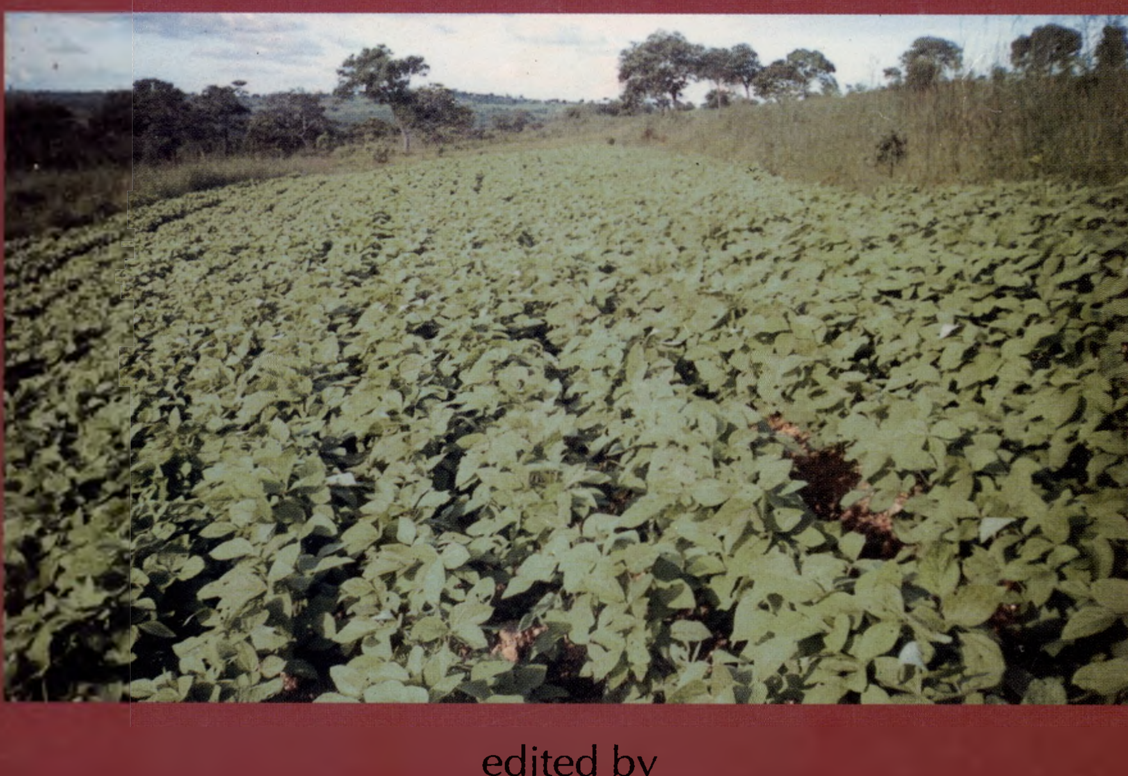
Harnessing Biological Nitrogen Fixation in African Agriculture

Challenges and Opportunities



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Sixth International Conference of the African Association
for Biological Nitrogen Fixation
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Selected Papers

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Preface

Biological Nitrogen Fixation (BNF) is a low-cost technology with potential to contribute to sustainability of African agriculture where low-input production systems are prevalent. The African Association for Biological Nitrogen Fixation (AABNF), founded in 1982, has as its main objective the promotion of scientific application of biological nitrogen fixation technologies in Africa to increase food production while reducing the need for expensive fertiliser inputs.

The first AABNF conference, organised by the Nairobi Microbiological Resource Centre (MIRCEN), was held in Kenya in 1984. The second was held in Egypt, and was organised by the Cairo MIRCEN in 1986. The third AABNF conference held in Dakar in 1988 was organised by the West African MIRCEN at Bambey, Senegal. The fourth conference took place in 1990 in Ibadan, Nigeria, and was organised by the International Institute for Tropical Agriculture (IITA). The fifth conference was organised by the Hassan II Institute of Agronomy and Veterinary Medicine and was held in Rabat, Morocco in 1992.

