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REGIMES

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# DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

## Comissão 2.1 - Biologia do solo

### MICROBIOLOGICAL PROPERTIES AND OXIDIZABLE ORGANIC CARBON FRACTIONS OF AN OXISOL UNDER COFFEE WITH SPLIT PHOSPHORUS APPLICATIONS AND IRRIGATION REGIMES<sup>(1)</sup>

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#### SUMMARY

Phosphorus fertilization and irrigation increase coffee production, but little is known about the effect of these practices on soil organic matter and soil microbiota in the Cerrado. The objective of this study was to evaluate the microbiological and oxidizable organic carbon fractions of a dystrophic Red Latossol under coffee and split phosphorus (P) applications and different irrigation regimes. The experiment was arranged in a randomized block design in a 3 x 2 factorial design with three split P applications (P1: 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, recommended for the crop year, of which two thirds were applied in September and the third part in December; P2: 600 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied at planting and then every two years, and P3: 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, the requirement for six years, applied at once at planting), two irrigation regimes (rainfed and year-round irrigation), with three replications. The layers 0-5 and 5-10 cm were sampled to determine microbial biomass carbon (MBC), basal respiration (BR), enzyme activity of acid phosphatase, the oxidizable organic carbon fractions (F1, F2, F3, and F4), and total organic carbon (TOC). The irrigation regimes increased the levels of MBC, microbial activity and acid phosphatase, TOC and oxidizable fractions of soil organic matter under coffee. In general, the form of dividing P had little influence on the soil microbial properties and OC. Only P3 under irrigation increased the levels of MBC and acid phosphatase activity.

**Index terms:** P cycling, soil microbial activity, acid phosphatase, soil organic matter.

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**RESUMO: ATRIBUTOS MICROBIOLÓGICOS E FRAÇÕES OXIDÁVEIS DO CARBONO ORGÂNICO DE LATOSSOLO CULTIVADO COM CAFEEIRO, SOB PARCELAMENTOS DE FÓSFORO E REGIMES HÍDRICOS**

*A adubação fosfatada e a irrigação aumentam a produtividade do café, mas pouco se sabe sobre o efeito dessas práticas na matéria orgânica e na microbiota de solos de Cerrado. O objetivo deste trabalho foi avaliar os atributos microbiológicos e as frações oxidáveis do carbono orgânico de um Latossolo Vermelho distrófico cultivado com café, sob parcelamentos de fósforo (P) e regimes hídricos. O delineamento experimental foi em blocos ao acaso em arranjo fatorial 3 x 2, com três parcelamentos de P (P1: 300 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>, recomendado para a cultura anualmente, sendo 2/3 aplicados em setembro e 1/3, em dezembro; P2: 600 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>, aplicado no plantio e a cada dois anos; e P3: 1.800 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>, aplicado somente no plantio, necessário para seis anos); dois regimes hídricos (sequeiro e irrigado durante todo o ano); e três repetições. A amostragem de solo foi feita nas camadas de 0-5 cm e 5-10 cm. Foram determinados o carbono microbiano (CBM), a respiração basal (RB), a atividade da enzima fosfatase ácida, as frações oxidáveis do carbono orgânico (F1, F2, F3 e F4) e o carbono orgânico total (COT). O regime hídrico irrigado do café aumentou os teores de CBM e a atividade microbiana da fosfatase ácida, do COT e das frações oxidáveis da matéria orgânica do solo. De maneira geral, a forma de parcelamento do P exerceu pouca influência sobre os atributos microbiológicos do CO do solo. Apenas no parcelamento P3, sob irrigação, obteve-se aumento dos teores de CBM e da atividade da fosfatase ácida.*

*Termos de indexação: ciclagem de P, atividade microbiana do solo, fosfatase ácida, matéria orgânica do solo.*

## INTRODUCTION

Ever since coffee (*Coffea arabica* L.) was introduced in Brazil, the crop has been highly relevant in agriculture and economy. In the marginal areas of expansion, where rainfall is insufficient or irregularly distributed throughout the year, as in the Cerrado, irrigation is necessary to ensure satisfactory yields (Fernandes et al., 2000; Coelho & Silva, 2005; Bonomo et al., 2008).

The chemical properties of the soils in this region are often limiting to the cultivation of most crops, e.g., by high acidity and low nutrient levels, especially of phosphorus (P) (Sousa et al., 2007). The P content in these soils is generally well below the critical level, around 8 mg dm<sup>-3</sup>, for clay soils (Sousa & Lobato, 2004; Reis et al., 2011). This limits crop growth, requiring the application of corrective fertilizer, in view of the high P demand of coffee for fruit production and the rapid initial growth (Nazareno et al., 2003).

In the Cerrado, the annual rainfall has a bimodal distribution, with a well-defined dry season between May and September, and dry spells, especially in January and February. To enhance the system of irrigated coffee production in the Cerrado, adjustments in crop management were proposed, by improving the irrigation management, applying controlled water stress to induce uniform flowering, and adjustments in the nutritional management of the crop (Guerra et al., 2007). These authors suggested an annual application of 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> in split doses, necessary due to the low P uptake efficiency of the coffee trees.

Thus, in an attempt to maximize P uptake, the supply in split doses is associated to the most demanding development stages of the crop (flowering, growth and grain filling), since, according to studies of Reis et al. (2011) and Silva et al. (2010), P application increases yield, dry matter production and growth of coffee plants.

