# BIOLOGICAL NITROGEN FIXATION AND NUTRIENT RELEASE FROM LITTER OF THE GUACHAPELE LEGUMINOUS TREE UNDER PURE AND MIXED PLANTATION WITH EUCALYPTUS

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**ABSTRACT:** *Pseudosamanea guachapele* (guachapele), a nitrogen fixing leguminous tree, is an alternative for mixed forest plantations in the tropics. As little information is available for guachapele (Mimosoideae) in mixed plantation with eucalyptus considering the Brazilian edaphoclimatic conditions, an experiment was carried out to evaluate the contribution of biological nitrogen fixation to guachapele and leaf litter decomposition rates and nutrient release of eucalyptus and guachapele residues (pure and mixed). The percentage of nitrogen derived from atmospheric $N_2$ (% Ndfa) was estimated by comparing the natural $^{15}N$ abundance ($\delta^{15}N$, ‰) in guachapele tissues with that of *Eucalyptus grandis*, a non-nitrogen fixing species, both with seven years after planting. Decomposition constants ($k$) and litter half-lives ($t^{1/2}$) were estimated by fitting a single exponential model to litter bag data. The estimation of %Ndfa for guachapele in pure stand fell within a narrower range (17-36 %) in relation to mixed conditions (35-60 %). Nitrogen concentration in leaf litter was positively related to the decomposition rate, decreasing from pure guachapele to pure eucalyptus. Half-lives ($t^{1/2}$) were significantly different ($p < 0.05$) among residues with 148, 185 and 218 days, for guachapele leaves, mixture of both species and for pure eucalyptus, respectively. Nutrient release rates followed the same sequence of $t^{1/2}$ due to the initial residues quality (mainly N). It was observed that a fast release of N, K and Mg occurred from the residues tested, mainly for guachapele and mixed stand. These results indicate that guachapele could benefit the mixed system from the N addition and a faster decomposition rate of a richer litter.

Key words: *Eucalyptus grandis*; litter decomposition; $N^{15}$ natural abundance; nutrients cycling; *Pseudosamanea guachapele.*

# FIXAÇÃO BIOLÓGICA DE NITROGÊNIO E LIBERAÇÃO DE NUTRIENTES DE FOLHAS DA SERAPILHEIRA DA LEGUMINOSA GUACHAPELE EM PLANTIO PURO E CONSORCIADO COM EUCALIPTO

***RESUMO:*** *A Pseudosamanea guachapele (guachapele), leguminosa arbórea fixadora de nitrogênio, é uma alternativa para plantios florestais mistos nos trópicos. Como são escassas as informações sobre a espécie em plantios mistos de eucalipto em condições edafoclimáticas brasileiras, foi conduzido um experimento no qual objetivou-se avaliar a contribuição da fixação biológica de nitrogênio para a guachapele e a velocidade de decomposição e de liberação de nutrientes de folhas senescentes de eucalipto e guachapele (oriundas dos plantios puros e consorciado). A porcentagem de N derivado da atmosfera (% Ndfa) foi estimada comparando-se a abundância natural de* $^{15}N$ *(*$\delta^{15}N$*, ‰) nos tecidos da guachapele com a observada nos tecidos do Eucalyptus grandis, espécie não fixadora, ambas com sete anos de idade. A constante de decomposição (k) e a meia-vida (*$t^{1/2}$*) de serapilheira foram estimadas utilizando-se o modelo exponencial aplicado aos dados oriundos de coletas de litterbags. A estimativa da %Ndfa para guachapele, em condições de plantio puro, variou de 17 a 36%, enquanto que, em condições de plantio consorciado, foi de 35 a 60 %. A concentração de N nas folhas senescentes estava positivamente relacionada com a taxa de decomposição, sendo essa decrescente da guachapele para o eucalipto. A* $t^{1/2}$ *dos resíduos diferiu significativamente (*$p < 0.05$*), sendo de 148, 185 e 218 dias para as folhas de guachapele, mistura das duas espécies e eucalipto, respectivamente. A liberação dos nutrientes (principalmente N, K e Mg) das folhas seguiu a mesma ordem da* $t^{1/2}$ *devido à qualidade inicial das mesmas. Os resultados indicam que a guachapele pode beneficiar o plantio misto pela adição de N e por meio da intensificação da decomposição da serapilheira.*

