Biological nitrogen fixation and soil fertility maintenance

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Introduction

Nitrogen compounds comprise from 40% to 50% of the dry matter of protoplasm, the living substance of plant cells. For this reason, N is required in large quantities by growing plants and is indeed the key to soil fertility. Non-N_2-fixing plants – for example, cereals – take all the N they need from the soil. In Senegalese conditions this uptake was estimated to be as follows: 79-132 kg N/ha/crop for pearl millet; 74-84 for rice; 134 for sorghum; and 121-138 for maize [3]. N_2-fixing plants, essentially legumes, take a part of the N they require from the atmosphere, but we shall see later that the N uptake from soil may be relatively high.

When N fertilizers are available, soil N levels are maintained or improved by applying these industrially fixed N sources. Such a technology, which allows continuous crop yields, is successfully used in intensive agricultural systems, but the following limitations have been progressively appearing: increasing cost especially in developing countries, low yields resulting from leaching and denitrification in many tropical soils, and pollution of underground water by nitrates. The other alternative for maintaining or improving the soil N status is to exploit biological N_2 fixation.

In the last decades a tremendous amount of work has been devoted to the study of this process. Very promising results have been achieved at the level of the molecular and genetic manipulations of N_2-fixing organisms, but agriculture and forestry have not yet benefited from these remarkable advances in our knowledge. Such a failure can be partly explained by the following attitudes of researchers: (1) most studies have been focused on the symbiont and have neglected the host; (2) the identification of the potential limiting factors acting in situ has been overlooked, probably because of the extreme difficulty of this task; (3) relatively few attempts have been carried out to precisely quantify the process of N_2 fixation, especially for trees; (4) finally, the transfer of fixed N_2 to soil and non-N_2-fixing plants has not been systematically investigated.

Improving the soil N status by exploiting the biological process of N_2 fixation should be based not only on the use of highly active N_2-fixing systems but also on methods facilitating the transfer of fixed N_2 to soil. Consequently, this paper will be divided into two sections. The first section will be devoted to the potential of the different N_2-fixing systems and to the improvement of this potential. In the second section we shall briefly present the information we have on the transfer of fixed N_2 to soil and on the maintenance of fertility in relation to different types of soil management.

Nitrogen-fixing potential of agronomically important systems and their improvement

Main N_2-fixing systems involved in agriculture and forestry

All major groups of symbiotic N_2-fixing systems occur in agrosystems and forests. They can be simply classified as follows:

- Legumes - *Rhizobium* symbioses.
- Actinorhizal plants - *Frankia* symbioses.
- Azolla.
- Symbioses with Gymnosperms (especially cycads).

In some ecosystems, N_2 fixation can also be achieved by free-living microorganisms:

- N_2-fixing blue-green algae (especially abundant in wetlands).
- Heterotropic N_2-fixing bacteria, for instance, *Azospirillum* thriving in the rhizosphere or on plant residues (such as straw).

Validity of quantitative data of N_2 fixation estimated in the field

Methods for evaluating N_2 fixation in the field have been described recently in the excellent treatise edited by Bergersen [2]. The methods most generally used are as follows:

1. The difference on N balance studies [39].
2. The difference method (the quantity of N_2 fixed being measured by the difference between the total N content of N_2-fixing plants and the total N content of non-N_2-fixing plants growing in similar conditions) [67].
3. ^{15}-N-based techniques, especially the direct isotope dilution method [4,51,64] and the A value method [21].
4. The acetylene reduction technique [2].

Other methods have been proposed; for example, with legumes and actinorhizal plants estimation of nodule numbers or weight has successfully been used to compare N_2-fixing potentials of clones or cultivars. It is not possible to discuss problems related to biological N_2 fixation in the field without checking the validity of the data that are reported. Unfortunately, a part of these data must be discarded because the authors did not take into account the limitations of the methods they used [69]. Some published, and often quoted, estimations have been calculated from wrong assumptions. Thus *Leucaena leucocephala* was credited with a very high N_2-fixing potential, ranging from 600 to 1,000 kg N_2 fixed/ha/year [10,30]. Such values, however, are not valid; they are related to the total N accumulation in the foliage fraction of the tree and thus cannot be attributed solely to N_2 fixation because soil also contributed to the plant N nutrition [31]. Quantification of N_2 fixation by trees is difficult indeed. The isotope methods, which are known for their reliability, may lead to insufficiently accurate estimations; with trees, it is difficult to label the total volume of soil explored by the roots and to get a stable soil enrichment throughout long-term experiments. Fortunately, relatively accurate estimations of N_2 fixation can probably be obtained by using the difference method, provided that the non-N_2-fixing and N_2-fixing systems exhibit equally efficient use of soil and/or fertilizer N [5].

