

Grass–legume intercropping in integrated crop-livestock systems: a strategy to improve soil quality and soybean yield in the Brazilian Cerrado

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Abstract

Aims

The integrated crop-livestock systems (ICLS) under no-tillage improves soil fertility of the Brazilian Cerrado. We aimed to evaluate the effect of different grass-legume intercropping compared to single grass cultivation in the off-season of an ICLS on (i) soil organic carbon (C) and nitrogen (N) pools, (ii) soil microbial biomass and activity, (iii) soil enzymatic activity, and (iv) soybean grain yield in succession.

Methods

The field study was conducted in an on-farm experiment in 2016/17 and 2017/18 cropping seasons. The soybean was cultivated in the summer season, with the subsequent treatments in the off-season, using two grasses (*Urochloa ruziziensis* and *U. brizantha*), single or intercropped with Cowpea (*Vigna unguiculata*) or Pigeon pea (*Cajanus cajan*). We evaluated soil organic C and N pools, microbial biomass and activity, enzyme activity, and soybean yield.

Results

Cowpea intercropping yielded 24% more soybeans than grasses single cropped. There was a higher microbial biomass and activity, and enzymatic activity in the soil under grass-legume intercropping. In addition to the lower basal respiration and microbial metabolic quotient (qCO_2), the greater microbial quotient ($qMIC$) and microbial biomass C indicate a higher soil microbial C utilization efficiency under grass-legume intercropping. The soil total organic C and N stocks increased under Pigeon pea intercropping by 16% and 27%, respectively, compared to single grasses.

Conclusions

Grass-legume intercropping in the pasture phase of ICLS is an additional tool to maximize soybean yields in the short term. The intercropping effects on soybean yield were directly related to soil quality improvements through soil biological and biochemical properties.

1. Introduction

The Brazilian Cerrado is the second largest biome in Brazil extending over 200 million ha^{-1} (Batlle-Bayer et al. 2010) and is the most important agricultural frontier in the world (PROBIO 2004). Represented by wet summers and dry winters (low and irregular rainfall), this region have large risks in growing a successful dry-season crop (thereafter named off-season) (Borghetti et al. 2013). Likewise, this region has reported problems due to lack of crop diversification (Petter et al. 2017; Trevisan et al. 2017), leading to decreased soil fertility and crop yield. This results in natural resources degradation and higher production costs (Freddi et al. 2017; Luciano et al. 2012; Tavanti et al. 2019).

Integrated agricultural production, such as ICLS, is a feasible option to increase food production during times of irregular rain and reduced pasture availability (Almeida et al. 2021; Moraes et al. 2019b; Soares et al. 2020), such as the Cerrado region. According to Salton et al. (2014), Sarto et al. (2020) and Reis et al. (2021), the ICLS adoption helps to improve soil quality and brings sustainability to the production system of the Brazilian Cerrado. The benefits of ICLS include increased soil fertility due to soil organic matter accumulation (Alves et al. 2020), improved nutrient cycling (Assmann et al. 2017; Carvalho et al. 2010), and increased fertilizer use efficiency (Denardin et al. 2020). The rotation of cash crops with livestock under no-till can also help to break pest, disease, and weed cycles, thus reducing production costs, increasing economic and environmental outcomes (Carvalho et al. 2021; Farias et al. 2020; Lazzarotto et al. 2009; Martha Junior et al. 2011).

Many studies indicate that ICLS under no-till are beneficial for sustainably intensifying crop production in tropical regions (Crusciol et al. 2021; Mateus et al. 2020; Moraes et al. 2019b; Salton et al. 2014). In Cerrado region, adoption of ICLS alone increases soil

