

THE ROLE OF BIOLOGICAL
NITROGEN FIXATION IN
LAND RECLAMATION,
AGROECOLOGY AND
SUSTAINABILITY OF
TROPICAL AGRICULTURE

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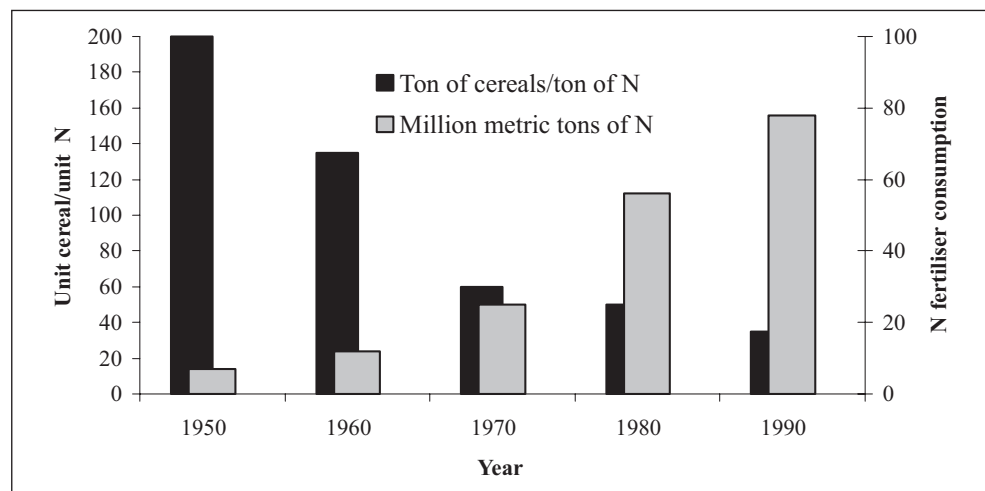
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INTRODUCTION

The great worries about the population growth of the last half century has been beaten by the increase of food production, not only on acreage but also on productivity. By mid 1990's, total food supply reached 2,740 calories per person per day, far beyond the 2,200 calories per day generally accepted as the nutritional bottom line to maintain human life. The United Nations predict that supply will continue to grow faster than population at least through 2010.

The increase in fertiliser use, especially nitrogen, has played a major role in these productivity gains. However, increasing the amount of nitrogen applied decreases its use efficiency (Figure 1) and a large amount of it is left causing environmental problems. This will add to the wastes left from intensive animal production already the biggest source of pollution.

FIGURE 1 – USE AND USE EFFICIENCY OF NITROGEN FERTILISER (WORLD BANK, 1992).



Plant products constitute 93% of the human diet and 66% of the world food supply is provided by about eight species of cereals (maize, wheat, rice, barley, sorghum and millet). The demand for cereals for the year 2025 as projected by Borlaug & Dowsell (1997) (Table 1) is likely to be around 4.0 billions metric tons, although this may be an overestimation since they have based their predictions on the demographic growth of the preceding decade.

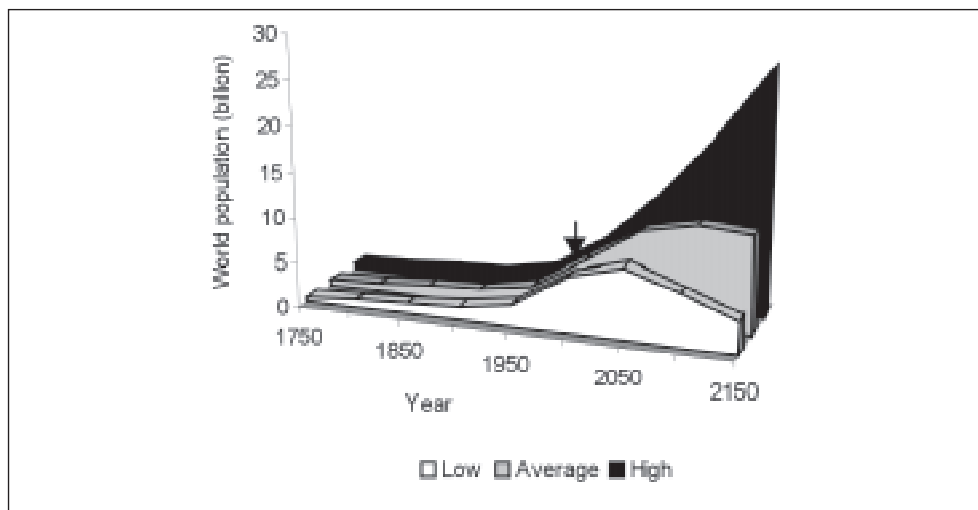
TABLE 1 – POPULATION AND WORLD CEREAL SUPPLY OF THE WORLD.

YEAR	POPULATION ¹ (MILLIONS)	FOOD SUPPLY ² (MILLION T)	PRODUCTIVITY ² (T/HA)
1990	5,284	1,970	2.5
2000	6,185	2,450	2.9
2025	8,303	3,970	4.5

¹World Development Resort (1992); ²Borlaug & Dowsell (1997)

This decade the population rate has decreased world-wide below what had been projected a few years ago, and may decrease even more than previously expected as shown in the bottom line of figure 2. Considering the lower rate of demographic growth, food demand will not increase much more than is produced today. Hunger today is not caused by lack of food but mainly as a consequence of lack of proper distribution and for political reasons. Preventing losses during harvest, post-harvest handling and storage also may contribute to increase the availability of food. In Brazil more than 300 million dollars are lost annually during the soybean harvest and storage. The Brazilian farmer has the capacity to storage only 5% of the total harvest, while in Argentine they may store 50% of the harvest and in the United States twice the amount harvested (Pavan, 1998).

FIGURE 2 - WORLD POPULATION - PRESENT AND PROJECTED.



Contamination and degradation of the environment, atmospheric CO₂ accumulation and lack of clean water may be foreseen as the main problems of the next century. Therefore, the focus on feeding the people of the world must be changed to the production of clean food on a clean and sustainable environment.

The remaining frontiers for crop acreage increases have predominantly acid soils and low natural fertility. The combinations of toxicity (Al, Mn, Fe and H) and deficiencies (N, P, Ca, Mg, K and some micronutrients such as Zn, Mo, B, Fe etc.) are serious plant growth-limiting factors. To maintain high crop yields on these soils with chemical and mineralogical characteristics that still impose strong limitations to phosphorus availability (Muniz et al., 1985; Ruiz et al. 1988; Villani et al., 1990; Novais & Smith, 1998) represents a great challenge. Selection and development of crops species and cultivars that grow and produce well at lower levels of available soil nutrients (high nutrient use efficiency) seems an important strategy for sustainability in tropical soil systems (Bernardo, 1995; Barros & Novais, 1996; Grespan, 1997; Baligar & Fageria, 1997; Novais & Barros, 1997).

The enhancement of soil productivity is a combination of appropriate tillage practices, crop rotations and planting time, the application of soil conservation measures to reduce loss of nutrients, the strategic use of organic matter and mineral fertilisers in doses tailored to match farmer's combination of crops, availability of organic materials and market opportunities.

