Quantitative Analysis of the Symbiotic N$_2$-Fixation, Non-Structural Carbohydrates and Chlorophyll Content in Sixteen Native Legume Species Collected in Different Savanna Sites

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Abstract

Three species of Caesalpinaceae, 8 species of Fabaceae, and 5 species of Mimosaceae, at flowering or fruiting stage, were collected during the rainy season, in 12 different savanna sites. The bulk of legume species was found in sandy soils, with pH between 4.2 to 4.6, containing low %Al saturation. Legume species were analysed in terms of their relative abundance of ureides (RAU), α-amino-N (RAA-N) and nitrate (RAN) in nodules (if present), basal roots, green shoots and mature leaves. According to RAU values in shoots, nodulated native species were categorized as fairly good, intermediate, or weak N$_2$-fixers; nodules from good N$_2$-fixers presenting the lowest RAU. Concomitantly, an inverse relationship was recorded between RAU and RAA-N, the highest RAA-N detected in non-nodulated plants. On the other hand, RAN in nodules was significantly lower than in shoots and roots. No detectable levels of nitrate were found in leaves. All species were shown to be ureide producers, except for *Sesbania sericea*. This species was tentatively classified as an amino-N exporter.

In ureide producer species, RAU values in shoots and roots of nodulated plants were significantly higher than those of non-nodulated individuals. This fact supports the use of the ureide technique for detecting and quantifying N_2-fixation in native legumes.

RAU in shoots was positively correlated with soil pH ($r=0.667$, $p<0.01$), and negatively correlated with soil Al contents ($r=0.558$, $p<0.01$). There was no correlation between shoot RAU values and the soil N, K, Ca, P, or Mg concentrations. RAU in shoots was also positively correlated with the leaf chlorophyll content ($r=0.597$, $p<0.01$) and inversely correlated with starch concentration in roots ($r=0.443$, $p<0.01$). In turn, no relationship was observed between leaves' or roots' total reducing sugars and the symbiotic process. The analysis of native rhizobial populations in superficial soil horizons (0–20 cm) indicated that the number of rhizobia g^{-1} soil was negatively correlated ($p<0.01$) with the % sand ($r=0.784$), but positively correlated ($p<0.01$) with soil cation exchange capacity ($r=0.790$) and Al contents ($r=0.884$). Maximum number of rhizobia was found in soils with pH 4.2, and no correlations were detected between number of rhizobia and soil N, P, K, Ca, and Mg concentrations.

1. Introduction

The *Trachypogon* savannas of the Guarico State of Venezuela are characterized by low soil fertility, strong seasonality in water availability and by the presence of a wide variety of physiognomic types of vegetation (Medina and Bilbao, 1991). In these savannas the diversity of grasses is smaller than that of legumes, and nodulation occurs in many of the native legume species (Barrios and Gonzalez, 1971; Medina and Bilbao, 1991).

It is known that the N_2-fixation capacity of symbionts depends mainly on the effective combination of the host and *Rhizobium*, but soil conditions are also of a great importance (Whitten and Ritchie, 1991; Wan Othman et al., 1991). Preliminary studies carried out in tropical savannas have indicated a large variability among several nodulated native legume species in terms of their ureide concentration and natural ^{15}N abundance (Medina and Bibao, 1991). However, to our knowledge a detailed comparative analysis of the effectiveness of the symbiotic process as well as the physiology of native legumes in tropical soils has not been reported. Therefore, the objective of this work was to examine the performance of 16 native legume species with respect to their relative abundance of ureides (RAU), α-amino-N (RAA-N) and nitrate (RAN) in relation to soil physicochemical characteristics of different savanna sites. Chlorophyll and non-structural carbohydrates content in collected legumes were also measured and the size of the soil native rhizobial populations determined.

2. Materials and Methods

Soil sampling and plant collection was carried out in the Estacion Experimental La Iguana, Guarico State, Venezuela (8° 25′N, 65°24′W), during the rainy seasons (August 1990 and September 1991).

Soil characterization

Triplicate soil samples to a depth of 20 cm were collected, at random, in representative areas of the 12 savanna sites. In each soil sample the number of rhizobia per gram of soil was estimated (Vincent, 1970). The physico-chemical characteristics of the soils were determined in air dried subsamples: pH, nitrogen, and organic matter content as in Jackson (1958); available phosphorus (P) was extracted with North Carolina solution (Nelson et al., 1953) and measured by the molybdate-ascorbic acid assay (Murphy and Riley, 1962); exchangeable cations were extracted in 1 N ammonium acetate, pH 7.0, and measured by atomic absorption spectrophotometry; exchangeable aluminum (Al) and exchangeable acidity were measured according to Juan (1959). Soil cation exchange capacity (CEC), Σ bases and %Al saturation were calculated as in Chapman et al. (1961). Soil texture was determined by the hydrometer method (Bouyoucos, 1927).

