Soil microbiological attributes under the cultivation of *Pennisetum purpureum* genotypes[1]

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10.1590/0034-737X2024710027

ABSTRACT

This study aimed to evaluate the biological quality of soil under the influence of different genotypes of elephant grass in the agreste region, which has a climate that marks the transition between a humid climate with a dry season and the semi-arid climate of the north-eastern hinterland. The study was conducted at the Experimental Farm of the Universidade Federal Rural de Pernambuco, Garanhuns, PE, Brazil. The treatments comprised a combination of two elephant grass cultivars (Elefante B and Mott), two irrigation regimes (with and without irrigation), and two climatic periods (dry and rainy). Biological indicators, microbial biomass carbon, soil basal respiration, metabolic quotient, enzymatic activity of soil β-glucosidase, acid and alkaline phosphatase, arylsulfatase, urease, and the hydrolytic determination of fluorescein diacetate were evaluated. The Mott genotype showed superior results, attributed to the biological indicators studied at different times and irrigation management, even during periods of drought, and Mott grass had significant effects microbial activities. This genotype constitutes one of the alternatives for soil quality in semiarid regions, with advantageous biomass and soil microbial activity, thus presenting the greatest complexity in biological attributes with microorganisms tolerant to climate change.

Keywords: enzymatic activity; bioindicators; soil quality; soil water content; temporal variability.

INTRODUCTION

Understanding the soil biology and ecology is important for ecosystem restoration and sustainability. In all ecosystems, soil microbes play important roles in organic matter breakdown, nutrient cycling, and nutrient availability to plants (Li *et al.*, 2022). Quantification of biomass carbon, basal respiration, its relationship with the metabolic quotient, and enzymatic activity, have been used as potential microbiological indicators, as they indicate changes in soil microbial biomass and community composition in this environment and are sensitive to small variations, allowing for a quick assessment of soil quality (Rodrigues *et al.*, 2022).

As an important indicator of soil fertility, enzymatic activity is fundamental for maintaining the availability of nutrients, including arylsulfatase, β-glucosidase, urease, acid, and alkaline phosphatase, which are involved in the transformation of organic compounds and as available inorganic sources for plants (Piotrowska-Długosz *et al.*, 2021).

Studies found that soil enzymes in various vegetation and grassland have different sensitivity to moisture and heat, while the sensitivity of the same soil enzyme was also different under vegetation types (Li *et al.*, 2022). However, little information is available on the effect of forage grasses on the diversity and activity of soil microbiota.

Submitted on June 23rd, 2021 and accepted on March 06th, 2024.
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The use of elephant grass (*Pennisetum purpureum* Schum.), a grass that is highly efficient in fixing atmospheric CO_2, could contribute significantly as an alternative source of nutrients to the soil-plant system with biological processes, as it acts as an easily obtainable source of energy and carbon, containing several substances that help the population development of microorganisms from composting processes (Adesemuyi *et al.*, 2020; Santos *et al.*, 2021). Owing to its well-developed root system, it can efficiently contribute to increasing soil organic matter content or carbon sequestration in the soil (Nguyen *et al.*, 2021).

In the semi-arid region of Northeast Brazil, which is characterised by high temperatures associated with irregular precipitation and low rainfall (Silva *et al.*, 2019), the cultivation of grass adapts well to the climatic conditions of the region; however, the seasonality of forage production has restricted its use, which can be supplied by the use of irrigation (Silva *et al.*, 2022). Irrigation during the dry season has been suggested as an option to minimise the seasonal effects on forage production (Souza *et al.*, 2020).

Therefore, considering the importance of biological attributes for the processes that occur in the soil, studies on the quantity and activity of microbial biomass can provide subsidies for the rational use of natural resources and promote the development of soil conservation practices. However, studies on the biological attributes of soil in plantations of *P. purpureum* genotypes subjected to different irrigation management regimes in semi-arid regions remain scarce.

Thus, this study aimed to evaluate the biological indicators of soil under the influence of different genotypes of elephant grass in the agreste region of Pernambuco, a region whose climate marks the transition between a humid climate with a dry season and the semi-arid climate of the north-eastern hinterland, under different irrigation regimes.