It is probably even more difficult to quantify N_2 fixation by blue-green algae and azolla in wetland rice fields. Many figures that have been reported are liable to criticism, especially evaluations based on acetylene reduction assay [65]. With these N_2-fixing systems, the isotope dilution technique has not yet been used in the field or in field-simulating conditions, probably because of the difficulty of obtaining a non-N_2-fixing control.

Most studies on rhizosphere N_2 fixation have been carried out by using acetylene reduction assays. These proved that N_2 fixation actually occurs in the rhizosphere of non-nodulating plants, but they were incorrectly used in quantifying rhizospheric N_2 fixation, since they ignored the limitations of the method [61,68]. The claim that rhizospheric N_2 fixation could be agronomically significant and the fact that some inoculation trials had effectively increased the yield of some crops [58] generated an explosion of investigations on this N_2-fixing system up to the end of the seventies. Recent studies, however, have shown that rhizospheric N_2 fixation is only in the range of 1-18 kg N_2 fixed/ha/crop in wetland rice fields [53], and the rate of N_2 fixation is certainly much lower in drylands, which are known to be a much less favorable habitat for N_2-fixing free-living bacteria. Extensive field experiments performed in different countries, especially in India [62] and in the United States [59], have clearly shown that if increases of crop yields resulted from inoculation with *Azospirillum* in some conditions, this response probably should not be attributed to N_2 fixation but rather to other mechanisms, namely, production of plant growth hormones, stimulation of nutrient uptake, or protection against diseases.

By contrast, free-living micro-organisms can probably fix N_2 actively when they have access to large amounts of energetic substrate, a situation that occurs when straw or other plant residues are generously incorporated into the soil. Estimations of the N input through this process are still scanty.

To summarize, one should always interpret with utmost caution N_2-fixation estimations, especially when they are related to systems that are difficult to explore, namely, perennial crops, trees, blue-green algae, azolla, and the rhizosphere of non-nodulating plants. By contrast, a number of correct estimations of N_2 fixation by annual legume crops are available [42], and these can be used with confidence. Most of the discussion in the following sections will be based on these last types of data.

Variations in the N_2-fixing activity of the different systems

The potential of N_2-fixing systems varies a great deal. Among legume crops, for example, it is well known that soybean fixes N_2 much more actively than does bean. Table 1 shows that among tropical N_2-fixing trees *Acacia mearnsii* and *Leucaena leucocephala* are very active in N_2 fixation, whereas *Acacia holosericea* is a poor fixer. However, such comparisons are often liable to criticism not only because of the lack of reliability of some estimations, as

Table 1 N_2 fixation by trees in the tropics

Species	Method of estimation[a]	N_2 fixed (kg/ha/yr)	Reference
Acacia mearnsii	B	200	[46]
Acacia holosericea	I	4-11	[8]
	A	6	[41]
Acacia pennatula	A	34	[54]
Gliricidia sepium	A	13	[54]
Inga jinicuil	A	35	[54]
Leucaena leucocephala	A	110	[34]
Prosopis glandulosa	C	25-36	[55]
Erythrina poeppigiana	D	57-66	[19]
Casuarina equisetifolia	B	58	[11]
Casuarina equisetifolia	I	40-60[b]	[23]

[a] A: acetylene reduction assay; B: nitrogen balance studies; C: ^{15}N abundance studies indicating that 50% of total plant N comes from nitrogen fixation; D: root nodule turnover; I: isotope method (A value).

[b] 11-month-old trees: 10,000 trees/ha.

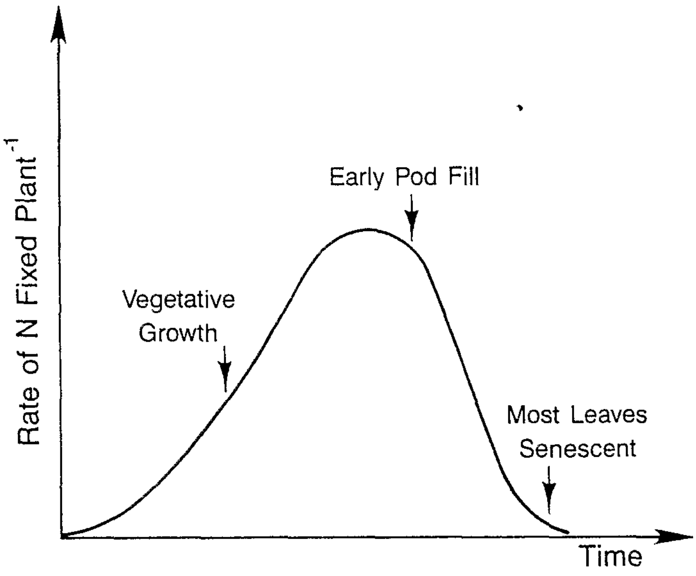

Figure 1. Generalized pattern of N_2-fixing activity for an annual legume.

mentioned earlier, but also because each individual N_2-fixing system may exhibit large variations in activity.

