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SOIL SCIENCE

Soil enzymatic activity under coffee cultivation with different water regimes associated to liming and intercropped brachiaria

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ABSTRACT: *This research evaluated the effects of coffee cultivation with two different water regimes associated or not with liming and the presence/absence of brachiaria as intercrop on the activities of the soil enzymes β-glucosidase, arylsulfatase and acid phosphatase. The study was carried out at the experimental farm of Embrapa Cerrados, using the cultivar IAC 144 (Coffea arabica L.), under a clayey dystrophic Cerrado Oxisol. Two water regimes (WR) were considered, WR1 with irrigation shifts throughout the year and WR3 with controlled water stress, for about 70 days, in the dry season. In each water regime, effects of lime application (with/without) and the presence/absence of brachiaria cultivated between the lines of coffee plants were evaluated. The activities of the enzymes β-glucosidase, arylsulfatase and acid phosphatase were evaluated during the rainy and dry seasons. Liming and intercropped brachiaria positively affected the activities of the three enzymes assessed in this study at varying degrees, depending on season and/or the WR. Our findings evidenced that intercropped brachiaria in coffee rows was the factor that most positively impacted soil enzymes activities.*
Key words: *Coffea arabica, Urochloa decumbens, β-glucosidase, arylsulfatase, acid phosphatase.*

Atividade enzimática de solo sob cultivo de café com diferentes regimes hídricos associados a calagem e braquiária nas entrelinhas

RESUMO: *O objetivo desse trabalho foi avaliar os efeitos do cultivo do café sobre a atividade das enzimas do solo β-glicosidase, arilsulfatase e fosfatase ácida em função de dois diferentes regimes hídricos associados ou não à calagem e ao cultivo de braquiária nas entrelinhas. Esse estudo foi realizado em um experimento conduzido no campo experimental do Centro de Pesquisa Agropecuária dos Cerrados, utilizando a cultivar IAC 144 (Coffea arabica L.), sob um Latossolo Vermelho distrófico argiloso. Foram considerados dois regimes hídricos (RH), RH1 com irrigação plena em turnos de rega ao longo do ano e RH3 com estresse hídrico controlado, por cerca de 70 dias, na época seca. Em cada regime hídrico foram avaliadas a aplicação ou não de calcário em cobertura e a presença ou ausência de braquiária cultivada nas entrelinhas das plantas do cafezal. As atividades das enzimas β-glicosidase, arilsulfatase e fosfatase ácida foram avaliadas durante as estações chuvosa e seca. A calagem e a presença de braquiária nas entrelinhas tiveram efeito positivo sobre a atividade das três enzimas avaliadas nesse estudo, em graus variáveis, dependendo da época de coleta das amostras e/ou do RH. Nossos resultados evidenciam que a braquiária nas entrelinhas do café foi o fator de maior impacto positivo sobre a atividade enzimática do solo.*
Palavras-chave: *Coffea arabica, Urochloa decumbens, β-glicosidase, arilsulfatase, fosfatase ácida.*

INTRODUCTION

Brazil is the largest producer and exporter of coffee in the world, with a 2020 estimated total production of 3.8 million tons, in a planted area of approximately 2 million ha (CONAB, 2020). The existence of some edaphoclimatic parameters that are favorable to the development of coffee cultivation contributed to the initial establishment of the crop in the Cerrado region, that is responsible for about 40% of the national production. However, limitations related to the low fertility of the soils and the rainfall distribution in the region, characterized by a long dry season and the occurrence of prolonged dry spell periods during the rainy season, conditioned the success of this activity to the management of soil fertility and mainly to the mandatory use of irrigation (COSTA et al., 2013).

Regarding irrigation management, the use of controlled water stress to standardize the flowering of irrigated coffee in the Cerrado is a strategy that allows optimizing the productivity and quality of the final product (VEIGA et al., 2019). This technology

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results in the harvest of more than 80% of cherry fruits, maximizing the production of special coffees and reducing the amount of water and energy cost by 33% (GUERRA et al., 2005).

Production can also be limited by physical and chemical factors that constrain root development and restrict the absorption of nutrients. The use of lime, for example, is an essential practice in the Cerrado, which allows increasing levels of calcium and magnesium, neutralizing aluminum, and reducing soil acidity, promoting better use of fertilizers with improved assimilation of nutrients, such as nitrogen, phosphorus, potassium and sulfur (CASTRO & CRUSCIOL, 2013).

Another practice that can be applied to reduce/overcome the limitations of areas used for coffee production is the use of cover crops, contributing to the maintenance and/or improvement of soils chemical, physical and biological parameters, with reflexes in the increase of crop production, with a more rational use of agrochemicals and greater environmental balance (CALEGARI, 2014).

The intercropping of coffee and brachiaria (*Urochloa* spp.) has been a very promising alternative to maximize production, improving soil structure and the capacity of storing water, ensuring higher levels of productivity and providing better use of the area (ROCHA et al., 2016). Brachiaria also have an incredible ability to keep the soil biologically active in Cerrado conditions. In a soybean/corn rotation experiment, MENDES et al. (2019) presented results that showed an increase in soil enzymatic activity in treatments with crop succession systems using brachiaria as a cover plant. This greater biological activity, resulting from the input of brachiaria biomass and its benefits over time, was correlated with the stability and resilience of these management systems, which could overcome a stress situation when compared to soils under monoculture.

