NITROGEN FIXATION IN LEGUMINOUS TREES

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ABSTRACT

Leguminous trees are major components of many tropical natural and agroecosystems. Although many woody legumes fix atmospheric nitrogen, little is known about the basic biology of nitrogen fixation by these species. Studies conducted, to date, have shown that, like herbaceous legumes, woody legumes can be quite specific in their rhizobial requirements, and exhibit variable responses to inoculation. Their expression of the nitrogen fixing symbiosis can be markedly affected by environmental characteristics such as soil type, nutrients, and climate, and man's actions to alter these environmental factors. Unlike herbaceous legumes, woody legumes exhibit different temporal patterns of nitrogen fixing activity and, in general, fix smaller amounts of nitrogen on an annual-areal basis. However, in the few instances when nitrogen inputs from fixation by woody species has been compared to other nitrogen imputs to a particular ecosystem, it has been found to be significant.

RESUMEN

Los árboles leguminosos representan unos de los mayores componentes de muchos sistemas naturales y agrícolas del trópico. Aunque muchos árboles leguminosos fijan nitrógeno atmosférico, muy poco se conoce acerca de las bases biológicas de la fijación de nitrógeno de estas especies. Los estudios conducidos hasta la fecha han demostrado que, como en el caso de leguminosas herbáceas, las arbóreas pueden ser muy específicas en sus requerimientos de rizobios, y pueden exhibir respuestas

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variables a la inoculación. La expresión de la fijación simbiótica de nitrógeno de estas especies puede ser marcadamente afectada por características del ambiente como tipo de suelo, nutrientes, y clima, y las acciones del hombre que alteran estos factores ambientales. Al contrario de las leguminosas herbáceas, las arbóreas exhiben diferentes patrones de tiempo de la actividad de fijación de nitrógeno y, en general, fijan cantidades más pequeñas de nitrógeno en una base año-área. Sin embargo, en los pocos casos en que el aporte de nitrógeno de la fijación por las especies arbóreas ha sido comparado con otros aportes de nitrógeno a un ecosistema particular, este ha sido significativo.

INTRODUCTION

Leguminous trees are abundant in many primary and secondary successional tropical forests (Knight, 1975; Rzedowski, 1978; Sylvester-Bradley et al., 1980). Recently, world interest in tree legumes has increased because many are fast-growing and can supply resources needed by developing tropical nations (N.A.S., 1980; Brewbaker et al., 1982).

Woody legumes can provide high-protein forage and fodder, nitrogen-rich green manures, fuel, timber, other wood products and help control soil erosion (N.A.S., 1977, 1979, 1980; Roskoski et al., 1982). In addition, many leguminous trees fix atmospheric nitrogen thereby increasing the nitrogen content of the ecosystems in which they occur. This has led to their widespread use in traditional tropical farming systems and their promotion as integral components of new agroforestry systems.

Despite the potential importance of the nitrogen inputs tree legumes can make to tropical agroecosystems, many aspects of nitrogen fixation by these species is poorly understood. It is only now as their use becomes more widespread that scientific investigations are focusing on this important aspect of their physiology. Studies conducted, to date, have pointed out some important similarities and differences in nitrogen fixation between woody legumes and their better-studied, herbaceous relatives.

OCCURRENCE OF NITROGEN FIXATION IN TREE LEGUMES

There are over 18,000 members of the family Leguminosae. Of this total about half are woody in nature. However, little is known about the biology of most of these woody species because they occur predominately in the tropics. A compilation of nodulation reports for over 1000 tree species (Halliday, 1984) revealed that 528 species were nodulated, 128 did not form nodules, and the remainder were of uncertain nodulation status. The lack of nodulation in some tree legumes set woody legumes somewhat apart from herbaceous members of the Leguminosae, where the majority of members nodulate.