Variations in the N_2-fixing activity of a given system

The activity of a given N_2-fixing system varies greatly with the age of the system and with the environmental conditions.

Variations with age

The pattern of N_2-fixing activity of annual plants is usually characterized by a peak just after flowering (Figure 1); the magnitude of this peak varies with environmental conditions and planting density. The seasonal pattern of N_2 fixation for perennial plants is probably not very different from that of annual plants: they also exhibit a peak sometime during the growing season. The N_2 fixation pattern related to the whole life span of the perennial plants probably follows the curve represented in Figure 2. When the perennial plants are still young, N_2 fixation is negligible, but it increases rapidly with age. Thus during the first 4 months of its life, a seedling of *Casuarina* fixes less than 0.05 g of N_2, but during the following 6 months it can fix up to 3-6

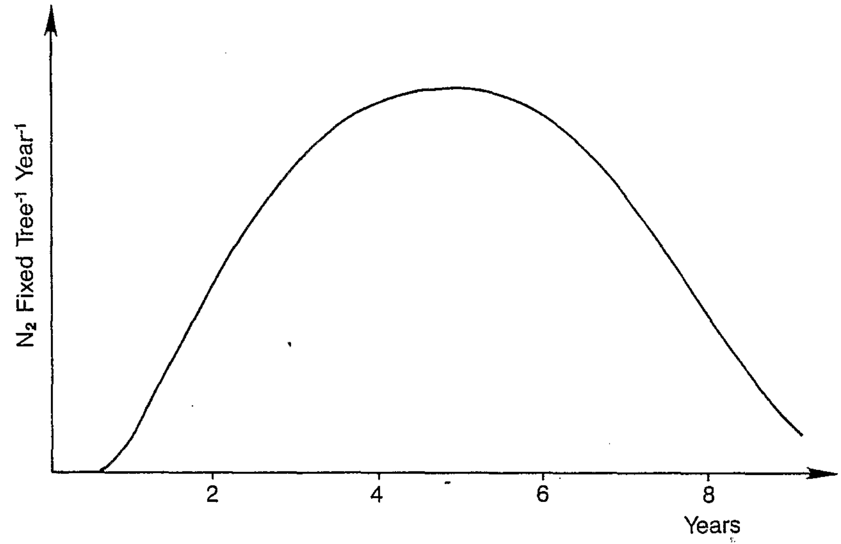

Figure 2. Hypothetical pattern of N_2 fixation during the first year of growth of a tree.

g of N_2. Such a surge in N_2-fixing activity probably lasts for 5-10 years. When the climax has been attained, the trees grow on the combined N accumulated in the soil through decomposition of leaf and root litter, and N_2 fixation is very low. During the growing season N_2 fixation of a perennial plant exhibits a seasonal pattern. The occurence of such variations should be kept in mind when comparing stands that differ especially in age and planting density.

Variations with environmental conditions

Large variations can be directly related to variations of the environmental conditions, which are chemical, physical, or biological in nature [12,25]. A consequence of the high sensitivity of the N_2-fixing systems to the environment is the need for caution in interpreting both short- and long-term estimates.

Technologies for improving N_2 fixation in the short term

Up to now these technologies have been mostly related to the improvement of the symbiont performance, a topic that we shall mention briefly. We shall focus our attention on the approaches based on the host plant studies and recall some of the elementary principles of agricultural management that are too often neglected. We shall not discuss here the technologies that are presently envisioned but not yet ready to be used. These include the generation of new N_2-fixing systems resulting from the transfer of *nif* genes (and other ancillary genes) to mycorrhiza [28], somatic hybridization of non-N_2-fixing and N_2-fixing plants, and introduction of expressible *nif* into the plant genome [48].

Improvement of the symbiotic micro-organism and inoculation

The recent advances in the molecular and genetic manipulation of micro-organisms offer the possibility of improving the strains of *Rhizobium* that are currently used. Broadening the host spectrum of specific strains has already been achieved by genetic manipulation [35]; competitiveness and/or effectiveness could probably also be improved by using the same technology. However, none of the strains of *Rhizobium* obtained by these means have yet been used in agronomic practice, and thus one must still rely upon strains that have been selected by the usual methods of screening [63]. *Frankia* was isolated and cultivated in vitro for the first time only in 1979 [7], which means that the genetics of this symbiont is still in its infancy. The isolation and culture in vitro of blue-green algae associated with azolla or cycads have not yet been achieved; thus, any attempt to initiate genetic studies is precluded.

