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Biomass and soil microbial activity in degraded pasture of *Brachiaria decumbens* after implantation of herbaceous legumes

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A B S T R A C T

N deficiency is one of the significant causes of tropical pasture degradation, and including legumes may be a sustainable strategy to prevent pasture degradation in tropical environments. The objective of this study was to investigate the use of forage peanut (*Arachis pintoi*), butterfly pea (*Clitoria ternatea*), calopo (*Calopogonium mucunoides*) and stylosanthes (*Stylosanthes* sp.) intercropped with signal grass to determine soil biomass and microorganismal activity in a tropical subhumid zone. Soil sampling was performed at the legume rows and, three meters away from them and in the soil of pastures with single signal grass with or without an application of N-fertilizer, two years after legume implantation and during the dry and rainy seasons. The estimate of carbon and nitrogen of microbial biomass, C-CO_2 emission, and soil metabolic quotient of each legume were compared between the dry and rainy seasons, at two distances and with both single signal grass treatments. Although the rainy season decreased soil basal respiration in all treatments, forage peanut (~332 mg kg^{-1}), and butterfly pea (~280 mg kg^{-1}) showed pronounced soil microorganismal biomass, surpassing 27% and 14% the estimate for single signal grass not fertilized, respectively. The soil microorganisms with forage peanuts and stylosanthes also emitted more C-CO_2 (~1.0 mg kg^{-1} h^{-1}) in the dry season, indicating an effective microorganismal activity in these soils, mainly due to the absence of disturbances, as noted in the low metabolic quotients in both climatic seasons (less than 1%).
Keywords: Soil respiration rate; Silvopastoral system; Soil microbial activity; Pasture.

Biomassa e atividade microbiana do solo em pastagem degradada de *Brachiaria decumbens* após implantação de leguminosas herbácea

Resumo

A deficiência de N é uma das principais causas da degradação de pastagens tropicais e a inclusão de leguminosas pode ser uma estratégia sustentável para prevenir a degradação de pastagens em ambientes tropicais. O objetivo deste trabalho foi investigar o uso de amendoim forrageiro (*Arachis pintoi*), cunhã (*Clitoria ternatea* L.), calopogônio (*Calopogonium mucunoides* Desv.) e estilosanthes (*Stylosanthes* sp.) consorciados com capim-braquiária para avaliar a biomassa e atividade microbiana do solo em uma zona subúmida tropical. A amostragem do solo foi realizada nas fileiras das leguminosas e a três metros de distância delas e no solo das pastagens com capim-braquiária com ou sem adubação nitrogenada, dois anos após a implantação da leguminosa e nas estações seca e chuvosa. A estimativa de carbono e nitrogênio da biomassa microbiana, emissão de C-CO_2 e quociente metabólico do solo de cada leguminosa foram comparados entre as estações seca e chuvosa, em duas distâncias e com ambos os tratamentos de capim-braquiária. Embora a estação chuvosa tenha diminuído a respiração basal do solo em todos os tratamentos, o amendoim forrageiro (~332 mg kg^{-1}) e a ervilha borboleta (~280 mg kg^{-1}) apresentaram pronunciada biomassa microbiana do solo, superando em 27% e 14% a estimativa para capim-braquiária não fertilizada, respectivamente. Os microrganismos do solo com amendoim forrageiro e estilosante também emitiram mais C-CO_2 (~1,0 mg kg^{-1} h^{-1}) na estação seca, indicando uma atividade microbiana efetiva nestes solos, principalmente devido à ausência de distúrbios, como indicado pela baixa quocientes metabólicos em ambas as estações climáticas (menos de 1%).
Palavras-chave: Taxa de respiração do solo; Sistema Silvipastoril; Atividade microbiana do solo; Pastagem.

Introduction

The global demand for food and fuel has triggered the replacement of forests by pastures, where non-conservation management practices are used often, driving the soil degradation processes. These practices directly interferes with the abundance and activity of microbial communities and in cycling Carbon and Nitrogen in the ecosphere (Cárdenas et al., 2019), exposing the global climate to intense temperature changes due to the emission of greenhouse gases arising from indiscriminate use of nitrogen fertilizers (Cerri et al., 2018).

