

Soil microbiological indicators in *Coffea arabica* crops under different weed management strategies

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Abstract

Microbiological variables can be sensitive indicators of changes in the physicochemical properties of soils resulting from disturbances caused by practices of weed management. The objective of this work was to evaluate the impact of weed management strategies on the soil, using microbiological indicators over four-year time measures (two in the dry and two during the rainy weather season). The experiment was carried out in a coffee plantation field (*Coffea arabica*), in a randomized block design with five treatments. The treatments corresponded to the cultivation of *Urochloa ruziziensis* and *Pueraria phaseoloides* managed by mowing, maintenance of spontaneous vegetation and control by mowing, control of spontaneous vegetation by application of herbicides (glyphosate or glyphosate + 2,4-D), and manual weeding using a hoe to maintain the soil without vegetation cover. Soil C – CO₂ evolution, basal respiration (RR), biomass carbon (C_{bio}), and metabolic quotient ($q\text{CO}_2$) were evaluated. Treatments with *U. ruziziensis* and *P. phaseoloides* presented lower $q\text{CO}_2$ values, indicating more stable systems and closer to equilibrium. C_{bio} was higher in the treatment with *P. phaseoloides*, indicating better soil fertility and carbon input conditions. The microbiological indicators allowed us to conclude that management methods that form a cover over the soil favor the maintenance of soil quality and the sustainability of the system.

1. Introduction

Coffee growing is an activity of great economic and social importance, being responsible for generating employment, and income and securing labor in the countryside. Coffee cultivation demands different agricultural practices, among which weed management stands out. The crop is extremely susceptible to competition for resources such as water, light, and nutrients, especially during the crop formation period and in the flowering and fruiting phases, impairing production (Melloni et al., 2018). Therefore, the need to manage the weed community is constant, and control strategies can be adopted through manual methods (weeding with a hoe), mechanized (mowing), and/or chemical (application of herbicides) (Ronchi & Silva, 2018).

However, when these strategies are improperly exploited, they can cause serious imbalances in the soil environment, leading to erosion and reduced soil water infiltration and storage capacity, associated with disturbances in the microbial community (Guimarães et al., 2017). Microbial biomass (BM), formed by microorganisms, such as bacteria, fungi, protozoa, algae, and microfauna, is the living part of soil organic matter, responsible for controlling essential functions such as waste decomposition and humus formation. (Totola & Chaer, 2002). BM is also involved in the processes of transformation and cycling of nutrients and the degradation of agrochemicals in the soil (Matos et al., 2019). For this reason, microbial biomass is an important indicator of the state and changes in total soil organic matter (Melloni et al., 2018).

Basal respiration rate (RR) and metabolic quotient ($q\text{CO}_2$) are also considered microbiological indicators of soil quality. The RR is a measure of the amount of CO₂ released by microorganisms' respiration per

unit of time and reflects their metabolic activity. High values of RR may indicate ecological disturbance, such as those resulting from the application of agrochemicals, or a high level of ecosystem productivity (Guimarães et al., 2017). The metabolic quotient ($q\text{CO}_2$) was proposed by Anderson and Domsch (1985) and corresponds to the respiratory rate per unit of biomass. This variable predicts that efficient biomass in the use of ecosystem resources causes less carbon to be lost in the form of CO_2 through respiration and a greater proportion of C to be incorporated into microbial tissues (Tótolá & Chaer, 2002). In general, soil disturbance and stress factors will induce changes in these indices, leading to reduced microbial efficiency.

Some weed control practices can be considered as stress factors in the soil, exerting an influence on physical, chemical, and biological properties (Babin et al., 2019). Conventional and conservationist weed control practices have been studied to contribute to the sustainability of the soil-plant system and the balance of the environment (Melloni et al., 2018; Babin et al., 2019).

Conventional weed control practices carried out through mechanical methods, such as manual weeding, and chemically with the application of herbicides, keep the soil unprotected and can result in erosive processes and loss of organic matter, reflecting directly on the biomass and microbial activity. While conservationist practices, which keep the soil covered with plant material (living cover and straw), reduce heating and increase the water storage capacity in the soil and tend to increase the population and activity of microorganisms, as well as favoring the attributes of soil physical and chemical properties (Simon, 2021). In coffee cultivation, few studies relate weed control strategies with soil conservation (Alcântara; Nóbrega; Ferreira, 2009; Glaeser et al., 2011; Zaidan et al., 2022), especially those carried out for longer periods. In this context, the objective of this work was to evaluate the impact of weed management strategies on the soil between the rows of coffee plantations over three years using microbiological indicators.

2. Material And Methods

2.1. Studied Area Characterization

The experiment was carried out in the field at the Teaching and Research Unit of “Horta Velha” on the Campus of the Federal University of Viçosa, in the municipality of Viçosa-MG, located in the Zona da Mata of Minas Gerais, at $20^{\circ}45'14''$, latitude S., $42^{\circ}52'54''$, longitude W and 680 m altitude. The climate of the region is classified in the Köppen-Geiger system as *Cwa*, with a cold and dry winter and a hot and rainy summer (Martins et al., 2018), with an average temperature (average of 20 years) of 19.4°C . (maximum 26.4°C and minimum 14.8°C) and average annual rainfall of 1221 mm (INMET, 2020). The soil in the area was classified according to the Brazilian Soil Classification System (SiBCS) as dystrophic Red Yellow Argisol, with a clayey texture (Table 1).

Table 1
Physical and chemical characterization of the soil in the experimental area.