Plant collection

Native legumes in flowering or fruiting stage were collected at 12 different savanna sites. A minimum of 10 individuals of each species were sampled. The plant species included: *Chamaecrista bauhiniaefolia* Benth, *C. calycioides* (DC) Gr., and *C. flexuosa* (L.) Gr. (Caesalpinaceae); *Crotalaria stipularis* Desv., *Desmodium barbatum* Benth. & Desv., *Galactia jussieuana* K., *Indigofera hirsuta* L, *Indigofera lespedeziodes* L., *Sesbania sericea* Willd., *Stylosanthes guianensis* (Aubl) Sw., and *Zornia curvata* Mohlb. (Fabaceae); *Mimosa debilis* H. & B., *M. camporum* Benth, *M. martensis* Br. & Rose, *M. orthocarpa* Spruce, and *M. pudica* L (Mimosaceae).

Plant harvesting consisted of the entire above-ground biomass and the roots contained in a soil volume of approximately 0.027 m^3. Separation of nodulated plants from those without nodules was carried out after washing the roots. Care was taken to distinguish root nodules from root malformations such as those caused by nematodes. Nodules from replicates of each plant species were dried, weighed and pooled. In some cases, minimum dry weight of 20 mg required for further analyses was not attained.

Determination of the relative abundance of N-compounds

Measurements of N-compounds was carried out in the first 5 cm of green shoots, in the basal regions of primary and lateral roots, in mature leaves, and in nodules. Plant samples were dried at 70°C up to a constant weight, and ground for further analyses. Dried subsamples were weighed and boiled for 15 min in 10 mM buffer phosphate, pH 7.2, containing 50% ethanol. After cooling, the volume made up to 25 ml was filtered through a Whatman No. 1 filter paper. Aliquots of 3 ml were used for ureide determinations (Vogels and van Der Drift, 1970), with the following modification: spectrophotometric readings at 520 nm were carried out before and after adding potassium ferricyanide to samples, to avoid interference in the ureide colorimetric assay of compounds with similar absorbance. The difference between absorbance values was used to calculate the ureide contents per mg dry weight. The α-amino-N contents was determined in aliquots of 0.1 ml (Herridge, 1984), and nitrate measured in dried subsamples (Cataldo et al., 1975). The values of the relative abundance of N-compounds in the different plant organs were calculated as in van Kessel et al. (1988). Non-structural carbohydrates were determined in dried subsamples (McCready et al., 1950) and the chlorophyll contents in well developed mature leaves (Hiscox and Israelstam, 1978).

Statistical analyses

Soil sites were grouped in three main soil types, based on principal component analysis. For statistical analyses data of soil parameters and number of rhizobia per gram of soil were transformed to $\log_{10}$, and data in percentage were transformed to degrees by angular transformation. Statistical differences for RAU, RAA-N, and RAN, between species growing in individual soil types, were analysed using one way analysis of variance. Statistical differences between means of each variable were determined using LSD (Least Significant Difference).

3. Results
Description of the savanna sites, and legume species composition

Chemical characteristics and texture of the soils collected in the 12 different savanna sites are presented in Table 1. A principal component analysis ran with data from Table 1 allowed the pooling of the savanna sites in 3 main soil types based on the % sand composition (r=0.986, p<0.01). Soil type 1, constituted by A to F sites, are sandy soils containing 64% to 84% of sand. Soil type 2, constituted by G to J sites presented intermediate percentages of

Table 1. Chemical properties and texture of the soil collected in 12 different savanna sites. Soil depth 0–20 cm.