Also in relation to P availability, soil microorganisms play a key role in the P cycle and availability to plants. The P flow is controlled by the microbial biomass, which solubilizes inorganic P, mineralizes organic P and associates plants and its mycorrhizal fungi (Plante, 2007). It is known that the lower the soil available P, the greater the reliance of the plant on organic P forms, including microbial biomass (Gatiboni et al., 2008). In this case, the importance of the activity of phosphatases in the soil is notable, since this enzyme is directly involved in the transformation of organic P to soluble P (Nahas, 2002).

There are several factors that influence the soil microbial activity and biomass and enzymatic activity, e.g., moisture (Gama-Rodrigues et al., 2005; Frazão et al., 2010), P concentration in soil (Ferreira et al., 2008), the soil layers (Figueiredo et al., 2007; Babujia et al., 2010), and soil organic matter (SOM) (Perez et al., 2004). There is, however, a lack of information about the behavior of microbiological and oxidizable SOM fractions under differentiated irrigation and P splitting systems.

In this sense, SOM plays a number of important roles in the soil, e.g., as substrate for the

establishment and activity of microorganisms, with consequences for the variation and availability of nutrients. When soils are cultivated, alterations are observed in the SOM quality, notably in the degree of oxidation and lability (Blair et al., 1995; Loss et al., 2009). Chan et al. (2001) proposed a modification of the classical method proposed by Walkley & Black (1934) to analyze the oxidizable fraction of soil organic carbon (OC) by separation of total organic carbon (TOC) in four fractions, based on the different degrees of oxidation, using increasing concentrations of sulfuric acid (F1, F2, F3, and F4).

The oxidizable organic carbon fractions, especially the most labile fractions represented by F1 and F2, are considered sensitive to impacts caused by soil management (Chan et al., 2001; Maia et al., 2007; Loss et al., 2009, 2010; Barreto et al., 2011). In addition, microbial biomass and soil enzymatic activity are also considered good indicators of the impacts caused by soil management (Perez et al., 2004; Nunes et al., 2009).

The purpose of this study was to evaluate the microbiological properties and oxidizable organic carbon fractions of a dystrophic Red Latossol under coffee and P applications and two irrigation regimes.

## MATERIAL AND METHODS

The study was carried out on an experimental field of Embrapa Cerrados, Planaltina, DF (latitude 15° 35' 30" S, longitude 47° 42' 30" W, 1,007 m asl). According to the Köppen classification, the climate is CWh1, the annual pluvial precipitation 1,460 mm and average annual temperature 21.3 °C. The soil was classified as a clayey dystrophic Red Latossol, with a moderate, 0.20 m thick A horizon.

Prior to the experiment, the chemical analysis of the soil (0-20 cm layer) showed: pH in water 5.2,  $\text{Al}^{3+}$  (4.3 mmol<sub>c</sub> dm<sup>-3</sup>);  $\text{Ca}^{2+}$  (22.9 mmol<sub>c</sub> dm<sup>-3</sup>);  $\text{Mg}^{2+}$  (8.3 mmol<sub>c</sub> dm<sup>-3</sup>); H+Al (76.0 mmol<sub>c</sub> dm<sup>-3</sup>); P (1.4 mg dm<sup>-3</sup>); K (61.2 mg dm<sup>-3</sup>); aluminum saturation (12 %). For particle-size analysis, the mean levels of clay, silt, and fine and coarse sand were 601, 116, 47 and 236 g kg<sup>-1</sup>, respectively, in the 0-20 cm layer.

Prior to the experiment, the area was covered by ungrazed pasture (*Brachiaria decumbens*). In December 2007, the experiment was initiated with the planting of coffee (*Coffea arabica* L. cv IAC144), spaced 3.50 m between rows and 0.70 m between plants. At planting, fertilization was applied as follows: 120 g triple superphosphate (TSP), 50 g magnesium thermophosphate (Yoorin®) and 24.5 g fritted trace elements (FTE) per planting hole. Base saturation was raised to 50 % by liming with 2 Mg ha<sup>-1</sup> dolomitic limestone, half of which was applied before plowing and half before harrowing.

In the year after planting, the trees received 61.25 g N per hole, as urea, corresponding to 136 g fertilizer. Similarly, 61.25 g K<sub>2</sub>O was applied as potassium chloride (KCl), corresponding to 102 g fertilizer per hole. In both cases, the total amounts were divided in four applications, between September and February. In the other years, annual doses of 272 g urea and at most 204 g KCl per hole were applied, divided in the same proportions; KCl varied according to the K soil reserve and was determined by chemical analysis. Micronutrients were fertilized, when necessary, via FTE. All fertilizations were applied by hand, under the tree canopy.

Then the experiment was set up in a randomized block design with three replications, in a 3 x 2 factorial arrangement with three P applications (P1, P2 and P3) and two irrigation regimes (R: rainfed and I: year-round irrigation).