A good option to prevent pasture degradation, enrich the soil with essential nutrients without using nitrogenous inputs, and ensure sustainable agricultural yield is the incorporation of legumes into the predominantly pure grass pastures. This system is associated with livestock and can provide economic advantages through income from the marketable woods or fodder (Lima et al., 2018). In addition, legumes can improve livestock diet, allowing an animal excretion rich in protein compound that enhances the soil organic matter quality (Fagundes et al., 2021).

The intercropping of legumes with grass has been used successfully in tropical regions, with emphasis on the works by Lira-Junior et al. (2020 a), which emphasized the increasing on biomass and microbial activity, enhancing soil organic matter quality and soil C and N stock, significantly after four years of system established. Others studies have also proposed that this system is efficient in replenishing soil nutrients and recovering degraded pastures promptly (Apolinário et al., 2016; Mosquera-Losada et al., 2015; Santana et al., 2016). On the other hand, some works evaluated each herbaceous legume individually in association with grasses (Kohmann et al., 2020; Menezes et al., 2019), reporting mainly the profit with the forage production and grain yield.

Particularly, herbaceous legumes also have the potential to fertilize the soil with nitrogen compounds, as there is an excelente symbiotic performance between their roots and diazotrophic bacteria. Forage production from *Arachis pintoi*, *Stylosanthes* sp., and *Calopogonium mucunoides* has been reported (Boddey et al., 2020; Kumar et al., 2018) as their effects on structural soil protection and nutrient input equalizing from decomposing microbial activity (Vazquez et al., 2020). In addition, the use of herbaceous has been well established in studies with legume green manure (Barros et al., 2020).

The objective of this work was to investigate different types of herbaceous legumes intercropped with signal grass after two years of system establishment in a tropical subhumid zone were investigated. The hypothesis of this work was that the system of herbaceous legumes intercropped with signal grass can increase microbial biomass and reduce C-CO2 emissions from soil microbial activity.

Material and methods

Site description and sampling strategy – The experiment was developed in a degraded pasture of signal grass (Brachiaria decumbens Stapf.) at the Experimental Station of the Instituto Agronômico de Pernambuco (IPA), in Itambé, Pernambuco, Brazil, at geographic coordinates 07°25' S and 35°06' W (Figure 1). The average annual rainfall, temperature, and relative humidity are 1,200 mm/year, 24 °C and 80%, respectively (CPRH, 2003), and the predominant soil is a dystrophic Ultisol, according to Jacomine et al. (1973).

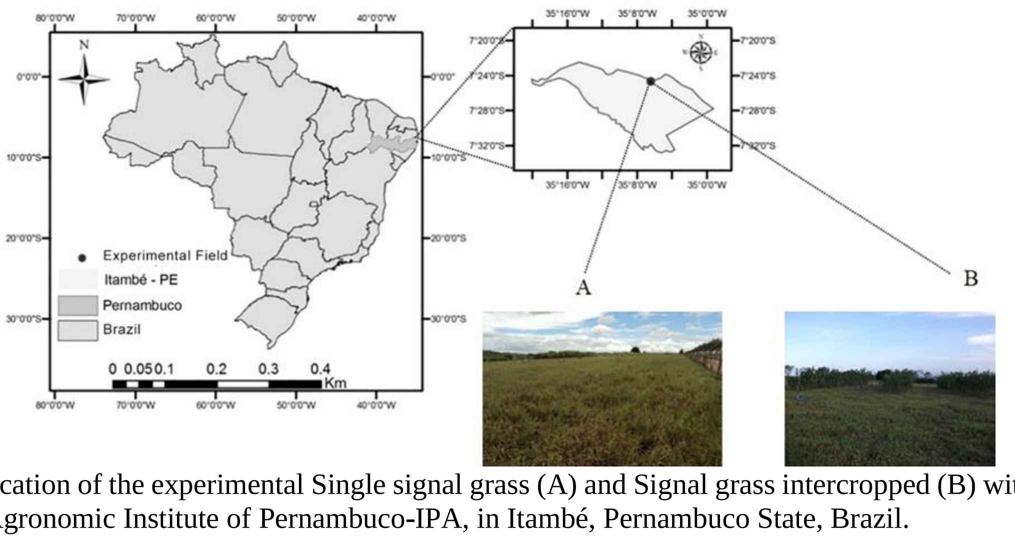

Figure 1. Location of the experimental Single signal grass (A) and Signal grass intercropped (B) with legumes area at the Agronomic Institute of Pernambuco-IPA, in Itambé, Pernambuco State, Brazil.

