



Soil organic carbon, microbial biomass and enzyme activities responses to natural regeneration in a tropical dry region in Northeast Brazil

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ABSTRACT

Natural regeneration may be a cost-effective method for recovering areas previously used for intensive agricultural purposes. Plant diversity in the succession periods has been well documented; however, less attention has been paid to the changes in soil attributes, which may work as an instrument for the validation of regeneration methods. The present study is part of a broader interdisciplinary research project assessing the effects of natural regeneration on biodiversity and the quality of the soil. We investigated the effects of natural regeneration on the physicochemical attributes of the soil, as well as on soil organic carbon (SOC), microbial biomass carbon (MBC) and enzyme attributes. We assessed three stages and five areas of each natural regeneration stage (early-ER, intermediate-IR and late-LR) in two layers: 0–5 and 5–10 cm. The present study found a 20% SOC increase due to natural regeneration. In the first layer, SOC, urease, acid phosphatase and arylsulfatase absolute activities were significantly higher in the two older natural regeneration stages (IR and LR) than those found in the ER stage. We found a reduction in specific enzyme activities per SOC unit in the ER areas. Natural regeneration influenced SOC and MBC, the absolute enzyme activities, and the specific enzymes per SOC unit, mainly in the surface layer. The present study provided some of the first data concerning the beneficial effects of natural regeneration on the quality of soil as measured through enzyme activity, SOC and MBC in a tropical dry region in Northeastern Brazil.

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1. Introduction

Significant changes in the processes and functions of ecosystems, such as carbon emissions and biodiversity losses, have happened as forests have been converted to intensive agricultural areas due to constant human interventions worldwide (Malhi et al., 2014). Tropical dry areas (TDA) constitute the most extensive terrestrial biome on Earth and cover approximately one-third of the planet's continental surface (Pointing and Belnap, 2012); these areas have suffered the most severity anthropic pressures (Quesada et al., 2009). México, for instance, had 73% of its tropical dry forest converted to other types of land use (Maass et al., 2005), and in Brazil, the conversion of tropical dry forest reached

46% (MMA, 2011). Deforestation and degradation have been responsible for 8 to 15% of the annual anthropogenic carbon emissions (Houghton et al., 2015).

However, little is known about the regeneration process in areas that have been intensively used for agricultural purposes. Accordingly, natural regeneration is an important strategy for reducing carbon emissions caused by deforestation and degradation (Chazdon et al., 2016).

Natural regeneration is the cheapest method available for providing habitat to improve the biodiversity of communities, sequester carbon (Gilroy et al., 2014) and increase carbon stocks (Moura et al., 2016). For instance, after 20 years of recovery, 3.05 Mg C ha⁻¹ yr⁻¹ of above-ground biomass uptake occurred (Poorter et al., 2016), whereas soil organic carbon (SOC) increased from 8.37 to 11.62 g C kg⁻¹ soil after 57 years of natural regeneration in a Brazilian tropical dry forest (Moura et al., 2016).

Additionally, there are many studies about natural regeneration concern the composition of tree species (Van Rensen et al., 2015; Barna and Bosela, 2015) or fertilization (Campo and Vázquez-Yanes, 2004). Nevertheless, litterfall and root activities of different tree species have a direct

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impact on soil properties (Neumann et al., 2013), such as nutrient availability, soil microbial community (Scheibe et al., 2015) and enzyme activities (Boeddinghaus et al., 2015). On the other hand, there are several studies related to soil attributes in TDA in México (Campo and Vázquez-Yanes, 2004) and Argentina (Palma et al., 2000; Abril et al., 2013). There are just a few studies related to the biological and biochemical indicators of TDA. For example, Pajares et al. (2009) studied an Acrisol cultivated according to different management practices. Medeiros et al. (2015) compared tropical dry forests to intercroppings and monocroppings. Although, important questions remain unanswered in the literature, such as what is the range of microbial biomass and enzyme activities and how is it affected by natural regeneration stages? Understanding the microbial and biochemical soil activities in TDA is relevant for planning strategies of conservation, recovery of ecosystems and the generation of data for different areas.

It is important to assess the composition and activity of microorganisms in order to find the potential quality and nutrient cycling and C sequestration capabilities of the soil since these microorganisms are a relevant source of soil enzymes and are responsible for organic matter transformations in the soil (Smith et al., 2015). Soil enzyme activities are a sensitive indicator of soil quality and may respond to changes in the soil faster than other soil properties (Medeiros et al., 2015). Many recent studies have recommended using specific enzymes activities per unit of SOC and microbial biomass carbon (MBC) (Wang et al., 2012; Raiesi and Beheshti, 2014; Medeiros et al., 2015) because specific enzyme activities per unit of SOC can express the nutritional status of the organic matter from the perspective of the microorganism (Wang et al., 2012).

Studies about soil enzyme activities in TDA under regeneration are scarce. Future studies must aim to determine how natural regeneration influences the quality of the soil by using sensitive and rapid indicators. A recent study about the successional and seasonal variations in wet post-agricultural regeneration areas detected differences between young and old secondary and primary forests by assessing the microbial community composition and activity via phospholipid fatty acid analysis (PLFA) and extracellular enzyme activity (Smith et al., 2015). Different studies about plant diversity, phenotypic plasticity and ecophysiological plant strategies were conducted in TDA to assess how these areas recover through natural regeneration (Cabral et al., 2013; Falcão et al., 2015; Moura et al., 2016; Chazdon et al., 2016; Poorter et al., 2016). Studies on soil properties may explain some of

the results described in many biodiversity studies (SISBIOTA Matas secas web- <http://tropi-dry.eas.ualberta.ca>). Therefore, determining soil attributes is crucial to understanding the efficiency of natural regeneration and to analyze whether these soils follow the same succession stage patterns in different plant species found in TDA. In this study, we have reported the effects of natural regeneration stages, based on different periods and plant species, on these areas have suffered the most severity anthropic pressures soil physicochemical properties, microbial biomass and enzyme activities in a reference Brazilian tropical dry area.

