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ENZYMATIC ACTIVITY OF CAATINGA BIOME WITH AND WITHOUT ANTHROPIC ACTION[1]

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ABSTRACT - The Caatinga is an exclusively Brazilian biome that has been under high anthropogenic action, which directly interferes in the quality of its soil. In order to evaluate and monitor the quality of the soil, chemical and biological indicators are used, with the determination of the enzymatic activity gaining prominence. The objective of this study was to evaluate the enzymatic activity of soils of the Caatinga biome in preserved and anthropized areas belonging to the Catimbau National Park, located in Pernambuco, Brazil, during the two periods of the year (rainy and dry), for three consecutive years, 2014, 2015 and 2016. Enzymatic activities of β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase in the soil were evaluated. Anthropic action caused reduction in the enzymatic activities of β-glucosidase, arylsulfatase and urease. Regarding the periods of the year, the dry one showed smaller values of enzymatic activity of β-glucosidase, arylsulfatase and urease. Among the evaluation years, when correlating accumulated precipitation and enzymatic activity, the highest correlations were observed in the preserved area in both periods of the year. The fragility of Caatinga soils under anthropic action was observed in both periods of the year, over time. The increasing anthropization of areas of the Catimbau National Park has a direct effect on soil quality, affects its ecological balance, causing degradation and reduction of its quality. Appropriate enforcement measures are urgently needed to ensure their environmental preservation.

Keywords: Enzymatic activity. Soil use. Soil content.

ATIVIDADE ENZIMÁTICA DE SOLOS DO BIOMA CAATINGA COM E SEM AÇÃO ANTRÓPICA

RESUMO - A Caatinga, bioma exclusivamente brasileiro vem sofrendo elevada ação antrópica, o que interfere diretamente na qualidade de seu solo. Para avaliar e acompanhar a qualidade do solo vem-se utilizando indicadores químicos e biológicos, com a determinação da atividade enzimática, ganhando destaque. Neste sentido, objetivou-se avaliar a atividade enzimática de solos do bioma Caatinga em áreas preservadas e antropizadas, pertencentes ao Parque Nacional do Catimbau, situado em Pernambuco, Brasil, nos dois períodos do ano (chuvoso e seco), por três anos consecutivos, 2014, 2015 e 2016. Foram avaliadas as atividades enzimáticas de β-glicosidase, urease, arilsulfatase, fosfatase ácida e alcalina de solos. A ação antrópica proporcionou redução das atividades enzimáticas de β-glicosidase, urease e arilsulfatase. Entre os períodos do ano, o seco apresentou menores valores de atividade enzimática de β-glicosidase, urease e arilsulfatase. Entre os anos de avaliação, ao correlacionar a precipitação acumulada e a atividade enzimática, as maiores correlações foram observadas na área preservada em ambos períodos do ano. Comprovou-se a fragilidade dos solos da Caatinga sob a ação antrópica em ambas as estações dos períodos do ano, ao longo do tempo. A antropização crescente de áreas do Parque Nacional do Catimbau apresenta efeito direto na qualidade do solo, afeta seu equilíbrio ecológico, causando a sua degradação e redução de sua qualidade. Tornam-se necessárias medidas urgentes de fiscalização adequada para garantir a sua preservação ambiental.

Palavras-chave: Atividade enzimática. Uso do solo. Teor de água no solo.

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INTRODUCTION

The Caatinga is the only exclusive Brazilian biome, of high plant and animal diversity, characteristic of the semi-arid region, occupying an approximate area of 844,453 km^2, covering 9.92% of the national territory (BRASIL, 2012; FIGUEIREDO et al., 2018). The annual precipitations oscillate between 700 and 2000 mm, between the beginning and end of the rainy period, with an average temperature of 25 ºC (MOURA et al., 2016).

The climatic variability found in this region results in a greater environmental degradation, making it more vulnerable to climatic changes, causing damage to both the agricultural production and the development of the native vegetation (ALTHOFF et al., 2016). It is estimated that about 16,141 km^2 of the Caatinga territory have already undergone intensive anthropic action (cultivation and livestock), which, combined with the effects of climate change, leads to an intense desertification process (LIRA et al., 2012; SOUZA; MENEZES; CÁMARA ARTIGAS, 2015). Even in the face of threats, the conservation of the Caatinga ecosystem in the form of integral protection occurs in less than 2% of this biome (VASCONCELOS et al., 2017).