*Palavras-chave: Eucalyptus grandis; decomposição de serapilheira; abundância natural de* $N^{15}$*; ciclagem de nutrientes; Pseudosamanea guachapele.*

## 1 INTRODUCTION

Eucalyptus plantations occupy nearly 3 Mha in Brazil and the wood is employed especially in the production of cellulose, pulp and energy. This fast growing tree accumulates from 10 to 20 Mg ha$^{-1}$ as trunk biomass per year, containing over 200 kg N ha$^{-1}$ at the final of rotation (with seven years). In Brazil, since most Eucalyptus

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plantations have been established on weathered soils, characterized by their low nutrient and organic matter contents, the imbalance for soil N generated by higher N outputs and low N fertilization is a potential threat for soil degradation. Moreover the plantations are generally monospecific. The use of leguminous tree species is advisable as $N_2$-fixing species and would help providing residues for soil organic matter accumulation and N enrichment in poor soils, with a high potential for benefiting from a companion species in the case of mixed forestry systems (FORRESTER et al., 2006; KHANNA, 1997). Mixtures can be used as a silviculture system to improve timber quality (DeBELL et al., 1997).

Although species and site attributes should be considered during the implementation of mixed plantation, the capacity of fixing $N_2$ could be considered a key factor for the selection of a leguminous tree, because the effects of the leguminous litter on the overall residue decomposition of the system must also be taken into account. Briones & Ineson (1996), evaluating the decomposition of eucalyptus leaves in litter mixtures with oak (*Quercus petraea*), ash (*Fraxinus excelsior*) and birch (*Betula pendula*), concluded that the litter decomposition in mixtures could not be readily predicted from the behavior of the component litter that decomposes in isolation. These authors observed a greater N mineralization from the mixture of eucalyptus + ash compared with oak, eucalyptus and birch alone and in mixture. According to O'Connell et al. (2003), the low-quality residues of eucalyptus affects the soil organic matter and reduces soil N supply.

Mixed plantations of eucalyptus with nitrogen fixing trees (NFT), although not popular in Brazil, has been studied in South and Southeast Brazil (BALIEIRO et al., 2002; COÊLHO et al., 2007; VEZZANNI et al., 2001). The dominance (in height) of *Eucalyptus grandis* over *Pseudosamanea guachapele* (ex. *Albizia guachapele*) and the input of nitrogen-rich litter from guachapele accelerate the residue decomposition rate and further the eucalyptus growth (BALIEIRO, 2002; BALIEIRO et al., 2004; FROUFE, 1999). As eucalyptus plantations have been associated with the reduction of litter consumption by millipedes (soil fauna) (CORREIA, 2003), flora activities (REZENDE et al., 2001) and anti-microbial effects (MOURA et al., 1996), low nutrient availability may be associated with the slow decomposition rate of its litter. Therefore, mixed plantations of eucalyptus with NFT could be an alternative to maintain the high eucalyptus productivity rate and to synchronize nutrient release with plant uptake, in addition to the increase in soil fertility.

The present study was conducted to: (a) estimate the contribution of biological nitrogen fixation to guachapele in pure and mixed systems, and (b) evaluate the patterns of litter decomposition and N, P, K, Ca and Mg release from residues.

## 2 MATERIAL AND METHODS

### 2.1 Site

This study was carried out in Seropédica, state of Rio de Janeiro (22º 46' of latitude South, 43º 41' of longitude West) at the experimental field of Embrapa – Agrobiologia (Agrobiology Research Center), located at 33 m above sea level. The mean annual rainfall is 1,250 mm, mostly concentrated in the summer, and the mean air temperature ranges from 16 ºC (June and July) to 32 ºC (January to March). The mean annual relative air humidity is 73 %.