2. Materials and methods

2.1. Study area and soil samples

The study area is located in Paraíba State, Northeastern Brazil (06°59'13" to 07°0'14" S and 37°18'08" to 37°20'38" W), with an altitude of 250 to 350 m (Fig. 1). The climate in the region is tropical semi-arid (Bsh) according to the Köppen classification system. The mean temperature and annual rainfall are 28 °C and 700 mm, respectively (Supplementary data). According to the classification system of the Food and Agriculture Organization (IUSS Working Group–FAO, 2006), the soil is a Cambisol. According to Lozano-García et al. (2016), these soils are characterized by low fertility, poor physical conditions, low organic matter (OM) contents due to climate conditions (semiarid climate) and sandy soil textures (Table 1).

Soil samples were collected in 2012 during the dry season (November) in areas presenting different natural regeneration stages, namely: early regeneration (ER), intermediate regeneration (IR) and late regeneration (LR). Five repetition plots of 1000 m² (50 × 20 m) in each natural regeneration stage were determined, totaling 15 areas. We collected samples of undisturbed soils using cylinders of 5 cm in diameter and 5 cm in height in two soil layers (0–5 and 5–10 cm).

The ER stage areas had been used for long fiber cotton (*Gossypium hirsutum*) cultivation and subjected to natural regeneration for over 15 years. Cabral et al. (2013) identified 6 species and 3 families of plants in these areas, namely: *Mimosa tenuiflora* (which represented 90.1% of all trees in the study area), *Poincianella pyramidalis*, *Croton blanchetianus*, *Cereus jamacaru*, *Caesalpinia ferrea* and *Bauhinia cheilantha*. The IR stage areas were also previously used for long fiber cotton cultivation and, thereafter, had been subjected to natural

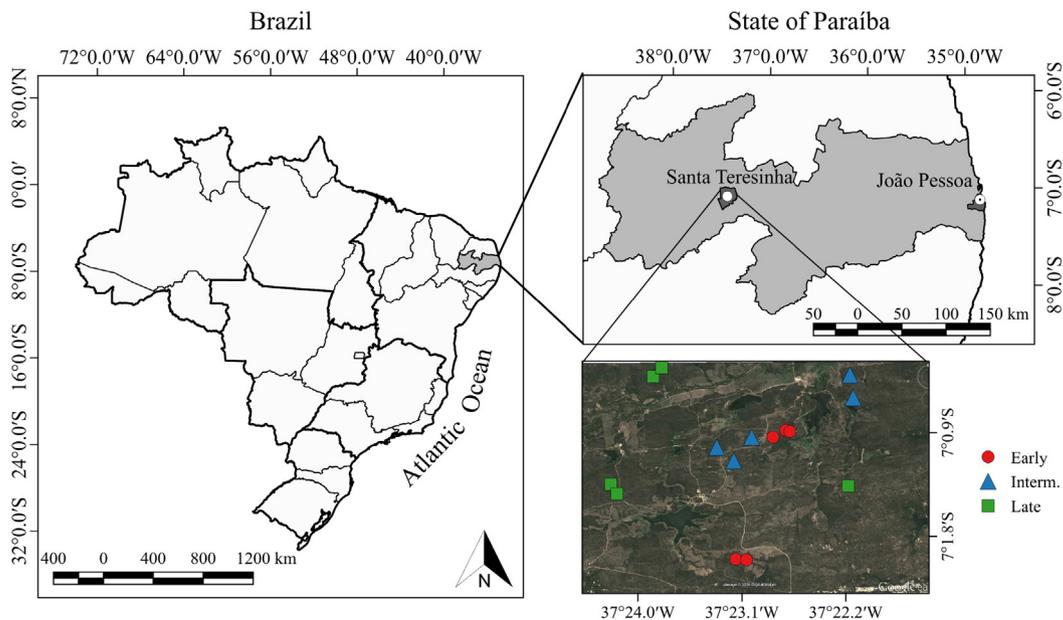


Fig. 1. Brazilian map showing the limits of Paraíba State and the regeneration stages (early, intermediate and late).

Table 1

Mean and standard deviation of physical and chemical characteristics of Cambisols in three stages of natural regeneration (early, intermediate, late) ($n = 5$) in tropical dry areas (NE Brazil). Different lower case letters indicate significant differences ($P < 0.05$) in the layer 0–5 cm and capital letters in the layer 5–10 cm by ANOVA followed by Student Newman-Keuls test.

	0–5 cm			5–10 cm		
	ER	IR	LR	ER	IR	LR
Sand (g kg^{-1})	764 (8.0) a	760 (5.2) a	757 (7.5) a	732 (10.7) A	760 (13.4) A	725 (15.9) A
Silt (g kg^{-1})	104 (14.5) a	80 (12.1) a	131 (6.1) a	120 (10.0) A	80 (6.9) A	155 (12.4) A
Clay (g kg^{-1})	132 (6.4) ab	160 (14.3) b	112 (4.3) b	148 (9.3) A	160 (14.3) A	120 (7.2) A
SBD (kg m^{-3})	1640 (19.6) a	1567 (7.1) ab	1558 (18.7) b	1662 (32.1) A	1609 (35.3) A	1598 (11.8) A
SP ($\text{m}^3 \text{m}^{-3}$)	0.38 (0.007) b	0.40 (0.005) ab	0.41 (0.007) a	0.37 (0.012) A	0.39 (0.013) A	0.40 (0.004) A
FD ($\text{m}^3 \text{m}^{-3}$)	0.13 (0.004) a	0.13 (0.002) a	0.18 (0.019) a	0.14 (0.004) A	0.13 (0.002) A	0.14 (0.020) A
WP ($\text{m}^3 \text{m}^{-3}$)	0.06 (0.004) a	0.06 (0.005) a	0.10 (0.013) a	0.06 (0.002) A	0.05 (0.005) A	0.06 (0.003) A
AW ($\text{m}^3 \text{m}^{-3}$)	0.07 (0.3) a	0.07 (0.3) a	0.08 (0.3) a	0.08 (0.1) A	0.08 (0.3) A	0.08 (0.8) A
pH (H_2O)	5.72 (0.1) b	6.29 (0.1) ab	6.57 (0.1) a	5.80 (0.1) A	6.22 (0.1) A	6.48 (0.1) A
Available P (mg kg^{-1})	1.21 (0.3) a	1.15 (0.1) a	1.12 (0.1) a	1.18 (0.3) A	1.10 (0.1) A	1.12 (0.2) A
Mg^{2+} ($\text{cmol}_c \text{kg}^{-1}$)	0.48 (0.05) a	0.25 (0.04) b	0.22 (0.04) b	0.46 (0.05) A	0.22 (0.05) B	0.19 (0.05) B
Ca^{2+} ($\text{cmol}_c \text{kg}^{-1}$)	5.75 (0.3) a	5.88 (0.4) a	5.67 (0.3) a	5.59 (0.4) A	4.75 (0.3) A	5.35 (0.2) A
Al^{3+} ($\text{cmol}_c \text{kg}^{-1}$)	0.07 (0.008) a	0.05 (0.002) a	0.05 (0.002) a	0.07 (0.008) A	0.06 (0.004) A	0.05 (0.003) A
Na^+ ($\text{cmol}_c \text{kg}^{-1}$)	0.17 (0.002) a	0.17 (0.009) a	0.17 (0.002) a	0.18 (0.01) A	0.17 (0.005) A	0.15 (0.006) A
K^+ ($\text{cmol}_c \text{kg}^{-1}$)	0.16 (0.01) a	0.18 (0.005) a	0.16 (0.01) a	0.16 (0.01) A	0.15 (0.007) A	0.16 (0.006) A
$\text{H}^+ + \text{Al}^{3+}$ ($\text{cmol}_c \text{kg}^{-1}$)	2.29 (0.1) a	2.15 (0.2) a	1.41 (0.1) b	1.97 (0.05) A	2.05 (0.2) A	1.37 (0.2) A

