NITROGEN-FIXING TREES IN AGROFORESTRY SYSTEMS: MYTHS AND REALITIES

David W. Odee,
Biotechnology Division,
Kenya Forestry Research Institute,

ABSTRACT

The need to increase crop production on sustainable basis is very crucial in the tropics because of poor soil fertility as characterized by low nitrogen and phosphorus status. Agroforestry entails inclusion of woody perennial (trees and shrubs) in a diversified and sustainable land-use system. Use of nitrogen-fixing trees (NFT) in agroforestry systems is specifically to conserve the production potential of such systems due to the inherent ability of these trees and shrubs to improve the nutrient status of the soil. Appropriate manipulation and management of the microsymbiont and macrosymbiont enhances biological nitrogen fixation (BNF) in an agroforestry systems that incorporates NFTs as tree/shrub components. This paper highlights the technologies available for ensuring optimal exploitation of BNF in the agroforestry context and also points out some of the misconstructions about the nitrogen-fixing symbioses.

NITROGEN FIXING TREES IN AGROFORESTRY SYSTEMS: THE MYTHS AND REALITIES

Introduction

The need to increase crop production on a sustainable basis has become crucial in the tropics, much so new, because of the ever growing population and the pressure it brings to bear on the diminishing arable lands. Crop production in the tropics is often constrained by poor soil fertility typified by low nitrogen and phosphorus status. Because of the prohibitive cost of chemical fertilizers, most farmers are adopting alternative crop production systems that require low resource inputs such as agroforestry and on-farm afforestation systems. A large number of trees and shrubs in these systems are often Nitrogen Fixing Trees (NFTs). Biological nitrogen fixation (BNF) is a characteristic of most legumes (and a few non-legumes) and it entails conversion and utilization of atmospheric nitrogen through the symbiotic association of these group of plants and certain taxa/groups of bacteria. Biologically fixed nitrogen is first and foremost important in the nutrition of the fixing plant; appropriate manipulation and management of such plants, as in agroforestry systems, can ensure that the fixed N is made available to other non-fixing plants crops. This paper highlights some of the assumptions made about BNF and NFTs and discusses ways of optimizing BNF and its utilization.

Nitrogen Fixing Symbioses

Many legumes and a group of non-legumes in symbiotic association with certain groups of bacteria are able to convert atmospheric nitrogen (hence the term "fix") into ammonia, a soluble form of nitrogen that after conversion to nitrate is readily utilizable by plants. The conversion is achieved by bacteria of the genus *Rhizobium* (general term) in the case of legumes and the non-legume *Parasponia* (Ulmaceae) (Trinick, 1979), and an actinomycete, *Frankia* with actinorhizal plants. The nitrogen fixing microsymbionts infect roots (and stems of certain legumes taxa) and form nodules (swellings). Once inside the nodules, the microsymbionts proliferate and fix atmospheric nitrogen.

The ability to form nodules is therefore a physical characteristic used to authenticate or determine the ability of a microsymbiont to be infective and/or effective. Infectivity simply implies ability to form nodules on a nitrogen fixing plant . Effectivity means actual ability of the nodules to fix nitrogen. The latter property is more important in establishing symbioses that are not a drain to the extant soil nitrogen.

Microsymbionts

There has recently been an explosion of *Rhizobium* (general term for all legume root-and stem-nodulating bacteria) taxonomy within the family Rhizobiaceae. There are 4 recognized genera: *Rhizobium (sensu stricto)*, with 6 *bona fide* and 3 unvalidated species;

Sinorhizobium, recently renamed from *Rhizobium*, with 4 species (de Lajudie *et al*, 1994); *Bradyrhizobium*, with 2 *bona fide* and 1 unvalidated species; and *Azorhizobium caulinodans*, the only species that forms nodules on both root and stem of certian legume taxa and can also fix nitrogen *ex planta* (see also table 1).

The second group of bacteria that is known to form root nodules with a group of higher plants known as actinorhizal plants is in the genus *Frankia*. This genus is taxonomically very different from *Rhizobium*. *Frankia* is a gram-positive bacteria within the family Frankiaceae in the order actinomycete. The genus *Frankia* is the only recognized taxonomic level and hence the term "type" is used widely to differentiate strains or isolates. This contrasts immensely with the taxonomic development of *Rhizobium* and is attributed to poor recovery of isolates from nodules and *in vitro* maintenance.

