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Reforestation of a Degraded Area with *Eucalyptus* and *Sesbania*: Microbial Activity and Chemical Soil Properties

Vanderlan de Oliveira Paulucio[(1)], Cristiane Figueira da Silva[(2)], Marco Antônio Martins[(3)], Marcos Gervasio Pereira[(4)*], Jolimar Antonio Schiavo[(5)] and Luciana Aparecida Rodrigues[(3)]

(1) Universidade Federal do Espírito Santo, Centro Agropecuário, Centro de Ciências Agrárias, Laboratório de Fitopatologia, Alegre, Espírito Santo, Brasil.
(2) Universidade Federal Rural do Rio de Janeiro, Departamento de Engenharia Florestal, Programa de Pós-graduação em Ciências Ambientais e Florestais, Seropédica, Rio de Janeiro, Brasil.
(3) Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Campos dos Goytacazes, Rio de Janeiro, Brasil.
(4) Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Rio de Janeiro, Brasil.
(5) Universidade Estadual de Mato Grosso do Sul, Departamento de Solos, Aquidauana, Mato Grosso do Sul, Brasil.

ABSTRACT: Mining activities generally affect soil quality, degrading it and creating the need for consistent environmental recovery efforts. This study evaluates the influence of monospecific and mixed stands of *Sesbania virgata* (S) and *Eucalyptus camaldulensis* (E) on the chemical properties and microbial activity of the soil in a degraded area by clay extraction in the northern part of the state of Rio de Janeiro, Brazil. Four treatments (100S:100 % *Sesbania*, 100E: 00 % *Eucalyptus*, 50S:50E: 50 % *Sesbania* + 50 % *Eucalyptus*, and DASV: a degraded area with spontaneous vegetation) were established according to a randomized complete block design with three replicates. Samples were collected in the 0.00-0.10 m layer in the rainy season (March) and the dry season (September). The properties evaluated were pH in water; contents of P, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, H+Al, N, and C; C/N ratio; total microbial activity (soil respiration - CO_2 emission); and total enzymatic activity (fluorescein diacetate hydrolysis). The reforestation of degraded areas by clay mining with the species *S. virgata* and *E. camaldulensis* either in monospecific or mixed stands increased the nutrient contents, C levels, and total microbial activity in the soil. It was possible to separate the planting systems (100S, 100E, 50S:50E) and the DASV using principal component analysis. In both seasons, soil C contents, chemical properties, and biological variables improved in the planted areas, in contrast with the DASV. The revegetation of degraded areas by mining improved the chemical and biological properties of the soil, leading to higher soil quality in revegetated areas compared to degraded areas with natural vegetation.

Keywords: soil respiration, fluorescein diacetate hydrolysis, clay extraction.

*** Corresponding author:**
E-mail: mgervasiopereira01@gmail.com

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INTRODUCTION

Clay mining for the production of ceramic tiles and bricks has great socioeconomic importance (more than 120 companies generating approximately 52 million dollars annually and directly creating 4,500 jobs) for the municipality of Campos de Goytacazes (RJ), Brazil. However, such activity degrades an area of approximately 3,500 m^2 per day at depths of 1 to 4 m (Ramos et al., 2006; Rodrigues et al., 2006; Valicheski et al., 2009). Some of these areas are being revegetated with single species and/or mixed commercial forest species (*Eucalyptus* spp.) and fast-growing nitrogen-fixing legumes (Mendonça et al., 2008; Santiago et al., 2009; Schiavo et al., 2010). The intention in planting such crops is to improve soil quality and produce wood (*Eucalyptus*) to be used as fuel in the manufacturing process of ceramics (Schiavo, 2005).

Planting legumes in combination with *Eucalyptus* has been suggested due to the unsatisfactory development of *Eucalyptus* stands in these revegetation areas compared to commercial plantations (Schiavo et al., 2010). These species can contribute to the nutrition and biomass production of *Eucalyptus* by increasing N availability in the soil and accelerating the nutrient cycling process (Rodrigues et al., 2003; Forrester et al., 2004; Voigtlaender et al., 2012; Silva et al., 2013; Koutika et al., 2014; Paula et al., 2015). In addition, legumes can favor the formation of humic substances in the soil, which increases the C stock and improves soil chemical properties (Vezzani et al., 2001; Garay et al., 2003; Mendonça et al., 2008; Schiavo et al., 2009; Forrester et al., 2013; Koutika et al., 2014).