ER = early regeneration; IR = intermediate regeneration; LR = late regeneration. SBD = soil bulk density; SP = soil porosity; FD = field capacity; WP = wilting point; AW = available water.

regeneration for over 30 years. The arboreal vegetation was denser in comparison to the previous stage (ER). Fifteen species and 10 families of plants have been identified in the (IR) areas: *Mimosa tenuiflora* (18.3% of all trees in the area), *Poincianella pyramidalis* (42.9%), *Croton blanchetianus* (24.8%), *Cereus jamacaru*, *Caesalpinia ferrea*, *Bauhinia cheilantha*, *Piptadenia stipulacea*, *Anadenanthera colubrina*, *Aspidosperma pyriformium*, *Commiphora leptophloeos*, *Tabebuia impetiginosa* (which was found in the IR stage, only), *Combretum leprosum*, *Cynophalla flexuosa*, *Myracrodruon urundeuva* and *Erythroxylum pungens*.

The LR stage areas have not been used for agricultural purposes, except for the mild selective cutting for firewood production, and have not undergone any clear cutting. These areas have underwent natural regeneration for over 50 years. The arboreal vegetation stands out for having a species diversity greater than the other stages, and includes several well-developed individuals belonging to climax species. Twenty-one species and 12 families of plants have been identified in the LR areas, including all of the species found in the other stages and *Amburana cearensis*, *Cnidoscolus quercifolius*, *Sapium glandulosum*, *Pseudobombax marginatum*, *Cochlospermum insigne*, *Ziziphus joazeiro*, *Jatropha mollissima* and *Bauhinia cheilantha*.

All of these areas presented similar climatic conditions, soil types, topographies and altitudes.

2.2. Physical and chemical attributes of the soil

Soil texture analysis was conducted in a hydrometer using sodium hexametaphosphate as the dispersing agent and according to Loveland and Whaley (1991). The soil bulk density (SBD) was determined through the core method using 5-cm-diameter and 5-cm-height cylinders. The soil porosity (SP) was calculated through SBD and PD, where $\text{SP} = 1 - (\text{SBD} / \text{PD})$, and the particle density (PD) is considered equal to 2650 kg m^{-3} .

The field capacity (FC) and wilting point (WP) were estimated using a Pressure plate (Richards, 1965) under tensions of 0.01 and 1.5 MPa, respectively. The available water capacity (AWC) was obtained through $\text{AWC} = \text{FC} - \text{WP}$.

The following chemical attributes were determined: pH in water (1:2.5), available P, exchangeable K^+ , Na^+ , Al^{3+} , Ca^{2+} and Mg^{2+} (EMBRAPA, 2009). The Na^+ , P and K^+ were extracted using Mehlich-I, and K^+ was quantified through the colorimetric method. The extractable inorganic P was quantified using the colorimetry method.

2.3. Soil organic carbon (SOC) and microbial biomass carbon (MBC) of the soil

The SOC was determined through wet oxidation using potassium dichromate and the titration of the remaining dichromate ammonium ferrous sulfate, according to Yeomans and Bremner (1988).

The soil MBC content was determined using the irradiation method (Mendonça and Matos, 2005). The biomass was determined by adding 80 mL 0.5 M K_2SO_4 , as the extractant to 20 g of each soil sample. The carbon content in the extracts was determined using the colorimetric method (Bartlett and Ross, 1988).

2.4. Absolute enzyme activities

The urease (URE) (EC 3.5.1.5), arylsulfatase (ARY) (EC 3.1.6.1) and acid phosphatase (Pac) (EC 3.1.3) enzymes activities were quantified according to the colorimetric analysis of the products released by the samples subjected to incubation in an adequate substrate (Sigma-Aldrich) under standard conditions.

The soil URE activity was determined using urea as a substrate, according to the method by Kandeler and Gerber (1988). The ARY activity was estimated in the ρ -nitrophenyl sulfate substrate using the Tabatabai and Bremner (1972) method. The Pac activity was determined in ρ -nitrophenyl phosphate through the method of Eivazi and Tabatabai (1977). Product absorbance was measured using a spectrophotometer (Libra S22, Biochrom, Cambridge, England).

2.5. Specific enzyme activities per SOC and MBC unit

Each absolute enzyme activity was divided by SOC (Acosta-Martínez et al., 2003) to determine the activities of specific enzymes per SOC unit. Data of all the enzyme activities were divided by MBC to set the specific enzyme activities per MBC unit, according to Raiesi and Beheshti (2014).

2.6. Data analysis

Data were statistically analyzed using ANOVA to investigate the significance of natural regeneration effects on soil attributes. Significant differences were compared using the Student Newman-Keul's test at 5% probability for each layer. Multivariate principal component analysis

(PCA) was used to identify the attributes most responsible for the variation between the different natural regeneration stages in tropical dry areas. A multivariate analysis was separately applied to each of the layers (0–5 and 5–10 cm). We included all chemical, microbiological and biochemical soil properties in the analyses conducted in STATISTICA 8.0 (Statsoft Inc., Tulsa, USA) software.