The legume-grass systems were implanted in 2008 legume-grass systems, proceeded by a chemical characterization of the degraded signal grass soil (Table 1), according to Silva et al. (2009). The soil correction was carried out annually with 1 Mg ha^{-1} of dolomitic limestone (80% PRNT), followed by 50 Kg ha^{-1} of superphosphate and potassium chloride.

The experiment was in a randomized block design, with six treatments and four blocks. The evaluated treatments were forage peanut (*Arachis pintoi* Krap & Greg cv. Amarillo); butterfly pea (*Clitoria ternatea* L.); calopo (*Calopogonium mucunoides* Desv.); Campo Grande stylo (a mixture of 80:20 ratio by weight of *Stylosanthes capitata* and *Stylosanthes macrocephala*, respectively), and two single singnal grass, either fertilized with 60 Kg ha^{-1} year^{-1} of urea or not. The evaluated treatments were abbreviated as follows: forage peanut (Ar); butterfly pea (Cl); callus (Ca); Campo Grande style (Sty) and two single grasses, fertilized (SSF) and unfertilized (SSN).

The plots were harrowed and received glyphosate. Before planting, legume seeds were inoculated with inoculants prepared with the recommended rhizobial strains (Table 2) and produced at 10 kg ha^{-1} for forage peanut and butterfly pea; 6 kg ha^{-1} for calopo and 5 kg ha^{-1} for stylosanthes. The seeds were planted in 3 m wide strips with 6 m of unharrowed signal grass between them on 540 m^2 (27 m x 20 m) plots with three legume strips per plot.

Table 1. Soil chemical characterization before the planting of herbaceous legumes intercropped with signal grass.

Conditions		Depth (cm)			VC
		0-10	10-20	20-40	(%)
pH	(water)	5.14	5.09	4.93	5.80
P	mg.dm^{-3}	4.46	1.96	0.87	68.3
Na^+	cmol$_c$.dm^{-3}	0.46	0.51	0.46	18.5
K^+	cmol$_c$.dm^{-3}	0.11	0.07	0.06	66.1
Mg^{2+}	cmol$_c$.dm^{-3}	1.79	1.48	1.00	33.7
Ca^{2+}	cmol$_c$.dm^{-3}	2.13	1.87	1.56	34.1
Al^{3+}	cmol$_c$.dm^{-3}	0.34	0.03	1.12	53.0
H^++Al^{3+}	cmol$_c$.dm^{-3}	6.31	7.00	7.20	9.30
OM	g.kg^{-1}	39.8	0.8	62.12	68.6

OM= Organic matter; VC= variation coefficient

Table 2. Strains recommended by the Ministério de Agricultura Pecuária e Abastecimento (MAPA) and Centro Nacional de Pesquisa em Agrobiologia (CNPAb) for herbaceous forage legumes, in Brazil.

Legume	Genera	Recommended strains
Arachis pintoi	*Bradyrhizobium* spp.	SEMIA 6439
C. mucunoides	*Bradyrhizobium* spp.	SEMIA 6152
Clitoria ternatea	*Bradyrhizobium* spp.	BR 2001 e BR 2801
Stylosanthes sp	*Bradyrhizobium* spp.	SEMIA 6154

Before the sampling period, all plots were grazed at a 1.2 kg green dry matter per kg of livestock weight grazing pressure to standardization. During the sampling period, animals were introduced into the fields to observe the animal effect on the pastures at the grazing pressure, without animal weighting. Sampling was carried out at the end of the dry and rainy season, two years after system establishment. The average rainfall during the experimental period is in Fig. 2. Three soil samples were collected at 0 and 3 m from the center of each legume strip and randomly in the treatment of both single signal grass.