Sesbania virgata is an important legume for recovery of clay mining sites because it occurs frequently in natural revegetation of abandoned pits, its seeds are highly available, and it is able to form symbioses with N_2-fixing bacteria (Samôr, 1999; Coutinho et al., 2006; Schiavo et al., 2010). In addition, some authors (Santos et al., 2013; Silva et al., 2013) have observed positive effects of this species on the chemical and biological properties of the soil.

The continuous build-up of litterfall on the ground is one of the main issues in forest management. This build-up is a consequence of long rotation periods, and, together with root death, increases the amount and permanence of soil organic matter (Gatto et al., 2010; Viera et al., 2014). Organic matter has been reported as one of the main soil properties affecting the size and activity of the microbial population (Klose and Tabatabai, 2000; Carneiro et al., 2009). The significant role played by microorganisms in the sustainability and productivity of terrestrial systems has been widely investigated (Silva et al., 2004; Schulz et al., 2013). Microorganisms are directly or indirectly responsible for a variety of microbiological and biochemical processes, especially in nutrient cycling and soil fertility (Carvalho et al., 2008; Schulz et al., 2013).

Microbial respiration and the hydrolysis of fluorescein diacetate (FDA) are some of the parameters most commonly used to evaluate microbial activity in soils; they define the total enzymatic activity in a soil where microorganisms are the main source of enzymes (Pereira et al., 2004; Green et al., 2006; Batista et al., 2008; Carvalho et al., 2008; Carneiro et al., 2009; Evangelista et al., 2012). Research has shown that these factors are significantly influenced by soil use and management methods (Pell et al., 1998; Stenström et al., 1998; Arshad and Martin, 2002; Schloter et al., 2003; Gil-Sotres et al., 2005; Batista et al., 2008).

Respiration rates are a key source of information regarding CO_2 emissions. It is known that respiration may be affected by any imbalance in soil biological components, such as organic matter levels and soil biota, as a consequence of the management practices adopted in a given area (Carvalho et al., 2008). Because residue decomposition increases the levels of nutrients available to plants, high short-term respiration rates may be interpreted as a desirable characteristic (Carter, 1986; Roscoe et al., 2006; Batista et al., 2008).

The hydrolysis of FDA measures the specific activity of proteases, lipases, esterases, and other enzymes that hydrolyze FDA (Guilbault and Kramer, 1964; Rotman and Papermaster, 1966; Taylor et al., 2002). These enzymes catalyze several reactions of central importance in the life cycle of microorganisms, the decomposition of organic residue during nutrient cycling, the formation of organic matter, and the establishment of a given soil structure (Burns, 1978). Therefore, recovery of the capacity to hydrolyze FDA may affect the energy cycle in the soil-plant system, and this capacity becomes a parameter of central importance in strategies for restoring areas under anthropic change (Carneiro et al., 2008).

For that reason, accurate assessment of soil microbiota is an important factor in the determination of the biological quality of soils because this assessment produces data relevant to the development of strategies for recovery of degraded areas (Li et al., 2014; Lal, 2015).

The hypothesis of this study is that monospecific and mixed planting of *Eucalyptus camaldulensis* (E) and *S. virgata* (S) contribute to improve the chemical and microbiological properties in areas where the soil is degraded by clay extraction. Thus, in this study, we evaluated the effects of monospecific and mixed stands of *Eucalyptus camaldulensis* and *Sesbania virgata* on the chemical properties of soils (namely nutrient and C levels) and on the microbial populations and total levels of soil enzyme activity as measured by the FDA assay in a degraded area by clay mining operations in the northern part of the state of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Location and characterization of the experimental site

This study was conducted in a clay pit owned by a ceramics company (Stilbe) located in the Poço Gordo district (21° 50' 28.5" S; 41° 14' 31.4" W), municipality of Campos dos Goytacazes, RJ, Brazil. The climate in the region is classified as Aw (tropical hot and humid), according to the Köppen system. Winter is the dry season and summer is the rainy season. Mean annual rainfall is approximately 1,020 mm. The climate data recorded during the study period are shown in figure 1.

