



## **Biological Attributes of the Soil with Intercaled Crops and Oil Palm (*Elaeis guineensis* Jacq.): Sustainable Management in the Amazon**

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### **Authors' contributions**

*This work was carried out in collaboration between all the authors. The authors SASM, ACCC, VFM, RHSS and SCPU designed the study, statistical analysis, drafting of the protocol and first draft of the manuscript. The authors SASM, IMF and AAMF managed the study analyzes, bibliographic searches and revision of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** In the Brazilian Amazon, the practices and use of agroforestry systems (SAF) are increasingly used, the proper management of them helps to improve soil properties and also to prevent their degradation. In this context, the objective of this work was to evaluate the biological attributes of the soil in the oil palm cultivation systems with intercropping.

**Study Design:** The experimental design used was completely randomized with four repetitions and six treatments: Oil palm (*Elaeis guineense* Jacq.) interspersed with pineapple (OPi), bean (OBe), banana (OBa), yucca (OYu) and *Brachiaria humidicola* (OPa), as well as adjacent area only with *Brachiaria humidicola* as a witness (Pa). The Tukey test was used at a level of 5% probability in samples analyzed at a depth of 0-0.0 m, to compare the means of the variables evaluated.

**Place and Duration of Study:** The experimental area is located of São João da Baliza, vicinal 26, km 12, with geographic coordinates of reference 00°51'13.3"N and 60°00'19.8"W, the altitude of 100 msnm and, distant to 352 km from the capital Boa Vista, state of Roraima realized in 2016.

**Results:** The TOC presented values between 4.70 and 9.45 g kg<sup>-1</sup>, being the highest values found in the interim systems OYu, Pa, OBa, highlighting the intermediate system OPi that presented the lowest levels. The highest basal respiration values of the soil (RBS) (23.50 mg C-CO<sub>2</sub> kg<sup>-1</sup> soil h<sup>-1</sup>) and carbon from microbial biomass (C-BMS) (116.0 mg C microbiano kg<sup>-1</sup> soil) were verified in the pasture system. Likewise, for the urease and acid phosphatase activity, the grass system stands out as a control with values of (148.42 g NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil 2 h<sup>-1</sup>) y (230 µg de p-nitrofenol g<sup>-1</sup> soil h<sup>-1</sup>) followed by palm with grass and yucca systems. However, the β-glucosidase activity (51.22 µg p-nitrofenol g<sup>-1</sup> h<sup>-1</sup>) it was positively influenced by the oil palm system with yucca. On the other hand, the system interspersed with pineapple showed a higher metabolic coefficient (qCO<sub>2</sub>) (0.36 mg C-CO<sub>2</sub> g<sup>-1</sup> C-BMS h<sup>-1</sup>). It can be concluded that the pasture system (Pa) is presented as a more stable environment, followed by interspersed systems of oil palm with grass (OPa) and yucca (OYu).

*Keywords: Agroforestry systems; family farming; soil biology; enzymatic activity.*

## 1. INTRODUCTION

The Brazilian Amazon region is characterized by environments with natural forest and areas in agricultural use [1], intense deforestation and conversion of forests to pasture areas [2-3]. The degradation of these systems (natural or anthropic) breaks a natural, dynamic and balanced cycle, with a consequence on the chemical, physical and biological attributes of the soils [4].

In this context, the agroforestry systems (SAF) the arboreal component helps to modify the soil environment, since in its constitution there is a greater diversity of root systems which provides greater contributions in the organic matter contents (OM) [5-6]. The deposition of the litter generated by the fall of leaves, stems and crop residues, appear as sources and maintenance of the organic matter in the soil [7-8], with subsequent availability and nutrient uptake by plants, especially in tropical soils with low natural fertility [9].

Several biological indicators can be recommended to assess changes in ecosystems and to sustain soil biological quality [10]. Of

these, the carbon of the microbial biomass (C-BMS) [11], basal breathing [12], the metabolic quotient (qCO<sub>2</sub>) and, microbial quotient (qCmic) derived from BMS [13-14], and, the enzymatic activity of the soil [15]. The BMS is one of the key components in soil, with functions in the decomposition of organic residues [16], defined as the living part of the soil organic matter [17], controlling the flows of C and N [18], this microbial biomass contributes, on average, 2 to 5% of the organic carbon in the soil [19] and between 1 to 5% of the total N of the soil [20-21], serving as a source of nutrients and energy intake [22].

Other indicators such as basal soil respiration [12], related to the degradation capacity of OM [23], bacteria and fungi are the major responsibility for the release of CO<sub>2</sub> via degradation of OM, in addition to algae, protozoa and, root respiration [24-25]. Related to the degradation capacity of OM [23], bacteria and fungi are the major responsible for CO<sub>2</sub> release via degradation of OM, as well as algae, protozoa and, root respiration [24-25].

The metabolic quotient (qCO<sub>2</sub>) [13-14] refers to the basal respiration of CO<sub>2</sub> incorporated per

gram of microbial biomass in a given time, being important in the studies that evaluate the effect of the environmental and anthropogenic conditions on the microbial activity of the soil [26], and may be a good indicator of stress in crop management when C-BMS is affected, as it infers in carbon gains, estimating the efficiency of substrate use by soil microorganisms [27-28]. In addition, the microbial quotient, providing information on the quality of the organic matter and the amount of carbon immobilized in the microbial biomass [14-29].

The BMS transformations are mediated and catalyzed by enzymatic processes [30] direct mediators in the functioning and catabolism biology of microorganisms [31].

In the decomposition of organic residues, the enzyme  $\beta$ -Glucosidase is associated with the breakdown of cellobiose, involved in the C cycle [32] the urease to the urea break in the N cycle, thus acting both in N and C of BMS [33] and phosphatases important in the phosphorus cycle, as they hydrolyze and transform organic P compounds into different inorganic P compounds, in the form of  $\text{PO}_4$  of phosphoric esters [34-35].