Although, plants and animals are also source of soil enzymes, the microorganisms are their primary source. These enzymes are responsible to control several processes, with key roles in soil organic matter (SOM) synthesis/decomposition and nutrients cycling (MOGHIMIAN et al., 2017). Because of this close relationship to several aspects of soil functioning, the activities of soil enzymes are considered excellent soil quality indicators (MENDES et al., 2021). Other advantages of using soil enzymes as biological indicators of soil quality include their sensitivity and faster response to shifts in agricultural management when compared to the SOM (MENDES et al., 2019, 2021; ARAGÃO et al., 2020).

In this context, we hypothesized that irrigation management associated with liming and intercropping with brachiaria influence enzymes activities and; therefore, the quality of soils under coffee cultivation. To test this hypothesis the present study evaluated the effects of coffee cultivation with two different water regimes associated or not with liming and the presence/absence of brachiaria as intercrop on the activities of the soil enzymes β-glucosidase, arylsulfatase and acid phosphatase.

MATERIALS AND METHODS

Site and experimental conditions

The experiment was carried out at the experimental farm of the Brazilian Agricultural Research Corporation (Embrapa), Centro de Pesquisa Agropecuária dos Cerrados (Embrapa Cerrados), in Planaltina, DF, Brazil (15º35'30" S and 47º42'00" W, at altitude 1175 m). According to the Köppen classification, the local climate is Cwa, which corresponds to the typical Cerrado climate, with an average annual rainfall of 1500 mm and two well-defined seasons, dry from May to September and rainy from October to April. The annual minimum and maximum temperatures are 15.9 ºC and 26.4 ºC, respectively. The monthly rainfall during the period in which this study was conducted is shown in figure 1.

The experiment was set in an area that, from January 2000 to December 2007, was covered with *Urochloa decumbens* (synonym *B. decumbens*), without grazing. Coffee trees (*Coffea arabica* L.), cultivar Catuaí Vermelho IAC 144, were planted in December 2007, under a clayey dystrophic Latosol (Typic Haplustox), with 583 g kg^{-1} of clay, 346 g kg^{-1} of sand and 71 g kg^{-1} of silt.

At planting, fertilization consisted of 120 g triple superphosphate, 50 g magnesium thermophosphate (Yoorin – Mineração Curimbaba Ltda., Poços de Caldas, MG, Brazil), and 24.5 g fritted trace elements (FTE), per plant. Previously, 2 t of dolomitic lime ha^{-1} was broadcasted and incorporated in the whole area, in order to increase soil base saturation to 50% (ROCHA et al., 2016). As maintenance fertilization 300 kg P_2O_5 ha^{-1} yr^{-1}, in the form of triple superphosphate, 2/3 in September and 1/3 in December, was applied according to VEIGA et al. (2019). The applications of nitrogen and potassium were 450 kg ha^{-1} of N in the form of urea and K_2O in the form of KCl, divided in four times a year from September to February. The application of micronutrients was carried out according to the results of chemical analyses of the soil and the plant.

