Nitrogen symbiotically fixed by cowpea and gliricidia in traditional and agroforestry systems under semiarid conditions

Júlio César Rodrigues Martins[1], Ana Dolores Santiago de Freitas[2], Rômulo Simões Cezar Menezes[1] and Everardo Valadares de Sá Barretto Sampaio[1]

[1]Universidade Federal de Pernambuco, Departamento de Energia Nuclear, Avenida Professor Luiz Freire, nº 1.000, Cidade Universitária, CEP 50740-540 Recife, PE, Brazil. E-mail: juliocesar_0407@yahoo.com.br, rmenezes@ufpe.br, esampaio@ufpe.br [2]Universidade Federal Rural de Pernambuco, Departamento de Agronomia, Avenida Dom Manoel de Medeiros, s/nº, Dois Irmãos, CEP 52171-900 Recife, PE, Brazil. E-mail: ana.freitas@depa.ufrpe.br

Abstract – The objective of this work was to estimate the amounts of N fixed by cowpea in a traditional system and by cowpea and gliricidia in an agroforestry system in the Brazilian Northeast semiarid. The experiment was carried out in a randomized complete block design, in a split-plot arrangement, with four replicates, in the semiarid region of the state of Paraíba, Brazil. Plots consisted of agroforestry and traditional systems (no trees), and split-plots of the three crops planted between the tree rows in the agroforestry system. To estimate N fixation, plant samples were collected in the fourth growth cycle of the perennial species and in the fourth planting cycle of the annual species. In the agroforestry system with buffel grass and prickly-pear cactus, gliricidia plants symbiotically fix high proportions of N (>50%) and contribute with higher N amounts (40 kg ha^{-1} in leaves) than in the traditional system (11 kg ha^{-1} in grain and 18 kg ha^{-1} in straw). In the agroforestry system with maize and cowpea, gliricidia plants do not fix nitrogen, and N input is limited to the fixation by cowpea (2.7 kg ha^{-1}), which is lower than in the traditional system due to its lower biomass production.

Index terms: *Gliricidia sepium*, *Vigna unguiculata*, biological nitrogen fixation, natural abundance, ^{15}N.

Nitrogênio fixado simbioticamente por feijão-caupi e gliricídia em sistemas tradicional e agroflorestal sob condições semiáridas

Resumo – O objetivo deste trabalho foi estimar as quantidades de N fixadas em feijão-caupi em sistema tradicional e em feijão-caupi e gliricídia em sistema agroflorestal, sob condições semiáridas do Nordeste brasileiro. O experimento foi conduzido em blocos ao acaso, em arranjo em parcelas subdivididas, com quatro repetições, no semiárido da Paraíba. As parcelas consistiram dos sistemas agroflorestal e tradicional (sem árvores), e as subparcelas, dos três cultivos entre as linhas das árvores, no sistema agroflorestal. Para estimar a fixação de N, amostras das plantas foram coletadas no quarto ciclo de crescimento das espécies perenes e no quarto ciclo de cultivo das espécies anuais. No sistema agroflorestal com capim-buffel e palma forrageira, a gliricídia fixa simbioticamente altas proporções de N (>50%) e adiciona quantidades maiores de N (40 kg ha^{-1} nas folhas) que no sistema tradicional (11 kg ha^{-1} nos grãos e 18 kg ha^{-1} na palhada). No sistema agroflorestal com milho e feijão-caupi, a gliricídia não fixa N e a adição de N é limitada à fixação no feijão-caupi (2,7 kg ha^{-1}), que é menor que no sistema tradicional em razão da sua baixa produção de biomassa.

Termos para indexação: *Gliricidia sepium*, *Vigna unguiculata*, fixação biológica do nitrogênio, abundância natural, ^{15}N.

Introduction

Agroforestry systems with trees or shrub alleys within annual crop fields can be an option to reduce the fragility of certain agricultural production systems (Wick & Tiessen, 2008; Foroughbakhch et al., 2009; Li et al., 2009; Kurppa et al., 2010; Sousa et al., 2010; Lima et al., 2011). Those systems produce a considerable amount of biomass that can be incorporated into the soil, transferring nutrients from the trees to the crops (Bertalot et al., 2010; Munroe & Isaac, 2014). When N_2-fixing legume trees are included in the system, symbiotically fixed N (SFN) can be an additional source of N to the associated crop (Paulino et al., 2009; Munroe & Isaac, 2014).