The sixth AABNF conference, whose proceedings are reported in this book, was organised by the Department of Soil Science and Agricultural Engineering in the Faculty of Agriculture at the University of Zimbabwe from 12 to 17 September, 1994. The conference was held under the theme 'Agronomic, Socio-economic and Environmental Benefits of Biological Nitrogen Fixation in Africa'. Specific objectives were to:

- bring together scientists working on BNF to share practical and research experiences of applying BNF technologies.
- highlight actual and potential agronomic, socio-economic and environmental benefits of BNF, especially in low input small-scale farming systems.
- identify areas requiring further research and consideration and draw up a framework for further collaboration and information sharing.

Altogether, 120 participants from 29 countries attended the sixth conference with five international organisations being represented.

The conference was organised into ten plenary sessions which covered the following topics:

- Ecology and bio-diversity in Biological Nitrogen Fixation
- Agronomic benefits of BNF
- Socio-economic and environmental benefits
- Use of N-15 methods in BNF
- Rhizobial inoculant technology
- Status of BNF technology use in Africa
- BNF in agroforestry systems
- BNF in non-legumes
- Genetic aspects of BNF
- Limiting factors of BNF in Africa

This book consists of selected papers from the conference proceedings focusing on the challenges and opportunities for exploiting biological nitrogen fixation in African agriculture.

Sheunesu M. Mpepereki
Fred Togarepi Makonese

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We also wish to thank the following:

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International
- Africa Centre for Fertiliser Development (ACFD)
- European Union (EU)
- Food and Agriculture Organisation (FAO)
- International Atomic Energy Agency (IAEA)
- International Science Foundations (ISF)
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- African Associated Mines (Pvt) Ltd
- Delta Corporation (Pvt) Ltd
- Olivine Industries (Pvt) Ltd
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The sixth AABNF conference was a great success. It brought together scientists from both Africa and abroad, who deliberated on relevant issues of BNF research and application. Mechanisms for future collaboration and communication were explored. A thorough review of the Association's past was undertaken and resolutions for future action adopted.

The AABNF has continued to draw its membership from both Anglo- and Francophone Africa and has maintained the international character of its conferences, thanks to funding support from various organisations.

Sheunesu M. Mpepereki and Fred T. Makonese

Foreword

Can biological nitrogen fixation meet the agronomic, socio-economic and environmental challenges facing Africa?

The Sixth International Conference of the African Association for Biological Nitrogen Fixation was held under the theme 'Agronomic, socio-economic and environmental benefits of biological nitrogen fixing systems in Africa'. This book reports on the deliberations of that conference which examined the activities of microorganisms that supply 'free' nitrogen fertiliser through a process of biological fixation. Given the rising costs of nitrogen fertilisers, any that comes 'free' is worthy of serious attention. Zimbabwe uses almost 25 per cent of its electricity to manufacture the same nitrogen fertilizer that microbes can supply 'free' through a process of biological fixation. The importance of harnessing biological nitrogen fixation to sustain agriculture in Africa cannot be overemphasised. The publication of this book is timely given that hunger and malnutrition are ravaging some parts of our continent, a situation accentuated by lack of affordable agricultural inputs, especially for smallholder farmers.

Much has been said about the benefits of biological nitrogen fixing systems. A technology can be impressive on paper. It can even perform extremely well in laboratories and controlled trials but until it brings food to the farmer's table and income into the farmer's pocket, it remains of academic interest, its merits being highlighted only to solicit for research funds. The book goes a long way to explore practical methods of harnessing BNF technologies for improved and sustainable agricultural production. Agricultural production at both subsistence and commercial levels underpins the economic development of our African countries. At the nutrition level, the rising costs of animal-derived protein means that more and more people must increasingly rely on protein from vegetable sources. This is why leguminous crops which can benefit from biological nitrogen fixation continue to dominate the farming systems in peasant agriculture. Several papers in the book discuss the use of nitrogen-fixing legume species in agroforestry systems to provide mulch, forage and soil nutrients and in rehabilitating mine dumps. Biological nitrogen fixation is shown to have the potential to help arrest and reverse the serious decline in the quality of the environment and its capacity to sustain ever-increasing human populations. This publication represents an important addition to the otherwise scarce literature on appropriate technologies for sustainable agriculture in Africa. I hope it will enjoy wide readership among agricultural students, researchers and policy makers.