ID	pH (H ₂ O)	H + Al ³⁺	Al ³⁺	Ca	Mg	K	P	M.O.
		 cmol _c dm ⁻³				 mg dm ⁻³		dag kg ⁻¹
0–20 cm	6,2	3,0	0	3,1	1,0	115	14,8	3,2
ID	SB	CEC(t)	CEC(T)	P-rem	V	m	Argila	Areia
		 cmol _c dm ⁻³		mg L ⁻¹	 %		 %	
0–20 cm	4,4	4,4	7,7	29,8	57	0	43	44
ID: identification; SB: Sum of exchangeable bases; CEC (t): Effective cation exchange capacity; CEC (T): Cation exchange capacity pH 7.0; V: Base saturation index; m: Aluminum saturation index.								

In the year of installation of the experiment, the crop was four years old after receiving, cultivar Oeiras of the species *Coffea arabica* L., with plants spaced 2.80 m between rows and 0.75 m between plants. Initially, a necessary liming was made to raise the base saturation to 70% and later coverage fertilizations were made with 750 grams of NPK per plant, divided into three times a year: November, December, and February, in the three years of experimenting. Being 150 g of N, 37.5 g of P₂O₅, and 150 g of K₂O per year. The coffee grain production was measured within experimental plots and converted to grain productivity in terms of kg ha⁻¹ (please see Zaidan et al., 2022 for more technical details).

2.2. Experimental Design and Soil Covering Treatments

The experiment consisted of five treatments arranged in a randomized block design with six replications. Adopting the experimental model in split plots in time, in which treatments × blocks were evaluated in the plot, in the time subplot (2017, 2018, and 2019). Each experimental unit consisted of three rows of coffee (two rows) with 7.5 m in length, where treatments were carried out involving weed management strategies: cultivation of brachiaria (*Urochloa ruziziensis*) managed using mowing; cultivation of *Pueraria phaseoloides* managed using mowing; control of spontaneous vegetation through the application of the herbicide glyphosate or glyphosate + 2,4-D; maintenance of spontaneous vegetation controlled by clearing and the soil kept “clean” by weeding (i.e., uncovered soil). Weed control in the planting row was performed by hand weeding using a hoe.

Before the implementation of the treatments, the crop, which had been managed using mowing for more than four years, was submitted to desiccation between the lines, using glyphosate (1.08 g ha⁻¹) and 2.4-D (0.536 g ha⁻¹), in November 2016, to homogenize the vegetation in the area and enable the implementation of treatments with *U. ruziziensis* and *P. phaseoloides*.

The sowing of brachiaria was carried out manually, in two furrows spaced 0.40 m apart, between the coffee rows, distributing one gram of seeds, with a cultural value of 40%, per linear meter of the furrow, in

December 2016.

The management strategy with *P. phaseoloides* was implemented through seedlings obtained from vegetative propagation via rooting herbaceous cuttings in polyethylene trays containing substrate (soil + sand + cattle manure), kept in a greenhouse with programmed micro-sprinkler irrigation. After the rooting period (approximately three months), they were taken to the field and transplanted at a spacing of 0.5 m in the center between the coffee rows in December 2016.

In the experimental plots where the control of spontaneous vegetation was carried out through the application of herbicides, glyphosate (1.08 g ha^{-1}) or glyphosate + 2,4-D ($1.08 \text{ g ha}^{-1} + 0.536 \text{ g ha}^{-1}$), applications were carried out twice a year, after the establishment of the plants at the beginning of the rainy season (November or December) and in the period before the harvest (February/March), which corresponds to the end of the rainy season. The application of the combination of the herbicide glyphosate + 2,4-D was carried out only once, this being to contain the increase in the density of *C. bonarienses*, the other herbicide applications were carried out with isolated glyphosate.

Applications were carried out using a CO_2 pressurized backpack sprayer, calibrated at a constant pressure of 250 kPa, equipped with a bar, with two air induction fan spray nozzles (TTI 11002) spaced 50 cm apart, which provided the application of 200 L ha^{-1} of solution. At the time of application, the air temperature ($25^\circ\text{C} \pm 2$), relative humidity ($80\% \pm 5$), and wind speed ($3 \text{ km h}^{-1} \pm 2$) were measured.

An average of four annual mowings were carried out in the experimental units maintained with spontaneous vegetation. These mowings were carried out only when the weeds interfered with the coffee plant and had sufficient fresh mass to cover the ground after cutting ($\pm 50 \text{ cm}$ in height) and the treatment where the manual weeding process was adopted, weeding was carried out whenever the weeds were between 5 and 10 cm high, to leave the soil clean.

2.3. Collection of Soil Samples

The first collection of soil samples was carried out in March 2017 (Season 1 – rainy season). After one year, the period necessary for the establishment of all treatments, the second collection was carried out in May 2018 (Season 2 - dry season), and the third and fourth collections were carried out in the months of December 2018 (Season 3 - rainy season) and August 2019 (Season 4 – dry season), respectively, according to the rainfall regime to identify the rainy and dry seasons. The data of average temperatures (maximum and minimum) and precipitation in the experimental area were registered during the months of soil collection during the experimental period.

Samples were randomly collected in each experimental unit's two central rows (between the coffee lines) (three per line). At each point, simple soil samples were taken at a depth of 0–10 cm using a sampling probe-type auger. The six simple samples were mixed, forming a composite sample of 200 g per plot, totaling 30 composite samples, properly identified and packed in polyethylene plastic bags and kept in

styrofoam boxes, for approximately two hours, at room temperature until they were taken to the processing in the laboratory.