The test consisted of experimental monitoring over a period of six years, after which the same amount of P had been applied in all treatments. Phosphorus fertilization was performed as defined for each treatment, beginning in the second year after planting. Treatments were based on a basic annual requirement of 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, corresponding to treatment P1 (annual application of 117 g TSP per hole, 78 g (2/3) in September, and 39 g (1/3) in December). In treatment P2, 600 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> was applied, corresponding to two basic fertilization requirements in a single application, equivalent to 233 g TSP per hole in September, which was repeated every two years. In treatment P3, 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> was applied at once to complete fertilization for six years of assessment, corresponding to 700 g TSP per hole. Thus, at the time of soil sampling, the treatments P1 and P2 had received 33.3 % and P3 100 % of the P scheduled for six years.

The irrigation regimes were regulated by a center pivot sprinkler, based on the monitoring of the water content in soil (ML1 probes Delta-T Devices). Irrigation was always applied when the soil moisture at a depth of 0.10 m corresponded to the consumption of 50 % of the available water.

Soil from the 0-5 and 5-10 cm layers under the coffee tree canopies was sampled in April 2011, when the trees were three years and four months old, in the fruiting period. Ten subsamples per plot were combined to form a composite sample of each soil layer, irrigation regime and split P application. Then the samples were ground, packed in plastic bags and stored at 4 °C in a refrigerator for about 15 days, until analysis.

Microbial biomass carbon (MBC) was determined by the fumigation-extraction method, as described by Vance et al. (1987), using a K<sub>EC</sub> factor of 3.8. Basal respiration (BR) was determined in the pre-incubation period when MBC was determined by the measurement of the CO<sub>2</sub> released from the no fumigated samples over the course of seven days (Alef



& Nannipieri, 1995). The metabolic quotient ( $qCO_2$ ) was calculated as the ratio of MBC by basal respiration (Anderson & Domsch, 1993) and the microbial quotient ( $qMic$ ) was determined as the ratio of MBC by total organic carbon. The acid phosphatase activity was determined according to Tabatabai (1994), based on the colorimetric determination of p-nitrophenol released from the action of phosphatases, after soil incubation in a buffered solution of  $0.05 \text{ mol L}^{-1}$  p-nitrophenyl phosphate.

For the chemical analysis, the soil was air-dried and sieved (2 mm). A subsample was sieved through 0.5 mm mesh to determine the oxidizable fractions of OC, subjected to C fractionation by degrees of oxidation, according to adaptations of Chan et al. (2001) and Mendonça & Matos (2005). The fractionation resulted in four fractions, with decreasing oxidation degrees: Fraction 1 (F1): C oxidized by  $0.167 \text{ mol L}^{-1} K_2Cr_2O_7$  in acidic medium with  $3 \text{ mol L}^{-1} H_2SO_4$ ; Fraction 2 (F2): the difference between C oxidized by  $0.167 \text{ mol L}^{-1} K_2Cr_2O_7$  in acidic medium with 6 and  $3 \text{ mol L}^{-1} H_2SO_4$ ; Fraction 3 (F3): the difference between C oxidized by  $0.167 \text{ mol L}^{-1} K_2Cr_2O_7$  in acidic medium with 9 and  $6 \text{ mol L}^{-1}$  of  $H_2SO_4$ ; fraction (F4): difference between C oxidized by  $0.167 \text{ mol L}^{-1} K_2Cr_2O_7$  in acidic medium with 12 and  $9 \text{ mol L}^{-1} H_2SO_4$ .

Data were subjected to analysis of variance (ANOVA) and means were compared by Tukey's test ( $p < 0.05$ ), using the statistical program SISVAR (Ferreira, 2003). In addition, the Pearson linear correlation was analyzed, grouping the individual data of split P applications, layers and irrigation regimes.

The data of all variables together were subjected to principal component analysis (PCA), based on linear combinations of the original variables on independent orthogonal axes. This analysis was performed to identify which factors (irrigation regime and P splitting) interfere most with the grouping of the variables (MBC, BR,  $qCO_2$ ,  $qMic$ , acid phosphatase, and oxidizable fractions). Statistical analyses were performed using software XLSTAT 2011.

## RESULTS AND DISCUSSION

The F values of analysis of variance for the effects of factors split P applications and irrigation regimes were significant and are shown with their interactions with the microbial and oxidizable OC fractions in both soil layers in table 1. No effect of the factor on the irrigation regime properties was verified, except for  $qCO_2$  and  $qMic$ .

### Microbiological properties

There was significant interaction between the effects of P splitting and the irrigation regime for MBC and acid phosphatase (Table 2). Regardless of the form of split P applications, the acid phosphatase activity

was always higher under irrigation than in the rainfed system, in both soil layers. Carneiro et al. (2004) found no difference in phosphatase activity between dry and rainy seasons in the Cerrado. However, differences in enzyme activity between management systems were identified in the rainy season only, indicating that water is an important factor for phosphatase activity. Nunes et al. (2009) assessed the effects of coffee monoculture on microbiological indicators of soil quality and observed that, in periods of higher water availability, acid phosphatase activity was stimulated, while in the driest time of year (July), the activity of this enzyme was drastically reduced. Also, phosphatase activity is higher in fertile soil with greater plant diversity, and with little or no soil disturbance (Sandoval-Pérez et al., 2009).

The different P splitting did not alter the phosphatase activity in the rainfed system in both soil layers. Conte et al. (2002) found that this enzyme activity was not influenced by higher P availability in the soil. However, in this study, in P3 under irrigation, greater phosphatase activity was stimulated, reaching higher levels than the other forms of P splitting in both soil layers.

