

## N<sub>2</sub>-fixation in tropical American savannas evaluated by the natural abundance of <sup>15</sup>N in plant tissues and soil organic matter

E. MEDINA<sup>1</sup> & M.L. IZAGUIRRE<sup>2</sup>

<sup>1</sup>Laboratory of Plant Physiological Ecology, Centro de Ecología and <sup>2</sup>Centro de Microbiología y Biología Celular; IVIC, Aptdo. 21827, Caracas 1020-A. Venezuela

**Abstract:** We measured the abundance of <sup>15</sup>N in herbaceous and woody plants and superficial soils of savannas in central and eastern Venezuela to assess the contribution of associative N<sub>2</sub> fixation to total plant N accumulation. In plants tissues we measured also the ureide content as an independent estimation of N<sub>2</sub>-fixing activity. Soils showed always positive δ<sup>15</sup>N values (averaging 2.8 to 5.2‰). Legumes had on average significantly more negative δ<sup>15</sup>N values (-0.8 to -1.2 ‰ against 2.4 to 3.1‰) and significantly higher N concentration than non-legumes leaf tissues. The relative abundance of ureides was high in legume species with negative δ<sup>15</sup>N values. *Trachypogon* spp. showed highly variable, frequently negative, δ<sup>15</sup>N values (0.6 ± 1.4 ‰), indicating an occasional significant contribution of N<sub>2</sub>-fixing bacteria associated with roots. On the contrary, Cyperaceae species measured had always relatively high positive δ<sup>15</sup>N values (6.3 ± 1.7 ‰). <sup>15</sup>N- and ureid abundance allowed identification of plants with associative N<sub>2</sub>-fixation.

**Resumen:** Medimos la abundancia de <sup>15</sup>N en plantas herbáceas y leñosas y en suelos superficiales de sabanas del centro y este de Venezuela para evaluar la contribución de la fijación asociativa de N<sub>2</sub> a la acumulación total de N en las plantas. En tejidos vegetales medimos también el contenido de ureidos como una estimación independiente de la actividad de fijación de N<sub>2</sub>. Los suelos siempre mostraron valores positivos de <sup>15</sup>N (con promedios de 2.8 a 5.2‰). Las leguminosas tuvieron valores promedio de <sup>15</sup>N significativamente más negativos (-0.8 a -1.2‰ contra 2.4 a 3.1‰) y una concentración significativamente más alta de N que los tejidos foliares de las no leguminosas. La abundancia relativa de ureidos fue alta en las especies de leguminosas con valores negativos de <sup>15</sup>N. *Trachypogon* spp. mostró valores muy variables de <sup>15</sup>N, con frecuencia negativos (0.6 ± 1.4‰), lo que indica que ocasionalmente las bacterias fijadoras de N<sub>2</sub> asociadas a las raíces pueden tener una contribución significativa. Por el contrario, las especies de Cyperaceae que fueron medidas siempre tuvieron valores positivos relativamente altos de <sup>15</sup>N (6.3 ± 1.7‰). La abundancia de <sup>15</sup>N y de ureido permitió identificar las plantas con fijación asociativa de N<sub>2</sub>.

**Resumo:** A abundância de <sup>15</sup>N nas plantas herbáceas e lenhosas bem como no solo superficial de savanas na zona central e oriental da Venezuela foi analisada para avaliar a contribuição da fixação associativa de N<sub>2</sub> na acumulação do N total nas plantas. Nos tecidos das plantas foram também medidos o teor em ureíde como uma avaliação independente da atividade de fixação do N<sub>2</sub>. Os solos mostraram sempre valores de <sup>15</sup>N positivos (oscilando entre 2,8 e 5,2‰). As leguminosas evidenciaram, em média, valores significativos mais negativos de <sup>15</sup>N (0,8 a 1,2‰ contra 2,4 a 3,1‰) e valores de concentração de N significativamente mais elevados do que os encontrados nos tecidos foliares de não leguminosas. A abundância relativa de ureídeos era mais alta nas espécies leguminosas com valores de <sup>15</sup>N negativos. A *Trachypogon* spp. mostraram valores de <sup>15</sup>N fortemente variáveis e frequentemente negativos (0,6 ± 1,4‰),

---

<sup>1</sup> Corresponding Author: Fax (582) 504-1088; email: emedina@ivic.ve

indicando uma contribuição ocasional significativa de bactérias fixadoras de  $\text{N}_2$  associados com as raízes. Pelo contrário, as espécies de Cyperaceae apresentaram sempre valores fortemente positivos de  $^{15}\text{N}$  ( $6,3 \pm 1,7\%$ ). A abundância de  $^{15}\text{N}$  e de ureídeos possibilitariam a identificação de plantas com fixação associativa de  $\text{N}_2$ .

