

N availability and mechanisms of N conservation in deciduous and semideciduous tropical forest legume trees

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RESUMO – (Disponibilidade de N e mecanismos de conservação de N em leguminosas arbóreas decíduas e semidecíduas de floresta tropical). Antes da abscisão, os nutrientes são removidos das folhas e redistribuídos para outras partes da planta. Quando se relaciona o reaproveitamento de nutrientes à fertilidade do solo, ou à longevidade foliar, os dados da literatura são controversos. Foram comparados mecanismos de conservação de nitrogênio (N) em quatro leguminosas arbóreas (*Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang., *Lonchocarpus guilleminianus* (Tul.) Malme, *Enterolobium contortisiliquum* (Vell.) Morong e *Peltophorum dubium* (Spreng.) Taub.), com longevidades foliares diferentes, de uma Floresta Semidecídua, remanescente da mata Atlântica. O objetivo do trabalho foi verificar se esses mecanismos são diferentes nas quatro espécies e se são afetados pela longevidade foliar e disponibilidade de N, tanto mineral, como proveniente de fixação simbiótica, no caso de *L. guilleminianus* e *E. contortisiliquum*, que apresentam associação com rizóbios. As plantas foram cultivadas em vasos contendo solo da mata, enriquecido (50 ou 100 mg de NH_4NO_3 planta⁻¹semana⁻¹) ou não com N mineral. *H. courbaril*, uma semidecídua que não fixa N e possui a maior longevidade foliar dentre as espécies analisadas, apresentou a maior eficiência de reaproveitamento de N (ERN), proficiência de reaproveitamento de N (PRN) e eficiência de utilização de N (EUN). O acréscimo de N no solo e a presença de fixação simbiótica de N levaram a decréscimos em ERN, PRN e EUN.

Palavras-chave: longevidade foliar, fixação de nitrogênio, eficiência de reaproveitamento de nitrogênio, proficiência de reaproveitamento de nitrogênio, eficiência do uso de nitrogênio

ABSTRACT – (N availability and mechanisms of N conservation in deciduous and semideciduous tropical forest legume trees). Prior to abscission, nutrients are redeployed from leaves and redistributed to other parts of the plant. Data comparing nutrient resorption to soil fertility and leaf life span remains controversial in the literature. We compared nitrogen (N) conservation mechanisms among four legume trees with different leaf life spans (*Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang., *Lonchocarpus guilleminianus* (Tul.) Malme, *Enterolobium contortisiliquum* (Vell.) Morong and *Peltophorum dubium* (Spreng.) Taub.), from a semideciduous tropical forest, remnant of the Atlantic Forest. We hypothesized that these mechanisms differ among the four species and are affected by their leaf life span and by the availability of N, both as a mineral in the soil and, in the case of *L. guilleminianus* and *E. contortisiliquum*, from symbiotic nitrogen fixation (SNF), as these species form associations with rhizobia. The plants were grown in a greenhouse using pots filled with forest soil, enriched (50 or 100 mg of NH_4NO_3 plant⁻¹week⁻¹) or not with nitrogen. *H. courbaril*, a semideciduous tree, without SNF, and with the highest leaf life span, presented the greatest N-resorption efficiency (NRE), N-resorption proficiency (NRP) and N-use efficiency (NUE). Increase in soil N and the presence of symbiotic N fixation led to a decrease in NRE, NRP and NUE.

Key words: leaf life span, nitrogen fixation, nitrogen resorption efficiency, nitrogen resorption proficiency, nitrogen-use efficiency

Introduction

Evergreen species have a lower concentration of leaf nutrients and a longer leaf life span than deciduous species. These are important mechanisms for nutrient economy, making possible the colonization of low fertility soils (Alerts 1996; Eamus & Prichard 1998). Removal of nutrients from leaves prior to abscission and their redeployment to other tissues is known as nutrient

resorption (Wright & Westoby 2003) and is considered an important adaptation of certain species to less fertile ecosystems (May & Killingbeck 1992; Pugnaire & Chapin 1993). Therefore, evergreen species are likely to show greater resorption efficiency in comparison to deciduous ones. However, consensus has not been reached in the literature comparing these two groups of plants (Aerts 1996; Lal *et al.* 2001) and attempts to relate nutrient resorption efficiency to soil fertility have

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led to controversy (Aerts 1996; Eckstein *et al.* 1999; Cordell *et al.* 2001; Carrera *et al.* 2003).