RESPONSE TO INOCULATION

Rhizobia for leguminous trees have been isolated from a wide range of soils and climates (Habish, 1970; Basak and Goyal, 1975; Aquiahuatl and Muñoz, 1983; Hansen and Munns, 1984). This evidence would seem to suggest that rhizobia for tree legumes are ubiquitous and therefore inoculation should be unnecessary. While this may be true for some species, others, for example *Leucaena leucocephala* Lam. (Trinick, 1968; Halliday and Somasegaran, 1982; Herrera and Olivares, 1983) and some *Acacia* species (Dreyfus and Dommergues, 1981a, 1981b; Habish and Khairi, 1970), require specific rhizobia which may not occur in all sites. In addition, examples from agriculture show that native rhizobia may not fix as much nitrogen as introduced stains that have been specifically selected for this characteristic (Weaver and Fredrick, 1974). Thus, available information suggests that some tree species, like Leucaena, may require inoculation to nodulate and that others may benefit from being inoculated with a more efficent strain than the indigenous rhizobia. When woody legumes have been examined with respect to inoculation response the latter has often proved to be true (Dutt et al., 1982; Dutt and Pathania, 1983, Moreno-Quiroz et al., 1983; Young and Chao, 1983).

For example, when rhizobia for *Leucaena leucocephala* and *Acacia pennatula* Schl. and Cham., collected from 17 different locations in México, were compared for nitrogen-fixing efficiency wide variation was found (Salo, 1985). The two most efficient Acacia isolates came from 2 different sites that were only 5 km distant from each other and had the same soil type, pH, and nutrient characteristics (Salo, 1985). The most efficient isolate for Leucaena came from a coastal site with a sandy soil with a pH of 8.0 but no efficient isolates were obtained from acid soils. When these three isolates plus strain TAL 1145 (NifTAL 1984) for Leucaena were field tested in two sites in Mexico, marked differences in growth and inoculation response were observed (Roskoski et al., 1986).

There were large, significant differences in the mean height of *L. leucocephala* between sites. Tree heights in La Balsa, the 600 meters elevation site, were 3 times those obtained in Xalapa, the 1500 meters elevation site, being 83 vs 267 cm, respectively. In addition, significant inoculation treatment differences were found in the high elevation site (Figure 1).

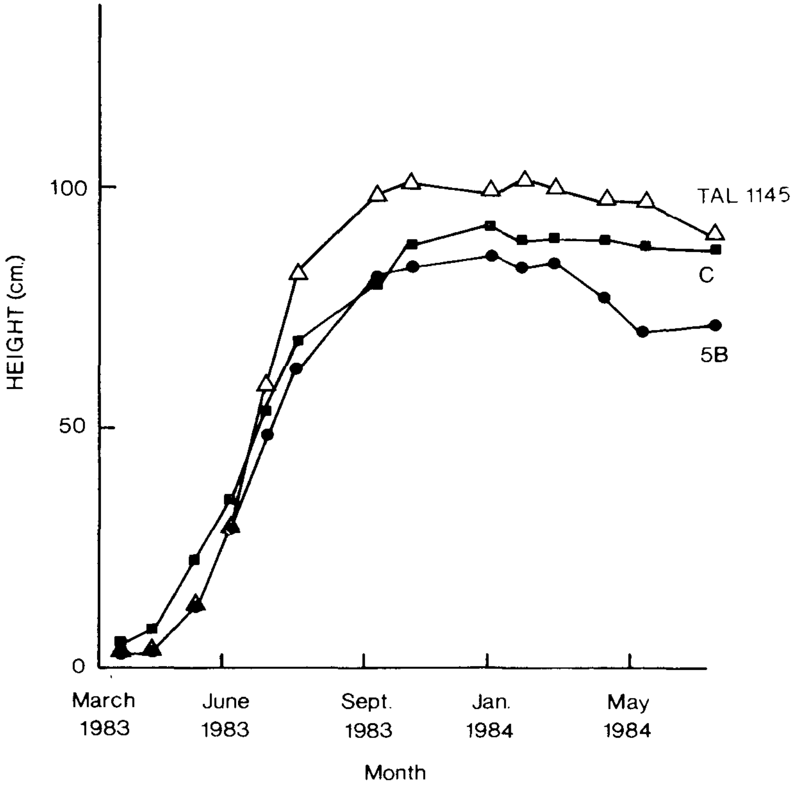


Figure 1. Mean heights of Leucaena leucocephala inoculated with Rhizobium strains TAL 1145, 5B, and uninoculated controls growing in Xalapa, Mexico.