**Key words:**  $\delta^{15}\text{N}$ , legumes,  $\text{N}_2$  fixation, tropical savannas, ureídeos.

## Introduction

South American savannas are particularly rich in legume species, and being the source of many cultivars currently used for pasture improvement throughout the world (Medina & Huber 1992). Their capability for  $\text{N}_2$ -fixation through *Rhizobium*-root symbioses is remarkable, and appears to be highly adaptive in soils rather poor in this nutrient (Barrios & González 1971; Medina & Bilbao 1991; Sicardi de Mallorca & Izaguirre-Mayoral 1993). However, grass species, mostly of the  $\text{C}_4$  photosynthetic pathway, dominate savanna landscapes in the tropics of all continents, their competitive capacity against legumes appearing to be enhanced by their relatively low N and, at least in South American savannas, low P requirements (Medina & Huber 1992; Medina 1996).

Shrubby and herbaceous legumes in tropical South American savannas have been shown to have consistently higher leaf N concentration than non-legumes dicots and grasses (Medina & Bilbao 1991). These higher concentrations have been also associated with active  $\text{N}_2$ -fixation as measured with the method of relative abundance of ureide (RAU) (Izaguirre-Mayoral *et al.* 1992; Sicardi de Mallorca & Izaguirre-Mayoral 1993). This method allows the measurement of the current  $\text{N}_2$ -fixation activity by *Rhizobium*-nodulated legumes (Herridge *et al.* 1990). Indeed, in several legume species under natural conditions, changes in nodule vigor determined by seasonal differences in water availability are accompanied by strong variations in RAU (Sicardi de Mallorca & Izaguirre-Mayoral 1993).

Natural abundance of  $^{15}\text{N}$ , expressed as  $\delta^{15}\text{N}$ , has been proposed as a reliable index of  $\text{N}_2$ -fixation in natural settings (Shearer & Kohl 1986). However, interpretation of field data may be difficult as  $\delta^{15}\text{N}$  tends to be affected by a number of factors such as life forms and mycorrhizal status

in addition to the  $\text{N}_2$ -fixation capability of the symbiotic system (Högberg & Alexander 1995; Pate *et al.* 1993).

A number of studies showed that  $\text{N}_2$ -fixing plants tend to have lower  $\delta^{15}\text{N}$  values and frequently higher N concentration than plants depending on the uptake of mineral soil N (Medina & Bilbao 1991; Schulze *et al.* 1991; Virginia & Delwiche 1982; Yoneyama *et al.* 1993). However, other studies report contradictory results using these criteria (Handley *et al.* 1994; Heaton 1987).

Detailed analysis of the natural abundance of  $^{15}\text{N}$  in tropical savannas are scanty and they failed to show a clear cut pattern differentiating  $\text{N}_2$ -fixing legumes from grasses and other plants without symbiotic  $\text{N}_2$ -fixing associations (Abbadie *et al.* 1992; Medina & Bilbao 1991; Sprent *et al.* 1996). We report here the  $\delta^{15}\text{N}$  values and N concentration of plants and soils from *Trachypogon*-savannas in Venezuela. The occurrence of associative  $\text{N}_2$ -fixation as measured by  $\delta^{15}\text{N}$  and the relative abundance of ureide in shoots was correlated in several legumes and non-legumes species.

## Materials and methods

Leaf material (for analysis of  $^{15}\text{N}$  natural abundance) or young stems (for the analysis of nitrogen compounds in xylem sap) from healthy plants were collected in *Trachypogon*-savannas in central and eastern Venezuela during the rainy season of 1992 (Table 1):

- (1) Urica, Edo. Anzoátegui  $\approx 9^\circ 40' \text{ N}$ ,  $64^\circ 00' \text{ W}$
- (2) Mata Negra, Edo. Monagas  $\approx 8^\circ 58' \text{ N}$ ,  $62^\circ 45' \text{ W}$
- (3) La Iguana, Edo. Guárico  $\approx 8^\circ 25' \text{ N}$ ,  $65^\circ 20' \text{ W}$ .

The sites selected were natural savannas submitted to annual fires, and only at La Iguana a light cattle grazing pressure of about 0.25 head  $\text{ha}^{-1}$  is regularly applied.

At each site soil cores (0-15 cm) between plants were also collected. Rainfall patterns are

**Table 1.** Set of species measured in the three savanna sites described in the text. Species were sampled in different savanna patches together with 0-15 cm depth soil samples.