In the 0-5 cm layer, irrigation promoted higher MBC values in treatment P3. Moreover, in the 5-10 cm layer, irrigation led to higher MBC values in all treatments of P splitting. The application of  $1,800 \text{ kg ha}^{-1} P_2O_5$  at once resulted in higher MBC under irrigation than in the rainfed regime, promoting a 2.3 and 1.87-fold increase in the layers 0-5 and 5-10 cm, respectively. The greater water availability increases microbial biomass (Nunes et al., 2009; Frazão et al., 2010), which responds intensely to the seasonal fluctuations of humidity (Gama-Rodrigues et al., 2005), and influences C cycling and nutrients.

It is known that rainfall, be it natural or artificial (via irrigation), is one of the factors that controls SOM decomposition, and consequently the microbial activity. Increased water availability leads to greater biomass production in coffee (Silva et al., 2008), to a greater accumulation of organic matter in the soil and therefore to an increased action of microorganisms, by the greater amount of available substrate, in the processes of nutrient mineralization and immobilization (Wardle, 1998). Freitas et al. (2009) also reported higher microbial carbon in soil with higher moisture (31.55 %), than in the dry season (21.74 %) and suggested that, under these conditions, the microbial abundance in the soil is greater.

The microorganisms and enzymes in the soil involved in P cycling have a dynamic activity. The microorganisms absorb and immobilize P in the soil solution, when the availability is higher in the soil-plant system, but gradually release P by adjusting the microbial population to the energy and P supply in the system (Conte et al., 2002; Martinazzo et al., 2007). This may have occurred in this study; the available P in the soil solution may have been

**Table 1. F value and significance of the analysis of variance of the effects of the factors and their interactions on microbial biomass carbon (MBC), basal respiration (BR), metabolic quotient ( $qCO_2$ ), total organic carbon (TOC), microbial quotient ( $qMic$ ) and acid phosphatase of soil under split P applications and irrigation regimes and their interactions, in the layers 0-5 and 5-10 cm**

SV	DF	MBC	BR	$qCO_2$	TOC	$qMIC$	Phosphatase
0-5 cm							
Split P application (P)	2	1.35 <sup>ns</sup>	0.331 <sup>ns</sup>	1.004 <sup>ns</sup>	10.069 <sup>**</sup>	2.093 <sup>ns</sup>	3.304 <sup>ns</sup>
Irrigation regime (IR)	1	20.81 <sup>**</sup>	8.403 <sup>*</sup>	0.639 <sup>ns</sup>	325.19 <sup>**</sup>	0.594 <sup>ns</sup>	115.64 <sup>**</sup>
P x IR	2	4.346 <sup>*</sup>	0.138 <sup>ns</sup>	0.237 <sup>ns</sup>	3.508 <sup>ns</sup>	2.414 <sup>ns</sup>	9.413 <sup>**</sup>
5-10 cm							
Split P application (P)	2	20.49 <sup>**</sup>	1.599 <sup>ns</sup>	8.983 <sup>**</sup>	2.074 <sup>ns</sup>	16.771 <sup>**</sup>	7.015 <sup>*</sup>
Irrigation regime (IR)	1	46.87 <sup>**</sup>	5.404 <sup>*</sup>	4.495 <sup>ns</sup>	155.41 <sup>**</sup>	0.768 <sup>ns</sup>	253.50 <sup>**</sup>
P x IR	2	4.141 <sup>*</sup>	6.683 <sup>*</sup>	5.273 <sup>*</sup>	0.251 <sup>ns</sup>	0.993 <sup>ns</sup>	6.851 <sup>*</sup>
		F1	F2	F3	F4	F1+F2	F3+F4
0-5 cm							
Split P application (P)	2	0.252 <sup>ns</sup>	0.904 <sup>ns</sup>	5.657 <sup>*</sup>	1.943 <sup>ns</sup>	1.121 <sup>ns</sup>	9.935 <sup>**</sup>
Irrigation regime (IR)	1	23.53 <sup>**</sup>	78.23 <sup>**</sup>	11.72 <sup>**</sup>	5.465 <sup>*</sup>	234.01 <sup>**</sup>	23.596 <sup>**</sup>
P x IR	2	4.172 <sup>*</sup>	1.989 <sup>ns</sup>	0.994 <sup>ns</sup>	1.734 <sup>ns</sup>	0.238 <sup>ns</sup>	1.610 <sup>ns</sup>
5-10 cm							
Split P application (P)	2	1.373 <sup>ns</sup>	10.33 <sup>**</sup>	30.21 <sup>**</sup>	0.441 <sup>ns</sup>	4.342 <sup>*</sup>	6.316 <sup>*</sup>
Irrigation regime (IR)	1	20.06 <sup>**</sup>	54.29 <sup>**</sup>	60.69 <sup>**</sup>	0.004 <sup>ns</sup>	136.97 <sup>**</sup>	18.051 <sup>**</sup>
P x IR	2	1.204 <sup>ns</sup>	1.410 <sup>ns</sup>	3.829 <sup>ns</sup>	0.084 <sup>ns</sup>	1.677 <sup>ns</sup>	1.638 <sup>ns</sup>

SV: source of variation; DF: degrees of freedom; P x IR: interaction between P splitting and irrigation regime (rainfed and irrigated); \*, \*\*: significant at 5 and 1 %, respectively, by the F test; <sup>ns</sup> : non-significant.