In arid and semiarid regions, N_2-fixing legume trees frequently obtain more than half of their N from symbiotic fixation (Freitas et al., 2010; Andrews et al.,

DOI: 10.1590/S0100-204X2015000200010

2011; Souza et al., 2012). However, the amounts of fixed N vary much, depending on the specificities of the symbiosis and on the environmental characteristics that affect biomass production and SFN. In Kenya, for instance, from 70 to 90% of the N in the biomass of *Sesbania sesban* and *Calliandra calothyrsus* was derived from SFN, amounting to 120 to 360 kg ha^{-1} N (Stahl et al., 2002), whereas in Sudan, 48% of the N in pure stands of *Acacia senegal* were fixed from the atmosphere (%Ndfa), introducing 36 kg ha^{-1} N per year to the agrosystem (Raddad et al., 2005). Therefore, SFN may represent relevant N inputs to soil-plant systems, decreasing the need of N fertilizer application, which is frequently the most expensive among commercial fertilizers.

In the semiarid region of Northeast Brazil, sole or intercropped maize and cowpea are the main cultures in an itinerant slash-and-burn system of a few years of cultivation followed by 10 to 15 years of fallow (Maia et al., 2007). Livestock is the most important activity, and the cattle feed on crop residues; on native vegetation growing in the fallow areas; on prickly-pear cactus plants, cultivated specifically as a fodder crop; and on pasture planted with introduced African grasses, such as buffel grass (Wick & Tiessen, 2008). Rows of maize and cowpea are sometimes interplanted within the rows of prickly-pear cactus. In recent years, agroforestry systems that combine rows of trees and maize and cowpea have been introduced, in which tree pruning is done shortly after sowing of the annual crops to increase fodder production, while maintaining some grain production (Pérez Marin et al., 2006, 2007). In the long run, this system could be more stable than the itinerant system, considering frequent crop failure due to low and erratic rainfall and the pressure to reduce fallow periods (Martins et al., 2013).

Few studies have been conducted in the Brazilian semiarid area to measure crop and tree productivities under agroforestry systems (Pérez Marin et al., 2006, 2007; Santos et al., 2010). Martins et al. (2013) evaluated the productivities of maize, cowpea, and gliricidia trees and found that the agroforestry system produced the largest amounts of biomass, indicating that it can be a viable system in the region. However, a proper management of the system requires the knowledge of the amounts of N introduced by symbiotic fixation of trees and cowpea, and this information is not available for agroforestry systems tested in the semiarid area of Northeast Brazil.

The objective of this work was to estimate the amounts of N fixed by cowpea in a traditional system and by cowpea and gliricidia in an agroforestry system with planting of maize and cowpea, prickly-pear cactus, or buffel grass.

Materials and Methods

The study was conducted in the municipality of Taperoá, in the state of Paraíba, Brazil (7º12'23"S, 36º49'25"W, at 532 m of altitude). The annual average temperature is 28ºC, with little daily and monthly variations, and the annual average rainfall is 600 mm, with large interannual variations. According to Köppen's classification, the climate is Bsh, semiarid and hot. The soil is a Neossolo Flúvico (Entisol) (Santos et al., 2006), and the top 20 cm layer has 677 g kg^{-1} sand, 147 g kg^{-1} silt, and 176 g kg^{-1} clay. Soil chemical characteristics were determined according to the procedures recommended by Silva (2009): pH in water of 7.3, measured in 2.5 soil:water (v/v); 9.1 g kg^{-1} organic C, obtained by the dichromate humid oxidation method; 6.3 and 0.98 $cmol_c$ kg^{-1} Ca^{+2} and Mg^{+2}, respectively, extracted with KCl and determined by atomic absorption; and 65.8 mg kg^{-1} P, 0.5 $cmol_c$ kg^{-1} K^+, and 0.19 $cmol_c$ kg^{-1} Na^+, extracted with Mehlich-1 and determined by colorimetry and flame photometry.

The experiment was carried out in a randomized complete block design, in a split-plot arrangement, with four replicates. Plot treatments consisted of the presence or absence of trees in the production system, whereas split-plot treatments consisted of three traditional production crops: 1, intercropping of maize (*Zea mays* L.) of the Sergipano variety and of cowpea [*Vigna unguiculata* (L.) Walp.] of the Moitinha variety; 2, perennial buffel grass (*Cenchrus ciliaris* L.) pasture; and 3, forage prickly-pear cactus [*Opuntia ficus-indica* (L.) Mill.]. Each split-plot of the treatment without trees had a 6x10 m area. Maize and cowpea were planted in the same row, with 1 m spacing between rows and 0.25 m spacing between plants; prickly-pear cactus was also planted in rows 1 m apart but with 0.5 m between plants; and buffel grass was planted in rows 0.5 m apart, with 0.1 m between plants. Each split-plot of the treatments with trees had an area of 10x30 m. Five rows of gliricidia [*Gliricidia sepium*

(Jacq.) Kunth], interspaced with *Manihot glaziovii* Mull. Arg., were planted 6 m apart with 1 m between trees, and the production crops were planted in five rows between the tree rows.