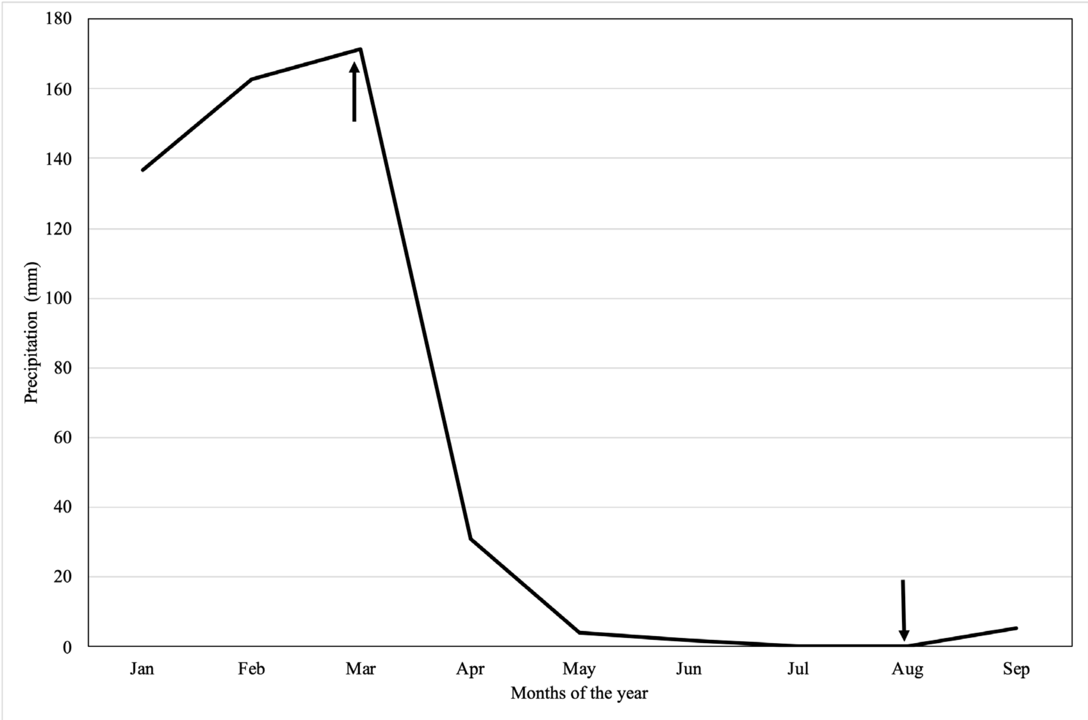

Figure 1 - Monthly rainfall during the period of the study at the experimental area of Embrapa Cerrados (2011). Arrows indicate the soil sampling time in the rainy season (March) and in the dry season (August).

The chemical properties of the soil (August / 2011) are shown in table 1.

Experimental design

Two water regimes (WR) were considered, WR1 with full irrigation, meaning irrigation shifts throughout the year, and WR3 with controlled water stress for about 70 days, from the end of June until the beginning of September (dry season), in order to induce uniform flowering after resuming irrigation (VEIGA et al., 2019). The Cerrado Irrigation Monitoring System (ROCHA et al., 2006) was used to calculate the soil water balance and to decide the irrigation management criterion. In each water regime, the application or not of lime on soil surface and the presence or absence of brachiaria (*U. decumbens*) cultivated between the rows of coffee plants were evaluated. In the treatments with liming, 2 t of dolomitic lime ha^{-1} were applied on the soil surface, two years after the beginning of the experiment (2009), at the dry season end. In the brachiaria-covered treatments, the grass was mechanically cut when they reached 0.60 m height. In the treatments without brachiaria, the soil was kept free of weeds by manual cleaning. Plant residues remained on the soil surface.

The experiment was carried out in a randomized complete block, in a 2 x 2 x 2 factorial design with six replications. The experimental plots consisted of nine coffee trees, of which five were used for collecting samples and the other four were considered border plants.

Soil sampling

Soil sampling (0-10 cm depth) was performed in the canopy projection of the five inner-plot plants and a composite sample of five subsamples from each plot was used. Sampling took place in March and August 2011, rainy and dry seasons, respectively.

Laboratory analysis

The β-glucosidase (E.C. 3.2.1.21), arylsulfatase (E.C.3.1.6.1) and acid phosphatase (E.C. 3.1.3.2) activities were determined according to TABATABAI (1994). Due to their short incubation periods (1 h), toluene was omitted from the assays.

Table 1 - Chemical properties of a typic Haplustox cultivated with coffee under two different water regimes associated with lime application and management system with brachiaria cover (0 to 10 cm depth).

------------------Treatments-----------------			Al	H+Al	Ca	Mg	pH	SOM	P	K
WR	Lime	Brachiaria	------------------(cmol$_c$ kg^{-1})-----------------					g kg^{-1}	------------mg kg^{-1}----------	
WR1	+	+	0.05	7.28	4.48	1.70	6.01	4.68	155.36	293.33
WR1	+	-	0.05	6.71	4.73	1.77	6.00	4.34	151.49	226.67
WR1	-	+	0.17	9.06	3.48	0.72	5.36	4.62	172.33	231.67
WR1	-	-	0.26	9.99	3.47	0.72	5.19	4.54	142.38	158.33
WR3	+	+	0.04	6.44	4.33	1.93	5.94	4.30	195.03	256.67
WR3	+	-	0.03	6.26	4.33	1.80	5.99	3.78	111.93	215.33
WR3	-	+	0.12	8.38	3.28	0.85	5.43	3.94	195.53	206.67
WR3	-	-	0.15	8.27	2.91	0.68	5.38	3.82	155.38	230.67

pH in H_2O; Ca, Mg and Al: extracted with 1N KCl; P and K: using the Mehlich 1 extractor (H2SO4 0.0125 M + HCl 0.05 M); SOM = soil organic matter by the Walkley & Black method. All according Teixeira et al. (2017).
WR = Water regime.
\+ = with.
\- = without.

These three soil enzymes were selected for their roles in the C cycle (β-glucosidase), S cycle (arylsulfatase) and P cycle (acid phosphatase). The methods are based on the colorimetric determination of the p-nitrophenol formed after the addition of specific colorless substrates for each enzyme.

Statistical analysis

Data normality was checked with the Shapiro-Wilk test, followed by analysis of variance (Anova). Tukey's test was used to compare the means, at 5% probability. Statistical analyses were carried out using the SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA).

The statistical software S-Plus v. 4.0 (MATHSOFT, 1999) was used to generate tree regression analyzes, with water regimes, lime application and management systems (presence or absence of brachiaria) as categorical explanatory variables. These analyses were performed, for each season separately, using standardized enzyme activity data. The final size of the trees was chosen using the 1-SE rule (BREIMAN et al., 1984).

RESULTS

Data from samples obtained in March, during the rainy season, revealed that the the β-glucosidase and arylsulfatase activities responded positively and significantly to the application of lime and to the management system (with and without intercropped brachiaria). Lime application was the only treatment that individually increased the activity of acid phosphatase. In the rainy season, the WR alone did not affect the activity of the evaluated enzymes (Table 2).