The thirty composite samples were air-dried and passed through 4 mm mesh sieves, and after this process, manual cleaning was performed to remove root remains. Going further, 50 g of soil was removed from each sample to determine the degree of moisture, by drying it in an oven at 105°C until constant weight (Claessen, 1997).

2.4. Microbiological Analysis

The microbiological analyzes were carried out at the Herbicide Laboratory in the Agronomy Department of the Federal University of Viçosa (UFV). Soil-evolved C-CO₂ was measured by the respirometric method in a continuous flow system as proposed by Curl and Truelove (1986).

To determine the evolution of soil CO₂, samples of 100 g of dry soil were weighed in six replicates per treatment, which were moistened up to 60% of the field capacity. The flasks containing the samples were connected to flasks with NaOH solution (0.5 mol L⁻¹) through silicone hoses and incubated for 10 days in hermetically sealed flasks. Pressure regulation registers were adjusted to release 15 to 20 bubbles per minute in 100 mL of NaOH solution (0.5 mol L⁻¹). To control the quality of the air carried, flasks without soil were used, being the samples of the “witness” in four replicates.

2.4.1. Basal Microbial Respiration Rate (RR)

After 10 days of incubation in the respirometer, 10 mL aliquots of NaOH solution (0.5 mol L⁻¹) from all samples placed in the bubble were transferred to Erlenmeyers, added with five mL of BaCl₂ and three drops of phenolphthalein to titration with HCl solution (0.5 mol L⁻¹) until the color changes from pink to colorless to estimate the evolved C – CO₂. The amount of CO₂ evolved from each sample was calculated in µg g⁻¹ C – CO₂/100cm³ of soil, according to the equation (Eq. 1) proposed by Stotzky (1972):

$$C - CO_2 = RR = (B - V) \times M \times 12 \times (v_1/v_2), \quad [\text{Eq. 1}]$$

wherein, B is the Volume of HCl consumed in the blank sample (mL); V is the Volume of HCl consumed in the sample (mL); M is the actual concentration of HCl (mol L⁻¹); the constant ‘12’ is the carbon equivalent weight; v_1 is the total volume of NaOH used to capture CO₂ (mL); and v_2 is the volume of NaOH used in the titration (mL).

2.4.2. Soil Microbial Biomass Carbon (C_{bio})

C_{bio} analysis was performed according to the fumigation-incubation methodology described by Vance et al. (1987) and modified by Islam and Weil (1998). From the 100 g of soil from the incubated samples,

subsamples (18 to 19 g) were taken in duplicate. These subsamples were weighed in Erlenmeyer flasks and divided into fumigated (placed in a microwave oven for two minutes) and non-fumigated.

After fumigation, 80 mL of K₂SO₄ extracting solution (0.5 M) was added to all samples, including the “blank” samples. Then, these samples with the K₂SO₄ solution were mixed and taken to shake at maximum rotation (240 RPM) on a Pendular Shaking Table with Timer NT 156, for 30 minutes. After this period, the flasks were left to rest for 30 minutes to decant the soil, then the supernatant was removed from the solution for filtration.

From each sample, 10 mL of filtered extract were removed and placed in test tubes, where 8 drops of potassium dichromate (K₂Cr₂O₇ – 0.06667 mol L⁻¹) and 10 mL of concentrated sulfuric acid were added. The test tubes were left to cool for 15 minutes, after which the contents of the tubes were transferred to Erlenmeyers, 10 drops of Ferroin were added, and taken for titration with a previously prepared solution of ferrous sulfate with sulfuric acid. The values of carbon present in the microbial biomass were calculated from the following equations (Eq. 2, 3, and 4):

$$C_{i,ni} = \frac{(3)(1000)(V_b - V_{am})(\text{ferroussulfatemolarity})(\text{extractorvolume})}{(\text{extractorvolume})(\text{soilweight})}, [\text{Eq. 2}]$$

wherein, V_b is the volume of titrant consumed in the blank sample (mL); V_{am} is the volume of titrant consumed in the sample (mL); the constant ‘3’ is the result of the ratio between the number of mols of Cr₂O₇, that react with C⁰ (3/2) multiplied by the equivalent weight of C (i.e., 12); and the constant ‘1000’ is the unit conversion factor.

$$C_{bio} = \frac{(C_i - C_{ni})}{Kc \mu g g^{-1} \text{soil} C}, [\text{Eq. 3}]$$

wherein, C_i is the irradiated sample; C_{ni} is the non-irradiated sample; and Kc was adopted as 0.33 for all irradiation methods to extract carbon from biomass.

2.4.3. Metabolic quotient or specific respiratory rate ($q\text{CO}_2$)

From the values obtained from the evolution of C – CO₂ and C_{bio}, the $q\text{CO}_2$ (μg⁻¹ CBM d⁻¹) of each sample was calculated, by the ratio between microbial activity, daily averages of C – CO₂ evolved from the evaluated soil samples, and estimated exchange rate of each sample, according to (Anderson; Domsch, 1985).

$$q\text{CO}_2 = \frac{C - \text{CO}_2 (\mu g C - \text{CO}_2 g^{-1} \text{soil} d^{-1})}{C_{bio} (\mu g C - g^{-1} \text{soil})}, [\text{Eq. 4}]$$

2.5. Statistical analysis

Analysis of variance (ANOVA) of the data was performed according to the model for split-plot experiments over time. In the plot, treatments × blocks were evaluated, in the time subplot (2017, 2018, and 2019). Management strategies within the year were compared using Tukey's test at 5% probability.