The experiment was established at the start of the rainy season of 2006 with the planting of buffel grass seeds, partially burying cladodes of prickly-pear cactus cut from a nearby field and transplanting three-month-old seedlings of gliricidia and *M. glaziovii* previously cultivated in the area. At the time of planting, dry cattle manure was spread over the soil surface in a dose equivalent to 10 Mg ha^{-1}. Maize and cowpea were planted for four years, from 2006 to 2009, always at the beginning of the rainy season, in February or March. Annual rainfall along these years totaled 987, 533, 725, and 756 mm, mostly concentrated from February to May.

Maize and cowpea were harvested every year after the end of the rainy season and their biomass was removed from the plots. The trees were cut at 1.5 m height in 2008 and 2009, one week before planting maize and cowpea, but not in 2007, when the trees were too small. The cut biomass was divided into tips (leaves plus twigs less than 1.5 cm in stem diameter) and wood (larger branches) and also removed from the plots. The forage cactus was only harvested in 2008 because of its long growing cycle, and its biomass was also removed.

To estimate N fixation, plant samples were collected in 2009 when the trees were over three years old and the planting of annual crops was in its fourth cycle. Fully expanded healthy leaves were collected from four plants of the N_2-fixing species (gliricidia and cowpea) and from four plants of one non-fixing reference species in each subplot. *M. glaziovii* was the tree reference species, and the weed *Alternanthera tenella* was the herb reference species. Maize and buffel grass were not taken as reference species because of reports of their possible association with N-fixing microorganisms (Roesch et al., 2007). The leaves of all four plants were pooled together to form a single composite sample of each species in each subplot. The samples were oven dried at 65ºC for 72 hours, ground, and analyzed for total N and ^{15}N concentrations (natural abundance).

N concentrations of leaf samples were determined in 0.25 g of the material digested with sulfuric acid using the standard Kjeldahl procedure (Silva, 2009). N concentration of the larger branches (1.51%) was equal to that determined by Alves et al. (2011) for these plants in the same location. The N concentration of cowpea grains (4.40%) was similar to that found by Arora & Das (1976). N contents were obtained by multiplying N concentration by the biomass. In the case of tips, the biomass of leaves plus twigs was multiplied by the N concentration of leaves. In the case of cowpea, the straw biomass was multiplied by the leaf concentrations.

The natural abundance of ^{15}N was analyzed by mass spectrometry and expressed using delta notation (‰): $\delta = (R_{sample}/R_{standard} - 1)\times 1,000$, in which R_{sample} and $R_{standard}$ are the $^{15}N:^{14}N$ ratios of the sample and the standard, respectively. When the difference between the $\delta^{15}N$ value of N_2-fixing legumes and the average $\delta^{15}N$ value of the reference plants in the subplot was significant ($p\leq 0.05$), the proportion of fixed N was estimated using the following formula (Shearer & Kohl, 1986): $\%Ndfa = [(\delta^{15}N_{(reference)} - \delta^{15}N_{(fixing)})/\delta^{15}N_{(reference)} - B]\times 100$, in which $\delta^{15}N_{(reference)}$ is the average value of the $\delta^{15}N$ of the reference plants (*A. tenella* for cowpea and *M. glaziovii* for gliricidia), $\delta^{15}N_{(fixing)}$ is the $\delta^{15}N$ value of each legume sample, and B is the $\delta^{15}N$ value for fixing plants cultivated in the absence of soil N. The B value for gliricidia was assumed to be -1.45‰ (Boddey et al., 2000), and for cowpea, -1.51‰ (Nguluu et al., 2002). The fixed amounts were obtained by multiplying the total N contents of the legume plants by the %Ndfa values of their samples.

The data on ^{15}N and total N concentration, total and fixed N amounts of gliricidia were subjected to analysis of variance, and the averages were compared by the Tukey test, at 5% probability. Data on cowpea were analyzed using the T test, because there were only two treatments (with and without trees).

Results and Discussion

There was no effect of the production system (traditional or agroforestry) on the ^{15}N concentrations of the herb reference species (*A. tenella*), but the reference tree species (*M. glaziovii*) had a lower signal value when intercropped with maize + cowpea (Table 1). This lower signal can indicate the occurrence of transference of some of the N fixed by cowpea to the associated trees, as reported by Kurppa et al. (2010).