Inoculating the host plant with its specific symbiont to increase N_2 fixation is often recommended as a general and unfailing method. In fact, the beneficial effect of inoculation can only be expected when the symbiont is absent or in very low numbers in the soil under study or when the strain to be introduced is both competitive and more effective than the native strains. A few illustrative examples follow.

Successful inoculations

– In most situations, including tropical soils, inoculating soybean with its specific *Rhizobium japonicum* significantly increases N_2 fixation and yields [1,6]. A less classical example is that of the inoculation of *Casuarina equisetifolia* with an effective strain of *Frankia.* Table 2 shows that inoculation increased the growth of the tree more than did the application of N fertilizer.

Unsuccessful inoculation

– In tropical soils, legumes of the cowpea cross-inoculation group show little or no response to inoculation. Thus in Nigeria little or no response has been obtained for cowpea, jack bean, lima bean, and pigeon pea [1]. Another example, related to trees, is that of an inoculation trial of *Acacia holosericea* carried out in Senegal. Inoculation with the effective strain did not significantly improve the height of the trees after transplantation, and it slightly reduced the survival percentage (Table 3, second and third lines).

Improvement of the host plant

Enhancing N_2 fixation by plant breeding has been advocated for a long time [32,44,45], but this approach has not yet been really exploited by plant genet-

Table 2 Influence of inoculation with Frankia ORSO21001 on height, dry weight, and N_2 fixation of 11-month-old Casuarina Equisetifolia

Treatments		Height (cm)	Dry weight (g/tree)	N_2 fixed	
Inoculation	N addition (g/tree)			% Ndfa[a]	g N_2/tree
0	0.5	170 a	295 a	0	0
0	2.5	192 a	409 a	0	0
+	0.5	216 b	525 b	39-53[b]	3.3-2.3[b]

[a] Nitrogen derived from atmosphere.

[b] First figure calculated from direct isotope dilution method, second figure from A value method.

Figures in same columns followed by same letter do not differ significantly, $P = 0.05$ (Duncan test).

Table 3 Influence of the treatment of acacia holosericea seedlings in the nursery on survival and growth after transplantating[a] Sangalkam, Senegal (unpublished)

Treatment in the nursery		11 months after transplantating		17 months after transplantating	
Sterilization[b]	Inoculation[c]	Survival (%)	Height (cm)	Survival (%)	Height (cm)
0	0	38.2 a	29.4 a	36.8 a	105.3 a
S	0	89.6 b	38.6 b	89.6 b	119.9 b
S	R	75.0 c	36.2 b	72.2 c	117.7 b
S	RM	84.7 bc	45.6 c	84.7 b	134.2 c

[a] Seedlings transplanted when 2 months old.

[b] S: soil sterilization with methylbromide (300 g/m^3).

[c] R: inoculation with *Rhizobium* strain ORS841; RM double inoculation with *Rhizobium* and *Glomus mosseae.*

Figures in same columns followed by same letter do not differ significantly, $P = 0.05$ (Duncan test).

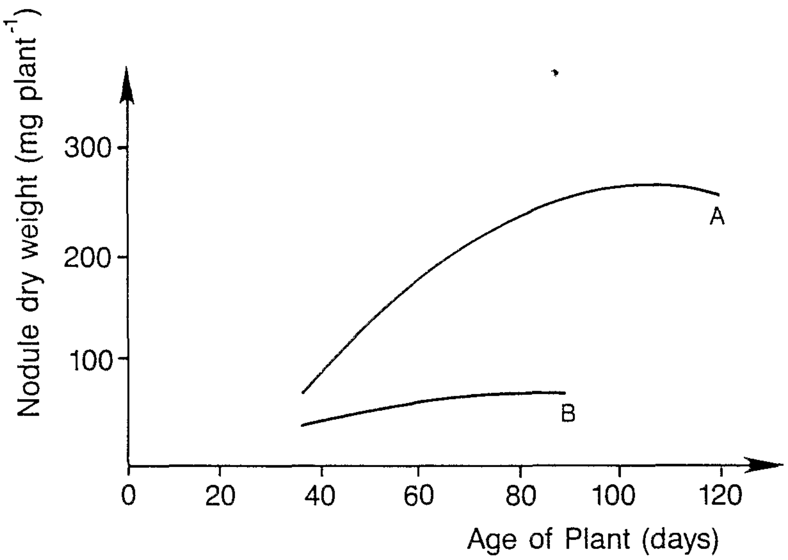

Figure 3. Time course of nodule dry weight of peanut expressed as milligrams per plant (A: cv. 28-206 and GH 119-20; B: cv. 55-437. Field experiment carried out at Patar, Central Senegal in 1977).

icists. In addition to plant breeding, screening of spontaneous or artificially induced mutants or screening followed by cloning of individuals with superior ability to fix N_2 within a heterogeneous population would probably lead to interesting applications in the short term. A few examples follow.

