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Scarcely Explored Means of Increasing the Soil N Pool through Biological N_2 Fixation

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Abstract

THE amount of N added to the soil through biological N_2 fixation varies a great deal. It depends not only on the amount of fixed N_2 but also on the efficiency of N transfer from the N_2-fixing system to the soil. To obtain maximum N_2 fixation, it is necessary to eliminate the effect of limiting factors such as P deficiency and excess of combined N. This objective is best achieved by simultaneously using biological and chemical technologies. The transfer of fixed N to soil is minimum with some crops such as grain legumes. This often leads to N deficit in the soil. By contrast, green manuring is the most efficient way of transferring fixed N_2 to soil. This practice, however, should be recommended only when the N_2-fixing system is very active, thus reducing to a minimum the period of time for its growth.

WHEN land is cultivated, the N content of most soils declines progressively to an equilibrium level which is characteristic of the climate, cultural practices and soil type. "At equilibrium, essentially all of the N required for plant growth must come from external sources, namely, through biological fixation and fertilizer N application" (Stevenson 1965). A major concern of the present-day agronomists, especially in the tropics, is (1) to decrease applications of N fertilizers because of their high cost and their hazardous environmental effects and (2) to promote the use and to increase the efficiency of N_2-fixing systems, namely, legumes, N_2-fixing non-legumes and *Azolla*.

For many years the general dogma has been that legumes, when introduced in any agricultural system, significantly contribute N to the soils. However, some data suggest that this dogma has to be revised. We intend here to assess the limits within which biological N_2-fixing systems may be expected to improve the N status of the soils.

The amount of N that is added to the soil pool through biological N_2 fixation depends on the amount of (1) fixed N_2 and (2) fixed N_2 that is transferred to the soil.

The amount of N_2 fixed by a given N_2-fixing system is related to the potential of this system, which is determined both by the host-plant and by the associated symbiont (*Rhizobium* or *Frankia*). However, this potential is often limited by environmental, chemical and biological factors, the influence

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of which should be minimized to increase actual N_2 fixation. We shall not discuss here the possibility of increasing N_2 fixation by exploiting the variation in host-plant × rhizobial strain interaction, which is being studied by several institutes (CIAT 1979; ICRISAT 1979). Rather we shall focus our attention on means of decreasing the impact of two major limiting edaphic factors: P deficiency and excess of inorganic N. We shall also compare the effectiveness of different cropping systems in transferring fixed N_2 to the soil.

Phosphorus Availability and N_2 Fixation

N_2-fixing systems have special nutritional requirements for microelements (Mo, Co, B, Zn, Cu) and also for P, S, K and Ca. Any soil deficiency affects not only the growth of the plant but also the functioning of the symbiotic system. A most common nutritional disorder is that resulting from P deficiency. Alleviating this soil defect by applying appropriate amounts of P fertilizers dramatically restores nodulation and N_2-fixing activity. Thus, by increasing P fertilization, N_2 fixation was improved in three out of four cultivars of *Phaseolus vulgaris* that were compared (Fig. 1). P is supplied to soil as soluble phosphate (e.g., superphosphate or triple superphosphate) or as rock phosphate. Soluble phosphates are expensive and it is desirable to reduce the rate of application by increasing their efficiency. Rock phosphates are more appealing because of their low cost in many countries, but one should devise methods for increasing their solubility.

Increasing the Efficiency of Soluble Phosphorus Fertilizers through Endomycorrhization

It is now well established that endomycorrhizal infection improves the P uptake by the host-plant. Recently Diem and Ollivier (unpublished) found that the response to endomycorrhizal infection varied according to the cultivar. It appears that the absence of response in some cultivars to mycorrhizal infection is closely related to the absence of response in the same cultivars to P fertilization. Table 1 clearly shows that in a non-sterile soil with 40 ppm available P, infection with *Glomus mosseae* markedly improved the P absorption by *Vigna unguiculata*, thus increasing its nodulation and N_2 fixation. The effect of endomycorrhizal infection appeared to be similar to that of the application of soluble P at the rate of 1000 kg/ha. Other experiments, not reported here, indicate that in typically P-deficient soils, a certain amount of soluble P fertilizer is necessary to obtain a significant response to endomycorrhizal infection. In other terms, it appears that endomycorrhizae cannot replace P fertilizer but increase fertilizer efficiency, thus allowing a reduction of their rate of application.