Soil organic matter has a very important influence on soil physical and chemical properties, on biological activities (Fassbender, 1987), and as a source of plant nutrients, especially nitrogen. The only exception to crops that are able to grow in soils depleted of organic matter, are those associated with diazotrophic bacteria, if nutrients other than nitrogen are available. Thus, instead of productivity alone, tropical agriculture in the next century must favour biodiversity, the preservation of soil organic matter and efficient cycling of nutrients. BNF may play an important role on restoring fertility and sustainability under those conditions.

Nitrogen, even though its abundance in the atmosphere, is the most limiting nutrient for crop yields in many soils, while phosphorus is the most limiting nutrient under natural conditions in the tropics and the second most limiting plant nutrient for farmers. Considering that the production of nitrogen fertiliser is the most costly both economically and energetically, that less than half of the nitrogen applied is recovered by the first crop under tropical conditions and that it strongly contributes to pollution, thus nitrogen availability must be a priority in any attempt to increase clean food in a clean environment. In this context BNF by micro-organisms free living in the soil or associated with plants have a great potential to contribute to food production. BNF uses energy derived from photosynthesis and does not accumulate excess nitrogen to cause pollution. The use of fertiliser has been increasing and is already higher in developing than in developed countries that are also using less fertiliser since 92/93 (Figure 3). This may be a consequence of an excess supply that is projected to increase even further in the next few years (Figure 4). The high surplus of nitrogen fertiliser will certainly result in price decline.

FIGURE 3 - CONSUMPTION OF FERTILISER IN THE WORLD (FAO 1998).

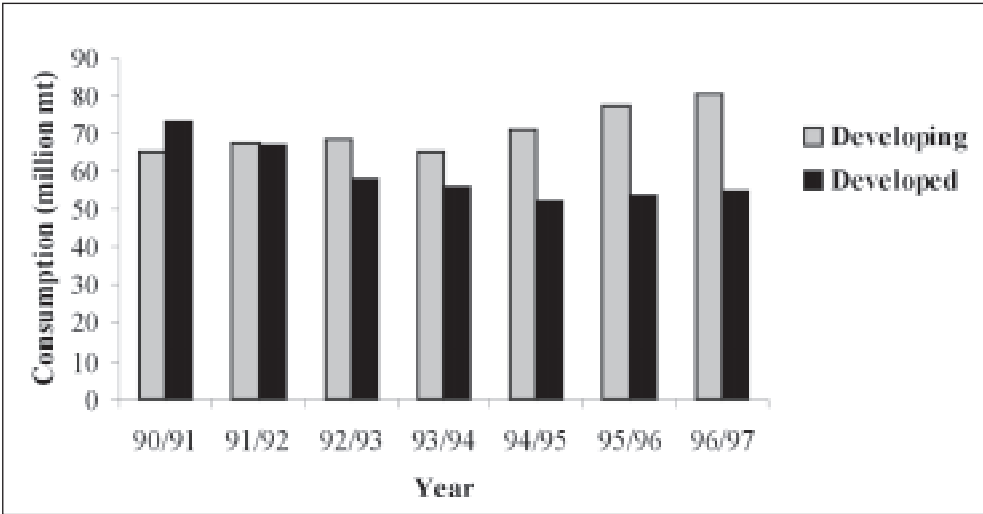
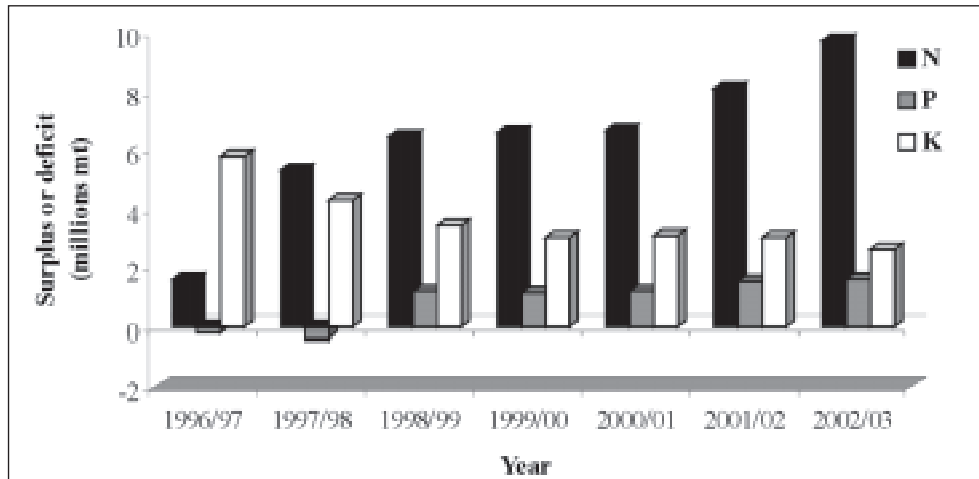


FIGURE 4- WORLD N, P₂O₅ AND K₂O SUPPLY AND DEMAND BALANCE (EXTRACTED FROM FAO, 1998).



Yamada et al. (1998) estimated that, from 1993 to 1996, 1.05 million tons of nitrogen (=US\$ 680 millions) were used per year in Brazil while BNF in the soybean crop alone has contributed to an estimated saving of US\$ 1.5 billions every year in replacing N that would be required in nitrogen fertilisers. Even though the soybean crop in Brazil may be used as the best example of the contribution of BNF to food production and the country economy, the total nitrogen acquired is still insignificant when compared to the estimated total BNF contribution in natural ecosystems. The annual contribution of BNF in terrestrial environments has been estimated around 175 million metric tons. At present costs this would be equivalent to 148.5 billion dollars in nitrogen fertilisers (Elkan, 1992).

BIOLOGICAL NITROGEN FIXATION IN AGRICULTURE

Many agronomic plant species associate with diazotrophic microorganisms able to fix atmospheric nitrogen. Among the legume species are the crops with highest potential for BNF already available to be used in the productive systems. Blue green algae, free-living in the soil or associated with the fern *Azolla* and endophytic associations between diazotrophic bacteria and gramineous and starchy species have also potential to be used in agriculture (Dobereiner et al., 1999). Table 2 presents some values on BNF in some crops. Even though the variability of conditions and methods where these values were obtained, they indicated the great potential of BNF already available. There are also experiments showing BNF contribution of 30 kg of N/ha.year in free-living cyanobacteria and up to 3 kg of N per ha per day in the symbiosis *Azolla* with cyanobacteria (Watanabe & Roger, 1984).

