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EFFECT OF VARIETY AND CLIMATIC SEASONALITY ON SOIL INTRACELLULAR ENZYMATIC ACTIVITIES IN COFFEE AGROFORESTRY SYSTEMS

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Abstract. Most soil physicochemical parameters do not respond immediately to changes in management when compared to microbiological and biochemical ones; the study of biological and biochemical quality of soils can serve as indicators of their general condition. Enzymatic activities are important in the biochemical functioning of soils. In this work, the activity of three intracellular enzymes of the soil was evaluated: dehydrogenase activity, fluorescein diacetate hydrolysis and arginine deaminase, its seasonal fluctuation and the effect of two coffee varieties Caturra and Catuaí on an agroforestry system. The soil samples were taken during the dry and rainy seasons in two contiguous plots sown with the two coffee varieties and in each one a non-systematic zigzag sampling was carried out. The physical, chemical properties and intracellular enzymatic activities of the soil were determined by the classical methods of analysis and following standard protocols. It was observed that the dehydrogenase activity and hydrolysis of fluorescein diacetate had greater activity in the rainy season regardless of the variety, whereas arginine deaminase showed more activity in the dry season and for the Caturra variety. The intracellular enzymatic activities showed sensitivity to the changes during the sampling period, in soils planted with coffee varieties Caturra and Catuaí.

Keywords: agroforestry system, coffee, dehydrogenase activity, fluorescein diacetate hydrolysis, arginine deaminase

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INTRODUCTION

Coffee (*Coffea arabica* L.) is widely distributed through the tropics with more than 70 species, being one of the most important agricultural export products in the world market (Pohlan and Janssens 2010). The Caturra and Catuaí coffee varieties are the most common varieties cultivated in different parts of the world, due to their high yields, compact size, and good cup quality (aroma and mild flavor). Consequently, it would be significant to establish if the cultivated variety influences the physicochemical and biological activities of the soils, which, in turn, would be reflected in its quality. Estimating the quality of the soil is important since it contributes to establishing the sustainability of the different management systems. High-quality soils are able to maintain high productivity and cause minimal environmental disturbance (Ferreras *et al.* 2009). On the other hand, microorganisms are fundamental in the decomposition process of organic soil residues through enzymes that catalyze the innumerable reactions necessary for the vital processes of microorganisms, and influence soil fertility, intervening both in the establishment of the biogeochemical cycles and the structure formation (García *et al.* 2003). Soil enzymes act as biological catalysts for specific reactions that depend on a variety of factors such as the presence or absence of inhibitors, climate, type of amendment, crop and/or vegetation coverage, among others, and can be considered as early indicators of the biological changes (Acosta-Martínez *et al.* 2007). According to Dick (1997), enzymes participate in important soil functions, among which the following can be mentioned: (i) decomposition of the contributions of organic matter to the soil through fallen leaves, radical exudates or organic amendments; (ii) native organic soil matter transformation; (iii) release of inorganic nutrients for plant growth; (iv) nitrogen fixation; (v) xenobiotics detoxification; (vi) nitrification; and (vii) denitrification. The plants, animals and microorganisms are enzyme sources in the soil but the microbial component, consisting of protozoa, fungi, actinomycetes and bacteria, are its main contributor (Tabatabai 2003) and its location in the soil can be: a) intra- or pericellular (those limited to the cell membrane); and b) extracellular, stabilized by soil particles, mainly clays and/or entrapped within the structures of the humic substances (Acosta-Martínez *et al.* 2007, Dick1997, Nannipieri *et al.* 2012, Tabatabai 2003).