In order to systematically monitor and evaluate the quality of Caatinga soils, one can use chemical and biological indicators, noting the interrelationship between them, as a function of changes in the environment over time (KUWANO et al., 2014). Among these indicators, it is possible to evaluate the carbon content, litter, water resources, as well as the density, diversity and functionality of microorganisms (CORDEIRO; CORÁ; NAHAS, 2013; MENDES; SOUSA; REIS-JUNIOR, 2015).

Soil assessments made from microbial biomass and enzymatic activity are considered the best way to study ecological stability (LV et al., 2014), due to its rapid responses to changes occurring in the soil (RAIESI; BEHESHTI, 2014). Enzyme activity analysis can be used to evaluate practical results in a variety of situations, covering the soil-plant system, including aspects related to fertility, remediation of contaminated soils, and impact management studies and agricultural soil quality (CARNEIRO et al., 2008).

The importance of the use of indicators is linked to the expression of soil functionality, evidencing the deficiencies of the evaluated areas, and guiding their recovery (FERREIRA; STONE; MARTIN-DIDONET, 2017). In this context, the objective of this work was to evaluate the enzymatic activity of soils of the Caatinga biome in preserved and anthropized areas belonging to the Catimbau National Park, in the municipality of Buíque, Pernambuco, Brazil, during the rainy and dry periods for three consecutive years.

MATERIAL AND METHODS

Soil samples were collected in the Caatinga Biome, belonging to the Catimbau National Park, located at the geographic coordinates 08°04’25” S and 37°15’52” W, in the municipality of Buíque, Pernambuco, Brazil. The collections were made in the preserved area (08°31’56.1” S and 37°15’03.2” W, elevation of 924 m) and in the anthropized area (08°34’5” S and 37°14’4.3” W, elevation of 744 m), with both areas featuring a *Neossolo Litólico* (Entisol). The predominant climate is the Hot-Summer Mediterranean climate (Cs'a), according to the Köppen-Geiger climatic classification (1928), with annual average temperature and precipitation of 23 °C and 300-500 mm, respectively (IPA, 2019). It should be noted that the term preserved area is being used for the Caatinga area without anthropic action, whose fauna and flora of the Caatinga biome are present.

The collections were carried out in preserved and anthropized areas, twice a year, covering the rainy period with the collection in the month of May, and dry period with the collection in the month of October, according to the rainfall data of the last 30 years, referring to the months of highest and lowest rainfall, respectively (IPA, 2019), for three consecutive years 2014, 2015 and 2016 (Table 1). Historically, the anthropic area evaluated has been used for grazing and browsing of small ruminants for more than five years. These small ruminants used were goats and sheep of different breeds, with stocking rate per hectare varying between times of year and years of evaluation. The Caatinga vegetation management consisted of thinning, enrichment and trimming.

The soil for analysis was collected from the 0.0-0.2 m depth, within nine transects of 4 x 25 m, in each of which 10 subsamples were collected and homogenized by transect and later homogenized in every set of three transects, totaling three composite samples per area, period of the year and year of evaluation. All samples were placed in sterile, cooled plastic bags at -4 °C. Subsequently, the soil was kept at room temperature, sieved through a 2 mm mesh, and stored at -20 ºC. The chemical and physical characteristics of the soils in the different collection areas, periods of the year and years of evaluation are expressed in Table 2, according to the recommendations of EMBRAPA (2009).

The determination of the enzymatic activity in μmol min^{-1} g^{-1} of soil was performed for β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase. The β-glucosidase activity was determined according to the methodology of Eivazi and Tabatabai (1988), with adaptations. Using spectrophotometry, 1 g of soil was incubated with 0.25 mL of toluene, 4 mL of the buffer (pH 6) and 1 mL of ρ-nitrophenyl-β-D-glucoside (0.05 mol L^{-1}) for 1 h at 37 °C. These procedures were followed by the addition of 1 mL of $CaCl_2$ (0.5 mol L^{-1}) and 4 mL of THAM buffer (pH 12); when the solution became yellowish, the reading was performed in a spectrophotometer at 410 nm.

