

Soil microbial biomass carbon and *Jatropha curcas* yield intercropping with forages and crop species

Carbono da biomassa microbiana do solo e produção de pinhão- manso consorciado com espécies forrageiras e culturas

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Abstract: The domestication of *Jatropha curcas* L. in the Brazilian territory has been boosted by its great potential in the production of grains, oil and, in particular, its adaptation in different soil and climatic conditions. The aim of this research was evaluate the soil biological quality through the soil microbial biomass carbon and its indices derivate (metabolic and microbial quotient) under *J. curcas* intercropping with many forages species, legumes and annual crop rotation systems, as well as the accumulated production of *J. curcas* grains and oil. The experiment was conducted in a commercial area in the randomized blocks experimental design with the treatments arranged in a 12 x 2 factorial design, with four repetitions, resulting in 12 treatments with cropping systems and 2 sampling times (February 2012 and May 2012). Soil sampling was performed in February and May 2012, at 0 at 10 cm layer and four composite samples were collected in

each cropping system, from five subsamples in each plot. *J. curcas* intercropping with *B. ruziziensis*, favors the maintenance of the community of soil microorganisms compared to the treatment with the species *P. maximum* cv. Massai and *Cajanus cajan*, which promoted decreasing in soil organic matter dynamics, when compared to the other cropping systems. The monocropping systems of *J. curcas* and intercropping in rotation systems 2 and 3 achieved higher yields of *J. curcas* grains and oil over three seasons.

Keywords: Soil microbial quotient. Soil microbiology. Soil basal breathing. Total organic carbon.

Resumo: A domesticação da *Jatropha curcas* L. no território brasileiro tem sido impulsionada pelo seu grande potencial na produção de grãos, óleo e, em particular, sua adaptação a diferentes condições de solo e clima. O objetivo foi avaliar a qualidade biológica do solo por meio do carbono da biomassa microbiana do solo e seus índices derivados (quociente metabólico e quociente microbiano) no consórcio de *J. curcas* com várias espécies de forragens, leguminosas e sistemas de rotação de culturas anuais, bem como a produção acumulada de grãos e óleo de *J. curcas*. O experimento foi conduzido em área comercial no delineamento experimental de blocos ao acaso com os tratamentos dispostos em um arranjo fatorial 12 x 2, com quatro repetições, resultando em 12 tratamentos com sistemas de cultivo e 2 momentos de amostragem (fevereiro de 2012 e maio de 2012). A amostragem do solo foi realizada em fevereiro e maio de 2012, na camada de 0 a 10 cm, e quatro amostras compostas foram coletadas em cada sistema de cultivo, a partir de cinco subamostras em cada parcela. O consórcio de *J. curcas* com *B. ruziziensis* favorece a manutenção da comunidade de microrganismos do solo em comparação com o tratamento com as espécies *P. maximum* cv. Massai e *Cajanus cajan*, que promoveram a diminuição na dinâmica da matéria orgânica do solo em comparação com os outros sistemas de cultivo. O sistema de monocultivo de *J. curcas* e os sistemas de consórcio em rotação 2 e 3 obtiveram maiores rendimentos de grãos e óleo de *J. curcas* ao longo de três safras.

Palavras-chave: Quociente microbiano do solo. Microbiologia do solo. Respiração basal do solo.

1 Introduction

The *Jatropha curcas* L. it is a species primarily exploited for the importance of its grains as raw material for oil production and biodiesel extraction (Souza *et al.*, 2013). The *J. curcas* can be cropped by smallholders and larger holders which can increment its economics gains, resulting in increasing the sustainability of the production system (Singh, Misha, Dixt, Gupta, 2019). *J. curcas* intercropping with forages and grain crops species can be a profitable alternative as cropping system which improve the efficiency of land use (Silva, Souza, Silva, Bottega, 2012; Silva, Souza, Silva, Fonseca, 2015).

Perennial crops intercropped with cover crops species can result in increasing of soil protection against erosion with higher straws amount on soil surface (Baumert, Vlek, Khamzima, 2014). Nevertheless, many cropping systems of land use can affect soil microbial biomass carbon content (SMB-C), which can be positive or negative for the process of soil organic matter mineralization (Singh *et al.*, 2019). The increment of organic residues in soil can conduct to increase the microbial population, which can increase the amount of carbon (C) and nitrogen (N) storage in microbial biomass, thus, addition in soil organic residues can affect the sensibility of soil microbial activity (Singh *et al.*, 2019).

As reported by Barreto, Gama-Rodríguez, Gama-Rodríguez and Barros (2008), SMB-C is the major active part, consequently is more sensitive that total organic C and total N to point the changes due to management and agricultural practices applied. However, according to Anderson and Domsch (1989), the ratio of SMB-C and total organic C present in the soil increases or decreases rapidly, as there is a rapid increase

or decrease of the organisms present in the soil in an ecological system, promoting thus a balance in the ecosystem.

The process of decomposition by soil microbial biomass is based on the action of the soil fauna, mainly the mesofauna and macrofauna, which generates the fragmentation of the vegetal residues, leading to the increase of the contact area of the decomposition (Simon *et al.*, 2019). As a determinant parameter, the SMB-C evaluation is of fundamental importance for soil extension studies, such as environmental monitoring (Santos and Maia, 2013).