In this study, three intracellular enzymes were evaluated, which are integral part of the microorganisms and do not accumulate extracellularly in the soil. Among these enzymes, the activity of the dehydrogenases (DHA) involved in the oxidation reactions, fulfill a fundamental role in the initial stages of the organic matter oxidation, which can be considered as a good measure of the microbial biomass oxidative activity and is commonly utilized as an indicator of biological activity in soils. Fluoriscein diacetate-hydrolyzing (FDA) enzymes are also related to organic matter degradation since it is a generic substrate for esterases, proteases and lipases and is, therefore, also a general potential indicator of soil microbi-

al activity (Schnürer and Rosswall 1982) and lastly, the activity of the arginine deaminase (AD) which is an enzyme that mineralized arginine to ammonium and is correlated with the microbial biomass measured by the fumigation-incubation method, the CO_2 released during respiration induced by substrate and the content of ATP (Alef and Kleiner 1986).

The objective of this research was to evaluate the activity of three intracellular enzymes and some physicochemical properties as quality indicators of the soils cultivated with two coffee varieties Catuaí and Caturra in an agroforestry system during the dry and rainy seasons, in the Venezuelan Coastal Range. It was hypothesized that moisture and coffee varieties affect the physicochemical properties and the biochemical and microbiological processes of the soil, causing changes in soil quality.

MATERIALS AND METHODS

Study site

The current research was carried out at the Jaime Henao Jaramillo Experimental Station of the Central University of Venezuela (formerly known as El Laurel Research Center), located in the municipality of Baruta, State of Miranda (Venezuela) at the geographic coordinates 10°22'24"N and 66°54'04"W in the Venezuelan Coastal Range (Fig. 1). It occupies an area of approximately 369 ha with altitudes from 1,050 to 1,400 m.a.s.l.; the relief is hilly, with rugged topography. The average annual rainfall is 1,322 mm of which 85% falls in seven months from May to November. The annual mean temperature was 19.1°C, with monthly average values of 17.5°C and 20.0°C during the coldest (January) and the hottest (May) months of the year (Fig. 2).

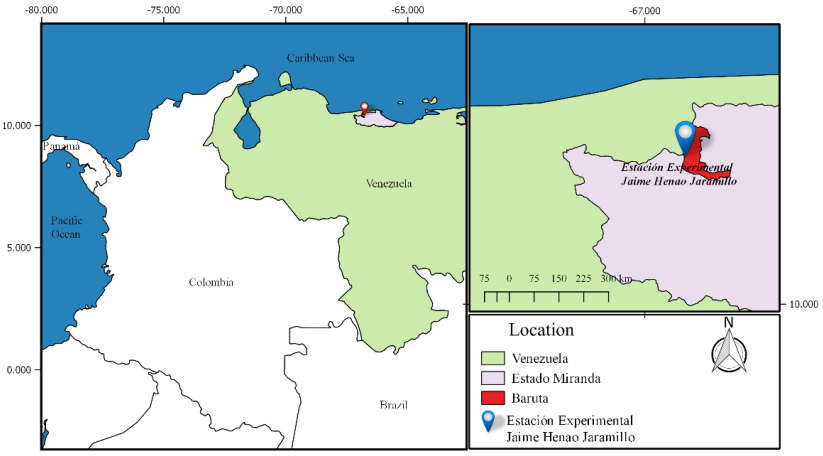

Fig. 1. Location of the study area

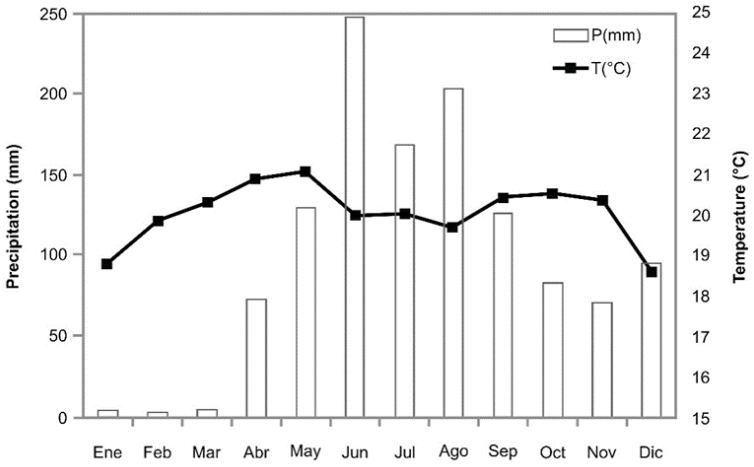

Fig. 2. Averages of the monthly precipitation (bars) and air temperature (line) recorded at the Jaime Henao Jaramillo Experimental Station, UCV