Table 1. Cumulative data of maximum (Max) and minimum (Min) temperature and precipitation (Prec) of the Catimbau National Park by quarter, the rainy period, extending from February to July, and the dry periods, from August to January, for three consecutive years, Buíque, Pernambuco, Brazil.

Quarter	2014			2015			2016		
	Temperature		Prec	Temperature		Prec	Temperature		Prec
	Max	Min		Max	Min		Max	Min	
	°C	°C	mm	°C	°C	mm	°C	°C	Mm
Feb to Apr	31.1	19.9	151.1	20.0	32.1	142.1	19.8	31.7	113.4
May to Jul	26.8	17.9	340.7	18.9	28.0	170.7	17.8	28.6	248.8
Aug to Oct	28.1	17.5	223.9	17.8	29.9	43.5	18.1	31.7	5.5
Nov to Dec	31.7	19.3	40.9	19.6	33.2	60.4	20.1	32.6	83.3

Table 2. Chemical and physical characteristics of soils, 0.0-0.2 m deep, belonging to the Catimbau National Park, in preserved and anthropized areas in the rainy period with the collection in April, and dry period with the collection in October, for three consecutive years, municipality of Buíque, Pernambuco, Brazil.

Chemical and physical characteristics		Preserved						Anthropized					
		2014		2015		2016		2014		2015		2016	
		Periods of the year											
		Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
pH	H_2O	4.15	4.07	4.15	4.19	4.19	4.15	7.40	7.50	6.50	6.32	5.80	6.37
P	mg kg^{-1}	0.28	0.17	0.18	0.12	0.28	0.20	0.11	0.93	0.10	0.10	0.89	0.13
Al^{2+}	$cmol_c$ kg^{-1}	0.15	0.01	0.18	0.02	0.05	0.02	0.40	0.20	0.20	0.20	0.10	0.23
Na^+	$cmol_c$ kg^{-1}	0.046	0.020	0.041	0.015	0.038	0.016	0.043	0.025	0.043	0.017	0.035	0.012
K^+	$cmol_c$ kg^{-1}	0.010	0.010	0.015	0.010	0.012	0.013	0.085	0.035	0.085	0.010	0.085	0.085
Ca^{2+}	$cmol_c$ kg^{-1}	1.30	0.60	1.30	0.70	0.90	0.80	0.50	0.80	0.70	0.50	0.60	0.50
Mg^{2+}	$cmol_c$ dm^{-3}	1.20	0.90	1.20	0.70	0.80	0.8	0.40	0.40	0.60	0.60	0.80	0.50
H+Al	$cmol_c$ dm^{-3}	3.15	3.01	3.15	2.45	3.13	2.65	1.37	0.82	0.95	1.43	0.99	1.34
OM	g kg^{-1}	19.72	13.84	9.95	7.14	15.84	10.04	13.78	8.87	4.80	3.83	8.81	4.48
Sand	%	71.7						69.3					
Silt	%	15.5						20.6					
Clay	%	12.8						10.1					

OM: organic matter.

Urease activity was determined according to the method described by Kandeler and Gerber (1988), with modifications. In 5 g of soil, 2.5 mL of urea were added and taken for incubation in a water bath for 2 h at 37 °C, followed by the addition of 50 mL KCl and stirring for 30 min at 140 rpm on a shaker table. Subsequently, 0.5 mL of the filtered solution was mixed with 4.5 mL of distilled water, 2.5 mL of dichloroisocyanuric acid solution and 1 mL of sodium salicylate, vortexed and after 30 min the spectrophotometer was read at 690 nm.

Acid and alkaline phosphatase activity was determined according to Eivazi and Tabatabai (1977), with modifications. 0.25 mL of toluene, 4 mL of Modified Universal Buffer (MUB) at pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase, and 1 mL p-nitrophenyl phosphate (15 mM), diluted in MUB with pH corresponding to the desired enzyme, were added to 1 g of soil. Subsequently, the samples were incubated at 37 °C for 1 h. After incubation, p-nitrophenol was extracted from the soil, with the addition of 1 mL of $CaCl_2$ (0.5 M), 4 mL of NaOH (0.5 M), followed by shaking and subsequent filtration of the suspension on Whatman nº1 filter paper. The reading was performed in a spectrometer at 400 nm.