MATERIAL AND METHODS

Soil samples were collected in an experimental area of elephant grass planting, belonging to the Experimental Farm of the Federal Rural University of Pernambuco, located at latitude 08°58'28" S, longitude 36°27'11" W, and altitude of 736 m, in the Municipality of Garanhuns, located in the Southern Agreste of Pernambuco, Northeast Brazil (Alvares *et al.*, 2013). The climate, according to the Köppen–Geiger classification, is tropical mesothermal (Cs'a) with dry and rainy seasons in summer and winter, respectively. The average annual precipitation is 660 mm, concentrated between May to August, and an average annual temperature of 21.7 ºC (Climate Data, 2022).

The collections were performed out in areas of cultivation of elephant grass genotypes Elefante B and Mott, with the presence or absence of irrigation twice annually, covering the rainy period with collection in July and the dry period with collection in December, according to rainfall and temperature data for the study period (INMET, 2018) in 2017 (Table 1).

Irrigation was managed by dripping at an average flow of 1.5 L h^{-1} using a spring located close to the experimental area as the water source. During winter, irrigation was managed in a variable irrigation shift, considering precipitation. During summer, irrigation was performed daily for 2 h. The study was conducted on treatments selected from a field trial arranged in a randomised block design, using two elephant grass genotypes, Elefante B and Mott, with and without irrigation.

Soil for analysis was collected from the rhizosphere, and within each treatment plot, the composite samples were homogenised from 10 subsamples of all plots of the Elefante B and Mott genotypes under different irrigation treatments, and the samples were analysed in triplicate. Soil samples were sieved through a 2 mm mesh and subsequently stored at 4 ºC.

Table 1: Accumulated data of maximum and minimum temperatures and precipitation of the Experimental Farm by quarter in the 2017, Garanhuns, Pernambuco, Brazil

Quarterly	Temperature (ºC)		Precipitation (mm)
	Maximum	Minimum	
January - April	28.3	19.2	55.0
May – July	21.5	15.9	300.2
August – October	25.6	17.2	149.7
November - December	31.1	19.9	5.5

Microbial biomass carbon (MBC) was determined using 20 g of the second soil sample (Islam & Weil, 1998). Then, the carbon was extracted with potassium sulphate (0.5 mol L^{-1}), followed by oxidation with potassium dichromate (0.066 mol L^{-1}) in the presence of sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) in a heating plate. After cooling, the solution was titrated with ferrous ammonium sulphate (0.033 mol L^{-1}) in the presence of a diphenylamine indicator (1%) (Vance *et al.*, 1987). The amount of MBC was determined by the difference between organic carbon extracted from fumigated and non-fumigated soil samples using a correction factor (Kec) of 0.35 and the results were expressed as mg g^{-1} of carbon on the ground.

Soil basal respiration (SBR) was quantified by incubating 20 g of soil in a hermetically sealed container for three days at 28 °C. The CO_2 released from the samples was captured by the NaOH solution (0.5 mol L^{-1}), which was titrated with HCl (0.5 mol L^{-1}) using phenolphthalein (1%) as an indicator (Anderson & Domsch, 1993). The difference of consumed volume of HCl between the treatment and the control in titration was used to calculate the quantity of CO_2 evolution from soil microbes, 1 ml 0.1 M consumed NaOH was equivalent to 2.2 mg CO_2 the results were expressed in mg carbon – CO_2. The metabolic quotient (qCO_2) was calculated using the SBR/MBC ratio, and the results were expressed in mg carbon – CO_2 (Anderson & Domsch, 1993).

β-Glucosidase activity was determined according to the methodology described by Eivazi & Tabatabai (1988) with some modifications. Using spectrophotometry, 1 g of soil was incubated with 4 mL of buffer (pH 6) and 1 mL of ρ-nitrophenyl-β-D-glucoside (0.05 mol L^{-1}) for 1 h at 37 °C. Following the addition of 1 mL of $CaCl_2$ (0.5 mol L^{-1}) and 4 mL of THAM buffer (pH 12), when the solution turned a yellowish colour, the reading was performed in a spectrophotometer at 410 nm.