The plantations age for the studied varieties are as follows: Catuaí was approximately 20 years old (planting density of 4,200 plants ha^{-1}) and Caturra was 8 years old (planting density of 6,410 plants ha^{-1}), in an area previously planted with the Catuaí variety; limenstone was added to the soil, throughout the area. The experimental site is a coffee agroforestry system known as polyculture or mixed shade systems in which the upper part was canopied by leguminous trees of the genera *Inga* spp. and *Erythrina poeppigiana*. Other crops include plantain (*Musa* spp.), citrus (*Citrus* spp.) and papaya or lechoza (*Carica papaya*) without the application of agricultural inputs (Fig. 3). The soil was classified according to the U.S. Soil Taxonomy as Typic Kanhaplohumults, clayey, mixed, isohipertermic (Soil Survey Staff 2006).

Fig. 3. Coffee agroforestry system (Jaime Henao Jaramillo Experimental Station, UCV)

Sampling

Soil samples were collected in contiguous plots planted with the two varieties. In each plot a total of six composite samples were collected in a zigzag non-systematic pattern using a metallic cylinder of 6.5 cm in diameter and 5 cm in height from the crown projection area of coffee plants. Composite soil samples were composed by pooling six-eight subsamples. The sampling was carried out during the dry season (January, March and April) and during the rainy season (June, July and September) of the year 2014. Such sampling depth (0–5 cm) was chosen since the highest contents of organic matter and biological activity are exhibited in the first centimeters of the soil. The samples were kept in plastic bags, protected from the light in thermal boxes and transported to the Soil Ecology Laboratory of the Instituto Venezolano de Investigaciones Científicas (IVIC) and kept under refrigeration for 24 h after sampling. Samples were sieved (<2 mm) and divided into two portions. One was stored in plastic bags in a refrigerator at 4°C until the enzyme analysis was performed within a lapse of 10 days. The other portion was air dried, for physicochemical analysis. Two (2) repetitions per sample were performed for each physicochemical and biochemical parameter evaluated. The analysis of real cation-exchange capacity (CECr) was carried out in the soil laboratory of the University of Córdoba (Montería, Colombia).

Laboratory analysis

The physical and chemical properties of the soils were determined by standard procedures described below: the pH in soil; water ratio of 1:2.5; soil bulk density (BD) – through the metal core method; soil moisture content (SM, % w/w) – by gravimetry; total nitrogen (TN) – by the Kjeldahl method; available phosphorus (P_{ava}) – by extraction in a solution of sodium bicarbonate (pH 8.2) and inorganic P in the extracts according to the molybdenum blue method (Wilke 2005); organic carbon (OC) – by wet digestion with potassium dichromate in sulfuric acid and measurement of Cr (III) – by spectrophotometry (Heanes 1984). The real cation exchange capacity (CECr) – through extraction with 1N ammonium acetate (IGAC 2006).

Dehydrogenase activity (DHA) was measured following the procedure described by Camiña *et al.* (1998). Briefly, the soil is incubated with an artificial electron INT [chloride 2- (*p*-iodophenyl) -3- (*p*-nitrophenyl) -5-phenyl-2H-tetrazolium] and buffer 1M Tris-HCl pH 7.5 for 2 hours at 40°C. The red-coloured formazan INTF (*p*-iodonitrotetrazolium formazan) produced was extracted with a mixture 1:1 (v:v) N,N-dimethylformamide:ethanol and measured spectrophotometrically at $\lambda = 490$ nm. The hydrolysis of fluorescein diacetate (3´,6´-diacetyl-fluorescein) (FDA) was assayed as described by Schnürer