Macrosymbionts

Rhizobial symbioses

The family Leguminoseae is divided into 3 sub-families:- Caesalpinioideae, Mimosoideae and Papiliniodeae. Nodulation, as a tell-tale feature for nitrogen-fixing symbioses, is less common in caesalpinioids. It is within this subfamily that we encounter the non-nodulating species such as *Cassia* (=Senna) *siamea* and *Parkinsonia aculeata* previously thought to be nitrogen-fixing due to their high leaf N-content and occurrence of nodule-like structures (pseudo nodules or false-nodules) on their roots. On the other hand, nodulation is prevalent in mimosoids and papilinoids with a few exceptions eg. in the largest legume genus *Acacia*: two species, namely the American *A. greggii* (Eskew and Ting, 1978) and the African *A. brevispica* (Odee and Sprent, 1992) have been well authenticated as non-nodulators, hence non-nitrogen fixers. In all, only 20% of known species in the family Leguminosae have been examined (Sprent and Sprent, 1990; Sutherland and Sprent, 1993). Thus, there is yet a lot of ground to be covered in terms of surveys for nodulation status in the family and statistics are bound to change between the sub-families as more species continue to be examined. *Rhizobium* nodulation is not exclusive to legumes; the species *Parasponia andersonii* (Ulmaceae) is known to nodulate in Papua New Guinea (Trinick, 1979).

Frankia symbioses

Frankia nodulates with actinorhizal plants which is a conglomerate of plants with no obvious taxonomic pattern covering 9 families and 19 genera. The families from which species/genera are known to have *Frankia* associations are Casuarinaceae, Betulaceae, Coriariaceae, Cycadaceae, Elaegnaceae, Rhamnaceae, Myriaceae, Rosaceae and Ulmaceae.

Important Nitrogen-fixing trees (NFTs)

In tropical agroforestry systems, the most widely used NFTs are within the genera *Leucaena, Calliandra*, *Sesbania* and *Prosopis* (legumes); and *Casuarina*, *Allocasuarina* and *Alnus* (actinorhizal plants). In Kenya, 27% of the species which were listed as putative NFTs in von Calowitz (1986) have been authenticated in indigenous soils as nodulating hence capable of fixing nitrogen (see also table 2).

Optimizing Nitrogen-Fixation

Environmental constraints

Nitrogen - fixation is optimized when environmental conditions are also optimum for plant growth. Environmental stress conditions such as extreme temperatures, drought, salinity, waterlogging, soil acidity, pests and diseases, nutrient deficiencies etc can adversely affect the process of nitrogen-fixation. The nitrogen-fixing symbiosis is affected by the effect on the host plant or microsymbiont or directly on the symbiotic process itself.

Plant viruses, diseases and pests, affect the structure, physiology and biology of both root nodules and host plants (Sprent and Sprent, 1990). It can also, via root exudates, selectively affect rhizobial strains hence affect the infection process necessary for nodulation.

Soil temperature has direct effects upon both nodulation and nodule activity: infection process by *Rhizobium* may be delayed at lower temperatures (Roughley *et al*, 1970).

Indigenous rhizobial populations may drastically fluctuate between rainy and drought conditions. A drastic drop in rhizobial populations to the magnitude of 1000 - fold has been observed from wet to dry seasons in arid lands of Kenya (Odee *et al*,1995). However, Nitrogen fixation by NFTs may take place even during drought. Most NFTs are phreatophytic (ie deep-rooted). Thus, nodulation and N-fixation can occur as deep down near the water table as reported by Dupuy *et al* (1992) for *A. albida* growing in the Sahel. Some species of *Sesbania* and *Aechynomene* have stem-nodules, a characteristic which facilitates nitrogen-fixation in waterlogged or flooded conditions.

