Variation in the Biological N_2 Fixation by Tree Legumes in Threé Ecological Zones from the North to the South of Senegal

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The utilization N_2-fixing trees (NFTs) in agroforestry, in semiarid regions, is often limited by weak N_2 fixation. This study was conducted to evaluate the N_2-fixing capacity of trees growing under natural conditions for 10 years with neither chemical fertilizer nor inoculum application. For this purpose, natural isotopic ^{15}N abundance ($\delta^{15}N$) of leaves harvested from NFTs and reference plants (ref) growing in three agroecological zones of Senegal were analyzed. At each site (one site per zone), average $\delta^{15}N$ ref was calculated N contents and $\delta^{15}N$ values increased from the north in the Sudano-Sahelian zone (Bambey and Nioro) to the south in the sub-Guinean zone (Djibelor). The percentage of fixed N_2 (%Ndfa) was low at both Bambey (Ndfa $\leq$ 22%) and Nioro (Ndfa $\leq$ 39%) and very high at Djibelor (76% $\leq$ Ndfa $\leq$ 95%). To explain the low levels of Ndfa observed at Bambey and Nioro, the hypothesis of lack of Bradyrhizobium *strains in these soils was made. This hypothesis was confirmed by results obtained from a tria1 conducted under controlled conditions, which showed absence of nodulation on* Gliricidia sepium *growing under optimal conditions of water and nutrient supply. Improvement of N_2 fixation of NFTs in the semiarid zone is a prerequisite for implementation of sustainable agroforestry systems.*

Keywords N_2-fixing trees, natural ^{15}N abundance, percent of fixed N_2, Sudano-Sahelian and sub-Guinean zones

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The establishment of sustainable agroforestry practices requires the understanding of several factors. First, the feasibility and the modes of implementation of these practices should be considered in Iight of the variability of the environment (soil, climate, and human pressure on land) and the diversity of farming practices (farmers' objectives, socioeconomic means and constraints). Then, atmospheric nitrogen fixation (N_2 fixation) and competition problems with associated crops should be addressed. The importance of N_2 fixation is well recognized (Bowen et al., 1990). However, few tests have been conducted to evaluate the N_2 fixation in natural ecosystems (Hansen & Paste, 1987; Domenach et al., 1989; Yoneyama et al., 1990a, 1990b; Sanginga et al., 1990, 1991; Mariotti et al., 1992). Many N_2-fixing trees (NFTs) have a potential use in agroforestry systems such as alley cropping. However, few have been evaluated (Yoneyama, 1987; Kang et al., 1990). Tree species such as *Leucaena* spp. and *Gliricidia* spp., which have been intensively investigated in the tropical zone of Nigeria, have shown high potential for N_2 fixation (Kang et al., 1990). Nevertheless, such leguminous species are difficult to establish in semiarid and arid zones for several reasons. Among them, besides socioeconomic reasons, one reason is the establishment and the difficult functioning of N_2 fixation of these species. Thus, the evaluation of the percentage of N_2 fixation (%Ndfa) in NFTs under natural systems is a primary requirement for the improvement of agroforestry techniques. It is indeed by improving the Ndfa, when it is below the N_2 fixation potential (NFP), that a sustainable agroforestry system may be established. The present study is devoted to the evaluation of the N_2 fixation by NFTs growing under natural conditions.

Materials and Methods

Determination of the Percentage of Fixed N_2 (%Ndfa) by the N_2-Fixing Trees (NFTs)

Three stations at the Institut Senegalais de Recherches Agricoles (ISRA), namely, Bambey (17°50′ N, 14°75′ W) in the northem Sudanian zone, Nioro (16°35′ N, 13°75′ W) in the southem Sudanian zone, and Djibelor (17°35′ N, 12°50′ W) in the sub-Guinean zone, representing three ecological zones, were selected. The definition of these zones was based on isohyets representing the rainfall probability of 5 years out of 10 in the 1970-1990 period. The isohyets were 400,600, and 1100 mm, respectively, at Bambey, Nioro, and Djibelor. Soils were mainly Ultisols and Alfisols (Soil Survey Staff, 1992; Singer & Munns, 1996), with a net predominance of kaolinite in the clayey fraction (Charreau & Nicou, 1971). The clay content ranged from 4 to 12% from Bambey to Djibelor. These soils presented mediocre physical properties, namely, weakly developed structure, very weak structural stability, and very poor water-holding capacity. The available water reserve ranged from 3 to 10% in the topsoil, and available P was less than 10 mg kg-‘.