3. Results

3.1. Physical and chemical attributes of the soil

The particle size did not significantly differ ($P < 0.05$) between plots in all regeneration stages in both layers. The plots ($n = 15$) presented a sandy soil in the different natural regeneration stages (Table 1), thus proving the homogeneity between the studied sites.

There was significant difference between soil bulk density (SBD) and total porosity (SP) in LR and ER stages in the first layer (Table 1). The regeneration stages did not influence the field capacity, the wilting point and available water in both layers.

The soil in the ER stage showed lower pH (5.72) lower than the soil of the others regeneration stages (6.29 in IR and 6.57 in LR) (Table 1), but only in the first layer. The chemical attributes (available P, Ca^{2+} , Al^{3+} , Na^+ and K^+) in the soil samples were not affected ($P < 0.05$) by the natural regeneration stages (Table 1), but the ER stage presented the highest Mg^{2+} ($0.48 \text{ cmol}_c \text{ kg}^{-1}$) and $\text{H}^+ + \text{Al}^{3+}$ ($2.29 \text{ cmol}_c \text{ kg}^{-1}$).

3.2. Soil organic carbon (SOC) and the microbial biomass carbon (MBC) in the soil

The SOC was significantly influenced ($P < 0.05$) by the natural regeneration stages (Fig. 2A). Higher SOC was observed in the LR and IR areas

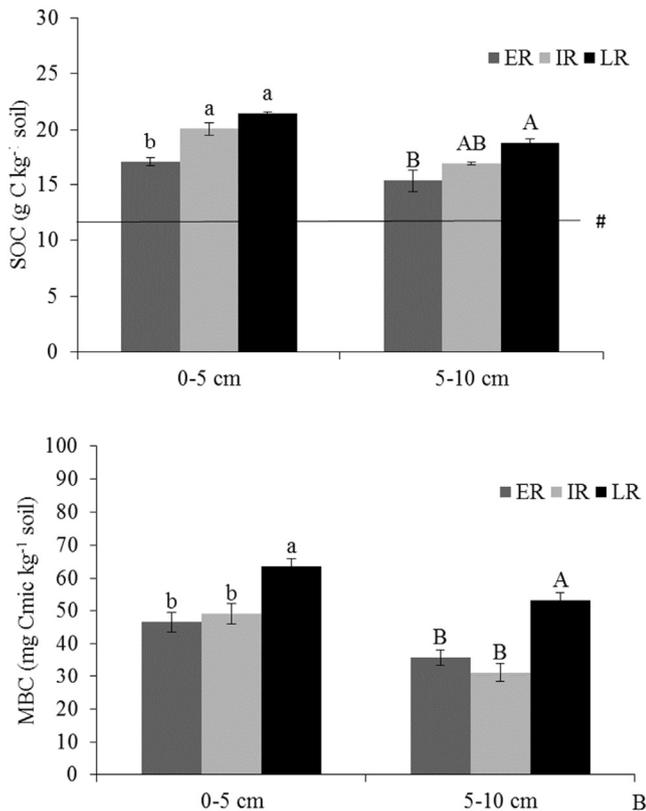


Fig. 2. (A) Soil organic carbon and (B) microbial biomass carbon of the two upper soil layers at three stages of natural regeneration (early, intermediate, late) of the tropical dry areas (NE Brazil, $n = 5$). Lower case and capital letters indicate significant differences ($P < 0.05$) in the layers 0–5 cm and 5–10 cm, respectively, using ANOVA followed by Student Newman-Keuls test.

in both layers. In the first layer, the SOC content for IR and LR was 20.07 and 21.40 g C kg⁻¹ soil, whereas the SOC content was 16.85 and 18.79 g C kg⁻¹ soil in the second layer, respectively.

Natural regeneration had a strong influence on the soil MBC content (Fig. 2B). The LR stage achieved a 26.75 and 22.85% increase when it was compared to the ER and IR stages in the first layer, respectively. Such results indicated microbial biomass accumulation with time.

3.3. Absolute enzyme activities

The absolute enzyme activities were significantly higher ($P < 0.05$) in IR and LR stages than in ER (Fig. 3). The URE activity increased >100% in LR in comparison to ER, ranging from 10.09 to 23.67 $\mu\text{g NH}_4\text{-N g}^{-1} \text{ soil h}^{-1}$