	Soil type 1						Soil type 2				Soil type 3	
	A	B	C	D	E	F	G	H	I	J	K	L
pH (1:5 water)	4.6	5.1	4.3	4.2	4.6	3.8	4.5	4.5	4.2	4.2	4.5	3.7
N (mg/g)	0.6	1.3	1.2	0.6	0.6	2.0	1.0	0.5	1.4	1.1	0.8	0.1
P available (μg/g)	1.9	0.3	0.4	0.2	0.5	1.6	0.2	2.6	1.2	0.2	0.1	1.8
Mg (meq/100 g)	0.2	0.7	0.7	0.2	0.5	0.3	0.6	0.3	0.3	0.5	0.3	0.2
K (meq/100 g)	0.3	0.8	0.5	0.3	0.4	0.3	0.2	0.7	0.7	0.4	0.3	0.2
Ca (meq/100 g)	0.3	0.4	0.1	0.2	0.8	0.1	0.8	0.1	0.8	0.3	0.2	0.8
Al (meq/100 g)	0.2	0.0	2.2	2.6	1.1	0.9	2.1	3.8	2.0	4.0	2.5	8.0
Exch. ac* (meq/100 g)	0.8	0.3	4.7	5.8	2.7	2.4	5.0	11.6	4.3	8.9	15.5	10.0
CEC**	1.8	2.2	8.2	9.1	5.5	4.0	8.7	16.5	8.1	14.1	18.8	19.2
$\sum$ bases	1.0	1.9	3.5	3.3	3.3	1.6	3.7	4.9	3.8	5.2	3.3	9.2
% organic matter	1.6	1.0	1.6	1.8	1.4	2.0	2.0	1.3	3.0	2.7	1.5	1.4
% sand	84.0	81.5	74.0	69.0	64.0	56.5	44.0	44.0	41.5	41.5	26.5	24.0
% clay	6.0	13.5	16.0	18.5	26.0	26.0	36.0	36.0	36.0	38.5	58.5	46.0
% silt	10.0	5.0	10.0	12.5	10.0	17.5	20.0	20.0	20.0	20.0	15.0	30.0
% Al saturation	11.1	0.0	26.8	28.6	20.0	22.5	24.0	23.0	24.7	28.3	13.3	39.6

* Exchangeable acidity
** Cation exchange capacity

sand (44% to 41.5%); while soil type 3 (K and L sites) was differentiated by low values of % sand (24% to 26.5%). The pH of soils ranged from 3.7 to 5.1 with no specific grouping as with texture. Concentrations of P, Ca, Mg, K, and organic matter in soils were also not associated with pH. All savanna sites were characterized by a low nutritional status.

The analysis of legume species diversity indicated a positive relationship between number of species and % of sand in the soils (Fig. 1A). The bulk of
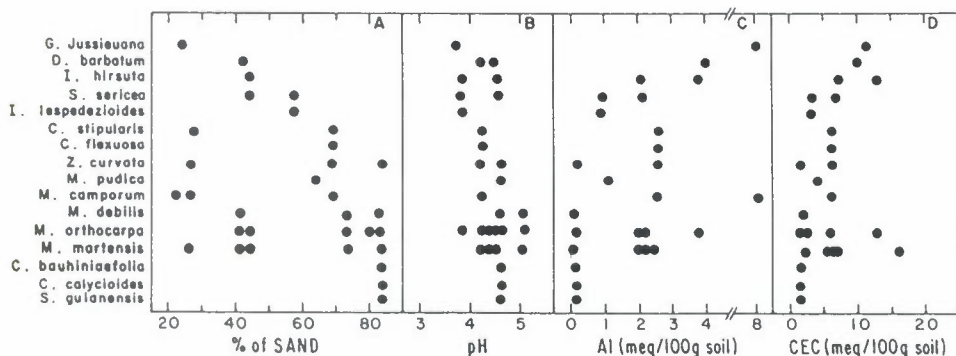

Figure 1. Legume species distribution in different savanna sites as influenced by (A) % Sand; (B) pH; (C) Al concentration, and (D) Cation Exchange Capacity of the soils.

legume species was found in soils with pH between 4.2 to 4.6 (Fig. 1B), the species number being also negatively associated with the increasing levels of Al (Fig. 1C) and soil CEC (Fig. 1D). *G. jussieuana* was detected in all savanna sites; however, plants in flowering stage were only found in soil site L (Table 1). *M. camporum* was also collected in this soil site. Legume species distribution could not be related to other soil chemical characteristics such as the P, K, Ca, N, and Mg concentrations.

