Role of Microorganisms in Sustainable Tropical Agriculture

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Introduction

In contrast to most tropical eastern countries, the South American continent is underpopulated. Many areas, mainly in the central regions, are available for development and represent possibilities for potential grain yield increases. More than two million square kilometers of savannas of the Brazilian, Venezuelan and Colombian central highlands, are covered with "cerrado" vegetation which could be cultivated by adding economically justified factors such as phosphorus, lime and minor elements (Freitas, 1971). This is a fascinating problem for agronomists and soil scientists since one half of this area could produce 30 million tons of soybean protein each year, enough to feed 25 percent of the actual world population by U.S. standards (100 g protein/person/day). These calculations are certainly hypothetical, but they may indicate the potential of this vast area.

In concentrating our efforts on the recuperation of unused land, preference should be given to the savannas while the forest reserves, for example, the Amazon region, should be maintained in its present state because the few years of rapidly degrading soil fertility in this environment will not compensate for ecological losses. Among the different savanna types, the edaphic savannas seem most suitable for agricultural use. In climatic savannas, indispensable irrigation costs are compensated only by highly intensive and sophisticated agricultural practices as, for example, the production of alfalfa hay in irrigated areas of northeast Brazil.

The economic importance of savannas as natural pastures might seem small if productivity per hectare is considered, but the immense areas involved in Brazil alone provide maintenance for 50 million cattle, which are fed almost exclusively on the natural pastures of Cerrado or Caatinga. In both types of savanna, herbaceous forage legumes are relatively unimportant, while legume shrubs and trees represent a major part of the vegetation and contribute to the non-deficient nitrogen economy of the soils. In the dry season, pods and browse of several shrubs, mainly Mimosaceae and Caesalpinoideae, represent a major protein supplement to the poor quality dry grass. The carrying capacity for cattle is about one animal per 10 hectares or more (Marques, 1965). Most regions with cerrado vegetation receive 6 months of heavy rain (more than 1000 mm) which is sufficient for the growth of many crops. In recent years a considerable part of the Cerrado area in Brazil has been cultivated, increasing from 1.4 million hectares in 1965 (Marques, 1965) to 11 million hectares in 1988 (J.R. Peres, personal communication). Consequently, the amount of grain produced in Brazil's Cerrado now comprises 27 percent of the country's total production. More intensive agricultural systems in those areas or in less extensive available regions of the humid tropics or subtropics require legumes as the main nitrogen source. However, agricultural development in the Cerrado has many problems. For example, chemical fertilizers are not only expensive and difficult to obtain, but their application is not feasible because of the large land area and heavy rains where several top dressings would have to be applied. Losses of mineral N through leaching are extensive.

Biological Alternatives

There are many possible alternatives to the development of agricultural systems which make use of the potential biological processes in the soil that may replace agrichemical input. These are especially important in the intensive, highly mechanized cropping systems of industrialized countries and often are assumed to be necessary in developing countries, especially on larger properties farmed by the more highly educated landholders. These farmers are exposed to intensive advertising campaigns by multinational firms which tend to ignore the fundamental differences between temperate and tropical agriculture. Tropical agriculture consists of much more extensive and diversified cropping systems.

At the other extreme, farmers in Latin and South America are exposed to mostly demagogic theories propagated by certain ecologists who have never critically analyzed properly planned experiments and, therefore, propose agricultural systems which usually are not economically or environmentally viable, especially over the long-run.

Agricultural cropping systems must be based on statistically-designed, scientific experiments which yield reproducible results and are economically viable. Very little of such information is available, especially for tropical agriculture. The most promising systems are based on crop rotations or agroforesty systems such as those considered "traditional" before agrichemicals became "traditional." Such systems make maximal use of biological dinitrogen fixation (BNF) and rock phosphates, and they are the most logical approach to biological control of plant diseases and pests. They also recognize the need for soil conservation.

Scientific investigations on crop rotations, and even more so on agroforesty systems, require long-term experiments which are costly and difficult to perform, especially in South America where funds for research are limited. Such experiments require perseverance and result in few published papers; few scientists remain in this region who think in those terms.

