B	Dommergues (1987) Habish and Khairi (1970)
A. tumida	R, B	Dreyfus and Dommergues (1981)
Albizzia lebbek	B	Ribero et al. (1987)
Calliandra calothyrus	R	Dommergues (1987)
Erythrina poeppigiana	B	Dommergues (1987)
Gliricidia sepium	R, B	Dommergues (1987) K.O. Awonaike, pers. com.
Leucaena leucocephala	R, B	Dommergues (1987)
Mimosa scabrella	R, B	Dommergues (1987)
Prosopis alba	R	Torres (1985)
P. chilensis	R, B	Olivares et al. (1988)
P. glandulosa	R, B	Jenkins et al. (1989)
Sesbania grandiflora	R	Dommergues (1987)

* B = *Bradyrhizobium*.
R = *Rhizobium*.

Table 4. Nodulation of *Acacia* species by *Rhizobium* and *Bradyrhizobium* (from data of Dreyfus and Dommergues, 1981)

	Nodulation						
	Rhizobium			Bradyrhizobium			
	Host of isolation			Host of isolation			
	A.se.*	A.bi.	A.f.	A.h.	A.si.	A.bi.	L.l.
Native African species							
Acacia albida	0	0	0	E	E	E	E
A. senegal	E	E	E	0	0	0	0
A. seyal	e	E	E	E	e	e	E
A. sieberiana	I	e	0	E	e	E	e
Introduced species							
A. bivenosa	I	E	I	E	e	E	I
A. farnesiana	E	E	E	0	I	0	0
A. holosericea	0	0	0	E	e	e	e
A. tumida	0	I	I	e	e	I	0

E = effective nodulation; e = partially effective; I = ineffective;
0 = no nodulation.
*A.se = *Acacia senegal*; A.bi. = *A. bivenosa*; A.f. = *A. farnesiana*; A.h. = *A. holosericea*; A.si. = *A. sieberiana*; L.l. = *Leucaena leucocephala*.

species evaluation, for both introduced and indigenous species. It may well be, for example, that Australian species of *Acacia* may perform poorly in Latin America or Africa, where indigenous rhizobia may be effective on indigenous species, but ineffective on introduced species.

The problem of competition between introduced effective strains of rhizobia and poorly effective indigenous strains (thus reducing the inoculation response) has not been examined with tree species. Bowen (1978) pointed out that species of legumes which nodulate at least partially effectively with a range of rhizobia may establish and grow reasonably well over large areas without inoculation and indeed this may be a major factor in the wide success of many agricultural legumes. However, inoculation of NFTs with highly effective rhizobia may not achieve the desired productivity due to competition by less effective indigenous strains. Specificity in nodulation requirements then becomes an advantage, allowing the full expression of the potential of the introduced *Rhizobium*/*Bradyrhizobium*. Many soils, for example, lack rhizobia for specific tree legumes, e.g. *L. leucocephala* (Sanginga et al., 1989b), and this would allow the full response to inoculation with effective rhizobia.

Management practices and environmental effects on BNF

Effects of management

We have indicated above that some 50% or more of the tree's nitrogen may be below ground. This may well be an underestimate because it neglects root turnover which can be extremely high, e.g. 40–92% of the standing crop in a forest ecosystem (Fogel, 1985). Some estimates on temperate forest species indicate the N allocated to fine roots can be up to 48% (Nadelhoffer et al., 1985) and it is highly likely that nitrogen does not retranslocate from senescing fine roots (Nambiar, 1987). As with severe pruning of herbaceous legumes (Bowen and Kennedy, 1959), after which a majority of roots and nodules senesce, severe pruning of *L. leucocephala* resulted in the death of approximately half of the nodules in the subsequent 3 weeks (Sanginga et al., 1990d) (root biomass was not studied). Thus, timing and severity of pruning may allow for some management of underground transfer of fixed nitrogen to associated crops, as well as regulating root competition between established hedgerows of NFT's and recently planted inter-row annuals. Reduction in

competition between the tree and the annual species and/or sustained transfer of N from the tree to the alley crop may be the reasons for the observation of Duguma et al. (1988) of increased maize and cowpea yields with increased pruning frequency and decreased pruning height of *L. leucocephala*, *G. sepium* and *S. grandiflora*.