Native rhizobial populations and nodulation in native legumes

The number of rhizobia per gram of soil was found to be negatively correlated with the soil % sand (r=0.784, p<0.01) (Fig. 2A), but positively correlated (P<0.01) with the soil CEC (r=0.790) (Fig. 2B) and Al concentration (r=0.884) (not shown). No significant correlations were obtained between the number of rhizobia and the levels of N, P, K, Ca, and Mg in the soil sites. The maximum number of rhizobia per gram of soil occurred at soil pH of 4.2, the size of rhizobial populations decreasing sharply at pH units above or below this value (Fig. 2C). In savanna sites A and L (Table 1), the rhizobial populations could not be detected. Preliminary experiments indicated that the
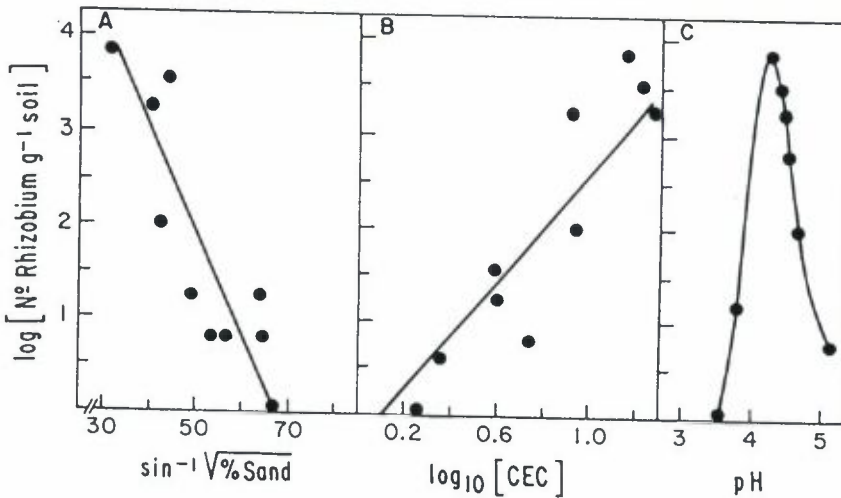
Figure 2. Effect of soil characteristics on the size of native rhizobial populations: (A) % Sand; (B) Cation Exchange Capacity, and (C) pH of soil sites.

native populations are mainly composed by *Bradyrhizobium* of the "cowpea miscellany".

Except for *Indigofera lespedeziodes*, all legume species collected in the 12 soil sites were nodulated even though some individuals did not have nodules on the harvested roots. Nodules were found mainly on lateral root regions hithermost to the crown. Nodules' form was either coralline, finger-like or spherical. However, each individual species presented a predominant nodule shape. The biggest nodules, of approximately 0.6 cm diameter, were harvested from *S. sericea*. The rest of the species presented collars and/or clusters of very small nodules.

Relative abundance of N-compounds in nodules, shoots, roots and mature leaves of native legumes

In Table 2 the legume species collected in the 3 main soil types are ordered according to RAU values in the shoots. Among nodulated species sampled in soil types 1 and 2, *S. sericea* showed the lowest RAU and highest RAA-N, similar to that of non-nodulated individuals. Statistical differences were also recorded among species collected in soil type 3 with respect to either RAU or RAA-N values. In soil type 1, nodulated *S. guianensis* and *S. sericea* showed the highest and lowest RAN, respectively. In soil type 2, high RAN was

Table 2. Relative abundance of ureides, α-amino-N and nitrate in shoots and leaves of native legumes

Species in:	Nodules	Shoots				Leaves		
		% ureide	% α-amino-N	% nitrate	Total N μgmg⁻¹dry wt	% ureide	% α-amino-N	Total N μgmg⁻¹dry wt
Soil type 1*								
C. flexuosa	+	32.9a	50.8ef	16.3cd	41.7±3.8	36.0a	64.0e	42.5±3.9
M. pudica	+	31.7a	48.0f	20.3bc	29.0±1.7	34.2e	65.8de	43.8±4.9
C. stipularis	+	30.7a	53.5ef	15.8cd	24.2±1.3	15.0cd	85.0bc	41.5±4.1
M. martensis	+	27.9ab	52.0ef	20.1bc	20.1±0.8	25.1b	74.9d	47.7±3.2
M. debilis	+	24.0bc	53.2ef	22.8bc	20.3±1.2	22.9bc	77.1cd	36.8±2.5
M. orthocarpa	+	23.1c	61.9bc	15.0de	30.6±1.1	26.9b	73.1d	57.7±3.1
C. calycioides	+	22.3c	58.0cde	19.7bc	21.0±1.3	16.6cd	83.4bc	41.9±2.3
Z. curvata	+	18.8d	58.2cde	23.0b	38.6±1.4	4.6e	95.4a	76.3±7.8
M. camporum	+	18.2d	60.9bc	20.9bc	42.3±3.2	20.6bc	79.4bcd	51.3±1.3
S. guianensis	+	16.4d	47.9f	35.7a	22.2±1.8	9.1d	90.9a	47.5±3.7
C. bauhiniaefolia	+	16.7d	70.2b	13.1ef	52.8±3.4	4.1e	95.9a	57.3±5.8
S. sericea	+	8.4e	83.5a	8.1g	61.4±3.5	12.7d	87.3b	67.6±3.2
I. lespedezioides	-	12.8e	66.3b	20.9bc	33.6±1.3	nd	nd	nd
C. bauhiniaefolia	-	8.5e	84.2a	7.3g	28.2±1.7	nd	nd	nd
Z. curvata	-	9.5e	79.6a	10.9f	30.6±3.1	nd	nd	nd
Soil type 2*								
M. orthocarpa	+	33.4a	44.5c	22.1b	29.2±1.5	38.7a	61.3d	49.8±3.8
D. barbatum	+	24.1b	49.3c	26.6a	22.1±1.5	23.5b	76.5c	32.2±2.8
M. martensis	+	22.9b	60.0b	17.1b	36.6±4.5	22.2b	77.8c	52.4±6.2
M. debilis	+	21.1b	46.1c	32.8c	20.6±1.8	nd	nd	nd
I. hirsuta	+	20.8b	63.7b	15.5b	38.4±2.5	17.3b	82.7c	28.7±3.0
S. sericea	+	9.1c	81.8a	9.1c	58.8±3.6	11.9c	88.1b	68.9±6.7
M. orthocarpa	-	1.2c	79.8a	19.0b	28.2±2.8	3.8d	96.2a	45.9±4.6
Soil type 3*								
M. martensis	+	23.9a	50.6c	25.5a	31.1±1.9	24.2a	75.8b	43.0±4.2
G. jussieuana	+	29.1a	57.8c	13.1c	25.3±1.2	15.4b	84.6a	33.8±2.9
Z. curvata	+	13.1b	63.0b	23.9a	16.7±2.5	nd	nd	nd
M. debilis	+	12.8b	67.6b	19.6b	24.2±2.5	nd	nd	nd
C. stipularis	+	10.9b	63.0b	26.1a	15.4±1.6	nd	nd	nd
M. camporum	-	2.9c	72.0a	24.1a	19.4±1.7	nd	nd	nd
G. jussieuana	-	1.7c	74.7a	23.6a	22.2±2.6	nd	nd	nd