Increasing the Solubility of Rock Phosphate

In some acid soils, rock phosphate can be used as a source of P for crops and especially legumes (CIAT 1979).

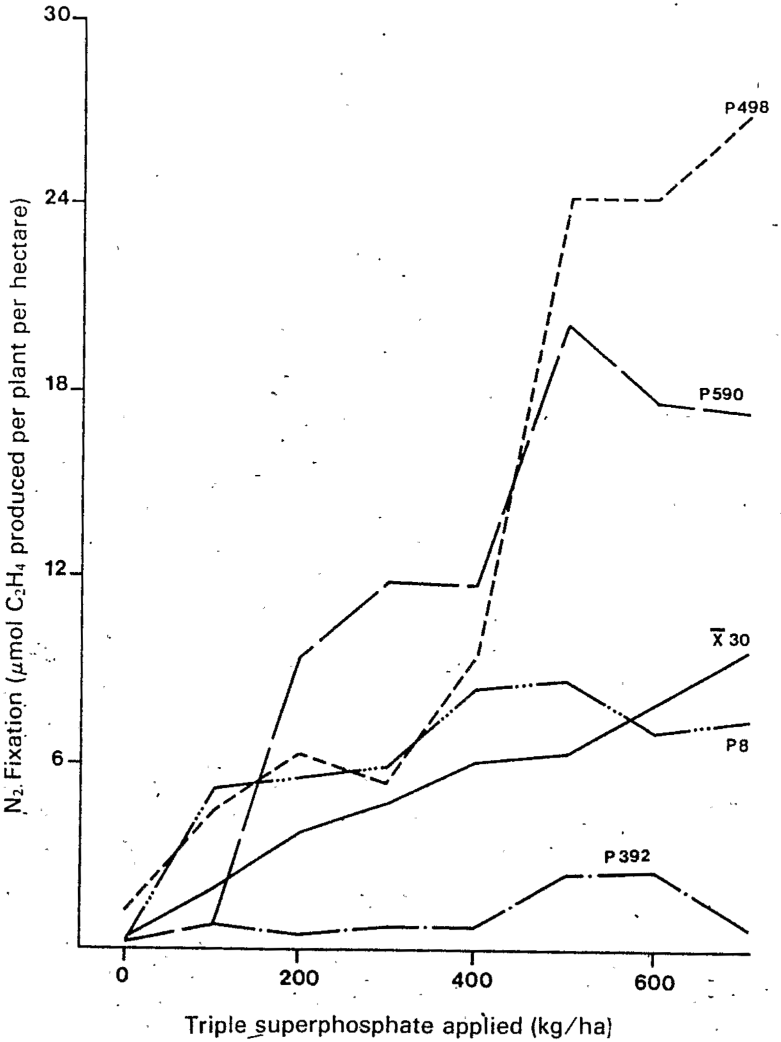

Fig. 1. N_2 fixation in selected cultivars of *Phaseolus vulgaris* as influenced by increasing P fertilization (data taken at flowering) (CIAT 1978).