As indicated above, the accession of nitrogen to soil by root sloughing, and addition of above-ground litter may decrease BNF in later seasons.

Environmental factors

The actual nitrogen fixed is conditioned by the plant's nitrogen fixing potential (in association with a highly effective Rhizobium/*Bradyrhizobium* strain) and by environmental factors. These may operate in two general ways: (i) by reducing plant growth and (ii) by direct effects on the symbiosis, e.g. by nodule physical conditions by affecting oxygen availability to bacteriods (Sprent et al., 1988) or by affecting the biochemistry of fixation, through e.g. molybdenum deficiency. Below, we indicate some of these factors.

Season

As with herbaceous legumes (e.g. Sprent, 1979), nitrogen fixation varies considerably within and between seasons due to plant age and environmental effects on plant growth.

Soil moisture

Soil moisture plays a dominant role in seasonality of nodulation and of nitrogenase activity. Some tree species have perennial nodules but many, if not most, shed their nodules and many fine roots when the soil becomes dry. For example, in the Mediterranean climate of Western Australia, Hansen et al. (1987) found nitrogenase activity per plant of two species of *Acacia* to be greatest in August/September (late winter/spring) due to a combination of increased specific acetylene reduction activity of the nodules and peak numbers of nodules. There was virtually no nitrogenase activity after November/December because of nodule senescence (decreasing soil moisture) through to the next March/April (arrival of rains), when nodulation recommenced. Habish (1970) recorded increased nodulation of *A. mellifera* in sand with increase in soil moisture to an optimum of 15% soil moisture.

Soil moisture may affect BNF indirectly through plant growth, and direct effects on infection (Sprent, 1979) and nodule characteristics, e.g. nodules with a great diffusion barrier to oxygen (Witty and Minchin, 1988).

Not much is known about nodulation of deep-rooted trees in arid and semi-arid areas, in which the surface layers of soil are dry for considerable periods, sometimes for years. *Prosopis glandulosa* has been recorded to nodulate at 10-m soil depth (Jenkins et al., 1988). Jenkins et al. (1989) found that *Rhizobium* dominated in the surface several metres but that at depth, *Bradyrhizobium* dominated. The nitrogen fixed by nodules at depth when the surface horizon is dry needs more quantitative study. Such nodules need only fix small amounts of N to be of significance in nitrogen-deficient soils. Rundel et al. (1982) suggested some 25–30 kg N ha^{-1} yr^{-1} may be fixed by *P. glandulosa*, largely by nodules at the capillary fringe of the groundwater table, as they could find no nodules in the surface layers of the dry soil.

Temperature

Various stages of nodulation and nitrogen fixation of herbaceous legumes are affected markedly by soil temperatures (Sprent, 1979) and this should hold for tree legumes. High soil temperatures may also affect survival of inoculated rhizobium in tropical soils (Bowen and Kennedy, 1959). *Casuarina cunninghamiana* plants supplied with nitrogen fertilizer grew moderately well at 15°C soil temperature, but plants dependent on *Frankia* showed no nitrogen fixation and extremely poor growth at that temperature (Reddell et al., 1985; Z. Zhang, G.D. Bowen and F. Zapata, pers. comm.). Nodulation and nitrogen fixation occurred at 20°C and above. The optimum soil temperature was 25°C for the symbiotic plant and 20°C for nitrogen fertilized plants. The much lower optimum temperature for symbiotic plants (25°C) than that reported for nitrogenase activity of detached nodules (40°C, Bond and MacIntosh, 1975a) suggests that the major determinant in the intact symbiotic system was the temperature effect on plant growth. For the two *Frankia* strains studied, the

nitrogen fixed g^{-1} nodule tissue was similar for 5 of the 6 temperature × *Frankia* combinations at 20°, 25° and 30°C, suggesting a major effect of assimilate distribution on nodule growth at the different soil temperatures. Reddell et al. (1985) suggested that such temperature requirements for significant nodulation and nitrogen fixation affects the natural distribution of casuarina species. *Casuarina* spp. occur in soils with low N and seem to be dependent almost entirely on BNF.