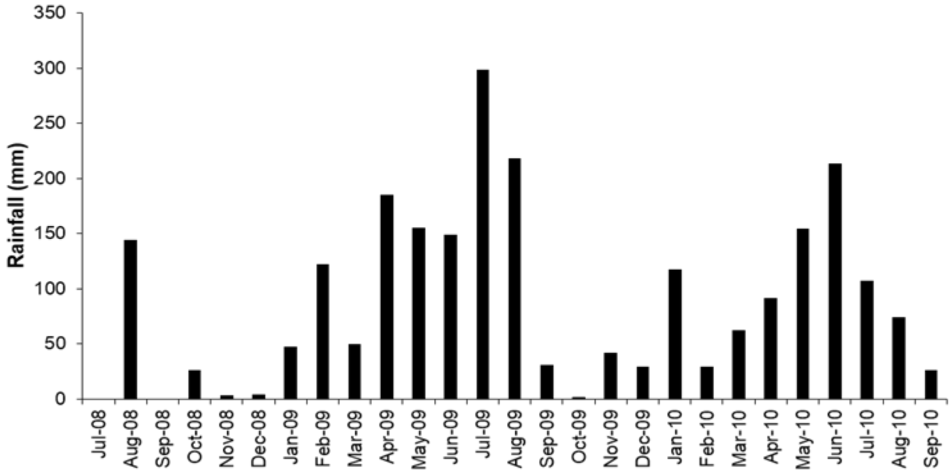

Figure 2. Average precipitation from July 2008 to September 2010 in the municipality of Itambé, Pernambuco, Brazil.

Variables analyzed - Microbial biomass carbon (C-mic) and Nitrogen (N-Mic) were obtained respectively by colorimetry, using the irradiation-extraction method and by sulfuric digestion followed by distillation and titration (Islam and Weil, 1998). Soil basal respiration (microorganismal C-CO_2 emission) was determined according to Alef and Nannipieri (1995), and the metabolic quotient was calculated according to Anderson and Domsch (1985) by the ratio between soil basal respiration and microbial biomass carbon.

Statistical Analysis – Initially, data were analyzed using SAS Mixed Procedure, considering each legume x distance combination as a separate treatment for a total of 10 treatments (four legumes at two distances each and two single signal grass treatments) with blocks as a random factor, and sampling season as repeated measures. When appropriate, a Dunnett test was conducted at 10% significance, considering SSN at the dry season as the control for the interaction or SSN for the pastures main effect.

A further analysis was performed considering only the legume treatments as a factorial 4x2 between legume species and distance from the legume row, again with the seasons as repeated measures and blocks as random effects. In this case, significant results were studied by the Tukey test, also at 10% significance.

In addition, Pearson's correlation and main component analysis were performed, and figure and graph was made using the Corrplot, Ggplot, and Ggcorrplot statistical package (Wei and Simko, 2017) of the R software (R Core Team 2017).

Results

All microbial attributes are in Fig. 3. Soil microbial biomass (C-mic) was significantly higher in the single signal grass fertilized roughly by 450 mg kg^{-1} and 340 mg kg^{-1} in the rainy and dry seasons, respectively. In the dry season, butterfly C-mic was 280 mg kg^{-1} at the legume strip, significantly higher than SSN (ca. 106 mg kg^{-1} or 38% higher) (Table 3). In the rainy season, there was a more significant difference in the mean value of this attribute between the distances for each treatment in 75, 86, 70, and 85 mg kg^{-1}, respectively, for the forage peanut, butterfly pea, stylosanthes, and calopo (Figure 3A).

The C-min in the forrage peanut strip was roughly 177 mg kg^{-1} (ca. 113%) higher in the rainy season than in the dry season. During the rainy season, it was also ~90 mg.kg^{-1} higher than SSN at the same season (ca. 27%), although there was no significant difference at 3 m from the legume strip in either case (Figure 3A). The butterfly pea, the gain was 14% compared to SSN, remaining second in the total C-mic increase in the rainy season.