K. M. Kangai
Hon. Minister of Lands and Agriculture, Zimbabwe

Introduction

Contribution of biological nitrogen fixation towards the African food and environmental challenges

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Summary

Efforts to increase food production for the rising population in Africa can benefit from the large pool of information on biological nitrogen fixation (BNF). The continued rise in the cost of chemical fertilisers and their unavailability to farmers have further propelled the quest for viable BNF technologies. Successes in BNF, especially the establishment of inoculant production in several countries in Africa is seen as a major step in the right direction. Similarly the acceptance of well nodulated legumes such as soybean, *Leucaena*, and the *Frankia*-nodulated plants like *Casuarina* provides positive bases for BNF demonstrations. Due to Africa's rich biodiversity, potential exists for exploiting newer plants and development of stress-tolerant plant hosts and their microsymbionts. Positive results on limited mycorrhiza-*Rhizobium* co-inoculation which alleviates phosphorus deficiency while facilitating N_2 fixation ought to be supported. At the same time, the search for acid-tolerant *Rhizobium* strains should be intensified. Many studies on response to inoculation have gained from regionally coordinated trials and a model which is now available to predict such response is likely to augment field studies provided chemical soil data is available. The need for an increased knowledge base on *Azolla, Cyanobacteria* and *Frankia* to augment the *Rhizobium* system is emphasised. Similarly soil management strategies aimed at improving soil fertility employing current BNF methodologies are examined. Further the need for broad host range *Rhizobium* strains, costing of BNF, and field demonstrations of BNF are discussed.

Introduction

As Africa approaches the year 2000, the continent is still grappling with the traditional challenge of providing enough food for each generation, to bring its children to maturity. Africa's current population of over 500 million people is growing at an average rate of 3% per annum while food production in most countries barely increases at 1,5% per annum. In the meantime, global food production problems have shifted from Asia to Africa with more famine in 1994 than in 1984 in the latter. Hundreds of millions of people suffer from a shortage of food and from malnutrition despite improved food production worldwide. The 1990s have continued to record environmental stresses manifested in periodic droughts, deforestation and soil degradation, all of which contribute to the declining trend of bio-productivity. Because of the complexity of the food problem, endeavours towards achieving sustainable production must take cognisance of areas such as agroforestry, multicropping, intensive gardening, green manuring, compost application, mulching, integrated crop protection, aquaculture and biological nitrogen fixation (Adelhem and Kitsch, 1985). It is for this reason that the African Association for Biological Nitrogen Fixation (AABNF) has, since its inception in 1984, nourished a multidisciplinary approach to addressing African food and environmental issues. Since nitrogen limits the growth of plants, BNF offers a viable alternative, especially to small-scale African farmers who cannot afford chemical fertilisers. This brief overview attempts to examine the efforts, successes and limitations of African scientists in harnessing biologically fixed nitrogen and also suggests some research imperatives for the future.

Microorganisms Implicated in Nitrogen Fixation

Microbially-mediated nitrogen fixation can account for 175 million t/yr in terrestial and aquatic environments (Burns and Hardy, 1975) compared to 80 million t/yr obtainable from industrial sources. The organisms that possess the enzyme nitrogenase have attracted considerable interest. Elkan (1992) remarked that the procaryotes in the Eubacteria and Archaebacteria kingdoms that are able to fix nitrogen are metabolically diverse, representing autotrophs, heterotrophs, photosynthetic aerobes, anaerobes, single celled and filamentous, free-living and symbiotic. The nitrogen-fixing process is similar in genetically unrelated procaryotes which possess the nitrogenase enzyme complex. The extent of biological nitrogen fixation by different bacterial systems in tropical soils has also been reviewed by Elkan (1992). Africa, with its rich biodiversity, can still take advantage of the array of organisms encompassing Legume-*Rhizobium, Frankia, Azolla-Anabaena,* associative bacteria, cyanobacteria and free-living N-fixing bacteria.