The soil belongs to the Ecologia Series, Class of Planosols (Abruptic Arenic Ochraquult). The upper horizon is sandy to 1.0 m depth in some locations (Table 1). A previous soil sampling (in 1993) before the experiment settlement, was performed at the 0-20cm layer and determined soil pH (5.3), $Al^{3+}$ content (0.2 $cmol_c/dm^3$), exchangeable $Ca^{2+}+Mg^{2+}$ (1.3 $cmol_c/dm^3$), extracted with 1mol/L KCl, and available P (10.3 $mg/dm^3$) and K (21 $mg/dm^3$), both extracted with Mehlich-1 (EMBRAPA, 1997). The site topography ranges from flat to a gentle slope and was used as a pasture for 10 years before trees were planted.

**Table 1** – Some physical and chemical properties of the experimental area (0-20 cm).

***Tabela 1*** – *Algumas propriedades físicas e químicas do solo da área experimental (0-20cm).*

| Bulk Density | Sand | Silt | Clay | pH | $Al^{+3}$ | $Ca^{+2} + Mg^{+2}$ | P | K |
|---|---|---|---|---|---|---|---|---|
| $g.cm^{-3}$ | ---------- | % | ----------- | | | --- $cmol_c.dm^{-3}$ --- | -- $mg.dm^{-3}$ | ---- |
| 1.39 | 92 | 2 | 6 | 5.3 | 0.2 | 1.3 | 10.3 | 21 |

## 2.2 Plant species

Three-months-old seedlings of guachapele (*Pseudosamanea guachapele*), a leguminous tree native of Central America and Mexico, and eucalyptus (*Eucalyptus grandis*), an Australian native tree, were produced at Embrapa Agrobiology Center and planted in pure and mixed stands in 1993. Guachapele seeds were inoculated with efficient *Rhizobium* sp. strains (BR6205 and BR6821 from Embrapa Agrobiology) at sowing. Both species were also inoculated with a mixture of mycorrhizal fungi *Glomus clarum* and *Gigaspora margarita*. Each stand occupied an area of 2.500 $m^2$, with plants spaced 3 m between rows and 1 m within rows (3,333 plants $ha^{-1}$). The mixed plantation was implemented by intercropping rows of guachapele with eucalyptus (same density, but 1,667 plants of guachapele and the same number of eucalyptus per ha). Each transplanted seedling received 100 g of “Araxá” rock phosphate + 10 g of fritted trace elements (FTE BR-12: B=1.8; Cu=0.8; Fe=3.0; Mn=3.0; Mo=0.1 and Zn=9.0 %). For eucalyptus, there was an application of 25 g ammonium sulfate per linear meter at 0.5 m from the planting rows. Evaluations were performed in the 1,000 $m^2$ central area of each stand. As eucalyptus and a large number o leguminous species have a low potassium requirement during initial stage, with critical level lower than this found at beginning at the experiment (BALIEIRO et al., 2001; DIAS et al., 2002; FERNANDEZ et al., 1996; NOVAIS et al., 1980) no fertilizer of this source was applied.

## 2.3 Sampling and Isotopic analysis

After seven years of growth, 12 trees, 3 from each species in each stand were sampled. The trees were selected as representing the mean diameters at breast height (DBH) of the trees from each stand (DBH of guachapele in pure and mixed system: 10.1 and 8.3 cm, respectively; and of eucalyptus in pure and mixed system: 12.4 and 16.0 cm, respectively). After weighted in the field, randomized samples of leaves (collected from total mass), branches (with different diameter and collected at different canopy parts) were taken together with 5 cm thick stems disks withdrawn at the heights of 90, 100 and 130 cm from the soil surface from both species and dried at 70 $^0$C under forced aeration up to constant weight. Bark samples was taken from stem discks. All plant samples were ground (<2 mm) and N concentration was determined by steam distillation after digestion with sulfuric acid plus catalyzing mixture (BREMNER, 1965). All plant samples were analyzed for $^{15}N$ natural abundance using a Finnigan Delta Plus continuous-flow isotope-ratio mass spectrometer interfaced with a Carlo Erba (Model EA 1108) automatic C N analyser (Finnigan- MAT, Bremen, Germany). None information of plant mortality existed until the moment of sampling.