SMB-C allows rapid information on changes in soil organic properties, detecting changes caused by inadequate cultivation or forest devastation (Barreto *et al.*, 2008). In this context, SMB-C and its activity have been highlighted as sensitive indicators to detect the environmental changes generated by the management adopted by the farmers, thus allowing guiding the planning of the most appropriate sustainable agricultural practices (Barreto *et al.*, 2008; Santos and Maia, 2013; Martins, Fernandes, Oliveira, Sampaio, Frazão, 2018). In order to evaluate the effect of soil biodynamics on the production of different forage species, a study of the soil biodynamics can be made as a more sustainable form of management, thus mitigating the environmental consequences of negative impacts (Fernandes *et al.*, 2013).

The recommendation of the cropping systems that promote increasing of the microbiological attributes in areas with different intercropping in the scope of the conservation of the productive systems directly interfere in the functioning of the soil and, consequently, in the sustainability of the agroecosystems, acting as an indicator of their degradation.

The aim of this research was evaluate the soil microbiologic quality through the soil microbial biomass carbon and its indices derivate (metabolic and microbial quotient) under *J. curcas* intercropping with forages species, and annual crop rotation systems, as well as the accumulated grains and oil production of *J. curcas*.

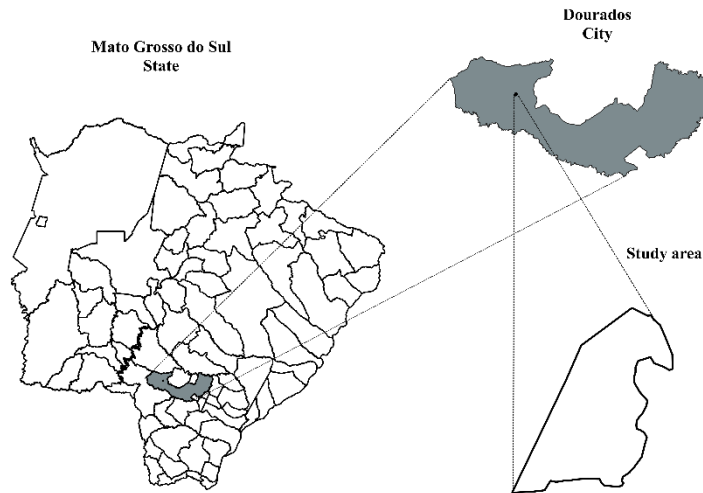
2 Material and Methods

Site description and soil

The experiment was carried out in the district of Itahum, city of Dourados, state of Mato Grosso do Sul, Brazil (22°05'44" S, 55°18'48" W; and 484 m altitude) (Figure 1), enabled by a partnership between Embrapa Western Agriculture and Paraíso Farm. The soil is classified as Typic Haplortox (Embrapa, 2018), with average clay content of 200 g kg⁻¹. The climate in the region, according to the classification system by Köppen-Geiger, is Cwa, humid mesothermal, with hot summers and dry winters (Fietz, Fisch, Comunello, Flumignan, 2017). According to data from Dourados meteorological station from Guia Clima (www.cpa0.embrapa.br/clima/), monthly rainfall mean and temperature during of the trial is presented in Figure 2.

Figure 1. Localização geográfica da área experimental.

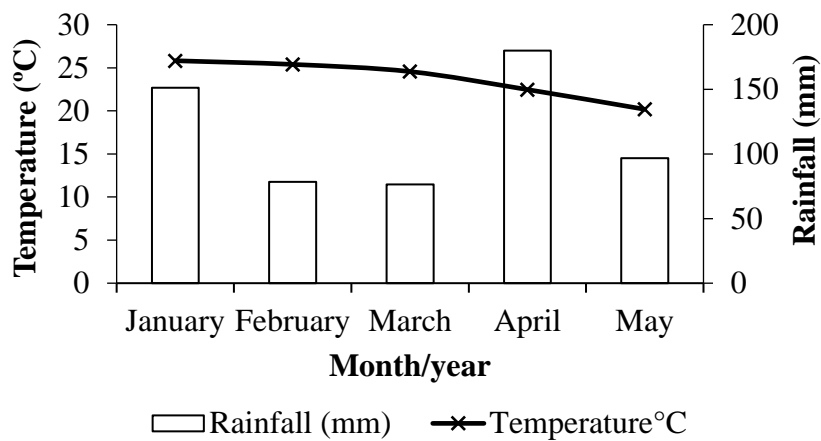
Figura 1. Geográfica localização of the experimental area.



Source: Prepared by the autor (2023). **Fonte:** Elaborado pelo autor (2023).

Figure 2. Monthly rainfall mean and temperature during of the trial in 2012. Source: Embrapa Western Agriculture.

Figura 2. Temperatura média e precipitação mensal durante o ensaio em 2012. Fonte: Embrapa Agropecuária Oeste.



Source: Prepared by the autor (2023). **Fonte:** Elaborado pelo autor (2023).

Experimental site and design

A randomized blocks experimental design was defined to evaluate the variables, with the treatments arranged in a 12 x 2 factorial design, with four repetitions, resulting in

12 treatments with cropping systems (Table 1) and 2 sampling times (February 2012 and May 2012).

