## Revista Brasileira de Ciência do Solo

Division - Soil Processes and Properties | Commission - Soil Biology

# **Biological Properties and Organic Matter Dynamics of Soil in Pasture and Natural Regeneration Areas in the Atlantic Forest Biome**

Leandro Ribeiro Nogueira<sup>(1)</sup>, Cristiane Figueira da Silva<sup>(1)</sup>, Marcos Gervasio Pereira<sup>(2)\*</sup>, João Henrique Gaia-Gomes<sup>(3)</sup> and Eliane Maria Ribeiro da Silva<sup>(4)</sup>

- <sup>(1)</sup> Universidade Federal Rural do Rio de Janeiro, Instituto de Florestas, Programa de Pós-Graduação em Ciências Ambientais e Florestais, Seropédica, Rio de Janeiro, Brasil.
- <sup>(2)</sup> Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Rio de Janeiro, Brasil.
- <sup>(3)</sup> Universidade Federal Rural do Rio de Janeiro, Instituto de Tecnologia, Programa de Pós-Graduação em Engenharia Agrícola e Ambiental, Seropédica, Rio de Janeiro, Brasil.
- <sup>(4)</sup> Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Agrobiologia, Seropédica, Rio de Janeiro, Brasil.

**ABSTRACT:** The removal of original vegetation for crops and pasture production and then followed by natural regeneration is a standard practice in the Atlantic Forest, which has produced patches with different degrees of degradation and regeneration across the landscape. The aim of this study was to evaluate the effects of replacement of native forest by pasture and natural regeneration of vegetation on soil and on soil organic matter (SOM) dynamics in the dry and rainy season in an Atlantic Forest fragment in Passa Vinte, Minas Gerais (MG), Brazil. Soil samples were collected in the rainy and dry season, at a depth of 0.00-0.05 m. The variables determined were total organic carbon (TOC) and particle-size fractions of SOM [particulate organic carbon (POC) and mineral-associated organic carbon (MOC)]; microbial activity by basal respiration (BR) and microbial biomass carbon (MBC); species richness (SR) and spore abundance (SA) of arbuscular mycorrhizal fungi (AMF); and total and easily extractable glomalin-related soil protein (T-GRSP and EE-GRSP, respectively). The conversion of native forest into pasture reduced TOC, POC, MOC, AMF-SA, T-GRSP, and EE-GRSP. However, it did not reduce MBC and BR. The fallow period in the area under natural regeneration was not long enough to restore soil TOC, POC, MOC, BR, MBC, T-GRSP, and EE-GRSP to levels approaching those observed in the forest area. Nevertheless, natural regeneration of vegetation stimulated the production of seedlings (spores) of arbuscular mycorrhizal fungi, which are important for the establishment of plant species and advance of ecological succession. Seasonality affected some of the biological soil properties and SOM dynamics.

**Keywords:** organic carbon, microbial biomass, arbuscular mycorrhizal fungi, biodiversity, forest fragment.



Received: September 26, 2015 Approved: January 12, 2016

How to cite: Nogueira LR, Silva CF, Pereira MG, Gaia-Gomes JH, Silva EMR. Biological Properties and Organic Matter Dynamics of Soil in Pasture and Natural Regeneration Areas in the Atlantic Forest Biome. Rev Bras Cienc Solo. 2016;40:e0150366.

**Copyright:** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.







### INTRODUCTION

Under balanced conditions, the Atlantic Forest provides continuous benefits to the environment, contributing to climate regulation, nutrient cycling, maintenance of biodiversity, and genetic variability (Lima et al., 2014). Despite the important social, economic, and environmental functions of this biome, it has been reduced to only 8 % of its original area, making it crucial to adopt efficient conservation and recovery measures (Carvalhal et al., 2015).

A common practice in Atlantic Forest areas has been substitution of natural forests for crops and pastures, followed by natural recovery of vegetation, which produces a landscape containing patches with different degrees of degradation and recovery. These practices can affect ecosystem equilibrium and the chemical, physical, and biological properties of soil, which can be considered indicators of environmental degradation and recovery (Machado et al., 2010; Silva et al., 2012a; Guareschi et al., 2014).

With transformation of forest to other types of land use, factors such as plant input, quality of residues, and decomposition rates lead to drastic changes in soil organic matter (SOM) and soil fractions (Loss et al., 2014). Organic matter determines the physical, chemical, and biological properties of soil and is considered one of the main indicators of its quality (Doran and Parkin, 1994; Reichert et al., 2003; Leite and Galvão, 2008; Braida et al., 2011). These changes also affect microbial biomass, one of the components that, along with macrofauna and plant roots, represent the live portion of SOM (Brookes, 2001; Moreira and Malavolta, 2004). Microbial biomass controls important soil functions, such as organic matter decomposition and accumulation, in addition to transformations involving nutrients (De-Polli and Guerra, 1999).

Changes in land use affect the community of arbuscular mycorrhizal fungi (AMF) (Silva et al., 2006; Ferreira et al., 2012), microorganisms that play an important role in plant development, especially in the first stages of ecological succession, because, in conjunction with plant roots, they improve water and nutrient uptake (Siqueira et al., 1998; Zangaro et al., 2000). In addition, they affect the physical properties of soil by mechanisms such as production of glomalin, a protein that serves as a binding agent for soil particles, promoting aggregate formation and stability (Wright et al., 1996; Morell et al., 2009). Given its role in aggregation, glomalin contributes significantly to ecosystem sustainability (Sousa et al., 2012), enhancing water infiltration, gas exchange, and root growth, decreasing erosion and increasing C storage capability (Rillig et al., 1999; Sousa et al., 2012).

Evaluation of glomalin as a gene product in soil is inaccurate, due to limitations of the Bradford and Elisa method, which is usually used for its extraction and quantification (Whiffen et al., 2007; Sousa et al., 2012). Thus, glomalin has been operationally assessed as a glomalin-related soil protein (GRSP) (Rillig, 2004), considered an indicator of soil quality with good sensitivity for detecting impacts from different soil use and management systems (Silva et al., 2012b; Silva et al., *in press*).