However, in most cases rock phosphate is not readily absorbed by the plants even when they are infected by endomycorrhizae since endomycorrhizae do not contribute to the solubilization of this form of P. This solubilization could probably be achieved, at least in tropical conditions, by the addition of elemental S (200 ppm) inoculated with thiobacilli to rock phosphate as suggested by Swaby (1975). Table 2 shows that such treatment applied to an annual legume, *Vigna unguiculata*, significantly increased the growth, total P and total N content of the plant. However, one should be

Table 1. Effect of endomycorrhizal (VAM) infection (*Glomus mosseae*) on growth and nodulation of *Vigna unguiculata*[1] cv. N-58-185 grown in a non-sterile Dior soil[2] (Ollivier and Diem, unpublished)

Treatments	Dry wt/plant			Total N (mg/plant)	Total P (mg/plant)	VAM infection intensity
	Shoots	Roots	Nodules			
Inoculation with *Glomus mosseae*	4.5 a	2.4 a	0.141 a	108 a	6.21 a	92 a
Phosphorus application (0.5 g K_2HPO_4/kg soil)	5.5 a	1.3 b	0.168 a	118 a	8.74 b	84 b
Control	3.4 b	1.0 b	0.098 b	77 b	3.40 c	84 b

[1] Sixty-day old plants; [2] Dior soil is an ultisol; total P: 268 ppm; available P (Truog): 40 ppm; pH (KCl) : 7.0

Table 2. Effect of soil inoculation with thiobacilli upon the growth and phosphorus uptake of a legume, *Vigna unguiculata*[1], grown in a Deck soil[2] amended with rock phosphate[3] (Ollivier and Diem, unpublished)

Treatment	Shoots and roots (g dry wt/plant)	Nodules (mg dry wt/plant)	Total N (mg/plant)	Total P (mg/plant)
Control	2.19 a	25 a	25 a	1.5 a
Rock phosphate + S[3]	3.72 b	46 b	39 b	2.2 b
Rock phosphate + S + thiobacilli[4]	5.00 c	64 c	55 c	3.1 c

[1] Plants grown in pots (1.5 kg sterile soil per pot) were inoculated with the strain of *Rhizobium* CB 756. They were harvested when 60 days old; [2] Deck soil is an Ultisol; total P: 190 ppm; available P (Truog) : 2 ppm; pH (KCl) : 6.2.; [3] Addition of rock phosphate (Taiba) was 40 ppm; addition of S (elemental) 200 ppm.; [4] Same treatment as (3) plus thiobacilli that had been obtained by enrichment of soil from Guadeloupe; Numbers not having same letter differ $P = 0.05$.

aware of the fact that elemental S is also expensive and more experimental studies are necessary in order to check whether the rate of S can be lowered down to 30-40 ppm (an economically valid rate) without significantly impeding the solubilization of rock phosphate.

N_2-Fixing Systems not Inhibited by Combined Nitrogen

It is well established that nitrate and, to a lesser extent, other forms of combined N retard nodulation and N_2 fixation. Thus, in a field experiment conducted in Ibadan, Nigeria, the application of 100 kg of N/ha significantly inhibited nodulation in all four cowpea cultivars that were used. Acetylene reduction activities at 45 days after sowing also reflected the inhibiting effect of this addition of 100 kg N/ha (Eaglesham 1981). Such an inhibition is obviously responsible for the deficit in the N balance of the plots which had received less (25 kg N/ha) fertilizer (Table 3). Though irritating, this problem of the inhibition of N_2 fixation has not yet been seriously studied. To the best of our knowledge two approaches exist that could lead to a solution, the first one is to find compatible fertilizers, the other one to discover and use N_2-fixing systems that are not sensitive to this inhibition process.

Table 3. Comparison of the influence of conventional fertilizers (NO_3^-, NH_4^+, Urea) and a compatible fertilizer, soybean meal, on N_2 (C_2H_2) fixation by soybean and on corresponding yields (Hardy *et al.* 1973)

Form*	Application Locus**	Age (days)	$N_2(C_2H_2)$ fixation	Yield
			Control %	
NO_3^-	S	0	43	98
NH_4^+	S	0	51	104
Urea	S	0	48	109
Soybean meal	S	0	112	123
NO_3^-	S	40-50	50	106
NH_4^+	S	40-50	37	106
Urea	S	40-50	44	97
Urea	F	50-71	29	100
Urea	F	78-99	53	100

*135 kg N/ha of indicated form; **S, soil; F, foliar.