Alternatively, to compare the variation of the means, the standard errors of the mean were also confronted and the overlaps between the intervals were verified. For this, the variables basal respiration rate (RR), microbial biomass carbon (C_{bio}), and metabolic quotient ($q\text{CO}_2$) were evaluated for each treatment and over four measurement moments.

To group the experimental units (treatments $\times$ repetitions) according to the three variables obtained through the respirometric method (C_{bio}, $q\text{CO}_2$, and RR), the Principal Component Analysis (PCA) method was applied. Three approaches were adopted: i) taking the three variables at the four measurement moments (12 variables); ii) only the variables measured in the rainy season (two seasons, thus accounting for 6 variables); iii) only the variables measured in the dry season (two seasons, thus accounting for 6 variables).

To evaluate the effect of microbiological indicators (C_{bio}, $q\text{CO}_2$, and RR) on coffee productivity, simple linear regression and LOESS regression (Locally Estimated Scatterplot Smoothing) was performed, to evaluate the linear relationship and the free trend relationship, respectively. Two moments were used: sampling in May 2018 (dry season) and immediately close to the productivity of the coffee harvested in May 2018; sampling in December 2018 (rainy season) and productivity of coffee harvested in May 2019. To assess significance, the F statistic for the slope or slope coefficient (i.e., the β_1) of the linear regression is shown.

3. Results And Discussion

The weed management strategies adopted did not lead to significant interactions with the basal respiration rate (RR) and metabolic quotient ($q\text{CO}_2$) variables (Table 2). Similar results have been reported in the literature (Melloni et al., 2018), indicating that these variables were little influenced by different weed community control methods. These results may indicate low soil microbiota dynamics or low sensitivity of microbiological indicators to the effects of the management strategies adopted (Melloni et al., 2018).

Table 2

Analysis of Variance (ANOVA) depicting the Mean Squares for the three microbiological indicators measured from the respirometry analysis, and considering a split-plot model in time

F.V.	D.F.	RR	Cbio	$q\text{CO}_2$
Block	5	0.1813	25007	4.183
Treatment	4	0.2135	16811	6.758
Time	3	3.0700 ***	178030 ***	45.670 ***
Time $\times$ Treatment	12	0.1278	39523 ***	6.230
Residual A	20	0.2388	12846	4.836
Residual B	75	0.2954	11293	6.970
Average		0.574	259.009	2.702
CV_A (%)		85.16%	43.76%	81.38%
CV_B (%)		94.72%	41.03%	97.70%
F.V.: Factor of Variance; D.F.: Degrees of Freedom; Residual A: Between plots; Residual B: Within plots (over time). RR: Basal respiration rate; Cbio: Carbon from microbial biomass; $q\text{CO}_2$: Metabolic quotient of soil microbiota.				

The treatment $\times$ time interaction was significant at the 5% probability level for the variable Cbio (Table 2). The treatment with *P. phaseoloides* showed the highest average of Cbio present in the soil at season 3 (Fig. 1). Higher Cbio values indicate temporary immobilization of nutrients, resulting in lower nutrient losses in the soil-plant system (Matos et al., 2019).

The accumulation of dry matter in the experimental units with *U. ruziziensis*, *P. phaseoloides*, herbicide application, and spontaneous vegetation (Table 3) generated the contribution of organic matter, which may have favored the greater development of microbial biomass in these experimental units when compared with to the management strategy by hand weeding (Fig. 1). Plant residues serve as a source of energy and nutrients for soil organisms and plant diversification in agricultural systems has been reported to have a positive influence on microbial biomass through the deposition of plant residues on the soil (Hoffmann et al., 2018).

Table 3

Number of weed species, total density (plants per m²), the value of the dry matter, and accumulated dry matter (g/m²) of weeds present between the coffee rows of each management strategy, measured at different times based on the pluviometric regime of the experimental area.

Measurement Periods				
TREAT	E1	E2	E3	E4
	Number of weed species			
UR	16	1	6	5
PP	18	1	7	4
HB	22	15	19	24
VE	20	9	15	17
CA	18			
	Density (plant m ⁻²)			
UR	173,67	3,33	60	21,33
PP	181,33	2	12	7
HB	180	66,33	32,33	219,67
VE	247	141	29	186,33
CA	178,67			
	Dry matter (g m ⁻²)			
UR	1.734,27 (1.093)	776,4	922,37 (604,89)	1.108,79 (941,65)
PP	752,21	(380,93)	(663,99)	(688,29)
HB	1.049,39	691,3	903,64	1.202,84
VE	1.176,37	666,29	626,42	718,28
CA	963,89			
	Accumulated dry matter (g m ⁻²)			
UR	1.734,27 (1.093)	7.903,39 (5.534,77)	9.543,77 (6.857,39)	10.652,56 (7.799)

UR: *Urochloa ruziziensis* managed by mowing; PP: *Pueraria phaseoloides* managed by mowing; HB: herbicide application; VE: spontaneous vegetation managed by mowing; CA: hand weeding. E1 and E3: periods in which soil collection was carried out in the rainy season; E2 and E4: periods in which soil collection was carried out in the dry season. The values in parentheses represent the amount of dry matter produced by the species *U. ruziziensis* and *P. phaseoloides* in their respective experimental units.