BIOLOGICAL NITROGEN FIXATION IN THE SOYBEAN CROP IN BRAZIL

Last year, 153 out of 274 million tons of oil-producing crops were soybean. Of this total 30.6 million tons were produced in Brazil, the second largest world producer. In spite of high taxation, inefficient structure of storage and transport, that reduces

Table 2 – Amount of nitrogen fixed (kg/ha/year or cycle) by some leguminous crops and tree species

PLANT SPECIE	BNF KG/HA/YEAR OR CYCLE	%NDDA	DAYS	COUNTRY
Grain crops				
Soybean (<i>glycine max</i>)	114-188	84-87	66	Nigeria ¹
	85-154	70-80	110	Brazil ¹
Beans (<i>Phaseolus vulgaris</i>)	25-65	37-68	60-90	Brazil ¹
	3-32	15-72	61	Brazil ¹
	11-53	19-53	-	Brazil ²
	18-36	32	47	Colombia ²
	92-125	69-73	-	Guatemala ²
Pigeon pea (<i>Cajanus cajan</i>)	68-88	88	-	India ¹
	168-208	-	-	³
Cowpea (<i>Vigna inguiculata</i>)	9-51	32-74	110	Brazil ¹
	73-354	-	-	³
	66-120	54-70	57	Nigeria ¹
Mung bean (<i>V. mungo</i>)	119-140	95-98	66	Thailand ¹
Chickpea (<i>Cicer arietinum</i>)	60-84	60-80	160	Australia ¹
Forage legume				
Leucaena (<i>Leucena leucocephala</i>)	500-600	-	-	³
Centrosema (<i>Stylosanthes pubescens</i>)	80-280	-	1 ano	Several ¹
Stylo (<i>Stylosantes spp.</i>)	20-263	-	1 ano	Several ¹
<i>S. capitata</i>	3-46	-	1 ano	Brazil ¹
Kudzu (<i>Pueraria phaseoloides</i>)	-	-	-	Colombia ¹
Tree species				
Acacia (<i>Acacia mearnsii</i>)	200	-	-	³
Gliricidia (<i>Gliridia sepium</i>)	-	26-75	-	Several ¹
<i>A. auriculiformis e mangium</i>	-	52-66	-	Several ¹

¹After Giller e Wilson (1991); ²After Hardarson (1993); Franco & Dobreiner, 1994; ³After Greenland (1985); Kang and Duguma (1985)

the value of Brazilian soybean by about 20% on the international market, it is still competitive (Silva, 1998; Pavan, 1998), mainly because it is grown with BNF.

The early history of the introduction of soybean in Brazil and the research that made it possible is described by Myasaka and Medina (1981), with the collaboration of most scientists that contributed to the success of the crop. The first attempt to introduce soybean in Brazil at the end of last century in Bahia State was a failure. Later an attempt was made in S. Paulo State, also without much success and finally in Rio Grande do Sul State where the lower latitudes had favoured the varieties available that were more sensitive to photoperiodism. The first export of 150 tons occurred in 1938. The official records started in 1941 with the production of 457 tons of grain in 702 ha. By 1947 the cultivated area had increased to 7,651 ha with a yield of 6,396 tons, with productivity of only 836 kg of grain per hectare. From this point on the soybean cropping area expanded throughout Rio Grande do Sul, Paraná and S. Paulo States. In 1960 was released the first cultivar bred in Brazil: cv. Pioneira, adapted to lower latitudes.

This expansion has been due to three main factors: a) the incorporation of the Central West region to cropping system; b) replacing of rice, *Phaseolus* bean, cassava, potato, onion, maize and coffee cropping areas in Central South areas, increasing 88% the soybean cropping area from 1970 to 1973 and mainly to c) the support given by a net of breeders, agronomist, soil scientists (soil fertility, plant nutrition and rhizobiologists) all over the country integrated by the National soybean cultivar trials and the National soybean versus inoculant trials. This network of research was co-ordinated by the Departamento Nacional de Pesquisa Agropecuária, later on transformed in the Brazilian Agricultural Research Corporation (EMBRAPA) from the Ministry of Agriculture and Food Supply. At the early seventies soybean crop gets into the cerrado region and up closer to the equator.

Together with breeding and adaptation of the plant to lower latitudes, studies on BNF on soybean were conducted in Rio Grande do Sul, S. Paulo and Rio de Janeiro States, not only to obtain *Bradyrhizobium* strains adapted to the new cropping areas but also to identify the main limiting biotic and abiotic limiting factors (Oliveira & Vidor, 1984a,b,c; Myasaka & Medina, 1981; Peres et al., 1984). At the same time several experiments were conducted in the cerrado region with the objective to test the effect of starter nitrogen and *Bradyrhizobium* strains adapted to the Cerrado soils and inoculum concentration (Vargas & Suhett, 1980; Vargas et al., 1982a; Vargas et al., 1982b, Scotti et al., 1993, 1997). It was again demonstrated that if soybean is well nodulated with efficient bradyrhizobia, even under low nitrogen availability, there is no response to nitrogen fertiliser with grain yields up to 3192 kg/ha.

Commercial inoculum was already available in 1949, at first in agar slants and from 1955 on using peat as carrier. Today the Brazilian inoculum industry has a turnover of US\$15 million annually. More than 95% of inoculant production is for soybean alone (Araújo, 1998).

The total amount of bradyrhizobia inoculum necessary for the 1997 crop was 600 metric tons, very little if compared with the amount of nitrogen fertiliser that would be required to meet the plant needs. Considering a 50% use efficiency of the fertiliser, 6 million tons of urea would be necessary to supply the crop demands. The figure 5 illustrates the annual economy in nitrogen fertiliser in relation to the total value of the crop, the value for internal use and the export. It is astonishing that BNF represents between 30 and 50% of the exported value, contributing in this way for the reduction of the ecological debt if all nitrogen used by the crop were from nitrogen fertilisers. To this should be added the costs of transport and application of the fertilisers and the costs derived from the problems caused the environment by the N not used by the crop. Other important point derived from this figure is that more that 50% of the soybean value is exported and greater effort should be made for soybean to contribute more to increase the availability of the protein to the population. In spite of the criticism about the precision in which those estimates of BNF were obtained, considering only the case of soybean, the economy derived from BNF represents much more than what has been invested in all research in agriculture in Brazil.

Last year the soybean cropping area was over 13 million hectares, with a productivity of 2,365 kg/ha and an export of 1,885 million metric tons of nitrogen (Table 3). In spite of the large expansion of the crop into harsh environments there was an increase of 182,8% in productivity since 1941, as a result of the technology that had been developed in Brazil during this period.

FIGURE 5 – ANNUAL CONTRIBUTION OF BNF ON BRAZILIAN SOYBEAN CROP IN RELATION TO THE TOTAL PRODUCTION AND EXPORT IN THE LAST TWO DECADES COMPARED TO THAT OF 1998.