Crop-legumes

– These legumes are knows to exhibit variations among cultivars. The variations affect nodulation (Figure 3) or the amount of N_2 fixed, and thus significantly change the soil N balance (Table 4). Using ethyl-methyl-sulfonate mutagenesis and M2 family pot selection, Gresshoff et al. [29] recently obtained soybean mutants showing excessive nodulation (up to threefold to fourfold increases in nodule number or mass) above that of wild plants. This supernodulation was also expressed in the presence of otherwise inhibitory levels (5 mM) of combined N (up to tenfold increases in nodule numbers). At the same time the nitrogenase activity of the soybean mutants was not affected by these inhibitory levels of combined N.

N_2-fixing trees

– Many N_2-fixing trees are cross-pollinated so that the genetic heterogeneity of individuals is considerable. This characteristic complicates germplasm exploration and selection, but it can be exploited by screening the best clones, a technique that implies that clonal propagation methods ara available. Investigations in the latter direction are under way at the Bureau for Overseas Scientific and Technical Research/National Center for Scientific Research laboratory in Dakar, and the preliminary results on *Acacia senegal* and *Casuarina* spp. are most promising.

Agricultural management

We have seen that the amount of N_2 fixed by a given N_2-fixing system is related to the potential of this system which, in turn, is determined both by the associated symbiont (*Rhizobium* or *Frankia*) and by the host plant. However, this potential is often limited by environmental, chemical, physical, and biological factors. In arid and semiarid conditions, water stress is a major limiting factor, as illustrated by Figure 4. The agronomist should attempt to minimize or eliminate the impact of these limiting factors. Since this topic has been adequately discussed already [13,26], we shall restrict our attention exclusively to the following two problems.

Phosphorus availability
– Any soil deficiency affects not only growth of the plant but also the functioning of the symbiotic system. A most common nutritional disorder is that

Table 4 Percent plant N derived from N_2 (% Ndfa) and N balances of cowpea and soybean cultivars when grown in a soil low in available N

Cultivars	N_2 fixed (kg N_2/ha)	% Ndfa	N balance (kg N/ha)
Cowpea[a]			
ER1	50	61	+2
TVu 1190	101	75	+52
Ife Brown	81	76	+24
TVu 4552	49	64	+3
Soybean[b]			
26/72	143	86	+7
22/72	126	80	+7
44/A/73	110	76	−1
Jupiter	108	75	−6
4/73	64	73	−6

[a] [18].
[b] (unpublished).
For each crop, comparisons between cultivars were carried out at the same time, in the same soil. Tops were returned to soil in both experiments.

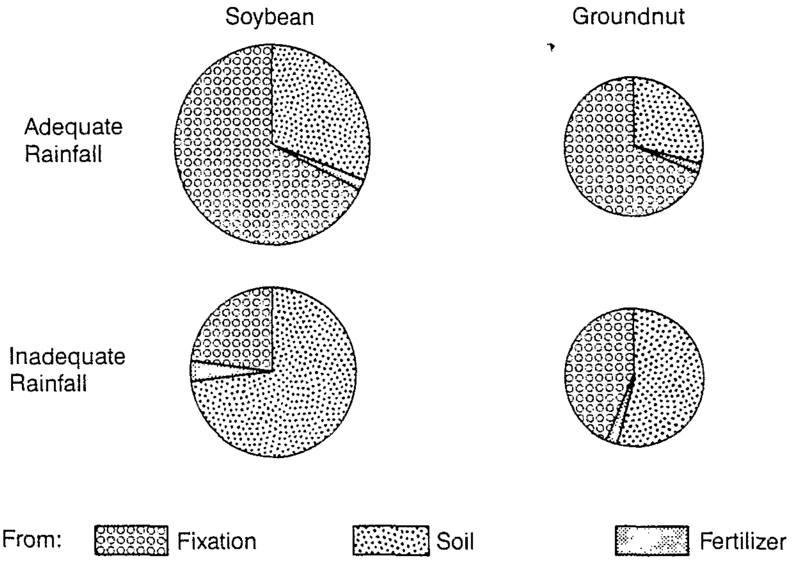

Figure 4. Left: N_2 fixation by soybeans inoculated with an effective strain of *Rhizobium* in a field experiment carried out in South Senegal (In 1982 [adequate rainfall] percentages of plant N derived from N_2 fixation [Ndfa] and N_2 fixed expressed as kg/ha were 70% and 109%, respectively, whereas in 1980 [inadequate rainfall] related figures were 24% and 34%).