Acid and alkaline phosphatase activities were determined according to the method of Eivazi & Tabatabai (1977), with some modifications. In 1 g of soil, 4 mL of modified universal buffer (MUB) solution at pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase, and 1 mL p-nitrophenyl phosphate (15 mM) were diluted in the MUB with a pH corresponding to the desired enzyme. The samples were incubated at 37 ºC for 1 h. After incubation, p-nitrophenol extraction from the soil was initiated by the addition of 1 mL of $CaCl_2$ (0.5 M) and 4 mL of NaOH (0.5 M), followed by stirring and subsequent filtering of the suspension on Whatman No. 1 filter paper. The reading was taken using spectrophotometry at 400 nm.

Arylsulfatase activity was measured following the methods proposed by Tabatabai & Bremner (1970), with some modifications. Initially, 1 g of soil was incubated in potassium p-nitrophenyl sulphate (50 mM) with 4 mL of acetate buffer (0.5 mol L^{-1}) at pH 5.8 for 1 h at 37 ºC. After incubation, 1 mL of calcium chloride solution (0.5 mol L^{-1}) and 4 mL of sodium hydroxide solution (0.5 mol L^{-1}) were added, followed by agitation and immediate filtering through Whatman No. 1 filter paper.

Urease activity was determined according to the methodology described by Kandeler & Gerber (1988) with some modifications. In 5 g of soil, 2.5 mL of urea was added per soil sample and incubated in a water bath for 2 h at 37 °C. Following the addition of 50 mL of KCl and stirring for 30 min at 140 rpm, on a shaking table, subsequently, 0.5 mL of the filtered solution was added to 4.5 mL of distilled water, 2.5 mL of dichloroisocyanuric acid solution and 1 mL of dichloride, gently vortexed, and after 30 min, the reading was performed using a spectrophotometer at 690 nm.

Enzymatic activity was determined in μg PNG g^{-1} soil h^{-1} for β-glucosidase and acid phosphatase. Alkaline phosphatase was expressed in μg PNP g^{-1} soil h^{-1}, arylsulfatase was expressed in μg PNS g^{-1} soil h^{-1}, urease in μg NH4-N g^{-1} soil h^{-1}, and fluorescein diacetate (FDA) was expressed in μg hydrolysates g^{-1} soil.

The total enzymatic activity of the soil was estimated by hydrolysis of fluorescein diacetate (Dick *et al.*, 1997), where 2.5 g of soil sample was incubated at 37 °C together with a fluorescein solution in sodium phosphate buffer (60 mmol L^{-1} at pH 7.0) for 24 h, under stirring at 50 rpm. The reaction was then interrupted with acetone (50%) and centrifuged for 5 min at 3,000 x *g*, the supernatant was filtered, and FDA was measured at 490 nm.

Data from biological variables were evaluated by analysis of variance using R statistical software (R Development Core Team, 2018). Statistical differences between the means were compared using Tukey's test at a 5% significance level. Principal component analysis was performed using the Past 4.03 software to present the ordering of microbiological attributes.

RESULSTS AND DISCUSSION

In this study, MBC increased significantly during the period of reduced rainfall; however, the magnitude of this effect differed according to the elephant grass genotype

(Table 2). The results showed that irrigation had a significant effect on the carbon content of the microbial biomass in the soil under the Elefante B genotype; however, in Mott grass, there was no significant difference between the irrigation treatments.

Plant diversity influences soil microbial biomass (Bargali *et al.*, 2018) and, according to Böhme & Böhme (2006), the root system of the plant can contribute to the microbial carbon content due to rhizodeposition, which results from the abundance of the root system that provides greater availability of organic substrate for the microbiota. Therefore, each genotype of the same species can differ in the quantity and quality of exudates supplied to the soil (Bargali *et al.*, 2018).

Water availability affects soil microbial communities in natural and agricultural ecosystems at the levels of microbial growth, biomass, and biogeochemical cycles (Morugán-Coronado *et al.*, 2019). However, water resources will be insufficient to support sustainable agriculture in semi-arid agroecosystems, and, as shown in the experiment, the Mott elephant grass genotype can obtain significant results in soil MBC in this place, both without and with the presence of irrigation.

Regarding soil basal respiration, considering the sampling time, higher respiration values were found in the Mott genotype in both irrigation management treatments when compared to the Elephant B genotype, and irrigation in the dry period contributed to an increase in the breathing rate.