Killingbeck (1996) concluded that plants which perform symbiotic N fixation (SNF) presented lower N-resorption proficiency (NRP), and N-resorption efficiency (NRE) than those which do not. Resorption efficiency is the difference between the nutrient concentration in green leaves and senescent leaves, given as a percentage (Distel *et al.* 2003), whilst resorption proficiency is the absolute value by which nutrients are reduced in senescent leaves. Thus, the lower the concentration of a nutrient in senescent leaves, the greater the resorption proficiency (Killingbeck 1996).

The presence of root nodules in legumes that have nitrogen-fixing potential is an indication that such plants possess another nitrogen (N) source in addition to that available from the mineral and organic fraction of the soil. However, these nodules may not be functional. The efficiency of the N₂ fixation process can be evaluated by the nitrogenase activity, presence of leghemoglobin and protein concentration within the nodules (Cresswell *et al.* 1992; Sprent 2001).

There are indications that plants with a long leaf life span produce more organic material per unit of mineral nutrient than those with shorter leaf life spans (Chapin 1980; Aerts *et al.* 1999). This ratio represents the nutrient use efficiency (Vitousek 1982). A number of studies have stressed that nutrient use efficiency also increases as soil nutrients decrease (Vitousek 1984; Distel *et al.* 2003). However, no differences were found in the N-use efficiency (NUE) between two evergreen species and two deciduous species from the Australian savanna (Eamus & Prichard 1998).

The aim of the present work was to compare N conservation mechanisms (NRE, NRP and NUE) among four Semideciduous Forest legume trees with different leaf life spans, growing under different availabilities of nitrogen (in mineral form or derived from SNF).

Material and methods

Characterization of species and site – Four legume tree species from the Santa Genebra Reserve, Campinas, São Paulo State, Brazil (22°49'45" S, 47°06'33" W) were chosen for this study. The site is a fragment of the Atlantic Forest (Morellato & Leitão Filho 1995) with an area of 251,000 m² and an altitude of 670 m. The soil is classified as Distrophic Purple Latossoil (Oliveira *et al.* 1979). The Santa Genebra Forest is classified as a Semideciduous Seasonal Forest as it harbors a diverse number of tree species that shed their leaves during the cold and dry season (Morellato & Leitão Filho 1995). Two of the species used in this study are classified as semideciduous species and the other two as deciduous. The semideciduous species shed their leaves during a definite time of the year but are never totally leafless, whilst the deciduous species shed all their leaves during a specific time of the year, remaining totally bare for a period that can vary from a week to several months (Morellato *et al.* 1989). The classification of the four legumes according to the subfamily, presence of nodules for SNF and leaf shedding can is found in Table 1.

Plant material – Seeds of *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang., *Lonchocarpus guilleminianus* (Tul.) Malme, *Enterolobium contortisiliquum* (Vell.) Morong and *Peltophorum dubium* (Spreng.) Taub. were collected in 2001 and stored at a temperature of 10 °C. Seeds from *H. courbaril* and *E. contortisiliquum* were scarified with concentrated sulphuric acid for 1 hour and those from *P. dubium* were scarified for 30 minutes. They were then washed with distilled water. Seeds from the four species germinated at a temperature of 25 °C within a 12-hour photoperiod. The plants were grown in a greenhouse, under natural temperature and sunlight conditions, in plastic pots (5 L

Table 1. Classification of the legume tree species according to subfamilies, presence (+) or absence (–) of symbiotic nitrogen fixation nodules and leaf deciduity.