The data of the enzymatic activities after transformation in square root were used to construct the dispersion chart in the principal component analysis using the statistical software PAST 1.9. In the form of a 2x2 factorial scheme the data were evaluated, considering two Caatinga areas (preserved Caatinga area and anthropized Caatinga area) and two periods of the year (rainy and dry), as three repetitions per area, period of year and year of evaluation. The comparisons of the means of each enzymatic activity were performed by Tukey test ($P \leq 0.05$), using the statistical software SISVAR 5.6.

RESULTS AND DISCUSSION

The anthropic action caused modifications in the enzymatic activity in soils of the Caatinga ecosystem of the Catimbau National Park, in the different years evaluated (Figure 1). Modifications in the Caatinga area caused by anthropic action reduced the enzymatic activity of β-glucosidase, urease and arylsulfatase as the values grouped for the soils of the anthropic area, unlike the preserved area, which is more dispersed, due to the greater and different enzymatic activities between the periods and years of evaluation (Figure 1 and Table 3), possibly indicating the higher soil quality.

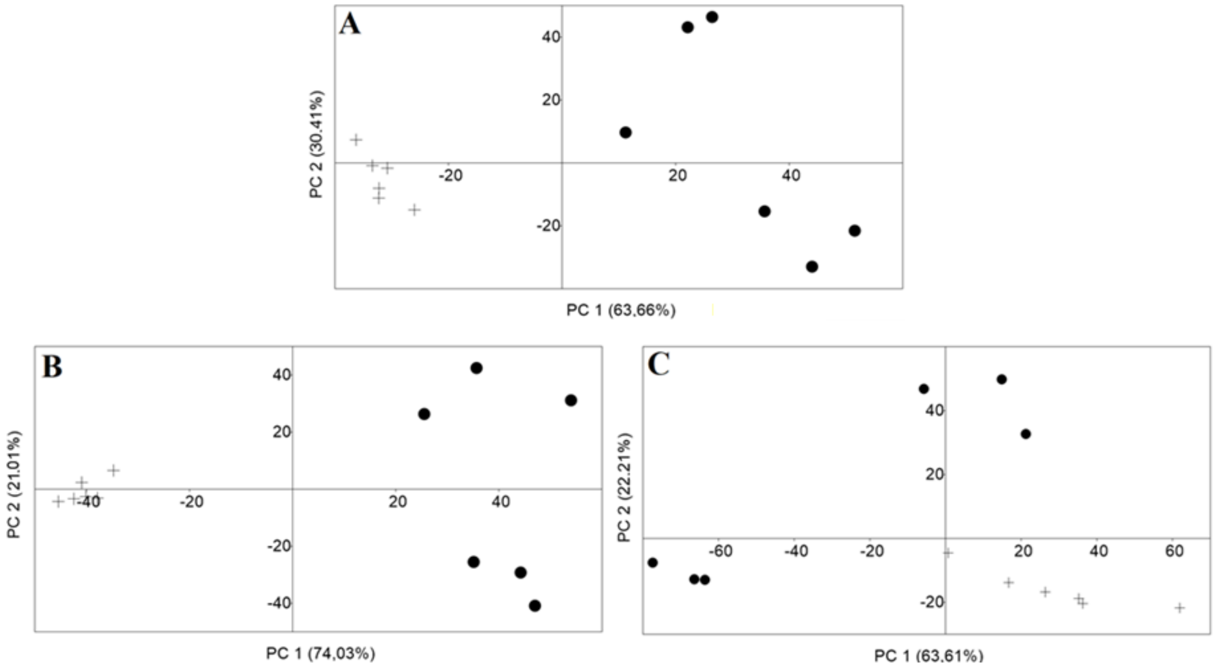


Figure 1. Principal component (PC) analysis of the enzymatic activities of β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase (μmol min^{-1} g^{-1} soil) of the Catimbau National Park soils in preserved (●) and anthropized (+) areas, in the rainy and dry periods, for three consecutive years (2014 (A), 2015 (B) and 2016 (C)), municipality of Buíque, Pernambuco, Brazil.