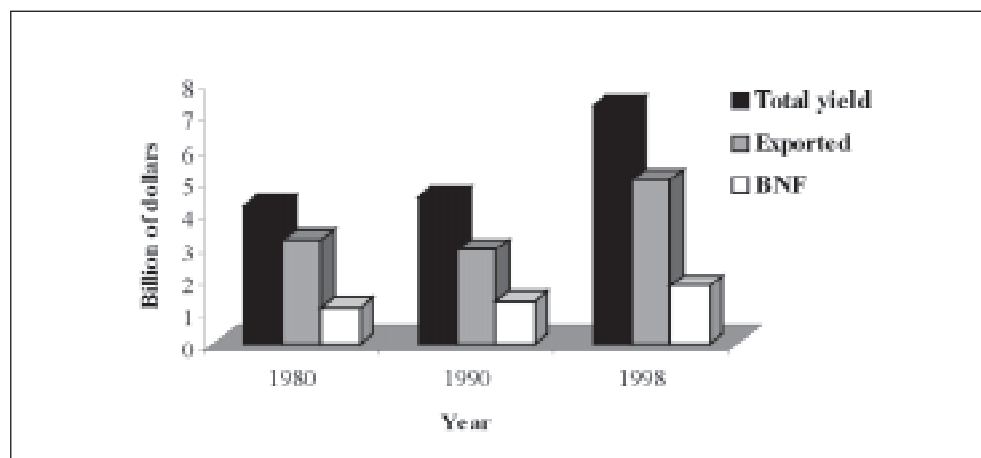


TABLE 3 – PLANTED AREA, TOTAL PRODUCTION, PRODUCTIVITY AND TOTAL NITROGEN EXPORTED IN THE GRAINS FROM 1970 TO 1998 IN BRAZIL.

Year	Cropping area (ha)	Total grain yield (t)	Productivity (kg/ha)	N exported ³ (thousand t)
1970-71 ¹	1,716,420	2,014,291	1,174	121
1980-81 ¹	8,501,169	15,007,367	1,765	900
1990-91 ¹	9,583,000	15,522,000	1,620	931
1997 ²	11,540,330	26,508,030	2,297	1,591
1998 ²	13,285,610	31,419,144	2,365	1,885

¹ IBGE (1992); ² IBGE (October 97, July 98), FIPE AGRÍCOLA (1997,1998); ³N Estimates considering 6% of N in the grain.

Today the Brazilian productivity, without using transgenic material, is higher than in USA and this represent an advantage for markets that restrict transgenic food. Not considering the other environmental and human hazard potential, for pure economical reasons, it is difficult to accept the present trend to introduce transgenic soybean in Brazil.

BIOLOGICAL NITROGEN FIXATION AND *PHASEOLUS VULGARIS*

The *Phaseolus* bean represents an important source of staple food protein to Latin America. Most of it is cropped by small farmers with very low productivity. In Brazil, the mean productivity has been around 500 kg/ha for decades (Table 4), even though, under some conditions it may be higher than 3,000 kg/ha. In contrast to soybean the nitrogen economy of beans is more due to nodulation with native rhizobia

than by inoculation with selected rhizobia. Beans nodulate with many native rhizobia - three species have already been described (*Rhizobium leguminosarum* bv. *phaseoli*, *R. tropici* and *R. etli*) and possible new ones currently under study (Mercante, 1998). A few data on biological nitrogen fixation in *Phaseolus* bean are presented in table 4. Even though it may obtain rates of BNF similar to that of soybean, the inoculation with selected rhizobia strains by farmers is not a guarantee of yields above that of the native populations. This constitutes an additional difficulty to convince extensionists and the farmers about the contribution of BNF to this crop.

TABLE 4 – CROPPING AREA, TOTAL YIELD, PRODUCTIVITY, TOTAL NITROGEN EXPORTED AND VALUE OF THE N THAT WOULD BE DERIVED FROM BNF IN PHASEOLUS BEAN FOR 1996 AND 1997, IN BRAZIL.

YEAR	AREA (HA)	TOTAL YIELD (T)	PRODUCTIVITY (KG/HA) (T)	TOTAL N IN THE GRAIN	VALUE ² US\$ x 10 ⁶
1996 ¹	2,707,890	1,334,830	493	53,393.2	45.3
1997 ¹	2,474,720	1,396,420	564	55,856.8	47.4

¹ FIPE AGRICOLA (1997); ² Considering US\$ 848.54 per metric ton of nitrogen.

Due to the short plant cycle preferred by the farmer, the delay for the crop to start benefiting from BNF and the decline of BNF in the early stages of grain filling stages (Franco & Dobereiner, 1994), what seems most important today is to work the plant genome for better performance as nitrogen fixers. Unfortunately, *Phaseolus* bean plant breeders have relegated BNF in their breeding programs and few effort have been made to increase BNF in this crop (Bliss et al. 1989, Bliss, 1993). For the *Phaseolus* bean cultivars in use or that are been released nowadays, the most import for improving BNF is to find the best nodulating cultivars and to remove the biotic and abiotic limiting factors to the symbiosis of the crop (Franco & Dobereiner, 1994).

Among the rhizobia that nodulate *Phaseolus* bean, *Rhizobium tropici* are the most tolerant to soil acidity (Vargas & Grahan, 1988) and to high temperature Mercante, (1993), some strains are competitive for nodulation (Vlassak, 1997) and have been recommended for the commercial inoculant production in Brazil.

Phaseolus bean is cultivated in the tropics even though it does have several characteristics of a temperate crop. It is sensitive to soil acidity and high temperature, and requires high levels of nutrients, especially P and Mo, especially when dependent on BNF (Franco & Dobereiner, 1994). The crop is also sensitive to several crop diseases and pests, including a larvae of an insect, identified as *Cerotoma arcuata* that eats nodules of several legumes but is especially detrimental to the *Phaseolus* bean crop because its short cycle (Teixeira et al.,1996).

The experiments conducted in Brazil with well nodulating type II and type III cultivars, with a cycle around 90 days, may yield up to 1,500 kg of grain per hectare in nitrogen poor soils, inoculated with selected strains of rhizobia but without nitrogen fertilisers. Yields above that may be obtained combining inoculation with rhizobia and nitrogen fertiliser applied 3 weeks after germination as demonstrated by Franco et al. (1979).

Results from Vidor et al., (1992), who conducted 64 experiments over 3 years in bean cropping areas in Rio Grande do Sul State, have indicated, that for a well nodulating cultivar, there was, on average, an increase of grain yield of 12%. For similar increases over the whole bean cropping area in Brazil, that would generate a net additional 19 million dollars in grain yield increase.

BNF AND FORRAGE CROPS

As early as the 1940s the Australians started on a program to develop mixed pastures based on grasses and legumes of the tropics. In the 1960s and 70s considerable success was achieved at selecting suitable grass/legume combinations suited the soil and climate of different regions of tropical and sub-tropical Australia such that animal performance was increased above that of the grass-only swards. This success encouraged Brazilian and other South American scientists to try to introduce these combinations of tropical grasses and legumes to the savannas and areas of cleared forest in their countries. These attempts met with little success as the legumes did not persist for more than a year or so and it became a general belief that mixed grass/legume pastures were not viable in South America. The main causes of failure were bad management of pastures and nutritional constraints to the legume species.

In recent years a large scale screening of many species and accessions of forage legumes was undertaken in many South American countries under the program RIEPT (Rede Internacional de Evaluación de Pastos Tropicales) organised by the Centro Internacional de Agricultura Tropical (CIAT) in Colombia. Many hundreds of accessions were screened and together with soil amendments some materials have shown a good ability to persist in mixed swards. A trial in the Atlantic forest region in the South of Bahia have shown that beef cattle grazing on mixed pastures of *Brachiaria dictyoneura* with the forage legume *Arachis pintoi* can make daily weight gains of over 500 g per head per day and yields per hectare of over 2 kg liveweight per day which compares to maximum yields recorded of 1.2 kg ha⁻¹ day⁻¹ for other grass-only *Brachiaria* pastures at the same site (Table 5).