organic C content, compared to using pasture as a cover crop (Gazolla et al. 2015; Sant-anna et al. 2017; Sarto et al. 2020). Current ICLS typically use a single grass species (e.g. *Urochloa brizantha* or *U. ruziziensis*) in the pasture phase (Carvalho et al. 2011; Rocha et al. 2020). However, diversifying botanical composition can lead to different inputs of residues and exudation of organic compounds (Chávez et al. 2011; Moujahid et al. 2017), activating the soil microbiota and nutrient cycling and improving soil fertility (Costa et al. 2015; Hungria et al. 2009; Martins et al. 2017). One way to increase botanical diversity is to intercrop the grass ley with a legume (Mead and Willey 1980). This leads to increased soil organic C and N (Louarn et al. 2015; Nascente and Stone 2018), mainly through increases in the labile C and N pools (Ball et al. 2020; Hungria et al. 2009; Laroca et al. 2018; Schipanski and Drinkwater 2012).

In addition to soil organic C and N pools, soil microbial biomass and activity are good short term indicators of anthropogenic interventions, and can be used to evaluate soil quality under different soil managements (Bending et al. 2000; Feng et al. 2021; Lopes et al. 2013; Zhao et al. 2015). Soil enzymatic activity is also being widely used as they allow of the measuring of the catabolism of organic and mineral components in the soil, because they are highly sensitive to provide information about changes in key soil functions (Bandick and Dick 1999; Sekaran et al. 2020; Verstraete and Voets 1977). In this regard, the basal respiration rate, qCO_2 , and the $qMIC$, also provide measures of the activity of microbial communities (Anderson and Domsch 1978; Wardle and Ghani 1995).

In general, an increase in the total enzymatic activity of soil is observed under grass-legume intercropping at the pasture phase (Curtright and Tiemann 2021; Laroca et al. 2018), mainly due to an increase in soil microbial community and activity (Mwakilili et al. 2021; Moraes et al. 2019; Pires et al. 2021; Zhao et al. 2015). Leguminous plants are capable of producing a wide variety of metabolic agents, such as flavonoids and strigolactones, increasing microbial biomass (Chang et al. 2017; Pires et al. 2021). According to Zhao et al. (2015), the fungal biomass is significantly greater under grass-legume intercropping than under grass monoculture. Likewise, the grass-legume intercropping positively affects the activity and diversity of arbuscular mycorrhizal fungi species (Pires et al. 2021).

The ICLS adoption with grass-legume intercropping can improve microbial biomass C and N (Dhakal and Islam 2018; Hurisso et al. 2013) and soil organic C and N stocks (Frasier et al. 2016; Li et al. 2016). Hence, intercropping systems may increase pasture production during the off-season and grain crop yields in succession (Dhakal and Islam 2018; Laroca et al. 2018; Mateus et al. 2020; Pires et al. 2021). From this, it is expected that grass-legume intercropping in ICLS will lead to a higher N availability, and greater microbial and enzymatic activity and soil organic C and N contents. As a consequence of the soil quality improvements, it is expected that grass-legume intercropping would increase grain yield of soybean in succession. The objective of this study was to evaluate the effect of different grass-legume intercropping compared to single grass cultivation on (i) soil organic C and N pools, (ii) soil microbial biomass and activity, (iii) soil enzymatic activity, and (iv) soybean grain yield in succession under ICLS in the Brazilian Cerrado region.

2. Material And Methods

2.1. Site description and historical characterization of the experimental area

A field experiment was conducted during the 2016/2017 and 2017/2018 soybean cropping seasons at "Gravataí Farm", Itiquira County, Mato Grosso State, Brazil (17°09'47" S, 54°51'45" W). The regional climate is a tropical climate, classified as Aw, according to Koeppen, with rainy periods from October to April and dry periods from May to September. The local annual average temperature was 24.8°C, and the annual cumulative rainfall was 1350 mm (**Supplementary Figure S1**). The soil is classified as Oxisol (Soil Survey Staff 2000), with a clay texture (48, 12, and 40% of clay, silt, and sand, respectively).

Prior to the experiment, the field was used for no-till soybean (*Glycine max*) - maize (*Zea mays*) rotations from 1993 to 2004; a cotton (*Gossypium hirsutum*) monoculture from 2005 to 2011; and a soybean-pasture (*Urochloa ruziziensis*) ley rotation from 2012 to 2015. The experiment began in 2016 and was conducted during the 2016/17 and 2017/18 cropping seasons.