Even though such systems attempt to recycle nutrients, the minerals removed with the harvest have to be replaced in order to maintain soil fertility. Natural vegetation, i.e., climax savannas and forests, are closed systems where few elements are lost and where the energy used for respiration is obtained by photosynthesis. Once man interferes, other elements, mainly nitrogen, are rapidly lost through leaching and runoff. This usually results in rapid multiplication of legumes, both annuals and trees, which can help to restore the natural vegetation if other elements are available.

In agricultural systems, leaching of soil nitrogen and rapid decomposition of soil organic matter are the main reasons for declining soil fertility. Fortunately, many diversified alternatives exist to obtain nitrogen from the non-1imiting reserves of atmospheric dinitrogen. There are no eucarionts which can directly use atmospheric dinitrogen and, therefore, the various symbiotic and associative bacterial systems with higher plants represent the major way of recycling nitrogen from the air. Biological nitrogen fixation is, therefore, the most important single item within the various potentially viable alternatives for replacement of agrichemicals. The tropics offer more favorable climatic conditions for biological processes during most of the year and also many other alternatives for the use of BNF. However, because of the same favorable climatic conditions, soil erosion and the concommitant loss of soil fertility and productivity can occur very rap-idly.

Even though there is an unlimited supply of nitrogen in the atmosphere as N_2, this element is the major limiting factor for agricultural production. In developing countries it can comprise more than 70 percent of the cost of chemical fertilizer. Crop rotations, which include pulses and green manure legumes, can replace nitrogen fertilizers to a large extent, but such systems require well-defined technologies. They are based on the exploitation of highly sophisticated symbiosis of plants of the Leguminosae family which harbor in their root nodules more or less specific strains of *Rhizobium* which are fed by the plant in exchange for "fixed" nitrogen that is essential for optimal yields.

N_2 Fixation in Legumes

Grain and forage legumes play important roles in tropical agriculture. After coffee, soybean is the most important export crop of Brazil and its growing area is advancing into the central high-land savannas (cerrados). This is possible because of plant breeding programs and the development of new *Rhizobium* inoculants. The Rhizobia had to be adapted to the specific microbial disequilibrium prevailing in newly cultivated soils. These new *Rhizobium* strains, however, are now being used. Even though they produce large nodule masses and fix a lot of N_2, they are rather inefficient in terms of the transference of fixed N to the grain crops. Recently, new strains were identified which increased harvest indexes from 60 to 82 percent (Neves *et al*., 1985). Grain yield increases in the field with these "super strains" can reach 45 percent (Table l) provided the strains can be established. In the cerrado soils, however, they can not be used because of competition with the established, less efficient strains. Various techniques to make these new strains more competitive are being studied.

Table 1. ***Rhizobium*** **Strain Effects on Nitrogen Fixation and Yields of Field Grown Soybeans.***

Strain	N_2 fixed (kg N ha^{-1})	Nodule Efficiency (mg N_2 fixed g^{-1})	Seed Yield (kg ha^{-1})	N Harvest Index
29W	243b	426b	1863c	60.1b
DF 395	214b	543b	1768cd	61.1b
SMb	204c	491b	1398de	56.0b
965	278a	899a	2898a	86.5a
CB 1809	239b	1005a	2682a	82.2a
DF 383	242b	851a	2284b	82.0a
Control	0	-	948f	49.1bc

*Calculations were based on the difference in total plant nitrogen between inoculated and control plants

$$HI = \frac{\text{Seed N}}{\text{Total N}} \times 100$$

Source: Neves et al. (1985)

Nitrogen fixation in beans has an additional problem related to the genetic instability of the NOD and FIX genes in the traditional *Rhizobium phaseoli* strains because of the frequent genetic rearrangement of these strains (Martinez *et al*., 1988). These authors observed two groups (possibly two species) of *Rhizobium* capable of nodulating beans. Group 1 is specific for *Phaseolus vulgaris* and is genetically unstable in relation to symbiotic growth; group 2 also nodulates *Leucaena leucocephala* and other tree legumes. Strains of group 2 have their NOD and FIX genes localized on two large plasmids, and show little or no rearrangement. Therefore, the strains of group 2 are much more stable.