The use of soil management studies using associated systems is a technique that is currently used with a boom to prevent soil degradation and at the same time prevent soil deterioration, being the biological indicators of soil used as a result of management. Equally, the product obtained from the palm-oil is considered as a suitable raw material for biodiesel production [36]. However, for tropical soils there are still many studies. In this sense, the objective of this work was to evaluate in an oil palm plantation, the effect of different associated sowing systems (pineapple, bean, banana, yucca and pasture) on the biological attributes (TOC, RBS, C-BMS,  $q\text{CO}_2$ ,  $q\text{Cmic}$  and enzymatic activity) to improve soil properties under agroecological production in a Red-Yellow Argisol of the Amazon (Brazil).

## 2. MATERIALS AND METHODS

### 2.1 Study of Area

The experiment was carried out in an experimental area of Embrapa (Brazilian Agricultural Research Company), located in the municipality of São João da Baliza, Roraima, Brazil. In the vicinal 26, Km 12, with geographic coordinates of reference  $00^\circ 51' 13,3''\text{N}$ ;  $60^\circ 00'$

$19,8''\text{W}$  and altitude of 100 m, distant to 352 km of the capital Boa Vista, state of Roraima (Fig. 1).

### 2.2 Soil, Vegetation and Climate of the Study Area

The experimental area is located in a type of dense Ombrophilous forest, with the dominant soil type of that area being argissol according to the Brazilian soil classification system [37-38], the relief according to the Köppen classification is undulated with an inclination between 2-10% and Aw, humid tropical climate, according to that type of region of the south of the State of [39-40], a precipitation varying from 1,800 to 1,900 mm, relative humidity with an annual average between 85 to 90%, luminosity of 1,500 to 3,000 hours per year and the average annual temperature is  $27^\circ\text{C}$  [41].

### 2.3 Management History of the Study Area

Before the implementation of the experiment, the system was established as a 12-year-old pasture of *Brachiaria humidicola*, used to graze cattle, and then intervened to install the experimental plots in the period of 2012, and an area of approximately  $1\text{ ha}^{-1}$  was demarcated and used, and, the practice of clearing and weeding.

After the Brazilian Agricultural Research Company (EMBRAPA), responsible for the experimental area, performed chemical analyzes for the 0 - 0.20 layer and 0.20 - 0.40 m with the following results: pH ( $\text{H}_2\text{O}$ ) = 5.4 - 5.0; organic matter content (%) = 2.6 - 1.3; exchangeable bases ( $\text{cmol dm}^{-3}$ ): Ca = 0.34 - 0.18; Mg = 0.10 - 0.05; K = 0.07 - 0.04; Al = 0.36 - 0.59; the sorptive complex: SB ( $\text{cmol dm}^{-3}$ ) = 0.52 - 0.28; CTCt ( $\text{cmol dm}^{-3}$ ) = 4.73 - 4.04; V (%) = 11 - 7; m (%) = 41 - 68; P-available ( $\text{mg dm}^{-3}$ ) = 1.54 - 0.94. Subsequently liming was performed throughout the area, with dolomitic limestone at a dose of  $1.5\text{ t ha}^{-1}$  (100% PRNT).

The planting of the oil palm was carried out with a density of  $143\text{ plants ha}^{-1}$ , in a system with the plants arranged in equilateral triangle with a spacing of 9 m of the side between the plants in the line and of 7.8 m between the lines, in pits of  $40 \times 0.40 \times 0.40\text{ m}$ . During the same period, intercropping was established between the lines of the palm tree, measuring  $36 \times 39\text{ m}$  (width x length), with 27 plants, making up an experimental area per plot of  $1,404\text{ m}^2$ . The pit was initially fertilized with 500 g of triple superphosphate (45%  $\text{P}_2\text{O}_5$ ).