Table 1. Cropping systems treatments relating *J. curcas* under monocrop and intercropping with crops and forages species.

Tabela 1. Tratamentos de sistemas de cultivo relacionados a *J. curcas* em monocultivo e consorciação com culturas e espécies forrageiras.

Treatments	Abbreviation	Cropping systems
1	JM	<i>Jatropha curcas</i> in monocrop
2	IJS	Intercropping of <i>J. curcas</i> with <i>Stylosanthes</i> spp
3	IJB	Intercropping of <i>J. curcas</i> with <i>U. ruziziensis</i> cv. Ruziziensis
4	IJBS	Intercropping of <i>J. curcas</i> with <i>U. ruziziensis</i> cv. Ruziziensis and <i>Stylosanthes</i> spp
5	IJBH	Intercropping of <i>J. curcas</i> with <i>U. humidicola</i> cv. Humidicola
6	IJP	Intercropping of <i>J. curcas</i> with <i>Panicum maximum</i> cv. Massai
7	IJCR-1	Intercropping of <i>J. curcas</i> with crop rotation system-1 (peanut/ <i>Crambe abyssinica</i> /cowpea/maize)
8	IJCR-2	Intercropping of <i>J. curcas</i> with crop rotation system-2 (maize off-season/ <i>Crambe abyssinica</i> /soybean/peanut)
9	IJCR-3	Intercropping of <i>J. curcas</i> with crop rotation system-3 (cowpea/radish/maize/cowpea)
10	IJCC	Intercropping of <i>J. curcas</i> with <i>Cajanus cajan</i>
11	IJCS	Intercropping of <i>J. curcas</i> with <i>Crotalaria spectabilis</i>
12	NV	Native vegetation

Source: Prepared by the autor (2023). **Fonte:** Elaborado pelo autor (2023).

J. curcas plantation was sowed in November 2006, on Paraíso Farm using a no-tillage system, by depositing three seeds per hill. After emerging, only the most vigorous seedling was left in each hill. Planting rows were spaced at 3 m and plants were spaced at 2 m within the row, being the density of 1.666 plants ha⁻¹ (Silva, Silva, Souza, Fonseca, 2013).

After the emergence of *J. Curcas*, a manual thinning was performed, leaving one plant per pit (Silva *et al.*, 2015). During the 2006/07 and 2007/08 harvest seasons, normal crop management practices were carried out, as commonly employed for the crop (Silva *et al.*, 2015). The experimental plots were established in three-year-old jatropha crops, with an area of 120 m² (12 x 10 m), consisting of four rows with five plants per row. The useful area comprised six plants, with three in each of the two central rows.

Jatropha received topdressing fertilization in the rows during the third and fourth harvest seasons, with 32 kg ha⁻¹ of N, 80 kg ha⁻¹ of P₂O₅, and 80 kg ha⁻¹ of K₂O, using the application of 400 kg ha⁻¹ of the formula 08-20-20 (Silva *et al.*, 2015). Fertilization was carried out manually and divided into two applications, with half applied in October 2009 and the other half in March 2010. Additionally, 50 kg ha⁻¹ of N in the form of urea was applied in January 2009 and January 2010 (Silva *et al.*, 2015).

The species *Stylosanthes macrocephala* + *Stylosanthes capitata*, *Urochloa ruziziensis*, *Urochloa ruziziensis* + *Stylosanthes macrocephala* + *Stylosanthes capitata*, *Urochloa humidicola*, *Panicum maximum* 'Massai', *Cajanus cajan*, and *Crotalaria spectabilis* were established in March 2009, and the annual species were cultivated as second or summer crops, according to the rotation system sequence (1, 2, and 3).

In the intercropping system, the plots with forage species were formed by three interrows of *jatropha*, each measuring eight meters in length. The forage species were cultivated with a spacing of 0.45 m between rows (Silva *et al.*, 2012). An approximate

distance of 0.5 m was maintained on each side of the jatropha row to prevent excessive competition between plants and to facilitate harvesting and crop management activities, as described in Silva *et al.* (2012).

For the *J. Curcas*, the doses and application method followed the indications of Laviola and Dias (2008) since there are no specific fertilizer recommendations for this crop. In the treatments of rotation systems 1, 2, and 3, fertilization and crop management for the intercropped species were carried out according to the recommendations for each specific crop. The cowpea received a basal fertilization of 350 kg ha⁻¹ of the formula 08-20-20 and 20 kg ha⁻¹ of N as topdressing in the form of urea. The forage turnip received a basal fertilization of 300 kg ha⁻¹ of the formula 08-20-20 (Silva *et al.*, 2013). The maize received a basal fertilization of 350 kg ha⁻¹ of the formula 08-20-20 and 50 kg ha⁻¹ of N as topdressing in the form of urea at 45 days after emergence (Silva *et al.*, 2015). The soybean received a basal fertilization of 300 kg ha⁻¹ of the formula 00-20-20. The forage species did not receive any fertilizer.

The management of forage and cover crops was carried out through mowing with a backpack mower when the plants reached the recommended grazing height for each species. Five mowing events took place from April 2009 to March 2010, according Silva *et al.* (2012). The resulting plant residue from mowing was evenly distributed over the plot, remaining in place to serve as soil cover.