In the tropics, the instability of bean *Rhizobium* strains is aggravated by excessively high soil temperatures. With two heat shocks of only 8 hours at 38C, well-nodulated beans become completely inefficient within 10 days (Hungria and Franco, 1988). However, nodulation and N_2 fixation of certain legume tree species (e.g., *Leucaena* or *Prosopis*) were unaffected by an 8 hour/day regime at 38C during the entire growth cycle (C.O. Cunha and A.A. Franco, personal communication). Selection from more than 100 *Rhizobium* strains isolated from 18 tree legume species provided 14 strains (isolated from *Leucaena, Gliricidia* or *Lonchocarpus*) which were able to nodulate beans effectively and fix N_2. When these strains were used to inoculate beans grown at an 8 hour /day 40C soil temperature regime, some of them were able to nodulate and fix N_2, thereby yielding plants equal or superior to those treated with N fertilizer (Table 2).

Table 2. Effect of High Soil Temperature on Nodulation and N_2 Fixation of Beans Inoculated with *Rhizobium* sp. Strains Isolated from Tree Legumes.

Origin of strains	Strain No.	Nodule (No./plant)	Total N (mg/plant)
Gliricidia sp.	BR 8801	8c	15d
	BR 8802	20cd	22cd
	BR 8803	12c	14d
Leucaena sp.	BR 814	46b	41ab
	BR 816	40c	41ab
	BR 817	68a	52a
Lonchocarpus sp.	BR 6009	14de	28c
	BR 6010	60ab	48ab
	BR 6011	20cde	22cd
Phaseolus vulgaris	CNPAF 146	2c	12d
P. vulgaris + 90 mg N/plant		0	35bc

Plants were grown in Leonard jars in a water bath with an 8 hour/day 40qC regime. Values are means of four replicatc jars. Source: Hungria and Franco (1988)

Legumes for Green Manure

Several tropical legumes are available which can be planted either as an intercrop or after the first crop is harvested. Several of them can fix large amounts of dinitrogen and some can assimilate phosphorus from rock phosphates which cannot be readily utilized by grain crops. For example, Silva *et al*. (1985) showed that *Stizolobium aterrimum* can obtain as much phosphorus from rock phosphate as from soluble phosphatic fertilizers. When such green manure cover crops are incorporated into the soil, phosphorus and nitrogen are present in slow release organic forms. These characteristics make green manure an economically viable and a valuable contribution to crop rotations. An estimate of the economic return of a crop rotation including such green manures in comparison with monocropping of maize is reported in Table 3.

Table 3. Effect of Crop Rotation on Maize Yield and Net Return for Five Years

	Maize Yield 1982/83 (kg/ha)	Maize Yield 1984/85 (kg/ha)	Net Return after 5 years (US S/ha)
Maize in monoculture	4480	1855	1178
Maize in crop rotation	3696	2703	1869
With rock phosphate	4808	2671	1780
With rock phosphate and green manure	5283	3023	1575

The crop rotation from 1981 to 1985 was *Phaseolus* beans-maize-peanuts-cassava intercropped with cowpea-majze. Green manure was *Stizolobium*.

Source: F.F. Duque and G.G. Pessanha (in preparation)

Forest Legumes

Brazilian reforestation projects, until recently, did not consider one of the most important characteristics of the native legume trees, i.e., their ability to fix N_2. The most valuable of the hardwood species and many native fast growing trees are legumes, but little is known about their capacity to nodulate or to fix N_2. Surveys in the northeastern dry regions, in the Amazon rain forest, and in southeastern Brazil discovered many economically important N_2-fixing trees not known before (Table 4). Mesquite (*Prosopis juliflora*), called algaroba in Brazil, is being planted on large government projects in the northeastern dry regions. Since 1982 it has been treated with commercially available inoculants developed by EMBRAPA.