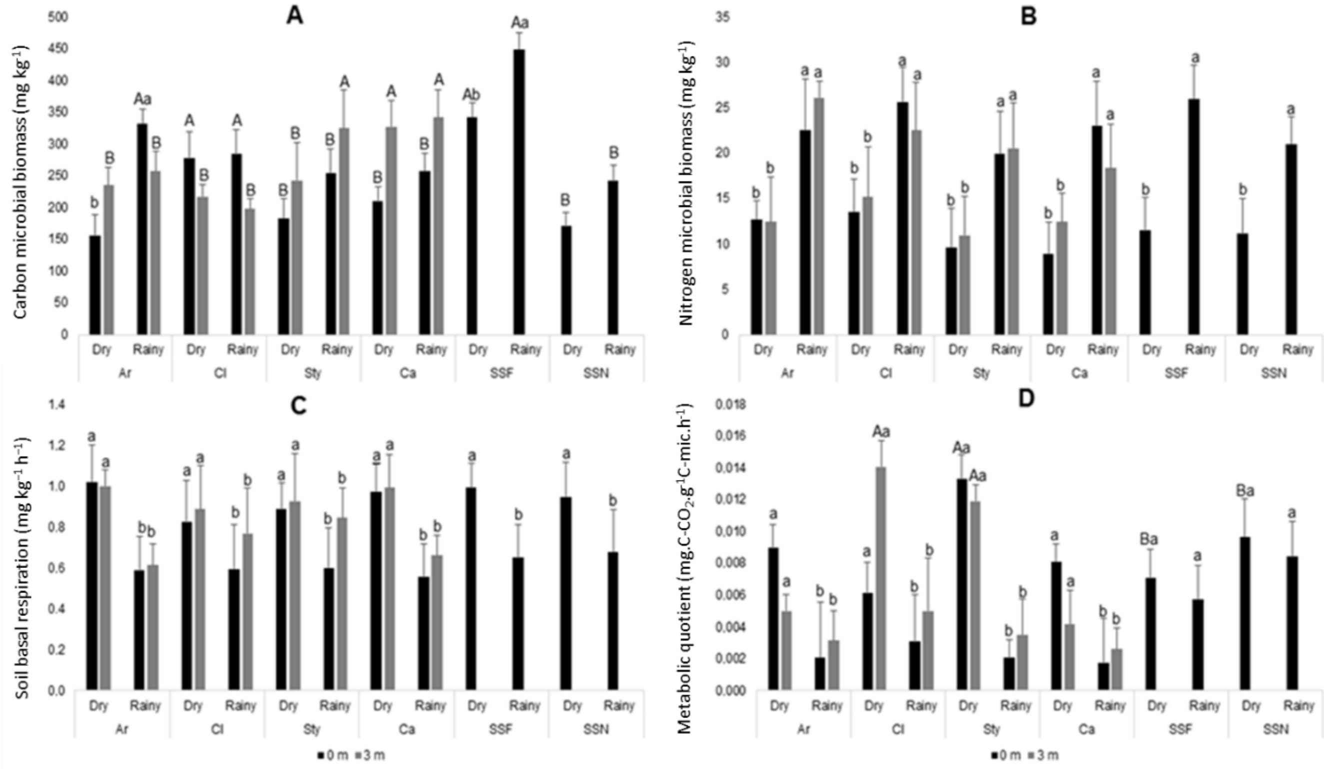

Figure 3. Carbon microbial biomass (A); Nitrogen microbial biomass (B); soil basal respiration (C); metabolic quotient (D) in systems of herbaceous legumes intercropped with signal grass. The upper case compares signal grass fertilized and other treatments and lower case is comparing treatments in different seasons. Different letters mean significant differences by the Dunnett test ($p < 0.1$). (Ar) *Arachis pintoi*; (Cl) *Clitoria ternatea*; (Sty) *Stylosanthes* sp.; (Ca) *Calopogonium mucunoides*; (SSF) signal grass fertilized; (SSN) signal grass not fertilized.

Table 3. Increase of soil microbial attributes in leguminous herbaceous systems intercropped with signal grass concerning single signal grass not fertilized with urea, in a tropical subhumid region.

Treatments	Distance	C-mic	N-mic	C-CO_2	$q$$CO_2$
		Dry season / Rainy season			
Ar	0	N / 91	1.6 / N	0.07 / N	N / N
	3	34 / N	1.8 / 3	0.09 / N	N / N
Cl	0	106 / 43	2.4 / N	N / N	N / N
	3	17 / N	4.5 / N	N / N	N / N
Sty	0	13 / 14	N / N	N / N	0.003 / 0.002
	3	42 / 24	0.3 / N	0.01 / 0.1	0.006 / 0.001
Ca	0	39 / 16	N / N	0.02 / N	N / N
	3	126 / 41	1.7 / N	0.08 / N	N / N
SSF	0	172 / 208	0.3 / N	0.04 / N	N / N
	3	131 / 104	0.6 / N	0.06 / N	N / N

C-mic = Carbon microbial biomass (mg kg^{-1}); N-mic = Nitrogen microbial biomass (mg kg^{-1}); C-CO2 = soil basal respiration (mg kg^{-1} h^{-1}); qCO_2 = metabolic quotient (mg.C-CO_2.g^{-1}C-mic.h^{-1}); N = no increase; SSF = singnal grass fertilized; Ar = Arachis pintoi; Cl = Clitoria ternatea; Sty = Stylosanthes sp.; Ca = Calopogonium mucunoides.