Leaf samples of NFTs and non-N,-fixing trees were harvested in May 1994 from t r-minal parts of tree branches in hedgerows of agroforestry trials. Working in fertilized *A a-cia*, Zakra et al. (1991, 1993) have found that a substantial amount of N in leaves may be from remobilization from other plant parts. For estimating the N_2 fixation by *Alnus gl ti-nosa* (L.) Gaertn. Using a ^{15}N labeling method, Domenach and Kurdali (1989) reco l-mended sampling for analysis from the most recently formed leaves at the end of the growing season to avoid the influence of nitrogenous reserves of other plant parts. It is known that the translocated N from root and trunk reserves can represent 10% of total N (Dommergues, 1997). To minimize the influence of remobilized N from root and trunk only newly formed leaves were sampled. Four to 5 trees per species or line within species were chosen at random and 4-l 0 branches per tree were sampled to have 4-5 fresh matter samples weighing 500 g each. This sampling was undertaken at the cutting or pruning period of the trees. After harvest, each sample was oven-dried at 80°C and then finely ground to obtain 10 g. Total N content and natural ^{15}N abundance of samples were measured by a double-introduction mass spectrometer at the Laboratoire Central du Centre National de la Recherche Scientifique de Lyon, France.

Soil samples were taken from topsoil (O-20 cm) at the three sites in NFTs growing area (three replicates) and outside of this area (three replicates). The total N content and natural ^{15}N abundance of these soil samples were determined using the mass spectrometer described earlier.

The natural ^{15}N abundance ($\delta^{15}N$: relative isotopic excess per thousand) of the sample was expressed as follows:

$$\delta^{15}N = 1000 \frac{^{14}N/^{15}N[sample] - ^{14}N/^{15}N[air]}{^{14}N/^{15}N[air]}$$

where $^{14}N/^{15}N[air] = 0.3663$, and $\delta^{15}N$ is expressed in parts per thousand.

The ^{15}N isotopic method for quantifying the N_2 fixation is based on the $\delta^{15}N$ variation. The $\delta^{15}N$ values in the N source and reactions by which plants metabolize N coming from this source define the average $\delta^{15}N$ values in the plant. Plants depending on soil exhibit positive $\delta^{15}N$ values, rather close to those of the soil (Domenach et al., 1989). Consequently, $\delta^{15}N$ values of plants cultivated on soil where minera1 N is available are lower if these plants fix N_2 (Amarger et al., 1979). Thus, based on reference plants (non-N,-fixing), which depend on soil N, the percentage of fixed N_2 in plants (%Ndfa) can be calculated using the following formula (Amarger et al., 1979):

$$\%Ndfa = 100 \frac{\delta^{15}Nref - \delta^{15}Nfix}{\delta^{15}Nref - \delta^{15}Np}$$

where $\delta^{15}Nref$, $\delta^{15}Nfix$, and $\delta^{15}Np$ are, respectively, $\delta^{15}N$ values of the reference plant, the N_2-fixing plant, and the N_2-fixing plant growing on an inorganic N-free medium. Reference plants were chosen close to NFTs in such a way that they feed on the same soil N pool.

Determination of $\delta^{15}Np$

Gliricidia sepium (Jack.) Kunth ex Walp. seeds were scarified in concentrated sulfuric acid for 15 min and rinsed several times with sterilized distilled water to remove acid. Seeds were then sown in plastic bags (3 seeds per bag, 4 bags), each filled with 650 g sterilized sand. Seedlings were thinned to 1 plant per bag 15 days after emergence and then inoculated with liquid inoculum of *Bradyrhizobium* TAL5 isolated on *Gliricidia* spp. in Nicaragua (provided by the Centre de Coopération International en Recherche Agronomique pour le Developpement/Cultures Annuelles, Montpellier, France). The TAL5 strain was cultured in liquid yeast extract mannitol broth (Vincent, 1970) at 28°C for 7 days. At thinning, immediately after strain cultivation, 1 ml of suspension containing approximately 10^9 cells was added to the soil near the seedlings. Bags were watered daily with an inorganic N-free medium (Vincent, 1970) to keep the moisture content near field capacity. At 100 days after planting, shoots were cut at soil level, roots were washed, and nodules were collected. Each plant part, oven-dried at 70°C, was ground and analyzed for nitrogen content and natural ^{15}N abundance.

