Tree nitrogen fixation in a tropical dry vegetation in Northeast Brazil

Ana Dolores Santiago de Freitas[A]**,** Leonardo Queiroz de Souza[B], Everardo Valadares de Sá Barretto Sampaio[B], Patrícia Maia Moura[B] and Rômulo Simões Cezar Menezes[B]

[A]Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros, s/n, Recife, PE, Brazil, 52171-900, E-mail: ana.freitas@depa.ufrpe.br
[B]Departamento de Energia Nuclear, Universidade Federal de Pernambuco, Av. Prof Luís Freire, 1000, Recife, PE, E-mail: leoqueiroz28@yahoo.com.br , esampaio@ufpe.br , patriciamaia5@hotmail.com ,rmenezes@ufpe.br

Abstract
Quantification of symbiotic nitrogen fixation is scarce, especially in tropical dry forests. We estimated the amounts of N fixed annually, measuring the proportions of fixed N (%Ndda) and the amounts of N in the leaf biomass of tree legumes in one area of mature caatinga in Northeast Brazil. The %Ndda was calculated comparing ^{15}N concentrations of legume and reference species. Leaf biomass was estimated using allometric equations based on the stem diameters at breast height. *Mimosa tenuiflora*, *Piptadenia stipulacea* and *Anadenanthera colubrina* had large proportions of their N derived from atmospheric N_2. The average sizes of the plants of the fixing species were not very large and the leaf biomasses corresponded to 5 to 10% of the total aboveground biomasses. The N content in the leaves was higher in legumes than in the non legume species. The low plant density and the low leaf biomasses of the fixing species contributed the most to the relatively low amounts of fixed N in the leaves (10.6 to 15.1 kg ha^{-1}). Although low, they are almost twice the amounts estimated for tropical rain forest and a nearby caatinga. Their accumulated inputs, along the years, are crucial to the nutrient balance of the systems.

Key Words
Biological nitrogen fixation, *Mimosa tenuiflora*, *Piptadenia stipulacea*, *Anadenanthera colubrina*, 15-N, caatinga

Introduction
Symbiotic nitrogen fixation is the main process of N entry in natural ecosystems and one of the most significant ecological processes (Cleveland *et al*., 1999). In spite of this importance, data on its quantification is rather scarce, especially in tropical vegetations. A suitable method to determine fixation in trees is the major limitation. The most reliable method, based on ^{15}N abundance, requires a significant difference between signals of legume and reference plants (Boddey *et al*., 200;, Högberg, 1997). This condition is not found in many vegetation systems (Roggy *et al*., 1999; Gehring and Vlek, 2004; Handley *et al*., 1994) but it is found in Brazilian tropical dry forests (caatinga) (Freitas *et al*., 2010), which covers about one million square kilometers in Northeast Brazil (Sampaio, 1995).

Based on the ^{15} N abundance method, the proportions of symbiotically fixed nitrogen to the total plant N have been determined for several tropical dry forest species, under different environmental conditions, in Africa (Sclulze *et al*., 1991; Ndiaye and Ganry, 1997) and America (Shearer *et al*., 1983). To estimate the amounts of N fixed in the plants, these proportions must be coupled with determinations of their amounts of produced N. This poses some difficult in evergreen forests but it is easier on deciduous mature forests, where leaves are renewed every year and compose most of the produced biomass (Machado *et al*., 1997). To estimate the N amounts fixed in a certain area, the quantities of N in all fixing legume trees in this area must be determined. This is also easier in dry forests than in humid ones because they tend to have fewer species. These integrated measurements have seldom been done and only a few papers have been published with estimates of fixation in native tropical vegetation (Roggy *et al*., 1999; Sylla *et al*., 2002). In spite of being easier to obtain, very few published data were found for tropical dry forests. Considering this lack of information, we estimated the amounts of N fixed annually by the leaves of trees and shrubs in one area of caatinga, in Northeast Brazil.

Material and methods
The study was conducted at Fazenda Tamanduá, Santa Teresinha municipality, Paraíba state, Brazil, around the coordinates 07º00’14" latitude South and 37º20’38" longitude West. Average annual rainfall is 600 mm, with large year to year variation and concentrated in three months, usually March to May. Average annual temperature is 26^{o}C, with little seasonal and daily fluctuations. Soils are Neossolos Litólicos (Leptosols), relatively shallow and of low fertility.

Three areas were chosen in the property, all covered by mature caatinga not disturbed for over 50 years In each area, one 50 x 20 m plot was established. Within each plot, all plants with stem diameter equal to or above 2 cm were marked, located, identified and had their stem diameter measured at breast height (DBH). The total and the leaf biomasses of each plant were estimated using allometric equations developed in a previous study (Sampaio and Silva, 2005). Since all legume species are deciduous, the estimated leaf biomasses were considered equal to the annual leaf biomass productions.