One of the most studied systems is the legume-*Rhizobium* association which fixes considerable amounts of nitrogen. Legumes are grown as subsistence crops by many African farmers. Although only 50 species are cultivated, less than 10% of the 19 000 species have been examined for their nodulation by the bacterial family *Rhizobiaceae*. Wester and Hogberg (1989) reported seven new nodulating legume species from Guinea Bissau, West Africa indicating that the list is still flexible. Earlier, Dreyfus *et al.* (1988) discovered stem nodulation in *Sesbania rostrata* growing in Senegal and named the microsymbiont *Azorhizobium caulinodans*.

The fascinating interaction of the root nodule bacteria and the legumes continues to challenge microbial taxonomists. For almost 100 years the term *Rhizobium* was used to designate those microorganisms capable of forming nodules with specific homologous legumes. Recently this classification has been revised based on literature which suggested that the differences between rhizobial species are more genetic than phenotypic. In their book, Giller and Wilson (1991) summarised the status of rhizobial taxonomy by listing five separate genera, namely: *Rhizobium, Bradyrhizobium, Azorhizobium, Sinorhizobium* and *Photorhizobium*. Refinement in classification is supported by the revelation of genetic differences. For example a new species, *Rhizobium tropici* that nodulates *Phaseolus vulgaris* and *Leucaena* spp. was proposed on the basis of multilocus enzyme electrophoresis, DNA-DNA hybridisation, analysis of ribosomal DNA organisation, a sequence analysis of 16S RNA and an analysis of phenotypic characteristics (Martinez-Romero *et al.*, 1991).

Symbiosis between *Rhizobium* and the nodulated legumes is further complicated in that formation of a nodule may result in an effective or ineffective association. The degree of effectiveness is dependent on the plant, environmental conditions and the predominant bacterial strain in the nodule. Recognising the role of *Rhizobium* strains in influencing nitrogen fixation, considerable efforts have been directed towards strain selection, identification, preservation and culture collection. Cherry (1981) reported that the Nitrogen Fixation by Tropical Agricultural Legumes (NifTAl) programme maintains a collection of over 3 000 strains at its headquarters in Hawaii. Similarly, various laboratories in Africa and elsewhere stock cultures of *Rhizobium*. The Microbiological Resources Centre (MIRCEN) network in environmental, applied microbiology and biotechnology which has been developed under the umbrella of UNESCO, UNEP and ICRO, concentrates heavily on culture collection.

Rhizobium, being a useful, non-pathogenic organism, has probably been exchanged by thousands of scientists across the world. Some of the elite cultures of *Rhizobium* are deployed for inoculant production. Again, *Rhizobium* is one of the very few organisms which, when added to natural soil by seed inoculation, can multiply to form nodules on target legumes. This age-old technology is the rationale behind inoculant production. Today, the status of inoculant production in Africa is much better than 10 years ago. For example, in Eastern and Southern Africa, there are now established production facilities in Zambia, Zimbabwe, Malawi, Tanzania, Uganda, Kenya, Rwanda and South Africa. Research intervention in rhizobiology shall not only subscribe to more efficient strains but also provide technology packages for the methods of inoculant application, protocol on the need for inoculation and quality control of inoculants.

Another system which can fix appreciable quantities of nitrogen is the *Frankia* symbiosis. The genera of dicotyledonous plants hitherto known to be nodulated by Actinorhizae (*Frankia*) are listed as 20 and cited by Elkan (1992). Some of the species such as *Purshia tridenta* are already an important forage component of rangelands in Africa. Similarly, *Casuarina,* a fast growing nitrogen- fixing tree is competing successfully as a commercial tree crop on the Kenyan Coast where it is used as building posts. The promising potential of *Casuarina equisetifolia* prompted the International Centre for Research in Agroforestry (ICRAF) in 1993 to initiate and coordinate a collaborative provenance trial of *Casuarina* established in 25 countries. The results of this experiment involving several African countries should be of interest to African BNF scientists. Meanwhile the knowledge on the physiology, genetics and taxonomy of *Frankia* ought to be improved so that it may be possible to mass culture the organism for inoculation as in the case of *Rhizobium*.

Rice consumption in Africa is generally on the increase yet production is dismal. It is known that yields of rice have been sustained in some regions of Asia at a moderate level of production of about 2 t/ha/yr even where no fertilisers are used. The role of the free-living heterotrophic N_2-fixing bacteria in these paddy soils has been recognised. Since cyanobacteria form symbioses with a greater number of diverse hosts than any other nitrogen-fixing system known, this hitherto overlooked group might be important in non-agricultural ecosystems.