Measurement Periods				
PP	752,21	1.695,63 (943,42)	2.359,62 (1.607,41)	3.855,07 (2.959,7)
HB	1.049,39	1.740,69	2.644,33	4.461,08
VE	1.176,37	4.082,26	4.708,68	6.149,36
CA	963,89			
UR: <i>Urochloa ruziziensis</i> managed by mowing; PP: <i>Pueraria phaseoloides</i> managed by mowing; HB: herbicide application; VE: spontaneous vegetation managed by mowing; CA: hand weeding. E1 and E3: periods in which soil collection was carried out in the rainy season; E2 and E4: periods in which soil collection was carried out in the dry season. The values in parentheses represent the amount of dry matter produced by the species <i>U. ruziziensis</i> and <i>P. phaseoloides</i> in their respective experimental units.				

The manual weeding treatment had the lowest mean Cbio value (Fig. 1). From the point of view of conservation of microbial biomass, weeding showed the worst result depending on the time of year, climatic conditions affected the balance of the ecosystem. The removal of weeds and the maintenance of the soil without cover, ie, without accumulation of dry matter (Table 3) combined with the climatic variations of the experimental period contributed to the sharp decrease in soil microbial biomass.

The condition of the soil maintained uncovered (i.e., “in the clean soil”) through manual weeding tends to generate an environmental condition of stress for the microbiota, resulting in less carbon entry into the soil and less microbial biomass. According to Silva et al. (2021), the variation in microbial biomass carbon occurs as a function of the type of vegetation cover used for phytomass production and its ability to protect the soil. Bare soils tend to damage microbial activity and reduce biomass carbon and nutrient cycling. The other treatments did not differ when compared within time 3 of sample collection (Fig. 1). The time of year was important for the response of microbiological indicators to weed control strategies (Fig. 1). In the seasons in which there was a shortage of rain (seasons 2 and 4), the variables basal respiration rate, biomass carbon, and metabolic quotient were statistically equal. The stress caused by the water deficit may have overcome the effects of the management strategies adopted (Fig. 1).

The short period between the implementation of the experiment and the performance of the first two samplings may have caused the absence of difference in microbial activity as a function of the management strategies adopted. Likely, the microbiological indicators did not show sensitivity to the disturbances caused by the treatments during the first year of evaluation due to the period of establishment of the cultures (Fig. 1).

The biomass carbon, basal respiration rate, and metabolic quotient variables did not show significant interaction between different cropping systems and evaluation times according to Guimarães et al. (2017). The adaptation of soil microbiota to environmental changes occurs gradually over time. Similar results were also obtained by Guimarães et al. (2017) who found no interaction between soil microbial activity and evaluation times and different organic coffee cultivation systems. In addition, in the first two

measurement moments, the weed diversity of the experimental units was higher than in seasons 3 and 4 (Table 3), which may also have contributed to the greater variability of the microbiological data.

Weeds can modify the structure of the soil microbial community, through the exudation of different combinations of organic compounds from the roots, the deposition of their plant material on the soil surface, and competition for nutrients (Matos et al., 2019). The high diversity and density of weeds may have caused greater competition among them, which may result in greater degradation of organic matter. The microbiota associated with the rhizosphere is sensitive to changes in the concentration of nutrients and carbon in the soil. Degradation of organic matter by weeds reduces the contribution of carbon to the soil, creating a stress condition for the soil microbial community (Matos et al., 2019).

In seasons 1 and 2 the weed density in the experimental units showed a higher value when compared to the density in seasons 3 and 4 (Table 3). The greater number of weeds per square meter can cause greater damage related to competition for water between weeds, culture, and microorganisms depending on environmental conditions, which can result in stress on the soil microbiota (Matos et al., 2019). Therefore, it is necessary to emphasize the importance of adopting integrated weed management, taking into account the rotation of control methods such as mowing, maintenance of vegetation between the rows in times of higher rainfall for the formation of cover, and the application of herbicide itself. at times when competition for water is greatest. The choice of the most appropriate method will depend on the dynamics of the weed population in the area.

In this research, two annual applications of the herbicide glyphosate between the rows of coffee plants did not affect the microbial activity of the soil (Table 2). Demonstrating that the use of this method as an alternative to the rotation of weed control methods does not make production unfeasible in terms of soil quality and crop sustainability. Microbiological indicators, biomass carbon, and microbial activity are extremely sensitive to the method of weed control by frequent applications of herbicides between the coffee rows (Marafeli et al., 2019; Silva et al., 2021). In other words, the use of chemical control can be a good alternative for weed management, as long as it is not adopted in an isolated and frequent way. Ideally, it should be used as an alternative strategy and in rotation of weed management methods.

The significance of qCO_2 in the time $\times$ treatment interaction was not detected (Fig. 1), as the mean square of the residue is overestimated due to the great variability of the mean values of basal respiration rate (RR), biomass carbon (C_{bio}) and metabolic quotient (qCO_2) of the management strategies evaluated in seasons 1 and 2 (Table 3). When the mean square of the residue is large, the F statistic of any treatment decreases (QM_{Inter}/QM_{Res}). A trend toward differentiation of microbiological indicators can be observed through the establishment of the management strategies adopted, starting at sampling time 3 (Fig. 1). As the mean standard error bars did not overlap, analysis of variance was performed only for this season. Given this, it can be observed that the management strategies with *U. ruziziensis* and *P. phaseoloides* showed lower mean values of qCO_2 at measurement time 3 (Fig. 1), demonstrating a lower imbalance of the soil microbiota. That is, low qCO_2 indicates savings in the energy use of the microbial cell, reflecting a more stable environment and closer to its equilibrium state (Fialho et al., 2020).