## 2.4 Calculation of biological nitrogen fixation

The natural abundance of $^{15}N$ of the $N_2$ in the air is constant (0.3663 at% $^{15}N$) (JUNK & SVEC, 1958). The small difference in $^{15}N$ abundance between soil N and air are usually expressed as $\delta^{15}N$ or parts per thousand (‰) relative to the $^{15}N$ composition of atmospheric $N_2$ (HÖGBERG, 1997):

$$\delta^{15}N\ (‰) = \frac{1000 \text{ x } (\text{at\% } ^{15}N \text{ sample} - 0.3663)}{0.3663},$$

hence, by definition, $\delta^{15}N$ composition of air is zero.

When plants are grown in conditions where they rely entirely on BNF for N supply, the resulting $\delta^{15}N$ value of the plants is usually negative.

The use of weighted $\delta^{15}N$ values for whole shoots of the $N_2$-fixing and control species for the quantification of BNF is highly advisable, when higher values of $\delta^{15}N$ is find in leaves of both species compared to other plant parts (BODDEY et al., 2000). The weighed mean $^{15}N$ abundance of the whole plant ($\delta^{15}Nw$) of eucalyptus and guachapele was calculated through the following equation:

$$\delta^{15}N_w = \frac{[(\delta^{15}N_L \text{ x } CNL) + (\delta^{15}N_B \text{ x } CN_B) + (\delta^{15}N_b \text{ x } CN_b) + (\delta^{15}N_S \text{ x } CN_S)]}{(CN_L + CN_B + CN_b + CN_S)},$$

where subscripts of $\delta^{15}N$ refer to leaves (L), branches (B), bark (b) and stems (S); and CN is the total N accumulated in L, B, b and S. CN data were extracted from Balieiro (2002).

It was assumed that the $\delta^{15}N$ of the eucalyptus plants represented the $\delta^{15}N$ of the N soil mineral available to guachapele.

The percentage of N derived from atmospheric $N_2$ (%Ndfa) was determined using the following equation:

$$\%\ Ndfa = \frac{(\delta^{15}N_{euc} - \delta^{15}N_{gua})}{(\delta^{15}N_{euc} - B)} \text{ x } 100,$$

where *B* represents the isotopic fractionation that occurs during the biological N fixation. For guachapele, there is no B value reported in literature. Thus, the extremes of the *B* values range listed in Boddey et al. (2000) for leguminous tree and shrub species of -2.89 and -0.34 were used. It was assumed that %Ndfa for guachapele would fall within this range.

The amount of N fixed (Nfixed) in guachapele was calculated with the equation:

$$\text{Nfixed} = \frac{\%\text{Ndfa}}{100} \times \text{Nyield},$$

where Nyield is the total N accumulated (kg $ha^{-1}$) by guachapele under pure (636,2 kg $ha^{-1}$) and mixed crop (167,8 kg $ha^{-1}$). These informations were extracted from Balieiro (2002).

### 2.5 Decomposition and nutrient release from litter

Samples of senescent leaves from pure and mixed stands of eucalyptus and guachapele were collected before reaching the soil in April and June of 2000, air-dried and stored until July, when the experiment started. As leaves are the most common component of the litter (approximately 60%) (BALIEIRO et al., 2004), this material was selected to be placed into litter bags.

Ten grams of dried leaf litter (65 °C for > 72 h) were placed into a nylon litter bag (25 x 25 cm with a 3.0 x 6.0 mm mesh) (ANDERSON & INGRAM, 1996). Fresh guachapele leaf litter was also tested and did not present any significant difference for the decomposition constant (*k*) when compared with the oven-dried material (data not shown). A total of 30 bags per plantation were placed on the top of the litter layer under field conditions, representing five replicates per collecting date in the corresponding stand on the 30th of July.