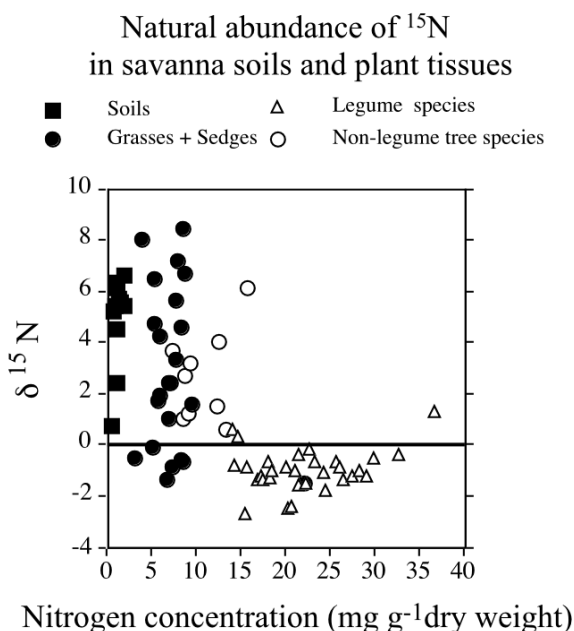
Species	No. samples	Mata Negra	Urica	La Iguana
<b>Legumes</b>				
<i>Aeschynomene</i> sp.	2	x		
<i>Bowdichia virgilioides</i> Kunth	3			x
<i>Chamaecrista chamaecrista</i> (L.) Britt.	2	x		
<i>Chamaecrista tetraphylla</i> (Desv.) Britt. & Rose	4		x	x
<i>Clitoria guyanensis</i> (Aubl.) Standl.	1			x
<i>Eriosema crinitum</i> (Kunth) G. Don	2	x		x
<i>Eriosema simplicifolium</i> (Kunth) G. Don	1			x
<i>Eriosema</i> sp.	1	x		
<i>Galactia jussiaeana</i> Kunth	8	x	x	x
<i>Indigofera suffruticosa</i> Mill.	1		x	
<i>Phaseolus diversifolius</i> Pittier	1		x	
<i>Phaseolus gracilis</i> Poepp. ex Benth.	2			x
<i>Phaseolus</i> sp.	1	x		
<i>Stylosanthes guyanensis</i> (Aubl.) Sw.	1			x
<i>Stylosanthes</i> sp.	1		x	
<i>Zornia</i> sp.	1	x		
<b>Non-legumes</b>				
<i>Aristida</i> sp.	1			x
<i>Bulbostylis capillaris</i> (L.) C.B. Clarke	3			x
<i>Bulbostylis paradoxa</i> (Spreng.) Lindm.	2	x		x
<i>Bulbostylis</i> sp.	1		x	
<i>Byrsonima crassifolia</i> (L.) Kunth	2			x
<i>Casearia silvestris</i> Sw.	1			x
<i>Curatella americana</i> L.	5	x		x
<i>Cyperus</i> sp.	1	x		
<i>Dichromena ciliata</i> Pers.	1	x		
<i>Eleocharis</i> sp.	1	x		
<i>Psidium</i> sp.	1			x
<i>Trachypogon plumosus</i> (H. & B ex Willd.) Nees	7	x	x	x
<i>Trachypogon vestitus</i> Andersson	5			x

similar in the three areas with about 80% of 1100-1300 mm falling within the period of May-October.

Measurement of  $\delta^{15}\text{N}$  was performed on homogenized 2 mg samples using an elemental analyzer (Carlo Erba) coupled to an isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany) at the University of Utah (USA) SIRFER facility. The nitrogen gas resulting from the oxidation/reduction reactions was collected on

silica gel using a trapping box and introduced to the mass spectrometer, operating in a dual inlet mode. All samples are referenced to atmospheric diatomic nitrogen. Sample precision was better than  $\pm 0.3\text{‰}$  (J. Ehleringer, University of Utah, personal communication).

Nitrogen concentration was measured using a micro-Kjeldahl technique (Jackson 1964). Ureides (allantoin plus allantoic acid),  $\alpha$ -amino compounds



**Fig. 1.** Distribution of organic nitrogen concentration and  $\delta^{15}\text{N}$  values (‰) of superficial soils (0-15 cm) and plant leaves from the three savanna areas. Plant species were separated in three groups: grasses+sedges, non-legume tree species and legume species.

and nitrate contents were determined in dried samples of shoots and leaves as described in Izaguirre-Mayoral *et al.* (1992). The relative abundance of ureides was calculated as in Sicardi de Mallorca & Izaguirre-Mayoral (1993).

