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Estimates of biological nitrogen fixation by *Pterocarpus lucens* in a semi arid natural forest park in Senegal using ^{15}N natural abundance method

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Nitrogen (N_2) fixation by *Pterocarpus lucens* in a natural semi arid ecosystem, in Ferlo, Senegal was estimated using ^{15}N natural abundance ($\partial^{15}N$) procedure. Other non-fixing trees accompanying *P. lucens* in the same area were also investigated as control. Results showed an important variation of $\partial^{15}N$ in leaves between the nitrogen-fixing tree (*P. lucens*) and reference plants, whereas no significant differences were recorded in amount of nitrogen (%). The relative $\partial^{15}N$ values (‰) were higher in non-fixing plants than in fixing plants considered. Calculations of %Ndfa gave rates ranging between 26% and 49%. The values of %Ndfa depend largely on soil and the reference plants. The contribution of nitrogen derived from fixation in leaves reached 28.9 kg.N/ha and 10.8 kg.N/ha in ferruginous and in sandy soil respectively.

Key words: *Pterocarpus lucens*, ^{15}N natural abundance, semi arid lowland, biological nitrogen fixation, natural ecosystem.

INTRODUCTION

The Leguminosae is one of the largest and most widely distributed families of flowering plants. Legumes habitats range from rain forests to arid zones throughout the world. These trees are well represented in Savannah and arid regions, where they grow in soils of low fertility unsuitable for most tree species. This dominance of leguminous trees reflects an ecological significance due to their capacity for biological nitrogen fixation. Through symbiosis with root nodule bacteria, legumes play a major role in the functioning of many ecosystems, from natural woodlands to agroforestry systems. Several reports have demonstrated the attractive role of nitrogen-fixing trees as sources of nitrogen and organic matter, and their potential in soil fertility improvement and sustainability (Danso et al., 1992; Sanginga, 1990).

Quantification of nitrogen from biological nitrogen fixation is a question of considerable relevance. This led to a demand for techniques to assess the contribution of biological nitrogen fixation to trees and shrubs ecosystems. Until now, most of the work undertaken on the evaluation of nitrogen (N_2) fixation by tree species has been performed in the framework of short-term experiments where seedlings were grown in containers (Ndoye et al., 1995; Sutherland et al., 2000; Sylla et al., 1998) or in field (Gueye and Ndoye, 2000). The data obtained from such experiments provide an estimation of the N_2-fixing potential in juvenile stage. Moreover, many environmental factors do interact with N_2 fixation of the trees from the juvenile to the mature stage in natural ecosystems. The N_2 fixation capacity of legume trees is often use to explain their ability to grow better than non-legume trees on nitrogen-depleted soils and to restore the fertility of such soils. Very few studies attempting to evaluate that potential in natural conditions are actually available, regardless of the estimation method used. The most promising technique, to quantify trees N_2 fixation contribution in natural ecosystems, is the "^{15}N natural

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abundance". It reflects the biological nitrogen fixation for long period for woody perennial plants in natural ecosystem (Boddey et al., 2000). This method does not require addition of costly ^{15}N fertilizers and it avoid disturbance of soil that often occurs when ^{15}N fertilizer is incorporated. Nitrogen used by higher plants for their growth comes from several sources. The $\partial\ ^{15}N$ values in the source nitrogen are reflected in $\partial\ ^{15}N$ values in whole tissues or individual metabolites in plants. The $\partial\ ^{15}N$ values of soil nitrogen are usually higher than those of atmospheric N_2, which is used as the standard to express the natural abundance in the samples using the following equation by permil (‰) deviation.

$$\partial^{15}N = [((^{15}N / ^{14}N \text{ sample}) : (^{15}N / ^{14}N \text{ standard})) - 1] \text{ X } 1000$$

The ^{15}N / ^{14}N ratio of atmospheric N_2 shows very small variation in the world (Mariotti , 1983; Yoneyama et al., 1987).

Plants that depend on soil nitrogen have positive $\partial^{15}N$ values, which is rather close to those of soil nitrogen. In temperate and cooler areas, however, some plants show very negative $\partial^{15}N$ values (Domenach et al., 1989; Virginia et al., 1989). In legumes, large fractions of plant nitrogen are derived from N_2, arising from biological nitrogen fixation. Therefore, the $\partial^{15}N$ values of plants grown in soils where nitrogen of positive $\partial^{15}N$ values is available, is diluted when these plants fix nitrogen using biological nitrogen fixation method. Significant success has been obtained when using the ^{15}N natural abundance in several studies with N_2-fixing trees including legumes (Hamilton et al., 1993; Ladha et al., 1993; Muofhe and Dakora, 1999; Peoples et al., 1997; Shearer and Kohl, 1986; Yoneyama et al., 1990, 1993) and actinorhizal trees (Domenach et al., 1989; Kurdali et al., 1990; Mariotti et al.1992).