Immediately before the installation of the experiment, the soil was sampled in the 0–10 cm layer and analyzed for chemical and physical characterization, being: pH-H₂O (1:1 soil/water ratio) of 5.3; exchangeable aluminum, calcium, and magnesium (KCl 1 mol

L⁻¹) of 0.2, 2.7, and 0.9 cmol_c dm⁻³, respectively; available phosphorus and potassium (Mehlich 1) of 28.2 and 91.0 mg dm⁻³, respectively; cation exchange capacity at pH 7.0 of 8.8 cmol_c dm⁻³; base saturation of 43.4%; and soil bulk density (pipette method) of 1.38 kg dm⁻³.

Soybeans were sown in October and harvested 110 days after sowing. Soybean sowing was carried out with a spacing of 45 cm between rows and 17 plants per meter, using TMG 1174 RR cultivar. The annual fertilization in the soybean sowing was 50 kg ha⁻¹ of monoammonium phosphate (12% of N; 52% of P₂O₅), 120 kg ha⁻¹ of potassium chloride (58% of K₂O), 290 kg ha⁻¹ of simple superphosphate (18% of P₂O₅), and 75 kg ha⁻¹ of elemental sulfur. For the desiccation, glyphosate (N-(phosphonomethyl) glycine) was used, divided into two sequential applications.

2.2. Experimental design and treatments

The experiment started after the soybean harvest in the 2015/2016 cropping season, with direct sowing of grasses in the pasture phase. The experiment, with a total area of approximately 60 ha, was divided into 18 plots, with an average plot area of 3.5 ha. The experiment was designed as randomized blocks, with three repetitions and subdivided plots. The main factors were two types of grasses (*U. ruziziensis* and *U. brizantha* cv. BRS Paiaguás), with subdivided plots representing the intercrop with legumes (Cowpea [*Vigna unguiculata*] cv. BRS Tumucumaque, or Pigeon pea [*Cajanus cajan*] cv. BRS Mandarin) and single grasses.

The pasture sowing was performed with seed rates of 4.0 and 21.0 kg ha⁻¹ of viable pure seeds, for grasses and legumes, respectively. Pasture sowing was performed after soybean harvest (at the beginning of February of each year). Grasses were sown by broad casting and for the intercrop with legumes was used precision seeders with a 45 cm spacing.

Yearling Nelore females (*Bos taurus indicus*) weighing around 235 kg were used for grazing. A variable number of cattle were used following the *put-and-take* technique (Mott and Lucas 1952) to maintain the recommended grazing intensities. Average adopted pasture height was 60 cm, and average stocking rate in the different treatments were (in kg live weight ha⁻¹): 1,170 in *U. ruziziensis* + Cowpea; 855 in *U. ruziziensis* + Pigeon pea; 675 in single *U. ruziziensis*; 1,665 in *U. brizantha* + Cowpea; 1,260 *U. brizantha* + Pigeon Pea; and 945 in single *U. brizantha*. The grazing period occurred from May to the end of August. Cattle's feeding was forage-based with only mineral salt being provided.

2.3. Soil and plant analyses

In March 2018, soil samples were collected after soybean harvesting. Five samples in representative areas of each plot were randomly collected in the 0–10 cm soil layer. Each sample was composed of five sub-samples collected from the nearby area. The soil samples were stored in plastic bags and transported to the laboratory. Part of the samples for the chemical analyses was dried in a forced-air circulation oven at 50°C, ground, and sieved through 2-mm mesh. Another part was stored in a refrigerated room and kept refrigerated (4°C) for soil microbial biomass and enzymatic activity evaluation.

2.3.1 Soil carbon and nitrogen stocks

Soil C and N contents were analysed by dry combustion in a Fisher Scientific FlashEA®. Soil bulk density was measured using the volumetric ring method (Blake and Hartge 1986), in the 0–10 cm soil layer. Then, the C and N stocks in the 0–10 cm soil layer were calculated by equivalent soil mass method (Ellert and Bettany 1995). The soil used as the reference was the one with the greatest mass.