Compatible N Fertilizers

To prevent the inhibition of N_2 fixation by combined N, Hardy *et al.* (1973) suggested the use of some forms of N fertilizers, such as soybean meal (Table 4), that do not inhibit N_2 fixation while providing the plants with the complementary N required for their growth. They designated such forms of fertilizers as compatible fertilizers. The possibility, though promising, has not yet been seriously explored.

Table 4. Effect of combined N on root and stem nodulation and N_2 fixation in *Sesbania rostrata*[1] (Dreyfus and Dommergues 1980)

Treatments		Nodules fresh wt. (mg/plant)		ARA[3]		Shoot	
Combined N	Inoculation	Roots	Stems	Roots	Stems	Fresh wt. (g/plant)	mg N/plant
0	0	0	0	0	0	0.5 a	0.9 a
0	+	246 a	91 a	5403 a	2939 a	3.4 b	15.8 b
+[2]	0	0	0	0	0	5.8 c	14.5 b
+[2]	+	23 b	133 b	95 b	3944 b	6.0 c	26.1 c

[1]Four-week old hydroponically grown plants. [2]NH_4NO_3 (3 nM); [3]Acetylene reducing activity (ARA) expressed as n moles C_2H_4 per hour per root system or per shoot. Numbers in columns not having same letters differ $P = 0.05$.

Non-inhibited N_2-fixing Systems

Up to now two N_2-fixing systems are known to keep fixing N_2 even in the presence of large amounts of combined N : *Azolla* and *Sesbania rostrata.*

Azolla. A water fern that lives in symbiosis with a N_2-fixing blue-green alga, *Azolla* is now widely used as green manure or introduced in more or less complex rotation systems (Lumpkin and Plucknett 1980; Watanabe 1981). This N_2-fixing system has the characteristic of not being strongly inhibited by ammonium, nitrate or urea. According to Ito and Peters (quoted by Watanabe 1981) *Azolla* growth is not affected by 2.5 m*M* ammonium; its N_2-fixing activity is only reduced by ca. 30% in these conditions. When *Azolla* is transferred to a N-free medium it quickly recovers its N_2-fixing activity.

Sesbania rostrata. It is a legume capable of forming nodules on both roots and stems, and also has the unique ability of simultaneously absorbing combined N with its roots and fixing N_2 with its stem nodules. Thus young plants (Table 5) the roots of which were continuously bathed in a 3 m*M* nitrate solution had few root nodules (23 mg/plant) exhibiting a very low acetylene reducing activity (ARA = 95 n moles C_2M_4 per root system) but had more stem nodules (133 mg/plant) which actively reduced acetylene (ARA = 3944 n moles C_2H_4 per stem). The stem ARA was higher in plants growing in a nutrient solution with combined N (3944) than in plants growing in a N-free nutrient solution (2639). It is interesting to emphasize the fact that the plant yield expressed as mg N/plant was significantly higher in the case of inoculated plants growing in the solution with combined N than in the case of plants growing in the N-free solution. Obviously, attempting to transfer the stem nodulation character from this plant to other *Sesbania* or even other legumes would really be a worthwhile venture.

It should be noted here that recently P. Dart of the International Crops Research Institute for the Semi-Arid Tropics found groundnut cultivars which nodulated on the hypocotyl and some others (cv. MK. 374) that nodulated "further up the stems beyond the crown of the plants." It would be interesting to know whether such nodules are sensitive or not to inibition by soil combined N.