Table 4. Nodulation of Brazilian Forest Legumes

	Subfamilies			
	Mimosoideae	Papilionoideae	Caesalpinoideae	Total
No. of species verified	102	119	100	321
No. of species with nodules	82	90	15	187
No. of species found for the first time with nodules	39	55	13	107
No. of genera found for the first time with nodules	2	6	2	10
No. of *Rhizobium* strains isolated	389	387	60	836

Source: Faria *et al*. (1987)

The development of agroforesty systems, which are included in the ten-year crop rotation periods for legume forests, supply the farm with energy and emergency fodder during dry years. Another almost unexplored prospect is that of rehabilitating degraded (eroded) soils by increasing their organic matter content with the large quantities of protein-rich leaves that fall oh the ground.

Cereals and Grasses

The extension of biological nitrogen fixation to cereals and grasses has been a major research challenge in the last two decades. Because plants, as with other eucarionts, cannot use molecular N_2, the most promising approach seems to be that of identifying some of the less symbiotic associations of bacteria which are able to fix N_2 in association with cereals. The transference of N_2 fixation genes into plant cells is a more pretentious alternative which, if successful, could become the most expedient solution. Unfortunately, progress in this area is very slow even though some promising naturally-occurring associations of N_2-fixing bacteria with cereals have already been identified.

A typical result which leads to the conclusion that nitrogen fixation must occur with rice is that of App *et al*. (1984). In this study, nitrogen analysis of long-term fertility plots at two sites in the Philippines were performed before and after 17 and 24 crops of paddy rice and showed positive N balances of 103 and 79 kg N/ha per year, respectively. Under temperature-controlled conditions, after 82 years of continuous wheat in the Rothamsted Broadbalk experiment, a positive N balance of 30 kg N/ha per year was estimated (Jenkinson and Rayner, 1977). Evaluations over shorter periods with forage grasses are in the same range (Jaiyebo and Moore, 1963; White *et al*., 1945). More precise estimates over short-term periods can be obtained by use of the ^{15}N iso-tope. In these experiments, either the incorporation of $^{15}N_2$ gas into plant material or soil, or the dilution by $^{14}N_2$ from the air of plants growing with ^{15}N-labelled fertilizer, was determined.

Substantial but variable amounts of N_2 fixation have been demonstrated with these methods in rice (Watanabe and Roger, 1984); sorghum (Wani *et al*., 1984); and forage grasses (De Polli *et al*., 1977; Boddey and Victoria, 1986). In recent experiments with sugarcane, N balance and ^{15}N dilution measurements have provided unequivocal proof that more than 50 percent of the plant nitrogen came from the air (Lima *et al*., 1987). More recent experiments with N balance studies in a large tank (Urquiaga and Dobereiner, 1989; Urquiaga and Boddey, unpublished data) indicated that sugarcane fertilized with PK alone can obtain most of the nitrogen needed for yields of 200 tons of cane/hectare from BNF (Table 5). There were pronounced differences between plant genotypes showing that the plant plays an important role and that plant breeding will be an important approach for increasing BNF.

Table 5. Effect of Sugarcane Genotype on N_2 Fixation.*

Sugarcane Cultivar	**Total N Accumulation (kg ha^{-1} yr^{-1})**	**Contribution of BNF (kg ha^{-1} yr^{-1})**	**(%)**
Saccharum oficinalis			
CB 45-3	258ab	164	60
CB 47-89	205bc	95	46
NA 56-79	193bc	83	43
Sp 70-1143	259ab	148	57
Saccharum espontaneum			
Krakatau	343a	233	68
Saccharum barberi			
Chunee	110cd	0	0
Brachiaria radicans			
Tanner grass	110cd	0	0

* N_2 fixation was measured in an 80-cm tank by total N accumulation and ^{15}N dilution compared with non-N-fixing control (*Brachiaria radicans*). Values are the means of three cuts.

Since it is known that considerable amounts of nitrogen can be fixed in association with cereals and other Gramineae, an understanding of the associated physiology is essential to manipulate and increase their efficiency. Many different N_2-fixing bacteria have been isolated from the rhizosphere and from roots of cereals, but only where plant-bacteria interactions exist is there an association. Pathogenic plant-bacteria associations have long been known and, therefore, effects of microorganisms are visible as damage to the plant tissue. Characteristic of these associations is the specificity that can be demonstrated at the strain or species level. Plant breeding for resistance to specific pathogens is one of the major objectives in agricultural research. Breeding for improved associations of plants with N_2-fixing bacteria will have to envisage different characteristics.