The contents of the litter bags were weighted and analyzed at 15, 30, 45, 105, 165 and 225 days after placement in the field. The decomposition constant *k* was estimated using the exponential decay equation below:

$$X_t = X_0 \,.\, e^{-kt},$$

where $X_t$ is the dry weight of the litter remaining in the litter bag after t days and $X_o$ is the litter dry weight at t = 0 days. The half life ($t^{1/2}$) of litter (and associated nutrients), or the time for half of the initial litter (or nutrients) to disappear (released), was calculated based on the expression:

$$t^{1/2} = \ln(2)/k,$$

where *k* is the decomposition constant estimated from the previous expression.

Oven-dried leaf litter samples (before and after field placement) were chemically characterized as described before for aboveground tissues. Polyphenols were determined only for the litter before incubation using the Folin-Denis method as described by Anderson & Ingram (1996).

Monthly rainfall and temperature means for the period of the experiment were recorded (Figure 1).

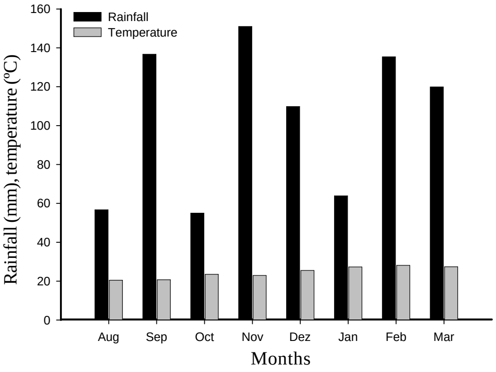

**Figure 1 –** Monthly means of the rainfall and temperature in Seropédica, Rio de Janeiro State, for the period of the litter bag experiment (2000/01).

***Figura 1*** *– Médias mensais da precipitação e da temperatura em Seropédica, Rio de Janeiro, durante o período de condução do experimento de litter bag (2000/01).*

### 2.6 Analysis of data

The $\delta^{15}N$ (‰) signals of the aboveground components of each species were compared through the t-test with 5% of probability. Data from litter decay and nutrient release were fitted to the single exponential function to determine decay constants and half-lives (a probability of 5 % was used to validate the half lives of the nutrient models). The curves of the remaining nutrient litter contents were fitted using the math module of the SigmaPlot software.

## 3 RESULTS AND DISCUSSION

### 3.1 Natural abundance of $^{15}N$ ($\delta^{15}N$) and biological nitrogen fixation

The $\delta^{15}N$ values ranged from –0.58 to +4.37 for guachapele and from +1.31 to +4.12 for eucalyptus depending on the aboveground tissue analysed (Table 2). Considering the different components of guachapele, bark presented the lowest $\delta^{15}N$ value followed by stems, branches and leaves, under both conditions. However, values for leaves were only significantly higher than those for bark and stem. The $\delta^{15}N$ in bark was only significantly lower in relation to branches. Higher $\delta^{15}N$ values were observed in leaves of eucalyptus both in pure or mixed stands, but no differences were observed between other plant tissues.

**Table 2 –** $\delta^{15}N$ (‰) values in different aboveground biomass compartments of guachapele and eucalyptus, under pure and mixed crops.

***Tabela 2** – Valores de $\delta^{15}N$ (‰) nos diferentes componentes da parte aérea de guachapele e eucalipto sob condições de plantio puro e consorciado.*

| Species-cropping systems | Leaf | Branch | Bark | Stem |
|---|---|---|---|---|
| | ‰ | | | |
| Guachapele – pure | 4.37 ± 0.40 | 1.71 ± 0.11 | 0.14 ± 0.27 | 0.44 ± 0.15 |
| Guachapele – mixed | 4.11 ± 0.47 | 0.51 ± 0.17 | -0.58 ± 0.21 | 0.26 ± 0.09 |
| Eucalyptus – pure | 3.10 ± 0.22 | 1.92 ± 0.32 | 1.97 ± 0.35 | 1.31 ± 0.32 |
| Eucalyptus – mixed | 4.12 ± 0.26 | 2.70 ± 0.12 | 2.23 ± 0.18 | 2.79 ± 0.74 |