The accumulation of dry matter by the species *U. ruziziensis* and *P. phaseoloides* (Table 3), as a result of the mowing, carried out during the experimental period, forms a protective layer that acts to maintain soil temperature and humidity, and suppresses weeds. Conditions that may have contributed to the decrease in qCO_2 averages in these control strategies. Guimarães et al. (2017) concluded that greater protection of the soil by vegetation covers decreases the incidence of sunlight, resulting in a decrease in soil temperature, which can decrease the level of microbial activity, suggesting greater ecosystem stability.

In the manual weeding management strategy in which there was no formation of cover due to the accumulation of dry matter on the soil (Table 3), the qCO_2 was higher when compared to the other treatments in season 3, demonstrating an imbalance in the system. The increase in qCO_2 in the dry season was probably not directly influenced by the increase in temperature, but by the water deficit (Fig. 1).

As previously mentioned, the availability of water in the soil affected microbial activity. From the results obtained by this research, it can be observed that water stress may have been determinant in the response of microbiological indicators (basal respiration rate, biomass carbon, and metabolic quotient) to the management strategies used (Fig. 1). Water stress may have been a determining factor in the definition of qCO_2 , more than the interactions of management strategies with the microbiota. In seasons 2 and 4 (dry seasons) the microbiological indicators were statistically equal (Fig. 1).

Management strategies with an accumulation of dry matter on the soil (RH and PP) showed a tendency toward higher qCO_2 averages about the averages of the other control methods at sampling time 4 (Fig. 1). This is because the species cultivated between the rows need water for their development, and because it is a period of water scarcity, the competition between cultivated species (UR or PP), culture, and microorganisms may have been accentuated, resulting in a more stressful for the soil microbiota, which tended to raise the qCO_2 value. The management strategy of spontaneous vegetation with mowing also tended to generate stress on the soil microbiota at a time of low rainfall because, with the low availability of water in the soil, competition between weed species and the microbiota is accentuated. Competition between plants can stimulate soil microbial activity, increasing the mineralization of organic matter and, as a consequence, raising the value of qCO_2 (Matos et al., 2019). Soil water availability was also a determining factor in the principal component analysis (Fig. 2).

At the time when there was water availability in the soil, the dimension measure presented a value close to 70% of the explanation for the grouping of management strategies according to microbiological indicators. Soil moisture in the rainy season (seasons 1 and 3) favored the grouping of management strategies: herbicide application (HB), *P. phaseoloides* (PP), and *U. ruziziensis* (UR) with mowing, reaffirming the importance of the availability of water in the soil for sampling and determination of microbiological indicators. It was found that the microbiological indicators showed no correlation with coffee productivity in the two dry seasons of sampling (Fig. 3). During the rainy season, microbial biomass showed an inverse correlation with productivity. At times when higher C_{bio} was observed, coffee

productivity was lower (Fig. 3). Generally, higher microbial biomass was observed where there was an accumulation of dry matter on the soil.

Soil microbial biomass tends to present higher levels in the summer period, which has higher temperatures and rainfall, due to the presence of plant residues on the soil (Rodrigues et al., 2018). Microbial biomass is an indicator that can be changed by the coexistence and competition with different plant species, the change in soil microbial activity may be due to this competition between plants that affects the microbial community (Fialho et al., 2020). The process of transforming soil organic matter and microbial biomass is directly related to maintaining the productivity of agricultural ecosystems. Microbial biomass is considered one of the most sensitive characteristics to changes in soil quality, which is directly related to crop productivity (Silva et al., 2021).

The microbiological indicator $q\text{CO}_2$ showed a positive correlation with the productivity of coffee beans. The higher value of $q\text{CO}_2$ indicates that the system is in disequilibrium, with that there greater mineralization of organic matter, more nutrients are released, resulting in greater productivity. To increase crop production, greater consumption of organic matter is necessary, so it is important to maintain plant residues on the soil for the formation of this organic matter and attenuation of soil carbon losses. The greater instability of microbial biomass can result in high $q\text{CO}_2$ values, due to the greater energy expenditure of microbial activity, resulting in lower efficiency of carbon incorporation in the microbial cell, leaving this carbon available for culture (Matos et al., 2019).

Basal respiration rate also showed a positive correlation with coffee bean productivity in the rainy season. High respiratory rates of soil microbiota may indicate both an ecological disturbance and a high level of ecosystem productivity (Silva et al., 2021). Although the microbiological indicators used in this research did not indicate differences between the weed management strategies, the idea of integrating control methods to obtain and maintain better quality soils that allow the sustainability of the system must be taken into account. coffee tree. In the medium and long term, management strategies enable the formation of vegetation cover on the soil, provide protection and conservation, avoid losses due to erosion, and increase the cycling of nutrients and the formation of organic matter, in addition to suppressing weeds.

4. Conclusions

The impact of weed management strategies on soil microbiological indicators was dependent on the sampling time. The availability of water in the dry season influences the microbiological variables more intensely than the different weed management strategies.

Weed management methods with the formation of vegetation cover and accumulation of dry matter on the soil provide better soil quality and greater sustainability of the system over time and for longer periods.

Declarations

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Author contributions Úrsula Ramos Zaidan conducted the experimental procedures, analysis, prepared all figures and wrote the manuscript; Maurício Dutra Costa wrote the manuscript; Ricardo Henrique Silva Santos - wrote the manuscript; Christiano da Conceição Matos wrote the manuscript; Francisco Cláudio Lopes de Freitas wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests The authors declare no competing interests.