In the presence of lime, the activities of β-glucosidase, arylsulfatase and acid phosphatase were 12%, 19% and 8% higher than those treatments without lime application, respectively. Activities of the β-glucosidase and arylsulfatase were, respectively, 33% and 46% higher in treatments with the presence of brachiaria compared to treatments without cover plants between the lines (Table 3).

For the activity of β-glucosidase and arylsulfatase enzymes, there was also an interaction between the application of lime and the management system (Table 4). Both enzymes showed increased activities in areas with the presence of brachiaria, regardless of the application of lime. Conversely, the increased activities of these enzymes as a function of liming were observed only in the presence of brachiaria (Table 4).

In the case of the acid phosphatase, there was an interaction between the WR and the management system. In the treatments with brachiaria, the activity of this enzyme was higher in WR3, while, in the absence of the forage, the greatest activities were observed in WR1. The activity of acid phosphatase in treatments with brachiaria was higher only under WR3 (Table 4). However, at that sampling time, there were no differences between the WR applied and; therefore, these effects were not expected and are difficult to interpret.

Table 2 - Summary of the analysis of variance of the β-glucosidase, arylsulfatase and acid phosphatase activity data from a typic Haplustox cultivated with coffee as a function of two different water regimes (WR), with and without lime application and brachiaria cover, in the rainy season (0 to 10 cm depth).

Sources of variation	-------β-glucosidase--------		--------Acid phosphatase--------		--------Arylsulfatase--------	
Principal Effects	F	p	F	p	F	p
WR	2.91	0.0982	0.33	0.5706	2.68	0.1117
Lime	21.30	0.0001	5.98	0.0206	20.63	0.0001
Management system	62.91	0.0001	0.53	0.4821	37.23	0.0017
---Interactions---						
A – WR x Lime	0.78	0.3836	0.00	0.9989	1.78	0.1925
B – WR x System	0.95	0.3363	13.34	0.0010	0.99	0.3284
C – Lime x System	8.01	0.0082	0.17	0.6816	4.91	0.0345
D – WR x Lime x System	6.13	0.0192	2.91	0.0984	0.86	0.3610

In the dry season, the WR, liming and intercropped brachiaria; individually, influenced the activity of the enzyme arylsulfatase, while the activity of β-glucosidase responded significantly only to the presence of brachiaria. At this sampling time, the activity of acid phosphatase did not respond to any of the sources of variation, in an isolated manner (Table 5).

Arylsulfatase activity was 47% higher in WR1 compared to WR3. When lime was applied, arylsulfatase activity was 24% higher when compared to areas without application. In systems with and without intercropped brachiaria, the β-glucosidase activity was 56% and arylsulfatase 58% higher with the presence of the forage (Table 6). These differences associated to brachiaria as intercrop were higher in the dry season than in the rainy season.

Interactions were observed between the WR and the management system for the activities of the enzymes arylsulfatase and acid phosphatase. Arylsulfatase showed increased activity in areas with brachiaria and under WR1. Regarding the activity of acid phosphatase, the effect of the presence of brachiaria as an intercrop was observed only under WR3. Still, in treatments without brachiaria, the activity of this enzyme was lower in WR3, when compared to WR1 (Table 7).

Tree regression models helped to graphically summarize the results obtained in this research. Figure 2 shows that the enzyme activities measured in the rainy season explained 63% of the data variability. Among the factors used as categorical explanatory variables for the construction of the tree, management system (presence/absence of brachiaria)

Table 3 - Effects of water regime (WR), lime application and management system with brachiaria cover on the activities of β-glucosidase, arylsulfatase and acid phosphatase enzymes in a typic Haplustox cultivated with coffee, in the rainy season (0 to 10 cm depth).

Sources of variation	β-glucosidase	Acid phosphatase	Arylsulfatase
	---µg *p*- nitrophenol g^{-1} soil h^{-1}---		
WR1	146^{ns}	618^{ns}	113^{ns}
WR3	140^{ns}	628^{ns}	106^{ns}
With lime	151 a	646 a	119 a
Without lime	135 b	600 b	100 b
With brachiaria	163 a	630^{ns}	130 a
Without brachiaria	123 b	616^{ns}	89 b

Within each source of variation, means followed by different letters in the columns are significantly different at P < 0.05 (Tukey's test). ns = Not significant by F test (P > 0.05).

Table 4 - β-glucosidase and arylsulfatase activities due to the interactions between the application of lime and the management system with brachiaria cover, and acid phosphatase due to the interaction between water regime (WR) and management system with brachiaria, in a typic Haplustox cultivated with coffee, in the rainy season (0 to 10 cm depth).