Similar results were reported by González-Ubierna & Lai (2019), corroborating the present study. They stated that irrigation practices contribute to increasing the soil respiration rate when applied in summer, suggesting that this result is related to the availability of water in the soil, as a soil with low water content tends to reduce microbial respiration, and water stress causes microorganisms to decrease their metabolic activity.

Kabiri *et al.* (2016) reported lower *q*CO2 values in arid and semi-arid soils; there was a decrease in *q*CO2, mainly due to the extent that microbial biomass becomes more efficient in the use of ecosystem resources; however, the increase in values indicates that there is a greater expenditure of energy to maintain the microbial community, and microorganisms tend to consume more substrate to survive (Ghosh *et al.*, 2020). However, the results of this study did not show significant differences in *q*CO2 among the evaluated treatments; thus, respiration and microbial biomass exhibited the same metabolic efficiency.

Regarding the Elephant B genotype, different periods of the year influenced the enzymatic activity of the soil, β-glucosidase, alkaline, and acid phosphatase, and the soil collected in the rainy season showed greater activity than that observed in the dry season (Table 3), indicating a strong correlation (Figure 1). Arylsulfatase, urease, and FDA activities showed greater activity during the dry season, and Figure 1 shows a correlation between arylsulfatase and urease activities.

Irrigation contributed to greater enzymatic activities of β-glucosidase, arylsulfatase, acid, and alkaline phosphatase, regardless of the collection period and genotype. However, in terms of urease and FDA activities in Mott grass, irrigation during the rainy season resulted in lower enzymatic activity.

It was verified that in the enzymatic activity of alkaline phosphatase and arylsulfatase, the genotypes did not differ significantly, whereas in relation to the other enzymes, the genotypes showed significant differences, where the most representative Mott grass had high levels of enzymatic activity in the soil.

Table 2: Biological analysis of the soil under the cultivation of *Pennisetum purpureum*, Elefante B and Mott genotypes, with and without irrigation systems, in different periods of the year: rainy (July/2017) and dry (December/2017)

Elephant grass genotypes	**Rainy**					
	Irrigated			**Non-irrigated**		
	MBC	SBR	qCO_2	MBC	SBR	qCO_2
Elefante B	13.280Aa[a]	7.285Bb[b]	2.754Aa[a]	8.1365Bb[a]	12.274Ba[a]	2.007Aa[a]
Mott	11.522Aa[b]	11.658Ab[b]	3.415Aa[a]	15.456Aa[b]	18.325Aa[a]	3.003Aa[a]
	Dry					
Elefante B	15.834Ba[a]	15.687Ba[a]	3.302Aa[a]	7.619Bb[a]	8.020Bb[b]	2.337Aa[a]
Mott	20.690Aa[a]	22.858Aa[a]	3.683Aa[a]	22.858Aa[a]	19.334Aa[a]	3.222Aa[a]

MBC: Microbial biomass carbon (mg g^{-1} of carbon in the soil); SBR: Soil basal respiration (mg carbon – CO_2); qCO_2: Metabolic quotient (mg carbon – CO_2); capital letters compare the elephant grass genotypes in each period of the year. Lowercase letters compare irrigation systems in each period of the year. Exponential letters compare genotypes between the periods of the year. Similar letters do not differ according to Tukey's test at the 5% level.

Table 3: Enzymatic activity of soil in the cultivation of Pennisetum purpureum, Elefante B and Mott genotypes, cultivated under different irrigation systems, in the rainy (July/2017) and dry (December/2017) seasons