Cowpea plants were isotopically depleted in both production systems in comparison to the reference plants, whereas gliricidia intercropped with maize + cowpea had a higher ^{15}N signal value than *M. glaziovii*. These patterns allowed to estimate the proportions of N derived from symbiotic fixation (%Ndfa) by cowpea in both the traditional and the agroforestry systems; however, those fixed by gliricidia could only be estimated when the tree was intercropped with prickly-pear cactus and buffel grass, since there was no apparent fixation in gliricidia when associated with the maize + cowpea intercropping.

High ^{15}N concentrations in reference species and concentrations several $\delta^{15}N$ (‰) units lower in legume species allow robust estimates of symbiotic fixation (Högberg, 1997). Similar conditions have been observed by other authors in native species of the semiarid area evaluated in the present study (Freitas et al., 2010; Reis Junior et al., 2010). The ^{15}N abundance of the tree species of the agroforestry system intercropped with maize + cowpea indicated no apparent fixation in gliricidia, as previously mentioned. The reason for this absence of fixation is not known and it is intriguing because gliricidia is considered a species with high N_2-fixing capacity (Paulino et al., 2009; Kurppa et al., 2010), and the trees fixed abundantly when intercropped with buffel grass and prickly-pear cactus.

Table 1. ^{15}N concentrations ($\delta^{15}N$) of tree and herb species – gliricidia (*Gliricidia sepium*) and *Manihot glaziovii*, and cowpea (*Vigna unguiculata*) and *Alternanthera tenella*, respectively – and nitrogen fixed from the atmosphere (%Ndfa) by legumes in agroforestry and traditional systems in the semiarid Northeast of Brazil[1].

Species	Agroforestry system/intercrop			Traditional
	Buffel grass	Prickly-pear cactus	Maize + cowpea	Maize + cowpea
	Biomass (kg ha^{-1})[2]			
Gliricidia (tips)	3,938a	2,213b	2,253b	-
Gliricidia (wood)	12,840a	9,767a	14,334a	-
Cowpea (grain)	36a	-	-	410a
Cowpea (straw)	61a	-	-	698a
	$\delta^{15}N$ (‰)			
M. glaziovii	11.98a	13.23a	8.78b	-
Gliricidia	4.95*	5.65*	10.35ns	-
A. tenella	-	-	12.14a	11.64a
Cowpea	-	-	2.80*	3.46*
	%Ndfa			
Gliricidia	52.35	51.62	0.0	-
Cowpea	-	-	68.42	62.21

[1]Means followed by equal letters in the lines do not differ significantly by the Tukey test for gliricidia and the T test for cowpea, at 5% probability. [2]Tips include leaves plus twigs less than 1.5 cm in stem diameter; wood consists of branches larger than 1.5 cm in diameter; and straw comprises leaves, stems, and pods (Martins et al., 2013).*Significant difference between means. nsNonsignificant in comparison to the values of the reference species (*M. glaziovii* for gliricidia and *A. tenella* for cowpea), at 5% probability.

Absence of fixation in plants belonging to species that are potentially high fixers has also been reported in the same (Souza et al., 2012) and in other semiarid areas (Faye et al., 2007). The legumes were not inoculated, and native rhizobia populations compatible with the legume species could be absent in the soil. This is unlikely to be the case in relation to cowpea, a promiscuous species (Jaramillo et al., 2013), which had high proportions of N derived from biological fixation when planted in the region without inoculation (Freitas et al., 2012). Indeed, cowpea plants had more than 60% N derived from fixation, both in the traditional and agroforestry systems, a performance similar to those already reported for most of the local varieties tested by Freitas et al. (2012) in single crop systems. Gliricidia nodulation is uncertain when it is planted outside its native region (Bala et al., 2003). However, the hypothesis of absence of compatible native rhizobia populations does not hold, because of the fixation of gliricidia in the same experiment when intercropped with buffel grass and prickly-pear cactus. Another possibility would be an inhibition of fixation due to the accumulation of N by the gliricidia plants in previous years. The intercropping of gliricidia, maize, and cowpea produced the highest amounts of aboveground biomass in the previous years (Martins et al., 2013) and probably also the highest amounts of belowground biomass. The decomposition of this biomass could increase the availability of mineral N, and it has been shown that high N availability reduces N fixation (Schipanski et al., 2010).