----β-glucosidase----		
----μg *p*- nitrophenol g^{-1} soil h^{-1}----		
----Lime----		
Management system	With	Without
With brachiaria	176 aA	151 bA
Without brachiaria	127 aB	120 aB
----Arylsulfatase----		
----μg *p*- nitrophenol g^{-1} soil h^{-1}----		
----Lime----		
Management system	With	Without
With brachiaria	145 aA	115 bA
Without brachiaria	94 aB	84 aB
----Acid phosphatase----		
----μg *p*- nitrophenol g^{-1} soil h^{-1}----		
----WR----		
Management system	WR1	WR3
With brachiaria	590 bA	671 aA
Without brachiaria	645 aA	586 bB

Means followed by different letters, lowercase in rows and uppercase in columns, are significantly different at P < 0.05 (Tukey's test).

was the one that most affected soil enzymes activities, separating the areas with and without brachiaria, responsible for explaining 51% of the model. In the areas with brachiaria lime application also had a significant effect, contributing with the remaining 12% to explain the entire model.

In figure 3 (dry season), it can be seen that the tree regression analysis explained 59% of the data variability. The presence/absence of brachiaria remained as the most important factor, accounting for 37% of the model. The water regime (16%) and liming (6%), with a significant effect only on WR1, also contribute to the model construction.

DISCUSSION

Arylsulfatase is responsible for the hydrolysis of organic sulphate esters in the soil

Table 5 - Summary of the analysis of variance of the β-glucosidase, arylsulfatase and acid phosphatase activity data in a typic Haplustox cultivated with coffee as a function of two different water regimes (WR), with and without lime application and brachiaria cover, in the dry season (0 to 10 cm depth).

Sources of variation	--------β-glucosidase-------		--------Acid phophatase------		----------Arylsulfatase--------	
Principal Effects	F	p	F	p	F	p
WR	0.86	0.3589	0.78	0.3845	52.51	0.0001
Lime	3.94	0.0550	1.39	0.2478	16.47	0.0003
Management system	70.89	0.0001	2.79	0.1559	50.26	0.0001
----Interactions----						
A – WR x Lime	2.65	0.1125	1.13	0.2954	1.65	0.2092
B – WR x System	1.98	0.1686	6.68	0.0148	7.60	0.0098
C – Lime x System	0.02	0.8754	0.59	0.4470	0.00	0.9488
D – WR x Lime x System	0.23	0.6320	3.89	0.0577	0.03	0.8691

Table 6 - Summary of the analysis of variance of the β-glucosidase, arylsulfatase and acid phosphatase activity data from a typic Haplustox cultivated with coffee in function of two different water regimes (WR), lime application and management system with brachiaria cover, in the dry season (0 to 10 cm depth).

Fontes de Variação	β-glucosidase	Acid phosphatase	Arylsulfatase
	---μg *p*- nitrophenol g^{-1} soil h^{-1}---		
WR1	123^{ns}	923^{ns}	141 a
WR3	118^{ns}	888^{ns}	96 b
With lime	127^{ns}	882^{ns}	131 a
Without lime	114^{ns}	929^{ns}	106 b
With brachiaria	147 a	957^{ns}	145 a
Without brachiaria	94 b	855^{ns}	92 b

Within each source of variation, means followed by different letters in the columns are significantly different at P < 0.05 (Tukey's test).
ns = Not significant by F test (P > 0.05).

(TABATABAI, 1994), while β-glucosidase hydrolyzes maltose and cellobiose leading to the production of glucose which serves as a crucial C energy supply for the microorganisms (MERINO et al., 2016), and acid-phosphatase hydrolysis organic esters and anhydrides of phosphoric acid mediating their transformations into free phosphates (RAO et al., 2017). Therefore, these three enzymes have crucial role in the sulfur (S), carbon (C) and phosphorus (P) cycling in the soil, respectively. These soil enzymes activities are often closely related to SOM (LOPES et al., 2013), exhibit strong links with the microbial communities and the functional gene abundance (PEIXOTO et al., 2010; TRIVEDI et al., 2016), and have shown positive correlations with soybean, corn (LOPES et al., 2013; MENDES et al., 2021) and coffee yields (ARAGÃO et al., 2020).

In this research, the average activities measured for arylsulfatase (114 μg p-nitrophenol g^{-1} soil h^{-1}) and β-glucosidase (132 μg p-nitrophenol g^{-1} soil h^{-1}) are higher or equivalent to the average values presented by ARAGÃO et al. (2020), for areas of high coffee yields in the Cerrado (cultivar Catuaí Vermelho IAC 144), for both enzymes, respectively. The average value for the acid phosphatase activity (764 μg p-nitrophenol g^{-1} soil h^{-1}) in the present study is around 40% of the observed by NAVES et al. (2020), also in a Cerrado Oxisol under coffee. This difference between the acid phosphatase activity in both studies could be explained by their differences in

Table 7 - Arylsulfatase and acid phosphatase activities due to the interactions between the water regime (WR) and management system with brachiaria cover, in a typic Haplustox cultivated with coffee, in the dry season (0 to 10 cm depth).

	---Arylsulfatase---	
	---μg *p*- nitrophenol g^{-1} soil h^{-1}---	
	---WR---	
Management system	WR1	WR3
With brachiaria	175 aA	114 bA
Without brachiaria	106 aB	79 bB
	---Acid phosphatase---	
	---μg *p*- nitrophenol g^{-1} soil h^{-1}---	
	---WR---	
Management system	WR1	WR3
With brachiaria	923 aA	990 aA
Without brachiaria	922 aA	787 bB

Means followed by different letters, lowercase in rows and uppercase in columns, are significantly different at P < 0.05 (Tukey's test).