The hypothesis of the present study is that the conversion of Atlantic Forest into pasture and then to areas of natural regeneration changes the biological properties and dynamics of SOM, and it is also affected by seasonality. The objective was therefore to assess the effects of replacing native forest by pasture and areas of natural regeneration on biomass activity and microbial biomass carbon (MBCn), AMF spore abundance (SA), AMF species richness (SR), GRSP, total organic carbon (TOC), and the particle size fractions of SOM in the dry and rainy season in an Atlantic Forest fragment in Passa Vinte, MG, Brazil.

## **MATERIALS AND METHODS**

The study was performed on the Palmital farm in Passa Vinte, MG (22° 12' S, 44° 12' W; 630 m altitude). Climate is characterized as humid subtropical (Cwa) according to the



Köppen classification system, with average annual temperature of 19.3 °C and rainfall of 1,494 mm. July is the driest month, with average rainfall of 16 mm, whereas the highest average rainfall of 269 mm occurs in January. Soil in the area is classified as *Argissolo Vermelho-Amarelo* (Ultisol) (Santos et al., 2013) and topography ranges from slightly rolling to rolling. Sampling was performed in secondary forest, natural pasture, and natural regeneration areas, and sampling points were located approximately 10 m apart.

The forest area was located in the upper third of a toposequence and corresponded to a fragment of Atlantic Forest biome, with an ecosystem characteristic of a Submontane Ombrophilous Dense Forest. Since the landowners acquired the property, cutting in the area has not been registered, but it appears to have occurred in secondary forest, based on the historical features, size, and distribution of forest fragments. The ecological features and the presence of ample biodiversity of large animals observed by the landowners indicate a prolonged period of formation. The natural pasture area was located at the top of the toposequence, facing west-southwest, with a slope of less than 45°. It is predominantly covered by low densities of brachiaria grass trampled by animals. The regeneration area, located in the lower third of the toposequence, was used as pasture until it was fenced to avoid cattle incursions. It was last cleared nearly 20 years ago.

Soil samples were collected during the dry season (September 2013) and at the end of the rainy period (April 2014). Chemical and physical properties of the soil samples were characterized according to Donagema et al. (2011) (Table 1).

A 1-ha plot was demarcated in each area, and five soil samples were collected in both seasons at a depth of 0.00-0.05 m, producing five compound samples per area and season. A portion of each sample was air dried, broken up, and sieved through 1-mm mesh to obtain air-dried fine earth (ADFE), which was used to determine C and particle-size fractions. The remaining fraction of the sample was processed immediately after collection for microbiological characterization.

Total organic carbon (TOC) was evaluated according to Yeomans and Bremmer (1988), and SOM particle size fractions were determined according to Cambardella and Elliott (1992), obtaining particulate organic carbon (POC) and mineral-associated organic carbon (MOC). Quantification of microbial activity was based on soil basal respiration (BR) and metabolic quotient (qCO<sub>2</sub>), carried out in accordance with Jenkinson and Powlson (1976). Microbial biomass carbon (MBC) was determined by fumigation/extraction, as proposed by Vance et al. (1987). The microbial quotient (qMIC) was calculated from the ratio between soil MBC and TOC.

Arbuscular mycorrhizal fungi (AMF) of each sample were evaluated in 50 cm<sup>3</sup> soil samples for spore extraction using the wet sieving method (Gerdermann and Nicolson, 1963). After spore counting to determine spore abundance (SA), species were identified according to Schenck and Perez (1988) and the descriptions provided by the INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, Morgantown,

**Table 1.** Chemical and physical properties of topsoil (0.00-0.05 m) in areas of natural regeneration, natural pasture, and a fragment of native forest on the Palmital farm in Passa Vinte, MG, Brazil, sampled in the dry and rainy season

Area	pH(H <sub>2</sub> O)		Ca <sup>2+</sup>		Mg <sup>2+</sup>		K <sup>+</sup>		Р		Sand	Silt	Clay
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy		Dry	
			cmol <sub>c</sub> kg <sup>-1</sup>			mg kg <sup>-1</sup>			g kg <sup>-1</sup>				
Forest	4.21 b	4.35 a	0.74 b	0.91 a	1.37 a	1.10 a	0.37 a	0.08 a	3.52 a	3.19 a	441 b	176 a	383 a
Pasture	5.07 a	4.28 a	1.84 a	0.75 a	2.12 a	0.09 b	0.36 a	0.04 a	2.53 b	1.98 b	460 ab	208 a	332 b
Regeneration	4.41 b	4.48 a	0.33 c	0.57 a	0.37 b	0.13 b	0.16 b	0.04 a	1.74 c	1.05 c	532 a	153 a	315 b
CV (%)	3.3	26	15.60		20.72		22.41		19.52		8.04	19.05	8.04

Means followed by the same uppercase letter in a row and the same lowercase letter in a column do not differ statistically (Bonferroni t test, p=0.05).

WV, USA - http://invam.caf.wvu.edu/). Based on AMF identification, we calculated species frequency (Fi) (Brower et al., 1990) (Fi = ji/Ke, where ji is the number of samples containing species i, Ke the number of soil samples, Fi the species frequency i); total species richness (SR); and the Margalef index ( $D\alpha = [(n-1)]/ln N$ , where  $D\alpha$  is diversity, n is the number of species recorded, N is the number of individuals of any species in the sample, and In is the natural logarithm of N).