The original soil of the pit area is an Inceptsol (*Cambissolo Háplico S*ódico gleico) to a depth of approximately 3 m. In pit operations, the surface soil layer was initially removed

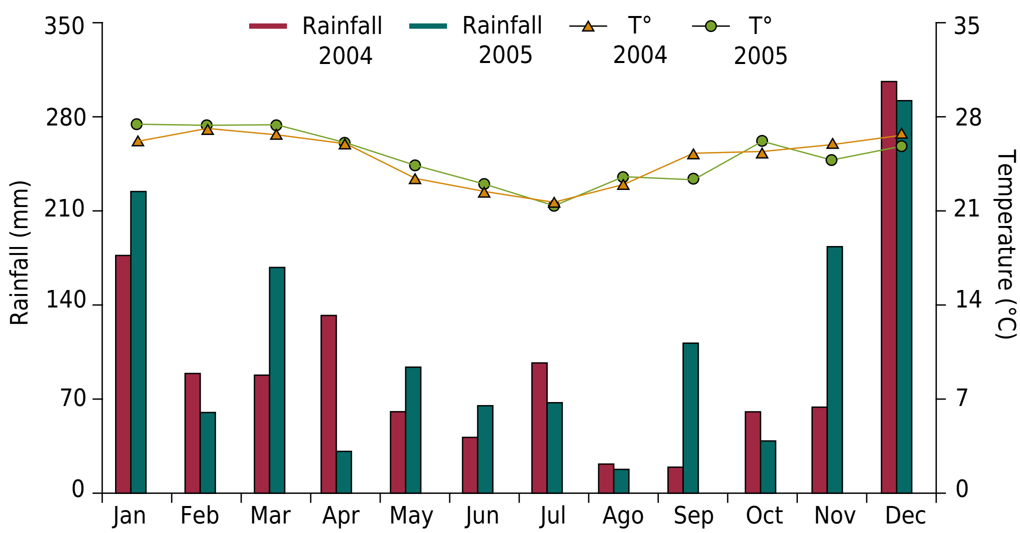

Figure 1. Monthly temperature and rainfall means for the years 2004 and 2005, recorded by the Leonel Miranda meteorological station in Campos dos Goytacazes, RJ, Brazil.

and set aside. Clay extraction proceeded down to a depth of approximately 2.5 m, at which point mining activities were interrupted; the surface layer was returned to the pit, mechanically leveled, and left to stand for two years. After this fallow period, the area was prepared by plowing and two harrowings. The pit areas were fertilized (except in the degraded area with spontaneous vegetation - DASV) with rock phosphate from Araxá (composition: 25.9 % Ca, 11.6 % total P, 5.9 % available P) at an application rate of 100 mg kg^{-1}.

The area within the pit shell was revegetated in August 2002 with *Sesbania virgata* (S) and *Eucalyptus camaldulensis* (E) in monospecific and mixed stands (Schiavo, 2005). After the seedlings were planted within the area defined by the pit shell, weed control, ant control, and irrigation were performed as needed (Schiavo, 2005).

Prior to tillage and planting in the area, chemical analysis of the 0.00-0.20 m layer of the substrate was conducted (Claessen, 1997), showing pH in H_2O 5.70, P 7.70 mg dm^{-3}, K 81.20 mg dm^{-3}, Ca^{2+} 3.65 $cmol_c$ dm^{-3}, Mg^{2+} 2.87 $cmol_c$ dm^{-3}, Al^{3+} 0.18 mg dm^{-3}, H+Al 3.40 mg dm^{-3}, and C 11.40 g kg^{-1} (Schiavo, 2005).

Experimental design and treatments

The experiment was carried out following a randomized complete block design with four treatments (three replicates), which were designated 100S (a monospecific *Sesbania* stand), 100E (a monospecific *Eucalyptus* stand), 50S:50E (a mixed *Sesbania* and *Eucalyptus* stand at identical proportions), and DASV (a degraded area with spontaneous vegetation in which *Brachiaria mutica* (Forsk.) Stapf. prevailed). Each quadrat included 16 plants spaced at 3 × 2 m. During seedling production, the plants were inoculated with arbuscular mycorrhizal fungi (*Glomus macrocarpum*, *Glomus etunicatum*, and *Entrophospora colombiana*) isolated from a clay pit that belonged to another ceramics company, Caco Manga Ltda., located in the Ururaí district, municipality of Campos dos Goytacazes, RJ, Brazil. The isolates, which came from the inoculum bank maintained by the Laboratory of Soil Studies, Universidade Estadual do Norte Fluminense, were multiplied on *Urochloa brizantha* grown in a 1:2 (v:v) mixture of soil and sand. Inoculation was performed at the time of placing the seeds in the containers with an inoculum of 5 mL of a mixture of rhizospheric soil and fungal spores (approximately 120 spores of *G. macrocarpum*, 100 of *G. etunicatum*, and 80 of *E. colombiana*). In addition to the fungi, the *S. virgata* seeds were inoculated with 5×10^7 cells mL^1 of the specific strain of *Rhizobium* (BR 5401) recommended as an inoculant for this species by Rede de Laboratórios para Recomendação, Padronização e Difusão de Tecnologia de Inoculantes Microbianos de Interesse Agrícola (RELARE), supplied by Embrapa Agrobiologia, Seropédica, RJ, Brazil.