Species	Subfamily	Nodulation (Reference)	Leaf deciduity (Reference)
<i>Hymenaea courbaril</i> L. var. <i>stilbocarpa</i> (Hayne) Lee et Lang	Cesalpinoideae	– (Faria <i>et al.</i> 1989)	Semideciduous (Lorenzi 1992)
<i>Lonchocarpus guilleminianus</i> (Tul.) Malme	Papilionoideae	+ (Faria <i>et al.</i> 1989)	Semideciduous (Morellato <i>et al.</i> 1989; Lorenzi 1992)
<i>Enterolobium contortisiliquum</i> (Vell.) Morong	Mimosoideae	+ (Faria <i>et al.</i> 1989)	Deciduous (Lorenzi 1992)
<i>Peltophorum dubium</i> . (Spreng.) Taub.	Cesalpinoideae	– (Faria <i>et al.</i> 1989)	Deciduous (Lorenzi 1992)

capacity) filled with soil from the Santa Genebra Forest. Irrigation was performed through daily sprinklings. The experiment was concluded after 419 days, when all plants (approximately 50 cm tall) presented senescent leaves for analysis.

The following soil treatments were applied from February 2002 to May 2003: control plants, in which soil from Santa Genebra Reserve was used, and plants grown in this same soil but enriched with mineral N (50 or 100 mg of N plant⁻¹week⁻¹) in the form of NH₄NO₃.

In order to ensure nodulation in species associated with rhizobia (with symbiotic nitrogen fixation-SNF), inoculation was carried out with slow growth rhizobia strains (2 mL/pot), previously isolated from root nodules (Vincent 1970), collected from the same species *in situ*.

Leaf characterization – Growth curves were produced based on the length of the central rachis, so as to verify when leaf growth was completed and nutrient resorption could start. The leaves were classified as mature green (when they had reached their maximum growth) and senescent (with a yellowish coloration and dropped when touched). The mean and standard deviation of the rachis length for mature green leaves of each species were as follows: *H. courbaril*: 8.8 cm ± 2.2; *L. guilleminianus*: 17.8 cm ± 3.5; *P. dubium*: 6.7 cm ± 0.4 and *E. contortisiliquum*: 13.9 cm ± 2.9. Leaf life span was monitored using dated plastic tape placed around 1cm long petioles of the same leaves described above.

Total chlorophyll concentration was determined in mature green and senescent leaves in order to find the point in which its concentration decreases and thus the beginning of senescence and N resorption processes (Smart, 1994 - data not shown).

Determination of N-resorption efficiency (NRE), N-resorption proficiency (NRP), N-use efficiency (NUE) and total N in tissues- Mature green leaves and senescent leaves were collected on a single occasion and at the same time for all four species. Subsamples of leaves weighing 100 mg per plant were dried at 65 °C for 7 days and total N determined according to Nelson & Sommers (1973). The N concentration in senescent leaves was used as an indicator of NRP (Killingbeck 1996).

NRE was calculated by: $NRE = (N_{\text{mature green}} - N_{\text{senescent}}) / N_{\text{mature green}} \times 100\%$, where: $N_{\text{mature green}}$ = N in mature green leaves, $N_{\text{senescent}}$ = N in senescent leaves (Pugnaire & Chapin 1993).

NUE was calculated by: $NUE (\text{g of dry mass mg}^{-1} \text{ of N}) = 1 / [N_{\text{mature green}} \times (1 - r)]$, where: $N_{\text{mature green}}$ =

total N concentration in mature green leaves and $r = \text{NRE}$ expressed in terms of a fraction (Aerts *et al.* 1999).

Determination of nodule activity – The leghemoglobin level was determined according to Becana *et al.* (1986) whilst total proteins were determined according to Bradford (1976). Concentrations of total proteins and leghemoglobin were calculated based on root dry mass. Specific nitrogenase activity was assessed by the acetylene reduction technique (Hardy *et al.* 1968) on intact plants (Gomes & Sodek 1984). SNF is evident in the two species (Tab. 2).

Table 2. Nodules dry mass (DM), leghemoglobin (LegHb) and total protein concentrations and nitrogenase activity (ARA), of semideciduous (*Lonchocarpus guilleminianus*) and deciduous (*Enterolobium contortisiliquum*) legume trees with symbiotic N fixation, cultivated in soil from the Santa Genebra Reserve. Data represent means ± standard deviation (n = 5).