Right: N_2 fixation by groundnut spontaneously nodulated by native *Rhizobium* in a field experiment carried out in central Senegal (In 1975 [adequate rainfall] Ndfa and N_2 fixed were 66% and 67%, respectively, whereas in 1974 [inadequate rainfall] related figures were 44% and 52%, respectively [unpublished]; diameter of circles is proportional to total N content of plants).

resulting from phosphorus deficiency. Alleviating this soil defect by applying appropriate amounts of soluble phosphorus dramatically restoes N_2 fixation. The example given in Table 5 is interesting because it shows that a modest application of P (22 kg/ha) combined with inoculation with *Glomus mosseae*, an endomycorrhizal fungus, significantly increased the percentage of N derived from atmospheric N_2 (% Ndfa) and the total amount of N_2 fixed (nearly a twofold increase) by field-grown soybean. Subsequently, the grain yield was significantly improved. The results of this inoculation trial with an endomycorrhizal fungus should not be extrapolated to other environmental conditions without caution. It should be emphasized that the successful effect of *Glomus mosseae* inoculation reported here was attributed mostly to the fact that the native endomycorrhizal populations were very low in the experimental site.

Inhibition of N_2-fixing systems by combined N
– It is well established that nitrate and, to a lesser extent, other forms of combined N retard nodulation and N_2 fixation. Such an inhibition is obviously responsible for the deficit in the N balance observed when the amount of N fertilizer applied to a given legume reaches inhibitory levels [18]. A first approach to circumventing this inhibition is to use plant-symbiont systems that are capable of continuing to fix N_2 even in the presence of large amounts of combined N. We have already given the example of mutant soybeans obtained by Gresshoff et al. [29]. *Sesbania rostrata,* a stem-nodulated legume, has been shown to have the unique ability to absorb combined N with roots and to fix N_2 with its stem nodules [15]. It has ben suggested that transferring the stem nodulation characteristic from this plant to other legumes would be a way to develop new, uninhibited N_2-fixing systems [13,14]. A second approach, which has not been seriously explored, is to use some forms of N fertilizers that do not inhibit N_2 fixation and yet provide the plants with the complementary N required for their growth. Hardy et al. [32] proposed designating such forms of fertilizers as compatible fertilizers. In a preliminary experiment they found that 'soybean meal' applied to soybean not only did not inhibit N_2 fixation but increased it by 12%. It would be unrealistic to propose the use of soybean meal as a compatible N fertilizer, but it would probably be possible to obtain new forms of N fertilizers with similar properties. To start with, it would be worthwile to study the effect of the existing slow-release N fertilizers on the N_2-fixing activity of symbiotic systems.

Fertility maintenance

The contribution of N_2-fixing systems to the soil N pool depends on their

Table 5 Effect of inoculation with glomus mosseae on N_2 fixation and grain yields of field-grown soybean with or without application of P fertilizer [22]

Treatments		N_2 fixed		Grain yield	
Inoculation	Superphosphate (kg P/ha)	% Ndfa	kg/ha	kg dry wt/ha	kg N/ha
Rhizobium	0	67 a	73 a	1,423 a	90 a
Rhizobium + Glomus	0	67 a	80 a	1,431 a	98 a
Rhizobium	22	70 a	109 b	2,017 b	134 b
Rhizobium + Glomus	22	76 b	139 c	2,290 c	155 b

Means followed by the same letter within columns do not differ significantly, $P = 0.05$.

N_2-fixing potential and on the amount of fixed N transferred to the soil. We have already discussed in detail the means of obtaining systems with the highest N_2-fixing potential. In this section we shall examine the problem of the transfer of fixed N_2 to soil and give examples related to different types of agricultural and forestry management.

Transfer of fixed N_2 to soil

J. Sprent [60] aptly recalled that 'legumes are not altruistic'. They fix N_2 for themselves, normally releasing little N into the soil during their growth. However, some N is transferred from the decomposition of dead tissues such as leaves, cladodes or phyllodes, fruit, roots, and nodules. A return of N in plant tops to the soil via leaching is likely to occur during rain, irrigation, dewfall, or spraying [66]. The amount of N transferred is usually low for grain annual crops, since much of the current research on improvement of grain legumes is aimed at improving the harvest index of grain and ensuring that it is indeed harvested [33]. In contrast, larger amounts of N are probably transferred when tops are incorporated into the soil or after being transformed into farmyard manure. N transfers are considerable for legumes that are ploughed in as green manure. The same is true for trees that shed large quantities of leaves and fruit.

Grain legumes

According to Henzell and Vallis [33], 60%-70% of the legume N may be harvested and residues may have a lower percentage of N than the material harvested. Consequently, it is not surprising to observe negative N balances in rotations comprising grain legumes. To improve the soil N status in such systems, the recommendation is to use cultivars that actively fix N_2 (Table 4), to ensure the return of tops, and to avoid or minimize the addition of N fertilizers, which are known to inhibit N_2 fixation. The main emphasis should be placed on rotations that ensure the replenishment of the N stock. Thus, maintenance of soil fertility in a soybean-maize rotation in Senegal has been achieved by returning the tops of both crops to the soil. Improvement of fertility was attributed not only to the increase in soil organic matter but also to the enhancement of N_2 fixation by soybean, as suggested by the increase of nodule numbers (Table 6). It is interesting to note that in this experiment a relatively high yield of maize (2,000 kg grain/ha) was obtained without the addition of N fertilizer in the framework of a 4-year rotation.