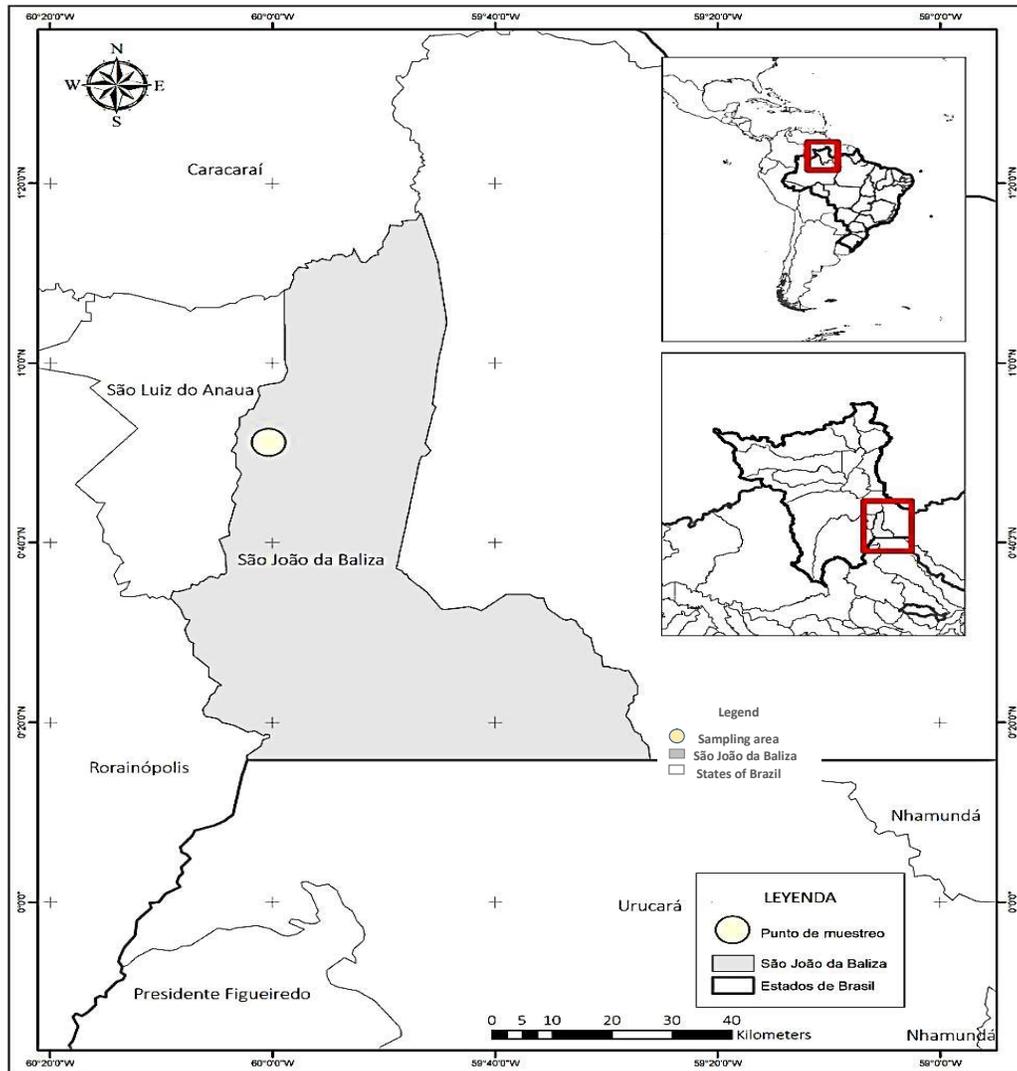


Fig. 1. Experimental area where the research was developed

## 2.4 Systems Evaluated

The systems evaluated were six; T1: OPa (Oil palm interspersed with pasture). In this system, the palm was intercalated with *Brachiaria humidicola*, with no fertilization in the pasture, only in the oil palm.

T2: OPi (Oil palm interspersed with pineapple). Before planting the pineapple, a planting of peanuts was carried out in 2012, with 11 lines (0.50 x 0.30 m) interspersed by a palm oil strip, with fertilization in the pit using 10 g of simple superphosphate and later harvested. The pineapple (Vitória) was then permanently implanted until the moment of the evaluation, being 4 double lines in the spacing of 0.40

between plants and 1 m between double lines, totaling 3,600 seedlings, with fertilization of 1 kg of the formulated 8-28-16 (NPK) per line and 10 g per plant.

T3: OBe (Oil palm intercalated with beans). It was initially composed by the consortium beans (Guariba) and maize (BR 106), established between each palm line, 3 lines of beans and 5 maize lines. The line spacing was 0.5 m, and 8 plants of linear bean and 5 plants of corn per linear meter. The planting fertilization was 10 g of the formulated 8-28-16 (NPK) and later planted only the bean culture.

T4: OBa (Oil palm intercalated with banana), 120 seedlings of the cultivar Japira were planted at

the centre of the palm interline, with a spacing of 1.50 m between plants, consorted with 25 rows of bean (Guariba), both sides of the banana line at a spacing of 0.50 m between rows and a density of 8 plants per linear meter. The fertilization consisted of 200 g of limestone and 100 g of simple superphosphate per well for the banana. Beans were used 10 g of NPK (8-28-16) per pit.

T5: OYu (Oil palm intercalated with yucca). Initially the system was confirmed with rows of maize in double rows spaced 0.50 m and density of 5 plants per linear meter (Cv.BR 106), spaced 1.00 m from single rows of yucca (3 rows) in the spacing of 1.00 x 1.00 m, following the arrangement of 2 rows of corn and 3 rows of yucca successively. The total area of the parcel had an arrangement of 20 maize lines combined with 15 lines of yucca, involving 1,440 corn pits and 135 yucca pits. Fertilization was performed in the pit, using 50 g of the formulation 8-28-16 (NPK).

T6: Pa (Single pasture as a control). The *Brachiaria humidicola* pasture 12 years after deforestation, without intervention or record of use of mechanization, corrective and fertilizers, with continuous pasture use.

The fertilization for the oil palm was carried out during the vegetative development from the first to the third year with the beginning of the production, according to the results of the soil analysis obtained and according to recommendations according to Embrapa-Roraima (Brazil), being carried out per year two maintenance fertilizers, with cover application per plant of: 200, 300 and 500 g of urea; 500, 600, 750 g of triple superphosphate; 200, 300 and 400 g of potassium chloride; 100, 100 and 200 g of magnesium sulfate; 30, 50 and 60 g of borax and 15, 15 and 50 g of zincop 101. Fertilizations were carried out in two periods at the beginning and end of the rainy season. During the conduction of the experiment to ensure the good development of the palm and the crops, cultural practices such as weeding, thinning, defoliation of intercropping, crowning of the oil palm and scrubbing of the spontaneous vegetation between the lines were carried out.

## 2.5 Soil Sampling and Biological Analyses

The experimental design was the completely randomized (DIC) with four replicates being the

readings made in triplicate. Each area consisted of the oil palm with the different intercrop cultures, distributed in plots with 27 plants. Twelve plants were randomly identified in each plot, in which sampling and evaluations were performed.

For the collection of the soil samples it was made in march 2016 in 3 mini-trenches were opened per experimental unit, with dimensions of 0.50 x 0.50 x 0.50 m and, allocated between the lines 0.5 m from the center of the palm frond, collected the samples only in the 0-0.10 m layer; after being conditioned, identified and kept in a thermal box, later transported to the soil laboratory of the Agricultural Sciences Center of the Federal University of Roraima, capital of Boa Vista, Brazil. Subsequently, a 2 mm Mesh analysis was carried out, the roots were removed and then the breathing experiment simulating the field conditions was mounted in the laboratory.

In the laboratory, the following biological analyzes were determined: Carbon from the soil microbial biomass (C-BMS), according to the fumigation-extraction methodology proposed by [42], with second adaptations [43]. Samples were then weighed and fumigated directly with 1 mL of chloroform and samples without the presence of chloroform (non fumigated samples). The calculation of the carbon released in the titration by the excess of dichromate with ammoniacal ferrous sulfate.