* see Table 1, nd = not determined
Means in each soil type were statistically compared by LSD. Numbers in columns followed by the same letter(s) are not statistically different at p<0.05.

detected in *D. barbatum* and *M. debilis*, while *S. sericea* had the lowest value. In soil type 3, except for nodulated *G. jussieuana* and *M. debilis*, the rest of the collected species showed similar RAN. Significant differences were recorded in RAU and RAA-N between nodulated and non-nodulated *C. bauhiniaefolia*, *Z. curvata* (soil type 1), *M. orthocarpa* (soil type 2), and *G. jussieuana* (soil type 3). However, between nodulated and non-nodulated plants, RAN values were not statistically different. When compared to data from soil type 1, total shoot organic N was lower in nodulated *Z. curvata* and *C. stipularis* from soil type 3.

Leaves of *M. pudica*, *C. flexuosa* (soil type 1), *M. orthocarpa* (soil type 2) and *M. martensis* (soil type 3) contained the highest RAU and lowest RAA-N values (Table 2). Leaf extracts from nodulated and non-nodulated species did not contain detectable levels of nitrate.

Analysis of N-compounds in roots indicated that among nodulated species collected in soil type 1, roots of *C. flexuosa* and *S. sericea* showed the highest and lowest RAU, respectively (Table 3). In these two species RAA-N values were opposite to those of RAU. The statistical comparison of RAN in species collected in soil type 1 showed no significant differences among means, the lowest value being recorded in roots of *S. sericea*. In soil type 2, except for *S. sericea*, there were no statistical differences among species with respect to RAU, RAA-N and RAN values in roots. However, in soil type 3, roots of *M. martensis* and *G. jussieuana* showed the highest RAU values. Results also indicated that RAU in roots of nodulated *G. jussieuana* was 19-fold higher than in non-nodulated plants of the same species, while roots of non-nodulated *G. jussieuana* presented the highest RAA-N values. In soil type 3, non-nodulated *M. camporum* showed the highest RAN. In general, results indicate that RAU in roots of nodulated plants was either similar or higher than in shoots.

Data on the relative abundance of N-compounds in nodules were not statistically analysed due to the absence of replicates (Table 3). Nevertheless, results indicate that nodules harvested from *S. sericea* (soil types 1 and 2) contained the highest and lowest RAU and RAA-N, respectively. The percentage of nitrate in nodules varied from 1.1 to 8.6, in contrast to the larger percentages detected in shoots and roots.