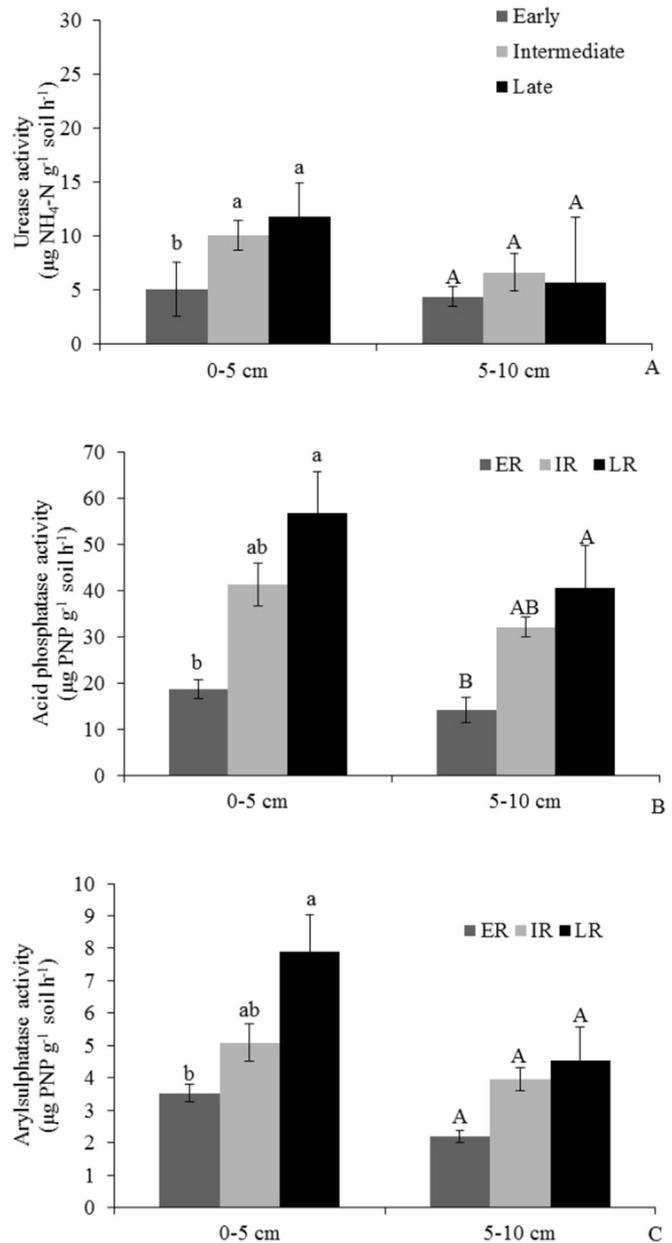


Fig. 3. Absolute enzyme activities in the two upper soil layers at three stages of natural regeneration (early, intermediate, late) of the tropical dry areas (NE Brazil, $n = 5$): (A) Urease; (B) acid phosphatase and (C) arylsulphatase activities. Lower case and capital letters indicate significant differences ($P < 0.05$) in the 0–5 cm and 5–10 cm layers, respectively, using ANOVA followed by Student Newman-Keuls test.

$\text{N g}^{-1} \text{ soil h}^{-1}$ (Fig. 3A) in the first layer (0–5 cm), and was similar in all regeneration stages in the second layer (5–10 cm), ranging from 8.80 to $13.21 \mu\text{g NH}_4\text{-N g}^{-1} \text{ soil h}^{-1}$.

The Pac and the ARY activities increased in the first layer of the LR (Fig. 3B and C). The Pac increased consistently with succession stage in both layers. The Pac ranged from $18.65 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ in the ER to $56.80 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ in the LR in the first layer, and 14.19 (ER) to $40.63 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ (LR) in the second layer. Only the Pac was influenced by the regeneration stages in the second layer. The ER stage presented the lowest Pac activity.

The ARY activities followed the same pattern of Pac in that it increased with succession stage (Fig. 3C), but only in the first layer. The ARY activities in ER, IR and LR areas were 3.53, 5.09 and $7.90 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ in the first layer (0–5 cm).

3.4. Specific enzyme activities per unit of SOC and MBC

The changes in specific enzyme activities per unit of SOC were caused by the natural regeneration stages and followed the same pattern as the absolute enzyme activities. All the specific enzyme activities per unit of SOC were higher in the LR stage areas (Fig. 4A), but only in the first layer (0–5 cm). These results are considered similar to the absolute enzymes because there were no differences in the second layer (5–10 cm).

Only the Pac/MBC showed significant natural regeneration effects when considering the specific enzyme activities per unit of MBC (Fig. 4B). High specific Pac/MBC in the LR and IR stages indicated increased enzyme production, and the release of such enzymes by microorganisms in the soil is dependent on the natural regeneration stage.

3.5. Multivariate analysis

The multivariate PCA analysis showed differences between the natural regeneration stages in tropical dry forests, but only in the first layer (Fig. 5A). The IR and LR natural regeneration stage areas were in the same group when the second layer was assessed (Fig. 5B). Data collected showed a clear difference between the soil parameters in the ER, IR and LR stage areas.

The PC1, PC2 and PC3 explained 40.73%, 15.82% and 11.79% of the variability in the first layer, respectively (Table 2). SOC, MBC, URE, Pac, ARY and pH had a positive weight in PC1, whereas the available P, Mg^{2+} and Al^{3+} had a negative weight. Only Ca^{2+} presented a negative weight in PC2, and Na^+ and K^+ had a positive weight in PC3. Thus, we confronted PC1 and PC3 because they clustered the different natural regeneration stages better (Fig. 5). The PC1, PC2 and PC3 explained 37.00%, 20.74% and 11.61% of variability in the second layer, respectively. The SOC, URE, Pac, ARY and pH had negative weight in PC1, and Mg^{2+} and Na^+ had positive weight in PC1. The available P, Ca^{2+} and K^+ had negative weight in PC2, whereas only MBC had a positive weight in PC3 (Table 2).

4. Discussion

4.1. Physical and chemical attributes of the soil

The assessed soils consisted mostly of sand and were similar to other tropical Cambisols (Mota et al., 2014; Martins et al., 2015). Soil texture is an important factor in controlling the balance between water and gases in the soil, and it becomes more stable in time, regardless of the soil

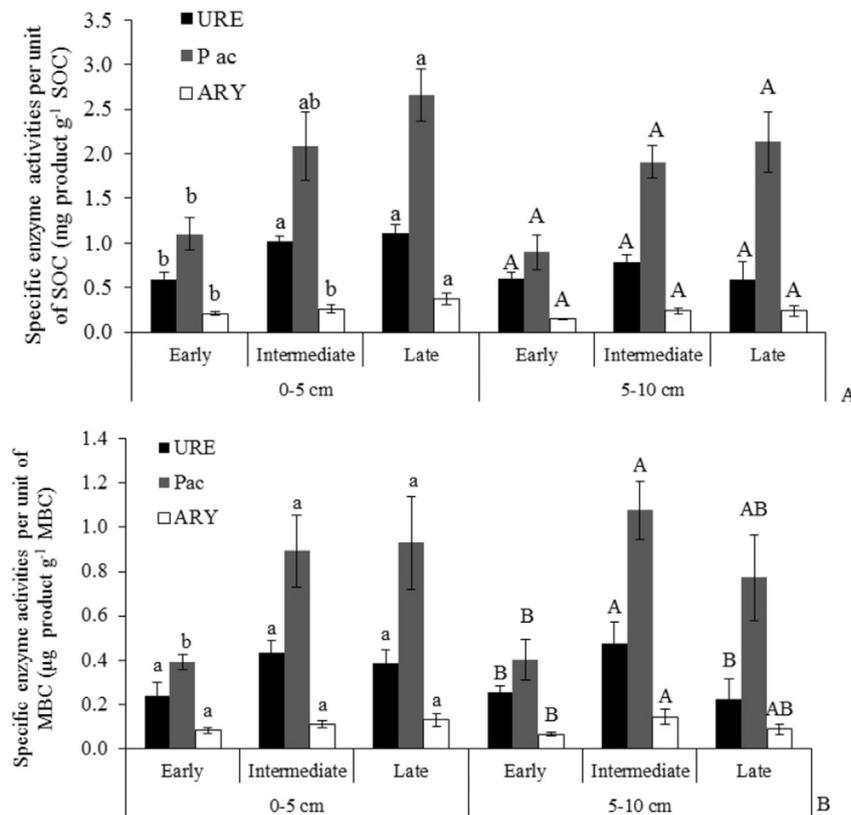


Fig. 4. Specific enzyme activities per unit of (A) soil organic carbon (SOC) and (B) microbial carbon biomass (MBC) of the two upper soil layers at three stages of natural regeneration (early, intermediate, late) of the tropical dry areas (NE Brazil, $n = 5$). Lower case and capital letters indicate significant differences ($P < 0.05$) in the 0–5 cm and 5–10 cm layers, respectively, using ANOVA followed by Student Newman-Keuls test.