Pterocarpus lucens is a small tree species of the family of Leguminosae, subfamily Fabacea. In Senegal, populations of *P. lucens* occupy a dominant part of ecosystems in the natural semi arid lowland of Ferlo. In this area, population of *Pterocarpus* has been studied in terms of structural position and density (Fredericksen and Lawesson, 1992). We have earlier reported that *P. lucens* is often spontaneously nodulated by rhizobia in its native environment in Senegal (Sylla, 1996). However the contribution of nitrogen fixation towards the nitrogen requirements of this species *in situ* has never been determined. In the present study, we investigated the variation in ^{15}N natural abundance between perennial woody plants and assessed the contribution of symbiotic nitrogen fixation by *P. lucens* in both sandy and ferruginous soil sites in Ferlo, Senegal.

MATERIALS AND METHODS

Sites of Investigation

The amount of nitrogen fixed by *P. lucens* in a forest park, Ferlo located in the sahelian region (15°-16°N; 14°-15°W) in Senegal was estimated. The annual rainfall means range is 400 mm and the temperatures vary slightly around 27°C. In this natural ecosystem, Fredericksen and Lawesson (1992) inventoried all woody plants higher than 1 m with a diameter ≥ 10 cm. A description of tree species, height, density and cover were reported, while phytogeographical affinities and morphopedological types were established. This study concerns two contrasted sites characterized by the vegetation type 4 and type 5 (Fredericksen and Lawesson, 1992). Site 1 is characterized by red ferruginous tropical soils, commonly called laterite. The vegetation type in this site includes a very high density of *P. lucens* with a significant presence of *Anogeissus leiocarpus*, *Balanites aegyptiaca, Combretum glutinosum*, and *C. aculeatum*. In site 2, the soil is sandy and the vegetation is dominated by *P. lucens* and *B. aegyptiaca*.

Field sampling

Sampling of leaves was done in November 1999 when *Pterocarpus* trees and most of the woody species are still bearing a maximum of foliage, and fructification is just beginning. Occurrence of root nodules has been previously checked in the surrounding soil at 15 cm depth for *P. lucens* trees. Because of the big size of trees and difficulty in whole plant sampling, the idea of sampling representative and more accessible plant organs were adopted. Sampling was limited to foliage and only young fully expanded leaves were collected. Leaves were obtained from the top of the trees in the upper canopy. Reference species were selected among the non-legume woody species occurring in the same area with *P. lucens*. These non-legume plants were *A. leiocarpus*, *C. acculeatum*, and C. *glutinosum* in site 1 and *B. aegyptiaca* in site 2. Ten individual samples from each tree species of both sites were randomly collected. The leaves from the same individual tree were pooled to form a single sample.

Sample analysis

The leaf samples were oven-dried at 60° to 70°C for 48h, ground into fine powder and packed. Analysis of

powdered samples was performed in stable isotope facility at the Department of Agronomy and Range Science, University of California Davis, USA. ^{15}N /^{14}N isotope ratio and total nitrogen content were determined using an automated nitrogen analyzer coupled to a mass spectrometer (EA-IRMS, PDZ Europe LTD, CW1 6ZA, UK). Results are reported in percent nitrogen in leaf (N%) and $\partial^{15}N$ ‰. The % Ndfa (%N derived from atmosphere) was calculated according to Shearer and Khol (1986) as follows:

$$\% \text{ Ndfa} = \frac{[\partial^{15}\text{N non fixing plant} - \partial^{15}\text{N fixing plant}]}{[\partial^{15}\text{N non fixing plant} - ß]} \text{ X } 100$$

$$‰\ \partial^{15}N = \frac{1000\ (\text{atom}\%^{15}\text{N sample} - \text{atom}\%^{15}\text{N standard})}{\text{atom}\%^{15}\text{N standard}}$$

where the standard is atmospheric N_2.