Habish (1970) recorded nodulation and nitrogen fixation by *A. mellifera* up to 35°C soil temperature.

Soil nitrogen

Inorganic N inhibits BNF, and appears important in NFTs. Sanginga et al. (1987) reported that 40 kg N ha^{-1} of applied fertilizer reduced BNF by *L. leucocephala* by some 50%. The suppressive effect of inorganic N on BNF has been shown in *Alnus* spp. and *Myrica* spp. (Stewart and Bond, 1961), *Coriaria* spp. and *Hippophäe* spp. (Bond and Mackintosh, 1975b), *C. equisetifolia* (Sougoufara et al., 1990), *L. leucocephala* (Sanginga et al., 1987, 1988) and *Acacia* spp. (Hansen and Pate, 1987a). Few studies have compared the relative abilities of different NFT species, provenances, isolines or clones to fix N_2 at different soil N levels. If large differences in BNF exist between genotypes within species in response to combined N, this may reflect on differences in BNF inhibition in second and subsequent seasons due to the build-up in soil N. This genotypic variation in sensitivity to soil nitrogen has been shown to be important in grain legumes (e.g. Hardarson et al., 1984). Domenach et al. (1989) reported that *Alnus incana* is capable of fixing N_2 in the presence of high soil N. Stewart and Bond (1961) reported that in *Alnus* spp., but not in *Myrica* spp., BNF was considerably enhanced by a low level of ammonium nitrogen, due to greater nodule development. Hansen and Pate (1987a) however observed that BNF in *A. pulchella*, *A. alata* and *A. extensa* was severely inhibited by inorganic N.

Soil toxicities

The effect of salinity on nodulation and nitrogen fixation by herbaceous legumes (Sprent, 1979) may also hold for tree species, many of which have potential for use in saline soils. For example, many species of *Prosopis* can withstand salinities generally considered too brackish for agricultural crops (Felker et al., 1981). Indeed, some can grow at salinity levels higher than that of sea water. Reddell et al. (1986a) found differences between two *Frankia* strains in their symbiosis with the extremely salt-tolerant species *Casuarina obesa*.

There is scope to combat many deleterious soil conditions (and to increase BNF) by selection of host genotypes and possibly by *Rhizobium*/*Bradyrhizobium* selection. Selection for tolerance of acidity may be especially relevant to the survival and persistence of inoculum, but the tolerance of the plant genome may play the dominant overall role in BNF. Habish (1970) recorded growth of *A. mellifera* with applied nitrogen at pH 3.8–4.2 but no nodulation and little plant growth at pH 5–5.5 in the absence of added nitrogen. More studies are needed to identify pH-related constraints, such as effects on rhizosphere growth of the bacterium or effects on infection and nodule development. Halliday and Somasegaran (1982) indicated that the effect of acidity on *Leucaena* itself, rather than on the *Rhizobium*, is the main factor in acidity tolerance of genotypes of *L. leucocephala*. However, many more studies are needed of the sensitivity of the symbiotic partners to soil toxicities (and other constraints) and the scope for genetic manipulation.

Nutrition

Low soil phosphate is an even more widespread constraint to plant growth than acidity. Sanginga et al. (1991) examined 23 provenances of *Gliricidia sepium* and 11 isolines of *L. leucocephala* for growth in low-phosphate soils; 2.30-fold and 2.10-fold differences in growth occurred between *L. leucocephala* and *G. sepium* genotypes, respectively, in a low-phosphate soil. Additions of phosphate had little effect on rhizosphere growth of *Rhizobium*, but reduced the time for nodulation and increased nodule numbers and nodule dry weights by 4- to 5-fold, while having little effect on % Ndfa. The constancy of % Ndfa indicates that effects on BNF

were probably via effects on plant growth (and assimilate available for BNF) rather than an effect specifically on the fixation process. The selection of genotypes of NFTs for high efficiency in uptake and use of phosphate should result in increased BNF.