**Table 2. Microbial carbon (MBC) and acid phosphatase of a dystrophic Red Latossol, under split P applications and irrigation regimes, in two layers**

Split P application	MBC		Acid phosphatase	
	S	I	S	I
	mg C kg <sup>-1</sup> soil		µg p-nitrofenol g <sup>-1</sup> dry soil h <sup>-1</sup>	
			0-5 cm	
P1	122.81 Aa	149.29 Aa	205.95 Ba	412.58 Ab
P2	95.67 Aa	130.32 Aa	220.40 Ba	379.05 Ab
P3	78.91 Ba	186.06 Aa	170.41 Ba	566.14 Aa
			5-10 cm	
P1	74.95 Ba	108.27 Ab	178.35 Ba	361.94 Ab
P2	59.45 Ba	99.93 Ab	202.14 Ba	416.77 Ab
P3	94.92 Ba	178.05 Aa	181.73 Ba	494.61 Aa

S: rainfed. I: irrigated. P1: 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied annually, 2/3 of the total amount in September and 1/3 in December. P2: 600 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, reapplication every two years. P3: 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied only at planting. Means followed by the same small-case letters in the column and same capital letters in the row, for each microbial property, did not differ from each other by Tukey's test (p<0.05).

immobilized and at this stage (three years and four months after soil P application), this nutrient may have been released gradually through increased phosphatase enzyme activity, as well as the higher MBC concentration, bearing in mind that, for this same P dose (1,800 kg ha<sup>-1</sup>), MBC was also higher than in the other P treatments in the 5-10 cm layer.

There was no difference between P fertilization in the 0-5 cm layer, but in 5-10 cm, the irrigation system fertilized with a single application of 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> resulted in higher MBC (178.05 mg C kg<sup>-1</sup> soil). Possibly, the P dose of P3 may have reached deeper layers and may therefore have stimulated the root system development and consequently, microbial biomass. Faria & Pereira (1993) studied the movement

of P after the application of 150 and 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> to the surface of five different soils and found that P moved down through the 4-6 and 6-8 cm layers in more clayey soils, and down to 14-16 cm in sandy soils. In a study on sugar cane, Bezerra et al. (2008) evaluated the microbial activity in the 0-20 cm layer of a cohesive dystrophic Oxisol, in the Coastal Plain region of Alagoas, fertilized with P rates, and concluded that MBC was highest at the highest P doses.

The water system also affected BR in both soil layers, i.e., irrigation increased the soil microbial activity (Table 3). As observed for phosphatase, greater water availability increases microbial activity, corroborating results of other studies (Nunes et al., 2009; Frazão et al., 2010). Also in relation to BR, no effect of P splitting on microbial activity was observed in either layer.

The microbial quotient showed the same trend as MBC, with higher values in P3 in the layer 5-10 cm (Table 3). However, qMic was always below 1 %, as found by Nunes et al. (2009) in 16 and 22 year-old coffee. According to Jenkinson & Lass (1981), MBC represents 1-4 % of TOC and when this ratio is below 1 %, the presence of some limiting factor to the activity of microbial biomass is presumed (Jakelaitis et al., 2008), due possibly to the quality of crop residues and/or the short experimental period.

An opposite behavior to other microbiological properties was found for qCO<sub>2</sub>, with lowest value in the irrigation system in the 5-10 cm layer, and no significant differences in the surface layer (0-5 cm). In P splitting, qCO<sub>2</sub> was similar in the 0-5 cm layer and highest in P2, in the 5-10 cm layer.

### Oxidizable organic carbon fractions

The TOC and oxidizable fractions of soil organic matter under different split P applications and irrigation regimes are listed in table 4. The values for the different oxidizable fractions are within the range of values published elsewhere in studies with different soils under different uses (Rangel et al., 2008; Loss et al., 2010; Barreto et al., 2011). With the exception of F4, in the 5-10 cm layer, irrigation resulted in higher contents of TOC and of the fractions in both layers. The effect of water on the increase in TOC and the different fractions may be due to the higher amount of organic material promoted by irrigation in dry periods in the Cerrado. As stated by Nazareno et al. (2003), irrigation in this region increases the biomass production of coffee, leading to a greater OC input into the soil.

The P3 split application led to higher TOC levels in the 0-5 cm layer, with no significant differences in the 5-10 cm layer. This treatment also increased the OC content in stabilized or more recalcitrant SOM fractions (F3+F4) than P1 and did not differ from P2, in both layers. The high amount of P applied at once (1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) at the beginning of the experiment may have induced a higher humification rate of SOM in the four experimental years, transforming labile to stabilized SOM. In P3, TOC consists of parts of balanced oxidizable fractions of SOM (F1+F2, 52 % of TOC and F3+F4, 48 % of TOC in both layers) tending to reach a balance. Loss et al. (2009) suggested that it would be interesting to use a management system tending to establish the same proportions between the C fractions, with one part of the organic matter being easily decomposable for nutrient mineralization and

**Table 3. Basal respiration (BR), metabolic quotient (qCO<sub>2</sub>) and microbial quotient (qMic) in soil under coffee with two irrigation regimes (rainfed and irrigated) in two layers**