## Results

### *Nitrogen concentration and $\delta^{15}\text{N}$ values*

Soils sampled at the three savanna sites were characterized by low N concentrations and positive  $\delta^{15}\text{N}$  values (Fig. 1), but there was a clear tendency for  $\delta^{15}\text{N}$  value to increase from Mata Negra to Urica and La Iguana (Table 2). Some sandy soils in Mata Negra showed comparatively low  $\delta^{15}\text{N}$  values (<1.0 ‰). Nitrogen content of soils was fairly homogeneous but all samples from La Iguana site had consistently higher N concentrations. Leaves of legume species showed significantly higher N concentration and lower  $\delta^{15}\text{N}$  values compared to leaves of non-legume species (Fig. 1). Among the latter grasses and sedges showed lower N concentrations than leaves of non-legume tree species. Notice that only 3 out of 33 legume samples had positive  $\delta^{15}\text{N}$  values (between 0.3 and 1.3 ‰). The averages ranged from -0.8 ‰ in La Iguana to -1.2 ‰ in Mata Negra (Table 2). The lowest  $\delta^{15}\text{N}$  values measured corresponded to *Eriosema crinitum* (-2.7 ‰) and *Aeschynomene* sp. (-1.8 ‰). Of the non-legume species only 6 out of 30 samples had negative values. Five of them corresponded to two species of the genus *Trachypogon*, the dominant grass genus in these savannas and the other corresponded to the sedge *Dichromena ciliata*. The highest values were found within the Cyperaceae, particularly in species of *Bulbostylis* (8.0 and 8.4 ‰). The  $\delta^{15}\text{N}$  values of non-legumes at each site were generally more negative than the soils, with the exception of the

**Table 2.** Average nitrogen concentration and  $\delta^{15}\text{N}$  values of the savanna sites investigated. In each column numbers followed by the same letter are not statistically different ( $P \leq 0.01$ ). (n) number of samples.

Site	(n)	Soils		(n)	Legumes		(n)	Non-legumes		Average $\Delta$ ( $\delta^{15}\text{N}_{\text{plant-soil}}$ )	
		N ( $\text{mg g}^{-1}$ )	$\delta^{15}\text{N}$ (‰)		N ( $\text{mg g}^{-1}$ )	$\delta^{15}\text{N}$ (‰)		N ( $\text{mg g}^{-1}$ )	$\delta^{15}\text{N}$ (‰)	Legumes (‰)	Non-legumes (‰)
Mata Negra	8	0.8a	2.8a	11	23.1a	-1.2a	10	8.7a	2.4a	-4.0a	-0.4a
Urica	2	0.6a	4.5a	5	24.8a	-1.1a	2	8.8a	3.1a	-5.6b	-1.4a,b
La Iguana	24	1.3b	5.2b	17	20.8a	-0.8a	18	7.3a	3.1a	-6.0b	-2.1b
Legumes vs non-legumes Statistics											
		N			$\delta^{15}\text{N}$			$\Delta$ ( $\delta^{15}\text{N}_{\text{plant-soil}}$ )			
Mata Negra		P<0.001			<0.001			<0.001			
Uric		P<0.001			<0.001			0.018			
La Iguana		P<0.001			0.02			<0.001			

Cyperaceae that were always more positive than the average value determined for soil samples of the same site. The difference  $\Delta(\delta^{15}\text{N}_{\text{plant-soil}})$  between legumes vs. non-legumes was highly significant (Table 2). It remained fairly constant among sites (3.6, 4.2, and 3.9) possibly indicating that the relative contribution of  $\text{N}_2$ -fixation to plant N is similar in the three sites.

We separated the data set in 4 groups in order to clarify the relationships between presumably symbiotically  $\text{N}_2$ -fixing plants and non-fixing plants (Table 3). All the legume species measured showed negative  $\delta^{15}\text{N}$  values, except *Clitoria*, with a value close to zero. Cyperaceae showed the most positive average  $\delta^{15}\text{N}$  values while the grasses and non-leguminous trees were highly variable.

#### *Ureides abundance and $\delta^{15}\text{N}$ values*

At the La Iguana site we collected several legume species previously shown to be active  $\text{N}_2$ -

**Table 3.** Average  $\delta^{15}\text{N}$  values of different plant groups in *Trachypogon*-savannas of Venezuela (n= number of samples).

	(n)	$\delta^{15}\text{N} \text{‰(s.d.)}$
Legumes		
<i>Aeschynomene</i>	2	-1.8 (1.0)
<i>Bowdichia</i>	3	-0.4 (0.8)
<i>Chamaecrista</i>	5	-1.0 (0.8)
<i>Clitoria</i>	1	0.3
<i>Eriosema</i>	5	-1.9 (0.7)
<i>Galactia</i>	8	-1.0 (0.6)
<i>Indigofera</i>	1	-1.0
<i>Phaseolus</i>	3	-0.7 (1.4)
<i>Stylosanthes</i>	2	-0.8 (0.9)
<i>Zornia</i>	1	-0.5
Cyperaceae	8	6.3 (1.7)
( <i>Bulbostylis</i> , <i>Cyperus</i> , <i>Eleocharis</i> , <i>Dichromena</i> )		
Grasses		
<i>Aristida</i>	1	4.2
<i>Trachypogon</i>	12	0.6 (1.4)
Non-Leguminous trees		
<i>Byrsonima</i>	2	3.0 (0.4)
<i>Casearia</i>	1	6.1
<i>Curatella</i>	5	2.2 (1.5)
<i>Psidium</i>	1	1.0