Wetland rice

Flooded soils appear to maintain higher N fertility than dryland soils. Moderate but fairly stable yields of rice (2 tonnes/ha) are maintained for many years without any addition of N fertilizer, a fact which is attributed to the input of N to the soil through spontaneous N_2 fixation. Four types of N_2-fixing systems can be involved: legumes used as green manure (and also, but only in China, a non-legume, *Coriaria sinica*); azolla, which is used in a similar way as legumes; blue-green algae; and heterotropic N_2-fixing microorganisms thriving on straw and, to a lesser extent, on living rice plants.

Green manuring based on the incorporation of N_2-fixing plants or azolla is certainly the most efficient technique, and it allows a substantial improvement of the N status of the soil. However, Roger and Watanabe [53] concluded their excellent review on the 'technologies for utilizing N_2 fixation in wetland rice' by stressing the socioeconomic limitations of green manuring: this management practice is labor intensive, and it can be used only where economics is not a major factor.

Since the ecology of blue-green algae is still poorly understood, Roger and Watanabe [53] would not confidently recommend algal inoculation in spite of the fact that this practice would probably require only little additional labor. According to these authors, the future for heterotrophic N_2 fixation is even less bright, except when it is associated with straw incorporation.

We are well aware that the practices based on the production and incorporation of a large biomass (legumes, azolla, and, to a lesser extent, straw) are limited by economic factors [33,53], but the use of stem-nodulated legumes

Table 6 Influence of the return of plant residues or addition of organic amendment (compost) on yields[a] of soybean and maize grown in a sequential rotation for 4 years (unpublished)

Treatments		Soybean		Maize
Return of maize straw	Addition of compost (t/ha)	Nodule number/ plant (day 55)	Grain yield (kg dry wt/ha)	Grain yield[b] (kg dry wt/ha)
0	0	7 a	1,650 a	900 a
+	0	27 b	2,000 b	1,300 b
+	1.5-2.0	26 b	2,250 c	2,000 c

[a] Mean yield for 3 successive years.

[b] No N fertilizer was applied at any time.

Figures in same column followed by same letter do not differ significantly, P = 0.05.

with a high N_2-fixing potential will probably facilitate the revival of composting and green manuring, such as practiced now or in improved versions. In fact, *Sesbania rostrata*, a stem-nodulated legume whose remarkable characteristics were discovered in 1979 [15,16], appears to be a good candidate for green manuring or composting [52].

N_2-fixing trees in mixed stands or associated with annual crops

The contribution of trees to the N status of soil is a priori more important than that of annual crops because of two specific habits.

1. Except for young trees (less than 1-2 years old), part of the N that trees take through their roots returns to the soil with litter; thus, N is continuously circulating in the ecosystem. The N returned in litter may be a high proportion of N derived from N_2 fixation (% Ndfa) or from soil uptake. An interesting consequence of this fact is that 'in low soil N conditions, leaf drop may be used as a very rough estimator of N_2 fixation, but it neglects the N stored in the plant during growth and N turned over underground' [57].
2. Through their extensive root system trees act as a 'nutrient pump', redistributing the nutrients, especially N, in the profile and contributing to their accumulation at the surface or the soil [50]. Thus trees appear to be good candidates for mixed cropping systems. However, one should be aware of limitations to their ue resulting from competition for light and water and, with some species, the production of phytotoxic compounds (allelopathy) excreted by roots or accumulated in the litter [36].

Mixed stands

In temperate areas, growing mixed stands of N_2-fixing and non-N_2-fixing trees has been attempted, but not with much success except for the reclamation of waste areas [43,56]. Although this approach has not yet been seriously studied in tropical and subtropical conditions, it should not be overlooked since spontaneous mixed stands do exist. For example, in Australian *Eucalyptus* forests, species of *Acacia* are widespread as understory shrubs with members of the Papilionoid tribe or the cycad *Macrozamia* (a thermophilic plant with coral-like nodules resulting from the association with an N_2-fixing blue-green alga) [60]. Mixed stands of *Eucalyptus sieberi* and *Allocasuarina littoralis* are not uncommon in southeast Australia [17]. Mixed stands of *Casuarina* sp. and *Anacardium occidentale* have reportedly been used to fix sand dunes in Orissa [47].