Data availability Not applicable.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication All authors reviewed the manuscript and agreed for publication.

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Figures

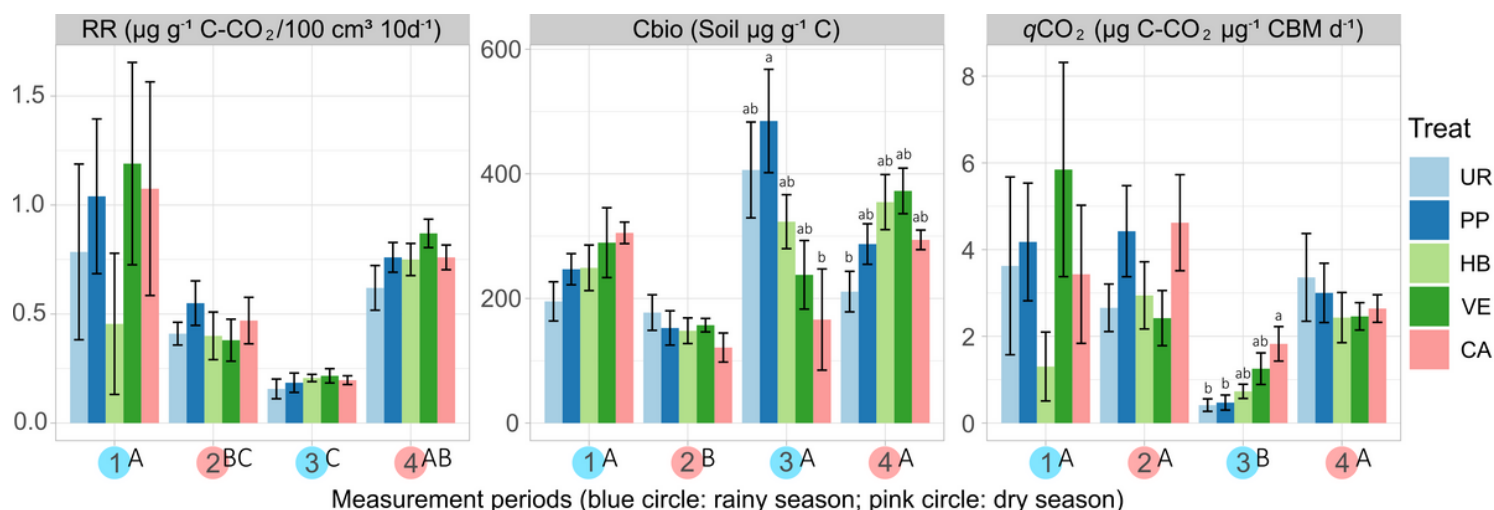


Figure 1

Averages and standard errors of means. The variables are shown: part A) basal respiration rate (RR); part B) biomass carbon (Cbio); and part C) metabolic quotient (qCO_2) for each management strategy and throughout the four measurement moments. The capital letters (A, B, AB, BC, and C) are the Tukey test results (with $\alpha=0.05$) for significant measurement epochs. Lowercase letters (a, ab and b) are comparing weed management strategies within each evaluated season and are the Tukey tests (with $\alpha=0.05$) for significant interactions.