The higher $\delta^{15}N$ values found in leaves of both species compared to other plant parts indicate a significant $^{15}N$ fractionation and that leaves would not be a valid sample for the BNF estimation (BODDEY et al., 2000). During plant growth, nitrate absorption and breakdown of organic N in leaves and its translocation to other parts of the plant result in $^{15}N$ fractionation leaving leaf tissues enriched in $^{15}N$ and sink organs like branches more depleted (EVANS, 2001). Under this condition, it is highly advisable to use weighted $\delta^{15}N$ values for whole shoots of the $N_2$-fixing and control species for BNF quantification (BODDEY et al., 2000).

The weighed $\delta^{15}N$ for the whole plant were +1.05 for guachapele in both pure and mixed stands while for eucalyptus the respective values were +1.84 and +3.12. The significant difference between guachapelle and eucalyptus for the $\delta^{15}N$ values suggests N contribution from biological nitrogen fixation (BNF) to guachapelle. Applying the extremes of B values (-2.89 to -0.34 ‰) (BODDEY et al., 2000) for the calculations of the %Ndfa for guachapele gave a potential contribution for the symbiotic process ranging from 17 to 36 % for the pure stand and from 34 to 60 % for the mixed stand with eucalyptus.

The amount of N fixed biologicaly in guachapele aboveground biomass under pure and mixed plantation ranged from 108.1 to 229.0 and 58.7 to 100.7 kg $ha^{-1}$ , respectively, when the data from Balieiro (2002) was used. It is important mentioning that the uncertainties related to the high heterogeneity of natural systems (GHERING & VLEK, 2004) would be attenuated in this study due to soil preparation for planting and the small number of species used, possibly reducing errors in the BNF estimation (HÖGBERG, 1997).

The higher contribution of BNF to guachapele in the mixed stand is explained by the competition between the legume and the associated eucalyptus plants for the soil N availability (GEHRING et al., 2005). It is known that litterfall and root turnover could contribute to the increase on the soil N availability and to the decrease on the need for biological nitrogen fixation, especially in adult stands and forests (FARIA, 1984; GEHRING et al., 2005; MOREIRA et al., 1992), and as under pure plantation of guachapele 248 kg $ha^{-1}$ of N reach the soil by litterfall whereas under mixed, only 66 kg $ha^{-1}$ (BALIEIRO et al., 2004), this hypothesis could be confirmed.

### 3.2 Leaf litter decomposition and nutrient release

Leaf litter decomposition was negatively related to the half-lives of the litter and decreased as follows: pure guachapele > mixed > eucalyptus. Half-lives were significantly different among residues with 148, 185 and 218 days, for leaf-litter of guachapele, mixture of both species and for pure eucalyptus, respectively. Decomposition constants (*k*) were estimated for guachapele, eucalyptus and the mixed stand: 0.0047, 0.0032 and 0.0038 g $g^{-1}day^{-1}$, respectively. Both parameters were related to the higher initial concentration of nutrients in guachapele, mainly N (Table 3). Also, low polyphenol contents furthered decomposition.

The faster decomposition rate of mixture residues in relation to pure eucalyptus stand could be explained by a stimulation of the soil microbial biomass in decomposing eucalyptus leaves caused by the nutrient released from the legume. Balieiro et al. (2004) also observed similar effects using the mass balance methodology under pure and mixed stands of both species.

Rainfall and throughfall are related to litter decomposition under forest plantations (LANDBERG & GOWER, 1997; LEITE, 1996), but the differences found between the residues tested could be specifically attributed to their chemical composition since each stand did not differ in throughfall percentage (BALIEIRO, 2002).

The apparent release or losses of nutrients during litter decomposition for guachapele, eucalyptus and a mixture of both are shown in Figure 2. Guachapele and

**Table 3 –** Chemical composition of litter leaves from pure stands of guachapele, eucalyptus and a mixed stands of both species.