Soil samples for chemical and microbial analysis

Soil samples were done in two sampling times: February 2012 and May 2012, in 0-10 cm depth, with four samples combined by each treatment. Each composed sample was combined by five soil samples. After the homogenization of samples, they were stored in plastic bags and deposited in cool camera (40°C), to accomplish the laboratory analysis. The soil chemical analyses were determined according to Embrapa (2017), a portion of soil analysis was used for microbial analysis. The chemical properties of soil experimental area are presented in the Table 2.

Table 2. Soil chemical characterization of the experimental area.

Tabela 2. Caracterização química do solo da área experimental.

pH (H ₂ O)	H + Al -----	Ca	Mg cmolc dm ⁻³	K	CEC -----	P mg dm ⁻³	SOM g dm ⁻³	BS %
6.15	2.87	2.12	1.16	0.15	6.28	5.50	16.87	56.46

H + Al: potential acidity; Ca: calcium; Mg: magnesium; K: potassium; CEC: cation exchange capacity; P: phosphorus; SOM: organic matter content; BS: base saturation; Source: Prepared by the autor (2023). H + Al: acidez potencial; Ca: cálcio; Mg: magnésio; K: potássio; CEC: capacidade de troca de cátions; P: fósforo; SOM: conteúdo de matéria orgânica; BS: saturação por bases. Fonte: Preparado pelo autor (2023).

Microbial soil measurement

The quantification of soil microbial biomass carbon content (SMB-C) was conducted in soil microbial lab in Embrapa Western Agricultural, Brazil. The SMB-C was assessed by extraction-fumigation method Vance, Brookes and Jenkinson (1987) and Tate, Ross and Feltham (1988). The soil samples were sieved (<2 mm) and separate in three replications, each one was processed by fumigation with chloroform previously purified, followed by extraction; the second part was passed by only extraction process. The extraction of SMB-C was conducted using K₂SO₄ 0,5 M. The samples were submitted to reading in a spectrophotometry with wave length of 495 nm.

The soil basal breathing (C-CO₂) obtained by incubation of samples with CO₂ capitation with NaOH (1N) during seven days by incubation-fumigation method (Jenkinson and Powlson, 1976). The microbial metabolic quotient for CO₂ (qCO₂) was determined through the relation of basal breathing values per microbial carbon (μ CO₂ /μg MBC h⁻¹) (Anderson and Domsch, 1990), being this attribute obtained by the relation to C-CO₂/C-SMB; the microbial quotient (qMIC), which is expressed in percentage (%), was obtained

by relation of (MBC/OrgC)x100. The soil organic matter (SOM) was determined in Embrapa West Agricultural following the method proposed by Embrapa (2017).

Jatropha Curcas Yield

To determine the productivity of *Jatropha Curcas* grains, six plants were manually harvested (useful area) in each experimental plot. After harvesting, the ripped fruits were stored in paper bags and taken to the oven at 55 °C until reaching constant weight (Horschutz, Teixeira, Alves, Silva, Silva, 2012), then after this period, threshing was performed and the dry mass of grains was determined, from which the grain yield was obtained, as described in Silva, Silva, Souza, Staut and Serra (2016). The analysis of the oil content of *Jatropha Curcas* grains was performed following the Soxhlet extraction method, according to Lara, Nazario and Pregolato (1985).

The cumulative grain yield (kg ha⁻¹) was obtained by the sum of the productivity in each of the evaluated harvests from 2008/2009, 2009/2010 and 2010/2011 seasons. For each harvest, the percentage of oil was determined. The average oil content (%) was obtained by the sum of the percentage of oil in each harvest divided by the number of evaluated harvests. The accumulated oil productivity (AOP, kg ha⁻¹) from these harvests was obtained by the sum of the products between the harvest grain yield (HGY, kg ha⁻¹) and its respective oil content (OC_{season}, %), divided by 100 (Equation 1).

$$AOP = ((HGY_1 \times OC_{season\ 1}) + (HGY_2 \times OC_{season\ 2}) + (HGY_3 \times OC_{season\ 3}))/100 \quad (1)$$

Statistical analysis

The database was submitted to analysis of variance (ANOVA) and in case of significant difference (p<0.05) the means were compared by Scott-Knott test of means with the assistance of the statistical software SISVAR (Ferreira, 2014). The parameters of soil microbial biomass carbon and indices derivate were submitted to cluster analysis, using the method of neighbor more distant (complete linkage) using Euclidian distance, in order to describe the similarity between studying systems. These analyses were accomplished by assistance of the software Statistica.

3 Results and Discussion

A higher soil microbial biomass carbon content was observed in areas under *J. curcas* intercropping with *Stylosanthes campo-grande*, *B. ruziziensis* and cropping rotation systems two (CRS-2) (maize off-season/Crambe abyssinica/soybean/peanut) and cropping rotation system three (CRS-3) (cowpea/radish/maize/cowpea) (Table 3). As observed by Fernandes *et al.* (2013), the *J. curcas* intercropping with forage grasses under desertified areas resulted in increment of soil organic carbon and N of soil microbial biomass C content (SMB-C) in 0-10 and 10-20 cm depth, resulting in increasing soil organic carbon.