Soil sampling and analyses

At 20 and 26 months after the species were planted in the pit (March 2004 and September 2004, in the rainy and the dry season, respectively), 12 simple soil samples were collected, in the interweaving of the plantations, with the aid of a Dutch auger at depths of 0.00-0.10 m in each quadrat and pooled for each quadrat. Total C and chemical properties (pH in H_2O, H^+, Al^{3+}, N, P, K^+, Ca^{2+}, and Mg^{2+}) were analyzed using air-dried soil obtained by drying the samples and passing them through a 2-mm mesh sieve. The C and N contents were determined in a simultaneous CHNS/O elemental analyzer (PE 2400 series II, Perkin-Elmer, Norwalk, CT, USA), and the data obtained were used to calculate the C:N ratio. The P contents were determined by colorimetry and the molybdate method, according to Malavolta et al. (1989). The K content (by flame emission spectrophotometry) and Ca and Mg contents (by mass absorption spectrophotometry) were determined as described in Claessen (1997).

The microbiological properties of freshly collected soil samples were identified using two methods: FDA hydrolysis (enzyme activity, as described by Chen et al., 1988) and respiration (CO_2 released, as described by Grisi, 1995). For fluorescein diacetate hydrolysis, 5 g samples of soil (two replicate analytical samples) were transferred to an Erlenmeyer flask (125 mL) along with 20 mL of sodium phosphate buffer and 0.2 mL of fluorescein diacetate stock (2 μg mL^{-1}). After incubation (25 °C, 20 min) under shaking (150 rpm), the reaction was interrupted by the addition of 20 mL of acetone. The soil suspensions were centrifuged (427 g) for 10 min, after which supernatant aliquots were removed for reading in a spectrophotometer at a wavelength of 490 nm. The standard curve was obtained by adding aliquots of FDA in quantities of 0-400 μg in 5.0 mL of phosphate buffer. The tubes were pre-hydrolyzed in boiling water for 60 min and transferred to flasks containing 5.0 g of soil and 15 mL of phosphate buffer. The results were expressed in μg of fluorescein per g of dry soil per hour.

For respiration analysis, two replicate analytical samples of 50 g of soil adjusted to 40 % field capacity were kept in hermetically sealed plastic containers containing 10 mL NaOH (1 mol L^{-1}). After incubation (25±2 °C) for 7 days, the absorbed CO_2 was determined by titration with HCl (0.5 mol L^{-1}), using phenolphthalein as the indicator. The values of accumulated CO_2 were expressed in μg of C per g of dry soil.

Statistical analysis

The chemical and microbiological data were evaluated for homoscedasticity by Cochran's test (Snedecor and Cochran, 1989) and the normal distribution of the residuals by the Lilliefors test. The data were then subjected to analysis of variance and the Scott-Knott test at 5 % significance using the Sisvar software. Principal component analysis (PCA) was carried out using the PAST software (Hammer et al., 2004). The PCA was used to reduce the size of the dataset and to consequently facilitate analysis through evaluation of how the variables are clustered in the plot (Herlihy and McCarthy, 2006).

RESULTS

There was no effect of the treatments (100S, 100E, and 50S:50E) on soil pH (active acidity) compared with the degraded area with spontaneous vegetation (DASV) (Table 1). However, all reforestation systems increased in H+Al (potential acidity) in both the rainy and the dry seasons compared with the DASV area (Table 1).

The planted areas (100S, 100E, and 50S:50E) were found to have higher contents of nutrients (Ca, Mg, P, K, and N) and carbon in the soil in relation to the DASV. In both seasons, the 100S planting presented higher levels of Ca, P, and K in the soil compared to the other planted areas, while the levels of N and C were higher in planted areas with the presence of the legume (100S - rainy season; 50S:50E - the dry and wet seasons) (Table 1).