Soil basal respiration (RBS), determined according to the methodology proposed [44], and modified [30]. The samples were weighed and incubated with 1 mol L<sup>-1</sup> NaOH for 24 days, with subsequent quantification of C-CO<sub>2</sub> released every 3 days, totaling 8 titrations (3, 6, 9, 12, 15, 18, 21 and 24 days), the values being summed, to obtain a general reference of accumulated C-CO<sub>2</sub> during the incubation period.

The metabolic quotient ( $qCO_2$ ), determined by the ratio of soil microbial activity (C-CO<sub>2</sub> released) in relation to microbial carbon biomass (C-BMS), following the methodology proposed [45], modified [43]. The microbial quotient ( $qCmic$ ) was calculated using the expression proposed [46] being necessary the determination of the TOC present in the soil and later calculated.

In the determination of  $\beta$ -glucosidase activity, 0.05 M p-nitrophenol- $\beta$ -D-Glucopyranoside (0.05 M PNG) was used as the substrate in the reaction described [47-48] with adaptations. The

determination of the amount of p-nitrophenol in each sample was performed on the basis of a standard curve prepared with concentrations of 0, 10, 20, 30, 40, 50  $\mu\text{g}$  of p-nitrophenol  $\text{mL}^{-1}$ . The readings were determined by molecular spectrophotometry, UV-Visible was used using a SHIMADZU UV-1800 model at an absorbance of 400 nm and expressed in micrograms of p-nitrophenol released per hour per gram of dry soil ( $\mu\text{g}$  p-nitrophenol  $\text{h}^{-1} \text{g}^{-1}$  dry soil).

The urease activity was determined by the quantification of the ammonium released by the urea hydrolysis (0.08 M solution), using the colorimetric method recommended [49] adaptations. Determination of the amount of  $\text{N-NH}_4^+$  in each sample was performed based on a standard curve with known concentrations of 0; 0.25; 0.5; 1.0; 2.0; and 4.0  $\mu\text{g}$   $\text{mL}^{-1}$  of  $\text{N-NH}_4^+$ , then the readings performed by UV-Visible spectrophotometry at an absorbance of 660 nm and, expressed in micrograms of  $\text{N-NH}_4^+$ , released for two hours per gram of dry soil ( $\mu\text{g}$   $\text{N-NH}_4^+ 2\text{h}^{-1} \text{g}^{-1}$  dry soil).

Likewise, acid phosphatase activity, determined using as a substrate in the reaction 0.05 M p-nitrophenol phosphate (0.05 M PNP) [47-48] adaptations. The determination of the amount of p-nitrophenol in each sample was performed based on a standard curve of p-nitrophenol (0, 10, 20, 30, 40, 50  $\mu\text{g}$  of p-nitrophenol  $\text{mL}^{-1}$ ), then the readings determined by spectrophotometry molecular UV-Visible at an absorbance of 400 nm, and values expressed in micrograms of p-nitrophenol released per hour per gram of dry soil ( $\mu\text{g}$  p-nitrophenol  $\text{h}^{-1} \text{g}^{-1}$  dry soil).

For the statistical analysis, the data were first tabulated in Excel spreadsheets and submitted to the verification of homogeneity of variances according to Levene test and normality of the errors by the Shapiro-Wilk test, after which variance and Tukey test were performed at level 5 % of probability, also performed Pearson and multivariate correlation analysis (PCA and HCA), with the aid of statistical software SAS and INFOSTAT.

### 3. RESULTS AND DISCUSSION

The results of the analyzes of variance obtained showed significant differences for the evaluated treatments (Table 1). The TOC presented values between 4.70 and 9.45  $\text{g kg}^{-1}$ , being the highest values found in the interim systems OYu, Pa, OBa, highlighting the intermediate system OPi

that presented the lowest levels. The intercalary systems contribute to the maintenance of the agrosystems, maintaining a balance of soil fertility [50] by promoting coverage, biomass production, carbon accumulation and nutrient removal from the deeper layers, in a cycling process that becomes more efficient reducing leach losses and erosion. The accumulation of biomass in these systems came mainly from the prunings made to the oil palm plants, as well as the fall of leaves and branches of the other species of the systems, contributing to the maintenance of the levels of organic matter and biological activity in these production models [51,52]. Another relevant factor to understand the best results presented in these areas of agricultural use is the response of limestone application, which may have favored, accelerating the decomposition process, mineralization and availability of organic matter with subsequent increase of biological activity in the soil generally.

For soil basal respiration (RBS), which relates the degradation capacity of the OM, being defined as the sum total of all the metabolic functions in which the  $\text{CO}_2$  is produced [53]. Variable results were observed after 24 days of incubation, with the highest values found in the Pa area (23.50  $\text{mg C-CO}_2 \text{ kg}^{-1} \text{ soil}^{-1}$ ) and the lowest values in the OBe and OPi intercalary systems (23.50; 19.65 and 19.31  $\text{mg}$  of  $\text{C-CO}_2 \text{ kg}^{-1}$ ). Although, without differences between the OBa and OYu systems (21.44 and 21.49  $\text{mg C-CO}_2 \text{ kg}^{-1} \text{ dry soil}^{-1}$ ) presenting lower values when compared to the Pa area and higher with respect to the OPa system (20.28  $\text{mg}$  of  $\text{C-CO}_2 \text{ kg}^{-1} \text{ soil}^{-1}$ ). Therefore, the microbial activity in the soil with intercalated cropping systems was lower when compared to the Pa system (Table 1).

Many works have been developed in the southwest of the Amazon, among them [54], evaluating biological attributes in an Argisol, found higher values of RBS in the pasture area compared to areas of agricultural use and native forest, observing a great similarity between values of respiration rates between native areas and pasture areas, with significant differences for areas used as agricultural systems. These results corroborate [55], observing higher values of RBS in the pasture area when they evaluated the biological activity of soils in organic, agroforestry and pasture systems in the southwest of the Amazon. Still, [56], when investigating RBS, verified the highest values in pasture areas and three forest fragments in

comparison with areas of annual and perennial crops. According to these authors, the factors responsible for the renewal of plant and microbial biomass and nutrient cycling may have promoted lower respiration rates in soils under annual and perennial crops, and in systems in which land use changes the dynamics of the organic matter, there were significant differences in the biological attributes.