Physiological parameters in native legumes

Values of total reducing sugars, starch and chlorophyll contents in nodules, roots and leaves are summarized in Table 4. For all legume species the sugar

Table 3. Relative abundance of ureide, α-amino-N, and nitrate in nodules and roots of native legumes

Species in:	nodule	Nodules				Roots			
		% ureide	% α-amino-N	% nitrate	Total N μg mg^{-1} dry wt	% ureide	% α-amino-N	% nitrate	Total N μg mg^{-1} dry wt
Soil type 1*									
C. flexuosa	+	4.7	89.5	5.8	316.0	49.8a	30.0e	20.2ab	24.6±2.1
S. guianensis	+	16.6	78.3	5.1	87.3	30.4b	58.7bc	10.9de	12.2±0.8
M. pudica	+	7.0	90.8	2.2	135.6	29.9b	47.7b	22.4d	26.0±1.9
M. orthocarpa	+	5.1	91.4	3.5	181.4	28.4b	56.5cd	15.1cd	15.7±1.2
C. stipularis	+	nd	nd	nd	nd	28.0b	48.5d	23.5a	17.1±0.7
M. martensis	+	7.2	86.6	6.2	110.2	28.2b	59.8bc	12.0d	18.6±0.6
M. debilis	+	8.4	85.4	6.2	211.1	26.7b	56.9cd	16.4bcd	17.2±1.8
C. calycioides	+	5.4	86.7	7.9	94.6	26.4b	55.0cd	18.6abc	22.5±0.3
Z. curvata	+	12.1	80.6	7.3	158.8	18.1c	67.8b	14.1cd	28.5±1.3
M. camporum	+	nd	nd	nd	nd	17.3c	63.9bc	18.8ab	35.0±1.8
C. bauhiniaefolia	+	16.5	81.6	1.9	136.5	17.4c	67.9b	14.7cd	22.5±0.3
S. sericea	+	36.2	62.7	1.1	112.7	12.2d	79.4a	8.4e	36.8±2.9
I. lespedezioides	-	-	-	-	-	10.3d	78.4a	11.3d	43.0±2.8
Soil type 2*									
M. orthocarpa	+	3.5	91.8	4.7	245.1	30.0a	56.2b	13.8a	22.8±1.5
D. barbatum	+	17.0	74.4	8.6	78.6	23.2a	61.9b	14.9a	23.2±1.5
M. martensis	+	11.0	84.7	4.3	115.5	21.9e	59.8b	18.3a	25.8±2.0
I. hirsuta	+	10.4	84.5	5.1	118.9	22.3a	61.6b	16.1a	20.1±2.3
S. sericea	+	33.7	64.6	1.7	129.0	14.2b	80.1a	5.7b	34.4±2.6
Soil type 3*									
M. martensis	+	9.1	86.6	4.3	161.1	23.0a	65.6b	11.4b	27.7±1.9
G. jussieuana	+	nd	nd	nd	nd	19.4a	69.8b	10.8b	26.0±0.2
M. camporum	-	-	-	-	-	3.4c	67.8b	28.8a	18.1±0.9
G. jussieuana	-	-	-	-	-	1.0c	83.5a	15.5b	25.7±1.6

* see Table 1, nd = not determined

Means in each soil type were statistically compared by LSD. Numbers in columns followed by the same letter(s) are not statistically different at p<0.05.

Table 4. Total reducing sugars, starch and chlorophyll content in nodules, roots, and leaves of native legume species. Values are the mean of six replicates ± standard error of the mean.

| | Sugars mg g^{-1} dry wt | | | Starch mg g^{-1} dry wt | | Chlorophyll μg mg^{-1} dry wt |
	nodules	roots	leaves	roots	leaves	leaves
Soil type 1*						
C. bauhiniaefolia	–	158.0±10	27.8±2	353.0±21	41.7±1	2.7±0.2
	96.4	41.6±1	58.1±2	64.9±5	50.0±3	8.6±0.2
C. calycioides	57.4	29.6±3	69.3±3	80.0±4	37.3±3	7.3±0.4
C. flexuosa	79.3	54.8±5	23.1±2	71.5±5	48.5±7	18.3±0.6
C. stipularis	nd	130.0±10	55.8±5	202.0±12	56.3±3	7.5±1.0
I. lespedezioides	–	160.0±10	67.9±1	102.0±9	115.1±10	5.6±1.3
M. camporum	nd	27.5±1	37.2±3	118.0±6	61.6±3	9.5±1.6
M. debilis	131.0	18.0±2	37.4±4	65.1±3	118.2±11	8.9±0.7
M. martensis	52.1	12.4±1	32.4±2	74.3±7	76.4±4	8.6±1.5
M. orthocarpa	82.6	24.9±3	51.2±4	65.8±4	87.6±13	15.1±1.2
M. pudica	86.8	28.0±3	53.0±8	63.1±14	99.0±6	7.7±1.0
S. sericea	10.8	150.0±14	51.7±5	76.0±4	72.5±7	11.2±1.4
S. guianensis	47.1	17.5±2	63.9±5	124.0±10	87.6±5	8.8±0.6
Z. curvata	–	47.3±1	33.6±8	155.0±23	65.0±4	3.4±0.4
	75.9	29.0±3	24.8±5	121.0±13	52.5±10	7.3±0.8
Soil type 2*						
D. barbatum	nd	42.4±4	50.2±3	118.0±9	52.0±5	7.7±0.7
I. hirsuta	50.3	35.9±11	48.8±3	165.0±20	99.1±18	10.2±0.9
M. martensis	50.0	32.1±5	25.5±1	64.1±5	82.6±13	6.8±2.1
M. orthocarpa	–	106.0±12	41.9±5	104.0±11	171.2±17	8.8±0.4
	49.9	13.4±2	42.2±4	70.1±7	112.1±11	15.3±0.8
S. sericea	13.6	49.5±4	46.1±2	110.0±18	34.7±1	17.6±0.9
Soil type 3*						
M. martensis	39.5	26.5±3	37.3±3	53.1±2	77.0±4	9.2±1.0
M. debilis	nd	nd	31.9±4	37.2±4	23.2±2	3.4±0.8
M. camporum	nd	50.4±3	56.9±8	42.8±5	44.5±4	4.9±0.5
Z. curvata	nd	64.4±5	38.8±4	172.0±25	33.7±5	6.5±1.4
C. stipularis	nd	81.9±16	66.8±11	161.1±16	509.3±9	4.1±0.5
G. jussieuana	nd	37.8±3	27.5±2	166.2±26	53.1±7	9.1±0.8
G. jussieuana	–	17.9±7	52.7±5	392.3±14	104.3±10	3.1±1.0