Both monospecific and mixed stands induced significant increases in total microbial activity in the soil when evaluated in terms of soil respiration (CO_2 production) and FDA hydrolysis (Table 2) compared to the DASV, with the exception of 100E (FDA) and 100S

Table 1. Chemical properties of soil samples from degraded area with spontaneous vegetation (DASV) and degraded area reforested with *Sesbania virgata* and *Eucalyptus camaldulensis* in monospecific (100S and 100E, respectively) and mixed (50S:50E) stands, 20 and 26 months after planting (rainy and dry seasons, respectively)

Stand	pH(H_2O)		Al^{3+}		H+Al		P		K		Ca^{2+}		Mg^{2+}		C		N	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
			mmol$_c$ dm^{-3}				mg dm^{-3}				cmol$_c$ dm^{-3}				g kg^{-1}			
100S	6.47 a	6.23 a	0.10 a	0.07 a	5.36 a	4.83 a	42.09 a	34.55 a	561 a	647 a	8.00 a	6.32 a	3.53 a	3.14 a	24.90a	20.40 b*	3.05 a	2.50 b*
100E	6.30 a	5.70 a	0.20 a	0.23 a	4.18 a	4.12 a	14.62 b	29.40 a*	202 b	286 b	4.55 b	3.85 b	3.73 a	2.78 a	16.45c	19.35 b	2.10 c	2.35 b
50S:50E	5.77 a	5.53 a	0.07 a	0.03 a	4.46 a	4.61 a	16.05 b	19.33 b	145 b	179 b	4.34 b	3.76 b	3.41 a	3.06 a	20.60b	24.10 a*	2.60 b	2.90 a*
DASV	6.10 a	6.03 a	0.00 a	0.03 a	1.76 b	2.14 b	12.26 b	14.60 b	55 c	58 c	1.30 c	1.37 b	1.79 b	1.39 b	5.20d	1.80 c*	0.30 d	0.35 c

Means followed by the same letter in the same column do not differ statistically in the Scott Knott test at 5 % probability. *: difference between seasons (rainy and dry) for each treatment by Scott Knott test at 5 % probability.

(respiration) plantations in the rainy season, which showed no significant difference. Higher FDA hydrolysis rates were obtained for the 100S and 50S:50E stands in the rainy season. In contrast, higher respiration values were recorded for 100S in the dry season (Table 2).

Regarding seasonal variation, only influences were observed in the 100E, 100S, and 50S:50E areas. The 100E planting showed higher contents of P and FDA in the dry season, whereas the 100S planting showed higher contents of C, N, and FDA in the rainy season and higher respiration in the dry season. The 50S:50E planting showed higher contents of C and N in the dry season.

The variables were distributed along two axes (PC1 and PC2), which explained 89.61 % and 84.30 % (rainy and dry season, respectively) of the total variation of the treatments (Figure 2). The treatments were found to have different clusters. The first group consisted of DASV, which is in the lower quadrant (Figure 2a) and the upper left quadrant (Figure 2b), that is, opposite the distribution of all the variables used in this analysis. The second

Table 2. Microbial activity of soil samples from degraded area with spontaneous vegetation (DASV) and degraded areas revegetated with *Sesbania virgata* and *Eucalyptus camaldulensis* in monospecific (100S and 100E, respectively) and mixed (50S: 50E) stands at 20 and 26 months after planting (rainy and dry seasons, respectively)

Stand	FDA		Respiration	
	Rainy	Dry	Rainy	Dry
	μg FDA g^{-1} h^{-1}		μg CO_2-C g^{-1} h^{-1}	
100S	85.34 a	63.60 a*	34.10 b	83.05 a*
100E	46.03 c	79.72 a*	52.06 a	48.40 b
50S:50E	65.44 b	70.32 a	41.44 a	41.19 b
DASV	27.98 c	21.88 b	25.52 b	15.38 c

FDA: Fluorescein diacetate. Means followed by the same letter in the same column do not differ statistically in the Scott Knott test at 5 % probability. *: difference between seasons (rainy and dry) for each treatment by Scott Knott test at 5 % probability.