The %Ndfa of *P. lucens* was calculated using the ‰ $\partial^{15}N$ mean value of each non-fixing tree species collected at the sites. The assumptions 1, 2, 3 correspond respectively to the calculations of %Ndfa of *P. lucens* in ferruginous soil with ‰ $\partial^{15}N$ values of *C. glutinosum*, *A. leiocarpus, C. aculeatum*, respectively. Assumption 4 corresponds to the calculations of %Ndfa of *P. lucens* in ferruginous soil using the average ‰ $\partial^{15}N$ of assumption 1 to 3. This process allowed integrating potential heterogeneity on ^{15}N uptake on the reference trees (IAEA Tec-doc, 1983). In sandy soil, the %Ndfa of *P. lucens*, was calculated, with the ‰ $\partial^{15}N$ mean value of *B. aegyptiaca* which was the only non fixing woody plant accompanying *P. lucens* in the site.

The fractionation factor ß equivalent to 0.8% represents the $\partial\ ^{15}N$ value of N_2 - fixing plants (*P. lucens*) when their only source of nitrogen is atmospheric N_2. The value of ß was determined in a separate experiment according to Ledgard (1989) and Unkovich et al. (1994). Seedlings of *P. lucens* were grown in hydroponical condition with nitrogen free nutrient solution (Vincent, 1970). Plants were immediately inoculated (10ml/pot) with a liquid inoculum (containing 10^9 cells/ml) of *Rhizobium* ORS 206, an effective slow growing strain isolated from *P. erinaceus* in Senegal (Sylla, 1996).

Plant biomass estimates

Plant leaf biomass (P) was assessed from diameter at breast height (dbh) according to the allometric equation where $P = k.dbh^a$ (Brown, 1997; Lescure et al., 1983; Puig et al., 1990).

With k = 0.00873 and a = 2.1360 for leaf biomass and k = 0.05635 and a = 2.7248 for total biomass.

Statistical analysis

Data were analyzed statistically. Separate one-way analysis of variance was performed. Means and standard deviation were calculated at the $P \leq 0.05$ levels.

Table 1: Total leaf nitrogen content %N, leaf natural ^{15}N abundance ($\partial^{15}N$ ‰) and estimates of the contribution of fixed-N_2 in *P. lucens* and non-fixing tree species in a ferruginous soil and in a sandy soil of Ferlo, Senegal.

Soil type	Plant species	N %	$\partial^{15}N$ ‰	%Ndfa			
Ferruginous soil				Assumption 1	Assumption 2	Assumption 3	Assumption 4
	P. lucens	2.18 ± 0.11	4.06 ± 0.57	35.25 ± 11	41.95 ± 10.2	49.43 ± 8.9	44.23 ± 9.77
	C. glutinosum	2.27 ± 0.36	5.83 ± 0.47	-	-	-	-
	A. leiocarpus	2.16 ± 0.28	6.51 ± 0.37	-	-	-	-
	C. aculeatum	1.98 ± 0.27	7.1 ± 0.66	-	-	-	-
	Non-fixing trees	2.32 ± 0.25	6.65 ± 1.33	-	-	-	-
Sandy soil							
	P. lucens	2.17 ± 0.25	5.54 ± 0.26	-	-	-	26.32 ± 5.91
	B. aegytiaca	2.57 ± 0.31	7.24 ± 0.37	-	-	-	-

Means values are followed by standard deviation (SD).

RESULTS

The %N and ‰ ∂^{15}N values of *P. lucens* leaves and that of different reference woody plants used are shown in Table 1. The %N in leaves does not seem to vary between the different woody species occurring in the two soil types. The ‰ ∂^{15}N in the leaves were significantly different between the woody non-fixing plants. In the ferruginous soil and sandy soil sites, the ‰ ∂^{15}N values of *P. lucens* were 4.06±0.57 and 5.54±0.26 respectively. These values are lower than those of neighboring non-fixing tree species (average value of 6.65±1.33 and 7.24±0.37, respectively). This indicates occurrence of N_2 fixation by *P. lucens* in both sites. *P. lucens* showed significant differences in ∂^{15}N between ferruginous soil and sandy soil. The %Ndfa values ranged from 35 to 49% for ferruginous soil under the assumptions 1, 2, 3 and 4 (Table 1). These values are higher compared to those for the sandy soil, 26% using assumption 4. In the ferruginous soil %Ndfa values were obtained by taking into account each reference plant ‰ ∂^{15}N (assumptions 1, 2 and 3) and the average of these values (assumption 4). In the sandy soil, only one species *B. aegytiaca* was used as reference plant. The standard deviation of the %Ndfa ranged from 6 to 11% for *P. lucens* (Table 1). These values are comparable to the 5-10% described in previous studies (Shearer and Kohl, 1986).