fixers and ureides producers (Sicardi de Mallorca & Izaguirre-Mayoral 1993) to examine the relationship between natural abundance of  $^{15}\text{N}$  and ureide concentration. Simultaneously, a series of non-legumes, presumably without any associative  $\text{N}_2$ -fixation activity, were sampled as references. The group of legumes had significantly higher percentages of ureide and  $\alpha$ -amino compounds than the reference group (non-legumes) (Table 4). As expected the difference between  $\delta^{15}\text{N}$  values among these groups was also highly significant. In the group of reference plants *Bulbostylis capillaris* showed, unexpectedly, high relative ureide content. This species, as the rest of the Cyperaceae sampled, had comparatively high  $\delta^{15}\text{N}$  values suggesting that it is not an  $\text{N}_2$ -fixer.

## Discussion

The upper 15 cm of soils reported here had an average  $\delta^{15}\text{N}$  of  $4.6 \pm 1.9 \text{‰}$  (n=34), well within the range reported for temperate soils (Shearer *et al.* 1978; Shearer & Kohl 1986). This value is similar to those reported by Abbadie *et al.* (1992) for tropical savannas in Africa and by Yoneyama *et al.* (1993) for Cerrado soils in Brazil. Although these values correspond to total soil N, it is expected that plants depending solely on soil N for their N nutrition should be also relatively enriched in  $^{15}\text{N}$ . This was the case for the majority of non-legume plants (Table 2).

To estimate the amount of atmospheric N that is being taken up by the plants we need to know the isotopic fractionation occurring during absorption of soil inorganic nitrogen compounds with different  $^{15}\text{N}$  content, and that taking place during the process of  $\text{N}_2$  fixation by associative or symbiotic systems. Mariotti *et al.* (1980) measured in the shoots of cultivated plants the isotopic fractionation that takes place during nitrate uptake by a range of non-legume species and non-nodulating legume species, and also the fractionation during  $\text{N}_2$ -fixation by nodulated legumes cultivated in N-free nutrient solutions. The average values indicated that the fractionation during  $\text{NO}_3$ -uptake by legumes and non-legumes is not statistically different and averaged  $-0.3 (\pm 0.6) \text{‰}$ , while the average discrimination by legumes grown in N-free solutions amounted to  $-1.1 (\pm 0.4) \text{‰}$ . In *Prosopis juliflora* cultivated under similar conditions Shearer & Kohl (1986) obtained a value of  $-1.3 \text{‰}$ .

**Table 4.** Percent composition of alcoholic extracts of branchlets or leaf bases and  $\delta^{15}\text{N}$  values of leaf material from legume and non-legume species at La Iguana (Edo. Guárico) during the dry season. RAU (relative abundance of ureids=  $[400 \times \text{Ureides}] / [4 \text{ Ureides} + \text{NO}_3]$ ). Each row represents a different sample. Significance of the differences tested with a factorial ANOVA using a Fisher's Protected Least Significant Difference test (StatView, Abacus Concepts Inc, California).

Species	Ureides %	$\alpha$ -amino %	$\text{NO}_3$ %	Total Leaf N $\text{mmol g}^{-1}$	$\delta^{15}\text{N}$ ‰	RAU %
Legumes						
<i>Bowdichia virgilioides</i>	10.5	41.1	48.3	1.11	-0.9	46
	0.0	57.9	42.1	0.99	0.6	0
<i>Chamaecrista tetraphylla</i>	13.5	64.4	22.2	1.66	-0.7	71
	11.9	70.2	17.9	1.65	-0.7	73
	10.4	66.1	23.5	1.72	-1.1	64
<i>Clitoria guyanensis</i>	6.6	78.6	14.8	1.67	0.3	64
<i>Eriosema crinitum</i>	6.9	74.2	18.9	1.19	-1.4	59
	7.5	65.1	27.4	1.10	-2.7	52
<i>Eriosema simplicifolia</i>	6.2	69.8	24.0	1.22	-1.2	51
<i>Galactia jussieuana</i>	6.1	81.7	12.2	1.31	-1.0	67
	5.2	84.1	10.7	1.86	-0.9	66
	4.9	83.5	11.6	1.52	-0.4	63
<i>Phaseolus gracilis</i>	8.8	77.5	13.7	1.88	-1.4	72
<i>Stylosanthes guyanensis</i>	8.2	61.9	29.9	1.23	-1.4	52
av.	7.6	69.7	22.7	1.4	-0.9	57
stdev.	3.4	11.7	11.3	0.3	0.8	18
Non-Legumes						
<i>Aristida</i> sp.	0.0	75.0	25.0	0.41	4.2	0
<i>Bulbostylis capillaris</i>	7.5	51.7	43.3	0.27	8.0	41
	1.9	60.5	37.6	0.60	8.4	17
<i>Casearia sylvestris</i>	0.0	60.0	40.1	1.11	6.1	0
<i>Curatella americana</i>	0.0	17.5	82.5	0.51	3.7	0
<i>Psidium</i> sp.	0.0	31.5	68.5	0.59	1.0	0
av.	1.6	49.4	49.5	0.6	5.2	10
stdev.	3.0	21.1	21.5	0.3	2.8	17
Significance of						
Legume vs non Legumes	0.001	0.012	0.001	<0.0001	<0.0001	<0.0001