Elephant grass genotypes	Rainy		Dry	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
β-Glucosidase (μg PNG g^{-1} solo h^{-1})				
Elefante B	21.710Aa[a]	17.280Ab[a]	10.897Aa[b]	5.900Ab[b]
Mott	17.796Ba[a]	11.903Ba[a]	8.997Aa[b]	5.347Ab[b]
Acid phosphatese (μg PNP g^{-1} solo h^{-1})				
Elefante B	17.797Aa[a]	11.833Ab[a]	7.547Ba[b]	2.270Ab[b]
Mott	4.903Ba[b]	2.577Bb[b]	10.480Aa[a]	4.330Ab[a]
Alkaline phosphatase (μg PNP g^{-1} solo h^{-1})				
Elefante B	13.380Aa[a]	8.826Ab[a]	8.570Aa[b]	2.687Ab[b]
Mott	10.037Aa[a]	8.763Ab[a]	7.690Aa[b]	2.127Ab[b]
Arylsulfatase (μg PNS g^{-1} solo h^{-1})				
Elefante B	19.726Aa[b]	12.366Ab[b]	37.693Ba[a]	18.116Bb[a]
Mott	20.216Aa[b]	13.263Ab[b]	42.763Aa[a]	24.396Ab[a]
Urease (μg NH_4-N g^{-1} solo h^{-1})				
Elefante B	14.880Bb[b]	17.226Ba[b]	26.500Ba[a]	24.300Bb[a]
Mott	18.353Ab[b]	21.586Aa[b]	29.950Aa[a]	26.043Ab[a]
FDA (μg hydrolysates g^{-1} de solo)				
Elefante B	28.063Aa[b]	27.356Ba[a]	45.176Ba[a]	23.730Bb[b]
Mott	29.286Ab[b]	37.733Aa[a]	51.750Aa[a]	28.350Ab[b]

Capital letters compare the elephant grass genotypes for each period of the year. Lowercase letters compare irrigation systems in each period of the year. Exponential letters compare genotypes between the periods of the year. Similar letters do not differ according to Tukey's test at the 5% level.

The elephant grass genotypes showed high dispersion in relation to the point of the axes (Figure 2), indicating that they were statistically different environments in terms of biological attributes according to the average test applied (Tables 1 and 2). When correlating irrigation management (Figure 3), the genotypes showed high correlations, thus showing that water contributes to better quality of microorganism activities. Notably, Mott grass showed high correlations under both irrigation treatments. The Mott genotype has a good ability to withstand different climatic conditions while maintaining a stable production level, thus releasing exudates throughout its production cycle and improving soil quality (Rupollo *et al.*, 2012).

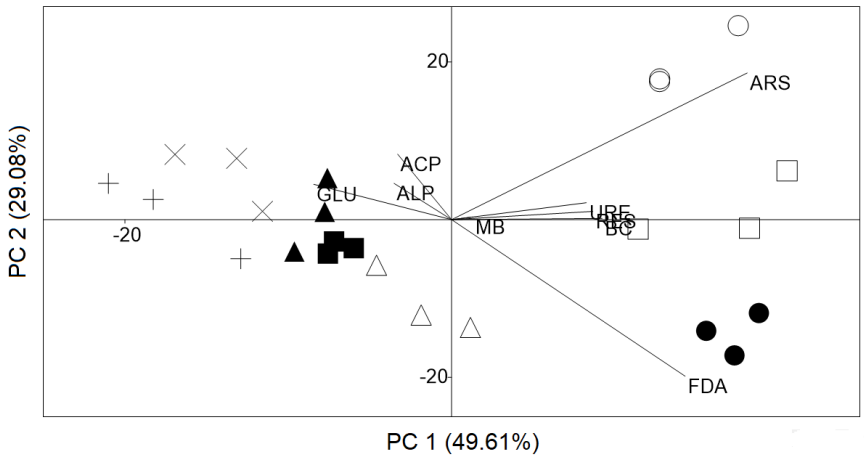

Figure 1: Principal component analysis of the enzymatic activities of soil in the cultivation of *Pennisetum purpureum*, Elefante B and Mott genotypes, cultivated under different irrigation systems, in the rainy (July/2017) and dry (December/2017) seasons. Mott, no irrigation, dry period = ●; Mott, with irrigation, dry period = ○; Mott, no irrigation, rain period = Δ; Mott, with irrigation, rainy period = ▲; Elefante B, no irrigation, dry period = ■; Elefante B, with irrigation, dry period = □; Elefante B, no irrigation, rain period = +; Elefante B, with irrigation, rainy season = X.