Leucaena grown in Xalapa and inoculated with TAL 1145 were significantly taller than plants inoculated with either 5B or the controls. Two additional facts warrant mention. Strain TAL 1145 is reported to perform well under acidic soil conditions (Halliday, 1981) and did so in the acid soil of Xalapa, surpassing 5B which was isolated from a plant growing in an alkaline soil, as well as the native rhizobia which formed nodules on the control plants. This indicates that although Leucaena could nodulate with the native rhizobia it benefited from inoculation with an introduced strain. The second point to note is that significant differences in plant height between inoculation treatments did not appear until the end of the rapid growth phase at the end of the rainy season. During most of the active growth phase, trees from all three inoculation treatments were similar in height and growth rates.

In contrast to Leucaena, Acacia heights were not significantly different between sites. Thus Acacia, which is native to the Xalapa region, grew equally well at the lower elevation site where it is not normally found.

As with Leucaena, there were significant inoculation treatment effects in Xalapa but not in La Balsa (Figure 2). Strain 16, originally isolated from the same site where the experimental plots in Xalapa were located, was superior to both strain 14 and the control. These differences translate into 2.0 metric tons/ha for plants inoculated with strain 16, and 1.5 and 1.0 tons/ha for strain 14 and the control, respectively. These findings indicate that even though Acacia formed an effective nitrogen fixing association with native rhizobia, it benefited when one of those native rhizobia was applied in large numbers as inoculant.

EFFECTS OF SOIL TYPE AND CLIMATE ON NITROGEN–FIXING ACTIVITY OF TREE LEGUMES

As with herbaceous legumes, soil type, nutrients and climate have been found to effect the establishment and expression of nitrogen fixation in woody legumes. In one study in Mexico, (Roskoski et al., 1982) a coastal sandy loam and an acidic, highland andosal were used to grow 9 species of leguminous