Statistical Analysis

The data were analyzed statistically using a statistical package (STAT-ITCF) for the PC computer (Beaux et al., 1988). Mean and standard deviation from mean were found for each species or line.

Results

N Content and $\delta^{15}N$ of Soils

The resulting N contents and $\delta^{15}N$ values of the soils are shown in Table 1. The variations of ^{15}N abundance of soils from areas with and without growing trees were similar, allowing the use of average values from six replicates. The N content values of soil were 0.280, 0.333, and 0.720 g kg-‘, respectively, at the Bambey, Nioro, and Djibelor sites. The $\delta^{15}N$ values were +6.58 at Bambey, +6.03 at Nioro, and +6.85 at Djibelor. These $\delta^{15}N$ values of soils are within the range observed in Brazil and Philippines soils, where a range of +5 to +1 1 was reported (Yoneyama, 1987). A similar range (+3.7 to +9.5) has been reported for Thailand soils (Yoneyama et al., 1993). The range (+7.7 to +8.2) observed by Snoeck (1995) for Burundi soils was, however, higher than that reported in the present study.

$\delta^{15}Np$ of N_2-Fixing Plants Growing on an Inorganic N-Free Medium

The $\delta^{15}N$ in leaves varied from -1.20 to -2.21 with an average value of -1.70 ± 0.32 (Table 2). Similar values have been reported by Domenach et al. (1989) in *Prosopis grandulosa* Torr. (-1.70) and by Shearer et al. (1983) in *Alnus* spp. (-1.80) but a superior value was found by Mariotti et al. (1992) in *Casuarina equisotifolia* (L.) (-1 .00). For this study, the $\delta^{15}Np$ value of -1.70 ± 0.32 has been chosen for estimating the Ndfa of tree species at the three sites.

$\delta^{15}N$ of Reference Plants

Reference plants included the nonlegumes *Azadirachta indica* Juss. and *Guiera senegalensis* J.F. Gmel., and non-N,-fixing legumes belonging to the Caesalpinoidose: *Cassia*

Table 1
Total N contient (g kg^{-1}) and $\delta^{15}N$ (parts per thousand) of soils

Soils	N (g kg^{-1})	$\delta^{15}N$
Bambey	0.280 ± 0.014	+6.58 ± 0.10
Nioro	0.333 ± 0.052	+6.03 ± 0.24
Djibélor	0.720 ± 0.034	+6.85 ± 0.10

Note. Soil sampling horizon = O-20 cm. Each value is the mean of six replicates. Numbers following ± are standard deviation from mean.

Table 2
Values of $\delta^{15}Np$ of *Gliricidia sepium* inoculated with the *Bradyrhizobium* strain TAL5 and grown on an inorganic N-free medium

Leaf samples	$\delta^{15}Np$
1	-1.20
2	-1.74
3	-1.76
4	-2.21
Mean	-1.70
Standard deviation from mean	±0.32

siamea, (Lam.) syn. *Senna siamea* (Lam.) H.S. Hirwin & Barneby, *Cassia sclerosperma* A. Cunn *ex* Vog, *Bauhinia rufescens* (Lam.), and *Piliostigma reticuhtum* (DC.) Hochst (Allen & Allen, 1981; Sprent & Sutherland, 1990). The $\delta^{15}N$ values of reference plants ($\delta^{15}N$ ref) were analyzed (Table 3). From the results, the average of the two reference plants that exhibited the closest values of $\delta^{15}N$ to that of the soil was used. Thus, the $\delta^{15}Nref$ values retained were +5.49 at Bambey, +5.57 at Nioro, and +6.89 at Djibelor. This $\delta^{15}Nref$ value was higher in the south (humid zone) than in the north (dry zone). Conceming *A. indica,* the $\delta^{15}Nref$ varied from +4.95 at Bambey to +8.22 at Djibelor, and took a intermediary value of +6.16 at Nioro.