and Rosswall (1982). The method comprises incubation of a soil sample with a FDA solution (acetone as solvent) and 60 mM phosphate buffer pH 7.6 at 30°C for 30 min and then fluorescein (reaction product) is extracted with acetone and measured spectrophotometrically at $\lambda = 490$ nm. Arginine deaminase or arginine ammonification (AD) was determined by a modification of the Alef and Kleiner (1987) method, where the soil is incubated at 37°C for 3 hours with an L-arginine solution, then the released ammonium was extracted with 2M KCl and determined spectrophotometrically at $\lambda = 690$ nm by the Berthelot reaction (formation of the indophenol blue with sodium salicylate and dichloroisocyanurate under alkaline pH conditions in the presence of sodium nitroprusside). All these measurements were performed using the field moist soils, and data are expressed on an oven-dried soil basis (105°C).

Statistical analysis

The effects of the varieties, the seasonality and their interaction on the enzymatic activities of the soil, were analyzed through two-way PERMANOVA (Permutational multivariate analysis of variance), after checking the homoscedasticity assumption of multivariate dispersion by PERMDISP (Permutational analysis of multivariate dispersions) (Anderson 2006). For all the analyses, the data were previously normalized and Euclidean distance matrixes were calculated. The program PRIMER 6. Version 6.1.13 & PERMANOVA + 1.0.3 was used to carry out these analyses. Statistically significant differences for all variables between treatments were established at the level of $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS AND DISCUSSION

Physical and chemical properties of the soil

It was observed that the soil moisture content varied significantly ($p < 0.05$) between the two varieties. Higher soil moisture content was recorded in the Catuaí variety, compared to the Caturra. The soil with the Catuaí variety (SM%) showed significantly higher values in the two climatic seasons – rainy (52.0±2.0% SM), and dry (41.9±2.1% SM), compared with the soil of the Caturra variety, whose moisture content was (46.3±2.3%) and (36.7±2.6%) in the rainy and dry seasons, respectively (Table 1). The difference in the moisture content percentage between the soils with the two varieties is related to the higher OC content in soils with the Catuaí variety (Table 2).

The BD showed statistically significant differences ($p < 0.01$) between the two varieties. The soil with the Catuaí variety presented a lower value in both seasons, dry (0.76±0.03 $g.cm^{-3}$) and rainy (0.75±0.03 $g.cm^{-3}$), in comparison

with the soil of the Caturra variety, which showed equal values in dry and rainy seasons (0.87±0.03 g.cm^{-3}) (Table 1). These results are related to the higher concentration of OC in the soil with the Catuaí variety (Table 2). Numerous investigations have shown that the soil organic matter improves the physical properties of the soil, such as structure, porosity, bulk density, aeration, and the movement-retention availability of water in the soil (Cardona and Sadeghian 2006, Weil and Magdoff 2004).

Table 1. Physical properties in soils cultivated with Catuaí and Caturra coffee varieties. Soil moisture content (SM) and bulk density (BD). (Mean ± standard error; n = 18)

Properties	Catuaí		Caturra		Variety	Climatic season
	Dry	Rainy	Dry	Rainy	P_{man}	P_{man}
SM (%)	41.9±2.1	52.0±2.0	36.7±2.6	46.3±2.3	*	***
BD (g.cm^{-3})	0.76±0.03	0.75±0.03	0.87±0.03	0.87±0.03	**	NS

Level of significance for each factor in PERMANOVA (P_{man}), *p < 0.05; **p < 0.01; ***p < 0.001; NS = non-significant at 95%.

Table 2. Chemical properties in soils cultivated with Catuaí and Caturra coffee varieties. pH; organic carbon (OC); total nitrogen (TN); real cation exchange capacity (CEC_r); available phosphorus (P_{ava}). (Mean ± standard error; n = 18).