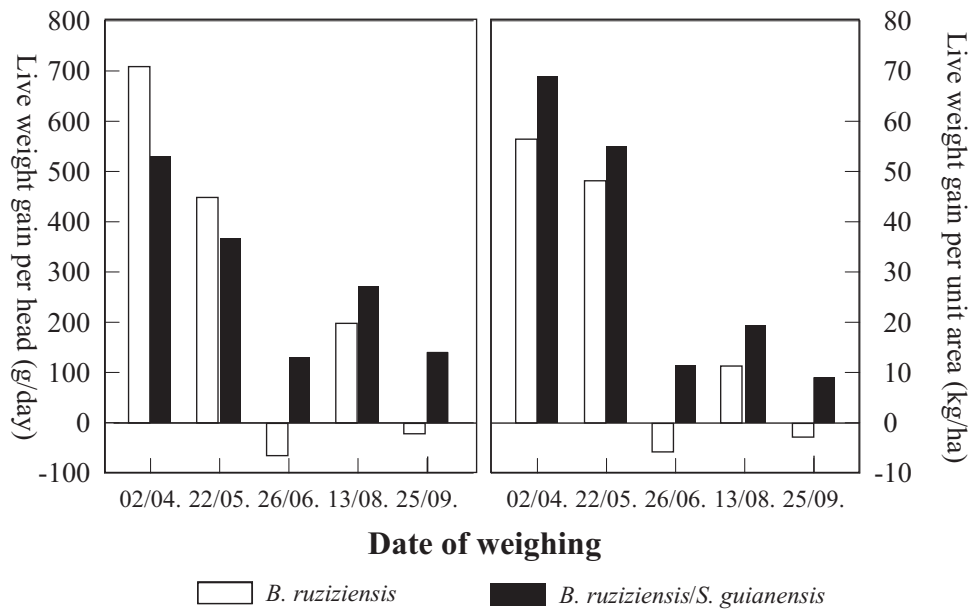
TABLE 5. LIVE WEIGHT GAINS OF NELORE CATTLE GRAZING ON A MIXED PASTURE OF *BRACHIARIA DICTYONEURA* AND *ARACHIS REPENS* IN THE ATLANTIC FOREST REGION, SOUTHERN BAHIA, BRAZIL. DATA FROM *SANTANA AND PEREIRA (1995).

Period	Stocking rate animal/ha	Live weight gain g/animal/day	g/ha/day
Nov. 92-May 93 (182 days)	1.6	549	879
	2.4	571	1,368
	3.2	575	1,841
	4.0	494	1,978
May 93-Feb. 94 (286 days)	1.6	499	797
	2.4	590	1,416
	3.2	509	1,629
	4.0	502	2,010
Oct. 94-May 95 (205 days)	1.6	688	1,100
	2.4	697	1,674
	3.2	688	2,201
	4.0	798	3,194

Recent studies on nutrient cycling and degradation of pastures have shown that in the Cerrado region the principal cause of pasture decline is overgrazing which causes a reduction in the proportion of nutrients recycled via the plant litter to the point where it becomes too low to support further forage growth (Boddey et al., 1996). Nitrogen has been shown to be the most critical nutrient followed by phosphorus (Oliveira et al., 1997), and the introduction of a strongly persistent N_2 -fixing legume, such as those cited above, should not only increase animal yields but also increase the resistance of the pasture to decline in productivity.

One serious problem in the Cerrado region is that cattle generally lose weight during the severe dry season. Recent results using the legume *Stylosanthes guianensis* (cv Mineirão) have shown that as the legume is of low palatability compared to *Brachiaria* in the wet season the cattle leave considerable quantities of legume for forage in the dry season. Data from a trial conducted near Uberlândia (MG), showed that where this legume had been introduced into a *B. ruziziensis* sward, the cattle continued to gain weight throughout the dry season in contrast to the weight loss experienced in the neighbouring grass-only sward (Fig. 6).

FIGURA 6. COMPARISON OF LIVE WEIGHT CHANGES OF NELORE CATTLE GRAZING A PURE *BRACHIARIA RUZIZIENSIS* AND A MIXED *B. RUZIZIENSIS*/*STYLOSANTHES GUIANENSIS* SWARD DURING THE DRY SEASON IN THE CERRADO REGION NEAR UBERLÂNDIA, MINAS GERAIS. DATA FROM AYARZA ET AL., 1998.



BNF ASSOCIATED WITH NON-LEGUME CROPS

Since the fifties it has been shown that the N_2 bacteria *Beijerinckia* were associated with grass roots (Dobereiner & Ruschel, 1958). In the seventies several species of *Azospirillum* were found to infect roots of maize, wheat, rice and other grasses (Boddey & Dobereiner, 1988). Even though the acetylene reduction assay has given indication of nitrogen fixation in these systems only in the last twenty years more

reliable data from nitrogen balance and isotopic dilution technique experiments, was it possible to quantify with more confidence the contribution of BNF on these systems. Since then, it has been observed that between 10 and 50% of the nitrogen incorporated were derived from the atmosphere in wetland rice, sugar cane and forage grasses (Boddey & Victoria, 1986, Miranda & Boddey, 1987, Boddey & Dobreiner, 1988, Urquiaga et al., 1992).

Soil core experiments in the field with *Brachiaria humidicola* and *B. decumbens* indicated that 30 to 40%, respectively 29 and 45 kg N/ha, of the nitrogen accumulated in the plant were derived from the atmosphere (Boddey & Victoria, 1986). Using similar procedure, Miranda and Boddey (1987) studying 10 *Panicum maximum* ecotypes, observed during the plant active growth rate, up to 30% of total nitrogen accumulated (10 kg of N/ha per month) were derived from the atmosphere. All these studies, even though indicated a good potential of BNF to be exploited in the long run, were obtained under low to median productivity conditions.

Approximately 20% of the fertiliser commercialised in Brazil is for the sugar cane crop, even though it receives less than 100 kg of N per hectare per year. Without water stress, sugar cane seldom responds to nitrogen fertiliser, especially after the first cutting. Urquiaga et al. (1992) in a three year experiment, where it was compared several cultivars of *Saccharum officinarum* with *S. spontaneum* growing in large tanks filled with 85 metric tons of soil containing 0.09% of ¹⁵N labelled N: Two varieties of *S. officinarum*, CB 45-3 and SP 70-1143 presented high yields up to the last harvest, accumulating, respectively 164 and 148 kg N/ha from the atmosphere. The other cultivars also accumulated significant quantities of nitrogen during the three successive harvests. These results are convincing evidence of the great importance of BNF in the sugar cane under high productivity conditions, at least three times the Brazilian current productivity.

If all cane sugar cultivars planted in Brazil were replaced by the two cultivars with high BNF, the economy in nitrogen fertiliser for this crop would be approximately half billion dollars per year (Table 6).

TABLE 6 - YIELD, COPPING AREA, POTENTIAL OF NITROGEN FIXATION AND ESTIMATED ECONOMY IN NITROGEN FERTILISER OF CANE SUGAR PLANTED IN BRAZIL.