Table 3. Enzymatic activities of β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase (μmol min^{-1} g^{-1} soil) of the Catimbau National Park in preserved and anthropized areas in the rainy and dry periods of 2014, 2015 and 2016, municipality of Buíque, Pernambuco, Brazil.

Periods of the year	Preserved area	Anthropized area
	β-glucosidase	
Rainy	99.36Aa	26.38Ba
Dry	67.07Ab	23.16Ba
	Urease	
Rainy	41.01Aa	26.17Ba
Dry	42.61Aa	22.38Ba
	Arylsulfatase	
Rainy	13.54Aa	8.34Ba
Dry	8.68Ab	9.87Aa
	Acid phosphatase	
Rainy	6.56Aa	3.71Aa
Dry	6.84Aa	4.36Aa
	Alkaline phosphatase	
Rainy	10.85Aa	17.04Aa
Dry	15.31Aa	11.77Aa

Uppercase letters compare the areas of Caatinga within each period of the year. Lowercase letters compare the periods of the year per area of evaluation. Means followed by the same letter do not differ statistically from each other by the Tukey test (P ≤ 0.05).

In both areas of the Caatinga, the periods of the year caused modifications in the enzymatic activities, but little grouping occurred (Figure 2). The grouping usually occurred between three points, referring to the repetitions of the areas, per year of evaluation, in both Caatinga areas and periods of the year (Figures 3A, B, C and D), evidencing the disparity of the enzymatic activity of the soil after the anthropic action and between the periods of the year.

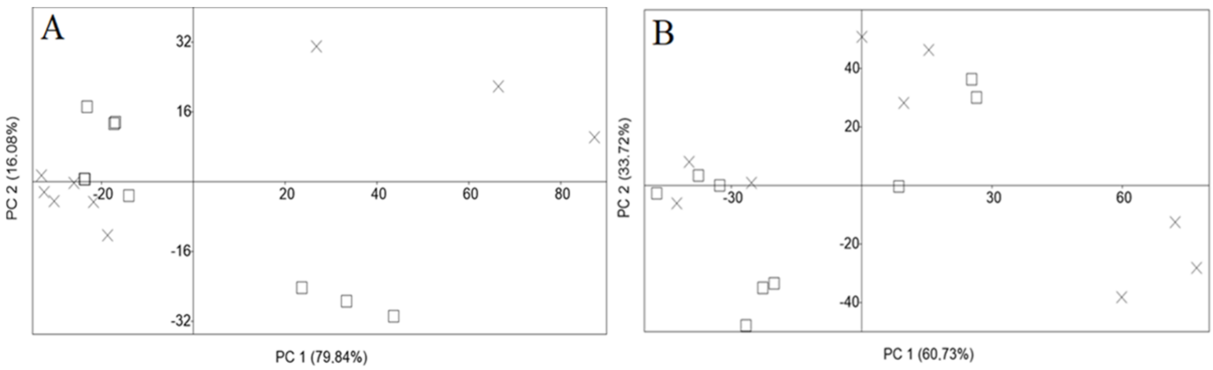


Figure 2. Principal Component (PC) analysis of the enzymatic activities of β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase (μmol min^{-1} g^{-1} soil) of the Catimbau National Park soils in preserved (A) and anthropized (B) areas in the rainy (×) and dry (□) periods, for three consecutive years, Buíque, Pernambuco, Brazil.