Soil glomalin was quantified as glomalin-related soil protein (GRSP). The two GRSP fractions, total (T-GRSP) and easily extractable (EE-GRSP), were distinguished as a function of extraction conditions (Wright and Upadhyaya, 1998). Both fractions were extracted in auclave - EE-GRSP with 1 g of soil in 8.0 mL of 20 mmoL L<sup>-1</sup> sodium citrate solution (pH 7.4) at 121 °C for 30 min, and T-GRSP with 1 g of soil in 8.0 mL of 50 mmoL L<sup>-1</sup> sodium citrate solution (pH 7.4) at 121 °C for 30 min, and T-GRSP with 1 g of soil in 8.0 mL of 50 mmoL L<sup>-1</sup> sodium citrate solution (pH 8.0) at 121 °C for 60 min. More than one autoclave cycle was needed to extract T-GRSP, and these cycles were repeated until the sample turned light yellow. After autoclave protocols, both GRSP fractions were centrifuged at 5,000 g for 20 min to quantify protein in the supernatant. GRSP was quantified using the Bradford method (Bradford, 1976), modified by Wright et al. (1996) (available at http://www.usda.gov), using bovine serum albumin (BSA) as a standard. The GRSP concentrations for both fractions were corrected to mg g<sup>-1</sup> of soil considering the total volume of supernatant and soil dry matter.

Homoscedasticity and data normality were tested before performing analysis of variance and test of means. The results obtained were analyzed for normality of error distribution using the Lilliefors test and homogeneity of variance by the Cochran and Bartlett test, performed with SAEG 5.0 software. Means were compared by the Bonferroni t test, conducted using Sisvar 4.6 software. Cluster analysis and principal component analysis (PCA) were performed using PAST software (Hammer et al., 2004).

## **RESULTS AND DISCUSSION**

#### Total organic carbon and particle size fractions of organic matter

Total organic carbon (TOC) levels in the dry and rainy season were higher in the forest, intermediate in pasture, and lower in the regeneration area (Table 2). Forest conversion into pasture and areas of natural recovery reduced soil TOC by nearly 40 %. Pasture and regeneration areas likely receive lower plant residue (leaves and branches) input on the soil surface, explaining the decline in TOC (Bernini et al., 2009). A decrease in TOC is a negative impact of pasture establishment because, according to Bernini et al. (2009), it can reduce nutrient cycling. The findings indicate that the fallow period in the recovery area was not long enough to return TOC levels to those observed in the forest area.

Area	тс	C	PC	DC	мос		
	Dry	Rainy	Dry	Rainy	Dry	Rainy	
			g k	(g <sup>-1</sup>			
Forest	45.42 aA	42.36 aB	10.24 aA	9.90 aA	35.52 aA	32.12 aB	
Pasture	26.02 bA	23.70 bB	7.83 bA	7.45 bA	18.57 bA	15.87 bB	
Regeneration	23.88 cA	20.82 cB	7.76 bA	5.38 cB	18.50 bA	13.06 bB	
CV (%)	2.2	LO	14	.22	7.	94	

Table 2. Total organic carbon (TOC), particulate organic carbon (POC) and mineral associated organic carbon (MOC) in topsoil (0.00-0.05 m) sampled in the dry and rainy season in forest, pasture, and natural regeneration areas on the Palmital farm in Passa Vinte, MG, Brazil

Means followed by the same uppercase letter in a row and the same lowercase letter in a column do not differ statistically (Bonferroni t test, p=0.05).



In both the rainy and dry seasons, the forest area also exhibited the highest POC levels. Only in the rainy season did POC levels differ between pasture and regeneration areas, with lower values in the latter. In the state of Acre, Bernini et al. (2009) observed that replacing original vegetation by pasture decreased TOC and POC, showing that these fractions are indicators of changes in land use in that ecosystem. Composed of fungal hyphae, plant roots, and animal and plant residues, POC corresponds to the labile fraction of SOM and is very sensitive to changes in land use and management (Blair et al., 1998).

Mineral-associated organic carbon (MOC) showed the same pattern as TOC and POC, with the highest levels detected in the forest area (Table 2), in both seasons. However, no difference was detected between pasture and regeneration areas (Table 2). Mineral-associated organic carbon, however, might not be a good indicator of the effects of land use systems on soil properties because it may take several years for changes in this SOM fraction to become detectable (Carmo et al., 2012). This occurs because of slow MOC cycling, interaction with the mineral fraction of the soil, and the formation of stable organomineral compounds (Bayer et al., 2004; Carmo et al., 2012).

Total organic carbon (TOC) declined by 10 % and POC by 18 % in the rainy season in comparison to the dry season. This is likely due to the increase in SOM mineralization that occurs in the rainy season as result of microbiota activity stimulated by high temperatures and rainfall (Gama-Rodrigues et al., 2005).

#### Carbon and microbial biomass activity

Microbial biomass carbon (MBC) did not differ between pasture and forest areas in either season, but in the dry season it was lower in the regeneration area than in the forest area (Table 3). Similar MBCs between native forest and pasture areas were also found in studies carried out in São Paulo (Marchiori Júnior and Mello, 1999) and Rio de Janeiro (Silva et al., 2012a).

The higher MBC in forest may be associated with the greater organic matter input and with high floristic diversity, which likely promotes the development of microbial biomass (Menezes, 2008; Silva et al., 2010, 2012a). In pasture areas, high MBC is associated with the root system of grasses, which is abundant and active throughout the year (Marchiori Júnior and Mello, 1999), and also with animal-derived organic matter (Toda et al., 2010), which likely enhanced the availability of organic substrates for soil microbiota (Oliveira et al., 2001). Toda et al. (2010) found that pasture areas exhibit higher content of animal-derived organic matter and MBC compared to different cropping systems. In addition, Vicente and Araújo (2013) observed that, over the years, microbial biomass tends to increase in land covered with pasture, due to the significant development of a grass root system and its cycling potential in topsoil. In the natural recovery area, the inputs of oxidizable C might not meet the demands for maintaining existing biomass (D'Andréa et al., 2002).