Therefore, we would expect that all the plants with  $\delta^{15}\text{N}$  values equal or lower than -1.1 ‰ have got most of their N from the atmosphere. Using average numbers of Table 2 to calculate the contribution of atmospheric  $\text{N}_2$  to the N-nutrition of the legumes with the model of Shearer & Kohl (1986) results in values of 100%. There was, however, a large variation of the  $\delta^{15}\text{N}$  values of the reference plants. Within the non-legume species  $\delta^{15}\text{N}$  values ranged from 1.0 (*Psidium* sp.) to 8.4 (*Bulbostylis capillaris*). In any case, the consis-

tency of negative values in legumes previously shown to be  $\text{N}_2$ -fixers, strongly supports the use of  $\delta^{15}\text{N}$  as an indicator of long-term  $\text{N}_2$  fixation by symbiotic legume-*Rhizobium* associations under natural conditions.

Yoneyama *et al.* (1986) demonstrated that the shoots of ureide-transporting legumes had negative  $\delta^{15}\text{N}$  values, and that the isotopic fractionation occurring during  $\text{N}_2$ -fixation in the same plants ranged from -0.2 to -2 ‰. The legumes reported here are ureide-accumulators and showed

generally negative  $\delta^{15}\text{N}$  values. However, the correspondence between these values is not simple, because  $\delta^{15}\text{N}$  values integrate  $\text{N}_2$ -fixation over the whole leaf life-span, and ureide concentration results from current nitrogenase activity. We do not have an explanation for the relatively high RAU values of *B. capillaris*.

Medina & Bilbao (1991) and Abbadie *et al.* (1992) reported that the majority of legume species measured in tropical savannas had negative  $\delta^{15}\text{N}$  values, approaching the minimum expected for plants depending exclusively on atmospheric  $\text{N}_2$  fixation. More recently Sprent *et al.* (1996) published a survey of  $\delta^{15}\text{N}$  values of legumes from Cerrado vegetation in Brazil. The authors called the attention to the frequency of negative  $\delta^{15}\text{N}$  values of legume species, something that is not common in other seasonal vegetation (Handley *et al.* 1994; Heaton 1987; Schulze *et al.* 1991; Virginia & Delwiche 1982; Yoneyama *et al.* 1993). This contrast requires further investigation.

In a large survey of  $\delta^{15}\text{N}$  values in South Africa, Heaton (1987) showed that there is an inverse relation between aridity and  $\delta^{15}\text{N}$  values. The negative relationship is very clear in the case of the grasses reported. This may explain why the values published for tropical savannas, with relatively high rainfall levels are more negative than those of semi-arid, subtropical areas.

The finding that *Trachypogon* spp. had often negative  $\delta^{15}\text{N}$  values, in the range of those measured in nodulated legumes, raises the question of the ecological significance of the associative  $\text{N}_2$ -fixation in these species. Abbadie *et al.* (1992) also found that several dominant grass species in Ivory Coast, Africa, had negative  $\delta^{15}\text{N}$  values in the range of the legumes measured in the same grasslands. Their explanation was that the grasses derive most of their assimilated N from the decay of root material with a  $\delta^{15}\text{N}$  value of  $-1.1\text{‰}$ . The fraction derived from associative fixation of atmospheric  $\text{N}_2$  was calculated to be  $\approx 17\%$ . However, Döbereiner and co-workers have reported that the association of free-living diazotrophs with grass roots may contribute a large fraction of the N requirements of native and cultivated grasses (see Boddey & Döbereiner 1995). In natural grasslands  $\text{N}_2$ -fixing bacteria associated with grass roots have been reported to fix annually several kg of N per ha (Balandreau *et al.* 1976; Santaella 1985). The  $\text{N}_2$ -fixation activity of grass root asso-