The N-mic estimate did not vary between distances or treatments within each season, but had a significant increase in the rainy season about the dry season roughly by 10, 12, 10, and 14 mg kg^{-1}, respectively, for the forage peanut, butterfly pea, stylosanthes and calopo in the legume row (Figure

3B). On the other hand, only peanuts and butterfly pea increased N-mic estimate compared to SSN in the dry season (~2 mg kg^{-1}) (Table 3).

The C-CO_2 emissions (Figure 3C) and qCO_2 (Figure 3D) were significantly higher in the dry season for all treatments or distances, whose maximum values were ~1.0 mg kg^{-1} h^{-1} for forage peanut and 0.01 mg.C-CO_2.g^{-1}C-mic.h^{-1} for stylosanthes and twice or more than found in the rainy season for the same system. Even in the dry season, significant variations were found for qCO_2 only between the distances of the legume rows, except for stylosanthes. At the same time, basal soil respiration remained constant in each treatment, even when compared to both single signal grass (Table 3). Stylosanthes was also the only treatment whose qCO_2 values indicate possible soil microbial community stress at both distances and seasons.

Sing the principal component analysis technique (PC), the individual results containing the respective eigenvectors, eigenvalues, and variance percentages are expressed in Table 4. In the analysis of the principal component for the simultaneous evaluation microbial around 68% of the total variation of the microbial attributes studied were explained by the first two principal components (PC1 and PC2). PC1 corresponded to 45% of this variation, while PC2 23% (Figure 4A). The analysis of principal components allows the joint visualization of the microbiological attributes of the soil in the different systems evaluated. The slight angle formed between the arrows representing qCO_2 and C-CO_2 attributes demonstrates the strong positive correlation between these attributes in pasture systems. These two attributes correlate significantly (Figure 4B).

Table 4. Principal Components (PC) soil microbial attributes in leguminous herbaceous systems intercropped with signal grass compared to single signalgrass not fertilized with urea, in a tropical subhumid region.

	Principal components	
	PC1	PC2
Eigenvalue(λ)	1,7916	0,9453
Explained Variance (%)	44,79	23,63
Cumulative Variance (%)	44,79	68,42
Variáveis	**Autovalores**	
	PC1	PC2
C-mic	0,5329	-0,1905
N-mic	0,2552	0,9639
C-CO_2	-0,4847	0,0691
qCO_2	-0,6448	0,1721

C-mic = Carbon microbial biomass; N-mic = Nitrogen microbial biomass; C-CO_2 = soil basal respiration; qCO_2 = metabolic quotient.

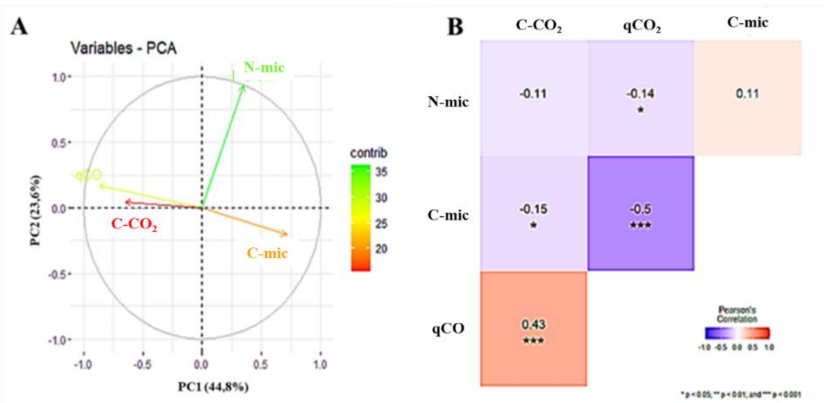

Figure 4. Analysis of principal components (A) and Matrices Pearson's correlation (B) between the variables microbial biomass carbon (C-mic); microbial biomasss nitrogen (N-mic); soil basal respiration (C-C02); metabolic quotient (qCO2) in leguminous herbaceous systems intercropped with signal grass, in a tropical subhmid region.