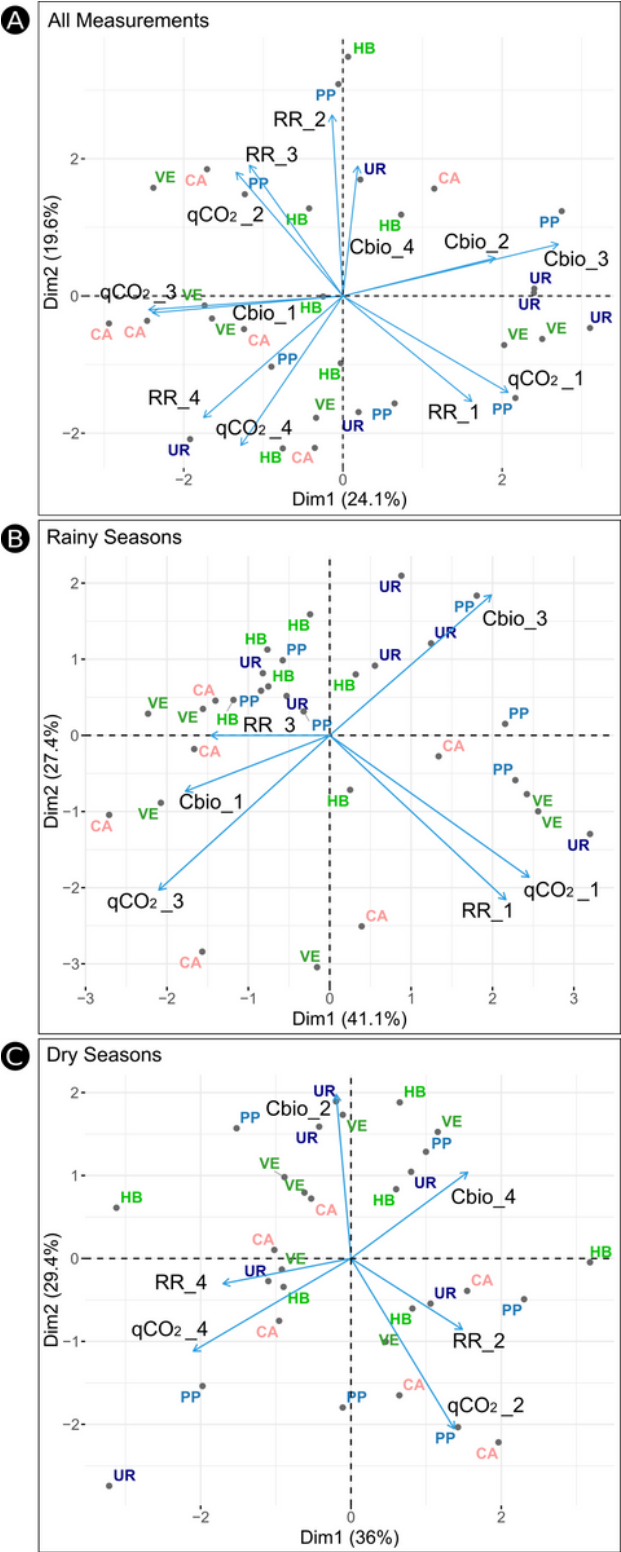


Figure 2

Principal Component Analysis (PCA) relating microbiological indicators in all measurements as well as compartmentalizing them in times of drought and rain. The numbers are the five management strategies, and the letters are the blocks, the combinations are the experimental units.

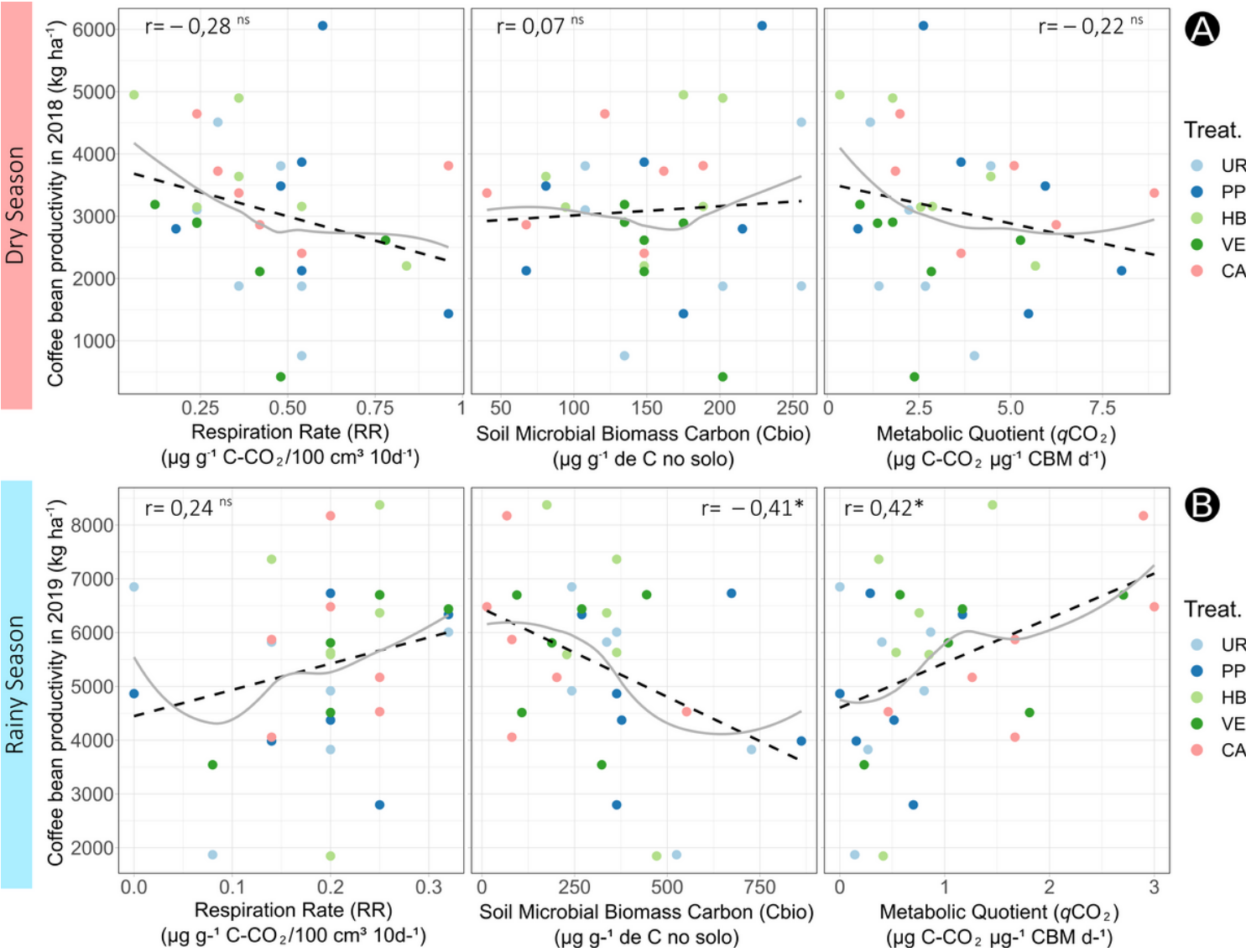


Figure 3

Relationship between microbiological indicators (Basal respiration rate, Cbio, and qCO₂) with coffee bean yield from experimental units (in kg and extrapolated to 1 hectare). Two sampling times were related, one in the dry season (upper part of the graph) and another in the rainy season (lower part of the graph) with the immediately following yields (2018 and 2019, respectively). The colors of the dots represent the management strategies adopted in each experimental unit. The coefficient "r" is the linear correlation followed by its statistical significance ("*" with $\alpha=0.05$).