Nitrogen-fixing plants need the same nutrients as other green plants; both macro- and micro-elements. They may need extra molybdemum (Mo) and Iron (Fe) for nitrogenase and haemoglobin. Mo is specifically an important micronutrient as it is a constituent of both the enzymes nitrate reductase, required for assimilation of nitrate from the soil, and nitrogenase, required during the conversion of atmosperic nitrogen in the nodules.

A common limiting nutrient is phosporus (P). Nitrogen-fixation is in most cases constrained by P-deficiency in tropical soils and it is no wonder that legumes growing in tropical regions are heavily dependent on mycorrhizas for efficient uptake of P and other poorly mobile nutrients in the soil. Phosphorus is essential during nodule metabolism . Phosphorus concentration is often higher in nodules than in other plant organs when are grown in P-deficient soils (O'Hara *et al*, 1988).

When is it necessary to inoculate ?

Three situations can be identified that may warrant inoculation with nitrogen-fixing microsymbionts to establish effective nodulation and hence nitrogen-fixation in legumes: (i) where infective microsymbiont strains are absent, (ii) where the indigenous populations are too low to cause effective nodulation; and (iii) where the indigenous populations are either ineffective or less effective in nitrogen-fixation with the host plant of interest. Most Kenyan soils are known to harbour indigenous rhizobial populations. These populations are often of mixed types (as characterized by phenotypic and genotypic traits) and the dominant type is in most cases effectively compatible with the native legume to the site (Odee *et al*, 1995). The population sizes of indigenous *Rhizobium* that are compatible with various NFTs range from under 10 to $10^5 g^{-1}$ soil (table 3). We have also established that soils with effective indigenous *Rhizobium* $>10^4$ may not need to be inoculated; it should be noted that this is always determined by the host. For example, in one site, soil may harbour an effective population for *Faidherbia albida*, but the population nodulates ineffectively with another legume. Thus the need to inoculate should be assessed in the field or use field soil using the legume to be planted in that field.

Inoculation technology

The simplest way of inoculating seeds/seedlings is by using soil collected from around root systems of nitrogen-fixing plants known to be effectively nodulated. This method is still actively used for inoculating actinorhizal plants eg *Casuarina* spp. using soil from well-nodulated plants from the coastal region known to harbour effective *Frankia* propagules.

Effective legume inoculation can be ehanced by inoculation witha pre-screened pure *Rhizobium* strain impregnated into a solid ground substrate known as a carrier. The carrier allows the inoculant to be coated onto the surface of the seed and also serves to protect the bacteria against desiccation. Different countries or regions use different carrier materials depending on the availability and suitability. In Northern America, Europe and Australia the common carrier material is peat. In East and Southern Africa, filter mud and bagasse (waste products from sugarcane refineries) are widely used. The main attributes of a good carrier material are a high water holding capacity, an ability to support *Rhizobium* growth and an ability to favour survival for longer durations. Likewise, a good inoculant should also be highly effective in nitrogen-fixation, competitive against indigenous *Rhizobium* and antogonistic microbes, have higher survival in the carrier and ideally be of broad-host spectrum.

Adoption of inoculant technology

Legume inoculant production has been active in East Africa (Kenya, Rwanda, Uganda and Tanzania) since the 1980s and Southern Africa (Zimbabwe, Zambia and South Africa) since the 1960s. In Southern Africa region, large scale inoculant production has been focussed on specific crops of economic importance eg. soybean. In Kenya, the most important legume crop is the common bean (*Phaseolus vulgaris*). Inoculants for most of the pulses and pasture legumes can be obtained from the Microbiological Resources Centre (MIRCEN), University of Nairobi. The Kenya Forestry Research Institute (KEFRI) also produces inoculants for most NFTs (*cf.* table 2).

However, it is worth noting that adoption of legume inoculant technology by the end-users (eg farmers, foresters, NGOs etc) has been very minimal. This scenerio is illustrated with the grim statistics of Marufu *et al* (1995) which gives inoculant utilization in Burundi as only 16% of the total land area under cultivation with common beans, pea, *Leucaena, Calliandra;* and in Kenya, only 24% of the total land area under common bean, soybean and lucerne. The low adoption is attributed to limited user awareness and technology transfer.

Fixed nitrogen: How available is it to the non-fixing plant?