YEAR	AREA (HA)	TOTAL YIELD (THOUSAND T)	BNF ² (THOUSAND T)	VALUE ³ U\$ (MILLION)
1996 ¹	4,827,320	325,929.07	596.17	505.9
1997 ¹	4,852,740	333,649.82	599.31	508.5

¹ FIPE AGRICOLA (1997); ²BNF: total nitrogen derived from the atmosphere considering the levels of fixation (123,5 kgN/ha) obtained by Urquiaga et al., 1992; ³Assuming US\$848.54 per metric ton of N.

BNF AND AGROECOLOGY

NITROGEN FIXING LEGUME TREES (NFT) AND AGROECOLOGICAL SYSTEMS

Nair et al. (1984) attributes two important functions for the trees in mixed cropping systems: production and protection. Cellulose, food, energy, wood, dyes, medicine, forage, poles, etc., are few of the products of N_2 -fixing legume trees. Erosion control, wind-breaks, shade, water quality, landscape stabilisation, nutrient storage and timing of release are some of the important attributes of protection of N_2 -fixing trees (NFT) in mixed cropping.

Several articles have stressed the importance of NFT as a way to insert BNF into productive systems (Peoples & Craswell, 1992; Budowski & Russo, 1997). Kass et al. (1997) have reviewed the importance of BNF on agroforestry systems indicating the several systems, natural or developed by men, where a combination of a NFT and other cash crops are used as source of nitrogen and sustainability. Alley cropping has been studied extensively but as yet has limited use.

The amount of N incorporated by the NFT is variable with plant species and growing conditions but may be as high as 231 kg N/ha.year in 3 months (Table 7).

TABLE 7 – N_2 -FIXED AND PROPORTION OF TOTAL N ACCUMULATED IN LEGUME TREE SPECIES DERIVED FROM THE ATMOSPHERE¹.

LEGUME SPECIES	COUNTRY	N ₂ FIXED		DURATION
		PROPORTION	AMOUNT (kgN/HA)	
<i>Acacia spp.</i>	Australia	-	12	annual
	Philippines	0.52-0.66	-	
	Senegal	0.3	3-6	6.5 months
	Mexico	-	34	annual
<i>Falcataria moluccana</i> (= <i>Albizia falcataria</i>)	Philippines	0.55	-	
<i>Cajanus cajan</i>	Australia	0.65	-	90 days
<i>Calliandra calothyrsus</i>	Australia	0.14	11	90 days
<i>Gliricidia sepium</i>	Australia	0.75	99	90 days
	Mexico	-	13	annual
	Philippines	0.6	-	
<i>Leucaena leucocephala</i>	Malasia	0.58-0.78	182-231	3 months
	Nigeria	0.34-0.39	98-134	6 months
	Tanzania	-	110	annual
	Thailand	0.59-1.00	-	
<i>Sesbania cannabina</i>	Phillipines	0.93	119-188	45-55 days
<i>S. grandiflora</i>	Indonesia	0.79	-	2 months
<i>S. sesban</i>	Senegal	0.13-0.18	7-18	2 months

¹ Peoples & Craswell (1992)

The use of a nodulating legume, however, is not a guarantee of high rates of BNF. Beyond good conditions for growth, it is necessary to appropriate conditions that favour the establishment of rhizobia in the soil, in the rhizosphere and the functioning of the symbiosis. It is also necessary to conform the land occupation with the main crop within the farmers social conditions and culture. This seems in reality the main difficulty to increase the participation of BNF as soil conditioner agent in farming systems. Pasture arborization with NFT seems a great possibility to increase BNF under tropical conditions (Carvalho, 1997, Carvalho et al. 1997).

The use of nodulating legume tree as live stakes is an example of a technology that may support sustainability. It is cheap to implement, in addition may produce wood for energy, forage, honey, incorporate N to the system, but above all, it does have a strong ecological appeal importance as it substitutes native hard wood depletion and deforestation (Kass et al.,1997). Fast growth and ease of rooting from cuttings are two important characteristics of nodulating legumes species for fast and simple establishment of the plant in the presence of animals, today restricted to only two genera. Special strategies must be also developed to allow establishment of these species from seedlings in the presence of animals to allow a greater diversity of species to be used.

BNF AND LAND RECLAMATION

It may be considered that at present at least 50% of all cultivated land has some degree of degradation and at current rates of land degradation a further 2.5 million km² of farm land could become unproductive by 2050. The build up of organic matter in the system is the main factor for land rehabilitation, especially in the tropics with prevalence of acidic soils, with high aluminium saturation and low phosphorus availability. However, the quality of the organic matter is important for nutrient cycling and availability for succeeding or intercropped species on these substrates. Nitrogen, lignin and polyphenols content present good relationships with the rate of mineralization and seem the most important indicators of organic matter quality (Fox et al., 1990; Palm & Sanches, 1991; Thomas & Asakawa, 1993).

Nodulating and mycorrhizal legume species have been found as the best colonisers of substrates without organic matter (Franco et al., 1992; Franco & Faria, 1997, Dommergues, 1997). The data summarised on table 8 were from several studies on land reclamation conducted in greenhouse and in the field and indicates the best species used on land reclamation under tropical conditions in Brazil.

Identification of nodulating legume species and isolation and screening of rhizobia to be used for inoculation in land reclamation projects have been done at Embrapa Agrobiologia since the eighties (Faria et al.1984, 1989). Franco & Faria (1997) summarised the results of more than 50 experiments listing the rhizobial strains most efficient for several legume species, including the species indicated for land reclamation: *Acacia mangium*, *A. auriculiformis*, *Sesbania exasperata*, *A. holosericea*, *M. caesalpinifolia*, *M. tenuiflora*, *Sclerolobium paniculatum*, *Albizia saman*, *A. lebbbeck*, *Pseudosamanea guachapele*, among others.

TABLE 8 – NUTRITIONAL REQUIREMENT AND TOLERANCE TO ACIDITY AND SOIL COMPACTION OF SOME FAST GROWING N₂-FIXING TREES USED IN LAND RECLAMATION. NUMBERS REPRESENT ARBITRARY VALUES, 5 LOWEST REQUIREMENT OR HIGHEST TOLERANCE AND 1 THE HIGHEST REQUIREMENT OR LEAST TOLERANCE.

SPECIES	NUTRITIONAL REQUIREMENT			TOLERANCE TO:	
	P	K	S	ACIDITY	SOIL COMPACTION
<i>Acacia mangium</i> ^{2,7,8}	3 – 5	2	2	4	5
<i>A. auriculiformis</i> ^{2,7}	3	2	2	3	5
<i>A. holocericea</i> ^{3,7}	3	3	3	4	5
<i>Sclerolobium paniculatum</i> ⁵	4	3	2	4	3
<i>Mimosa tenuiflora</i> ^{6,7}	5	3	3	2	2
<i>M. caealpinifolia</i> ⁷	-	-	-	4	4
<i>Enterolobium contortisiliquum</i> ⁷	-	-	-	4	5
<i>Leucaena leucocephala</i> ⁷	-	-	-	2	2
<i>Samanea saman</i> ¹	-	-	-	4	5
<i>Stryphnodendrum guianensis</i> ¹	-	-	-	4	4
<i>Acacia angustissima</i> ¹	-	-	-	3	4
<i>Acacia crassicarpa</i> ¹	-	-	-	-	3
<i>Falcataria moluccana</i> ¹	4	-	-	4	4

¹FRANCO et al. (1996); ²DIAS et al.(1990); ³BALIEIRO et al. (1995); ⁴BALIEIRO et al (1999); ⁵DIAS et al. (1991); ⁶PAREDEZ et al. (1996); ⁷FERNÁNDES et al. (1994); ⁸FARIA et al. (1996).