**Table 3.** Microbial biomass carbon (MBC), and activity (soil basal respiration - BR, metabolic quotient -  $qCO_2$ , and microbial quotient - qMIC) in topsoil (0.00-0.05 m) sampled in the dry and rainy season in forest, pasture, and natural regeneration areas on the Palmital farm in Passa Vinte, MG, Brazil

Area	MB	MBC		BR		<b>D</b> <sub>2</sub>	qMIC		
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	
	mg kg <sup>-1</sup>		mg CO <sub>2</sub> -C kg <sup>-1</sup> h <sup>-1</sup>		– mg CO <sub>2</sub> -C mg <sup>-1</sup> CBM h <sup>-1</sup> –		%		
Forest	365 aB	543 abA	4.49 aA	3.42 aB	14.32 abA	6.84 aB	0.80 aB	1.28 bA	
Pasture	296 abB	635 aA	4.48 aA	3.54 aA	17.14 aA	5.74 aB	1.14 aB	2.68 aA	
Regeneration	207 bB	474 bA	2.15 bA	1.98 bA	11.88 bA	4.35 aB	0.87 aB	2.28 aA	
CV (%)	20.	20.77		21.39		17.46		14.46	

Means followed by the same uppercase letter in a row and the same lowercase letter in a column do not differ statistically (Bonferroni t test, p=0.05).

Microbial biomass carbon levels were higher in the rainy season than in the dry season, likely because increased temperature and rainfall produced conditions favorable to microbial biomass development (Espíndola et al., 2001). Basal respiration (BR) was also higher in forest and pasture areas, in both seasons, indicating higher rates of CO<sub>2</sub> emission compared to the regeneration areas (Table 3). In the native forest area, this pattern may be related to constant litter deposition and residue incorporation, prompting organic matter build up and producing high biomass and biological activity in this material (Silva et al., 2012a; Islabão et al., 2015). Litter incorporation can lead to a five-fold increase in the biological activity of soils (Della Bruna et al., 1991), a desirable feature, given that microbial activity may represent rapid transformation of organic residues into plant nutrients (Azar et al., 2013). Nutrient cycling in pasture areas may be faster because of high plant biomass renewal and intense biological activity, which increases BR (Silva et al., 2012a).

The metabolic quotient  $(qCO_2)$  did not differ among the study areas, indicating similar response to mineralization (Martins et al., 2010). However,  $qCO_2$  was higher in the dry season in all areas. This response suggests higher energy use by the microbiota, likely because of stress or disturbance in the dry season (Tótola and Chaer, 2002).

The microbial quotient (*q*MIC) was similar among the areas in the dry season, but lower in the forest in the rainy season. Sparling (1992) underscores that *q*MIC is related to the efficiency of organic C conversion into microbial C and organic C loss and organic C stability in the mineral fraction of soil. Therefore, from the dry to the rainy season, a *q*MIC increase represents higher organic C content available to soil microbiota in the rainy season, that is, C that was stable in the organic form became unstable in the microbial form (Anderson and Domsch, 1989).

#### Arbuscular mycorrhizal fungi

The 10 AMF species observed in soil sampled in the dry and rainy season consisted of five families and six genera (Table 4). Three species belonged to the genera *Glomus* and *Acaulospora*, while the genera *Claroideoglomus*, *Gigaspora*, *Scutellospora*, and *Ambispora* were represented by a single species each. The species *Ambispora leptoticha*, *Acaulospora laevis*, *Claroideoglomus lamellosum*, and *Glomus macrocarpum* were observed in all the areas, in both the dry and rainy season, the last species being the most frequent (100 %) (Table 4). The species *Acaulospora foveata* and *Glomus clavisporum* were exclusive to the regeneration area, whereas *Acaulospora scrobiculata* was observed only in the pasture area (Table 4). One noteworthy aspect is that species not identified in a determinate area are not necessarily absent in that environment since they can be present in other forms (e.g. hyphae, colonized roots, and auxiliary cells), which are not detectable by the methods adopted in the present study (Santos et al., 2014).

In both seasons, the values of total SR and the Margalef diversity index were very similar among the areas (Table 5), indicating similar composition of AMF communities, ranging from 50 to 66 %. In the dry season, the AMF community had greater similarity between pasture and forest areas, while in the rainy season, the AMF community of the regeneration area had greater similarity to that of the forest fragment (Figure 1). This pattern suggests that the AMF community is affected not only by the host, but also by climatic conditions, and possibly by soil conditions (Silva et al., 2007).

Spore abundance (SA) did not differ between the forest and regeneration areas in either season evaluated, but lower values were observed in the pasture area (Table 5). In contrast, both EE-GRSP and T-GRSP levels were generally lower in regeneration and pasture areas compared to the forest area (Table 5), following the same pattern observed for TOC and particle size fractions of organic matter (Table 2). Many studies have emphasized the relationship between GRSP and organic C (Fokom et al., 2012; Silva et al., 2012b) and the importance of this protein for soil C pools (Nobre et al., 2015). The higher EE-GRSP



**Table 4.** Relative frequency of arbuscular mycorrhizal fungus species in topsoil (0.00-0.05 m) sampled in the dry and rainy season in forest, pasture, and natural regeneration areas on the Palmital farm in Passa Vinte, MG, Brazil

	Relative frequency							
Family/Specie	Fo	rest	Pasture		Regeneration			
	Dry	Rainy	Dry	Rainy	Dry	Rainy		
				% ———				
Archaeosporaceae								
Ambispora Leptoticha (Schenck and Smith) Walker, Vestberg and Schüssler	20	40	40	20	20	40		
Acaulosporaceae								
Acaulospora laevis Gerderman and Trappe	40	60	100	80	100	100		
Acaulospora foveata Trappe and Janos	-	-	-	-	20	20		
Acaulospora scrobiculata Trappe	-	-	60	20	-	-		
Gigasporaceae								
Gigaspora sp.	40	60	20	-	-	40		
Scutellospora scutata Walker and Dieder	40	20	-	20	-	-		
Claroideoglomeraceae								
Claroideoglomus lamellosum (Dalpé, Koske and Tews) Walker and Schüßler	60	80	20	20	60	60		
Glomeraceae								
Glomus clavisporum (Trappe) Almeida and Schenck	-	-	-	-	20	40		
Glomus macrocarpum Tulasne and Tulasne	100	100	100	100	100	100		
Glomus tortuosum Schenck and Smith	40	60	-	-	40	60		