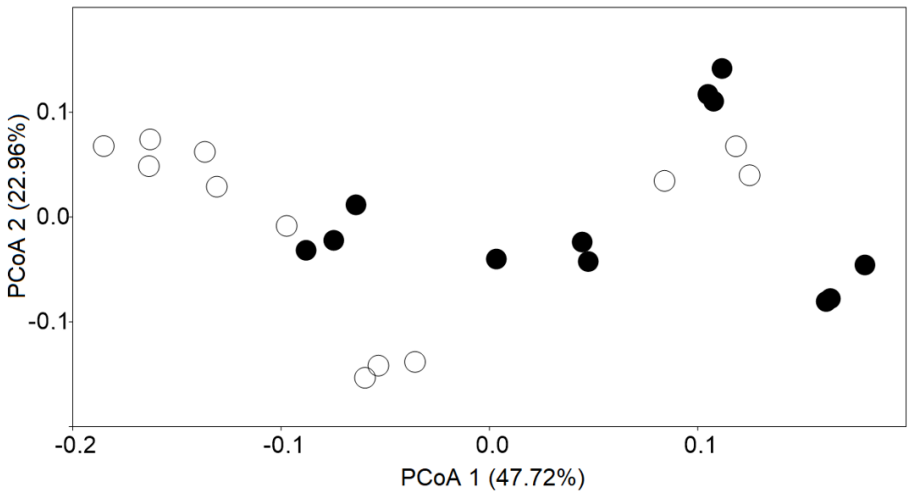

Figure 2: Principal component analysis of Elefante B = ○ and Mott = ● genotypes grown under different irrigation systems in the rainy (July/2017) and dry (December/2017) seasons.

As shown in Figure 4, the climatic seasons exert a greater influence on the biological attributes of the genotypes, in which it is possible to perceive the existence of two distinct groupings: the dry period (warm months) on the left and the rainy period (cold months) on the right, considering that the rainy period is more favourable to the biological attributes in both genotypes and the dry period, and the level of soil quality is more intense in Mott grass.

Enzymatic activity in soil is considered a potential indicator of fertility because of its rapid reaction to changes caused by management and environmental variations (Puissant *et al.*, 2018). Enzymes are strongly influenced by soil physicochemical properties such as pH, temperature, moisture, texture, mineralogy, carbon availability, and composition (Dotaniya *et al.*, 2019).

The results of this study indicate that climatic conditions caused by different seasonal periods and soil moisture directly influence enzymatic activity. According to Li *et al.* (2018), changes in soil environmental conditions, such as moisture and temperature, are correlated with changes in enzymatic activities, microbial communities, and consequently, nutrient cycling.

The area subjected to low water availability showed a reduction in β-glucosidase activity, which suggests less decomposition of organic matter because the activity of this enzyme is correlated with the organic matter content in the soil. According to Taketani *et al.* (2015), the restriction of water content may be related to the reduction in activity, since microbial activity tends to decrease in dry soil, and consequently, enzymatic activity is slowed down.

The results suggest that the activities of acid and alkaline phosphatases were influenced by soil humidity, with better activity in the rainy period and in the presence of irrigation. According to Baldrian *et al.* (2008), the activity

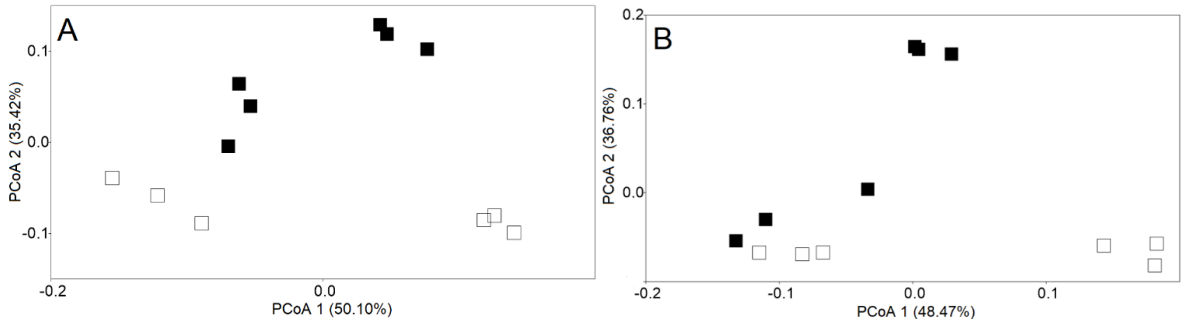

Figure 3: Principal component analysis of Mott and Elefante B genotypes grown under different irrigation systems. A = Mott and B = Elefante B; ■ = With irrigation and □ = Without irrigation.