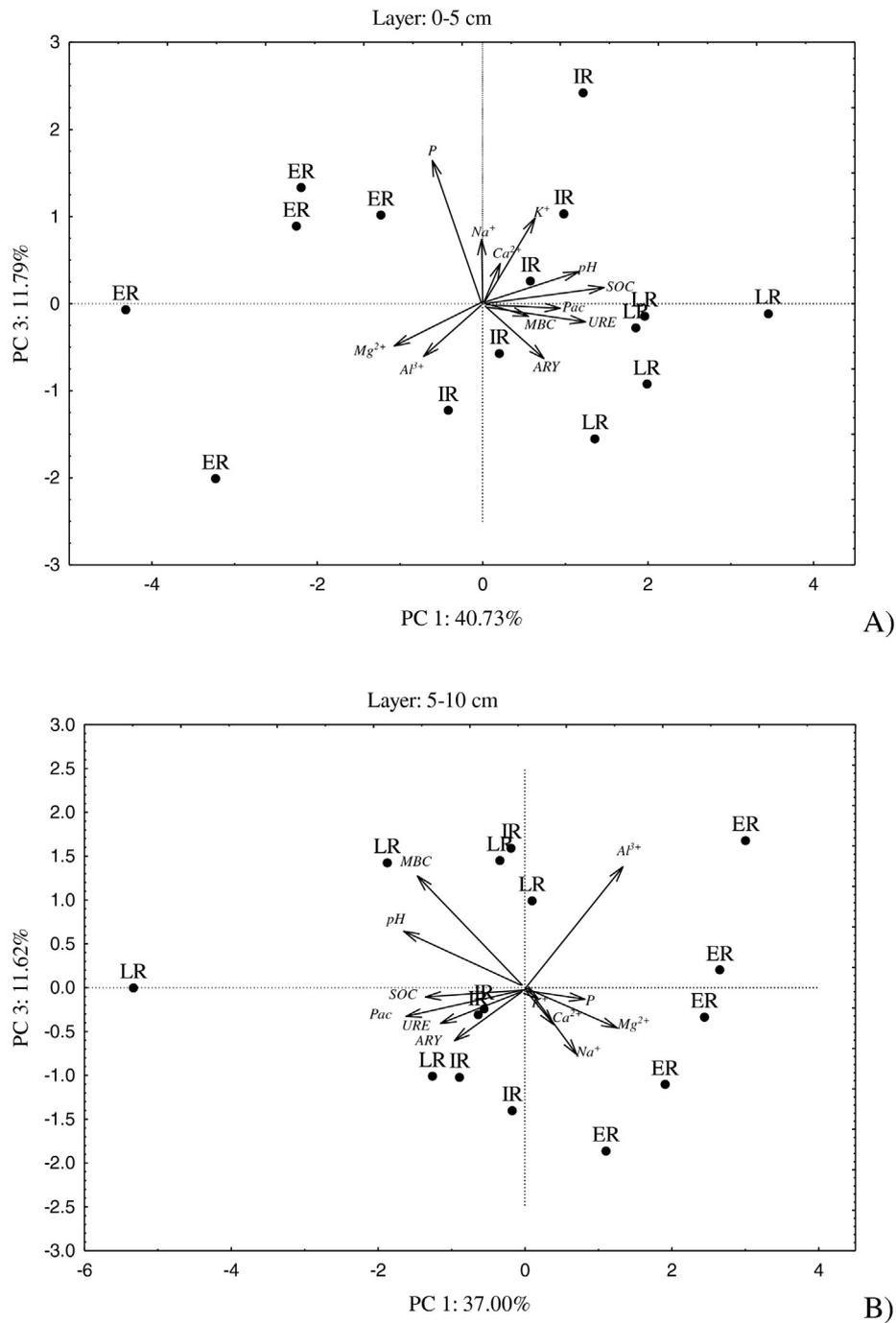


Fig. 5. Biplot of a principal components analysis (PCA) of A) Layer 0–5 cm and B) layer 5–10 cm showing the soil organic carbon (SOC), microbial biomass (MBC) and absolute enzymes activities (URE, Pac and ARY) and the clusters points as three stages of natural regeneration (early, intermediate, late) of the two upper soil layers from tropical dry areas (NE Brazil).

management system. Accordingly, the different natural regeneration stages did not change the soil texture and were similar among Brazilian TDA plots.

The soils in the ER stage showed the highest SBD and the lowest SP in the first layer. Values in the second layer (5–10 cm) were similar to the IR and LR stages. According to Beutler et al. (2002), these two variables may express the effects of land use and management on the soil. Lower SBD, as seen in the LR areas, has been generally observed in soils under low anthropogenic interference (Bini et al., 2013) because the organic matter in the soil enables its particles to better aggregate, improving soil structure (Quraishi and Mouazen, 2013). The higher SBD observed in the first layer (0–5 cm) may be due to the conventional tillage system used in the ER stage areas. These areas were cleared of

native Caatinga by slash and burn in 1965, and then cultivated with cotton [*Cenchrus ciliaris* L. (buffelgrass)] in 1970 and used as pasture up to 1992.

This tillage system may cause soil compaction and increased SBD and SP reduction (Hamza and Anderson, 2005). The different natural regeneration stages did not affect some chemical attributes of the soil. Overall, the TDF showed nutrient limitations because present water limitations prevented the plant uptake of nutrient from the soil and reduced the release of nutrients during decomposition (Campo et al., 2007). Therefore, TDA's regeneration capacity is more limited than humid environments because of reduced mineral nutrient supply and the low availability of species of shrubs and trees (Rico-Gray and García-Franco, 1992). In this Brazilian TDA, others studies showed that

Table 2

Correlation between the variables and principal components, the Eigenvalues and the percentage rate of explained variability of each component.