**Table 5.** Total species richness (SR) and spore abundance (SA) of arbuscular mycorrhizal fungi (in 50 cm<sup>-3</sup> soil), Margalef index (D $\alpha$ ), easily extractable (EE-GRSP) and total (T-GRSP) glomalin-related soil protein in topsoil (0.00-0.05 m) sampled in the rainy and dry season in forest, pasture, and natural regeneration areas on the Palmital farm in Passa Vinte, MG, Brazil

Area	SR		SA		Dα		EE-GRSP		T-GRSP	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
							mg g <sup>-1</sup>			
Forest	7	7	222 abA	311 aA	1.11	1.04	3.99 aA	2.73 aB	9.53 aB	23.86 aA
Pasture	5	6	182 bA	139 bA	0.77	0.81	2.85 bA	1.49 bB	5.96 abB	16.72 bA
Regeneration	7	8	364 aA	411 aA	1.02	1.00	1.93 bA	1.27 bA	4.68 bB	12.29 cA
CV (%)		25.57					26	.12	20.33	

Means followed by the same uppercase letter in a row and the same lowercase letter in a column do not differ statistically (Bonferroni t test, p=0.05).

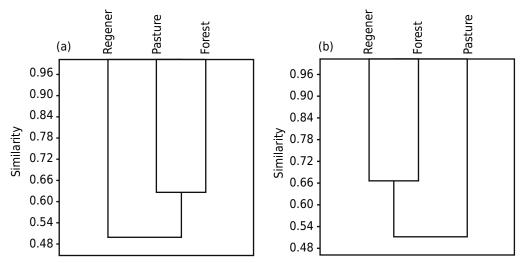
and T-GRSP levels in the forest area may be associated with the accumulation of this protein over time. This T-GRSP pattern was also observed by Silva et al. (2013) in native Atlantic Forest areas.

#### **Multivariate analysis**

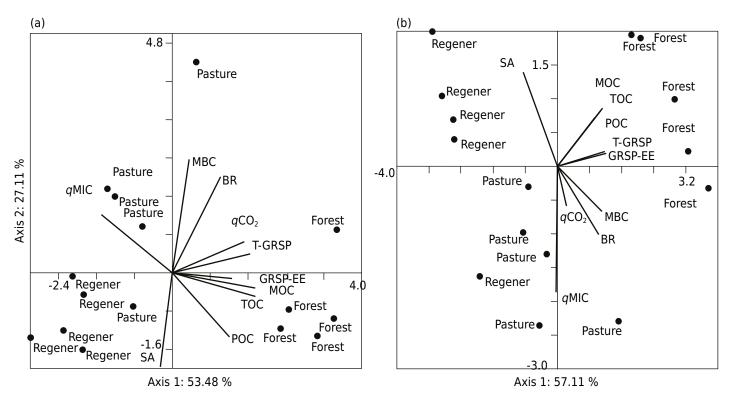
Principal component analysis (CPA) of soil properties (TOC, POC, MOC, MBC, BR,  $qCO_2$ , qMIC, SA, T-GRSP, EE-GRSP) showed that 78.85 and 80.59 % of dry and rainy season data variability, respectively, were explained in the first two components (Figures 2a and 2b). The forest area was separated from regeneration and pasture areas in axis 1 in both the dry season (principal axis, A1 = 57.11 %) and rainy season (A1= 53.48 %) (Figures 2a and 2b). Most variables evaluated contributed to separating the areas (EE-GRSP, T-GRSP, MBC, BR, TOC, MOC, and POC in the dry season; EE-GRSP, T-GRSP,  $qCO_2$ , TOC, MOC, and qMIC in the rainy season) and showed correlation coefficients with axis 1 above 0.70. The variables were more associated with the forest area, likely because of the higher

values exhibited in this area. This result shows the changes in these soil properties during native forest conversion into natural pasture and in a natural regeneration area. These findings corroborate a study on the fertility and organic matter content of forest and pasture soils in Acre (Loss et al., 2014).

The higher association between the forest area and the different C fractions and biological variables is explained by their particular situation. The variables under study are favored by the continuous input of organic materials, particularly plants at different degrees of



**Figure 1.** Dendrogram showing the frequency of arbuscular mycorrhizal fungi species in topsoil (0.00-0.05 m) from areas of natural regeneration (Regener.), natural pasture, and a fragment of native forest on the Palmital farm in Passa Vinte, MG, Brazil, sampled in the dry (a) and rainy (b) season (Jaccard similarity index was used)



**Figure 2.** Ordination diagram produced by principal component analysis of topsoil properties (0.00-0.05 m) associated with organic matter and microorganisms in areas of natural regeneration (Regener.), natural pasture, and a fragment of native forest on the Palmital farm in Passa Vinte, MG, Brazil, sampled in the dry (a) and rainy (b) season. T-GRSP: total glomalin-related soil protein; EE-GRSP: easily extractable glomalin-related soil protein; BR: soil basal respiration; MBC: microbial biomass carbon, *q*CO<sub>2</sub>: metabolic quotient; *q*MIC: microbial quotient; POC: particulate organic carbon; MOC: mineral associated organic carbon; TOC: total organic carbon; SA: spore abundance.



decomposition and susceptibility to degradation, in addition to the lack of disturbance by anthropogenic activity, which results in lower rates of water erosion and SOM mineralization (Cunha et al., 2012; Silva et al., 2012a)

One aspect to consider is the occurrence of outliers among the observation points in the diagram, in both seasons and especially in the pasture area. This pattern results from the higher spatial variability of pasture properties, which is likely caused by animal activity, given that it makes the pasture more heterogeneous through discontinuous and non-uniform grazing and excrement distribution (Salton and Carvalho, 2007). In a review study, Dias Filho and Ferreira (2008) report that animals tend to change nutrient cycling, affecting litter quality, nutrient concentration, organic matter, and the biological activity of soil by feces and urine deposition in the areas where they remain for longer periods (Franzluebbers et al., 2000; Rook et al., 2004; Salton and Carvalho, 2007). These processes can increase soil heterogeneity and are likely associated with the variability observed in the results, given that sample collection was random.