Another factor that increases the potential of these species for land reclamation is its symbiosis with arbuscular mycorrhizae fungi (AMF). The main function of the fungi is to increase the acreage of the plant roots to harvest nutrients and water (Siqueira, 1996). The effect of inoculation with rhizobia or arbuscular fungi varies with nitrogen or phosphorus availability and plant species, and frequently show a synergistic effect. An example of this response may be observed in table 9 for *Leucaena leucocephala* growing in greenhouse in a latosol where the responses to mycorrhizae were greater than to rhizobia, indicating that plant growth was more limited by P than N (Table 9).

TABLE 9 - RESPONSE OF LEUCAENA LEUCOCEPHALA TO INOCULATION WITH RHIZOBIA AND MYCORRHIZAE^{1,2}.

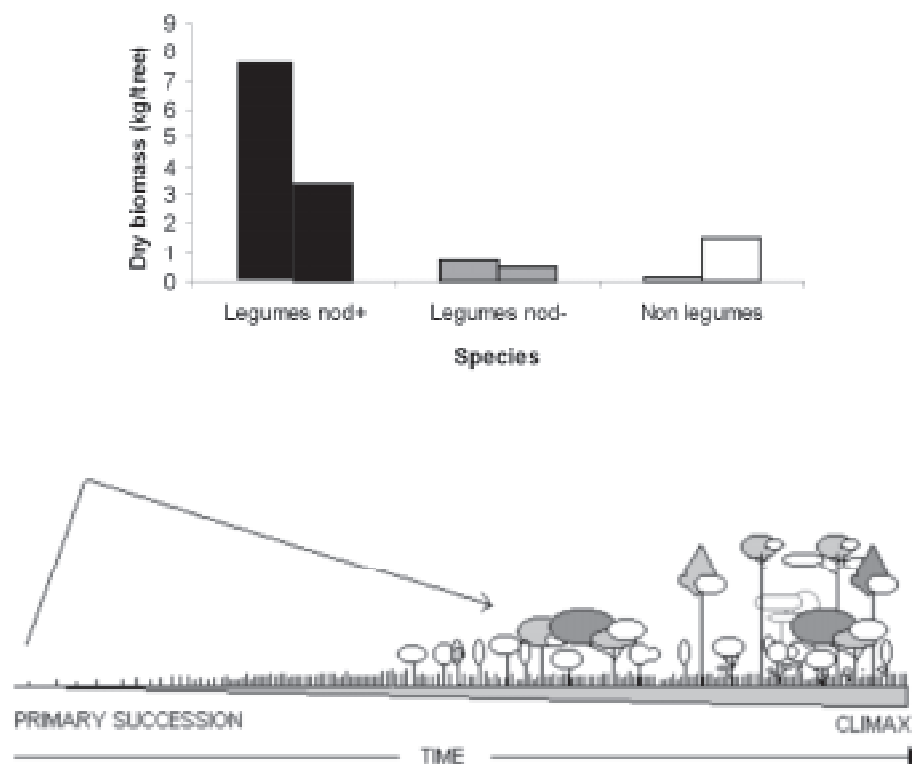
INOCULATION TREATMENTS	NODULE DRY WEIGHT	ROOT INFECTION AMF	PLANT DRY WEIGHT	ACCUMULATED IN THE SHOOT	
				N	P
	(g/pot)	(%)	(g/pot)	(mg/pot)	
None	0	0	4.3 d	116 d	5 c
Rhizobia (R)	81.7	0	5.7 c	200 c	8 c
Arb. Mic. Fungi (AMF)	0	55b	8.1 b	243 b	14 b
(R) + (AMF)	143.2	70a	10.4 a	406 a	25 a

¹From Costa & Paulino (1992).

²Values in the same column followed by different letters differ at 5% by Tukey test. different

The inoculation with AMF also increases water uptake and the survival of the seedlings transplanted to the field (Awotoye et al., 1992; Santos et al., 1994). This is critical in land reclamation projects where the substrates are frequently devoid of organic matter and have poor structure. The addition of two litters of cow manure on an acid substrate devoid of organic matter and poor in nutrients ($0.4 \text{ cmol/dm}^3 \text{ Ca+Mg}$; $3 \text{ mg/dm}^3 \text{ K}$; $0 \text{ mg/dm}^3 \text{ P}$ and $\text{pH}=4.6$), favoured the establishment of nodulating legume, non nodulating legume and others species (Franco et al., 1996). However, even without addition of organic matter, the two best fast growing NF-species accumulated, 22 months after transplanting to the field, more than 8 metric tons/ha of dry matter. These results indicated that the period of land reclamation could be considerable abbreviated by using nodulating and mycorrhizal NF species as indicated in figure 7. The top figure represents the biomass accumulated in 22 months of two nodulating and two non nodulating legume, and two non leguminous tree species growing in a substrate without detectable nitrogen and carbon (Franco et al., 1996). By using the nodulating and mycorrhizal species the time of land recovery could be substantially reduced without much investment.

FIGURE 7 - COLONISATION OF A SUBSTRATE DEVOID OF NITROGEN - NATURAL X USING NF-SPECIES. INSERT EXTRACTED FROM FRANCO ET AL. (1996).



Nitrogen availability is of utmost importance for plant colonisation, however, the organic matter quality may affect the intensity and quality of the colonisation plants. Legumes, in general, present litter fall with higher nitrogen content than non legume species. Table 10 presents some data on litter analyses of *Acacia mangium* compared with *Eucalyptus pellita* in land reclamation of a latosol sub soil in a bauxite mining in the Amazon (Dias et al. 1994). The results indicate the superiority of the legume species over *Eucalyptus* in dry matter and nutrients accumulated in the litter.

TABLE 10 – DRY MATTER AND NUTRIENTS ACCUMULATED IN THE LITTER OF ACACIA MANGIUM AND EUCALYPTUS PELLITA, GROWN IN AN EXPOSED SUBSTRATE IN BAUXITE MINING SITE IN PORTO TROMBETAS-PA

PLANT SPECIES	LITTER DRY MATTER	NUTRIENTS ACCUMULATED IN THE LITTER (KG/HA)					RATIO C/N
		P	K	Ca	Mg	N	
Eucalyptus	4,664.4	0.56	2.75	45.86	4.86	27.45	93.41
Acacia	7,844.6	1.37	4.90	29.43	5.46	94.47	38.72

From: Dias et al. (1994)

The chemical composition of the litterfall will determine the velocity of decomposition and the cycling of nutrients regulating the growth of the succeeding species. The legume species may also increase the action of the soil fauna, but there are differences among species. Correia et al., (1995), observed a greater increase of saprophagous group under *Mimosa caesalpiniiifolia* than under *A. mangium*. The *M. caesalpiniiifolia* litter contained higher levels of lignin and nutrients (N, P and K) while the levels of soluble polyphenols and C/N ratio were lower than that of *A. mangium* litter (Table 10). Froufe et al., (1999) have also observed higher rates of decomposition of *Pseudosamanea guachapele* than under *A. mangium* that was superior to *Eucalyptus grandis*.