The *Azolla-Anabaena* symbiosis which has been extensively exploited in South East Asia is yet to be maximised in Africa. This technology which may be new to African farmers has potential for biological nitrogen fixation, green manure supplement and animal feed. Nevertheless, *Azolla* is a heavy feeder on other nutrients, particularly phosphate, hence careful management is required.

Plant Factors in N_2 Fixation

Plants are the immediate beneficiaries of the nitrogen fixed biologically. Yet the host plant can influence nodule initiation, development and function hence controlling the proper performance of the partnership under field conditions. It is also known that size, shape, number of nodules and longevity of nodules is plant-related while the environmental stresses affect the plant as much as the microsymbiont. It is the plant that accumulates nitrogen as protein, the later improving human nutrition or soil fertility. In crop rotations and multiple cropping, the selection of host plant is crucial in managing soil fertility. The plant also furnishes carbohydrates via photosynthesis. The plant's ability to enhance nitrogen fixation can be manipulated through plant breeding as summarised by Giller and Wilson, (1991); Sanginga *et al.*, (1990) and Piha and Munns, (1987).

Environmental Constraints

Since an in-depth review of the factors limiting nitrogen fixation can be found in Giller and Wilson (1991; see also Wollum, this volume), it is not my intention to delve into the same. However, it suffices to remind ourselves that individual physical, chemical and biological factors singly or in multiple ways affect the capacity of free-living or symbiotic N_2 fixers in field situations. For example, excessive soil temperature can kill the majority of bacteria in the plough layer. In general, bacteria are less tolerant to high temperature in moist than dry soil. In the tropics high temperatures tend to be accompanied by droughty conditions. High temperatures can prevent nodulation, or inhibit N_2 fixation. Adaptation to high temperature as demonstrated by laboratory experiments showed that cowpea rhizobia isolated from Sahelian Savanna in Niger grew in media at 40°C (Eaglesham and Ayanaba, 1984). At the Nairobi MIRCEN, Gitonga *et al.* (1989) reported that thermo-tolerant strains of *Rhizobium leguminosarum* bv *phaseoli* were isolated from nodules of *Phaseolus vulgaris* grown in Kenyan soils where high surface temperatures often exceed 40°C during the day. Two of the isolates grew at 40°C on yeast extract mannitol agar. The strains also survived in moist clay soil at 40°C and 42°C for seven days.

Most tropical soils are predominantly acidic, low in phosphate while aluminium and manganese can be present in toxic quantities. Research that will provide solutions to overcome these factors is still needed. It is encouraging to note that inoculation of legumes with mycorrhiza has been shown to facilitate the uptake of phosphorus and nitrogen fixation (Paula and Sequiera, 1987; Daft, 1991). Phosphorous pelleting was also reported to be a potentially promising strategy for circumventing phosphorus constraints in reforestation of marginal grasslands (Manguiat and Padilla, 1982). In nursery soils in Sudan, irregular nodulation of *Casuarina, Acacia* and *Prosopis* spp. was attributed to salinity of irrigation water (Miettinen *et al.*, 1992). *Rhizobium* isolates tolerant to salinity were isolated in Haryana, India, presenting a possibility of using such isolates under sodic and saline soil conditions (Neeru-Bala *et al.*, 1990).

It should be borne in mind that when dealing with stresses in field situations, different factors may come into play at the same time or at different times. Limitations during one growing season might recur or change depending on the prevailing weather conditions in the next season.

Methodologies for the Study and Measurement of Biological Nitrogen Fixation

Research on biological nitrogen fixation has been accompanied by efforts to quantify the amounts ot nitrogen fixed from the atmosphere. During the first conference of the AABNF, Danso (1984) presented a detailed coverage of eight such methods. Recently, Peoples *et al.* (1989) have reviewed several techniques currently used under field and laboratory conditions. Despite the advances in methodologies, there is as yet no ideal or simple way of measuring N_2 fixation in the field. It is also equally difficult to find a method that precisely differentiates N gained from the atmosphere from N absorbed from the soil by a plant. A method chosen for each research agenda must consider the objective and priority of each hypothesis. In certain instances, the benefits derived from N_2 fixation might be measured by improved yield of subsequent crops or N accumulation in the growing crops. Considering the various shortcomings, isotope based techniques are still superior to the rest for integrated measurements of the amounts of N_2 fixation in plants, so long as the assumptions of the methods are understood and met.