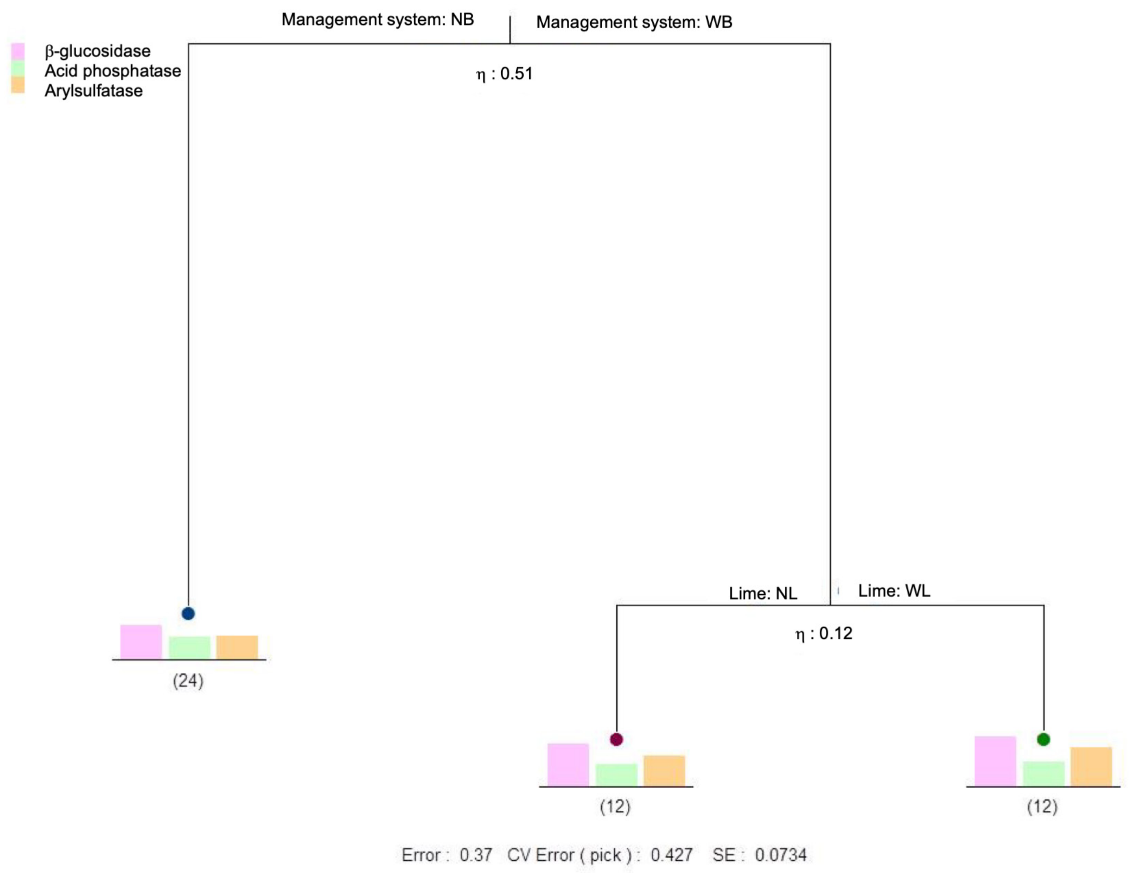

Figure 2 - Tree regression model build with the enzyme activity data (β-glucosidase, acid phosphatase and arylsulfatase), soil sampling in the rainy season (March 2011), taking into account the factors management system (NB - without brachiaria; WB - with brachiaria), lime application (NL - without lime; WL - with liming) and water regime (WR1 - full irrigation; WR3 - controlled water stress). ( ) = number of repetitions. η = explained variability.

soil's P content, which was more than 100 times higher in our experiment. The addition of phosphate fertilizers increases P levels in soil and affect acid phosphatase activity, since orthophosphate is a competitive inhibitor of this enzyme (TABATABAI, 1994).

In general, it was observed that the sensitivity gradient of the enzymes studied in this research was arylsulfatase > β-glucosidase > acid phosphatase. These results revealed that arylsulfatase was the most sensitive indicator for detecting changes in the soil due to the treatments evaluated, being able to respond to the water regime (in the dry season), lime application and presence of brachiaria.