In agreement with this work, in all the evaluated systems the highest respiration rates were observed in the Pa area compared to the areas of agricultural use (Table 1). Soils under anthropic interference as in cultivated areas undergo changes in their composition and metabolic activity, and the microbial population is under stress [57]. Thus, the highest rate of respiration may or may not be desirable and may indicate both disturbance and high level of ecosystem productivity and should be analyzed in each context [58]. It can thus be interpreted as a desirable characteristic when it is considered that the decomposition of organic residues will provide nutrients to the plant, in addition, it promotes processes such as: aggregation, cation exchange capacity and water retention [59]. These increases in the rate of respiration in the pasture area can also be explained by the preference of the microorganisms for certain types of organic materials, besides the presence of feces and urine of the cattle promoting a high metabolic activity, being also that the grasses release C in the form of CO<sub>2</sub> because they present a larger root system, more aggressive and exploratory, with a high respiratory activity.

In relation to the soil microbial biomass carbon (C-BMS), which represents the amount of carbon that the microbial biomass of the soil immobilizes in its cells, it is verified that the different systems evaluated promoted variations in these contents, area Pa (116.00 mg C microbial kg<sup>-1</sup> alone). OPa was the system that presented the highest values of 105.75 mg C microbial kg<sup>-1</sup> soil, followed by OYu with 95.93 mg C microbial kg<sup>-1</sup> soil, OBa and OBe presented close values and no significant statistical differences (85.22 and 81.35 mg C microbial kg<sup>-1</sup> alone). Taking into account the interim system of OPi that presented the lowest values of 53.36 mg C microbial kg<sup>-1</sup> soil, differing statistically from all other treatments, and the Pa and OPa systems were two times higher (Table 1).

Factors such as clay content, moisture and water dynamics, types of cover with the continuous

addition of residues, modifications of the microclimate of the area, root distribution, incorporated organic carbon contents directly influencing the activity and biological diversity and management practices such as incorporation of inorganic fertilizers explain the results of the present study. In this context, research developed by several authors in the southwest of the Amazon also reported the highest values of C-BMS in the pasture area compared to areas of agricultural use and native forest. These results were attributed to fine root biomass as a factor that can influence the response of microbiological attributes in the Pa system, in addition, the age of establishment as a source of carbon accumulation [54-55].

In pasture areas, this effect occurs because the root system is abundant and extensive, presenting a continuous renewal and a strong rhizospheric effect, promoting greater biological activity [60]. Similarities between pasture and native areas may be related to equivalent carbon stock, while the differentiated behavior presented by the areas in agriculture may be related to the soil management and the different types of crops that are found in the region (pineapple, banana, beans, corn, rice, cocoa, coffee, among others). It should be noted that the evaluation of C-BMS or RBS alone provides only limited information on certain responses in the ground interference to stress or disturbances. Therefore, it is necessary to follow other evaluations and can be conducted together with the determination of these characteristics, such as the metabolic quotient ( $qCO_2$ ), microbial carbon quotient ( $qCmic$ ) and the enzymatic activity of the soil.

Thus, the metabolic quotient ( $qCO_2$ ) was estimated from the values of RBS accumulated in 24 days and C-BMS, being verified the highest value in the agricultural use system OPi (0.36 mg C-CO<sub>2</sub>. g<sup>-1</sup> C-BMS. h<sup>-1</sup>) differing from the other systems, followed by OBa, OYu and OBe, which did not differ statistically from each other (0.25, 0.22 and 0.24 mg C-CO<sub>2</sub>. g<sup>-1</sup> C-BMS. h<sup>-1</sup>), and the lowest values were found in the Pa and OPa areas (0.20 and 0.19 mg C-CO<sub>2</sub>. g<sup>-1</sup> C-BMS) (Table 1). According of [61-62], low values of  $qCO_2$  indicate a more stable environment with better quality in the physical, chemical and biological attributes, which demonstrates a more balanced ecosystem in the pasture areas, however, in the cultures intercalated with the replacement of the cover occurs more rapid decomposition of the vegetal residues, increasing the metabolic quotient [63].

**Table 1. The values of Total Organic Carbon (TOC), Basal Respiration of soil accumulated in 24 days (RBS), Soil Microbial Biomass Carbon (C-BMS), Metabolic Quotient ( $qCO_2$ ) and the Quotient of Microbial Carbon ( $qCmic$ ), in a Red-Yellow Argisol with different systems of use in the 0-0.10 m layer, São João da Baliza, Boa Vista - Roraima, Brazil, 2017.**

Systems of use	Total organic carbon and microbial attributes				
	TOC (g kg <sup>-1</sup> )	RBS (mg of C-CO <sub>2</sub> Kg <sup>-1</sup> soil h <sup>-1</sup> )	C-BMS (mg C microbial Kg <sup>-1</sup> soil)	$qCO_2$ (mg C-CO <sub>2</sub> g <sup>-1</sup> BMS-C h <sup>-1</sup> )	$qCmic$ (%)
OPa	8.02 bc	20,28 c	105,75 b	0,19 c	1,34 a
OPi	4.70 d	19,31 d	53,36 e	0,36 a	1,15 ab
OBe	7.19 c	19,65 d	81,35 d	0,24 b	1,16 ab
OBa	8.41 abc	21,44 b	85,22 d	0,25 b	1,02 b
OYu	9.45 a	21,49 b	95,93 c	0,22 bc	1,02 b
Pa	8.64 ab	23,50 a	116,00 a	0,20 c	1,36 a

*The average value for the readings made in triplicate is presented. Means followed by the same lowercase letter in the columns and upper case in the rows do not differ statistically from each other by the Tukey test at a 5% probability level.*

On the other hand, [64], report that this attribute serves to estimate the efficiency of substrate use by soil organisms, so the high value found in agricultural areas and more specifically in the pineapple (OPi) palm system indicates the occurrence of disturbances where the microbial population is oxidizing of their own cells, and these high values correspond to the need for a high energy demand for their maintenance, survival and adaptation to the soil, therefore, the microbial population is in adverse or stressful conditions.