ciations may be comparable to that of nodulated legumes, as reported by Massdorp (1987) for *Paspalum urvillei*. In addition, several reports indicate important nitrogenase activity in soil cores of savannas in Africa and South America (Berkum & Day 1980; Zietsman *et al.* 1988), although the contribution of this activity to the accumulation of available nitrogen in the soil may be of little ecological significance (Giller & Day 1985). The N budget of *Trachypogon* grasslands requires between  $6\text{--}9\text{ kg N ha}^{-1}\text{ yr}^{-1}$  in order to maintain the level of soil N (Chacón *et al.* 1991; Medina 1982). This amount of N may be supplied by  $\text{N}_2$ -fixation by soil blue-green algae and by grass-soil systems as measured by Santaella (1985) and reevaluated by Chacón *et al.* (1991). Using the Shearer & Kohl (1986) model to calculate potential contribution of associative  $\text{N}_2$ -fixation results in values of 40–95% with an average of 68%. The numbers used in this calculation were: *Trachypogon* spp. ( $n=12$ )  $\delta^{15}\text{N}=0.56 \pm 1.41\text{‰}$ ; reference plants ( $n=19$ )  $\delta^{15}\text{N}=4.05 \pm 2.67\text{‰}$ .

We suggest as a tentative conclusion that the negative  $\delta^{15}\text{N}$  values of *Trachypogon* spp. and other tropical grasses result from rhizospheric and/or endophytic fixation of atmospheric  $\text{N}_2$ , that in the long-term decrease the concentration of  $^{15}\text{N}$  in shoot and root litter. This  $\text{N}_2$  fixation activity mediated by root-bacterial associations in tropical savannas may have interesting implications for the analysis of terrestrial-atmospheric interactions. Increasing atmospheric  $\text{CO}_2$  concentrations resulting from burning of fossil fuels and deforestation in the tropics may increase the photosynthetic capacity of both legumes and grasses. This in turn may stimulate  $\text{N}_2$ -fixation by plant root-microorganism associations, since their activity is limited by energy supply in form of soluble sugars released by the plant hosts.

Research on the nitrogen regulation of biomass production in natural savannas should emphasize the differentiation in nitrogen sources available to plant groups such as the Cyperaceae, the dominant tuft grasses, and the herbaceous, shrubby, and tree legumes. A comprehensive approach using extensive  $^{15}\text{N}$  measurements of living and dead biomass, throughout the year, together with a direct assessment of nitrogenase activity, will be essential to reach a definitive conclusion on the significance of different associative  $\text{N}_2$  fixation pathways by legumes and grasses in tropical savannas.

## Acknowledgments

We are thankful to the University Simón Rodríguez for the access to the facilities of La Iguana station (Edo. Guárico). Marta Francisco (Centro de Ecología, IVIC) and Octavio Carballo (Centro de Microbiología, IVIC) provided skillful technical assistance in the laboratory determinations.