## CONCLUSIONS

Forest conversion into natural pasture changes the biological properties of soil and organic matter dynamics, reducing total levels of organic carbon and particle size fractions (POC and MOC), abundance of arbuscular mycorrhizal fungus spores, and glomalin-related soil protein.

Microbial biomass carbon and basal respiration in the soil were not changed by the use of land as natural pasture, suggesting higher resilience of these indicators.

The fallow period in the natural regeneration area was not long enough to recover total organic carbon levels and particle size fractions of organic matter (POC and MOC), microbial biomass activity, and carbon and glomalin-related soil protein to levels close to those observed in the forest area.

Natural regeneration stimulated spore production by arbuscular mycorrhizal fungi, microorganisms with an important role in establishing and advancing the ecological succession of plant species.

Seasonal conditions affect biological properties and organic matter dynamics in soil. From the dry to the rainy season, total organic carbon, mineral-associated organic carbon, metabolic quotient, soil basal respiration, and easily extractable glomalin-related soil protein decreased, whereas microbial biomass carbon, microbial quotient, and total glomalin-related soil protein increased.

## ACKNOWLEDGMENTS

Our thanks to CAPES for financial support and to UFRRJ and Embrapa Agrobiologia for providing the structure and equipment for soil analyses.

#### REFERENCES

Anderson TH, Domsch KH. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol Biochem. 1989;21:471-9. doi:10.1016/0038-0717(89)90117-X

Azar GS, Araújo ASF, Oliveira ME, Azevêdo DMMR. Biomassa e atividade microbiana do solo sob pastagem em sistemas de monocultura e silvipastoril. Semina: Cienc Agrár. 2013;34:2727-36. doi:10.5433/1679-0359.2013v34n6p2727

Bayer C, Martin-Neto L, Mielniczuk J, Pavinato A. Armazenamento de carbono em frações lábeis na matéria orgânica de um Latossolo Vermelho sob plantio direto. Pesq Agropec Bras. 2004;39:677-83. doi:10.1590/S0100-204X2004000700009



Bernini TA, Loss A, Pereira MG, Coutinho FS, Zatorre NP, Wadt PGS. Frações granulométricas e oxidáveis da matéria orgânica do solo em sucessão floresta - pastagem no Acre. Rev Bras Agroecol. 2009;4:4334-8.

Blair GJ, Chapman L, Whitbread AM, Ball-Coelho B, Larsen P, Tiessen H. Soil carbon changes resulting from sugarcane trash management at two locations in Queensland, Australia and in Nort-East Brazil. Aust J Soil Res. 1998;6:873-82. doi:10.1071/S98021

Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. doi:10.1016/0003-2697(76)90527-3

Braida JA, Bayer C, Albuquerque JA, Reichert JM. Matéria orgânica e seu efeito na física do solo. Tópicos Cienc Solo. 2011;7:222-7.

Brookes PC. The soil microbial biomass: concept, measurements and applications in soil ecosystem research. Microbes Environ. 2001;16:131-40. doi:10.1264/jsme2.2001.131

Brower JE, Zar JH, von Ende CN. Field and laboratory methods for general ecology. 3rd ed. Dubuque: W. C. Brown; 1990.

Cambardella CA, Elliott ET. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Sci Soc Am J.1992;56:777-83. doi:10.2136/sssaj1992.03615995005600030017x

Carmo FF, Figueiredo CC, Ramos MLG, Vivaldi LJ, Araújo LG. Frações granulométricas da matéria orgânica em Latossolo sob plantio direto com gramíneas. Biosci J. 2012;28:420-31.

Carvalhal F, Rodrigues SS, Berchez FAS. Mata Atlântica [internet]. 2015. [acesso: 14 Jul 2015]. Disponível em: http://www.ib.usp.br/ecosteiros/textos\_educ/mata/index.htm.

Cunha EQ, Stone LF, Ferreira EPB, Didonet AD, Moreira JAA. Atributos físicos, químicos e biológicos de solo sob produção orgânica impactados por sistemas de cultivo. Rev Bras Eng Agríc. Amb. 2012;16:56-63. doi:10.1590/S1415-43662012000100008

D'Andréa AF, Silva MLN, Curi N, Siqueira JO, Carneiro MAC. Atributos biológicos indicadores da qualidade do solo em sistemas de manejo na região do cerrado no sul do Estado de Goiás. Rev Bras. Cienc Solo. 2002;26:913-23. doi:10.1590/S0100-06832002000400008

Della Bruna E, Borges AC, Fernandes B, Barros NF, Muchovej RMC. Atividade da microbiota de solos adicionados de serapilheira de eucalipto e de nutrientes. Rev Bras Cienc Solo. 1991;15:15-20.

De-Polli H, Guerra JGM. C, N e P na biomassa microbiana do solo. In: Santos GA, Camargo FAO, editores. Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Porto Alegre: Genesis; 1999. p.389-411.

Dias Filho MB, Ferreira JN. Influência do pastejo na biodiversidade do ecossistema da pastagem. In: Pereira OG, Obeid JA, Fonseca DM, Nascimento Junior D, editores. In: Simpósio sobre manejo estratégico da pastagem; 2008; Viçosa, MG. Viçosa, MG: Universidade Federal de Viçosa; 2008. p.47-74.