Properties	Catuaí		Caturra		Variety	Climatic season
	Dry	Rainy	Dry	Rainy	P_{man}	P_{man}
pH	5.1±0.0	4.6±0.1	5.8±0.1	5.4±0.2	***	***
CEC_r (cmol^{+}.kg^{-1})	32.5±0.9	45.4±1.7	27.2±2.1	30.3±1.9	***	***
OC (g C.kg^{-1})	58.7±1.9	58.3±2.5	44.3±2.0	42.1±2.0	***	NS
TN (g N.kg^{-1})	6.1±0.1	5.8±0.2	5.0±0.2	5.3±0.2	***	NS
P_{ava} (mg P.kg^{-1})	70±12	50±5	32±5	23±3	***	*

Level of significance for each factor in PERMANOVA (P_{man}), *p < 0.05; ***p < 0.001; NS = non-significant at 95%.

Regarding pH, highly significant differences were observed (p < 0.001), both among the varieties and in the seasonality, the lowest pH values appear in soils with the Catuaí variety in both climatic seasons (dry: 5.1±0.0 and rainy: 4.6±0.1), compared to soils of the Caturra variety in the dry (5.8±0.1) and rainy (5.4±0.2) seasons (Table 2) (the latter were limed at the time of establishment of the plot). In both soils that were cultivated with the two coffee varieties, the pH was observed to be lower in the rainy season. This supports the study by Dilly and Munch (1996) who found a drop and lower pH values in humid sites when compared to dry sites.

The CECr recorded significantly higher values in soils with the Catuaí variety and in the rainy season (45.4±1.7 cmol^{+}.kg^{-1}), compared to the Caturra

variety measured during the same time period (30.3±1.9 $cmol^{+}.kg^{-1}$), $p < 0.001$ (Table 2). These results can be explained by the higher content of OC in the soils cultivated with the Catuaí variety. Cardona and Sadeghian (2006) found similar CECr trends in coffee plantations under shade of *Inga* spp. which they attributed to a higher percentage of organic matter.

Regarding OC, significant differences were observed ($p < 0.01$), among the varieties, with higher values in soils with the Catuaí variety both in the dry season (58.7±1.9 $gC.kg^{-1}$) and in the rainy season (58.3±2.5 $gC.kg^{-1}$), compared to the Caturra variety in the dry (44.3±2 $gC.kg^{-1}$) and rainy (42.1±2 $gC.kg^{-1}$) seasons (Table 2). These results are possibly due to the fact that the plants of this variety have higher vegetative growth and greater establishment age (approximately 20 years) than the Caturra variety, whose plants are smaller and were planted approximately 8 years ago, which shows greater disturbance in soils cultivated with the Caturra variety. Studies carried out by Durango *et al.* (2015) found greater values of OC in coffee soils (57 $gC.kg^{-1}$), and soils with coffee–banana association (39 $gC.kg^{-1}$) from samples collected from 0 to 10 cm depth, in the Turrialba region of Costa Rica.

On the other hand, the TN and the P_{ava} registered higher values in soils with the Catuaí variety ($p < 0.001$). The values found for P_{ava} varied between 23±3 $mgP{\cdot}kg^{-1}$ corresponding to the rainy season of the soils cultivated with the Caturra variety, and 70±12 $mgP{\cdot}kg^{-1}$ in the dry season, for the soils cultivated with the Catuaí variety (Table 2). According to Cortés and Malagón (1983), these values are considered to be medium to high. The highest P_{ava} values were obtained in the dry season. For TN, the values varied between 5.0±0.2 $gN.kg^{-1}$ of soil corresponding to the dry season in the soils cultivated with the Caturra variety, and 6.1±0.1 $gN.kg^{-1}$ of soil obtained in the dry season in soils with the Catuaí variety (Table 2). These TN values are considered very high for agricultural soils (Rioja 2002). Similarly, they are higher than those observed by Durango *et al.* (2015), who reported values between 4.6 $gN.kg^{-1}$ and 4.3 $gN.kg^{-1}$ ultisols grown with coffee and coffee–banana, respectively, in Turrialba (Costa Rica). The highest TN content in soils cultivated with the Catuaí variety is probably related to the higher OC contents.