Still, [65], explain that the higher values of  $qCO_2$  found in the systems indicate more carbon losses in the system per unit of microbial biomass and are related to the microbial biomass mineralization response. Where lower values of  $qCO_2$  and higher value of C-BMS suggest that microbial biomass was more efficient in the use of organic compounds, releasing less carbon, like CO<sub>2</sub> and incorporating more to the microbial tissues [66]. In this way, in relation to the mineralization of the microbial biomass, it can be inferred that, in comparison to the Pa system, the OPa, OYu and OBe systems were very similar in the use of the organic compounds, incorporating C to their tissues and releasing less as CO<sub>2</sub>. It can also be concluded that the soil microbial population in these areas of agricultural use OPa, OYu and OBe, demanded similar amounts of energy to maintain, indicating that the intercalated systems studied can reduce the emission of CO<sub>2</sub> over time, since they are more stable environments for the soil microbial community.

In addition, it is also observed that the OPi system is undergoing some environmental stress due to its relationship with  $qCO_2$  and low

microbial activity, unlike the Pa, OPa and OYu systems, which besides stimulating microbial development (C-BMS), present a good quality of the organic matter ( $qCmic$ ), increasing the efficiency of use of the substrates (Table 1). In this aspect, the microbial quotient ( $qCmic$ ) is an important indicator of impacts [67]. Providing information on the quality of organic matter and the amount of C immobilized in microbial biomass [68-69]. The highest values of  $qCmic$  observed in the Pa and OPa environments (1.36 and 1.34%) were higher than the values found in the areas destined to the conventional use OPi and OBe (1.15 and 1.16%) and, with the lower values observed in OBa and OYu systems (1.02 and 1.02%) (Table 1). High levels of  $qCmic$  indicate that there is an increase in C-BMS against the amount of organic C available, that is, a greater efficiency in its use by microorganisms, being reported as an indicator of the quality of OM, allowing to accompany disturbances promoted by ecological imbalance and variations in OM levels caused [70].

In an experiment carried out [54], when assessing soil biological attributes in southwestern Amazonia, observed higher  $qCmic$  values in pasture areas compared to areas of agricultural use and native forest. In this study, the lowest values were found in the systems of agricultural use in relation to the systems submitted under pasture, indicating a greater disturbance in these environments, either by type of management or by anthropic intervention. [71-72], establish normal values of  $qCmic$  between 1 and 4%, being the values observed in this study in this range, for the different areas studied.

On the other hand, it was also evaluated the enzymatic activity  $\beta$ -glucosidase, urease and

phosphatase for the different systems studied (Table 2).

In the OYu system with the highest activity of  $\beta$ -glucosidase  $51.22 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$ , followed by Pa and OPa systems with values of  $47.83$  and  $39.59 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$ , behaving statistically the same. On the other hand, lower values were observed in the OBe and OBa systems ( $37.90$  and  $32.16 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$ ) and the OPi system with values much lower than  $22.97 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$  (Table 2). The incorporation of OM in these systems provides the necessary substrate for the action of  $\beta$ -glucosidase, maintaining and protecting the enzymes in their active forms, due to the formation of complex enzymes-humic compounds [73].

In addition, areas formed by grasses have a dense root area exploring greater depths when compared to agricultural areas, favoring the microbial biomass of the rhizosphere and consequently stimulating a greater activity of the microorganisms. Regarding the type of cover, in the PS area besides forming these complexes, the incorporation and decomposition of the organic residues become slower due to chemical conditions, presenting lower pH values when compared to the intercalated areas, allowing to maintain material for periods long time with more action and biological breathing activity. In addition, the quality of the incorporated residue influences the enzymatic activity, the intercalary systems with the use of cover plants, present material with recalcitrant constituents to the microbial decomposition, such as lignins, waxes and phenolic compounds of high molecular weight, with greater difficulty to be broken, and less amount of easily decomposed and incorporated material such as carbohydrates [66].

According with [74], the land use systems that provide greater diversity and quantity of organic waste favor the development of microorganisms and promote an increase in enzymatic activity. This explains the greater activity found in the OYu, Pa and OPa systems, being that these systems presented the highest TOC levels in the soil (Tablet 1), and, the greater activity of the enzyme  $\beta$ -glucosidase. According with [75], in experiments developed establish values of variation for the enzyme  $\beta$ -Glucosidase of  $38$  to  $720 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$ , observing in this range the values found in the different systems evaluated in this work [76], when evaluating the

fauna and microbiological attributes of an Argisol under cover systems in southern Brazil, reported maximum values of  $72.4 \mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$  soil for  $\beta$ -Glucosidase activity and values of  $23.6 \mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2h}^{-1}$  for the urease activity, in a consortium of lablab (*Dolichos lablab*) with corn. In this sense [77], when studying the effect of fertilizers and crop rotation on the enzymatic activity in the soil, establish activity values of  $40$  to  $270 \mu\text{g NH}_4^+ \text{ g}^{-1} \text{ solo 2 h}^{-1}$ . Results found in this research are within this range of variations in urease activity (Table 2).

These results indicate that OM, besides serving as a substrate for the microbiota, may be protecting this enzyme against the action of proteolytic enzymes naturally present in the soil, maintaining the potential of urease activity for longer periods of time. In the Pa area there is consequently a greater root system when compared to the areas with intermediate cultures OYu, OPa, OBa, OBe and OPi, which increases the rhizosphere stimulating the activity of the organisms in these places, in addition, there is a continuous contribution of organic residues, even in areas under agricultural use, low activity may also be related to changes in the composition of microbial communities present. Although, organic nitrogen can be found as urea occurring in the natural form through animal excretions and as a product of nucleic acid mineralization [78], indicating that the ammonification is normally occurring in the Pa area, and the chemical conditions are favorable with low values of pH, clay contents that may be influencing the retention of the ammonium cation, by adsorption processes to the soil colloids making it relatively stationary. It should be pointed out that the interim systems OYu, OPa, OBa, OBe did not present significant differences between them in the urease activity, with values very close to the Pa system, but it is also important to highlight that the coverage has a great influence in this process, stable conditions of pH, as well as a more diversified coverage that provides a better source of energy for the microorganisms, thus favoring the activity of this enzyme.