Mycorrhizas

Mycorrhizal fungi enhance the uptake of phosphorus and many other nutrients which also enhance biological nitrogen fixation, e.g. Zn, Mo, Cu, etc. (Bowen, 1980). Tree growth is stimulated by the 'tripartite' symbiosis of plant-nitrogen-fixing microorganism-mycorrhiza. For example, inoculation of *L. leucocephala* by species of *Glomus* doubled plant growth, increased nodule fresh weight and specific acetylene reduction (nitrogenase) activity each by 50% (Purcino, et al., 1986). In a phosphate-deficient soil, De la Cruz et al. (1988) obtained little increase in the N per plant of *A. auriculiformis*, *A. mangium* and *Albizzia falcata* from the inoculation with *Rhizobium* alone, but 8- to 25-fold increases when inoculated both with *Rhizobium* and selected vesicular arbuscular mycorrhizal (VAM) fungi. These were paralleled by acetylene reduction increases. Mycorrhizal fungi differed in their effectiveness, and there is considerable scope for selection of highly efficient mycorrhizal fungi.

Vesicular arbuscular mycorrhizas are by far the most dominant form of mycorrhiza, especially on leguminous trees. Some trees, e.g. *Allocasuarina* and *Casuarina* are infected by both ectomycorrhiza and VAM (Reddell et al., 1986b), ectomycorrhiza increasing in occurrence in extremely low-phosphate soils in both casuarinas and in leguminous trees (Högberg, 1986).

Technology of inoculation with ectomycorrhizal fungi is now fairly well established (Marx et al., 1984) mainly because they are easy to grow in artificial media. The inability to grow VAM fungi in artificial media makes inoculation on a broad scale difficult because inoculum must be raised on living plant roots. However, inoculation with appropriately raised root inoculum is quite feasible in nursery situations. Thus inoculation of the tree species with selected mycorrhizal fungi before outplanting is quite feasible. Additionally, such outplanting of infected tree stock will supply an inoculum source for interplanted legumes and cereals, although more research is needed on rates of spread of the fungus to such crops. One may, however, not always get a mycorrhizal response as many soils already contain populations of effective mycorrhizal fungi. Identifying the soils/plant species which will respond to inoculation is a high research priority.

Conclusion and future research needs

The rising demand for increased food and fuelwood production by resource-poor farmers and concern for environmental degradation under intensive agriculture have increased our interest in agroforestry and nitrogen-fixing trees (NFTs).

Large differences in the biological nitrogen-fixing (BNF) capacities of different NFTs have been noted, and have been classified broadly into high- and low-NFT species. While species like *Leucaena leucocephala* and *Sesbania rostrata* can accumulate up to 500 (or more) kg N ha^{-1} yr^{-1} of N_2 fixed in their biomass, others like many *Acacia* species and *S. sesban* derive substantially less (often <50 kg N ha^{-1} yr^{-1}). Also, within each species of NFT examined, large differences in BNF capacities exist. Such genotypic differences should be exploited for enhancing the BNF contribution in agroforestry. The ^{15}N isotope labelling techniques are useful for assessing genotypic differences in BNF.

Inorganic N inhibits BNF in NFTs. This raises some important questions, such as (i) does the rapid litter and N turnover commonly associated with tree growth limit the period of N_2 fixation in NFTs to their first few years of growth and (ii) are there substantial differences in the tolerance of BNF to inorganic N in different NFTs? These important questions have received scanty attention and should be examined. Differences in the tolerance of different NFT genotypes to inorganic N could be exploited to extend the duration of BNF contribution to soil and plants.

Management practices like pruning intensity and frequency will not only affect BNF in trees, but may increase the rate of N release from senescing roots and nodules, and thus the soil N status and N transfer to companion crops. These management aspects have been little studied.

The ^{15}N isotope labelling technique will be very useful in such studies.

It is necessary to examine soils for the presence of effective *Rhizobium* and Frankia strains for NFTs to be used in agroforestry, particularly when these trees are being newly introduced. The few available data suggest that in many soils *Rhizobium* or Frankia inoculation would increase BNF and growth of many NFTs. Inoculation with highly effective strains at the nursery stage appears to be an effective way of enhancing nodulation success prior to field establishment, and is highly recommended. This can be complemented by mycorrhizal inoculation to increase NFT growth and BNF in low-P soils.

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