Donagema GK, Campos DVB, Calderano SB, Teixeira WG, Viana JHM, organizadores. Manual de métodos de análise do solo. 2ª ed. rev. Rio de Janeiro: Embrapa Solos; 2011.

Doran JW, Parkin TB. Defining and assessing soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA, editors. Defining soil quality for a sustainable environment. Madison: SSSA; 1994. p.3-21.

Espíndola JAA, Almeida DL, Guerra JGM, Silva EMR. Flutuação sazonal da biomassa microbiana e teores de nitrato e amônio de solo coberto com *Paspalum notatum* em um agroecossistema. Flor Amb. 2001;8:104-13.

Ferreira DA, Carneiro MAC, Saggin Junior OJ. Fungos micorrízicos arbusculares em um Latossolo Vermelho sob manejos e usos no Cerrado. Rev Bras Cienc Solo. 2012;36:51-61. doi:10.1590/S0100-06832012000100006

Fokom R, Adamou S, Teugwa MC, BegoudeBoyogueno AD, Nana WL, Ngonkeu MEL, Tchameni NS, Nwaga D, TsalaNdzomo G, AmvamZollo PH. Glomalin related soil protein, carbon, nitrogen



and soil aggregate stability as affected by land use variation in the humid Forest zone of south Cameroon. Soil Till Res. 2012;120:69-75. doi:10.1016/j.still.2011.11.004

Franzluebbers AJ, Stuedemann JA, Schomber HH. Spatial distribution of soil carbon and nitrogen pools under grazed tall fescue. Soil Sci Soc Am J. 2000;64:635-9. doi:10.2136/sssaj2000.642635x

Gama-Rodrigues EF, Barros NF, Gama-Rodrigues AC, Santos GA. Nitrogênio, carbono e atividade da biomassa microbiana do solo em plantações de eucalipto. Rev Bras Cienc Solo. 2005;29:893-901. doi:10.1590/S0100-06832005000600007

Gerdermann JN, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Brit Mycol Soc. 1963;46:235-44. doi:10.1016/S0007-1536(63)80079-0

Guareschi RF, Pereira MG, Menezes CEG, Anjos LHC, Correia MEF. Atributos químicos e físicos do solo sob pastagem e estádios sucessionais de floresta estacional. Rev Fac Agron Univ Nac La Plata. 2014;113:47-56.

Hammer O, Harper DA, Ryan PD. PAST – Paleontological Statistics ver.1.12, 2004 [internet]. [accessed on: 20 Apr 2015]. Available at: http://www.folk.uio.no/ohammer/past.

Islabão GO, Timm LC, Castilhos DD, Prestes RB, Bamberg AL. Carbono da biomassa e atividade microbiana em solos cultivados com morango no município de Turuçu/RS. [Acesso: 02 Jun 2015]. Disponível em: http://www.ufpel.edu.br/cic/2008/cd/pages/pdf/CA/CA 00507.pdf.

Jenkinson DS, Powlson DS. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem. 1976;8:209-13. doi:10.1016/0038-0717(76)90005-5

Leite LFC, Galvão SRS. Matéria orgânica do solo: funções interações e manejo em solo tropical. In: Araújo ASF, Leite LFC, Nunes LAPL, Carneiro RFV, editores. Matéria orgânica e organismos do solo. Teresina: EDUFIP; 2008. p.11-46.

Lima GC, Silva MLN, Oliveira MS, Curi N, Silva MA, Oliveira, AH. Variabilidade de atributos do solo sob pastagens e Mata Atlântica na escala de microbacia hidrográfica. Rev Bras Eng Agríc Amb. 2014;18:517-26. doi:10.1590/S1415-43662014000500008

Loss A, Pereira MG, Bernini TA, Zatorre NP, Wadt PGS. Fertilidade do solo e matéria orgânica em Vertissolo e Argissoloso sob cobertura florestal e pastagem. Comunic Sci. 2014;5:1-10.

Machado RL, Resende AS, Campello EFC, Oliveira JA, Franco AA. Soil and nutrient losses in erosion gullies at different degrees of restoration. Rev Bras Cienc Solo. 2010;34:945-54. doi:10.1590/S0100-06832010000300036

Marchiori Júnior M, Mello WJ. Carbono, carbono da biomassa microbiana e atividade enzimática em um solo sob mata natural, pastagem e cultura do algodoeiro. Rev Bras Cienc Solo. 1999;23:257-63. doi:10.1590/S0100-06831999000200009

Martins CM, Galindo ICL, Souza ER, Poroca HA. Atributos químicos e microbianos do solo de áreas em processo de desertificação no semiárido de Pernambuco. Rev Bras Cienc Solo. 2010;34:1883-90. doi:10.1590/S0100-06832010000600012

Menezes CEG. Integridade de paisagem, manejo e atributos do solo no médio Vale do Paraíba do Sul Pinheiral-RJ [tese]. Seropédica: Universidade Federal Rural do Rio de Janeiro; 2008.

Moreira A, Malavolta E. Dinâmica da matéria orgânica e da biomassa microbiana em solo submetido a diferentes sistemas de manejo na Amazônia Ocidental. Pesq Agropec Bras. 2004;9:1103-10. doi:10.1590/S0100-204X2004001100008

Morell F, Hernández A, Borges Y, Marentes FL. La actividad de los hongos micorrízicos arbusculares em la estructura Del suelo. Cult Trop. 2009;30:25-31.

Nobre CP, Lázaro ML, Espirito Santo MM, Pereira MG, Berbara RL. Agregação, glomalina e carbono orgânico na chapada do Araripe, Ceará, Brasil. Rev Caatinga. 2015;28:138-47.

Oliveira JRA, Mendes IC, Vivaldi L. Carbono da biomassa microbiana em solos de cerrado sob vegetação nativa e sob cultivo: avaliação dos métodos fumigação-incubação e fumigação-extração. Rev Bras Cienc Solo. 2001;25:863-71. doi:10.1590/S0100-06832001000400009



Reichert JM, Reinert DJ, Braida JA. Qualidade dos solos e sustentabilidade de sistemas agrícolas. Rev Cienc Amb. 2003;27:29-48.