In relation to acid phosphatase activity, the Pa system with the highest values of  $230.33 \mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$  is also highlighted, followed by the OYu and OPa systems ( $168.83$  and  $145.85 \mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$ ), but without significant differences. Already, decreasing values were observed in the OBa and OBe intercalated systems ( $132.04$  and  $119.42 \mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1} \text{ soil}$ ) and also lower values in

the OPi system with 47.42  $\mu\text{g p-nitrophenol g}^{-1}$  soil  $\text{h}^{-1}$  (Table 2). The activity of acid phosphatase is influenced mainly by the chemical characteristics of the soil, OM has beneficial effects since it increases the microbial community. In addition, the highest activity of this enzyme is found with pH of the soil around 5.0 with tendency to decrease with increases of pH to values of 7.0 [79]. Being strictly controlled by biological demand, so that the lower the amount of inorganic phosphorus the greater activity of the enzyme [80-81]. This was verified in the systems with OYu, OPa, OBa, OBe, OPi, where the acid phosphatase activity was lower, in this sense, observing ideal conditions in native areas and pasture areas when compared to cultivated areas (Table 2).

It is also known that the higher activity of acid phosphatase is related to the decrease of soil P contents [82], corroborating with the results found in the Pa area, as control with the highest activity without the presence of inorganic fertilizers, followed by the OYu and OPa intercalated systems. Consequently, in the cultivated areas, localized applications of phosphate inorganic fertilizers cause a decrease in the enzymatic action, occurring in zones of low activity and concentration in this enzyme, contrary in the native and pasture areas, maintaining this dynamic balance. These observed differences between the different evaluated systems can also be explained to the qualitative changes in the diversity of the microbial communities and the stress caused by the incorporated inorganic fertilizers. Like this [83], when evaluating microbial biomass and enzymatic activity in soils of the state of São Paulo under native and cultivated vegetation, found values for acid phosphatase activity of 8.61  $\mu\text{g p-nitrophenol kg}^{-1}$  soil  $\text{h}^{-1}$  in culture condition at 191.79  $\mu\text{g p-nitrophenol kg}^{-1}$  soil  $\text{h}^{-1}$  in grazing condition. For forest soils found values of 19.63 mg p-nitrophenol  $\text{kg}^{-1}$  soil  $\text{h}^{-1}$  at 158.22  $\mu\text{g p-nitrophenol}$ .

In this sense, (Table 3), it presents the correlation between the OM contents and the soil biological attributes, for the different evaluated treatments.

Thus, a positive and highly significant correlation between RBS and OM was observed (r) of 0.60 ( $p \leq 0.01$ ). Thus, the availability and continuous addition of organic residues to the soil increases

the OM content, favoring and promoting greater biological activity with a subsequent increase in the metabolism of microorganisms and respiratory rate. Positive and significant correlation of C-BMS with OM and RBS, (r) 0.74 and 0.74 ( $p \leq 0.01$ ). [84], reported a significant and positive correlation between C-BMS and OM, indicating that the microbial attribute is influenced by the OM contents, being an expected fact since, the greater incorporation of organic residues increases the biological activity, releasing C as  $\text{CO}_2$  by microbial metabolism (Table 3).

Highly significant but negative correlation was observed between  $q\text{CO}_2$  and OM, (r) of -0.76 ( $p \leq 0.01$ ), and a highly significant and negative correlation between  $q\text{CO}_2$  and RBS and C-BMS with (r) -0.55 and -0.94 ( $p \leq 0.01$ ) (Table 3), data corroborated by [85-87]. Positive and significant correlation between the OM, C-BMS with the enzyme  $\beta$ -glucosidase, (r) of 0.70 and 0.74 ( $p \leq 0.01$ ). The higher the soil carbon content in the studied areas, the higher the  $\beta$ -Glucosidase activity found. [88-89], also report the same sequence in their studies.

Positive and highly significant correlation between OM, C-BMS and, urease activity, (r) of 0.59 and 0.84 ( $p \leq 0.01$ ). Research carried out by different authors as [90] in soils of the Mediterranean region in Spain; [91], evaluating the quality of agricultural soils of Rio Grande do Sul, also observed a positive and highly significant correlation between organic matter and urease activity. Positive and significant correlation between OM, C-BMS and, the enzyme acid phosphatase, (r) of 0.71 and 0.90 ( $p \leq 0.01$ ) respectively. Data that corroborate with those obtained by [83-92], when they found significant correlations between OM and acid phosphatase activity. These types of correlations are important from the point of stability of the soil fertility of the different systems.

Furthermore, analyzes of the main components were carried out jointly for the different OPa, OPi, OBe, OBa, OYu and Pa systems in the 0-0.10 m layer in order to evidence and analyze the interdependence of the variables between carbon (TOC), and the biological attributes studied, trying to find with minimal loss of information, a new set of variables (main components) that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).

**Table 2. Activity of  $\beta$ -glucosidase, urease and acid phosphatase, in a red-yellow Argisol with different systems of use in the 0-0.10 m layer, São João da Baliza, Boa Vista - Roraima, Brazil, 2017.**

Systems of use	Microbial attributes		
	$\beta$ -Glucosidase ( $\mu\text{g p-nitrophenol. g}^{-1}$ soil $\text{h}^{-1}$ )	Urease ( $\mu\text{g NH}_4^+ \cdot \text{g}^{-1}$ soil $^{-1}$ )	Acid phosphatase ( $\mu\text{g p-nitrophenol. g}^{-1}$ soil $\text{h}^{-1}$ )
OPa	39.59 abc	121.11 b	145.83 bc
OPI	22.97 d	104.49 c	47.42 d
OBe	37.90 bc	117.15 b	119.42 c
OBa	32.16 cd	118.34 b	132.04 c
OYu	51.22 a	126.45 b	168.83 b
Pa	47.83 ab	148.42 a	230.33 a

The average value for the readings made in triplicate is presented. Means followed by the same lowercase letter in the columns and upper case in the rows do not differ statistically from each other by the Tukey test at the 5% probability level.