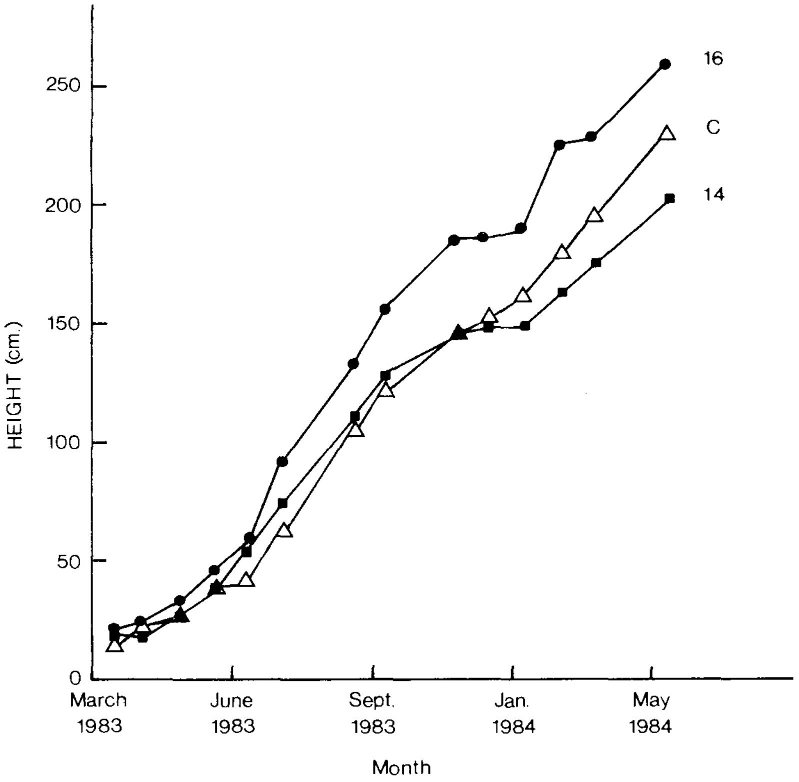


Figure 2. Mean height of Acacia pennatula inoculated with Rhizobium strains 16, 14, and uninoculated controls growing in Xalapa, Veracruz. Mexico.

trees. Half the trees were grown at 1500 meters elevation and the other half at sea level. Results showed that both soil type and climate affected nitrogen fixing activity (Table 1). *Gliricidia sepium* Jacq., for example, did not exhibit highest nitrogen-fixing activity in the sandy soil where it naturally occurred but in the high elevation andosol. It did, however, yield highest activity when grown under its regular climate at sea level. *Acacia pennatula* on the other hand, exhibited maximum activity in both its native upland soil and climate, despite the fact that overall growth was much greater at sea level.

TABLE 1. Nitrogen fixing activity of tree legumes grown in different soils and climates*.

Species	Experimental Treatments**			F text***
	Sandy Soil Sea level	Upland Soil Sea level	Upland Soil 1500 m	
Gliricidia sepium	2.33	18.40	8.57	.01
Acacia pennatula	4.79	6.55	9.40	.05

* μ mol acetylene produced/ g nodules/ hr.

** Sea level, mean annual temperature = 24 C, annual rainfall = 1340 mm;
1500 m, mean annual temperature = 19 C, annual rainfall = 1957 mm;
Sandy soil, pH = 8.1, total o/o N = .14. ppm P = 9.2;
Upland soil, pH = 5.5, total o/o N = .33, ppm P = 29.0.

*** F—test, values indicate significance level.

EFFECTS OF SOIL NUTRIENTS AND MANAGEMENT ON NODULE BIOMASS AND DISTRIBUTION

Man can also affect nitrogen fixation by tree legumes by altering the environment in which they grow. In a study of nitrogen fixation in a Mexican coffee plantation, it was found that nodules of *Inga jinicuil* Schl., a leguminous tree employed for shade, were not uniformly or randomly distributed throughout the coffee plantation but were concentrated around the base of coffee trees close to the trunk (Roskoski, 1981).

The highest concentration of nodules was located within 20 cm of the coffee trunks. Beyond 1 meter very few nodules were found and no nodules were found around the base of Inga trees. This unusual distribution pattern led to an examination of plantation management practices for an ex-planation.

Several times during the year N—P—K fertilizer is applied around the base of the coffee trees close to the trunks. Since nodulation and nitrogen fixation are inhibited by nitrogen and stimulated by phosphorus, it seemed likely that management practices related to fertilization might be responsible for the observed nodule distribution pattern.

Soil analysis (Van Kessel and Roskoski, 1981) revealed that both phosphorus and nitrogen levels were higher directly under the coffee trees than further away from the trunk (Table 2). A follow-up study (Van Kessel and Roskoski, 1983) which isolated the effects of applied nitrogen and phosphorus found that applied nitrogen inhibited and phosphorus stimulated nodulation and nitrogen fixation by *Inga jinicuil*. Although these results are consistent with those found for herbaceous legumes, they do not explain how such a high nodule biomass could occur in soils with high nitrogen levels.

TABLE 2. Analysis of soils sampled at varying distances from coffee trunks.