Table 5. The net N balances of cowpeas at two levels of mineral N availability[1] (Eaglesham 1981)

Fertilization	Cultivar	Mineral N uptake	Fixed N_2	Grain N removed	Residue N returned	N balance
25	ER-1	32	50	48	34	+ 2
	TVu 1190	33	101	49	85	+52
	Ife Brown	25	81	57	49	+24
	TVu 4552	27	49	46	30	+ 3
100	ER-1	66	28	54	40	−26
	TVu 1190	69	49	49	69	0

Transfer of Fixed N_2 to Soil in Different Types of Cropping Systems

Legumes can be introduced in the many different cropping systems which are currently in use or which can be devised. Their contribution to the soil N pool varies widely and we shall attempt here to give an idea of the range of this contribution in a few typical cropping systems.

Green Manure

The maximum N transfer from the plant occurs when the N_2-fixing plant is ploughed in as green manure. In tropical conditions, up to 60% fixed N_2 can be mineralized for use by a subsequent crop (Henzell and Vallis 1977). Unfortunately this system is generally not appealing to the farmer since it does not yield any food or cash. However, the system may have real economic justification when the buildup of soil N is large enough. This latter situation is well known to occur in Asia where N_2-fixing plants, namely *Astragalus sinicus* or *Sesbania cannabina*, are used as green manure alone or included in rotation patterns with *Azolla* (Lumpkin and Plucknett 1980). According to Dao The Tuan (personal communication) the incorporation of *Sesbania* in paddy soils is equivalent to the addition of 50-150 kg N/ha.

Sesbania rostrata, a stem nodulated legume already mentioned, appears to be a very promising plant as a green manure. In a preliminary experiment conducted at the ORSTOM station in Dakar, Senegal, we compared the effect of three treatments upon the yield and N content of rice grown in $1m^2$ irrigated microplots: (1) *Sesbania rostrata* ploughed in as green manure, when it was 52 days old, (2) N fertilization (60 kg N/ha) and (3) control. Treatment (1) dramatically increased grain and straw yield compared to the control (ca. + 100%). They also improved the quality of grain and straw, significantly increasing their N content (ca. 50%) (Rinaudo *et al.* 1981). A careful analysis of the soil N content after harvesting the rice showed that the soil N pool in the plots which had received green manure was 176.8 ± 14.4 g N/m^2, whereas in the plots without green manure it was only 132.4 ± 12.2 g N/m^2. Extrapolated on a 1-ha basis, the increment due to green manuring was 444 ± 266 kg N/ha. Even if we take into account the lower estimate, the soil N pool after harvesting rice was $444 - 266 = 178$ kg N/ha; this is an impressive figure since the increase of N exportation through the crop was 135 ± 46 kg/ha, which means that the amount of N_2 that was fixed by *Sesbania rostrata* during its cultivation (52 days) and transferred to the soil was at least $178 + 89 = 267$ kg/ha or approximately 280 kg/ha. This figure can be explained by the high N_2-fixing potential of *Sesbania rostrata* and its unique ability to simultaneously absorb soil nitrogen and fix atmospheric N_2. On the other hand, the application of a chemical fertilizer as ammonium sulphate, did not improve the soil N content.

According to the Microbiology group of the Hunan Soil and Fertilizer Institute, *Coriaria sinica*, a N_2-fixing non-legume perennial deciduous bush, is successfully used as a fertilizer for rice in West Hunan. *Coriaria sinica* grows quickly and has N content in the stems and leaves similar to that of *Astragalus sinicus*. More information on *Coriaria sinica* is required before it is recommended as a green manure in other locations (Watanabe, personal communication).

Grain Legumes Grown as Sole Crops

Many species of grain legumes are grown as sole crops, but the contribution of these crops to the soil N pool has not been studied very much. Since the improvement of legumes has mostly been aimed at increasing the N-harvest index (seed N as a proportion of total crop N), it can be predicted that the addition of N through the return of crop residue after grain harvest is low.