2.3.2 Soil microbiological and biochemical analysis

The microbiological and biochemical determinations were performed in fresh soil samples, preserving the soil field moisture. Soil C and N microbial biomass contents were determined according to the fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987), with a soil extractor ratio of 1:2.5 (Tate et al. 1988) and a correction factor of 0.33 and 0.54 for C and N, respectively (Brookes et al. 1985). Extraction in fumigated and non-fumigated samples was performed with potassium sulfate 0.5 mol L⁻¹. The C and N were measured according to Tedesco et al. (1995). Basal respiration was determined according to the methodology proposed by Jenkinson and Powlson (1976). The *q*CO₂ was calculated by dividing the basal respiration (mg CO₂-C kg⁻¹ dry soil day⁻¹), by the

microbial C content (Anderson and Domsch 1993). The $qMIC$ was calculated as the ratio of microbial C by total soil organic C (Sparling 1992).

The hydrolysis of fluorescein diacetate (FDA) was determined by the method proposed by Dick et al. (1996). Urease enzyme activity assayed by the method of Tabatabai and Bremner (1972). β -Glucosidase enzyme activity was assayed by the method of Eivazi and Tabatabai (1988). Acid phosphatase was quantified following the method of Dick et al. (1996). Arylsulfatase activity was obtained by the method proposed by Tabatabai (1994).

2.3.3 Plant analysis

In 2016 and 2017 growing seasons, the pasture shoot biomass was sampled before the start of grazing, according to the methodology proposed by Crusciol et al. (2005). Six random points were collected per plot, using a metallic square with an area of 0.25 m². The samples were dried in an oven to determine the dry matter weight. In the 2016/2017 and 2017/2018 cropping seasons, soybean yield was evaluated by collecting soybean in the entire plot 110 days after sowing. Harvesting was performed by an automated harvester. The samples were threshed, cleaned, and weighed. The grain moisture was determined and adjusted to 130 g kg⁻¹.

2.3.4 Statistical analyses

Analyses were performed using the Sisvar (version 5.6) and SigmaPlot (version 10.0) statistical software's, and the data were assessed using analysis of variance (ANOVA). The model considered the two types of grasses as the main factors, with the intercrops as the subdivided plots. Model residual assumptions were checked with the Shapiro–Wilk normality test and Levene's homogeneity of variance test. When a factor was deemed significant in the ANOVA, least squared means were calculated and compared using Tukey's adjustment. All the tests were performed at a significance level of $\alpha = 0.05$.

Person correlation was done (SigmaPlot for Windows v. 13.0, Systat Software, Inc., San Jose, CA) using all the data, and models were assessed using both their statistical significance and coefficient of correlation (r). Principal component analysis (PCA) was conducted by the Statistica 7.0 program, to discriminate the indicator attributes associated to productivity which best characterize the productive potential of each treatment in two-dimensional plans. The principal components were generated from the eigenvalues of the covariance matrix obtained from the original variables. Only eigenvalues greater than one were considered.

3. Results

3.1. Soil carbon and nitrogen pools

The total organic C and N stocks were affected by both the grasses and the legumes intercropping (Table 1). The use of *U. ruziziensis* increased the total organic C stock in 0.79 Mg ha⁻¹ compared to *U. brizantha*. On the other hand, the stock of total organic N was 1.47 Mg ha⁻¹ higher when *U. brizantha* was used. The total organic C stock on Pigeon pea intercropping was 14.9% and 16.0% higher than Cowpea intercropping and single grass cultivation, respectively (Table 1). The intercropping with Pigeon Pea also increased the total organic N stock by 27.1% compared to the single grass cultivation, with intermediate N stock in intercropping with Cowpea (6.17 Mg ha⁻¹).