**Table 3. Pearson's correlation between Total Organic Carbon (TOC) and biological attributes, studied in a Red-Yellow Argisol with different systems of use in the 0-0.10 m layer, São João da Baliza, Boa Vista - Roraima, Brazil, 2017.**

	TOC	RBS	C-BMS	$q\text{CO}_2$	$q\text{Cmic}$	$\beta$ -Glu	Urease	Acid phos
TOC	~							
RBS	0.60**	~						
C-BMS	0.74**	0.74**	~					
$q\text{CO}_2$	-0.76**	-0.55**	-0.94**	~				
$q\text{Cmic}$	-0.32ns	0.19ns	0.38ns	-0.30ns	~			
$\beta$ -Glu	0.70**	0.58**	0.74**	-0.71**	-0.06ns	~		
Urease	0.59**	0.88**	0.84**	-0.68**	0.33ns	0.69**	~	
Acid phos	0.71**	0.87**	0.90**	-0.79**	0.27ns	0.75**	0.91**	~

Where: n.s. - not significant ( $p > 0.05$ ); \* - significant at the 5% level ( $p \leq 0.05$ ); \*\* - significant at the 1% level ( $p \leq 0.01$ ).

Being represented in the (Fig. 2), the results of the analysis of the main components (PCA) of the evaluated variables: TOC and microbiological and biochemical attributes, the sum of the variability retained in these components explained 90% of the original variability of the data.

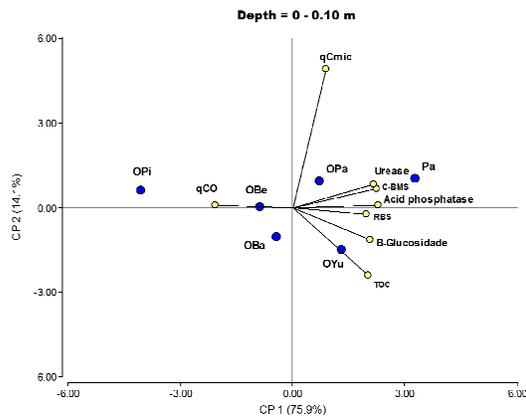
The first major component (CP 1) contributed with 75.9% of the total variance explained, however, most of the variables that were strongly affected, among them: Urease, C-BMS and acid phosphatase, contributing positively to the CP 1, and inverse with the variable  $q\text{CO}_2$ . In addition,  $q\text{Cmic}$  did not influence all the different systems evaluated in this component. These results indicate that CP 1 allowed to distinguish the cultures that are associated to these variables, being the Pa, OPa and OYu systems that contributed the most to improve the microbiological and biochemical conditions of the soil. However, the second main component (CP 2) explained 14.1% of the total and was

related to the variable  $q\text{Cmic}$ , in negative projection with the variables RBS,  $\beta$ -glucosidase and, TOC. The analysis showed that the OPI system was more related to this component and, with lower effects for OBe and OBa (Fig. 2).

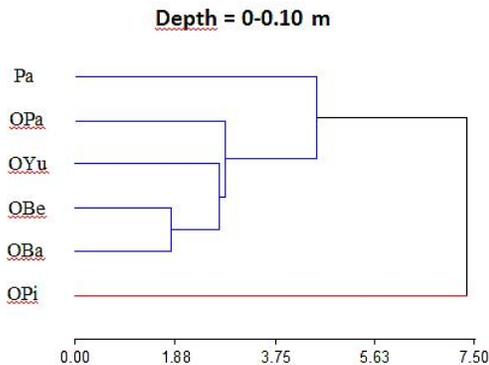
In the HCA hierarchical dendrogram (Fig. 3), the variables that were grouped are OBe and OBa with a Euclidean distance of 1.88; a second grouping between OYu and the group formed by OBe and OBa, a third group formed by OPa, a larger group composed of Pa together with the other groups (OBa, OBe, OYu and OPa) can still be visualized. We can say that the variable OPI has no correlation with the other elements of the group, because there is a separation distance very high.

In this case, the analyzes of PCA and HCA showed a great sensitivity to the categorical selection of the variables in the different evaluated systems, suggesting that the continuous incorporation and decomposition of

residues and the exudates released by the roots, promote an increase in microbial activity as a final result in greater biochemical cycles, as observed in the Pa, OPa and OYu areas. In addition, the  $qCmic$  attribute was negatively correlated with the  $qCO_2$  attribute, with the OPi system being more strongly associated with this component and, with smaller effects for the OBe and OBa systems, indicating that the systems were strongly affected, implying a high metabolic activity ( $qCO_2$ ) with higher energy supply ( $qCmic$ ) (Figs. 2 and 3).



**Fig. 2. Distribution of the original variables between the total organic carbon (TOC) and the biological attributes in the 0-0.10 m layer on the first and second main component (CP 1 and CP 2)**



**Fig. 3. Analysis of hierarchical components (HCA) for the variables studied in the different systems evaluated**

**4. CONCLUSIONS**

In agreement with the results obtained in this work, the interleaved systems improve soil

conditions in relation to the Pa control system. The balance and the dynamics in the systems modified by the anthropic action, present high values for the OPa, OYu systems and, intermediate values for the OBe, OBa systems, related to the microbiological and biochemical attributes. The OPi system presents as the less stable environment, with a high stress and lower quality of microbiological and biochemical attributes. All attributes analyzed in the different agricultural systems are influenced by the organic carbon contents.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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