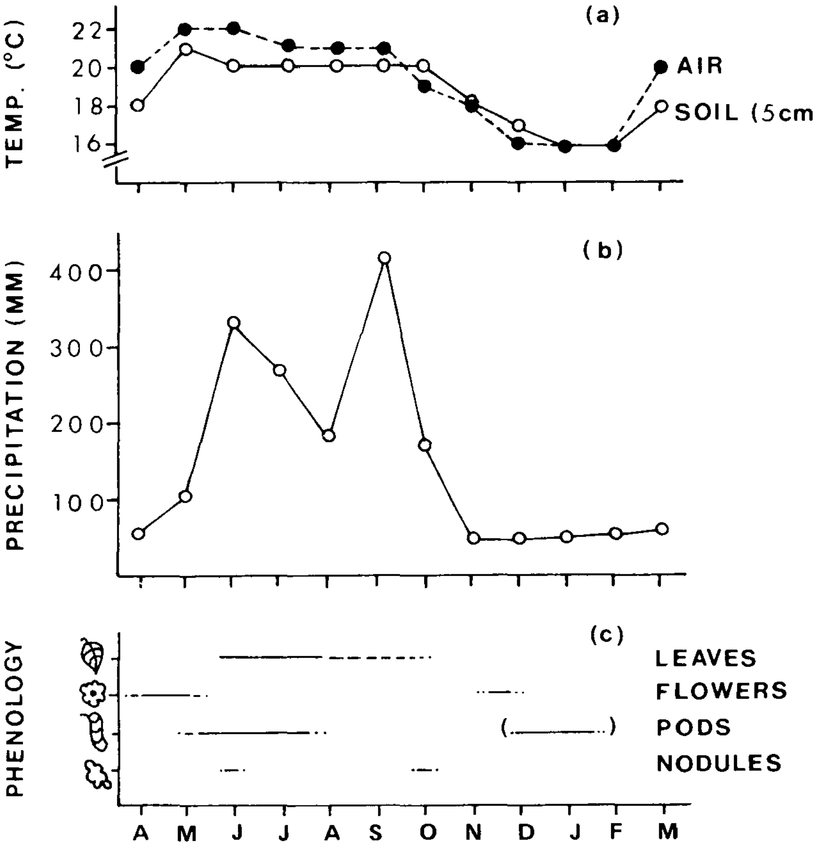
Distance from coffee trunk in cm	pH	o/o N	ppm P	o/o Organic matter
0–30	4.8	0.16	64	3.1
30–60	5.2	0.15	54	2.6
60–90	5.8	0.12	44	2.3

TEMPORAL VARIATION IN NITROGEN FIXING ACTIVITY BY LEGUMINOUS TREES

Because tree legumes are perennial organisms, marked temporal variation in nitrogen fixing activity might be expected. However, few studies had examined this aspect of nitrogen fixation by woody legumes. A three and one half year study of nitrogen fixing activity by *Inga jinicuil* (Roskoski and Van Kessel, 1985) revealed several interesting facts about temporal variation in nitrogen fixing activity by this species.

Year to year variation was quite pronounced, varying by as much as 100o/o from one year to the next; or from 23.4 to 44.6 kg nitrogen fixed/ha/yr in 1979 and 1980, respectively. This unexpected variation in nitrogen-fixing activity by 30-year-old trees, whose nitrogen demand might be expected to be constant, may have been caused by a change in the plantation management regime; specifically the cessation of fertilization in 1980. The lack of readily available nitrogen may have stimulated the trees to fix more nitrogen to meet their nitrogen requirements. At the same time, an insect infestation that led to complete defoliation of the Inga trees in 1980 may have stimulated an increase in nitrogen fixing activity to produce nitrogen for new leaf production.

Seasonal variation in nitrogen fixing activity was also pronounced. Highest activity occurred in July and October, one month after the two annual peaks in precipitation in June and September and coincident with major phenological events (Figure 3). Thus nitrogen fixing activity increased when demand for nitrogen was highest. This bimodal peak in activity has not been documented for other perennial legumes and would not be expected in annual legumes which have only one phenological cycle.