Leaf biomass per plant and leaf biomass per hectare (Table 2) were estimated according to the allometric equation of Lescure et al. (1983). The leaf biomass per hectare found for *P. lucens* was 3.000 t/ha and 2.286 t/ha in ferruginous and sandy soils, respectively. *P. lucens* roots were checked for nodulation, and root nodules were observed only on those growing in the ferruginous soil; no nodule was recorded in *P. lucens* growing in sandy soil at 15 cm depth.

Table 2: Leaf biomass and density of trees in a ferruginous soil and in a sandy soil in Ferlo, Senegal.

	Diameter (cm)	Leaf biomass plant^{-1} (kg)**	Plants dens ha^{-1}	Leaf biomass ha^{-1} (t)
Ferruginous soil	16.91*	2.496	1202	3.000
Sandy soil	15.04*	1.974	1158	2.286

*Datas are based on literature (Lawesson, 1990; Fredericksen and Lawesson, 1992).
**Leaf biomass values were estimated according to the allometric equation of Lescure et al. (1983) and Puig et al. (1990).

The average amount of nitrogen accumulated in leaves was 0.054 kg N/plant and 0.043 kg N/plant for *P. lucens* in the ferruginous and sandy soils, respectively (Table 3). Taking together all the *P. lucens* plants in this study, the amount of nitrogen contained in leaves was 66.65 kg N/ha and 49.66 kg N/ha in the ferruginous and sandy soils, respectively (Table 3). Significant differences in Ndfa were recorded when different species were used as reference trees (Table 3). In the ferruginous soil, the Ndfa value varies from 23 to 32 kg N/ha, depending on the reference plant. In the sandy soil, where only one reference plant was used, the Ndfa was 13 kg.

DISCUSSIONS

The study was undertaken in a natural park in Senegal, where *Pterocarpus* exploitation is prohibited. No data are available in literature on the values of leaf biomass of adult *P. lucens* trees in their natural habit. To avoid tree destruction, our method of estimation of tree leaves biomass was similar to those of Lescure et al. (1983) and Puig et al. (1990). Other authors have also used this method to estimate total leaf and or total above ground biomass of trees in tropical forest ecosystems (Roggy et al., 1999; Scatena et al., 1993). The amount of leaf biomass in their studies ranges from 3.7 to 6.4 t/ha. In our study, which was conducted in a tropical semi-arid ecosystem, values of leaf biomass were about 3.0 t/ha. Due to the non-variation of the average values of diameter of woody vegetation on both sites of this study (Lawesson, 1990), results obtained by using allometric equation showed uniform values of leaf biomass per plant within each site. Therefore, the leaf biomass per hectare changes with the plant density per hectare.

Many authors recommend that if possible entire plants or at least whole shoots should be sampled to assess overall ^{15}N abundance for estimating biological nitrogen fixation. With trees, sampling entire plant is usually extremely difficult. And from the limited data available, variation between tree plant organs may often be less than that observed in annual legume species. Ladha et al. (1993) found that there were no significant differences in ^{15}N abundance of trunk, branch and leaves taken from nodulated *Gliricidia sepium* or from *Senna spectabilis*. Data indicates that in many nitrogen-fixing trees (both greenhouse and in-field studies), %Ndfa did not differ significantly between different plant parts and the whole tree (Danso et al., 1995; Gueye et al., 1997; Sylla et al., 1998). Leaves are the most easily accessible part of tree plants. Consequently, we

Table 3: Assessment of leaf nitrogen per plant and contribution of fixed-N_2 by *P. lucens* in a ferruginous soil and in a sandy soil of Ferlo, Senegal.

Soil type	Total N (kg)		Ndfa (kg)							
			Assumption 1		Assumption 2		Assumption 3		Assumption 4	
	plant^{-1}	hectar^{-1}	plant^{-1}	hectar^{-1}	plant^{-1}	hectar^{-1}	plant^{-1}	hectar^{-1}	plant^{-1}	hectar^{-1}
Ferruginous	0.054±0.02	66.65±3.22	0.019±0.06	23.0±7.1	0.023±0.05	27.4±6.3	0.027±0.04	32.4±5.4	0.024±0.05	28.9±6.0
Sandy soil	0.043±0.02	49.66±2.84	-	-	-	-	-	-	0.011±0.03	12.7±2.7

Means values are followed by standard deviation (SD).

measured the %Ndfa in leaves to estimate the contribution of *P. lucens* adult trees in their natural ecosystem.