Table 1

Total organic carbon and nitrogen stocks, particulate organic carbon and nitrogen stocks two years after the adoption (March 2018) of an integrated crop-livestock system under no-tillage with intercrops at the pasture stage

Grasses	Intercrops			Average
	Cowpea	Pigeon Pea	Single	
Total organic carbon stock (Mg ha⁻¹)				
<i>U. Ruziziensis</i>	32.7	36.4	32.9	34.00 A
<i>U. brizantha</i>	31.5	37.4	30.7	33.21 B
Average	32.12 b	36.90 a	31.80 b	
Total organic nitrogen stock (Mg ha⁻¹)				
<i>U. Ruziziensis</i>	5.23	5.87	4.60	5.23 B
<i>U. brizantha</i>	7.10	7.27	5.73	6.70 A
Average	6.17 ab	6.57 a	5.17 b	
Particulate organic carbon stock (Mg ha⁻¹)				
<i>U. Ruziziensis</i>	2.60 Ab	4.00 Aa	1.53 Bc	2.71
<i>U. brizantha</i>	2.47 Ans	3.10 B	2.40 A	2.65
Average	2.53	3.55	1.97	
Particulate organic nitrogen stock (Mg ha⁻¹)				
<i>U. Ruziziensis</i>	0.30	0.50	0.30	0.37 B
<i>U. brizantha</i>	0.53	0.60	0.73	0.62 A
Average	0.42 ns	0.55	0.52	
Tukey's test (p < 0.05) = Uppercase letters indicate differences between grasses. Lowercase letters indicate differences among intercropping. ns = not significant.				

Whereas particulate organic C was 29.0% higher in *U. ruziziensis* when intercropped with Pigeon Pea, when the grass was single-cropped the particulate organic C was 56.9% higher using *U. brizantha* (Table 1). In Cowpea intercropping the particulate organic C was independent on the grasses. Using *U. Ruziziensis*, the Pigeon pea intercropping had the highest particulate organic C (4.0 Mg ha⁻¹), followed by Cowpea intercropping (2.6 Mg ha⁻¹) and the single grass cultivation (1.5 Mg ha⁻¹). However, using *U. brizantha* the particulate organic C was independent on legumes intercropping. Particulate organic N was 67.6% higher using *U. brizantha* grass compared to *U. ruziziensis*, and similar among legumes intercropping (Table 1).

Regarding microbial biomass C and N, the grasses effect was different among the legumes intercropping. When intercropped with Cowpea and Pigeon pea the *U. brizantha* had a microbial biomass C 17.1% and 55.2%, respectively higher than *U. ruziziensis* (Table 2). On the other hand, when single-cropped *U. ruziziensis* had 51.7% higher microbial biomass C than *U. brizantha*. Regardless of the grasses used, soil microbial biomass C was higher when intercropped with Cowpea. However, when using *U. ruziziensis* the microbial biomass C was similar between the other intercropping, and under *U. brizantha* the Pigeon pea had 86.4% more microbial biomass C than the single crop (Table 2).

Table 2

Soil microbial biomass carbon and nitrogen, basal respiration, metabolic quotient ($q\text{CO}_2$), and microbial quotient ($q\text{MIC}$) two years after the adoption (March 2018) of an integrated crop-livestock system under no-tillage and different grasses and intercrops at the pasture stage