Discussion

Soil moisture stimulates the microbial biomass through increased plant biomass, and this trigger the degrading of organic compounds, providing C and N later immobilized by microorganisms (Ren et al., 2017). Over time, these elements are mineralized and incorporated into the fractions of soil organic matter (humic, fulvic and humic acids), contributing to soil C and N storage (Lira Junior et al., 2020b).

Butterfly pea legumes had the lowest C-mic variation between seasons and higher N-mic compared to single fertilized signal grass. Thus, the butterfly pea treatment seems to stimulate soil microbiota development, even where the pastures are subject to degradation, mainly due to irregular rainfall and incorrect soil management during changes in land use (Santos et al., 2020). This legume has na efficient symbiotic relationship with N-fixing bacteria (Somtrakoon and Chouychai, 2018) due to it has good root and shoot structure, with leaves, flowers, and fruits that stimulate the soil microorganisms and consequently the organic matter quality in senescence (Oguis et al., 2019).

Forage peanut is well adapted to signal grass intercropping. It can also increase soil total C and N contents and microbial biomass (Kumar et al., 2018), mainly due to its high forage yield (Teixeira et al., 2011) and for providing a palatable, high-protein diet to cattle, whose excreta then enrich the soil with nitrogenous nutrients (Hussainy et al., 2020). In the present study, forage peanut was the legume intercropped with signal grass that probably triggered a greater abundance of fungi in the soil since its C:N ratio of microbial biomass was high. This also favored a greater availability of microbial C in the rainy season in legume row, as most of this biomass is formed by this type of microorganism which, together with actinobacteria, have enzymes that degrade more recalcitrant organic matter (Javed et al., 2021).

Both forage peanut and butterfly pea were effectively increased microbial biomass when intercropped with signal grass, after 2 years. This reduces the possible effect of soil preparation for the legumes during their implementation, as seen by the higher C and N-mic compared to single signal grass not fertilized. On the other hand, both stylosanthes and calopo only had a significant increase of microbial C and N in the soil 3 m away from the legume crops, where there was no soil disturbance during the planting. This is possibly due to the more incredible speed of calopo and stylosanthes to cover sample plots with their forages compared to forage peanut and butterfly pea (Espíndola et al., 2005; Martuschello et al., 2011), although this coverage is composed of more resistant materials (Martuschello et al., 2011).

Comparing the C-mic obtained in the present study with those obtained by Barros et al. (2020) for legume green manure soils with the implementation of peanut, stylosanthes, and calopo after corn harvesting, we conclude that these same herbaceous used here had an increase of ~50, 30 and 25 kg ha^{-1}, respectively, in the rainy season, two years after the start of cultivation, revealing the effectiveness of these legumes in stimulating the soil microbial community the soil. On the other hand, our highest C-mic results are similar to that reported by Lira-Junior et al. (2020a) in the dry season, and the legume row found silvopastoral system based on shrub-tree legume *Gliricidia sepium* and *Mimosa caesalpinifolia*, but *ca*. 300 kg ha^{-1} below to that found in the rainy season, indicating the herbaceous legume system might need a more extended evaluation period.

Usually, the microbial biomass is more abundant and active during the rainy season. Thus respiration is also higher (Lira-Junior et al., 2020a), but the relatively short period after legume implantation might have led to the observed higher values during the dry season. It is also possible that these legumes have not completed an entire life cycle; hence, we understand that not all shoot dry matter and root exudates production have been fully utilized by the microbial biomass in these environments yet.

Despite the possibility of stress by the soil microbiota in stylosanthes, the soil basal respiration was invariable or relatively low compared to other legumes; thus the higher metabolic quotient observed in this treatment is due to the low estimate of microbial carbon produced as a result of more remarkable recalcitrance of the organic compounds generated during the life cycle of this legume (Martuschello et al., 2011).

Final considerations

Butterfly pea and forage peanut legumes intercropped with signal grass stood out in stimulating soil microbial biomass in dry and rainy seasons, respectively, after two years of system establishment.

Forage peanuts also had a higher microbial activity based on C-CO_2 emissions without signs of disturbance by the microbial community.

Two years of herbaceous legume establishment was not enough to balance the biomass microbial estimate or C-CO_2 emissions values between the legume row and three meters away from them.

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