Analyses	<i>L. guilleminianus</i>	<i>E. contortisiliquum</i>
DM (g)	0.13 ± 0.02	0.52 ± 0.17
LegHb (mg g ⁻¹ DM)	2.46 ± 0.17	3.61 ± 0.44
Total protein (mg g ⁻¹ DM)	12.58 ± 1.30	17.12 ± 1.79
ARA (ethylene μmoles g ⁻¹ DM h ⁻¹)	8.73 ± 0.70	27.97 ± 7.69

Experimental design and statistical analysis – Statistical analyses were performed with SAS/STAT software (1990). The normality of data distribution was determined with the Kolmogorov-Smirnov test. The data were subjected to an analysis of variance and the means compared for significant differences by Duncan's Multiple Range test at 5% probability level. Percentage data in NRE analyses were converted into arcsine, and analysis of variance was then applied. A total of five replicates (each represented by one leaf for each plant) was used for each species, in a completely randomized design, for chlorophyll, total protein, leghemoglobin and nitrogenase activity analyses, whereas three replicates were used for NRE, NRP and NUE calculations, and ten replicates for leaf life span and leaf growth curves.

Results and discussion

Semideciduous species (mean ± standard deviation for *H. courbaril*: 419 ± 54.8 days and for *L. guilleminianus*: 340 ± 9.7days) presented leaf life spans at least twice those of the deciduous species

(*P. dubium*: 152 ± 23.1 days and *E. contortisiliquum*: 139 ± 11.9 days). A longer leaf life span is regarded as a mechanism for conserving nutrients since it reduces the loss of minerals during leaf abscission (Aerts 1995; 1996; Carrera *et al.* 2003; Eckstein *et al.* 1999).

Nodules were found only in plants growing in soil without the addition of N (control) (Tab. 2) which could be explained by the fact that mineral N applications hinder nodule formation. Studies have shown that applications of NH_4^+ and NO_3^- inhibit the association of rhizobia during the formation of the nodules (Streeter 1988; Guo *et al.* 1992; Waterer & Vessey 1993; Gonçalves *et al.* 1999).

Figure 1 shows the N-resorption efficiency (NRE) results for the four species. When comparing the species without symbiotic nitrogen fixation (SNF), the semideciduous species, *H. courbaril*, had a greater NRE than the deciduous species, *P. dubium*. Regarding the relationship between NRE and N availability from symbiotic fixation, the species with SNF (*L. guilleminianus* and *E. contortisiliquum*) presented a lower NRE in relation to the others. Adding N to the soil also resulted in a decrease in NRE (Fig. 2).

The decrease in NRE given a greater availability of N is further evidence of the existence of a negative feedback mechanism between resorption efficiency and soil fertility (Berendse 1994; Jones *et al.* 1994; Aerts 1995; Feller *et al.* 1999; Enoki & Kawaguchi 1999; Anderson & Eickmeier 2000). Moreover, NRE is reportedly lower in species with SNF than in species that do not fix N_2 (Côte & Dawson 1986; Côte *et al.* 1989; Killingbeck 1993). However, the relationship between soil fertility and NRE should not be generalized. Feller *et al.* (2003) found that the addition of N to the soil did not alter the NRE of *Metrosideros*

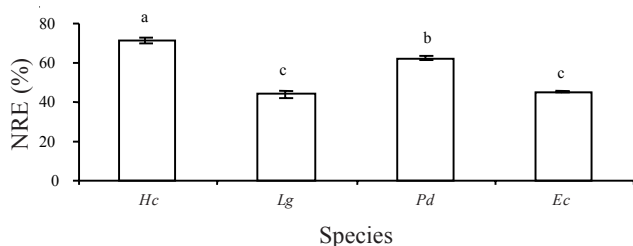


Figure 1. N resorption efficiency (NRE) in semideciduous and deciduous legume trees with and without symbiotic N fixation (SNF): *Hc* - *Hymenaea courbaril* (semideciduous - without SNF), *Lg* - *Lonchocarpus guilleminianus* (semideciduous - with SNF), *Pd* - *Peltophorum dubium* (deciduous - without SNF), and *Ec* - *Enterolobium contortisiliquum* (deciduous - with SNF). Bars indicate standard deviation ($n = 3$). Different letters indicate differences between species (Duncan 5%).