The inputs of intercrops increase the amount of SMB-C with higher quality of SOM activity able to increase the SOM mineralization and consequently increases in nutrients recycle in soil (Dhakai, Parajuli, Jian, Li, Nandwani, 2022).

The treatments with lower values of SMB-C content were observed in *J. curcas* monocrop, besides the intercropping with *P. maximum* cv. Massai. Silva *et al.* (2015) evaluated the growth and production of forages species under intercropping with *J. curcas* in the same region, observed that in the same growth stage *P. maximum* cv. Massai showed higher yield of dry matter, resulting in higher accumulation of biomass with higher C/N ratio. As reported by Dhakai *et al.* (2022), the biomass accumulation under soil results in increment of SOM, consequently increasing in SMB-C activity.

Soil microbial biomass carbon content showed higher amount when sampled in February, 2012 (Table 3). Such result can be attributing to greater average temperature (25°C) and rainfall associated with increasing in crops species growth under intercropping, which were cut and released the biomass aboveground. The fertilizer in the annual crops

under rotation cultivated in intercropping can be results in increasing the SMB-C activity and crop biomass production, majority because better soil fertility conditions in comparison to the absence of fertilizers and crop rotations.

Table 3. Mean test for agricultural systems and evaluation times for soil microbial biomass carbon (SMB-C), basal breathing (C-CO₂), metabolic quotient (qCO₂), microbial quotient (qMIC) and soil organic matter (SOM).

Table 3. Teste de média para sistemas agrícolas e tempos de avaliação para carbono da biomassa microbiana do solo (SMB-C), respiração basal (C-CO₂), quociente metabólico (qCO₂), quociente microbiano (qMIC) e matéria orgânica do solo (SOM).

	SMB-C	C-CO ₂	qCO ₂	qMIC	SOM
Cropping systems	µg C g ⁻¹ dried soil	µg C-CO ₂ g ⁻¹ soil day ⁻¹	µg C-CO ₂ µg ⁻¹ SMB-C h ⁻¹	%	g kg ⁻¹
<i>J. curcas</i> monocrop	124.7 c	11.1 c	37.3 b	1.5 b	13.8 b
IJS	209.6 a	20.2 a	43.4 b	2.3 a	15.7 a
IJB	205.6 a	12.6 c	27.1 b	2.1 a	16.4 a
IJBS	188.1 b	21.7 a	50.2 b	1.9 a	16.3 a
IJBH	170.8 b	16.6 b	44.1 b	1.7 b	16.9 a
IJP	109.3 c	18.6 b	77.5 a	1.2 c	15.9 a
IJCC	106.7 c	17.9 b	77.3 a	1.1 c	15.2 a
IJCS	126.7 c	18.0 b	64.2 b	1.3 c	16.4 a
IJCR-1	169.7 b	14.9 c	37.1 b	2.1 a	14.2 b
IJCR-2	205.9 a	13.4 c	32.1 b	2.5 a	14.4 b
IJCR-3	205.7 a	11.0 c	23.9 b	2.3 a	15.2 a
Native vegetation	409.7	23.6	25.9	3.4	21.4
-----Sampling times-----					
February 2012	175.8 a	14.8 b	40.2 b	2.0 a	14.9 b
May 2012	155.5 b	17.2 a	53.3 a	1.6 b	16.1 a

Means followed by the same later do not differ by Scott-Knott test of means at 5% of probability.

Source: Prepared by the autor (2023). Médias seguidas de mesma letra não diferem pelo Teste de Scott-Knott a 5% de probabilidade. **Fonte:** Preparado pelo autor (2023).

These results demonstrated the dynamic of soil microbial in function of intercropping and growing seasons. As reported by Gomes *et al.* (2021), during the dry season, part of microbial biomass died, and with the rainy season return and increment in soil moisture, the biomass survived use the organic matter accumulated in soil, resulting in higher microbial activity during rainy season.

The intercropping with *Stylosanthes campo-grande* and *Brachiaria ruziziensis*+*Stylosanthes* promoted the highest soil basal breath (C-CO₂) (Table 3). The plot cultivated with *J. curcas* intercropping with *B. humidicola*, *P. maximum* cv. Massai C. cajan and *C. spectabilis* showed medium values of C-CO₂. The soil of plots with *J. curcas* monocrop or intercropping with *B. ruziziensis* and crop rotation system 1, 2 and 3, showed the lowest values of C-CO₂ (Table 3). According to Vargas and Scholles (2000), the presence of residues aboveground, in general promote the increasing of heterotrophic microorganisms activity, where the values more expressive of C-CO₂ results in higher

biologic activity, which show a short relation to SMB-C content. However, Silva *et al.* (2015) observed significant effects in relation to basal breathing, between cover crops interaction and the sampling time, when it was evaluated the microbiologic attributes of soil under cover crops effects. According to Li, Liu, Luo and Zhang (2022), throughout the improvement of soil microbial activity efficiency, less carbon is lost with C-CO₂ by soil basal breathing, because a significant fraction of carbon is incorporated into microbial biomass tissue.