Split P application	BR	qCO <sub>2</sub>	qMic
	mg C kg <sup>-1</sup> soil day <sup>-1</sup>	mg C-CO <sub>2</sub> mg <sup>-1</sup> MBC day <sup>-1</sup>	%
		0-5 cm	
P1	8.17 a	0.063 a	0.69 a
P2	8.73 a	0.085 a	0.53 a
P3	7.70 a	0.065 a	0.54 a
Irrigation regime			
Rainfed	6.70 b	0.076 a	0.61 a
Irrigated	9.70 a	0.065 a	0.56 a
		5-10 cm	
P1	7.03 a	0.078 b	0.46 b
P2	9.06 a	0.128 a	0.36 b
P3	8.22 a	0.060 b	0.62 a
Irrigation regime			
Rainfed	7.02 b	0.103 a	0.46 a
Irrigated	9.19 a	0.074 b	0.49 a

P1: 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied annually, 2/3 of the total amount in September and 1/3 in December. P2: 600 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, reapplication every two years. P3: 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied only at planting. Means followed by the same small letters in the column did not differ from each other by Tukey's test (p<0.05).

**Table 4. Carbon fractions of oxidizable organic matter and total organic carbon (TOC) in soil under coffee with irrigation regimes (rainfed and irrigated) and split P applications in two layers**

Split P application	TOC	F1	F2	F3	F4	F1+F2	F3+F4
g kg <sup>-1</sup>							
0-5 cm							
P1	20.55 b	6.45 a	6.90 a	5.70 b	1.50 a	13.35 a	7.20 b
P2	21.83 b	6.65 a	5.91 a	7.60 ab	1.67 a	12.56 a	9.27 ab
P3	23.96 a	6.25 a	6.31 a	8.82 a	2.56 a	12.56 a	11.38 a
Irrigation regime							
Rainfed	16.50 b	5.33 b	3.72 b	6.06 b	1.35 b	9.05 b	7.42 b
Irrigated	27.74 a	7.57 a	9.03 a	8.68 a	2.46 a	16.60 a	11.14 a
5-10 cm							
P1	20.1 a	6.53 a	6.25 a	5.78 b	1.58 a	12.78 a	7.37 b
P2	21.8 a	6.60 a	6.35 a	6.83 b	1.98 a	12.95 a	8.82 ab
P3	21.7 a	7.45 a	3.80 b	9.35 a	1.13 a	11.25 a	10.48 a
Irrigation regime							
Rainfed	16.6 b	5.73 b	3.55 b	5.82 b	1.54 a	9.29 b	7.37 b
Irrigated	25.8 a	7.98 a	7.38 a	8.82 a	1.58 a	15.37 a	10.41 a

P1: 300 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied annually, 2/3 of the total amount in September and 1/3 in December. P2: 600 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, reapplied every two years. P3: 1,800 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, applied only at planting. Means followed by the same small-case letter in the column do not differ by Tukey's test (p<0.05).

the other more resistant part as reserve in the soil to improve and/or maintain the soil physical properties.

There was no effect of P splitting on the labile OC fractions (F1+F2). In general, the results of research with fractions of OC oxidation show that management systems and/or crops that favor frequent additions of organic material to the soil tend to have a higher proportion of C in this fraction at the expense of the more recalcitrant fractions (F3 and F4) (Chan et al., 2001; Andrade et al., 2005; Rangel et al., 2008; Loss et al., 2010; Barreto et al., 2011).

Two principal components (PC1 and PC2) were generated, whose loads in relation to the various variables associated with the principal components are presented in table 5. These components were created as tools to discriminate the effects of irrigation and rainfed systems, considering the microbial and oxidizable fractions of SOM (MBC, BR, qMic, qCO<sub>2</sub>, acid phosphatase, F1, F2, F3, F4, F1+F2 and F3+F4) together for the layers 0-5 cm (Figure 1a) and 5-10 cm (Figure 1b).

Similar behaviors were observed in both layers. The distribution of selected variables showed cumulative variance of 73.41 and 71.66 % for the sum of the principal components PC1 and PC2 in the layers 0-5 and 5-10 cm, respectively. For both layers, the PC1 axis separated two groups: irrigated and rainfed, corroborating the results for the absolute variables, as discussed above. This means that, considering all properties together, the factor irrigation regime has a dominant effect, forming distinct environments for microbial survival and activity, and for the accumulation of TOC and the different oxidizable fractions.