Grasses	Intercrops		
	Cowpea	Pigeon Pea	Single
	Microbial biomass carbon (mg kg^{-1} soil)		
<i>U. Ruziziensis</i>	705.7 Ba	352.3 Bb	445.0 Ab
<i>U. brizantha</i>	826.3 Aa	546.7 Ab	293.3 Bc
	Microbial biomass nitrogen (mg kg^{-1} soil)		
<i>U. Ruziziensis</i>	51.7 Aa	50.0 Aa	16.7 Ab
<i>U. brizantha</i>	54.0 Aa	40.7 Bb	14.7 Ac
	Basal respiration ($\text{mg C-CO}_2 \text{ kg}^{-1} \text{ soil h}^{-1}$)		
<i>U. Ruziziensis</i>	6.46 Ac	12.53 Ab	17.20 Aa
<i>U. brizantha</i>	5.30 Ab	3.33 Bb	8.03 Ba
	$q\text{CO}_2$ ($(\text{mg C-CO}_2 \text{ mg}^{-1} \text{ C-MB h}^{-1}) \times 10^{-3}$)		
<i>U. Ruziziensis</i>	12.67 Ac	26.00 Ab	104.33 Aa
<i>U. brizantha</i>	10.33 Ab	8.67 Bb	57.67 Ba
	$q\text{MIC}$ (%)		
<i>U. Ruziziensis</i>	2.83 Aa	1.37 Ab	1.76 Ab
<i>U. brizantha</i>	3.40 Aa	1.93 Ab	1.20 Ab
Tukey's test ($p < 0.05$) = Uppercase letters indicate differences between grasses. Lowercase letters indicate differences among intercropping.			

The microbial biomass N was 22.8% higher in *U. ruziziensis* only when intercropped with Pigeon pea, and similar in the other intercropping (Table 2). In both grasses the legume intercropping had higher microbial biomass N than the single crop. However, using *U. ruziziensis* the microbial biomass N was similar between intercropping and under *U. brizantha* the Cowpea intercropping had 32.7% more microbial biomass N than the Pigeon pea intercropping (Table 2).

3.2. Microbial and enzymatic activity

The basal respiration and the $q\text{CO}_2$ had significant interaction between grasses and legumes intercrops (Table 2). The basal respiration and the $q\text{CO}_2$ indicated that using *U. ruziziensis* single-cropped leads to higher biological activity. The lowest basal respiration and $q\text{CO}_2$ were found under *U. brizantha* intercropped with pigeon pea. Both basal respiration and $q\text{CO}_2$ were higher in single grass than in intercrops, and the use of *U. brizantha* decreased the basal respiration and the $q\text{CO}_2$ by 53% and 45%, respectively, compared to *U. ruziziensis* (Table 2). The use of Pigeon pea intercropped with *U. ruziziensis* increased the basal respiration and the $q\text{CO}_2$ by 94% and 106%, respectively, compared to Cowpea intercropping. However, using *U. brizantha* both the basal respiration and the $q\text{CO}_2$ were similar between the intercropped legumes. The $q\text{MIC}$ was similar between grasses, but it was 88% and 113% higher under Cowpea intercropping, compared to Pigeon pea intercropping and single cropping, respectively (Table 2).

Regardless of the intercropping, the β -glucosidase activity was higher with *U. brizantha*. Similarly, in both grasses the β -glucosidase activity was 31% and 65% higher when intercropped with Pigeon pea, compared to Cowpea and single cropping, respectively (Fig. 1A). The FDA release was similar between the grasses intercropped with Cowpea, but 77% and 98% higher in *U. ruziziensis*

compared to *U. brizantha*, when intercropped with Pigeon pea and single-cropped, respectively (Fig. 1B). Using *U. ruziziensis*, the FDA release was higher in Pigeon pea intercropping than in single-cropped, followed by Cowpea intercropping. However, the intercropping with both legumes were superior to single cropped of *U. brizantha* (Fig. 1B).

The arylsulfatase activity was higher under *U. ruziziensis* than *U. brizantha*, in all the intercropping (Fig. 1C). Under *U. ruziziensis* the Pigeon pea intercropping had higher arylsulfatase activity than the single cropping, with an intermediate level in the Cowpea intercropping. On the other hand, under *U. brizantha*, the Pigeon pea intercropping had 62% and 53% lower arylsulfatase activity than Cowpea intercropping and single cropping, respectively (Fig. 1C). The activity of acid phosphatase and urease were independent of the grasses and legume intercrops (Figs. 1D and 1E).