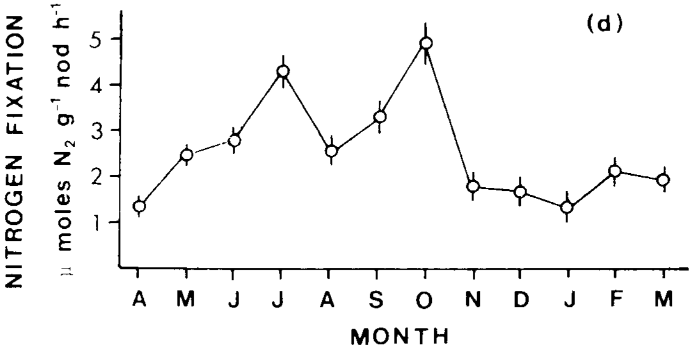


Figure 3. Temperature (a), precipitation (b), Inga phenology (c), and nitrogen fixing activity (d) vs. months April to March. Data for air temperature and precipitation are from Jimenez and Martinez (1979). Phenological data are as follows: heavy lines represent maximum activity, dotted lines signify less activity. Lines for leaves and flowers show the months when leaf and flower production occur. The line for pods indicates the time of pod development, and the line for nodules indicates the time of the year when new pink nodules were observed in the field. Figure 1d plots mean nitrogen fixing activity for each month ± 1 $S\bar{x}$.

Diel variation in nitrogen fixing activity also varied from that normally reported for herbaceous legumes. Highest activity was observed at 7 pm and not at noon. This difference, however, may be related to the physiological activity pattern of trees which is normally highest later in the afternoon immediately prior to shoot elongation and diameter growth (Kramer and Kozlowski, 1960) and not at midday when high ambient temperatures may suppress photosynthetic activity required for nitrogen fixation.

IMPORTANCE OF NITROGEN FIXATION BY TREE LEGUMES

To assess the importance of the nitrogen inputs from fixation by tree legumes in agro or natural ecosystems it is essential to know 1) how much nitrogen is actually being fixed by these species, and 2) what is the magnitude of other nitrogen inputs to the system.

The size of trees and their extensive root systems, makes it difficult to measure the amount of nitrogen fixed using the standard difference harvest methods employed for crop species. Most investigators have estimated annual-areal fixation from changes in nodular biomass and activity through time.

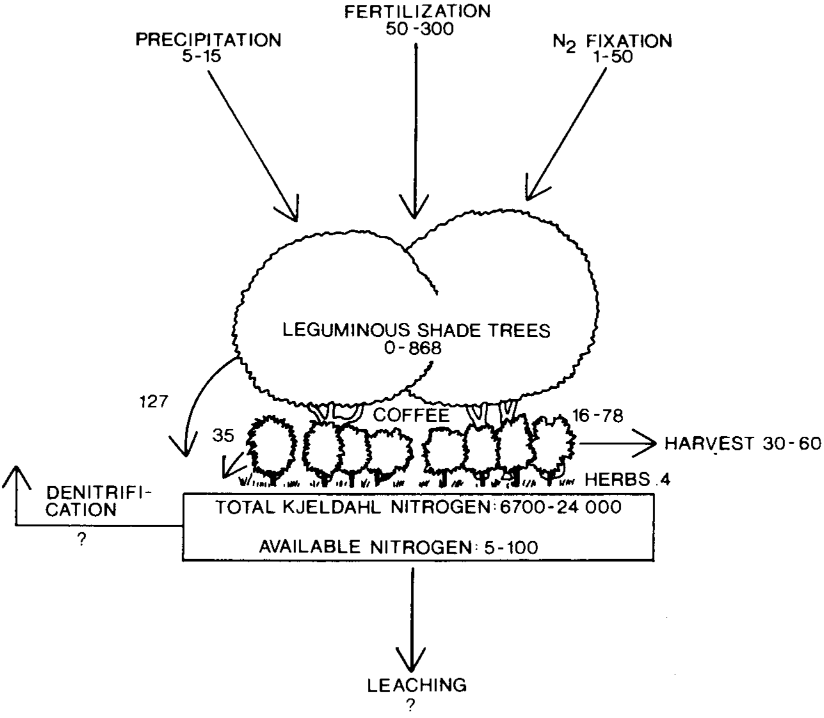
Shearer et al. (1983) reported that *Prosopis glandulosa* fixed up to 60o/o of its annual nitrogen requirements, or about 30 kg/ha/yr (Rundel et al., 1982). Higher amounts, 110 kg N fixed/ha/yr were reported by Hogberg and Kvarnstrom (1982) for *Leucaena leucocephala* in Kenya. Roskoski (1981) estimated that *Inga jinicuil,* a shade tree in Mexican coffee plantations, fixed about 40 kg nitrogen/ha/yr, which amounted to 20o/o of its total nitrogen demand and that annual fixation by *Acacia pennatula* and *Gliricidia sepium* were 35 and 13 kg nitrogen/ha/yr, respectively (Roskoski et al., 1982).