**Table 5. Loads of the different variables associated with the principal component (PC) of soils under irrigation regimes and split P applications**

Variable	PC1	PC2	PC3
0-5 cm			
MBC	0.721	0.626	0.121
BR	0.626	-0.115	0.731
qCO <sub>2</sub>	-0.250	-0.820	0.437
TOC	0.986	-0.111	-0.069
qMic	-0.083	0.912	0.219
Acid phosphatase	0.967	0.121	-0.011
F1	0.682	-0.216	0.363
F2	0.845	0.261	-0.051
F3	0.709	-0.383	-0.064
F4	0.627	-0.189	-0.585
F1+F2	0.907	0.110	0.107
F3+F4	0.801	-0.373	-0.283
5-10 cm			
MBC	0.877	0.406	-0.105
BR	0.507	-0.308	-0.666
qCO <sub>2</sub>	-0.385	-0.626	-0.502
TOC	0.934	-0.290	0.201
qMic	0.407	0.833	-0.246
Acid phosphatase	0.956	-0.143	-0.095
F1	0.839	-0.058	0.094
F2	0.485	-0.734	0.076
F3	0.882	0.315	-0.171
F4	-0.027	-0.143	0.790
F1+F2	0.753	-0.579	0.101
F3+F4	0.832	0.225	0.264

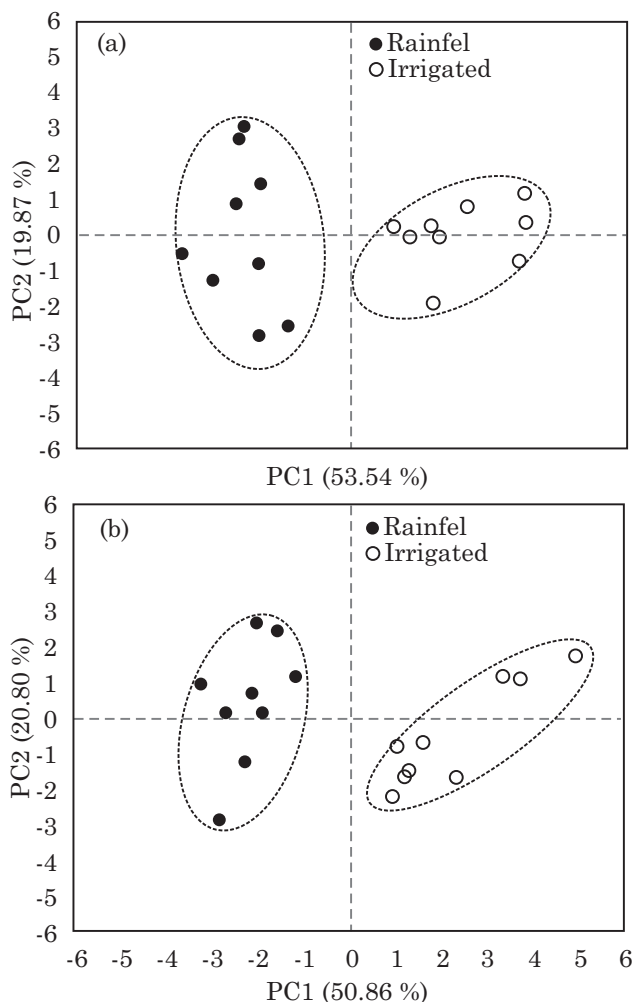


In both layers, as represented by the presence in quadrants in the diagram, MBC and  $qCO_2$  behaved conversely in the soil, under the conditions studied (Figure 2a,b). Moreover, the direction of the vector indicated that of all properties,  $qCO_2$  occupies the quadrant most closely related to the rainfed system (Figure 1a,b), indicating that for rainfed coffee grown in the Cerrado, the soil represents a stressful environment in terms of C use by microorganisms.

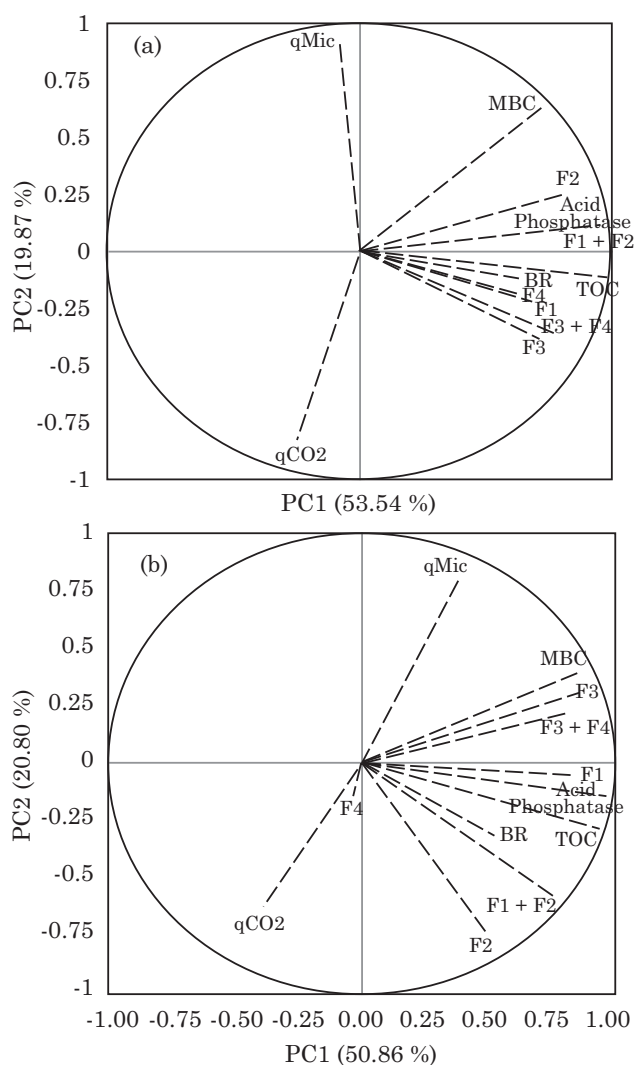
The correlations of the more labile SOM fractions (F1, F2 and F3) with MBC, BR and acid phosphatase were positive and highly significant (Table 6), demonstrating the close relationship between OC and the activity of soil microorganisms (Cunha et al., 2011; Araújo et al., 2007), especially of the most easily oxidizable fractions of organic matter. According to Gama-Rodrigues & Gama-Rodrigues (2008), microbial biomass represents the labile SOM fractions that are dynamic and easily influenced by biotic and abiotic

factors, which is why microbiological variables are closely related to the more bioavailable SOM fractions.