The metabolic quotient (qCO₂) is an index that expresses the relationship between soil basal breathing (C-CO₂) and SMB-C content. In this study, the values obtained in *P. maximum* cv. Massai and *C. cajan* intercropped with *J. curcas* showed higher indices of qCO₂, which was significantly higher than the other cropping systems (Table 3). Under stress conditions increases the emission of C-CO₂. However, in this research the results obtained showed lost in C-CO₂ to atmosphere, which indicated such perturbation in the environment (Álvarez-Arteaga, Fajardo, Hernández, Lezama, Matínez, 2017). Lower qCO₂ values were observed in *J. curcas* monocrop, *J. curcas* intercropping with *Stylosanthes campo-grande*, *B. ruziziensis*, *B. ruziziensis* + *Stylosanthes*, *B. humidicola*, *C. spectabilis*, crop rotation system-1, crop rotation system-2 and crop rotation system-3. According to Silva *et al.* (2015), lower values of qCO₂ indicated a more stable agroecosystem. As far as SMB-C content become more efficient in the utilization of ecosystems resources, lower C-CO₂ lost by basal breathing and more proportion of carbon incorporated into the microbial biomass tissue, consequently lower qCO₂ (Koudahe, Allen, Djaman, 2022). Among the sampling times, the evaluation conducted in May, 2012 indicated higher values of metabolic quotient (qCO₂) in relation to February, 2012. The highest values in this ratio were observed in May, according to Souza *et al.* (2020), due to the rise in microbial biomass carbon and basal respiration, which were influenced by the presence of the cover crops.

The microbial quotient (qMIC), index obtained by the relation between SMB-C/total organic C, has been used to evaluate the quality of SOM. In *J. curcas* intercropping, the cropping systems with the forages species (*Stylosanthes campo-grande*, *B. ruziziensis*, and *B. ruziziensis* + *Stylosanthes*) and the crop rotation system-1 (peanut/Crambe abyssinica/cowpea/maize), crop rotation system-2 (maize off-season/Crambe abyssinica/soybean/peanut) showed higher values of qMIC, followed by *J. curcas* monocrop and intercropping with *B. humidicola*. The lowest values were observed in the soil under *J. curcas* intercropping with *P. maximum* cv. massai, *C. cajan* and *C. spectabilis* (Table 3). All the cropping system, as well as, native vegetation showed microbial quotient above 1%, indicating possible increment in soil C throughout time, which C values are in accordance to (Arantes *et al.*, 2020). According to these authors, an expected outcome for a soil in equilibrium. Lowest values were observed in the soil under *J. curcas* intercropping with *P. maximum* cv. massai, *C. cajan* and *C. spectabilis*, according Souza *et al.* (2020), possibly reflect higher efficiency of the microbial biomass in the use of organic C, meaning that less C is lost as CO₂ through respiration and more C is incorporated into microbial cells. However, this outcome may not directly reflect an increase in agricultural productivity (Souza *et al.*, 2020).

Among the soil samples, in the evaluation conducted in the months of February, 2012, the qMIC was significantly higher in relation to the evaluation conducted in May, 2012, indicated higher potential of SOM accumulated during rainy season and when there are higher biomass production by intercropping systems.

The *J. curcas* intercropping with *P. maximum* cv. Massai and *C. cajan* promoted decreasing of SOC, proved by higher values of qCO₂ and a little ratio of qMIC, that associated showed lower soil microbial activity and lower nutrient cycle. In previews research conducted in the same experimental area, Silva *et al.* (2012) observed that the species of *C. cajan* under intercropping with *J. curcas* resulted in lower dry matter production in comparison to the other cropping systems evaluated. Due to lower C/N ratio, the SOM degree of decomposition from legumes species are faster in comparison to forage grasses (Silva *et al.*, 2015).

As reported by Simon *et al.* (2019), the features of cover crops in biomass production and its effects resulted from soil residues are important to determine the strategy of management to achieve sustainable production systems. The highest values of SOM were observed in the treatments under *J. curcas* intercropping with *Stylosanthes campo-grande*, *B. ruziziensis*, *B. ruziziensis* + *Stylosanthes*, *B. humidicola*, *P. maximum* cv. Massai, *C. cajan*, *C. spectabilis* and crop rotation system-3 (Table 3), directing that these treatments can show the balance of required nutrients in soil for plant growth. The highest part of SOM shows a stable fraction and resistant to alterations, which changes in this soil fraction can take long years to observe changes. However, in the second sampling time showed higher values than the first, because the highest biomass production occur in spring and summer seasons associated with the period that *J. curcas* shows the highest leaf area, right after the *J. curcas* leaves die and fall on the ground resulted in faster process of SOM mineralization with the biomass of the intercropping, which increased the SOM from 14.9% to 16.1%.