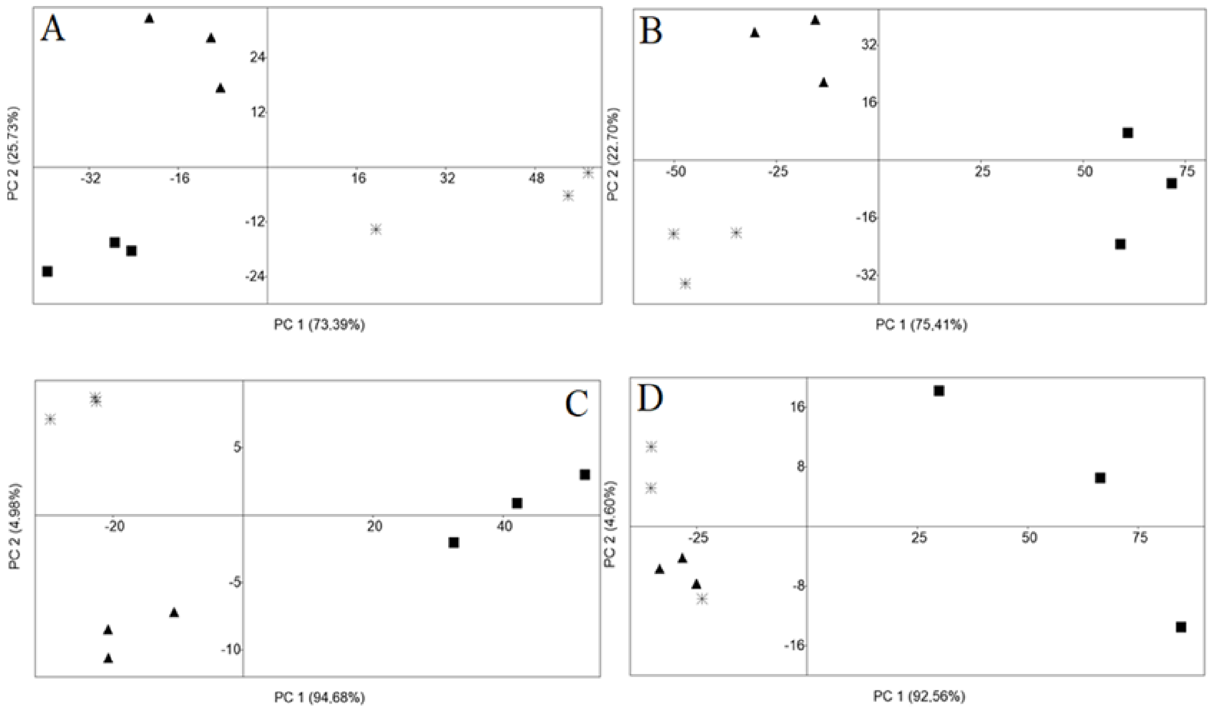
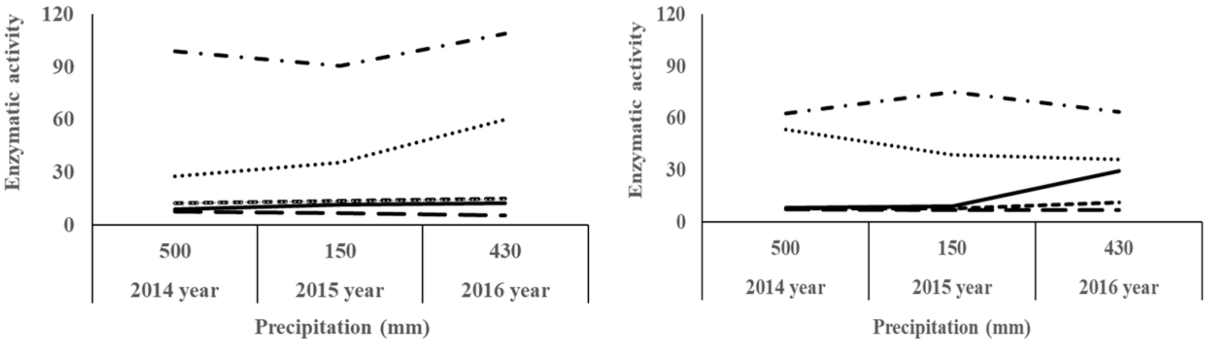


Figure 3. Principal component (PC) analysis of the enzymatic activities of β-glucosidase, urease, arylsulfatase, and acid and alkaline phosphatase (μmol min^{-1} g^{-1} soil) of the Catimbau National Park soils in preserved areas during rainy periods (A) and dry periods (B) and in anthropized areas in the rainy periods (C) and dry periods (D), for the years 2014 (▲), 2015 (■) and 2016 (*), in the municipality of Buíque, Pernambuco, Brazil.