Nitrogen concentrations in the legume plants (Table 2) were high in all systems, close to 4%, and the contents of N reflected the predominant effect of biomass production (Martins et al., 2013). Quantification of N in the straw is important, although seldom done in the region (Sampaio et al., 2004), because of its use as forage or because it is incorporated into the soil where it constitutes a net input to soil organic matter.

The highest amount of N accumulation in gliricidia tips occurred when it was intercropped with maize + cowpea, in spite of the absence of fixation, corresponding to double the accumulation in the other associations. Considering the harvest index of 0.37, obtained by Freitas et al. (2012) for local cowpea varieties in the Brazilian Northeast, the straw biomass (leaves, stems, and legume pods) was estimated at 61 kg ha^{-1} in the agroforestry system and at 698 kg ha^{-1} in the traditional system. Nitrogen accumulation in cowpea was about 13 times higher in the traditional system than in the agroforestry one, and the accumulation in this last system corresponds to a low cowpea biomass production (Table 1), probably due to the severe competition of the trees for water and nutrients. Considering the total N contents, the presence of trees provided N accumulation several times higher in the agroforestry system.

The amount fixed by cowpea (straw + grains) in the traditional system was close to 30 kg ha^{-1} N but was much reduced in the presence of trees, reaching only 2.7 kg ha^{-1} N in the agroforestry system (Table 2). The gliricidia trees did not contribute to the amount of symbiotically fixed N in the agroforestry system with maize + cowpea intercropping; however, in the associations with buffel grass and prickly-pear cactus, the tree leaves added about 40 kg ha^{-1} of fixed N, obtained from the atmosphere. These inputs may be translated into significant reductions in the cost of fertilizers to intercropped crops, since they correspond from 40 up to 100% of the amounts of N in buffel grass and prickly-pear cactus produced in the systems. The incorporation of N in the agroforestry system is particularly interesting in the association with buffel grass, since pasture fertilization is almost never practiced in the region and soils tend to be deficient in this nutrient (Sampaio et al., 2004). The low productivity of cowpea and the absence of fixation by gliricidia trees in the agroforestry system resulted in a very low addition of fixed N (>1 kg ha^{-1}), indicating that more research on this system is needed before it can be recommended for the region.

Table 2. Nitrogen concentration (%), total and fixed N (kg ha^{-1}) in gliricidia (*Gliricidia sepium*) and cowpea (*Vigna unguiculata*) in agroforestry and traditional systems in the semiarid Northeast of Brazil[(1)].

Legume species[(2)]	Agroforestry system/intercrop			Traditional
	Maize + cowpea	Buffel grass	Prickly-pear cactus	Maize + cowpea
	Nitrogen concentration (%)			
Gliricidia (tips)	4.03a	3.73a	3.68a	-
Gliricidia (wood)	1.51	1.51	1.51	-
Cowpea (grain)	4.40	-	-	4.40
Cowpea (straw)	3.79a	-	-	4.24a
	Total nitrogen content (kg ha^{-1})			
Gliricidia (tips)	158.70a	82.54b	76.15b	
Gliricidia (wood)	193.88a	147.48a	216.44a	
Cowpea (grain)	1.58b	-	-	18.04a
Cowpea (straw)	2.31b	-	-	29.60a
	Fixed nitrogen (kg ha^{-1})			
Gliricidia (tips)	0.00b	43.2a	39.3a	-
Gliricidia (wood)	0.00b	77.21a	111.75a	-
Cowpea (grain)	1.08b	-	-	11.22a
Cowpea (straw)	1.58b	-	-	18.41a

[(1)]Means followed by equal letters in the lines do not differ significantly by the Tukey test for gliricidia and the T test for cowpea, at 5% probability. Tips include leaves plus twigs less than 1.5 cm in stem diameter; wood consists of branches larger than 1.5 cm in diameter; and straw comprises leaves, stems, and pods (Martins et al., 2013).

Conclusions

1. In the agroforestry system with buffel grass (*Cenchrus ciliaris*) and prickly-pear cactus (*Opuntia ficus-indica*) in the semiarid of the state of Paraíba, Brazil, gliricidia (*Gliricidia sepium*) plants symbiotically fix high proportions of N and contribute with higher N amounts than in the traditional system.
2. In the agroforestry system with maize (*Zea mays*) + cowpea (*Vigna unguiculata*) intercropping, gliricidia plants do not fix nitrogen, and the N input is limited to the fixation by cowpea, which is lower than in the traditional system due to its lower biomass production.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process numbers 478138/2007-5 and 574893/2008-3) and to Inter-American Institute for Global Change Research (IAI, process number CRN2-014), for financial support; and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for scholarship granted.

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Received on July 4, 2014 and accepted on January 20, 2015