Dehydrogenase activity

DHA is an intracellular oxidoreductase that participates in the processes of oxidative phosphorylation of all soil microorganisms, and is thus a good indicator of the oxidative activities of the soil and in general its microbiological activity (García *et al.* 2003). According to the results obtained, the DHA did not show significant differences between coffee varieties, but differences were observed between the climatic seasons of sampling ($p < 0.001$), being greater in the rainy season for both soils. The DHA varied between 100.3±6 $\mu gINTF.g^{-1}ss.h^{-1}$ in the

dry season and 274.2±14 μgINTF.g^{-1}dw.h^{-1} in the rainy season, for soils with the Catuaí variety and between 114.4±9 to 281.4±13 μgINTF.g^{-1}dw.h^{-1} during dry and rainy seasons, respectively for soils with the Caturra variety (Table 3). The increase in dehydrogenase activity in the rainy season may be related to the increase in soil moisture which stimulates the proliferation of microorganisms (due to the greater availability of energy and carbon sources), since fallen leaves are left on the soil since the dry season (Arellano *et al.* 2004), caused by the hydric stress of both shade trees and coffee plants and the mechanical action of the winds, with which there is a greater availability of organic substrates for their degradation. Additionally, insofar as the soil is saturated with water, the proliferation of anaerobic microorganisms is favored, which are important contributors to the secretion of dehydrogenases (Brzezińska *et al.* 1998).

Table 3. Intracellular enzymes in soils cultivated with Catuaí and Caturra coffee varieties. DHA: dehydrogenase activity (DHA); FDA: hydrolysis of fluorescein diacetate activity (FDA); arginine deaminase activity (AD). (Mean ± standard error; n = 18).

Properties	Catuaí		Caturra		Variety	Climatic season
	Dry	Rainy	Dry	Rainy	P_{man}	P_{man}
DHA (μgINTF.g^{1}dw.h^{-1})	100.3±6.0	274.2±14	114.4±8.7	281.4±13	NS	***
FDA (μgFluoriscein.g^{1}dw.h^{1})	387.7±28.3	609.8±33.4	409.4±19.2	492.1±14.7	NS	***
AD (μgN-NH_4 g^{1}dw.h^{1})	8.9±0.9	6.4±0.5	12.0±1.0	7.0±0.5	*	***

Level of significance for each factor in PERMANOVA (P_{man}), *$p < 0.05$; ***$p < 0.001$; NS = non-significant at 95%.

The results of this study, with regard to the increase in dehydrogenase activity with a higher soil moisture content, concur with Quilchano and Marañón (2002) in a study on Mediterranean cork oak forests (*Quercus suber*) in Spain; with Sánchez-Arias *et al.*'s study (2010) on mangrove forests (*Rhizphora mangle*) in the Margarita Island (Venezuela) in both saline and hypersaline locations; and with Araújo *et al.*'s publication (2013) on native forests in recovery of Northeast Brazil. In contrast, Lammel *et al.* (2015), when comparing several management systems in coffee plantations in the south of the state of Minas Gerais (Brazil), did not detect significant differences between climatic seasons.

With respect to the literature on tropical soil, there is not much data available for DHA through the INT method; however, we can cite some, e.g. Paolini *et al.* (2010) with reference to semi-arid soils of Falcón state (Venezuela), who compared native vegetation with cultivated soils, finding greater DHA under native vegetation (87.9 μgINTF.g^{-1}dw.h^{-1}) and lower under water melon cultivation (22.3 μgINTF.g^{-1}dw.h^{-1}). Such results are associated with lower OC contents and, therefore, lower microbiota activity in semi-arid soils. Pajares *et al.* (2010)

found values between 0.59 and 1.25 μgINTF.g^{-1}dw.h^{-1} in degraded Acrisols of the Mexican transvolcanic axis in Michoacán, poor in organic matter and rich in clays. Henríquez *et al.* (2014), who analyzed 12 farms located in different parts of Costa Rica under different agronomic management and in different types of soils of the Inceptisol and Ultisol orders, taking samples from 0 to 20 cm depth, found that DHA varied from 0.13 to 4.46 μgINTF.g^{-1}dw.h^{-1} with an average of 1.17 μgINTF.g^{-1}dw.h^{-1}. These were very low values due to a greater depth of sampling and, in addition, they were collected in most cases in the fertilizer band. It is known that a high level of chemical fertilization negatively affects the activity of dehydrogenase (Rossel *et al.* 1997). All the works mentioned above showed lower values than the coffee-growing systems studied.