Estimation of the Percentage of Fixed N_2 (%Ndfa)

The %Ndfa values of leaves harvested from trees growing in different zones of Senegal are indicated in Table 3. Among NFTs analyzed at Bambey, *Gliricidia sepium* (Jacq.) Kunth ex Walp. showed a $\delta^{15}N$ value (+4.29) close to the $\delta^{15}Nref$ (+5.49), indicating a low level of N_2 fixation (Ndfa = 17%). In contrast, the values of *Hardwickia binata* Roxb. (+3.90) and *Prosopis cineraria* (L.) Druce (+3.97) were low, suggesting respective N_2 fixations (Ndfa) of *22* and 21%. However, *G. sepium* showed the highest N content among the three species while having the lowest %Ndfa.

Percentages of fixed N_2 (%Ndfa) at Nioro showed that all species, except *Acacia holosericea* Cunn. ex G. Don. and *Prosopis cineraria,* had $\delta^{15}N$ values similar to the $\delta^{15}Nref$ value considering the experimental error (Table 3). These two species showed respective Ndfa values of 39 and 21%. As found at Bambey, the highest value of N content *(3.96%)* was observed in leaves of *G. sepium* (ILG 50), which fixed only 6% of its total N.

At Djibelor, the $\delta^{15}N$ values for G. *sepium* lines were significantly lower than the average $\delta^{15}N$ ref value (+6.89). Their $\delta^{15}N$ values varied from -1.30 for ILG61 to +0.34 for ILG58. Contrarily to Bambey and Nioro, the contribution of N_2 fixation (Ndfa) to N nutrition of plants was very high at Djibelor, ranging from 76% for ILG58 to 95% for ILG61.

Improvement Test of the N_2 fixation of G. sepium *at Nioro*

The low levels of Ndfa observed at Nioro prompted us to define the best way to improve the N_2 fixation. Water and minera1 nutrients such as P are the main limiting factors of the

Table 3
Total N content (%), $\delta^{15}N$ (parts per thousand), and Ndfa (%) of plants

Species	N (%)	$\delta^{15}N$	Ndfa (%)
	Bambey site		
Nonnodulating legumes			
Cassia siamea	22.2 ± 0.12	4.29 ± 0.11	N:D
Cassia sclerosperma var1	1.58 ± 0.04	4.84 ± 0.09	ND
Cassia sclerosperma var2	1.58 ± 0.05	4.73 ± 0.10	ND
Cassia sclerosperma var3	1.62 ± 0.03	4.66 ± 0.18	ND
Bauhinia rufescens	1.81 ± 0.08	6.03 ± 0.42	NID
Nonlegumes			
Azadirachta indica	1.82 ± 0.05	4.95 ± 0.25	NID
Mean for reference plants retained"	1.81	5.49	NID
Nodulating legumes			
Gliricidia sepium	3.93 ± 0.07	4.29 ± 0.27	1'7
Hardwickia binata	1.66 ± 0.03	3.90 ± 0.18	22
Prosopis cineraria	2.09 ± 0.05	3.97 ± 0.14	21
Mean for nodulating plants	2.56	4.05	20
	Nioro site		
Nonnodulating legumes			
Piliostigma reticulatum	1.49 ± 0.06	4.73 ± 0.16	ND
Cassia siamea	2.60 ± 0.09	4.58 ± 0.04	ND
Nonlegumes			
Azadirachta indica	2.99 ± 0.11	6.16 ± 0.59	ND
Guiera senegalensis	1.29 ± 0.04	4.99 ± 0.49	ND
Mean for reference plants retained[b]	2.34	5.57	ND
Nodulating legumes			
Acacia holosericea	1.52 ± 0.03	2.74 ± 0.09	39
Prosopis cineraria	'2.10 ± 0.06	4.01 ± 0.10	211
Gliricidia sepium ILG50	3.96 ± 0.06	5.11 ± 0.09	6
Gliricidia sepium ILG55	3.65 ± 0.12	5.39 ± 0.04	2
Gliricidia sepium HYB	3.68 ± 0.13	5.48 ± 0.16	1l
Hardwickia binata	1.46 ± 0.05	5.66 ± 0.04	0
Leucaena leucocephala	3.82± 0.10	5.69 ± 0.12	0
Mean for nodulating plants	2.89	4.87	10
	Djibélor site		
Cassia siamea	3.22 ± 0.12	+5.57 ± 0.10	ND
Azadirachta indica	2.45 ± 0.09	+8.22 ± 0.43	ND
Mean for reference plants! retained[c]	2.83	+6.89	ND
G. sepium ILG 50	4.90 ± 0.15	-0.55 ± 0.11	87
G. sepium ILG 52	5.06 ± 0.19	-0.08 ± 0.23	81
G. sepium ILG 54	4.69 ± 0.13	+0.27 ± 0.29	77