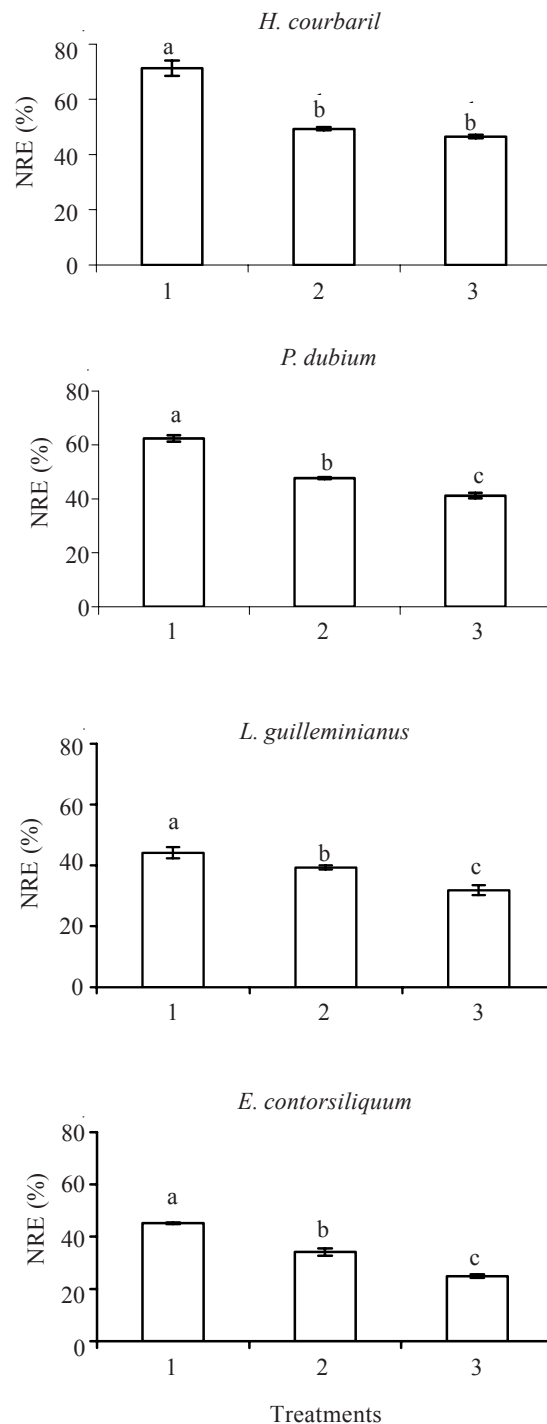


Figure 2. N resorption efficiency (NRE) in semideciduous and deciduous legume trees with and without symbiotic N fixation (SNF): *H. courbaril* (semideciduous - without SNF), *L. guilleminianus* (semideciduous - with SNF), *P. dubium* (deciduous - without SNF), and *E. contortisiliquum* (deciduous - with SNF). Plants were grown in different soils: 1 - soil from the forest, 2 - soil from the forest enriched with $50 \text{ mg of NH}_4\text{NO}_3 \text{ plant}^{-1} \text{ week}^{-1}$ and 3 - soil from the forest enriched with $100 \text{ mg of NH}_4\text{NO}_3 \text{ plant}^{-1} \text{ week}^{-1}$. Bars indicate standard deviation of means ($n = 3$). Different letters indicate differences between treatments (Duncan 5%).

polymorpha. Likewise, Distel *et al.* (2003) did not find any difference in the NRE of *Stipa gynerioides*, a species restricted to a less fertile environment, and *Stipa brachychaeta*, a species that grows in a fertile environment. The authors concluded that the different distribution of the two species cannot be explained by NRE efficiency.