* see Table 1, – = nodules absent, nd = not determined

and starch contents in roots were significantly lower in nodulated when compared to non-nodulated plants. No such relationship was detected between non-structural carbohydrate contents in leaves and the presence or absence of nodules on roots. Nevertheless, leaves of nodulated plants showed the highest chlorophyll contents regardless of site of collection. On the other hand, total reducing sugars in nodules were 2 to 7-fold higher than in roots, except in those of *S. sericea*.

Correlation analysis between plant variables and soil characteristics

For all legume species RAU in shoots was positively correlated with soil pH (r=0.667, p<0.01), and negatively correlated with soil %Al saturation (r=0.558, p<0.01). There was no significant correlation between RAU in shoots and soil CEC, N, P, K., Ca, or Mg concentration. A very significant positive correlation between RAU in shoots and leaves was only detected in Mimosaceae (r=0.646) and in Caesalpinaceae (r=0.772). In turn, a significant positive correlation (p<0.01) was found between RAU in shoots and

roots of Mimosaceae (r=0.508), and Caesalpinaceae (r=0.910). Within the Fabaceae, only *S. sericea* showed the same correlation (r=0.508, p<0.01). A positive correlation, albeit weak, was detected between chlorophyll contents and leaf RAU (r=0.336, p<0.01). Concomitantly, a significant negative correlation (p<0.01), was obtained between the starch content in roots and RAU in shoots (r=0.443). However, no correlations could be detected between leaf non-structural carbohydrates and the symbiotic process. Due to the distinct results obtained in *S. sericea*, data from this species were not included in correlation analyses carried out between RAU and either chlorophyll or starch contents.

4. Discussion
Legume species and native rhizobial population in savanna soils

The distribution of legume species in savanna sites was found to be determined mainly by the % of sand. %Al saturation, CEC, and pH of the soils. It was also observed that as the % of sand increased, the size of the native rhizobial populations decreased in the superficial soil horizons. In those soils, large rhizobial populations were detected at greater depth (not shown), probably due to the nonrestricted downward water movement within the sand bed (Worral and Roughley, 1991).

The higher number of rhizobia detected in soils with pH 4.2, as well as the positive correlation found between number of rhizobia and soil Al contents point toward a tolerance of these native populations to adverse soil factors. However, previous reports have shown that native rhizobia are not necessarily tolerant to overall soil acidic factors (Richardson and Simpson, 1989). Rhizobial cells may survive in protective soil microsites of higher pH and lower Al contents (Wood and Shepherd, 1987). The observation that nodulation occurs in plants collected in soil type L, in which no rhizobia were detected and the most constrained conditions were observed, may indicate that the host rhizosphere could also offer a better environment for rhizobial growth and nodulation (Velageti and Marsh, 1989). The present results also seem to indicate that in savanna soils, growth of free living rhizobia is more sensitive to low pH than to high Al concentrations, in agreement with previous observations (Taylor et al., 1991).