In 20 years of studies with bio indicators in the Cerrado region, enzymes arylsulfatase and β-glucosidase were the indicators that consistently showed greater sensitivity to detect changes in the soil due to the management system and for this reason have been recommended for soil bioanalysis (MENDES et al., 2019; 2021). MENDES et al. (2005) had highlighted the activity of arylsulfatase as one of the most sensitive bioindicators to differentiate agricultural areas under no-tillage (NT) and conventional tillage. These authors observed significant and expressive increases in the activity of this enzyme in all areas under NT. Moreover, these changes could be detected rapidly, only one year after the adoption of NT system. These results showed the ability of this enzyme to detect changes in the biological functioning of the soil, even before significant changes in the levels of microbial biomass carbon (MBC) and SOM can occur. MENDES et al. (2019) suggested that this decoupling of arylsulfatase activity in relation to MBC is an

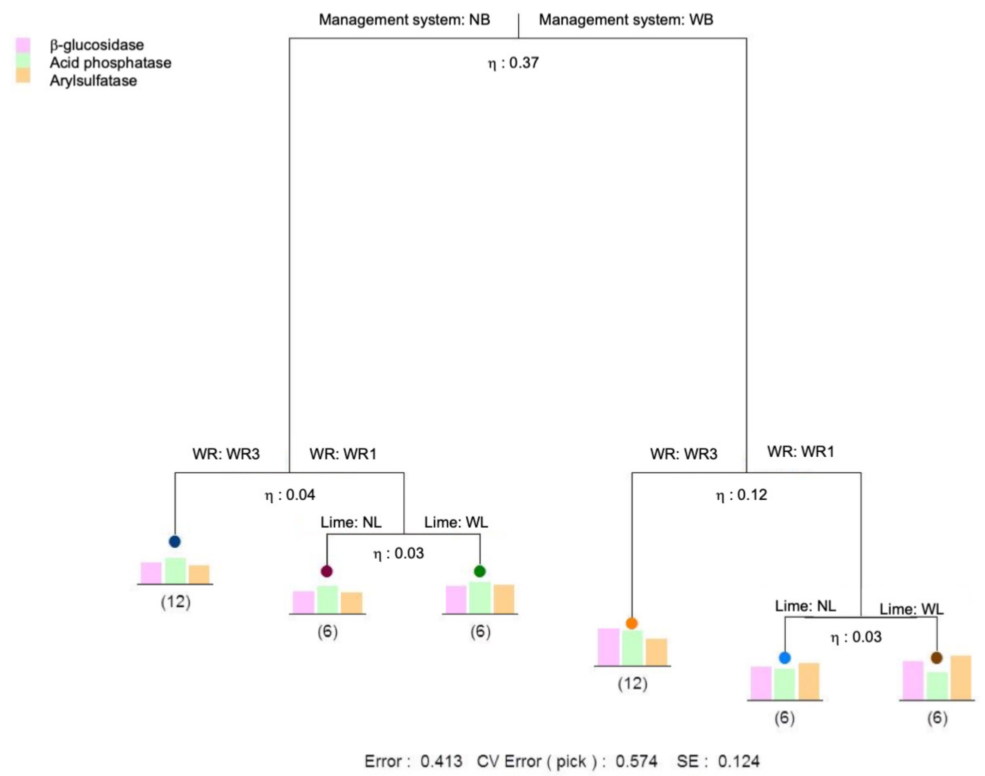

Figure 3 - Tree regression model build with the enzyme activity data (β-glucosidase, acid phosphatase and arylsulfatase), soil sampling in the dry season (August 2011), taking into account the factors management system (NB - without brachiaria; WB - with brachiaria), lime application (NL - without lime; WL - with liming) and water regime (WR1 - full irrigation; WR3 - controlled water stress). ( ) = number of repetitions. η = explained variability.

indication that conservationist agricultural systems, such as NT, integrated crop-livestock system and integrated crop-livestock-forestry system, provided conditions that favored the predominance of abiontic activity of this enzyme in the soil. In this case, the enzymes are associated with the non-living fraction of the soil and accumulate protected from the action of proteases, through their adsorption in clay particles and in the organic matter (WALLENSTEIN & BURNS, 2011).

In the present study, as can be seen in table 1, in general, where lime was not applied, the soils had higher concentration of Al^{+3}, higher potential acidity (H + Al) and a slightly lower pH. However, these values are in a range where significant impacts for microbial activity and the development and productivity of most agricultural crops would not be expected. Additionally, the concentration of Ca and Mg, were higher in the treatments with lime application. These observations, together, can help to explain the effects of liming on the activity of the β-glucosidase and acid phosphatase in the rainy season (Tables 3 and 4), and arylsulfatase in both seasons (Tables 3, 4 and 6).

In acidic soils, liming can create better environmental conditions for the development of acid-intolerant microorganisms, resulting in increased microbial biomass and soil respiration (SOUZA et al., 2016). The increase in pH stimulates the deprotonation of organic substances and, as a result, the bond between organic compounds and soil particles decreases, making these substances more available for microbial consumption (AYE et al., 2016). According to ACOSTA-MARTÍNEZ & TABATABAI (2000) the addition of lime, in general, also increases the activity of glucosidases and sulfatases. These authors claimed that the optimum pH for the activity of these enzymes is presumably achieved after liming. Although, in the case of β-glucosidase, the substrate quality e seemed to be more important (MENDES et al., 2019). Acid phosphatase activity could be negatively affected by liming, with an increase in pH above the optimum for this enzyme (5.0 - 6.0) (ACOSTA-MARTÍNEZ & TABATABAI, 2000). Conversely, studying the relationship between enzymatic activity and different soil characteristics altered by liming, FERNANDES et al. (1998) observed a positive response of acid phosphatase, which was determined by the increase in the levels of SOM, calcium and magnesium. In this research, the positive responses of the activities of arylsulfatase and β-glucosidase to lime application were observed only in the presence of brachiaria as a cover plant (Table 4). Probably, this is due to the nutritional and physiological effect of liming to the system with brachiaria and its positive impact on the soil biology.