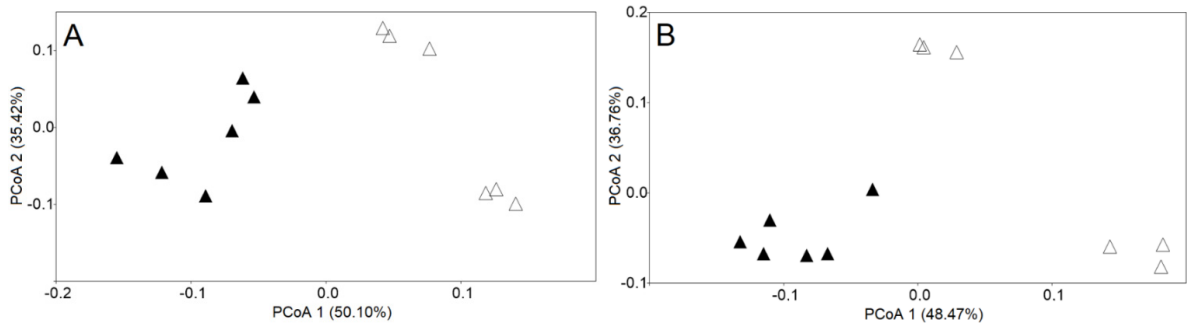

Figure 4: Principal component analysis of the Mott and Elefante B genotypes in the rainy (July/2017) and dry (December/2017) seasons. A = Mott and B = Elefante B; ▲ = dry period and Δ = rainy period.

of acid and alkaline phosphatases is sensitive to seasonal changes, showing greater activity in the rainy season and lower activity in very dry soils, which is considered common because of the reduction in the metabolism of microorganisms and nutrient transport.

Arylsulfatase activity can be used as a direct indicator of the presence of fungi in the soil. Fungi are the only microorganisms present in the microbial biomass that have sulphate esters as substrates for the activity of this enzyme (Bandick & Dick, 1999). During the dry period with irrigation, higher activity levels of this enzyme were observed, which can be attributed to favourable humidity and temperature conditions for these microorganisms.

Urease is produced by plants and microorganisms, mainly bacteria, and is directly related to the nitrogen cycle, which is responsible for hydrolysing urea and releasing CO_2 and ammonia into the soil (Alizadeh *et al.*, 2017). Weitao *et al.* (2018) observed a negative correlation between urease activity and moisture content, and saw that the urease concentration was lower at higher soil moisture indices, which corroborates our results.

One method to evaluate the biological changes occurring in the soil is to determine the total enzymatic activity in the soil, as evaluated using FDA. The amount of hydrolysed fluorescein is related to the greater amount of enzymes released by microorganisms, which may be directly associated with the abundance of soil organic matter (Barbieri *et al.*, 2019). It was verified that the collection performed in the dry period the FDA was superior to that in the rainy period, and these results confirm the data of Pereira *et al.* (2004) and Barbieri *et al.* (2019), in which higher FDA hydrolysis values were found in soils in the dry period.

CONCLUSIONS

Elephant grass genotypes show a good ability to establish the biological attributes of the soil. Among the genotypes studied, Mott grass stood out the most, constituting one of the alternatives for soil quality in the semi-arid region, favouring biomass and soil microbial activity, and presenting greater complexity in biological attributes with microorganisms tolerant to climate change. It is feasible to plant Mott grass during periods of high and low rainfall because it resists variations in irrigation.

Studies on bioindicators have shown that soil microorganisms, owing to their characteristics such as abundance and biochemical and metabolic activity, in addition to providing faster responses to changes in the environment, have a high potential for use in assessing soil quality.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

All authors declare that there is no conflict of financial interest or personal relationship that could have influenced the work reported in this article. Was extracted from the dissertation, from the Graduate Program in Agricultural Production/UFRPE, by the first author, Euzanyr Gomes da Silva, scholarship holder Fundação Fundação de Amparo a Ciência e Tecnologia de Pernambuco – Process PBPG-0083-5.01/17.1.

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