While these amounts are not large when compared to the amounts fixed by crop species, they probably do not represent the maximum fixation possible by woody species, which have not undergone genetic improvement as have many crop species. In addition, higher amounts might be obtained from the existing genetic material if all other limiting factors are optimized as is normally done with crop species.

Despite the low absolute amounts of nitrogen fixation reported in the above studies, the true significance of the nitrogen input by these species can only be assessed in relation to other nitrogen inputs and outputs in the ecosystems in which they occurred.

In the case of the Mexican coffee plantation, it appears that the nitrogen input from the leguminous shade tree of 40 kg/ha/yr represents a significant nitrogen input to the system (Figure 4).

Since nitrogen inputs via fertilizers range from 50 to 300 kg nitrogen/ha/yr, and inputs in precipitation vary from 5 to 15 kg/ha/yr, it appears that inputs from fixation from Inga shade trees equals from 20 to 100o/o of the nitrogen inputs via fertilizers and 3 to 10 times as much as inputs in precipitation. Thus the data indicate that fixation by leguminous trees in one tropical agroecosystem adds a significant amount of nitrogen to that ecosystem (Roskoski, 1981).



NITROGEN IN A COFFEE PLANTATION

Figure 4. Nitrogen inputs, outputs, transfers, and standing crop in a Mexican coffee plantation.

It is important to note, however, that although *Inga jinicuil* fixed a significant amount of nitrogen, little of this nitrogen was immediately available to coffee plants. Legumes, studied to date, do not leak significant quantities of nitrogen into the environment unless severely stressed. Never-the-less, the part of the coffee plantation with Inga shade trees had higher coffee yields than adjacent sites with other species of non-fixing shade trees. (Jimenez and Martinez, 1979). The most likely explanation for this phenomenon is the sparing effect of the Inga on the applied nitrogen fertilizer which left more nitrogen for the coffee plants.

CONCLUSION

The existing evidence suggest that nitrogen fixation by leguminous trees is in some ways similar to and in other ways different from fixation by herbaceous species.

Inoculation of some species of woody legumes with superior effective rhizobia can result in greater height growth and consequently increased biomass in some sites. Unfortunately, to date, there is no reliable way to predict in what sites or with what species an inoculation response is likely. The only way to determine if inoculation would be efficacious is to run an inoculation trial.

Fixation by tree legumes also appears to be sensitive to the same environmental factors, such as soil type, nutrients, and climate as other members of the Leguminosae.

However, unlike annual legumes, perennial legumes can exhibit marked changes in nitrogen fixing activity on a daily, seasonal, and yearly basis. Any attempts to assess the importance of nitrogen fixation by woody species in a particular ecosystem will have to take such temporal variability into account.

Finally, the finding that nitrogen fixation by a leguminous shade tree in a Mexican coffee plantation made a significant nitrogen input to that system suggests that other agroecosystems with leguminous trees should be similarly investigated. The impact of tree legumes in other tropical agroecosystems is poorly documented yet may be quite important to long term sustained productivity. If so, these agroecosystems warrant increased attention by scientists interested in understanding and managing agricultural production in the tropics.

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