Resorption proficiency is considered a more stable indicator of the plant capacity to reuse nutrients than resorption efficiency (Killingbeck 1996). An increase in the availability of N for the plant, due to SNF, leads to a decrease in N-resorption proficiency (NRP), that is, there is an increase in the concentration of N in the senescent leaves (Killingbeck 1996; Killingbeck & Whitford 2001). The data from the present study agree with these observations, given that the N concentrations found in senescent leaves for species without SNF were much lower than those with SNF (Fig. 3). After comparing the NRP of many species, Killingbeck (1996) established that this parameter can be considered efficient when the concentration of N in senescent leaves is lower than 7.0 mg g⁻¹ of dry mass. Our results show that species without SNF had NRP values that were much lower than 7.0 mg g⁻¹ of dry mass (2.7 mg g⁻¹ of dry mass for *H. courbaril* and 3.6 mg g⁻¹ of dry mass for *P. dubium*) whilst the NRP values of the species with SNF had values that exceeded this threshold (9.0 mg g⁻¹ of dry mass for *L. guilleminianus* and 7.1 mg g⁻¹ of dry mass for *E. contortisiliquum*).

Distel *et al.* (2003) verified that species which inhabit less fertile soils present high NRP values when compared to species growing in fertile soils. Our results agree with this observation since an increase in the availability of N by adding it to the soil, led to an

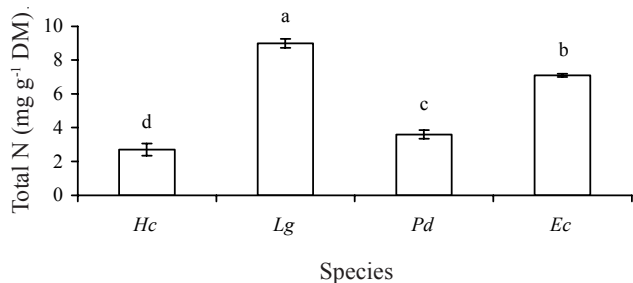


Figure 3. Total N in senescent leaves of semideciduous and deciduous legume trees with and without simbiotic N fixation (SNF): *Hc* - *Hymenaea courbaril* (semideciduous - without SNF), *Lg* - *Lonchocarpus guilleminianus* (semideciduous - with SNF), *Pd* - *Peltophorum dubium* (deciduous - without SNF), and *Ec* - *Enterolobium contortisiliquum* (deciduous - with SNF). Bars indicate standard deviation (n = 3). Different letters indicate differences between species (Duncan 5%). DM = dry mass.