The introduction of N-free semi-solid media is the key to the identification of several additional new root-associated, microaerobic, N_2-fixing bacteria, six of which were discovered in Brazil (four *Azospirillum* spp, *Herbaspirillum seropedicae*, *Bacillus azotofixans*, *Acetobacter diazotrophicus* and three poorly defined *Pseudomonas* species) (Table 6). This medium apparently resolved the complicated problem of microaerobic, N_2-fixing bacteria which need O_2 for respiration and generation of ATP, but can not fix N_2 in the presence of oxygen. In the semi-solid medium, these microorganisms move actively, aided by chemotactic attraction, to the zone where their respiration rate equals the oxygen diffusion rate. There they have optimal access to O_2 but none accumulates in the medium, and, therefore, selective growth occurs with molecular N_2 as sole nitrogen source. Characteristics of these diazotrophs are summarized in Table 6.

Table 6. Comparison of New N_2 Fixing Bacteria which Occur in Association with Plant Roots.

Characteristic	Azospirillum brasilense, A. lipoferum	A. amazonense	A. halopraeferans	Herbaspirillum	Acetobacter	Bacillus	Pseudomonas
Growth under air	+	+	+	+	+	+	+
Microaerobic N_2 fixation	+	+	+	+	+	-	+
Growth with N_2 as sole N source	+	+	+	+	+	+	-
N_2 fixation unaffected by 10 mM NO_3	+	+	+	+	+	+	-
Use sucrose	-	-	-	-	+	+	-
Optimum pH	6.0-7.0	5.8-6.6	6.8-8.0	5.3-8.0	3.8-5.5	6.5-7.5	6.5-7.5
Optimum temperature (C)	35	35	41	35	30	32	30
Isolated from surface sterilized roots	+	+	-	+	+	+	+
Isolated from stems	+	+	-	-	+	-	+

Source: Baldani *et al.* (1986); Barraquio *et al*, (1983); Cavalcante and Dobereiner (1988); Reinhold *et al.* (1987); Seldin *et al.* (1984); Tarrand *et al.* (1978); Watanabe *et al.* (1987).

Many inoculation experiments have been performed with a few strains of *Azospirillum brasilense* and *A. lipoferum*, but the more recently discovered organisms have not yet been tested. Thus far, the effects observed repeatedly on yield and total N incorporation are highly significant but can not be attributed to BNF. Hormonal effects (Okon, 1985) and more efficient soil N assimilation (Boddey *et al*., 1986; Ferreira *et al*., 1987) seem to be the major causes in most cases.

In only a few of these experiments have the responsible N_2-fixing bacteria been unequivocally identified, or has the mechanism of association been determined. While legumes form nodules as a response to infection with their N_2-fixing symbionts, grasses show no visible bacterially-induced structures. However, there is attachment to the root hairs (Umali-Garcia *et al*., 1980), infection and multiplication within the root and sometimes in the stem tissues by *Azospirillum* spp, *Herbaspirillum* and the recently discovered *Acetobacter diazotrophicus* (specifically in sugarcane) (Cavalcante and Dobereiner, 1988; Gillis *et al*., 1989). Certain strains of *Azospirillum* were established by inoculation specifically in the roots or on the root surface of certain cereals (wheat and sorghum) in fields where large natural populations were present, but the increases in observed N incorporation were again attributed to more efficient fertilizer use rather than to BNF (Ramos Pereira *et al*., 1988; Baldani *et al*., 1987).

Conclusions

The challenge, therefore, is to enhance BNF in cereals and grasses even though many new bacteria are now known which fix N_2 in laboratory cultures, and which are associated with and infect cereal and grass roots. Modern biotechnological methods, as well as traditional plant breeding and strain selection and adaptation, will have to be explored in order to reach this goal.

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