A field experiment conducted at the International Institute of Tropical Agriculture, Ibadan, Nigeria (Table 3), using four cowpea (*Vigna unguiculata*) cultivars that had received two rates of N fertilizer (25 and 100 kg N/ha) showed that the soil N balances were positive only when the rate of application of N was low and that they depended on the N_2-fixing ability of cultivar and the fractions of the total plant N removed with grains (harvest index) (Eaglesham 1981).

A budget study of a single irrigated soybean crop carried out at the Agricultural Research Centre in Tanworth, Australia, showed that the overall effect of this crop on the N status of the soil was a loss of 64-164 kg N/ha (Herridge 1981).

Recently, Eaglesham (1981) drew attention to the fact that "two factors are important in addition to N_2-fixing potential : mineral N availability, and the harvest index for N. Field-grown soybeans in the USA fixed 76 kg N/ha and absorbed 219 kg N/ha from the soil (Hardy and Havelka 1976). Assuming a N-harvest index of 70% (a relatively low figure for soybeans), 206 kg N/ha would be removed in the grain and 89 kg N/ha would be added back as plant residues, a net depletion of 130 kg N/ha (i.e., 219 − 89 kg N/ha)."

These examples show clearly that growing grain legumes may lead to a significant decrease in the soil N pool. When legume crops are grown for several years, soil N mineralized after the first crop reduces N_2 fixation by the subsequent crops or may also lead to losses through denitrification. Thus in Northern Australia, at a site where the N losses of the soil were low, the second and third successive groundnut and cowpea crops fixed less N_2 than the first one, thus increasing the N deficit of the soil (Wetselaar 1967).

Mixed Cropping and Rotations

In the tropics, grain legumes are often grown as a component of crop mixtures, which gives the farmer the main advantage of minimizing the risk of crop failures in marginal conditions. In soils with low levels of available nitrogen, mixed cropping probably improves agricultural productivity (Eaglesham *et al.* 1981; Eaglesham 1981; Wetselaar and Ganry 1981), but the effect of mixed cropping upon the soil nitrogen status has not yet been seriously studied. There is some information on the effect of rotation systems. The example we give here is related to a system involving a legume (groundnut) and a cereal (pearl millet) widely used in western Africa. The study carried out at the Bambey Research Station, Senegal, by Ganry (personal communication), indicates that this rotation causes a deficit of 71 kg N/ha for a 2-year period. The deficit results not only from losses through leaching and denitrification but also from N exportations by the crops and especially from the fact that N exportation by groundnuts (109 kg/ha) is larger than the amount of N_2 fixed (82 kg/ha). It may be added that this experiment was conducted during a year with climatic conditions favourable to N_2 fixation. The N deficit would have probably been larger if the rainfall had been less, which is most often the case in the semi-arid tropics.

Pastures

When comprising N_2-fixing legumes, pastures increase N content in the soil. Thus, Russell (1960) calculated that N in the 0.15 cm horizon of a pasture with more than 30% clover accrued at a rate of 52 kg N/ha/year.

A long-term field experiment conducted by Bruce (quoted by Sanchéz 1979) in Australia showed that after clearing a tropical rain forest the total nitrogen content of the 0.15 cm horizon was maintained at the initial level when the pasture was a grass/legume mixture, whereas with grass alone there was a sharp decline in the soil N content (Fig. 2).

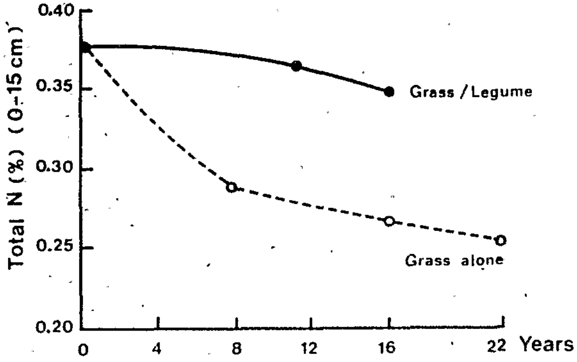

Fig. 2. Long-term effects of unfertilized guinea grass (*Panicum maximum*) pastures, with and without *Centrosema pubescens*, on the topsoil N content after clearing a rainforest in South Johnstone, Australia (Bruce quoted by Sanchez 1979).