Regarding the periods of the year, the dry one had lower values of enzymatic activity of β-glucosidase and arylsulfatase (Table 3). Among the evaluation years, when correlating accumulated precipitation (Table 1) and enzymatic activity, the highest correlations were observed in the preserved area in both periods of the year (Figure 4). This is evidence of the fragility of Caatinga soils under the interference of man in the two periods of the year, over time. However, it should be noted that the activity of arylsulfatase in both Caatinga areas and periods of the year showed a high correlation with precipitation.

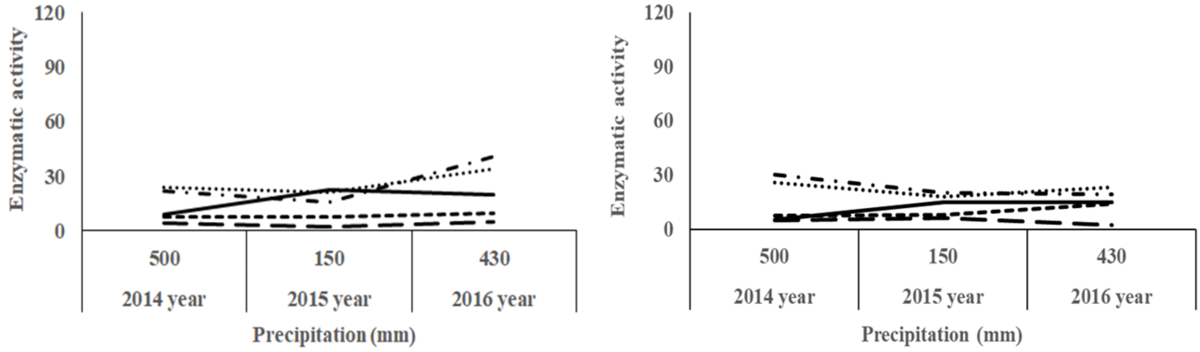
The enzymatic activity is an indicator of soil quality, playing a fundamental role in the cycling of nutrients in the various biogeochemical cycles (FERREIRA; STONE; MARTIN-DIDONET, 2017). These results are influenced by the chemical and physical characteristics of the soil, with special importance for the organic matter content, as well as the amount of water present in the soil, soil solution (SANTOS, MAIA, 2013).

Glucosidases are an enzymatic group whose activity is associated with the C components present in the soil, with the ability to hydrolyze cellulose and other polymers (DONI et al., 2012). According to Santos and Maia (2013), among the glucosidases, the β-glucosidases, the main enzyme of this group, are shown to release nutrients from the organic matter present in the soil. As observed in this study, the preserved area had a higher amount of organic matter (Table 2), which justifies the greater enzymatic activity of β-glucosidases in comparison to the soil of the anthropized area.



Preserved area

		Rainy period		Dry period	
β-Glucosidase	– · ·	y = 5.0735x + 89.215	R^2 = 0.298	y = 0.328x + 66.421	R^2 = 0.002
Urease	....	y = 16.021x + 89.717	R^2 = 0.920	y = -8.7085x + 60.028	R^2 = 0.866
Arylsulfatase	- - -	y = 1.1639x + 11.217	R^2 = 0.998	y = 1.6158x + 5.4426	R^2 = 0.705
Acid phosphatase	— -	y = 1.629x + 7.592	R^2 = 0.917	y = -0.227x + 7.2973	R^2 = 0.870
Alkaline phosphatase	——	y = -12195x + 9.001	R^2 = 0.961	y = 10.669x - 6.0263	R^2 = 0.775



Anthropized area

		Rainy period		Dry period	
β-Glucosidase	– · ·	y = 9.521x + 7.34	R^2 = 0.527	y = -5.6365x + 34.447	R^2 = 0.809
Urease	....	y = 5.0125x + 16.15	R^2 = 0.557	y = -1.354x + 25.097	R^2 = 0.307
Arylsulfatase	- - -	y = 1.3023x + 5.7439	R^2 = 0.836	y = 3.1921x + 3.4915	R^2 = 0.800
Acid phosphatase	— -	y = 0.341x + 3.030	R^2 = 0.085	y = -1212x + 6.7843	R^2 = 0.403
Alkaline phosphatase	——	y = 5.5685x + 5.8917	R^2 = 0.600	y = 4.7475x + 2.2813	R^2 = 0.734

Figure 4. Correlation between the enzymatic activity (μmol min^{-1} g^{-1} of soil) and precipitation of preserved and anthropized Caatinga areas of the Catimbau National Park, in the rainy and dry periods, for three consecutive years, Buíque, Pernambuco, Brazil.