Activity of hydrolysis of fluorescein diacetate

FDA activity has been used as an indicator of the activity of soil hydrolytic enzymes, because it is hydrolyzed by a set of enzymes: proteases, lipases and esterases; therefore, it is considered a parameter that measures the heterotrophic activity of the soil (Gómez and Paolini 2006). Other researchers have found greater FDA activity with the increase in the organic matter content of the soil (Tripathy *et al.* 2014). In our study, it was observed that the FDA activity was higher in the rainy season for both soils, varying from 387.7±28 to 609.8±33 μgFluoriscein.g^{-1}dw.h^{-1} in soils with the Catuaí variety, and 409.4±19 to 492.1±15 μgFluoriscein.g^{-1}dw.h^{-1} in soils with the Caturra variety, showing significant differences with reference to climatic season ($p < 0.001$) (Table 3). Partelli *et al.* (2012), in Minas de Gerais (Brazil), did not find a clear trend when comparing FDA activity in conventional (chemical fertilization) and organic coffee plantations with respect to the climatic season (summer, hot and humid vs. winter, cold and dry). Although the organic system showed higher values (172±12 μgFluorisceing^{-1}dw.h^{-1}) when compared to the conventional one (136±7 μgFluoriscein.g^{-1}dw.h^{-1}), these values turned out to be lower than those in the present work. In a study conducted on savanna soils in the eastern Venezuelan plains by Gómez and Paolini (2008), it was found that FDA activity was significantly affected by seasonal variation ($p < 0.001$). Values ranged from 38.7 to 51.1 μgFluoriscein.g^{-1}dw.h^{-1} in the native savannah and from 40.8 to 44.7 μgFluoriscein.g^{-1}dw.h^{-1} under the cover of *Brachiaria brizantha*. The highest values were found in the rainy season. These values are much lower than those found in our study for coffee-growing soils. However, they coincide in the seasonal variation, observing the highest values in the rainy season, possibly due to a higher microbiota activity favored by soil moisture. Furthermore, Sánchez-Arias *et al.* (2010) found higher values in *Rhizophora mangrove* forests on Margarita Island (Venezuela) in the rainy season (440 and 516 μgFluoriscein.g^{-1}dw.h^{-1} for hypersaline and saline localities, respectively) compared to the

dry season (217 and 229 µgFluoriscein.g^{-1}dw.h^{-1} for hypersaline and saline localities, respectively). According to the literature, FDA enzyme values between 68 and 99 µgFluoriscein.g^{-1}dw.h^{-1} are reported in coffee plantations in Minas Gerais (Brazil), where different types of weed control (manual, mechanical and chemical) were performed between the lines and among coffee plants, but no differences were detected between treatments (Melloni *et al.* 2013). In another study conducted in Karnatakala (India), the differences among organic management (46 µgFluoriscein.g^{-1}dw.h^{-1}) and conventional management (34 µgFluoresceein.g^{-1}dw.h^{-1}) were detected. Both studies cited indicate lower values than the current study (Velmourougane 2016).

Activity of the enzyme arginine deaminase (arginine ammonification)

Ammonification is a process through which ammonia is released from nitrogen-containing organic compounds, which serve as a source of carbon and nitrogen that occurs within the cell (Alef and Kleiner 1986). The results obtained indicated that the activity of arginine deaminase (AD) showed significant differences ($p < 0.05$) at the variety level. The highest activity was found in the soils with the Caturra variety, both in the dry season (12.0±1.0 µgN-NH_4.g^{-1}dw. h^{-1}) and the rainy season (7.0±0.5 µgN-NH_4.g^{-1}dw.h^{-1}), compared to soils with the Catuaí variety in the dry season (8.9±0.9 µgN-NH_4.g^{-1}dw.h^{-1}) and the rainy season (6.4±0.5 µgN-NH_4.g^{-1}dw.h^{-1}). In addition, significant differences were found due to the climatic season ($p < 0.001$) (Table 3).