3.3. Pasture and soybean yield

The pasture shoot biomass production of *U. ruziziensis* was higher than that of *U. brizantha*, both intercropped with Cowpea and single-cropped (26% and 16%, respectively) (Fig. 2). The grasses yielded similar amounts of shoot biomass when intercropped with Pigeon pea (5.3 Mg ha^{-1}). Similarly, whereas *U. ruziziensis* yielded more biomass when intercropped with Cowpea or single cropped, *U. brizantha* had similar shoot biomass productions among the intercrops (Fig. 2).

The highest soybean yield was found under *U. brizantha* intercropped with Cowpea (3.9 Mg ha^{-1}), on average for both cropping seasons (Fig. 3A). Soybean yielded 5% more after *U. brizantha* + Cowpea than after *U. ruziziensis* + Cowpea. On the other hand, when intercropped with Pigeon pea or single-cropped, soybean yields were 9% and 8% higher, respectively, using *U. ruziziensis* compared to *U. brizantha* (Fig. 3A). Regardless of the grasses evaluated, Cowpea intercropping yielded 18% and 24% more soybeans than Pigeon pea intercropping and single-cropped, respectively. Regarding the cropping seasons, soybeans yielded 20% more in 2017/2018 crop than in 2016/2017 (3.1 Mg ha^{-1}) (Fig. 3B).

3.3. Relationship among the variables

Positive and significant correlation was found between soybean yield and microbial biomass C ($r = 0.74$), microbial biomass N ($r = 0.50$) and $q\text{MIC}$ ($r = 0.70$) and inversely proportional to $q\text{CO}_2$ ($r = -0.40$) (Table 3). In addition to microbial biomass N ($r = 0.60$), basal respiration ($r = -0.50$), $q\text{CO}_2$ ($r = -0.53$) and $q\text{MIC}$ ($r = 0.96$), microbial biomass C was also correlated with urease activity ($r = 0.39$). Microbial biomass N had a correlation with basal respiration ($r = -0.47$), $q\text{CO}_2$ ($r = -0.84$), $q\text{MIC}$ ($r = 0.63$), acid phosphatase ($r = 0.60$), and urease ($r = 0.60$) (Table 3). In addition to acid phosphatase ($r = -0.56$) and urease ($r = -0.46$), $q\text{CO}_2$ correlated with β -glucosidase activity ($r = -0.68$). The $q\text{MIC}$ correlated only with urease activity ($r = 0.42$).

Table 3

Pearson correlation coefficients among soil attributes in an integrated crop-livestock system under no-tillage with intercrop at the pasture stage

	TOC	TN	MBC	MBN	BR	qCO_2	$qMIC$	β	A-Phos	Aril	Urease	FDA
TN	0.36 ^{ns}											
MBC	-0.10 ^{ns}	0.34 ^{ns}										
MBN	0.22 ^{ns}	0.35 ^{ns}	0.65*									
BR	-0.12 ^{ns}	-0.70*	-0.50*	-0.47*								
qCO_2	-0.26 ^{ns}	-0.64*	-0.53*	-0.84*	0.83*							
$qMIC$	-0.33 ^{ns}	0.20 ^{ns}	0.96*	0.63*	-0.42*	-0.47*						
β	0.20 ^{ns}	0.70*	0.06 ^{ns}	0.38 ^{ns}	-0.68*	-0.68*	0.02 ^{ns}					
A-Phos	0.19 ^{ns}	0.21 ^{ns}	0.33 ^{ns}	0.60*	-0.37 ^{ns}	-0.56*	0.29 ^{ns}	0.36 ^{ns}				
Aril	-0.02 ^{ns}	-0.48*	-0.01 ^{ns}	0.25 ^{ns}	0.51*	0.12 ^{ns}	0.03 ^{ns}	-0.46*	0.60*			
Urease	0.03 ^{ns}	0.51*	0.39*	0.60*	-0.46*	-0.63*	0.42*	0.64*	-0.09 ^{ns}	-0.09 ^{ns}		
FDA	0.20 ^{ns}	-0.21 ^{ns}	-0.08 ^{ns}	0.17 ^{ns}	0.52*	0.14 ^{ns}	-0.05 ^{ns}	-0.27 ^{ns}	0.03 ^{ns}	0.60*	0.19 ^{ns}	
Yield	-0.05 ^{ns}	0.30 ^{ns}	0.74*	0.57*	-0.29 ^{ns}	-0.40*	0.70*	0.12 ^{ns}	0.16 ^{ns}	0.20 ^{ns}	-0.02 ^{ns}	-0.02 ^{ns}