N_2-fixing Trees

The N_2-fixing potential of trees varies a great deal. Thus within the genus *Acacia*, *A. mearnsii* is an actively fixing tree whereas *A. albida* is not. The accumulation of N in the 0.20 cm horizon soil under the canopy of *Acacia albida*, which has been reported many times (e.g., Jung 1969), is not due to N_2 fixation but to other processes, namely, (1) concentration of soil nutrients, especially N, extracted from the deeper soil horizons and eventually from the water table and returned to the soil surface with the leaf litter, and (2) accumulation of wind-blown organic residues near the trunk. Moreover, one should be aware of the fact that the N_2-fixation rate of trees tends to decrease with age of the stand, since the N content of the soil progressively increases, impeding nodulation and N_2 fixation.

By comparing the soil total N content under a plantation of *Acacia mearnsii* in six different soil types with the total N content under adjoining virgin veld (mixed natural pasture), Orchard and Darby (1956) found that this N_2-fixing tree enriched the 0.23 cm horizon by 200 ± 44 kg N/ha/year over a period of 30 years, which is an impressive figure.

Casuarina equisetifolia, a N_2-fixing non-legume tree which has been introduced in many parts of the world, can fix 58-280 kg N_2/ha/year (Dommergues 1981).

This ability of *Casuarina* to increase the soil N content is exploited in Papua New Guinea where this tree is planted in food gardens as fallow, the increment in soil N being larger than that observed under *Albizia* or *Crotolaria* (Thiagalingam and Fahmy 1979). Current studies of other N_2-fixing trees, such as *Glyricidia sepium* (Okigbo 1975; Roskoski *et al.* 1981), *Acacia* sp. or *Inga jinicuil* (Roskoski *et al.* 1981) indicate that extension of the use of such trees in agroforestry is most desirable, but much more research is necessary to exploit this promising way of improving soil fertility.

Conclusion

Restoration and maintenance of the N status in soils can be achieved by growing N_2-fixing plants provided (1) that these systems exhibit a high N_2-fixing potential, (2) that no environmental factor limits their activity and (3) that the largest portion of the fixed N_2 is transferred to the soil.

The first condition is fulfilled when one has chosen the best host × rhizobial or actinorrhizal strain association.

The second condition is fulfilled only when limiting factors are excluded. Two of them play a major role: P deficiency and excess of combined N in the soil. In P-deficient soils, it is necessary to apply P fertilizers; this application can be minimized if the N_2-fixing plants are infected with mycorrhizae and satisfactorily respond to this infection. Even soils with a low or relatively low total N content may have a relatively high content in inorganic N at least at some time in the year. In such a situation where the activity of most N_2-fixing systems is inhibited, two solutions have been envisioned here: adding compatible fertilizers to the soils or using N_2-fixing systems that are not inhibited by inorganic N. The third condition is currently fulfilled with only some cropping systems especially systems including green-manure or N_2-fixing trees.

Finally one should note that restoration and maintenance of the soil N content through N_2 fixation cannot be obtained solely by the introduction of N_2-fixing plants; they also require the addition of fertilizers. In other words, it may be necessary to combine biological and chemical technologies. This combination would probably be successful in the case of P fertilization, since inoculation with endomycorrhizae has been shown to increase the efficiency of P fertilizers, thus allowing a decrease in their rate of application. Simultaneous benefit from the input of N from fertilizers and from N_2 fixation is the objective which should be reached in the future. To achieve it, it will be necessary to use either compatible fertilizers or N_2-fixing systems uninhibited by combined soil N. This should provide high yields with a minimum consumption of N fertilizers.

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