Table 3 (*Continued*)

Total N content (%), $\delta^{15}N$ (parts per thousand), and Ndfa (%) of plants

Species	N (%)	$\delta^{15}N$	Ndfa (%)
G. sepium ILG 55	5.45 ± 0.20	-0.55 ± 0.11	87
G. sepium ILG 56	4.99 ± 0.22	-0.75 ± 0.22	89
G. sepium ILG 57	4.68 ± 0.11	-0.54 ± 0.09	86
G. sepium ILG 58	4.61 ± 0.12	+0.34 ± 0.01	76
G. sepium ILG 59	4.84 ± 0.28	-0.34 ± 0.12	84
G. sepium ILG 60	4.94 ± 0.09	-0.41 ± 0.26	85
G. sepium ILG 61	4.84 ± 0.13	-1.30 ± 0.12	95
G. sepium ILG 62	4.34 ± 0.08	+0.27 ± 0.14	77
G. sepium ILG 63	4.40 ± 0.09	-0.07 ± 0.07	81
G. sepium HYB	4.44 ± 0.12	+o.oo ± 0.09	80
Mean for nodulating plants	4.78	-0.20	83

Note. Each value is the mean of four replicates. Numbers following ± are standard deviation from mean. ND, not determined.

[a]Mean of δ^{15}Nref of *B. rufescens* and *A. indica.*

[b]Mean of δ^{15}Nref of *G. senegalensis* and ***A. indica.***

[c]Mean of δ^{15}Nref of *C. siamea* and *A. indica.*

N_2 fixation in this zone. This was why we have cultivated G. ***sepium*** in plastic bags under greenhouse conditions by providing water and mineral nutrients (except N). Two treatments-Tl (G. sepium growing on Nioro soil where tested trees have been planted) and T2 (G. ***sepium*** inoculated with a ***Bradyrhizobium*** strain TAL5 and growing on Nioro soil)—were compared in a completely randomized design with four replicates. The results obtained after three months of cultivation (Table 4) showed a higher $\delta^{15}N$ value for G. ***sepium*** than those obtained in situ (Table 3). It can be deduced from these test results that water and mineral elements (N excluded) were not the limiting factors of N_2 fixation at Nioro. The major limiting factor was probably a deficiency of the symbiosis due to absence of specific native strains of ***Bradyrhizobium*** or to other constraints different from lack of water or minera1 elements. Inoculation with an effective strain of ***Bradyrhizobium***

Table 4

Inoculation effect of the ***Bradyrhizobium*** TAL5 strain on dry matter (g plant-‘) and $\delta^{15}N$ (parts per thousand) of ***Gliricidia sepium***

Treatment	Dry weight (g plant-‘)		$\delta^{15}N$	
	Stems + leaves	Nodules	Stems + leaves	Nodules
T1: G. ***sepium*** +Nioro soil	0.53 ± 0.08	nil	9.09 ± 2.59	nil
T2: ***G. sepium*** + Nioro soil + TAL5 inoculum	4.87 ± 0.96	0.61 ± 0.24	-1.99 ± 0.08	12.69

Note. Each value is the mean of four replicates. Numbers following ± are standard deviation from mean.

such as TAL5, which should be tested under field conditions, is a possible improvement technique of the N_2 fixation (Ndfa).