Acid phosphatase was also significantly positively correlated with MBC (0.75), BR (0.58) and TOC (0.92), reflecting the contribution of microorganisms to the activity of this enzyme. Acosta-Martínez et al. (2007) and Balota et al. (2004) also found a significant positive correlation between SOM and acid phosphatase activity. Wang et al. (2010) observed a strong positive correlation between phosphatase activity and TOC (0.72) and MBC (0.68). These authors also verified an increase of phosphatase activity with increasing soil C, suggesting that C was a limiting factor for P mineralization in waterlogged soils. According to Tabatabai (1994), the enzymatic activity is generally correlated with the SOM content, for playing a key role as a precursor of enzymatic synthesis (such as



**Figure 1. Ordination diagram derived from principal component analysis of the scores of treatments under split P applications and irrigation regimes: (a) 0-5 cm, (b) 5-10 cm.**



**Figure 2. Ordination diagram derived from the principal component analysis of microbial and soil organic carbon under split P applications and irrigation regimes: (a) 0-5 cm, (b) 5-10 cm.**

**Table 6. Pearson bivariate correlation between variables of a dystrophic Red Latossol under coffee with three split P applications, two irrigation regimes and two soil layers**

	MBC	BR	qCO <sub>2</sub>	TOC	qMic	Phos.	F1	F2	F3	F4	F1+F2	F3+F4
MBC	1.00	0.42**	-0.55**	0.63***	0.69***	0.75***	0.42**	0.47**	0.59***	0.12 <sup>ns</sup>	0.54**	0.55***
BR	-	1.00	0.41*	0.49**	0.08 <sup>ns</sup>	0.58***	0.49**	0.34*	0.47**	-0.11 <sup>ns</sup>	0.47**	0.35*
qCO <sub>2</sub>	-	-	1.00	-0.25 <sup>ns</sup>	-0.54**	-0.27 <sup>ns</sup>	-0.13 <sup>ns</sup>	-0.16 <sup>ns</sup>	-0.22 <sup>ns</sup>	-0.15 <sup>ns</sup>	-0.18 <sup>ns</sup>	-0.25 <sup>ns</sup>
TOC	-	-	-	1.00	-0.08 <sup>ns</sup>	0.92***	0.71***	0.78***	0.71***	0.43**	0.90***	0.81***
qMic	-	-	-	-	1.00	0.135 <sup>ns</sup>	-0.101 <sup>ns</sup>	-0.04 <sup>ns</sup>	0.05 <sup>ns</sup>	-0.24 <sup>ns</sup>	-0.07 <sup>ns</sup>	-0.07
Phos.	-	-	-	-	-	1.00	0.63***	0.76***	0.72***	0.26 <sup>ns</sup>	0.84***	0.73***
F1	-	-	-	-	-	-	1.00	0.36*	0.48**	0.17 <sup>ns</sup>	0.70***	0.49**
F2	-	-	-	-	-	-	-	1.00	0.27 <sup>ns</sup>	0.23 <sup>ns</sup>	0.92***	0.34*
F3	-	-	-	-	-	-	-	-	1.00	0.07 <sup>ns</sup>	0.42*	0.89***
F4	-	-	-	-	-	-	-	-	-	1.00	0.25 <sup>ns</sup>	0.53**
F1+F2	-	-	-	-	-	-	-	-	-	-	1.00	0.47**
F3+F4	-	-	-	-	-	-	-	-	-	-	-	1.00

MBC: microbial carbon; BR: basal respiration; qCO<sub>2</sub>: metabolic quotient; TOC: total organic carbon; qMic: microbial quotient; Phos: Acid phosphatase; F1, F2, F3, and F4 oxidizable fractions of organic matter. \*, \*\* and \*\*\* significant at 5, 1 and 0.1 %, respectively; <sup>ns</sup>: non-significant.

increased microbial biomass) and their physical stabilization.

In general, the irrigation regime raised the levels of SOC (total and in the different fractions), consequently forming a favorable environment for an increase in microbial biomass and activity in the soil. The form of P splitting had little influence on the microbiological properties and components of SOC. However, P3 splitting, under irrigation, increased MBC levels and acid phosphatase activity.

## CONCLUSIONS

1. Irrigation of the coffee produced in the Cerrado increased the levels of microbial carbon, microbial activity and acid phosphatase, total organic carbon and oxidizable organic matter fractions.

2. In general, the form of splitting P applications had little influence on the microbial and organic carbon in soil. Only treatment P3 under irrigation increased the levels of MBC and acid phosphatase activity.

3. Regardless of the soil layer, the irrigation regime and P splitting, the interaction between the microbiological properties with the levels of total organic carbon and the more easily oxidizable fractions of soil organic matter was strong.

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