***Tabela 3*** *– Composição química de folhas senescentes de plantios de guachapele, eucalipto e da mistura das duas espécies.*

| Stands | N | P | K | Ca | Mg | Polyphenols |
|---|---|---|---|---|---|---|
| | | | $g.kg^{-1}$ | | | % |
| Guachapele (n=4)[1] | 25.34 | 0.76 | 5.38 | 13.38 | 7.13 | 8.52 |
| Eucalyptus (n=4) | 9.62 | 0.51 | 4.88 | 9.38 | 3.04 | 14.27 |
| Mixed[2] (n=6) | 13.69 | 0.61 | 3.58 | 9.83 | 4.49 | 6.38 (gua)[3]<br>15.57(euc) |

[1]Number of samples collected during the months of May, June and July 2000; 2 Leaves of both species were collected at the same proportion that it fall on soil; [3] Average values of sub-samples analyzed.

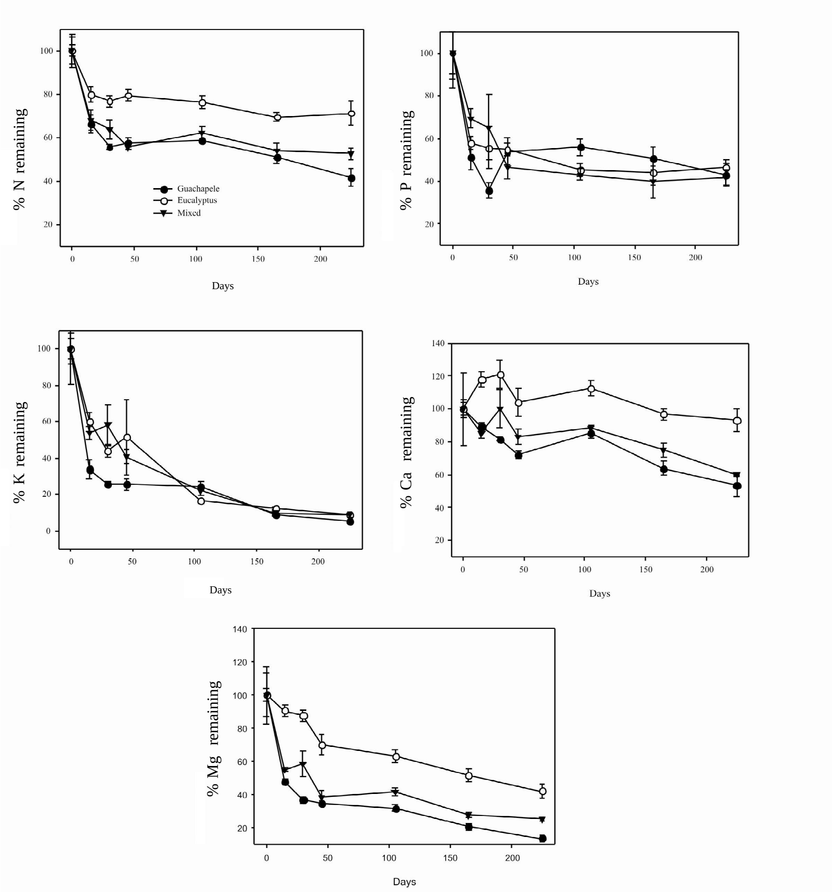

**Figure 2 –** Percentage of N, P, K, Ca and Mg remaining in guachapele and eucalyptus leaf litter during experimental period (225 days) using litter bag methodology. Bars represent the standard error (SE).

***Figura 2*** *– Porcentagem do N, P, K, Ca e Mg remanescentes em folhas senescentes de eucalipto e guachapele durante o período de experimental (225 dias) usando a metodologia do litter bag. Barras representam o erro-padrão (SE.).*

mixed litters presented similar nutrient loss pattern. K and Mg were the nutrients with the fastest releases: guachapele litter lost over 50 % of K within 2 weeks, but the half-lives of K and Mg in mixed and eucalyptus litter were 6-7 weeks. The faster potassium and magnesium releases are well documented and are related to their function in the plant (MARSCHNER, 1995) (Table 4).