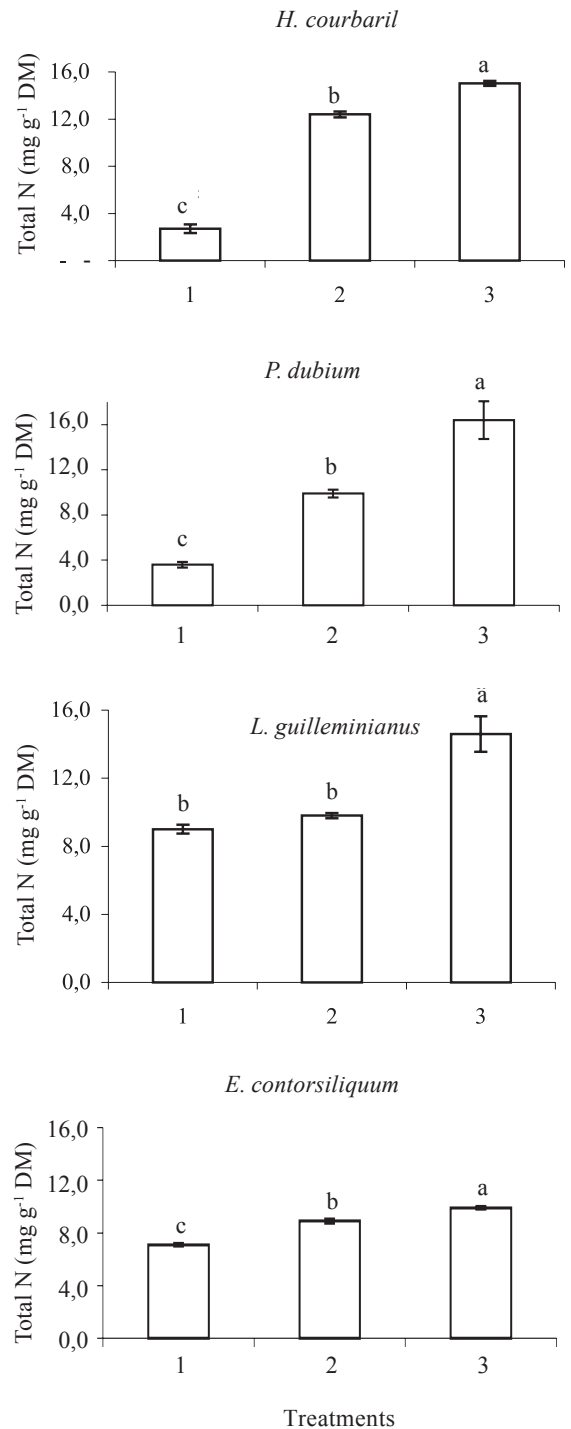


Figure 4. Total N in senescent leaves of semideciduous and deciduous legume trees with and without simbiotic N fixation (SNF): *Hymenaea courbaril* (semideciduous - without SNF), *Lonchocarpus guilleminianus* (semideciduous - with SNF), *Peltophorum dubium* (deciduous - without SNF), and *Enterolobium contortisiliquum* (deciduous - with SNF). Plants were grown in different soils: 1 - soil from the forest, 2 - soil from the forest enriched with 50 mg of NH₄NO₃ plant⁻¹ week⁻¹ and 3 - soil from the forest enriched with 100 mg of NH₄NO₃ plant⁻¹ week⁻¹. Bars indicate standard deviation of means (n = 3). Different letters indicate differences between treatments (Duncan 5%). DM = dry mass.

increase in the concentration of N in the senescent leaves of the four species (Fig. 4).

N-use efficiency (NUE) for the two species without SNF was higher for the semideciduous species than for the deciduous ones (Fig. 5). Similar data were obtained by Haddad *et al.* (2004) when comparing *H. courbaril* with another deciduous species, *Croton priscus*, from the Santa Genebra Reserve.

An increase in the availability of N through SNF (Fig. 5) or by the addition of this mineral to the soil (Fig. 6) resulted in lower NUE values, confirming previous reports (Vitousek 1982; Knops *et al.* 1997; Cordell *et al.* 2001; Distel *et al.* 2003). According to Feller *et al.* (1999) and Hiremath *et al.* (2002) the NUE value is inversely proportional to soil nutrient availability. They concluded that this parameter helps to explain the species distribution in natural communities, since the species with a greater efficiency in using minerals are more adapted to colonize less fertile environments.

In conclusion, the N conservation mechanisms (NRE, NRP and NUE) of the four legume trees species decreased with the increase of N concentration in the soil and with SNF. When comparing the species without SNF, the species with a longer leaf life span (*H. courbaril*) had more efficient N conservation mechanisms. No relationship was found between leaf life span and N conservation mechanisms in the species that fix N₂. It is possible that *L. guilleminianus* is more efficient at conserving N if it grows in environments with a low availability of N and/or in the absence of nitrogen fixing bacteria. Since only four species were compared in this study, further studies with a larger number of semideciduous forest species, with different leaf life spans, are necessary in order to obtain a more

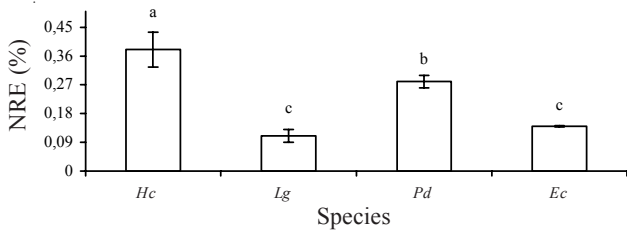


Figure 5. N use efficiency (NUE) of semideciduous and deciduous legume trees with and without symbiotic N fixation (SNF): *Hc* - *Hymenaea courbaril* (semideciduous - without SNF), *Lg* - *Lonchocarpus guilleminianus* (semideciduous - with SNF), *Pd* - *Peltophorum dubium* (deciduous - without SNF), and *Ec* - *Enterolobium contortisiliquum* (deciduous - with SNF). Bars indicate standard deviation of means (n = 3). Different letters indicate differences between species (Duncan 5%). DM = dry mass.