Methodologies that aid in the ecological study of the bacteria in the field and laboratory have advanced in recent years. Methods such as Enzyme-linked Immunosorbent Assay (ELISA), intrinsic antibiotic resistance, fluorescent antibodies as well as serological techniques have been useful for the detection and identification of rhizobial strains. The above advanced techniques which require sophisticated equipment should not be allowed to overshadow the commonly used methods such as growth pouches, Leonard jars and related pots. Improvement of each method as better equipment becomes available is a continuous process. Even a method such as Most Probable Number (MPN) which has been in use for over 60 years to enumerate microorganisms has recently been refined using a computer programme. In the case of plant infection technique for counting rhizobia, the improved MPN method by Woomer *et al.* (1990) makes it possible to adjust for inoculation volume and initial dilution ratios. In this way, MPN experiments can now be designed to better measure the organisms of interest rather than have the design dictated by published tables.

Institutional and Socio-economic Framework

Research on N fixation is carried out by individuals or groups of research scientists based at institutions and supported by resources from within and without. The continued interest in this subject has benefitted from training of personnel, strengthening of existing institutions and at times creation of new ones. The question one might ask is whether we have an adequate human resource base to sustain biological nitrogen fixation activities in Africa. Noting the gravity of the food and environmental problems, the answer would be, no. Therefore, the quest for more support is backed by the fact that Africa suffers from a brain drain, disincentives to professionals and the high turn over of skilled staff. The African food syndrome can best be tackled by people largely trained with the tropical environment in mind. The socio-economic setting under which BNF thrives best has to be put into proper perspective if faster adoption of proven technologies is to be achieved. Promotion of excellence ought to be the goal while the need to pass on useful results to the consumers must not be forgotten. Capacity building which has been started should not be left to disintegrate. For the future, a policy framework conducive to the application of biological nitrogen fixation ought to be encouraged. It is gratifying that old organisations and even younger ones such as Round Table on Science-led Development for Africa (FRANDFORUM) recognize BNF as a crucial technology for increasing crop yields. The research and training activities carried out by the International Institute for Tropical Agriculture (IITA), The International Livestock Centre for Africa (ILCA), International Centre for Research in Agroforestry (ICRAF), MIRCENs, Tropical Soil Biology and Fertility (TSBF) and the National Institutions have subscribed to the need for capacity building. Networking among such institutions and scientists must thrive in order to cope with the formidable task illustrated by the nature of BNF itself. These interactions will benefit immensely from the personnel and the infrastructure already in place.

Conclusion

The major players in the nitrogen fixation theme are the microorganisms bearing the nitrogenase enzyme, plants closely associated with the fixed nitrogen and the environment under which the biological processes operate. Our manipulation of each component requires an ecological approach which considers the variability of the environment, the complexity in biodiversity of the plants as well as the microorganisms of choice. Whereas a lot of information has been generated on *Rhizobium*, our knowledge base is thin with respect to *Frankia*, *Cyanobacteria*, management of *Azolla* and *Rhizobium-Mycorrhiza* interactions. Future research ought to target microsymbionts which can fix more nitrogen in the presence of soil N and broad host range cowpea-type organisms such as *Rhizobium* species strain NGR 234 which nodulates at least 35 diverse genera of legumes as well as the non-legume *Parasponia* (Lewin *et al.*, 1990). Although past achievements include availability of stress tolerant rhizobia and acid tolerant cultivars, these have been based more on a single-stress format rather than multiple constraints.