Mature, fully developed leaves of the fixing legume species were collected in each plot, together with leaves of a non-fixing legume (*Caesalpinia pyramidalis* Tul.) and leaves of a non-legume species (*Aspidosperma pyrifolium* Mart.), to be used as reference plants. A maximum of five plants of each species were selected in each plot. A random selection of the five sampled plants was made when more than five of a species occurred in one plot. The leaves were oven dried, ground and analyzed for their N and ^{15}N contents (by mass spectrometry).

The proportion of fixed N in each plant was calculated using the formula (Shearer and Kohl, 1986):

$$\%Ndfa = (\delta^{15}N_{(reference)} - \delta^{15}N_{(diazotrophic)} / \delta^{15}N_{(reference)} - B) \times 100$$

Where $\delta^{15}N_{(reference)}$ is the mean value of the $\delta^{15}N$ of the reference species of each site, $\delta^{15}{}_{(diazotrophic)}$ is the mean $\delta^{15}N$ value for the plants of each specie identified as diazotrophic and B is the $\delta^{15}N$ value for fixing plants cultivated in the absence of a mineral N supply. Due to the high $\delta^{15}N$ values found for non fixing plants of the caatinga and methodological complications for estimating this value in arboreal species (Högberg, 1997; Boddey *et al*., 2000), the B values in this work were not estimated. However, according to the suggestion by Högberg (1997), the importance of using extreme B values in the %Ndfa calculations was tested. With the absence of data for the studied species, values of 0‰ and -2‰ were used which are commonly found in studies of tree legumes (Schulze *et al*., 1991; Raddad *et al*., 2005; Roggy *et al*., 1999).

The quantity of fixed N in the leaves was estimated multiplying the leaf biomass of each plant in one plot by the average of the proportion of fixation of the species in the plot.

Results and discussion

The ‰ $\delta^{15}N$ in the leaves were significantly different between the fixing (averages from 1.02 to 1.74‰) and reference plants (4.48 and 4.70‰) (Table 1). All fixing species had large proportions of their N derived from atmospheric N_2. The high capacity of fixation of *Mimosa tenuiflora* (Willd.) Poir. and *Piptadenia stipulacea* (Benth.) Ducke had already been reported, in a nearby site (Freitas *et al*., 2009) but *Anadenanthera colubrina* (Vell.) Brenan plants had shown no fixation in this same nearby site. The proportions of fixation by these species are among the highest already reported for native legume trees in natural vegetation (Schulze *et al*., 1991; Ndiaye and Ganry, 1997; Roggy *et al*., 1999; Sylla *et al*., 2002; Gehring *et al*., 2005).

The average sizes of the plants of the fixing species were not very large (Table 1) but followed the usual pattern of the vegetation in the site, limited by water availability during five to seven months every year. The leaf biomasses corresponded to 5 to 10% of the total aboveground biomasses, within the range already reported for caatinga plants (Silva and Sampaio, 2008).

The N content in the leaves was higher in legumes than in the non legume species (Table 1). The amounts of fixed N in the leaves of *A. colubrina, M. tenuiflora* and *P. stipulacea* reached 12.8 or 18.3 kg ha^{-1}, according to the B value adopted. Among the variables that compose the calculation of fixation of on a plant basis - leaf biomass, N content and proportion of fixed N – only the leaf biomass has low values in relation to other vegetation types. On an area basis, the low plant density and the low leaf biomasses of the fixing species contributed the most to this low amounts of fixed N (Table 1). These amounts are lower than those reported for cultivated legumes (*Mucuna pruriens*), which may reach 87 to 177 kg/ha (Hauser and Nolte, 2001), but almost two times the values estimated by Roggy *et al*. (1999), in a tropical rain forest in Guyana, and by Freitas *et al*. (2009), in a nearby caatinga. Although low, their accumulated inputs, along the years, are crucial to the nutrient balance of the systems.

Table 1, Leaf biomass, leaf N content, N amount, foliarδ^{15}N, nitrogen derived from atmosphere and fixed N in tree species of one area of dry forest (caatinga) in Northeast Brazil.

Species	Leaf biomass	Leaf N content	N amount	δ^{15}N	Ndfa		Fixed N	
					B=0	B=-2	B=0	B=-2
	kg ha^{-1}	%	kg ha^{-1}	‰	%		kg ha^{-1}	
Fixing legumes	738		20.2				15.1	10.6
Anadenanthera colubrina	106	2.72	2.9	1.84	60.4	42.3	1.7	1.2
Mimosa tenuiflora	141	3.24	4.6	1.02	78.0	54.6	3.6	2.5
Piptadenia stipulacea	491	2.70	12.7	1.05	77.5	54.2	9.8	6.9
Non-fixing legumes	1931	2.77	53.5	4.79	0	0	0	0
Other species	2773	2.04	56.7	4.48	0	0	0	0

Conclusion

Mimosa tenuiflora, *Piptadenia stipulacea* and *Anadenanthera colubrina* fixed large proportions of their nitrogen, but their low plant density and their low leaf biomasses contributed to the relatively low amounts of fixed N in the leaves (10.6 to 15.1 kg ha^{-1}). Although low, their accumulated inputs, along the years, are crucial to the nutrient balance of the systems.

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