It is important to emphasize the point that plants that fix nitrogen do so for their own needs. There is very little evidence of direct transfer of fixed nitrogen to associated non-fixing plants other than the insignificant amounts which may occur through mycorrhizal 'bridges' bridges'. Fixed nitrogen may be made available from the fixing plant by natural nutrient flows within the soil system arising from nutrients stores such as literfall (leaves, twigs, residues, nodules, roots etc) after mineralization. NFTs have dual advantage over annual legumes: they enhance nutrient lift from lower horizons and can fix nitrogen throughout the seasons because of their phreatophytic nature. The amount of fixed nitrogen is dependent on prevalent environmental conditions (see section on optimization of nitrogen fixation). Table 4 shows some of the reported estimates of nitrogen-fixation by NFTs which range from as low as 7kg ha^{-1} yr^{-1} for *A. holosericea* to as high as 600 kg $ha^{-1}yr^{-1}$ for *S. sesban*. Thus, if nitrogen from fixation is to be released into the soil, the NFT tissue must die, so that normal mineralization can occur (Sutherland and Sprent, 1993). Many agroforestry practices have the potential to hasten this process and make the fixed nitrogen available to non-fixing plants. Agroforestry technologies such as alley-cropping accompanied by management strategies as mulching and green manure will ensure that the fixed nitrogen locked up in the above-ground biomass is judiciously harnessed and returned to soil in forms readily availabe to the non-fxing plants.

Conclusions

Inoculation of seeds/seedlings destined for sites where there is lack of information on microsymbiont status should be the norm and not the exception. Biological nitrogen fixation has a crucial role to play in sustaining soil fertility and productivity in tropical agroforestry systems. However, there is an urgent need to create greater awareness on BNF and establish efficient medium on its technology transfer to the end users.

References

de Lajudie, P., Willems, A., Pot, B., Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M.D., Dreyfus, B., Kersters, K. and Gillis, M. 1994. Polyphasic taxaonomy of rhizobia: Emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. *International Journal of Systematic Bacteriology* **44:** 715 - 733.

Dupuy, N. C. and Dreyfus, B. L. 1992. *Bradyrhizobium* populations occur in deep soil under leguminous tree *Acacia albida*. *Applied Environment and Microbiology* **58**: 2415 - 2419.

Eskew, D. L. and Ting, I. P. 1978. Nitrogen fixation by legumes and blue-green algal-lichen crusts in a Colorado desert environment. *American Journal of Botany* **65**: 850 - 856

Giller, K. E. and Wilson, K. J. 1991. Nitrogen fixation in tropical cropping systems. CAB International, Wallingford, UK.

Marufu, L., Karanja, N. and Ryder, M. 1995. Legume inoculant production and use in East and Southern Africa. *Soil Biology and Biochemistry* **27**: 735 - 738.

Nair, P. K. R. 1993. An introduction to Agroforestry. Kluwer Academic Publishers and ICRAF Netherlands.

Odee, D. W. and Sprent, J. I. 1992. *Acacia brevispica*: A non-nodulating mimosoid legume? *Soil Biology and Biochemistry* **24:** 717 - 719.

Odee, D. W., Sutherland, J. M., Kimiti, J. M and Sprent, J. I. 1995. Natural rhizobial populations and nodulation status of woody legumes growing in diverse Kenyan conditions. *Plant and Soil* **173**: 211 - 224.

Roughley, R. J. 1970. The influence of root temperature, *Rhizobium* strain and host selection on the structure and nitrogen-fixing efficiency of the root nodules of *Trifolium subterraneum*. *Annals of Botany* **34**: 631 - 46

Sprent, J. I. and Sprent, P. 1990. Nitrogen fixing organisms. Pure and applied aspects. Chapman and Hall, London. UK.

Sutherland, J. M. and Sprent, J. I. 1993. Nitrogen fixation by legume trees. In: Subba Rao, N. S. and Rodriguez - Barruecco, C. (eds.), *Symbioses in nitrogen-fixing trees,* pp 33 - 63. Oxford and IBH publishing Co. Ltd., New Delhi, India.