Areas of preserved Caatinga possibly possess greater diversity and density of organic matter in their soils, due to the greater floristic diversity, formed largely by plants of the genus *Fabaceae* (FABRICANTE; ANDRADE, 2015). Plant species of this genus have a higher capacity to fix N and greater potential to add nutrients to the systems, thus favoring conditions that are more favorable to macro and micro fauna of the soil, due to the decrease in the C:N ratio (TAVARES-JÚNIOR et al., 2015).

Such conditions, besides favoring the greater activity of β-glucosidases, contribute to the increase of microorganisms linked to the nitrogen cycle. According to Melloni et al. (2001), it is possible to observe higher values for the enzymatic activity of urease in ecosystems without human interference, as observed in the present study (Table 3). The activity of arylsulfatase, involved in the metabolism of S, catalyzes the hydrolysis of sulfate esters, one of the organic forms of S (YADA et al., 2015). According to Nogueira and Melo (2003), the activity of arylsulfatase in the soil decreases with the reduction of the organic matter content, as observed in this study with the soils of the anthropized Caatinga area (Table 2 and 3).

Phosphatases are a group of enzymes responsible for catalyzing the hydrolysis of esters and anhydrides releasing orthophosphate. These enzymes are produced when the soil has a low level of P, which is essential for plants and microorganisms (BALOTA et al., 2014). According to Yada et al. (2015), reductions in phosphatase activity levels may occur due to the increase in P in the soil solution. This may explain the absence of significant results in the rainy period of both the anthropized and the preserved areas (Table 3).

The alkaline phosphatase activity values were higher in both Caatinga areas and evaluated periods

(Table 4), showing that soil pH influences the synthesis and release of enzymes by microorganisms, as well as the stability and conformation of these substances, as demonstrated by Herbien and Neal (1990). According to Wang et al. (2012), the highest concentration of alkaline phosphatase in forest areas is related to the abundance of plant species, which form a significant layer of litter, increasing the nutrient content in the soil after its decomposition, which can lead to a greater activity of this enzyme. However, no differences were observed between the areas in this study (Table 3).

Table 4. Average of enzymatic activities of acid and alkaline phosphatase (µmol min^{-1} g^{-1} soil) from Catimbau National Park soils in preserved and anthropized areas during the rainy and dry periods of 2014, 2015 and 2016, Buíque municipality, Pernambuco, Brazil.

Periods of the year	Preserved area	Anthropized area
	Acid phosphatase	
Rainy	6.56A	3.71B
Dry	6.84Aa	4.36B
	Alkaline phosphatase	
Rainy	10.85A	17.02A
Dry	15.31A	11.77A

The letters compare the acid and alkaline phosphatases by area, periods and years of evaluation. Means followed by the same letter do not differ statistically from each other by the Tukey test ($P \leq 0.05$).

The main biogeochemical cycles can be analyzed by the enzymes β-glucosidase, phosphatases and urease (ROLDÁN et al., 2003). Other enzymes such as arylsulfatase are related to the cycle of S, which is widely distributed in soil and absorbed by plants (YADA et al., 2015). The study of these enzymes in the soil is a way of evaluating the ecological stability of the different biomes (LV et al., 2014), due to their rapid responses to changes occurring in the soil (RAIESI; BEHESHTI, 2014; SANTOS; MAIA, 2013), guiding the possible management actions to revitalize it (FERREIRA; STONE; MARTIN-DIDONET, 2017).

CONCLUSIONS

Anthropic action causes a reduction in the enzymatic activity of β-glucosidase, urease and arylsulfatase, as well as in the availability of water in the soil during the rainy period, and between the years of evaluation increases the enzymatic activity of Caatinga soils of the Catimbau National Park.

The ecological imbalances caused by anthropic action in the Caatinga soils of the Catimbau National Park, evidenced by divergent enzymatic activities, emphasize the need for urgent adequate inspection measures, as well as the reforestation of the affected areas of the park, in order to guarantee their environmental preservation.

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