Discussion and Conclusion

The validity of the estimation of the N_2 fixation based on the ^{15}N technique lies in the choice of the reference plant, which is generally considered the main source of error (Fried et al., 1983). Its choice is particularly difficult for NFTs for which nonnodulating lines are not yet available. *Cassia siamea* (Lam.) and *Eucalyptus grandis* Hill ex Maiden have already been used as reference plants for estimating the N_2 fixation of *Leucaena leucocephala* (Lam.) de Wit. and *Acacia albida* (L.) syn. *Fuidherbia albida* (Del.) A. Chev. (Sanginga et al., 1990). Another difficulty of the $\delta^{15}N$ method is the requirement that reference plants be very close to NFTs. To reduce the possible errors in choosing the reference plants, Domenach et al. (1989) have proposed an average $\delta^{15}N$ value of several reference plants (rather than one) growing in the same environment as that of NFTs. In the present study, the average of the two closest $\delta^{15}Nref$ values to that of the soil was used at each site. Our results showed that the $\delta^{15}N$ values of the soil were high enough (>4 parts per thousand) and homogeneous enough within a site to allow the utilization of the $\delta^{15}N$ method (Peoples et al., 1988).

At Bambey, the $\delta^{15}Nref$ (particularly that of *C. siamea*) was lower than that of the soil, but $\delta^{15}Nref$ values found at Nioro and Djibelor were very close to or identical to those of soils. These $\delta^{15}Nref$ increased with an ecological "north–south" gradient (Bambey, +5.49; Nioro, +5.57; Djibelor, +6.89).

The percentage of fixed N_2 was very low at Bambey (Ndfa $\leq$ 22%) and Nioro (Ndfa $\leq$ 39%) and very high at Djibelor (76% $\leq$ Ndfa 195%).

Based on the results of the experiment on Nioro soil under controlled conditions, we advanced the hypothesis of lack of N_2 fixation due to the absence of *Brudyrhizobium* strains or the inability of these strains to express themselves in the Nioro environment. In contrast, specific strains of *Bradyrhizobium* were present and were able to efficiently fix N_2 at Djibelor. Therefore, we obviously showed that the two zones (Sudano-Sahelian and sub-Guinean) are strongly contrasting in respect to N_2 fixation. In the Sudano-Sahelian zone, the main constraints are the low clay content of soils ($\leq$7%), and low rainfall ($\leq$600 mm of precipitation per year) over a duration not exceeding 4 months, and secondarily high concentration of minera1 N in the soil solution induced by marked drying and rewetting cycles (Blondel, 1971; Sprent, 1976). These environmental conditions are not favorable to the establishment and expression of *Bradyrhizobium* strains because the nodulation of host plant is insufficient and inadlequately repeated on time for developing and maintaining specific population of native strains in the soil. However, these strains can be present deep in the soil and form effective nodules (Felker, 1986; Virginia et al., 1986; Dupuy & Dreyfus, 1992). In contrast, under a more humid climate like that at Djibelor ($\geq$1 000 mm of precipitation per year), abundant nodulation is observed (Dupuy & Dreyfus, 1992), thus favoring the buildup of efficient *Bradyrhizobium* strains when nodulations are repeated. The findings of Ducousso et al. (1995) are in agreement with our explanation. Working in *Acacia albida*, they have shown that most *Bradyrhizobium* strains isolated from khe Sahelian zone are ineffective. They further demonstmted that the proliferation of *Bradyrhizobium* populations is strongly dependent on the durable presence of the host tree. The low values of %Ndfa found in the Bambey and Nioro sites showed that NFTs evaluated in these zones were more dependent on soil N than on the N_2 fixation, because of major constraints affecting N_2 fixation. To fix N_2, Ieguminous trees need at least 700 mm of rainfal 1.

The $\delta^{15}N$ values observed in C. *siamea* (+4.29 at Bambey, +4.58 at Nioro, and +5.57 at Djibelor) were very close to those of the $\delta^{15}N$ of NFTs reputed to fix N_2 (Allen & Allen, 1981). Nevertheless, the hypothesis of N_2 fixation by C. *siamea* cannot be confirmed due to a number of reasons, including morphological factors (immobilization of *Rhizobium* at the absorbent root hair base, reduced cortex) and biochemical factors (production of antibacterial compounds, phenolic compounds, tannins and quinones in root cells) that militate against the nodule formation on C. *siamea* roots (Allen & Allen, 198 1). Based on our results, no valuable hypothesis can be formulated to explain the low $\delta^{15}N$ values in C. *siamea.* This induces interesting fields of investigation in order to find causes and determining factors of this phenomenon.

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