Trinick, M. J. 1979. Structure of nitrogen-fixing nodules formed by *Rhizobium* on roots of *Parasponia andersonii* Planch. *Canadian Journal* of *Microbiology* **25**: 565 - 78.

von Calowitz, P. G. 1986. *Multipurpose Tree* and *shrub Seed Directory*. ICRAF, Nairobi, Kenya.

Young, A. 1989. *Agroforestry for soil conservation*. CAB International, Walling, UK.

Table 1. Updated *Rhizobium* taxonomy

***Rhizobium* species**	**Host legume genera**
Rhizobium leguminosarum bv. phaseoli	*Phaseolus*
Rhizobium leguminosarum bv. trifolii	*Trifolium*
Rhizobium leguminosarum bv. viciae	*Pisum, vicia*
Rhizobium tropicii	*Phaseolus, Leucaena*
Rhizobium etli	*Phaseolus, Acacia, Chamaecrista*
Rhizobium loti	*Lotus*
Rhizobium huakuii	*Astragalus*
Rhizobium galegae	*Galega*
?*Rhizobium ciceri*	*Cicer*
?*Rhizobium tianshanense*	*Glycine*, several other taxa
?*Rhizobium hainanensis*	*Phaseolus, Leucaena*
**Sinorhizobium fredii*	*Glycine, other taxa*
**Sinorhizobium meliloti*	*Medicago, Melilotus, Trigonella*
**Sinorhizobium teranga*	*Acacia, Sesbania*
**Sinorhizobium saheli*	*Sesbania*
Bradyrhizobium elkanii	*Glycine*
?*Bradyrhizobium liaoningensis*	*Glycine*
Azorhizobium caulinodans	*Sesbania, Aechynomene*

? Not yet recognized as *bona fide Rhizobium* species
* Recently proposed genus

Table 2. Nodulating species in Kenyan soils and sources of cultures and inoculants

Species	Sources of cultures /inoculants
Acacia albida (Syn. *Faidherbia albida*)	KEFRI
Acacia elatior	KEFRI
Acacia mellifera	KEFRI
Acacia nilotica	KEFRI
Acacia nubica	KEFRI
Acacia polyacantha	KEFRI
Acacia reficiens	KEFRI
Acacia senegal	KEFRI
Acacia seyal	KEFRI
Acacia sieberiana	KEFRI
Acacia tortilis	KEFRI
Acacia xanthophloea	KEFRI
Acacia zanzibarica	KEFRI
Acacia aneura	KEFRI
Acacia auriculiformis	KEFRI, UQ
Acacia holosericea	KEFRI, UQ
Acacia karoo	KEFRI
Acacia arenaria	KEFRI
Acacia mangium	KEFRI
Acacia mearusii	KEFRI
Acacia melanoxylon	KEFRI
Acacia salicina	KEFRI
Acacia victoriae	KEFRI, UQ
Albizia falcatania	KEFRI
Albizia lebbeck	KEFRI
Albizia procera	KEFRI
Cajanus cajan	KEFRI, UoN, NifTAL
Calliandra calothyrsus	KEFRI, UoN, NifTAL
2*Casuarina cunninghamiana*	KEFRI
Casuarina equisetifolia	KEFRI
Casuarina glauca	KEFRI
Casuarina junghuriana	KEFRI
Casuarina stricta	KEFRI
Dalbergia melanoxylon	KEFRI
Gliricidia sepium	KEFRI, NifTAL
Leucaena leucocephala	KEFRI, NifTAL, UoN
Leucaena diversifolia	KEFRI, NifTAL
Milletia dura	KEFRI

Pithecellobium dulce	NifTAL
Prosopis chilensis	KEFRI, NifTAL
Prosopsis juliflora	KEFRI, NifTAL
Prosopsis pallida	KEFRI, NifTAL
Sesbania grandiflora	KEFRI, NifTAL
Sesbania sesban	KEFRI, NifTAL, UoN

1 Sources: KEFRI , Kenya Forestry Research Institute; UoN, University of Nairobi MIRCEN project; UQ, University of Queensland P. Dart's Group; NifTAL, Nitrogen fixation for tropical agricultural legumes, Hawaii.

2 Inoculum for *Casuarina* spp. in the form of soil and nodules known to contain effective *Frankia* propagules.