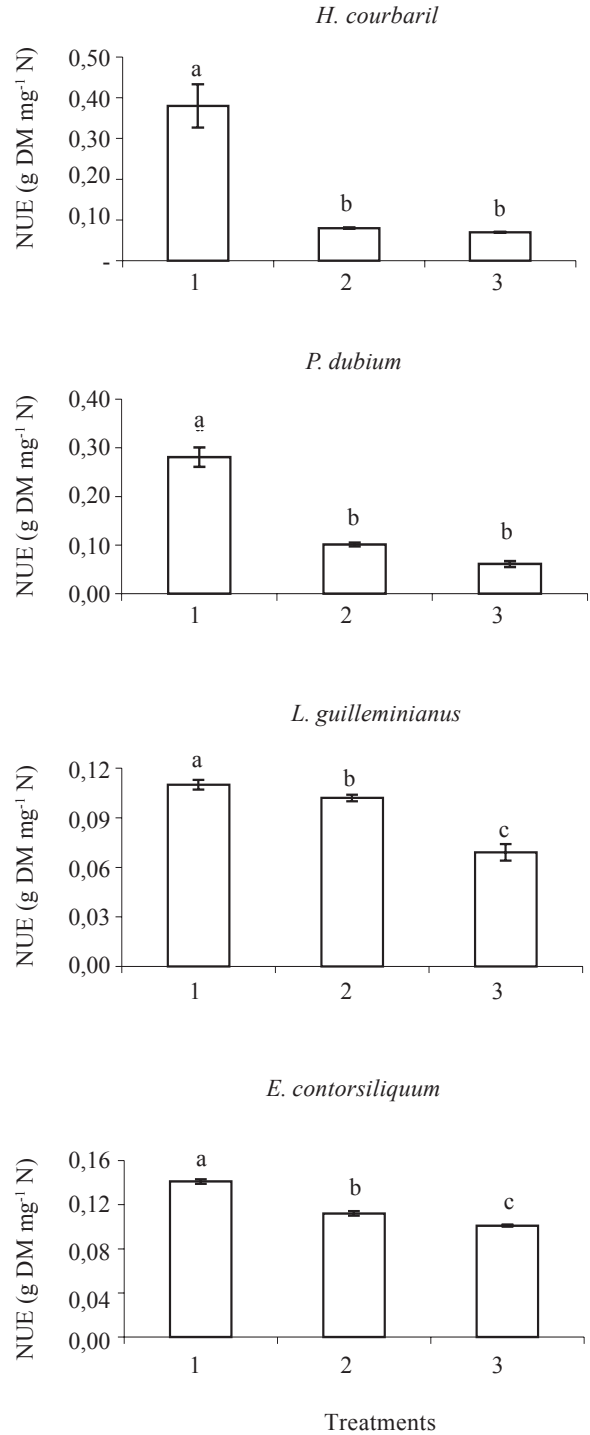


Figure 6. N use efficiency (NUE) of semideciduous and deciduous legume trees with and without symbiotic N fixation (SNF): *H. courbaril* (semideciduous - without SNF), *L. guilleminianus* (semideciduous - with SNF), *P. dubium* (deciduous - without SNF), and *E. contortisiliquum* (deciduous - with SNF). Plants were grown in different soils: 1 - soil from the forest, 2 - soil from the forest enriched with 50 mg of NH₄NO₃ plant⁻¹ week⁻¹ and 3 - soil from the forest enriched with 100 mg of NH₄NO₃ plant⁻¹ week⁻¹. Bars indicate standard deviation of means (n = 3). Different letters indicate differences between treatments (Duncan 5%). DM = dry mass.

general picture of the relationship between N conservation mechanisms and leaf life spans in this type of environment.

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