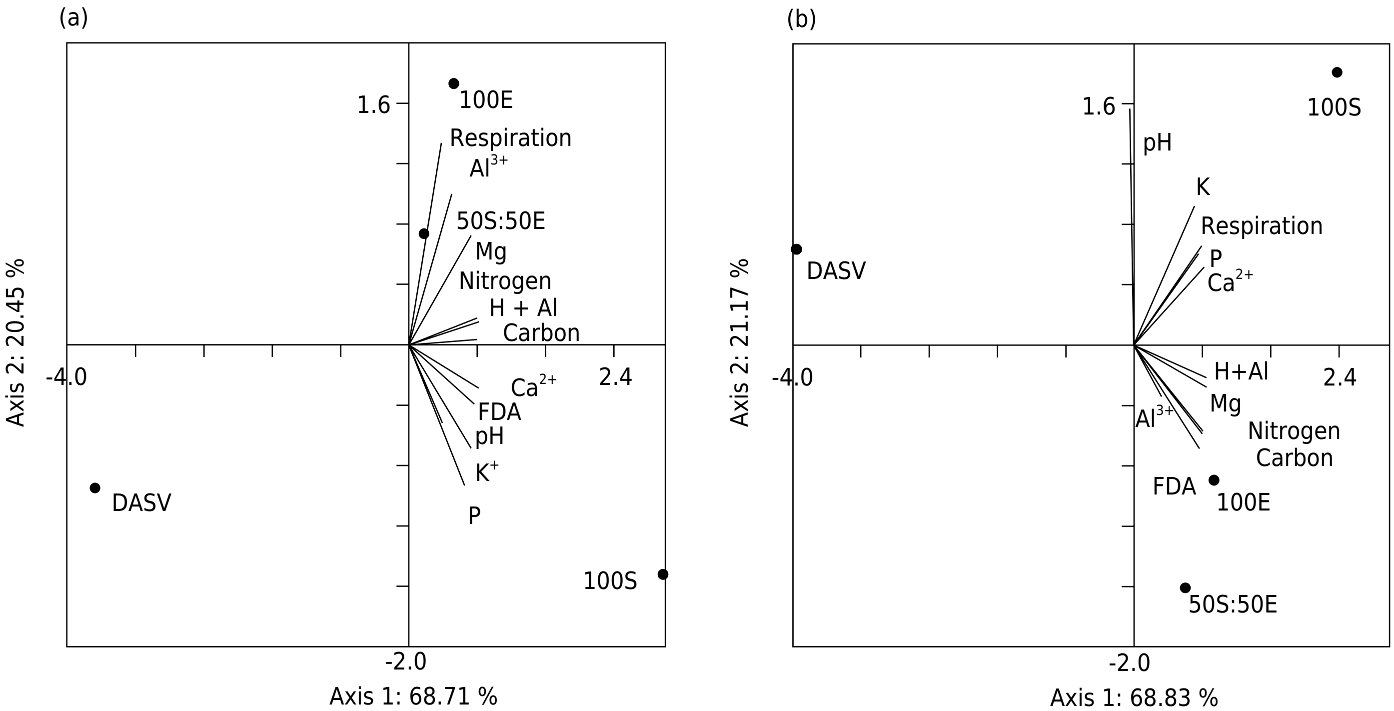

Figure 2. Principal component analysis integrating chemical and microbiological characteristics of degraded area with spontaneous vegetation (DASV) and degraded areas reforested with *Sesbania virgata* and *Eucalyptus camaldulensis* in monospecific (100S and 100E, respectively) and mixed species (50S:50E) stands in the rainy (a) and dry (b) seasons.

group, made up of the different planting systems (100S, 100E, and 50S:50E), was located between the upper and lower right quadrants. This group was strongly correlated with the chemical and biological variables of the soil (Figures 2a and 2b)

DISCUSSION

Although no significant variation was observed in the active acidity (pH) of the soil samples collected from the regenerating forest stands investigated, the potential acidity (H+Al) was higher than the values measured for the DASV. This higher value may be due to the increase in the H^+ contents in the stands since the contents of Al^{3+}, although very low, did not differ among the 100E, 100S, and 50S:50E treatments (Table 1). One of the reasons for this pattern may be the high contents of organic matter in the reforested areas compared to the DASV and the biological N_2 fixation (BNF) mediated by *Sesbania*, a leguminous species that establishes symbioses with *Rhizobia*. In addition, BNF requires proton (H^+) extrusion by the roots of leguminous species in order to maintain intracellular pH (Marschner and Römheld, 1983). Another hypothesis is that the rapid growth of arboreal species causes high absorption of cations to sustain growth of biomass, which promotes the high extrusion of protons (H^+) to the rhizosphere to compensate for the electrochemical imbalance (Yamashita et al., 2008; Koutika et al., 2014).