TABLE 11 – CHEMICAL CHARACTERISTICS OF LITTER OF ACACIA MANGIUM AND MIMOSA CAESALPINIIFOLIA

Plant species	C	N	P	K	Ca	Mg	Lignin	Polif.
				dg/kg				
<i>A. mangium</i>	42.61	1.399	0.023	0.22	0.91	0.28	12.28	5.21
<i>M. caesalpiniiifolia</i>	43.94	1.655	0.048	0.36	0.68	0.13	18.84	4.66

From: Correia et al. (1995)

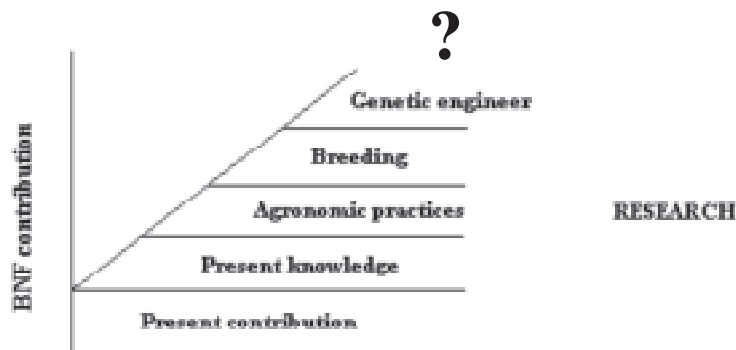
This approach of land reclamation by using BNF and using minimum quantities of fertilisers has been showing good results in the field. Franco et al., (1994) are recommending the application of 100g rock phosphate per plant with success in several soils of Brazil while Dart (1995) is recommending 200g of rock phosphate per plant. Gypsum, potassium sulphate and a mixture of rock phosphate and superphosphate or any local cheap fertiliser may also be used as a source de P, K and S, according to soil analysis and fertiliser availability. The application of small amounts of organic matter close to the seedling at planting may replace, in some situations the need of adding micronutrients, however the application of 10 g per seedling of a mixture of fritted trace elements (FTE) will guarantee the micronutrients during the colonisation of the land. This technology has already been used with great success in several municipalities and mining areas in Brazil.

BNF AND FOOD PRODUCTION IN THE NEXT CENTURY

In absolute terms N_2 fixation is small compared with total soil N reserves ($105,000 \times 10^6$ t N), but it is at least several fold greater than inputs of N from N fertiliser (65×10^6 t N/yr).

There is no doubt about the importance of BNF for soybean, other grain legumes, forage legumes and land reclamation. Plant sources commonly provide 90% of the calories and up to 90% of human dietary protein in tropical regions (Peoples & Craswell, 1992). There are also several agroforestry systems, in use or under study, in which BNF may be of economic importance (see reviews of Bohlool et al., 1992, Peoples and Craswell, 1992, Giller & Cadisch, 1995 and Kass et al., 1997). Nitrogen fixing trees are an important component of the production systems for coffee and cacao, two very important cash crops. Studies in the Amazon with fallow-rotation systems incorporating fast growing nodulating legume trees have shown great advantage over the traditional fallow-rotating systems (Brienza et al., 1997). Agrosilvicultural systems are also being studied in the Amazon region (Wanderlli et al., 1997). Incorporating the knowledge already available and by a little of research efforts the present BNF levels may certainly be more than doubled. With more research removing the limiting factors and on the genetic of the bacteria and the plant, it is difficult to predict the limit of BNF contributions (Figure 8).

FIGURE 8 - PRESENT AND FUTURE POTENTIAL OF BNF (MODIFIED FROM GILLER AND CADISH, 1995)



A good example of a combined sustainable production system has been developed at CATIE in which *Erythrina*, a nitrogen fixing tree, associated with the non-nitrogen fixing *Morus spp.* and King grass. The *Erythrina* trees are pruned periodically and the foliage applied to the soil. Goats are fed a combination of only King grass, *Morus spp.*, mineral salts and water. The goat manure is applied to the shrubs and grass associated with *Erythrina*. Over three years, 1,200 m² of land that contained 800 trees of *Erythrina beteroana* and two goats supported production of an average of 12,000 kg of milk per year with the mineral salts fed to the goats as the only input from outside the system. Profit was US\$4,800 per hectare per year (Oviedo et al. 1994). The productivity in this system was high, profit was high, whereas the principal input of nitrogen to the system was through BNF and the system sustainable.

Comparing benefits and constraints in favour of BNF or the use of nitrogen fertilisers (Table 12) may favour either sources depending on the scenario of the moment which can not be predicted due to the uncertainties of the next century.

TABLE 12 – BENEFITS AND CONSTRAINTS IN FAVOUR OR AGAINST BNF AND THE USE OF NITROGEN FERTILISERS.

Nitrogen fertilisers (NF)	Biological Nitrogen Fixation (BNF)
<ul style="list-style-type: none"> -Plants prefer combined nitrogen -Use fixed energy for production and distribution: increase atmospheric CO₂ and global heating - May generate acidity or alkalinity, depending on source - Crops less sensitive to biotic and abiotic stresses - More prone to cause eutrophication, HNO₃ in aerosols in the rain, ozone layer destruction (NO_x) from denitrification - Health problems: Methamoglobin caused by excess NO₃ and NO₂; Cancer caused by nitrosamines; and Respiratory diseases caused by NO₂ and HNO₃ - Expensive 	<ul style="list-style-type: none"> - BNF occurs under low N availability - No fixed energy necessary, no atmospheric CO₂ increase no global heating - Generate acidity: may be used for rock phosphate solubilization on the rhizosphere - Crops more sensitive to biotic and abiotic stresses - Equilibrated system less prone to cause problems to the environment - No known health problems – possibly produces a more equilibrated food - Less expensive

Even though, as less agricultural land became available, pressure increases toward higher yields and the use of nitrogen fertilisers, the understanding and development of more efficient NF-systems may favour BNF. Unfortunately, the increasing nitrogen surplus will certainly be pushing toward adding nitrogen fertiliser to the production systems. It is certainly the quality of food and the problems caused by excess use of nitrogen fertiliser the driving forces that in the next century may increase the use of BNF in crop production.

ACKNOWLEDGEMENTS

The authors acknowledge the National Development and Scientific Council (CNPq) for financial support and Dr. Robert M. Boddey for the chapter on forage legumes and together with Dr. M. C. P. Neves and Dr. S. M. de Faria for reviewing this paper.

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