With respect to methodologies, adequate techniques are at hand. What is missing is putting together a set of operational packages which can enable each laboratory to carry out work that meets the academic goals of institutional mandates yet yields useful output for extension agents. The approaches hitherto employed for crop plants may not always be suitable for N-fixing trees which have already assumed importance in agroforestry and silviculture. The outcry that BNF has yet to get out of the laboratory to the field is still relevant for Africa and probably justifies the necessity for aggressive demonstrations on farmers' fields or at open days. The BNF workers should incorporate the socio-economists where response to nitrogen fixation is likely to be positive so that adoption rates by farmers are increased.

A lot has been said about the potential role of biological nitrogen fixation but what is now required for Africa is harnessing of that potential using the existing microbial cultures, selected plants and the available database for the tropical environment. We should not wait until we have a perfect germplasm since the improvements in agricultural productivity that Africa expects from BNF are already overdue.

References

ALEXANDER, M. 1977. Introduction to Soil Microbiology, Wiley and Sons, New York.

EAGLESHAM, A.R.J. 1987. The Use of Intrinsic Antibiotic Resistance for Rhizobium Study. In: *Symbiotic Nitrogen Fixation Technology*, Elkan, G.H. (ed.), Marcel Dekker, New York,

ADELHEMM, R. AND J. KOTCH, 1985. Development and Introduction of Self Sustaining Agricultural Practices. In: *Tropical Smallholder Farms,* 19:17-20 (English Summary)

BOHLOOL, B.B., J.K. LADHA, D.P. GARRITY AND T. GEORGE, 1992. Biological Nitrogen Fixation for Sustainable Agriculture: A Perspective. *Plant and Soil*, 14:1–11

BURNS, R.C. AND R.W.F. HARDY, 1975. Nitrogen Fixation in Bacteria and Higher Plants. Academic Press Inc., New York.

CHERRY, M. 1981. The Technology of Nitrogen Fixation: Encouraging its Use in the Tropics. *Plant and Soil,* 24:114–116.

DANSO, S.K.A., 1984. Methods of Estimating Biological Nitrogen Fixation in Biological Nitrogen Fixation (BNF) in Africa. In: *Biological Nitrogen fixation in Africa*. Ssali, H. and S.O. Keya (eds.). MIRCEN Publications, University of Nairobi, Kenya.

DAFT, M.J. 1991. Influence of Genotypes Rock Phosphate and Plant Densities on Mycorrhizal Development and Growth Responses of Five Different Crops. *Agriculture Ecosystems and Environment,* 35:151-169.

DREYFUS, B., D. ALAZARD AND Y.R. DOMMERGUES, 1984. Stem Nodulating Rhizobia. pp 161–169. In: *Current Perspectives in Microbial Ecology*, Klug, M.C and C.E. Reddy, (eds.). American Society for Microbiology, Washington D.C.

DREYFUS, B., J.L. GARCIA AND M. GILLIS, 1988. Characterization of *Azorhizobium caulinodans* gen. nov. sp. nov., a Stem Nodulating Nitrogen-Fixing Bacterium Isolated from *Sesbania rostrata, Int. J. Syst. Bacteriol.* 38: 89–98.

EAGLESHAM, A.R.J. AND A. AYANABA. 1984. Tropical stress ecology of rhizobia: Root nodulation and nitrogen fixation. pp 1-35. In: *Current developments in Biological Nitrogen Fixation*. Subba Rao (ed.). Oxford and IBH, New Delhi.

EL-DIN, S.M.S.B. AND H. MOAWAD, 1988. Enhancement of Nitrogen Fixation in Lentil, Faba Bean, and Soybean by Dual Inoculation with Rhizobia and Mycorrhizae. *Plant and Soil*, 108:117–123.

ELKAN, G.H. (ed.), 1987. Symbiotic Nitrogen fixation Technology. Marcel Dekker, Inc. New York and Basel.

ELKAN, G.H. (ed.), 1992. Taxonomy of the rhizobia. *Canadian Journal of Microbiology,* 38:446–450.

GITONGA, N.M., D. WIDDOWSON AND S.O. KEYA, 1989. Interaction of *Phaseolus vulgaris* with Thermotolerant Isolates of *Rhizobium leguminosarum* bv *phaeoli* from Kenyan Soils. *Mircen Journal* 5:493–504.

GIBBS, M. AND C. CALSON, (eds.), 1985. *Crop Productivity Research Imperatives Revisited* .