In both sampling times, increased activities of β-glucosidase (Tables 3, 4, 6) and arylsulfatase (Tables 3, 4, 6 and 7) were observed in the treatments with brachiaria as intercrop between of rows of coffee trees. It can be explained by the observations made in studies such as those conducted by BRANDAN et al. (2017), which emphasized the improvement of microbiological activity and metabolic efficiency in areas with the presence of this forage grass due to the high root density and greater amounts of litter provided by the brachiaria. The presence of a cover plant leads to the continuous addition of C, litter and roots, on the surface and sub-surface of soils (BALOTA & CHAVES, 2010). This explains the differences reported for enzymatic activity, which are directly related to the quantity and quality of SOM. In addition to the availability of organic matter, the presence of a living cover crop between the coffee rows helps to prevent sudden changes in temperature and maintain moisture, factors that also positively influence soil biology. The increase in plant residues, greater amount of root exudates and soil protection, especially during the dry season, also favor an edaphic environment biologically more active. This is likely why the differences in β-glucosidase and arylsulfatase activities in the presence or absence of brachiaria as a cover crop reported in our study were more pronounced in the dry season than in the rainy season.

MENDES et al. (2019) showed results of an experiment with the objective of evaluating grain production systems, under no-tillage (NT), with and without the presence of brachiaria. In this experiment, the chemical characteristics, including SOM, did not allow to differentiate the treatments with and without brachiaria. However, the presence of brachiaria increasead activity levels of arylsulfatase and β-glucosidase (eight and four fold, respectively), meaning that; although, the soils in all treatments were chemically similar, their biological functioning was different due to the presence of brachiaria.

Effects related to the WRs were observed in the samplings carried out in the dry season. In this situation, the treatments under controlled water stress (WR3) had been without irrigation for approximately 60 days, in a period without precipitation (Figure 1). This explained the responses observed in areas with full irrigation (WR1), which showed greater activity of the enzyme arylsulfatase (Tables 6 and 7). Also interesting was the response of acid phosphatase, with lower activity under WR3 only in the absence of brachiaria (Table 7), which reinforces the importance of this cover crop in maintaining moisture, with a positive effect on soil biology.

As soil moisture is considered one of the main factors that govern the dynamics of biomass and microbial activity, seasonal changes or irrigation practices can affect soil functions (RAO et al., 2017). Soil moisture influences the activity of various enzymes, such as endocellulases, endoxylanases, catalases and phosphatases, both in litter and in the soil (BALDRIAN et al., 2010). Moisture can affect soil enzymes indirectly, by increasing microbial growth and substrate availability (SINSABAUGH et al., 2008). COSTA et al. (2013) showed, in an oxisol under coffee cultivation, that irrigation increased MBC, basal respiration and acid phosphatase activity.

Intra-annual seasonal fluctuations in the bioindicators are directly linked to the quality, quantity and availability of the substrate in the different seasons and vary according to the ecosystem, the parameters studied and the soil properties (MENDES et al. 2019). In our study, in general, it was observed that activity of acid phosphatase was higher in the sampling performed in the dry period compared to the rainy period. This behavior was not expected; however, as P applications are made at the beginning of the rainy season, this practice may have inhibited the activity of acid phosphatase at this time of sampling, having a greater influence on the results than the season itself.

The tree regression models, built for each sampling period, confirmed a) the importance of lime application, especially in areas with brachiaria coverage, for the biological quality of the soil in the first sampling (Figure 2); b) WR as one of the important factors to explain the data variability in the second sampling, in a season marked by water deficit

(Figure 3); c) the presence of brachiaria as the main factor to explain the variability of the data, in both samplings (Figures 2 and 3).

This last observation evidences the importance of brachiaria as an intercrop in coffee plantations. Even though the levels of SOM have not yet been influenced (Table 1), the increased levels of enzymes activities under brachiaria clearly showed that the presence of deep-rooted grasses enhance biological activity, being important for the maintenance and / or improvement of soil quality.

Soil's capacity to protect enzymes is related to its ability to store and stabilize SOM and other associated structural properties (aggregation and porosity), whose changes are difficult to detect in short periods of time, unlike the enzymatic activity (RAO et al., 2017; MENDES et al., 2019; ARAGÃO 2020). For this reason, the increase in enzyme activity may foreshadow if the agricultural system or management adopted is favoring the accumulation of SOM and; consequently, the soil quality.

CONCLUSION

Brachiaria intercropped between the rows of coffee plants was the factor that most positively impacted β-glucosidase, arylsulfatase and acid phosphatase activities. Lime application in association with intercropped brachiaria increased β-glucosidase and arylsulfatase activities, in the rainy season. Lime application alone increased acid phosphatase and arylsulfatase activities, in the rainy and dry seasons, respectively. In the dry season β-glucosidase activity was not influenced by the water regime, whereas the use of controlled water stress (WR3) reduced arylsulfatase activity, and the presence of intercropped brachiaria prevented reductions in the acid phosphatase activity.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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