	Layer					
	0–5 cm			5–10 cm		
	PC1	PC2	PC3	PC1	PC2	PC3
SOC	0.92 ^a	0.09	0.08	−0.80 ^a	−0.37	−0.14
MBC	0.50 ^a	0.25	−0.12	−0.36	−0.45	0.53 ^a
URE	0.88 ^a	−0.18	−0.13	−0.63 ^a	0.28	−0.21
Pac	0.74 ^a	−0.41	−0.12	−0.87 ^a	−0.11	−0.21
ARY	0.63 ^a	−0.59	−0.32	−0.80 ^a	0.17	−0.37
PH	0.86 ^a	0.18	0.15	−0.74 ^a	−0.32	0.25
Available P	−0.52 ^a	−0.08	0.50	0.45	−0.65 ^a	−0.19
Mg ²⁺	−0.74 ^a	−0.55	−0.10	0.75 ^a	−0.25	−0.27
Ca ²⁺	0.09	−0.85 ^a	0.39	0.24	−0.85 ^a	−0.17
Al ³⁺	−0.59 ^a	−0.32	−0.32	0.57 ^a	0.30	0.47
Na ⁺	0.01	0.22	0.56 ^a	0.41 ^a	0.29	−0.66
K ⁺	0.36	−0.26	0.64 ^a	0.11	−0.74 ^a	−0.11
Eigenvalue	4.89	1.90	1.41	4.44	2.49	1.39
% total	40.73	15.85	11.79	37.00	20.74	11.62
Cumulative	40.73	56.58	68.37	37.00	57.74	69.36

^a Significant effect.

water availability resulting from rainfall is lower than in other dry forests (Chazdon et al., 2016). These TDA have low water retention due to their shallow soil profiles (Moura et al., 2016) and sandy texture (Table 1), all of which have contributed to the low nutrient supply in the present study.

4.2. Soil organic carbon (SOC) and microbial biomass carbon (MBC) in the soil

The two late natural regeneration stages (IR and LR) showed the highest SOC value. In a study in the same areas reported similar results regarding C, N and the C/N ratio in soil samples collected every month from June 2007 to May 2009 (Moura et al., 2016). We did not determine soil N, but Moura et al. (2016) showed 0.78, 1.18 and 1.06 g N kg^{−1} of soil TN in ER, IR and LR, respectively. The C/N ratio was 11.04 (ER), 11.94 (IR) and 10.96 (LR), and was not sensitive enough to detect differences between the different stages of natural regeneration. The sites presenting the highest SOC values corresponded to the largest amount and diversity of dominant tree species. According to Cabral et al. (2013), the ER stage areas had 343 trees belonging to three different families and six species, the IR stage areas had 545 trees distributed in ten different families and 15 species and the LR stage areas had 700 trees distributed in 12 different families and 21 species. This higher tree density in LR helped achieve higher root biomass (Kaur et al., 2000) and organic residues, as well as higher C values above and below the ground. Litterfall and root turnover are major nutrient cycling components between plants and soil (Campo et al., 2007). We did not determine litter, but in a study in the same area, Moura et al. (2016) determined carbon and nutrient fluxes through litterfall and showed that the nutrient concentration in litter was in the range of other tropical dry forests. The litterfall C fluxes in ER, IR and LR were 1.25, 1.10 and 0.91 t C ha^{−1} year^{−1}, respectively. The concentration of nutrients in litter in these areas (ER, IR and LR) were 19.4, 16.7 and 14.4 g of N kg^{−1} of litter, 0.81, 0.67 and 0.71 g of P kg^{−1} of litter and 4.94, 6.55 and 5.57 g of K kg^{−1} of litter, respectively (Moura et al., 2016). The ER stage increased the SOC value >20% compared to cultivated areas with pineapple plants in the same soil and similar conditions (Mota et al., 2014). It was not surprising to find the highest SOC content in natural regeneration areas, rather than in agricultural lands. There is vast literature on how land use often causes SOC decreases in relation to forests (Wang et al., 2012) in TDA (Medeiros et al., 2015; Chazdon et al., 2016). Our results indicated that 15 years of natural regeneration (ER stage) led to consistent SOC increases in cultivated areas. It corroborated the evidence that natural regeneration is an efficient method to restore biochemical

attributes of agricultural TDA (Chazdon et al., 2016). The SOC contents in Cambisols sites in the studied areas ranged from 15.32 to 21.41 g C kg^{−1} of soil and was higher than other dry areas, such as the Cambisols cultivated with *Acacia* spp. (7.5 to 12.8 g C kg^{−1} of soil) in Eastern Burkina Faso (Traoré et al., 2007) and in the semiarid region in India (3.8 g C kg^{−1} of soil) (Kanchikerimath and Singh, 2001). However, SOC was close to Cambisols in the Apodi Plateau in northeastern Brazil (about 16 g C kg^{−1} of soil) (Mota et al., 2014). Nevertheless, SOC in this study was lower than Cambisols in the humid forests of southern Brazil (43 g C kg^{−1} of soil) because moisture in this region can strongly affect the SOC in native bush (De Oliveira et al., 2015). The Brazilian semiarid region has peculiar features, such as the distinct phenological patterns in plant growth and litter decomposition. These patterns are due to climate seasonality, which, in turn leads to C losses due to soil respiration (Ribeiro et al., 2016) and low vegetation biomass, which is mainly composed of annual species that dry and disappear with the dry season (Moura et al., 2016). Both temperature and moisture strongly affect organic matter decomposition rates; therefore, such decomposition processes appear to be extremely sensitive to different climate conditions (Steinweg et al., 2013). Additionally, the Brazilian semiarid region is affected by extensive deforestation (Jiménez et al., 2011).

The natural regeneration stages showed MBC accumulation through time in the Brazilian TDA. This observation is in agreement with research about different succession stages in secondary forests in Ziulin, Northwestern China (Jia et al., 2005), which showed increased MBC in a 17-year-old secondary forest. The differences came from plant diversity and composition in the LR stage, which changed the litterfall and the exudate compositions. Such changes helped the microbial community release nutrients in the soil and promoted vegetation development, as observed in other studies (Zak et al., 2003; Notaro et al., 2014; Smith et al., 2015). Eisenhauer et al. (2012) observed that areas with high plant diversity influence the microbial community in different soil types, and the effect was most remarkable after four years.