Regarding temporal variability, this enzyme is sensitive to changes in soil moisture as demonstrated by Gómez and Paolini (2006) in the Eastern Plains of Venezuela under native savanna vegetation of *Trachypogon* spp. (rain 1.7–3.7 µgN-NH_4.g^{-1}dw.h^{-1} and drought 1.0–1.1 µgN-NH_4.g^{-1}dw.h^{-1}) and for introduced pastures of *Brachiaria brizantha* and *Stylosanthes capitata* (rain 1.9–2.11 µgN-NH_4.g^{-1}dw h^{-1}; drought 1.4–1.6 µgN-NH_4.g^{-1}dw.h^{-1}). In their another study on the same subject, Gómez and Paolini (2011) evaluated the effect of livestock on AD in native and introduced savannas, and they also observed that this activity turned out to be greater in the rainy season (native savanna 3.4 µgN-NH_4 g^{-1}dw h^{-1} and for introduced pastures 4.6 µgN-NH_4.g^{-1}dw.h^{-1}) when compared to the dry season (native savannah 1.8 µgN-NH_4.g^{-1}dw.h^{-1} and for introduced pastures 1.9–2.1 µgN-NH_4.g^{-1}dw.h^{-1}). Moreover, Sánchez-Arias *et al*. (2010) confirmed the same pattern in *Rhizophora mangle* forests on Margarita Island (Venezuela) where the highest values were observed in the rainy season (7.3 and 5.1 µgN-NH_4.g^{-1}dw.h^{-1} for hypersaline and saline locations), compared to the dry season (4.1 and 2.5 µgN-NH_4 g^{-1}dw h^{-1} for hypersaline and saline locations, respectively). The values obtained from this study exceed those indicated by Romillac (2019) in a recent review which indicates that values between 1 to 5 µgN-NH_4.g^{-1}dw.h^{-1} are frequent in agricultural soils, although values close to 15 µgN-

$NH_4.g^{-1}dw.h^{-1}$ are usually found, for instance, under *Lupinus* vegetation of the legume genus. In Mexico, Pajares *et al.* (2010), in the study conducted in the Atécuaro basin, Michoacán state, examined agricultural soils of the order Ultisol (Acrisol) derived from volcanic ash and under different agronomic management (traditional, organic, mulch and fallow). The values were between 1.0 and 1.7 $\mu gN\text{-}NH_4.g^{-1}dw.h^{-1}$. The same authors conducted the study on in a volcanic origin soil toposequence located in the Transversal Neovolcanic Axis with grassland and oak pine vegetation, and reported values of 1.4 and 1.5 $\mu gN\text{-}NH_4.g^{-1}dw.h^{-1}$, respectively (Pajares *et al.* 2011).

CONCLUSIONS

1. The highest activities of the enzymes dehydrogenase and fluorescein diacetate hydrolysis were observed during the rainy season, for both soils cultivated with the Catuaí and Caturra coffee varieties. These results are related to the higher moisture content of the soil, which, in turn, stimulates the proliferation of microorganisms, due to the greater availability of energy and carbon sources during this period.
2. The activity of the enzyme arginine deaminase was sensitive to both the climatic period of sampling and the coffee variety. The highest levels of arginine deaminase activity were found in soils cultivated with the Caturra coffee variety during the dry season.
3. The soils cultivated with the Catuai variety showed the higher observed values of physicochemical properties associated with its higher organic matter content and its consequent mineralization, which indicates that this variety improved the physicochemical properties of the studied soils. The only physicochemical parameters that showed seasonal variations were pH, available phosphorus and cation exchange capacity.

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