Even though N release for all residues and P release for guachapele and eucalyptus litter did not fit well to exponencial decay model, a tendency to faster release from mixed litter was observed for P (154 days) when compared to eucalyptus (224 days) and guachapele residues, and for N from guachapele and mixed. Calcium presented the lowest release pattern among the nutrient, independent of the residues (Figure 2).

Patterns of N and P concentrations in decomposing litter were probably affected by precipitation just after the beginning of the experiment (Figure 1) and by nutrients immobilized there after by the soil microorganisms. The N immobilization tended to be more intense for eucalyptus litter and P for guachapele and mixed residues (Table 4).

On the other hand, Ca concentrations presented a tendency to increase from the beginning of the experiment as Ca-rich structural components are the last to be attacked by soil microrganisms (MARSCHNER, 1995). As a constituent of the more complex cellulose, hemi-cellulose and lignin molecules, the access and decomposition by the soil microorganisms was limited.

## 4 CONCLUSION

The presence of guachapele in the mixed stand represents a source of N derived from BNF. Even considering the low BNF values detected after 7 years of establishment (17-36 % in pure and 36-60 % in mixed stand)

**Table 4 –** Changes in nutrient concentrations (g.kg$^{-1}$) during the litter decomposition of guachapele, eucalyptus and mixture of both.

***Tabela 4 –*** *Mudança na concentração (g.kg$^{-1}$) de nutrientes durante a decomposição de folhas senescentes de guachapele, eucalipto e sua mistura.*

| Litter | Nutrient | Concentration (g.kg$^{-1}$) | | |
|---|---|---|---|---|
| | | Initial | Final | Change (%) |
| Guachapele | N | 24.96 ± 1.48[1] | 32.8 ± 0.58 | +31.4$^{***}$ |
| | P | 0.74 ± 0.09 | 0.99 ± 0.04 | +33.7$^{*}$ |
| | K | 5.30 ± 0.44 | 0.87 ± 0.06 | -83.62$^{***}$ |
| | Ca | 13.60 ± 0.56 | 22.60 ± 0.91 | +66.2$^{***}$ |
| | Mg | 7.10 ± 0.28 | 3.02 ± 0.20 | -57.5$^{***}$ |
| Eucalyptus | N | 9.62 ± 0.26 | 14.62 ± 0.49 | +52.0$^{***}$ |
| | P | 0.60 ± 0.10 | 0.59 ± 0.02 | -0.7$^{n.s.}$ |
| | K | 4.09 ± 0.79 | 0.78 ± 0.05 | -80.8$^{**}$ |
| | Ca | 7.69 ± 1.7 | 15.3 ± 0.63 | +99.0$^{**}$ |
| | Mg | 2.62 ± 0.46 | 2.34 ± 0.13 | -10.7$^{n.s.}$ |
| Mixed | N | 13.78 ± 1,05 | 18.11 ± 0,95 | +31.4$^{*}$ |
| | P | 0.65 ± 0.06 | 0.68 ± 0.05 | +4.0$^{n.s.}$ |
| | K | 3.70 ± 0.20 | 0.82 ± 0.11 | -77.8$^{***}$ |
| | Ca | 9.60 ± 0.53 | 14.4 ± 0.61 | +50.0$^{***}$ |
| | Mg | 4.49 ± 0.59 | 2.84 ± 0.11 | -36.8$^{*}$ |

[1] Means of five replicates ± standard error. $^{n.s., *, **, ***}$ not significant, significant at $p<0.05$, $p<0,01$ and $p<0,001$, respectively.

it would represent from 59 to 108 kg N $ha^{-1}$ $y^{-1}$ accumulated in the leguminous biomass that would be gradually introduced into the soil through litter decomposition. Apart from the extra source of N, leguminous litter can contribute to accelerate eucalyptus litter decomposition. A faster nutrient recycling other than N was also demonstrated as beneficial effect of using guachapele in mixed stand with eucalyptus.

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