While the effects of forest plantations on the chemical characteristics of the soil normally only begin to be observed several years after planting (Schiavo et al., 2009; Forrester et al., 2013), in this study, higher nutrient (Ca, Mg, P, K, and N) contents were observed 20 and 26 months after planting in the 100E, 100S, and 50S:50E areas than in the DASV area. Because the plantations are considered to be young and the contents of nutrients in the soil are quite high, it can be inferred that a residual effect of the soil fertilization performed during the planting of the species has occurred. However, we cannot disregard the contribution of the litter from these species to nutrient cycling. Munawar et al. (2011) evaluated litter production and decomposition in areas that had been mined and then revegetated with *Albizia* and *Sesbania* and their effects on some soil properties a year after planting. These authors observed higher contents of N in the two plantations in relation to the original soil (before planting), highlighting the importance of revegetation for the improvement of soil properties.

The deeper root system of the tree species may be an explanation for the improved soil fertility in the restoration area because such root systems can take in nutrients from the soil layers that are out of reach of the roots of forage plants (in this study, there was a predominance of *B. mutica* in the DASV), which are usually more shallow, and bring these nutrients to the soil surface through litter deposition (Carvalho and Xavier, 2005). Increases of 63 and 172 % in Ca and Mg levels, respectively, were reported by Schiavo et al. (2009) in soils in 5-year-old *Eucalyptus* stands in comparison to a clay pit left to regenerate with spontaneous vegetation. In another study carried out in a clay pit, Batista (2006) observed that the P and K levels rose by 470 and 460 % with the establishment of monospecific and mixed stands of a variety of *Eucalyptus* species and sabiá (*Mimosa caesalpiniifolia* Benth.) 36 months after planting.

The comparatively higher Ca, K, and P levels in the 100S treatment in relation to the other plantations may be a consequence of the quality of the litterfall that, according to Paulucio (2007), has a lower C:N ratio and higher contents of P and K (that is, the litterfall mostly composed of leaves) than the 50S:50E and 100E stands. In general, the release of nutrients by plant residue with a low C:N ratio occurs more quickly than that of plants with higher C:N values (such as *Eucalyptus*), since the amount of N affects the decomposition rate. In addition, in this same experiment, Paulucio (2007) observed that the 100S planting had a leaf litter decomposition constant (*k*) that was higher than the one in the 100E and 50S:50E plantations and therefore exhibited a lower half-life, i.e., a more rapid decomposition rate.

The higher C contents observed in the plantations may have been caused by the high amounts of litterfall caused by the species in each stand compared to the area covered with spontaneous vegetation (Schiavo et al., 2007; Freitas et al., 2013). Another important factor is that the denser cover of the stands may help protect the soil more effectively against the variety of processes through which nutrients and carbon can be lost to the environment (Cunha Neto et al., 2013), such as erosion, leaching, decomposition, volatilization, and mineralization (Blum, 1997). In addition, the higher contents of C in the plantations containing the N_2-fixing legumes can be related to the presence of more protected or complexed organic matter, hindering the action of decomposer organisms, as suggested by Forrester et al. (2013). These authors found an increase in C in the soil with a higher proportion of N_2-fixing species and observed that decomposition rates, evaluated as the percentage of acid hydrolyzable molybdenum, tended to decrease with an increase in the proportion of *Acacia mearnsii*.

The BNF promoted by *Sesbania* may explain the high N contents observed in the soils covered by the 50S:50E and 100S stands (Vezzani et al., 2001; Laclau et al., 2008) compared with the value observed for the monospecific *Eucalyptus* stands (100E). Freitas et al. (2013) detected higher N contents in the litter layer of a mixed stand composed of *Eucalyptus* and *Acacia* (a leguminous genus) than in a monospecific *Eucalyptus* stand. Additionally, Vezzani et al. (2001) and Forrester et al. (2004) concluded that the N contents in the soil increased in a mixed stand of *Eucalyptus* and another leguminous species, *Acacia decurrens* (the Brazilian teak), compared with the monospecific *Eucalyptus* stand. Higher N contents were found in leaf litter from the same *Sesbania* stands analyzed in the present study (50S:50E and 100S) than in the 100E treatment (Paulucio, 2007).