Effect of soil factors on the relative abundance of N-compounds in native legumes

The sampling of legumes at the flowering or fruiting stage allowed the comparison of symbiotic N_2-fixation among species of similar physiological

conditions. Herridge et al. (1990) have shown that for plants in reproductive stage and growing in unamended soils, RAU is a good indicator of the symbiotic process and nodulation status of plants. Thus, in soil type 1, *C. flexuosa*, *C. stipularis*, *M. pudica* and *M. martensis* can be considered as fairly good N_2 fixers. Intermediate RAU values were recorded in *M. debilis*, *M. orthocarpa* and *C. calycioides*, while *Z. curvata*, *M. camporum*, *C. bauhiniaefolia* and *S. guianensis* ranked as weak fixers. In soil type 2, *M. orthocarpa* showed the highest RAU as compared to the intermediate values recorded in the other collected species. In soil type 3, the contrasting soil chemical characteristics apparently exerted a negative effect on the RAU of nodulated *M. debilis*, *C. stipularis*, hindering the nodulation of *M. camporum*. This observed effect was reflected in the correlation obtained between shoot RAU and soil pH (positive) and Al contents (negative). According to previous results, the functioning of the symbiotic N_2-fixation is less sensitive than that of the host plant to specific soil elements (Lindstrom et al., 1985; Schubert et al., 1990). Therefore, the apparent non-response of *M. martensis* and *G. jussieuana* to soil type 3 conditions might indicate that the tolerance of the host to soil factors is indeed the major prerequisite for nodulation and N_2-fixation in acidic soils.

In general, the apparent low N_2-fixation detected in nodulated species as compared to that of commercially cultivated legumes (Izaguirre-Mayoral et al., 1992) might be the result of the inherent low nutritional status of these savanna soils (Medina and Silva, 1990). The dependence of the symbiotic process on the supply of nutrients is well-documented (O'Hara et al., 1988). Nevertheless, the range of RAU values measured in nodulated native legumes was similar to that of several nodulated soybean genotypes growing under field conditions (Herridge et al., 1990).

Physiological aspects of nodulated and non-nodulated native legumes

Previous work indicated that legumes, in contrast to grasses, are able to translocate more photosynthates to roots (Oaks, 1992), and that carbohydrates are efficiently used in the N_2-fixation process (Day and Copeland, 1991). The lower concentration of non-structural carbohydrates detected in roots of nodulated native plants as compared to non-nodulated ones, as well as the observed sugar partitioning between roots and nodules, are consistent with these results: N_2-fixing nodules, therefore, constitute by far the strongest sink in legumes. In turn, the observation that nodulated native legumes had similar or higher RAU in roots as compared to shoots may be associated with the legume ability to accumulate a large proportion of their biomass and nutrient

resources in underground organs (Medina and Bilbao, 1991). The importance of the symbiotic process for legume growth in tropical savannas was furthermore manifested in the higher chlorophyll contents of nodulated plants, as compared to non-nodulated individuals. The significant positive correlation detected between RAU in shoots and leaf chlorophyll contents has been also reported for soybean nodulated with different strains of *Bradyrhizobium japonicum* (Mirza et al., 1990).

Another aspect regarding N_2-fixation of native legumes was the observation that nodulated *S. sericea* always showed the lowest RAU and the highest RAA-N values in shoots and roots, regardless of soil type. The low RAU measured in this species was not related to either the high chlorophyll contents, nodule abundance (456.4 ± 70 mg dry weight per plant), or height (average 2 m) of the plants. These results might suggest that the symbiotic process in *S. sericea* takes place via synthesis and export of amino-N compounds, as shown in *Sesbania rostrata, S. cannabina* and in *S. sesban* (Yoneyama and Kondo, 1990). The low RAA-N values detected in nodules of this species also support this assumption, since nodules are not likely to accumulate the main products of the N_2-fixation. On the other hand, RAU values in nodules harvested from this species eliminate, at present, the possibility that the observed low RAU in shoots and roots of *S. sericea* is due to the strong sink demand of aerial organs for nodule exported ureides. Certainly this point requires further investigation because not all nodulated *Sesbania* species seem to behave in a similar manner (Vaughn et al., 1982; van Kessel et al., 1988).

Finally, the high RAA-N values detected in non-nodulated individuals has been also previously found in non-nodulated leguminous trees (van Kessel et al., 1988), and in other legume species in which ureides are absent or present at low levels (Pate et al., 1980). Yet, in non-nodulated native plants the percentage of ureides in shoots ranked from 1.2 to 12.8% in contrast to the higher percentages reported in non-nodulated leguminous trees (van Kessel et al., 1988). The observed significant differences in RAU values between nodulated and non-nodulated individuals suggest that the ureide technique can be useful for detecting and quantifying the symbiotic N_2-fixation in native legumes.

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