Rillig MC, Field CB, Allen MF. Soil biota responses to long term atmospheric CO<sub>2</sub> enrichment in two California annual grasslands. Oecologia.1999;119:572-7. doi: 10.1007/s004420050821

Rillig MC. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J Soil Sci. 2004;4:355-63. doi: 10.4141/S04-003

Rook AJ, Dumont B, Isselstein J, Osoro K, Wallisdevries MF, Parente G, Mills J. Matching type of livestock to desired biodiversity outcomes in pastures – a review. Biol Conserv. 2004;119:137-50. doi: 10.1016/j.biocon.2003.11.010

Salton JC, Carvalho PCF. Heterogeneidade de pastagem - causas e consequências. Dourados: Embrapa Agropecuária Oeste; 2007.

Sparling GP. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Aust J Soil Res. 1992;30:195-207. doi:10.1071/SR9920195

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha TJF, Oliveira JB. Sistema brasileiro de classificação de solos. 3ª ed. Brasília, DF: Embrapa; 2013.

Santos RS, Barreto PAB, Scoriza RN. Efeito da sazonalidade na comunidade de fungos micorrízicos arbusculares em um fragmento de mata de cipó em Vitória da Conquista, Bahia. Rev Bras Biocienc. 2014;12:46-51.

Schenck NC, Perez Y. A manual for identification of vesicular-arbuscular mycorrhizal fungi. 2nd ed. Gainesville: University of Florida; 1988.

Silva CF, Pereira MG, Miguel DL, Feitora JCF, Loss A, Menezes CEG, Silva EMR. Carbono orgânico total, biomassa microbiana e atividade enzimática do solo de áreas agrícolas, florestais e pastagem no médio Vale do Paraíba do Sul (RJ). Rev Bras Cienc Solo. 2012a;36:1680-9. doi:10.1590/S0100-06832012000600002

Silva CF, Pereira MG, Santos VL, Miguel DL, Silva EMR. Fungos micorrízicos arbusculares: Composição, comprimento de micélio extra radicular e glomalina em áreas de Mata Atlântica, RJ. Cienc Flor. *In press*.

Silva CF, Pereira MG, Silva EMR, Correia MEF, Saggin-Júnior OJ. Fungos micorrízicos arbusculares em áreas no entorno do Parque Estadual da Serra do Mar em Ubatuba (SP). Rev Caatinga. 2006;19:1-10.

Silva CF, Simões-Araújo JL, Silva EMR, Pereira MG, Freitas MSM, Saggin Júnior OJ, Martins MA. Fungos micorrízicos arbusculares e proteína do solo relacionada à glomalina em área degradada por extração de argila e revegetada com eucalipto e acácia. Cienc Flor. 2012b;22:749-61. doi:10.5902/198050987556

Silva CF, Tavares PD, Pereira MG, Saggin-Júnior OJ, Silva EMR. Fungos micorrízicos arbusculares em solo sob sistemas agroflorestais, mata nativa e agricultura anual em Paraty (RJ). In: Anais do 34º Congresso Brasileiro de Ciência do Solo; 2013; Florianópolis. Florianópolis: SBCS; 2013.

Silva LX, Figueiredo MVB, Silva GA, Goto BT, Oliveira JP, Burity HA. Fungos micorrízicos arbusculares em áreas de plantio de leucena e sábia no estado de Pernambuco. Rev Árvore. 2007;31:427-35. doi:10.1590/S0100-67622007000300008

Silva RR, Silva MLN, Cardoso EL, Moreira FMS, Curi N, Alovisi AMT. Biomassa e atividade microbiana em solo sob diferentes sistemas de manejo na região fisiográfica Campos das Vertentes - MG. Rev Bras Cienc Solo. 2010;34:1585-92. doi:10.1590/S0100-06832010000500011

Siqueira JO, Carneiro MAC, Curi N, Rosado, SCS, Davide AC. Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in South Eastern Brazil. For Ecol Manage. 1998;107:241-52. doi:10.1016/S0378-1127(97)00336-8

Sousa CS, Menezes RSC, Sampaio EVSB, Lima FSL. Glomalina: Características, produção, limitações e contribuição nos solos. Semina: Cienc Agrár. 2012;33:3033-44. doi:10.5433/1679-0359.2012v33n6Supl1p3033

12



Toda FE, Vasques T, Araújo FF. Biomassa microbiana e sua correlação com a fertilidade de solos em diferentes sistemas de cultivo. Colloq Agr. 2010;6:1-7.

Tótola MR, Chaer GM. Microorganismos e processos microbiológicos como indicadores de qualidade dos solos. Tópicos Cienc Solo. 2002;2:195-276.

Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem. 1987;19:703-7. doi:10.1016/0038-0717(87)90052-6

Vicente GCMP, Araújo FF. Uso de indicadores microbiológicos e de fertilidade do solo em áreas de pastagens. Semina: Cienc Agrár. 2013;34:137-46. doi:10.5433/1679-0359.2013v34n1p137

Whiffen LK, Midgley D, McGee PA. Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. Soil Biol Biochem. 2007;39:691-4. doi:10.1016/j.soilbio.2006.08.012

Wright SF, Franke-Snyder M, Morton JB, Upadhyaya AA. Time course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil. 1996;181:193-203. doi:10.1007/BF00012053

Wright SF, Upadhyaya AA. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil. 1998;198:97-107. doi:10.1023/A:1004347701584

Yeomans JC, Bremner JM. A rapid and precise method for routine determination of organic carbon in soil. Commun Soil Sci Plant Anal. 1988;19:1467-76. doi:10.1080/00103628809368027

Zangaro W, Bononi VLR, Trufem SB. Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil. J Trop Ecol. 2000;16:603-22. doi:10.1017/S0266467400001607

13