The reference plant is unquestionably the 'Achilles' heel' of the methodology within the context of isotopic non-uniformity. According to Danso et al. (1992), the so-called 'selection criteria' do not permit identification of a single valid reference plant. We adopted the strategy which used several reference plants as advocated by a number of authors (Bremer et al., 1983; Boddey et al., 1995, 2000).

Root nodules occur more often in ferruginous soil conditions than in sandy soil conditions in *P. lucens*. Moreira et al. (1992, 1994) mentioned similar observation in the frequency of leguminous tree root nodules between the oxisols and the spodsols in Brazilian primary rain forest. Our observation is also comparable to the N_2 fixation variability of some nitrogen-fixing tree species reported among habitats in Sonorian desert and Tanzania (Högberg, 1986; Shearer et al., 1983). Johnson and Mayeux (1990) postulated that, in a number of sites, nodules cannot be detected in some nitrogen-fixing tree roots even when compatible rhizobia are present in the soil because they decomposed too rapidly, are too small to be detected, or are located at 5-10 m depths. In the case of *Pterocarpus* trees in Ferlo (Senegal), these hypotheses require further investigation.

The fractionation factor, ß for *P. lucens* was 0.8‰. This value appear to range between -2.0 and + 1.0 ‰, for most tree legume species (Boddey et al., 2000). Indeed estimates may vary between and within plant species. Significant differences in ∂^{15}N values were obtained for the different reference trees. Likewise, we observed differences in ∂^{15}N for *P. lucens* in the ferruginous and sandy soils. The ∂^{15}N value of plant-available soil nitrogen, which is inferred by that of non-fixing (reference) plants, may differ depending on soils, soil profiles where roots are developing (Ledgard, 1989; Virginia et al., 1989), and the forms of nitrogen available to plants (Yoneyama et al., 1990). We did not measure the exact rooting depth of the different species. The increase in ∂^{15}N in the sandy soil could indicate that there was a considerable loss of nitrogen from the soil.

Among the plants investigated, *B. aegyptiaca* in sandy soil and *C. aculeatum* in ferruginous soil have the highest ∂^{15}N values (7.24 ‰ and 7.1 ‰) in their leaves. *P. lucens* (the fixing plant), possessed the lowest values of ∂^{15}N in leaves (4.06 ‰, in ferruginous soil and 5.54 ‰ in sandy soil). This suggest that *P. lucens* is less dependant on soil nitrogen nutrient than the non-leguminous trees, due to the potentialities of this tree species to fix nitrogen in symbiosis with rhizobia. In natural condition, as in the semi arid zone of Ferlo, the calculation of nitrogen derived from atmosphere in *P. lucens* tree leaves indicate that Ndfa may represent 35-49 % of total nitrogen accumulated. Using the soil ^{15}N labeling method in a greenhouse, we found Ndfa of 35% in the whole plant of *P. erinaceus* and *P. lucens* at the juvenile stage, in earlier work (Sylla et al., 1998). As previously reported by Högberg (1990) and Shearer and Kohl (1986), the %Ndfa values calculated depends on the reference plant. The estimated %Ndfa for *P. lucens* is 44.23% in ferruginous soil and 26.32% in sandy soil. Schulze et al. (1991) estimated %Ndfa ranging from 10 to 30% in woody *Mimosaceae* species along an aridity gradient in Namibia. The % Ndfa obtained for *P. lucens* in ferruginous soil exceeded most of what is reported for other N_2-fixing species occurring in tropical regions. Shearer et al. (1983) reported that N_2 fixation might contribute 43-61% to the nitrogen content for *Prosopis* under drastic conditions of very low mineral fertility. In Tanzania 44% of the nitrogen content of potentially N_2-fixing species originated from N_2 fixation (Högberg,

1986). Coté and Camire (1984) estimated fixed- N_2 to be 53 kg N/ha in both mixed and pure stands of alder grown in Canada. Under field conditions, Sougoufara et al. (1990) estimated fixed nitrogen to be 40-60 kg N/ha for *Casuarina equisetifolia*. Our results compare very well with these reports. Roggy et al. (1999), however, found very low estimates (7 kg ha-1) for tree species in a rain forest in French Guyana.

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