In relation to cluster analysis, which was possible to arrange cropping systems based on its similar features, resulted in formation of two interpretable groups (A and B) (Figure 3). These groups showed any similarity each other, once its linked distance is 100%. In group (A), there was formation of two levels of clusters, the first englobe (B2) the *J. curcas* intercropping with *Stylosantes campo grande* (JIS), *B. ruziziensis* (JIBR), crop rotation system-2 (CRS-2), crop rotation system-3 (CRS-3), *B. ruziziensis* + *Stylosanthes* (JIBRS), *B. humidicola* (JIBH), crop rotation system-1 (CRS-1) with 92% of similarity. Based on cluster analysis, these cropping systems (JIS, JIBR, CRS-2, CRS-3, JIBRS, JIBH and CRS-1) favored the SMB-C content, resulting in similarity with the native vegetation. Panico *et al.* (2022) in evaluation of soil microbial variables confirmed this similarity of cover crops with native vegetation. Thus, the residues on the ground affect positively the soil microbial activity and soil quality. The second cluster (B1) composed by *J. curcas* intercropping with *C. spectabilis* (JICS), *P. maximum* cv. Massai (JIPM), *C. cajan* (JICC) and *J. curcas* monocrop (JM). The formation of this cluster (B1) indicated that the effects of these cropping systems (JICS, JIPM, JICC and JM) show SMB-C content equally compared *J. curcas* monocrop, which was covered just by spontaneous vegetation, showing higher stress condition of SMB-C content. As reported by Luan *et al.* (2023), the monocrop over time results in decreasing of soil quality. Consequently results in losing of nutrients and become the system with low sustainability. The principal components analysis (PCA), ordering the data in the biplots (Figure 4), including cropping systems and microbial properties, explained 93% of the original variability, where PC-1 and PC-2 retained 57% and 36%, respectively. Thus, it was possible to observe in the biplots different positions of the cropping systems. The native vegetation grouped with the soil organic matter (SOM) and with the basal breathing (C-CO₂), which probably, in the absence of the anthropogenic interferences, allied to the accumulation of litter on the soil surface, may have contributed to this result.

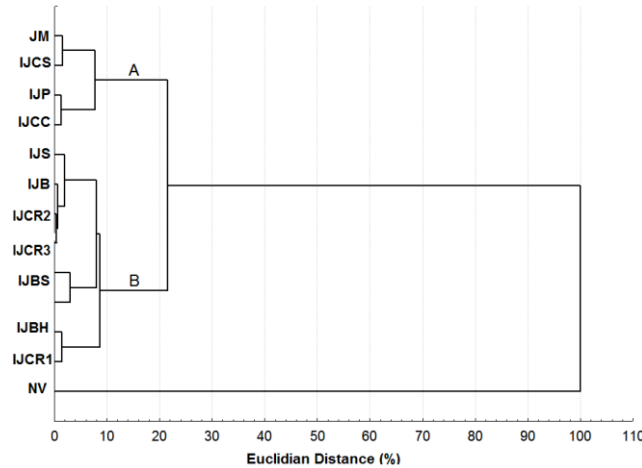
Therefore, it can be inferred that different *J. curcas* intercropping with forages and grain crops species favored the dynamic equilibrium of the soil microbial biomass carbon content and consequently the conservation of the environment.

The influence of the systems with forage crops and grain-producing crops under intercropping with *J. curcas*, in three harvests, was observed on the accumulated grain yield and oil yield (Table 4).

The lowest cumulative grain yields of *J. curcas* were observed in the intercropping systems with *Stylosantes*, *U. ruzizienses*, *U. ruzizienses* + *Stylosantes*; *Panicum maximum* 'Massai', *U. humidicola*, *Stylosantes*, *Crotalaria spectabilis*, rotation system 1, with *Cajanus cajan*, while the *J. curcas* (single), rotation system 2 and rotation system 3 stood out with higher cumulative grain yields.

Figure 3. Dendrogram of dissimilarity of soil microbial biomass carbon content (SMB-C), and derivate indices in intercropping with forages and crops species under two sampling times.

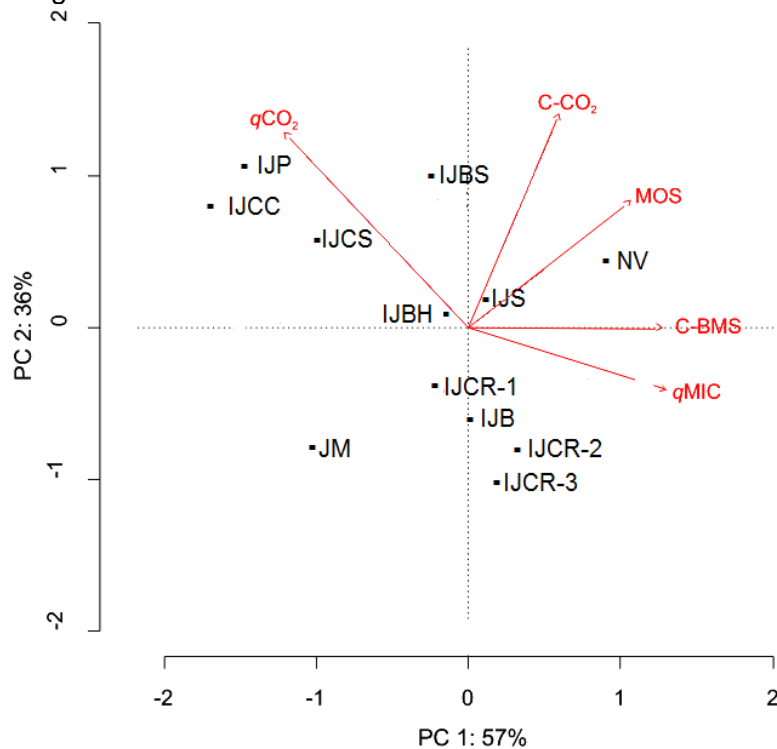
Figura 3. Dendrograma de dissimilaridade do conteúdo de carbono da biomassa microbiana do solo (SMB-C) e índices derivados em consórcio com espécies forrageiras e culturas em duas épocas de amostragem.