## References

- Abbadie, L., A. Mariotti & J.-C. Menaut. 1992. Independence of savanna grasses from soil organic matter for their nitrogen supply. *Ecology* **73**: 608-613.
- Balandreau, J., R. N'Dri Allou, G. Villemin, P. Weinhard & P. Villecourt. 1976. Fixation rhizosphérique de l'azote en Savane de Lamto. *Revue d' Ecologie et Biologie du Sol* **13**: 529-544.
- Barrios, S. & V. González. 1971. Rhizobial symbiosis on Venezuelan savannas. *Plant and Soil* **34**: 707-719.
- Berkum van, P. & J.M. Day. 1980. Nitrogenase activity associated with soil cores of grasses in Brazil. *Soil Biology and Biochemistry* **12**: 137-140.
- Boddey, R.M. & J. Döbereiner. 1995. Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future. *Fertilizer Research* **42**: 241-250.
- Chacón, P., I.D. López-Hernández & M. Lamotte. 1991. Le cycle de l'azote dans une savane à *Trachypogon* au centre du Venezuela. *Revue d' Ecologie et Biologie du Sol* **28**: 67-75.
- Giller, K. E. & J.M. Day. 1985. Nitrogen fixation in the rhizosphere: significance in natural and agricultural systems. pp.127-147. *In*: A.H. Fitter (ed.) *Ecological Interactions in Soils*. Blackwell Scientific Publishers, London.
- Handley, L., D. Odee & C.M. Scrimgeour. 1994.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  patterns in savanna vegetation: dependence on water availability and disturbance. *Functional Ecology* **8**: 306-314.
- Heaton, T. H. E. 1987. The  $^{15}\text{N}/^{14}\text{N}$  ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia* **74**: 236-246.
- Herridge, D.F., F.J. Bergersen & M.B. Peoples. 1990. Measurement of nitrogen fixation by soybean in the field using the ureide and natural  $^{15}\text{N}$  abundance methods. *Plant Physiology* **93**: 708-716.
- Högberg, P. & I.J. Alexander. 1995. Role of root symbioses in African woodland and forest: evidence from  $^{15}\text{N}$  abundance and foliar analysis. *Journal of Ecology* **83**: 217-224.
- Izaguirre-Mayoral, M. L., O. Carballo, S. Flores, M. Siccardi de Mallorca & T. Oropeza. 1992. Quantitative analysis of the symbiotic  $\text{N}_2$ -fixation, non-structural carbohydrates and chlorophyll content in sixteen native legume species collected in different savanna sites. *Symbiosis* **12**: 293-312.
- Jackson, M. L. 1964 *Análisis Químico de Suelos*. Ed. Omega. Barcelona.
- Maasdorp, B.V. 1987. Contribution of associative  $\text{N}_2$ -fixation (acetylene reduction) in some grassland ecosystems in Zimbabwe. *Soil Biology and Biochemistry* **19**: 7-12.
- Mariotti, A., F. Mariotti, N. Amarger, G. Pizelle, J.-M. Ngambi, M.L. Champigny & A. Moysse. 1980. Fractionnements isotopiques de l'azote lors des processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plantes. *Physiologie Vegetal* **18**: 163-181.
- Medina, E. 1982. Nitrogen balance in the *Trachypogon* grasslands of central Venezuela. *Plant and Soil* **67**: 305-314.
- Medina, E. 1996. Biodiversity and nutrient relations in savanna ecosystems: interactions between primary producers, soil microorganisms and soils. pp. 45-57. *In*: O.T. Solbrig, E. Medina & J. Silva (eds.) *Biodiversity and Savanna Ecosystem Processes*. Ecological Studies 121. Springer Verlag Berlin.
- Medina, E. & B. Bilbao. 1991. Significance of nutrient relations and symbiosis for the competitive interaction between grasses and legumes in tropical savannas. pp. 295-320. *In*: G. Esser & D. Overdieck (eds.) *Modern Ecology: Basic and Applied Aspects*. Elsevier Publishing Company. Amsterdam.
- Medina, E. & O. Huber. 1992. The role of biodiversity in the function of savanna ecosystems. pp. 139-158. *In*: O.T. Solbrig, H.M. van Emden & P.G. W.J. van Oordt (eds.) *Biodiversity and Global Change*. International Union of Biological Sciences, Monograph No. 8, Paris.
- Pate, J.S., G.R. Stewart & M. Unkovich. 1993.  $^{15}\text{N}$  abundance of plant and soil components of a *Bankisia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and  $\text{N}_2$ -fixing abilities of component species. *Plant, Cell and Environment* **16**: 365-373.
- Santaella, S.E. 1985. *Fijación Biológica de Nitrógeno en Sabanas de Trachypogon spp.* Trabajo Especial de Grado. Escuela de Biología, Universidad Central de Venezuela. Caracas.
- Schulze, E.-D., G. Gebauer, H. Ziegler & O.L. Lange. 1991. Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. *Oecologia* **88**: 451-455.

- Shearer, G. & D.H. Kohl. 1986. N<sub>2</sub>-fixation in field settings: estimations based on natural <sup>15</sup>N abundance. *Australian Journal of Plant Physiology* **13**: 699-755.
- Shearer, G., D.H. Kohl & S. Chien. 1978. The nitrogen-15 abundance in a wide variety of soils. *Soil Science Society of America Journal* **42**: 899-902.
- Sicardi de Mallorca, M. & M.L. Izaguirre-Mayoral. 1993. A comparative evaluation of the symbiotic N<sub>2</sub>-fixation and physiological performance of thirty-six native legume species collected in a tropical savanna during the rainy and dry seasons. *Symbiosis* **16**: 225-247.
- Sprent, J.I., I.E. Geoghegan, P.W. Whitty & E.K. James. 1996. Natural abundance of <sup>15</sup>N and <sup>13</sup>C in nodulated legumes and other plants in the cerrado and neighbouring regions of Brazil. *Oecologia* **105**: 440-446.
- Virginia, R.A. & C.C. Delwiche. 1982. Natural <sup>15</sup>N abundance of presumed N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing plants from selected ecosystems. *Oecologia* **54**: 317-325.
- Yoneyama, T., K. Fujita, T. Yoshida, T. Matsumoto, I. Kambayashi & J. Yazaki. 1986. Variation in natural abundance of <sup>15</sup>N among plant parts and <sup>15</sup>N/<sup>14</sup>N fractionation during N<sub>2</sub> fixation in the Legume-*Rhizobia* symbiotic system. *Plant and Cell Physiology* **27**: 791-799.
- Yoneyama, T., T. Muraoka, T. Murakami & N. Boonkerd. 1993. Natural abundance of <sup>15</sup>N in tropical plants with emphasis on tree legumes. *Plant and Soil* **153**: 295-304.
- Zietsman, P.C., N. Grobbelaar & N. van Rooyen. 1988. Soil nitrogenase activity of the Nylsvlei Nature Reserve. *South African Journal of Botany* **54**: 21-27.