The capacity of some *Eucalyptus* and leguminous species to incorporate C into the soil has also been reported by Garay et al. (2003), Schiavo et al. (2007), and Mendonça et al. (2008). Several studies have shed light on the possible beneficial effects of introducing extra N through BNF by leguminous species on the stabilization of C soil contents (Resende et al., 2006; Balieiro et al., 2008; Simões et al., 2010). The availability of N is essential to stabilization of C because it promotes the synthesis of more humified substances (richer in N), ensuring better structural stability of the soil organic matter. In addition, the N of legumes (organic N) is preferred in the processes of microbial synthesis of humic substances (Ribeiro et al., 2011). This may also explain the greater increase in C contents in the mixed stands analyzed in the present study (50S:50E) compared to the monospecific *Eucalyptus* stands (100E).

The low contents of C and nutrients observed in the DASV compared with the monospecific and mixed stands analyzed in the present study likely led to lower microbial activity (soil respiration and FDA). A number of studies have described the considerable importance of factors such as the composition of the plant community and the chemical properties of the soil for the maintenance of microbial populations in soils (Rachid et al., 2013; 2015). Moreover, CO_2 production in the surface soil layers is directly influenced by both the amount and quality of the organic matter available (Peña et al., 2005; Souza et al., 2010).

The introduction of *Eucalyptus* and *Sesbania* likely increased the amount of litterfall accumulating on the soil, which promoted microbiological activity (Andrade et al., 2000; Simões et al., 2010), as observed in the soil respiration and FDA activity of the soil samples analyzed. According to Biederbeck et al. (1984), nutrients such as P and N play important roles in residue decay, improving the conditions for microbial growth. Rachid et al. (2013) found that mixed plantations of *Eucalyptus* and *Acacia* can ensure the integration of microorganisms adapted to decomposing the litter of each species individually, and this has positive influences on P and N contents in the soil compared to the respective monocultures.

In a study conducted by Carneiro et al. (2008), enzymatic activity expressed as FDA hydrolysis was found to increase rapidly with the reforestation of an area degraded by bauxite extraction. The authors claim that FDA activity represents the heterotrophic potential of soils because it represents the activity of enzyme groups that decompose organic matter (Dick et al., 1996). Other studies (Evangelista et al., 2012; Silva et al., 2012) have shown that the activity of several enzymes is influenced by the levels of organic matter in soils.

The higher contents of C and N in the soil during the rainy season compared to the dry season in the 100S plantations may be associated with higher total microbial activity (FDA) in the soil at that time, which can increase litter decomposition rates and the incorporation of C and N into the soil (Batista et al., 2008; Silva et al., 2012). The same pattern may help explain the higher content of P during the dry season for the 100E planting, when there was greater FDA activity. Although there was no significant difference in FDA between the dry and rainy seasons for the 50S:50E stands, there was a tendency toward higher values of total enzyme activity in the dry season, in which the highest C and N levels were observed. Silva et al. (2012) found high correlations between the activity of different enzymes (β-glucosidase, arylsulfatase, and acid phosphatase) and C in soils of forest systems at different stages of succession. The higher FDA activity in the 100E plantation and respiration in the 100S plantation in the dry season may be related to greater litter deposition rates at that time, which can stimulate microbial activity. Silva (2009) found higher litter deposition rates in the dry season in those plantations until they reached four years of age.

The analysis of the chemical and microbiological properties of the soils investigated in the present study allowed the effects of each of the clay pit reforestation approaches to be differentiated. This differentiation was clear mainly in PC1, which showed separation of the stands and DASV in both seasons. Additionally, PC1 was positively correlated with most of the variables analyzed, which were more consistently associated with the stands, suggesting positive changes in the chemical and biological properties of the soil as a result of reforestation of the clay pit, as also observed by Batista et al. (2008) and Schiavo et al. (2009). Thus, there is a negative influence of DASV based on the properties analyzed. This may be related to the lower deposition rates of plant material by this system, resulting in lower incorporation of organic matter into the soil, which may reflect the lower contents of C and biological activity represented by the FDA and respiration in this study (Costa et al., 2004; Cunha et al, 2012). Moreover, greater association of the microbiological variables, C, and chemical properties with the plantations (100S, 100E, and 50S:50E) may reflect the particular components of the soil in such systems; the components are not only stimulated by the continuous supply of organic materials that originate from the tree species, with different degrees of susceptibility to decay, but are also benefited by possible reduction in water erosion caused by the greater ground cover from litter (Cunha et al., 2012).

CONCLUSION

The reforestation of clay pits with *Sesbania virgata* and *Eucalyptus camaldulensis*, whether in monospecific or mixed species stands, improves the chemical and biological properties of soils and increases nutrient (Ca, Mg, K, P, and N) and carbon contents, promoting the expansion of biological activities in soils.

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