Source: Prepared by the autor (2023). **Fonte:** Elaborado pelo autor (2023).

Figure 4. Biplots of soil of microbial attributes in the treatments with *J. curcas* intercropping with forages.

Figura 4. Biplots de atributos microbiológicos do solo nos tratamentos com *J. curcas* em consórcio com forrageiras.



Source: Prepared by the autor (2023). **Fonte:** Elaborado pelo autor (2023).

Table 4. Average of accumulated productivity of grains and accumulated oil productivity in different systems of intercropping with *J. curcas* in the harvests 2008/2009, 2009/2010, 2010/2011.

Tabela 4. Média de produtividade acumulada de grãos e produtividade acumulada de óleo em diferentes sistemas de consórcio com *J. curcas* nas safras 2008/2009, 2009/2010, 2010/2011.

Cropping systems	Cumulative grain yield		Accumulated oil productivity	
	kg ha ⁻¹		kg ha ⁻¹	
JM	947	a	272	a
IJS	712	b	228	b
IJR	576	b	211	b
IJBS	660	b	217	b
IJBH	612	b	213	b
IJP	585	b	204	b
IJCC	738	b	247	b
IJCS	775	b	254	a
IJCR-1	799	b	264	a
IJCR-2	1012	a	300	a
IJCR-3	923	a	275	a
Average	758		244	
C.V. (%)	16,8		15,4	

Means followed by the same later do not differ by Scott-Knott test of means at 5% of probability.

Source: Prepared by the autor (2023). Médias seguidas de mesma letra não diferem pelo Teste de Scott-Knott a 5% de probabilidade. **Fonte:** Preparado pelo autor (2023).

This decrease in accumulated grain yields of *J. curcas* in intercropping system can be attributed to competition for water, light and nutrients (Silva *et al.*, 2015; Silva *et al.*, 2016). On the other hand, in Rotation 2, higher cumulative *J. curcas* grain yield was observed (1,012 kg ha⁻¹), although it is statistically similar to that obtained for single *J. curcas* (947 kg ha⁻¹); this productive potential is in agreement with results obtained by Maftuchah *et al.* (2020) when evaluating the performance of 6 cultivars during 5 harvests. However, soil properties can affect the grain yield of *J. curcas*, which can result in high diversity of grain yield in different regions (Silva *et al.*, 2016). It is emphasized that there are several factors that affect the level of *J. curcas* production and those most dominant are altitude and rainfall per year. Rainfall is the most important factor in *J. curcas* sowing, and the level of rainfall suitable for the cultivation of this crop is about 300 - 1000 mm yr⁻¹ (Maftuchah *et al.*, 2020), however this did not represent a limiting factor during the period of conducting this study, since the accumulated rainfall in the three harvests was total precipitation was 2.607,2 mm, according to Guia Clima (www.cpa0.embrapa.br/clima/).

It is noteworthy that the higher productivity of *J. curcas* achieved in Rotation 2 system is possibly due to the lower competition with the corn crop in summer, which had low biomass accumulation and grain productivity, a result of competition for light, when in consortium with *J. curcas* in 3 x 2 m spacing (Silva *et al.*, 2015).

When analyzing the accumulated oil productivity, in three harvests, there was an influence of the cropping systems with the highest value for *J. curcas* in monoculture, the three rotation systems and *C. spectabilis*. It is noteworthy that the lowest value in the intercropping with *Panicum maximum* 'Massai' can be attributed to the fact that this forage has greater productive capacity and tolerance to shading than other species, when intercropped with *J. curcas* (Silva *et al.*, 2015), therefore, competing with this crop. Other factors are related to the productivity of the crop, including soil fertility, and it is possible that during the three harvests the cycling of nutrients or the effects of soil cover by cultural residues have been little significant in relation to the management performed in the rotation systems, mainly. In addition, it should be noted that the *J. curcas* is characterized

by senescence and leaf abscission in the drier or colder periods of the year, and, from the third year of cultivation, there may be the deposition of 1,216 kg ha⁻¹ of dry phytomass, and the amount of nutrients deposited between the rows, by the abscised leaves is about 1.5; 15; 23 and 14 kg ha⁻¹ of P, K, Ca and Mg, respectively (Kurihara, Kikuti, Binotti, Silva, 2016).

4 Conclusion

The *J. curcas* intercropping with forage and grain crops species change the soil microbial biomass carbon content, increasing the soil organic matter dynamic. *J. curcas* intercropping with *P. maximum* cv. Massai, *C. cajan* and *C. spectabilis* promoted decreasing in SOM dynamic in comparison to the others cropping systems.

Intercropping with *Stylosanthes campo-grande*, *B. ruziziensis* and crop rotation system-2 with maize off-season/Crambe abyssinica/soybean/peanut and crop rotation system-3 cowpea/radish/maize/cowpea stimulated the maintenance of microorganisms' community in soil.

The mono cropping systems of *J. curcas* and intercropping in rotation systems 2 and 3 achieved higher yields of *J. curcas* grains and oil over three harvests.

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