GILLER, K.E. AND K.J. WILSON, (eds.), 1991. *Nitrogen Fixation in Tropical Cropping Systems*, C.A.B. International.

HENZELL, E.F. 1988. The Role of Biological Nitrogen Fixation Research in Solving Problems in Tropical Agriculture. *Plant and Soil*, 108:15–21.

LEWIN, A., E. CERVANTES, C.H. WONG, W.J. BROUGHTON, 1990. Two New *Nod* Genes of the Broad Host Range *Rhizobium* Strain NGR 234 Encode Host Specific Nodulation of the Tropical Tree *Leucaena leucocephala*. *Molecular Plant-Microbe Interactions* 3:317-326.

MANGUIAT, I.J. AND V.M. PASILLA, 1982. Rhizobium Inoculation and Phosphorus-Lime Pelleting of a Promising Leguminous Reforestation Species. *Int. J. of Ecol. Environ. Sci.* 159–166.

MARTINEZ-ROMERO, E., L. SEGOVIA, F.M. MERCANTE, A.A. FRANCO, P. GRAHAM AND M.A. PARDO, 1991. *Rhizobium tropici*, Anovel Species Nodulating *Phaseolus vulgaris* L. Beans and *Leucanea* sp. Trees. *Int. J. Syst. Bacteriol.* 3:417–426.

MIETTINEN, P., M. KARSISTO AND M.G. MUSA, 1992. Nodulation of Nine Nitrogen-fixing Tree Species Grown in Central Sudan. *For. Ecol. and Mgmt.* 48:107–119.

MULONGOY, K., M. GUEYE AND D.S.C. SPENCER, (eds.), 1990. Biological Nitrogen Fixation and Sustainability of Tropical Agriculture, Chichester, pp488.

NEERU-BALA, P., K. SHARMA AND LAKSHMINARAYANA, 1990. Nodulation and Nitrogen Fixation by Salinity Tolerant Rhizobia in Symbiosis with Tree Legumes. *Agric. Ecosystems and Environ.* 33:33–41.

PAULA, M.A. AND J.O. SIQUEIRA, 1987. Effects of Soil Moisture on Endomycorrhizal Symbiosis in Soyabean 1. Root Colonization, Sporulation, Nodulation and Nitrogen Accumulation. *Plant Soil* 11:283–287.

PEOPLES, M.B., A.W. FIZAH, B. RERKASEM AND D.F. HERRIDGE, 1989. Methods for Evaluating Nitrogen Fixation in Nodulated Legumes in the Field. ACIAR Monograph No. 11, ACIAR Canberra.

PIHA, M.I. AND D.N. MUNNS, 1987. Nitrogen Fixation Potential of Beans *(Phaeolus vulgaris* L.) Compared with Other Grain Legumes Under Controlled Conditions. *Plant and Soil* 98:169–182.

SHU, F.Y., H.J. SUN, W.G. LI, AND Q. LEI, 1990. Influence of Eco-environment on the Symbiotic System of *Rhizobium-Robinia, Journal of Ecology* 9:51–53.

SANGINGA, N., G.D. BOWEN AND S.K.A. DANSO, 1991. Intra-Specific Variation in Growth and P Accumulation of *Leucaena leucocephala* and *Gliricidia Sepium* as Influenced by Soil Phosphate Status. *Plant and Soil* 133: 2, 201–208, 21.

SANGINGA, N., G.D. BOWEN AND S.K.A. DANSO, 1990. Assessment of Genetic Variability for N_2 Fixation Between and Within Provenances of *Leucaena leucocephala* and *Acacia albida* Estimated by ^{15}N Labelling Techniques. *Plant and Soil* 127: 169-178.

SSALI, H. AND S.O. KEYA, (eds.), 1984. Biological Nitrogen Fixation in Africa., Nairobi *Rhizobium Mircen* Publication, University of Nairobi.

WOOMER, P., J. BENNETT, AND R. YOST, 1990. Overcoming the Inflexibility of Most Probable Number Procedures. *Agronomy Journal* 82: 349–353.

WESTER, J. AND P. HOGBERG, 1989. New Nodulating Legume Tree Species from Guinea Bissau, West Africa, *Forest Ecology and Management* 29:311–314.