4.3. Absolute enzyme activities

The absolute enzyme activities measurements applied to the soil in different natural regeneration stages showed that the URE, Pac and ARY analyses were strongly differentiated between the regeneration stages. Areas presenting older natural regeneration stages, which were separated by regeneration time and plant diversity, showed higher absolute enzyme activity and MBC. In turn, it led to higher enzyme activities (Wang et al., 2012), since the absolute enzyme activities reflects microbial activities. The functional diversity involved in decomposition played a crucial role in biogeochemical nutrient release (Sinsabaugh et al., 2008). However, the enzyme activities in the soil have different responses to soil use (Trasar-Cepeda et al., 2008) and forest age (Lucas-Borja et al., 2016). The highest absolute enzyme activities in the oldest natural regeneration areas reinforced the hypothesis that such activity is improved throughout long non-tillage periods. Thus, it may be a suitable instrument to measure changes in the quality of the soil and, therefore, become an appropriate microbial function, soil fertility, and ecological stability indicator (Caldwell, 2005).

The higher URE activity in the two late regeneration stages was attributed to microbial biomass improvement, the high quality of the substrates and the longer periods without soil disturbance (Sinsabaugh et al., 2008). In addition, the plant diversity in the LR stage, in the current study, influenced the microbial populations and, consequently, the enzyme activity (Boeddinghaus et al., 2015) because many bacteria, fungi, archaea and plants present in the soil produce urease (Fisher et al., 2017).

The URE activity in the current study was higher than that in the Cambisol areas of the subtropical forests in China (Lv et al., 2014) because they were coniferous forests (dominated by *Pinus massoniana*). The Brazilian forests are tropical and present higher plant diversity

than the Chinese coniferous forests. It corroborated the hypothesis that there is a strong link between plant diversity, microbial biomass (Smith et al., 2015) and activity in the soil because the majority of organic matter and nutrients required for plant production in the soil of the forest comes from the decomposition of litterfall and other plant inputs (Palma et al., 2000). However, some studies have stated that ER stages may produce more litterfall than late regeneration stages, as shown in a study conducted in the same site as the present study (Moura et al., 2016). The diversity was more important to evidence this difference because the higher absolute enzyme activities in LR and IR stages followed the same pattern of SOC values in the year of sampling.

The Pac and ARY activities increased and were dependent on regeneration time due to the high organic residue input that improved the microbial biomass, as also observed in the secondary forest succession in Ziulin, Northwestern China (Jia et al., 2005). However, Smith et al. (2015) found no influence of regeneration time on the Pac activity in tropical post-agricultural forests. Generally, phosphatase appeared to be higher when there was a higher shortage of available P in the system (Lemanowicz, 2011).

Our results showed that the absolute enzyme activities, as well as the nutrients, could reflect the quality of the soil. However, when they are individually assessed, this activity may result in errors due to their interaction with other soil factors.

4.4. Specific enzyme activities per unit of SOC and MBC

The changes in the specific enzyme activities per unit of SOC resulted from the natural regeneration stages. They followed the same pattern as the absolute enzyme activities. It corroborated other studies stating that changes in the soil enzyme activities might occur regardless of the SOC (Raiesi and Beheshti, 2014). Some authors have shown that the decrease in SOC in the deeper layers may be one of the main causes of reduction in microbial abundance and activities, and may due to the close relationship between SOC and MBC (Stone et al., 2014).

The enzyme activities per unit of MBC provided important ecological information because they showed the physiological capacity of viable microorganisms (Waldrop et al., 2000) and the efficiency of the enzyme (Allison et al., 2011). The higher specific Pac/MBC in the two late regeneration stages indicated increased enzyme production and its release by microorganisms in the soil, dependent on the natural regeneration stages. Previous studies have indicated that the increased specific enzyme activities per unit of MBC indicates greater metabolic activity by microorganisms in the soil (Lagomarsino et al., 2011; Raiesi and Beheshti, 2014). Such ratios can be used as an instrument to detect the metabolic status of microorganisms in the soil and the variability of the stabilized enzyme activities (Lagomarsino et al., 2011).

4.5. Multivariate analysis

The PCA results have revealed that all plots in each regeneration stage were grouped together in the first layer. The separation between the IR and LR areas in the first layer and the similarity between them in the second layer reinforced the significant effect of natural regeneration on the surface layer. The deposition and input of organic residues resulting from plant density and diversity (Cabral et al., 2013) improved the chemical, microbiological and biochemical soil attributes. These results have demonstrated that forest regeneration is a long-term process in tropical dry and Mediterranean areas (Lucas-Borja et al., 2016). Because plant diversity between the IR and LR stage areas was different (Cabral et al., 2013), it had a direct effect on the soil attributes in the first layer, as was found in previous studies about the upper soil layers of a *Quercus petraea* forest (Šnajdr et al., 2008).

The data showed that the spatial distribution of natural regeneration areas in PCA graphs can be better explained by SOC and absolute enzyme activities than by other soil attributes. These data are consistent with the reported results (Raiesi and Beheshti, 2014; Medeiros et al.,

2015). These SOC and absolute enzyme activities attributes are directly or indirectly linked to organic matter in the soil, which is related to sustained productivity, biodiversity and soil quality status. The findings reinforced our hypothesis that soil enzyme activity is one of the most sensitive instruments to detect changes in soils subjected to natural regeneration in tropical dry forests in Northeastern Brazil (Medeiros et al., 2015).

5. Conclusions

These results suggested that the soil chemical, microbiological and biochemical parameters responded to natural regeneration stages in the Brazilian tropical dry areas. They showed that the SOC, the MBC, absolute and some specific enzymes activities increased with natural regeneration stages according to time and plant diversity. The effect was validated by the PCA and showed three discriminated groups (ER, IR and LR) in the first layer (0–5 cm). The PCAs supported that the tropical dry regeneration effects were most evident in the superficial layer.

The late natural regeneration stages, as it was expected, presented higher soil quality, suggesting that the regeneration of Brazilian tropical dry forest is a long term process, with observed increases up to 20% in soil organic carbon after 15 years of natural regeneration (ER).

These results are important in understanding how natural regeneration influences soil attributes. We provide some of the first data related to the beneficial effects of natural regeneration on the quality of the soil, which was measured through enzyme activity, SOC and MBC, in a tropical dry region in Northeastern Brazil. These data may be the baseline for future studies about the reference Brazilian tropical dry area.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.catena.2016.12.012>.

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