Intercropping tree legumes with annual crops

Intercropping can be defined as growing two or more crops simultaneously in the same field. Ideally one of the associated plants should be able to fix N_2. Some examples show that this type of intercropping can result in improved soil fertility and often increased yields. The classical example is that of *Acacia albida* associated with millet, sorghum, or groundnut. *Acacia albida* is a tree legume that has the unusual habit of growing new foliage during the dry season and losing its leaves during the early part of the rainy season. Crops can be grown around and under the tree without suffering from light and water competition; thus, they derive benefit from the topsoil enrichment [9,20,27,38]. In this association it is not very clear whether the improved fertility of the soil under the trees is due mainly to N_2 fixation or to the redistribution of nutrients, especially N, in the profile (pumping effect). Intercropping *Leucaena leucocephala* with maize is probably an interesting method. Experiments carried out that a population of 10,000 to 20,000 plants/ha is adequate to provide sufficient foliage to cover the soil and supply substantial quantities of nutrients without significantly competing with maize. Table 7 shows that maize yields were only slightly reduced by the highest population of *Leucaena leucocephala* [49]. Except for a few clear-cut examples, such as that of *Acacia albida*, 'we have as yet very little experimental evidence on tree-crop associations that tells us whether the species mixtures will interact mutual inhibition, cooperatively or through one form or

Table 7 Maize height reductions and grain yields when grown in association with leucaena at CIAT, Colombia [49]

Leucaena	Maize	Maize	
Population plants/ha	Population plants/ha	Height[a] reduction (%)	yield[b] (t/ha)
0	25,000	0	4.6
0	50,000	0	5.2
10,000	25,000	2	4.7
10,000	50,000	11	4.6
20,000	25,000	0	4.2
20,000	50,000	12	4.9
40,000	25,000	10	3.5
40,000	50,000	15	4.9

[a] Forty days after planting compared to check.

[b] Corrected to 15% moisture content.

another of compensation' [36]. This type of information is obviously required if a particular tree-crop association is to be encouraged.

N_2-fixing tree-annual crop or N_2-fixing-non-N_2-fixing tree rotations

In New Guinea the following shifting cultivation system is currently practiced with success. *Casuarina oligodon* is planted in wasted, N-deficient soils and grown for 5-10 years. It is then harvested for wood or charcoal, after which the land is planted with various crops [57]. The same system based on buildup of an N reserve by the N_2-fixing tree is used in India [40]. Soil-improving properties of casuarinas have also been exploited in alternating rotation with *Anacardium occidentale* for the production of cashew nuts in India (J.C.G. Ottow, personal communication).

Conclusion

Restoration, maintenance, and improvement of the N status in soils can be achieved through biological N_2 fixation provided that the N_2-fixing systems used exhibit a high N_2-fixing potential, that no environmental factor limits this activity, and that the largest portion of fixed N_2 is transferred to the soil. Most often these conditions are far from being fulfilled. Thus there is a tremendous need for research in three major fields.

1. Improving the N_2-fixing potential of existing systems or obtaining new systems is obviously a major requirement. There is a need for increased emphasis on the plant rather than on the associated symbiont. It is high time that we orient our efforts toward exploiting the genetic variability of the host plants. For trees, one should attempt to select the best 'soil improvers,' that is, trees that will fix the largest amounts of N_2 and also return N (and P absorbed together with N) to the soil via leaf fall or lopping [36,37]. The host plant and microbial approaches should be used to identify N_2-fixing systems that would not be too sensitive to combined N.
2. Identification of limiting factors, though often extremely difficult, should be carefully carried out at each site. This type of investigation is a prerequisite to any attempt to exploit biological N_2 fixation. It would be ineffectual to develop powerful N_2-fixing systems without first attempting to identify and eliminate potentially limiting factors.
3. The problem of improving the transfer of fixed N_2 to soil should probably be undertaken by agronomists and economists; however, plant ecologists should study some aspects, such as those related to the plant-to-plant interactions in agroforestry systems.

Biological N_2 fixation is probably the only alternative source of N in some specific situations: in countries where N fertilizer is unavailable or unaffordable[1] or where intensive labor is cheap and in agroforestry systems or rice fields where only moderate yields are expected. By contrast, intensive agriculture usually consumes large amounts of N fertilizer without attempting to benefit from the input of N from N_2 fixation. Such a situation should be corrected not only for the sake of saving N fertilizers but also to avoid the pollution hazards that are linked to their use. We have already noted that the combination of biological N_2 fixation and N fertilizers is not a utopian dream,since the possibility exists of processing compatible fertilizers or obtaining N_2-fixing systems that are not inhibited by combined N.

Notes

[1] In Casamance, 1 kg of urea costs 150 FCFA (0.33 US $) during the summer of 1984; this price was considered as prohibitive by the farmers

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Ci-joint la communication
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