^{ns}: Not significant. *: Significant at 5% by t-test. Total organic carbon stock (TOC), total nitrogen stock (TN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), basal respiration (BR), metabolic quotient (qCO_2), microbial quotient ($qMIC$), β -glucosidase (β), acid phosphatase (A-Phos), arylsulfatase (Aril), urease and fluorescein diacetate hydrolysis (FDA) and soybean yield (Yield).

The PCA results showed that PC1 (principal component 1) retained 40.84% and PC2 (principal component 2) retained 35.96% of data inertia, accumulating in the graph 76.8% of the information of the original matrix (Fig. 4). It is possible to verify clearly the individual effects of the treatments on the soybean yield and soil biochemical properties. From the PCA analysis it is possible to verify that the highest soybean yields were found using *U. ruziziensis*, intercropped with Cowpea or Pigeon pea. These yields were obtained by higher $qMIC$ and microbial biomass C and N (Fig. 4). On the other hand, the cultivation of single *U. brizantha* has lower soybean yields.

4. Discussion

The grass-legume intercropping improved soil quality through soil biological and biochemical properties and increased soybean yields. The Cowpea intercropping yielded more soybeans than Pigeon pea intercropping and single-cropped. According to Laroca et al. (2018), the grass-legume intercropping with Cowpea and Pigeon pea resulted in higher soybean yields (416 and 338 kg ha⁻¹, respectively), compared to single cropping of grasses. The higher soybean yields were associated with higher microbial biomass C and N contents, $qMIC$ and lower basal respiration and qCO_2 (Table 2).

The basal respiration rate and the qCO_2 are variables used to measure the metabolic activity of microbial biomass in the soil. Higher qCO_2 values indicate C losses by the soil, showing stress conditions for microbiota according to the large amount of energy for its maintenance (Nunes et al. 2011). Low- qCO_2 values are related to environments that are more stable or closer to their equilibrium state, as they indicate energy savings (Silva et al. 2010; Wardle and Ghani 1995). In addition, microbial biomass and $qMIC$ are commonly used for soil quality assessment (Bastida et al. 2008; Mader et al. 2002; Salinas-García et al. 2002). Therefore, the lower basal respiration and qCO_2 , and the greater $qMIC$ and microbial biomass C may indicate higher soil microbial C utilization efficiency (Andersen et al. 2013; Anderson and Domsch 1978; Pleisner et al. 2016) under grass-legume intercropping (Table 2). This may be

due to the higher contribution of labile C and N (with easy oxidation) from treatments under grass-legume intercropping (Crème et al. 2016) with residue inputs with low C/N ratio.

The residue inputs with lower C/N ratios under grass-legume intercropping contributes to an increase in microbial biomass and activity (Leite et al. 2013; Moraes et al. 2019). This indicates a higher conversion efficiency of the C and N in plant residues into C and N in microbial biomass (Balota and Chaves 2011; Spohn 2015; Veloso et al. 2019). Therefore, the grass-legume intercropping in ICLS improve soil microbial properties, which may contribute in the long run to achieve the equilibrium in the production system, since it showed low- qCO_2 values, but maintained high $qMIC$ values (Table 2), highlighting that the microbial biomass is not under stress and is able to use organic C efficiently (Fang et al. 2018; Hartman and Richardson 2013; Silva et al. 2010).

The microbial biomass C and N improvements under grass-legume intercropping (Table 2) were also reported by Hurisso et al. (2013) and Almeida et al. (2016). The microbial biomass N increments may be due to leguminous plants insertion, which favors soil N availability through the biological N fixation (Crème et al. 2016). On the other hand, in single grasses the soil microbiota consumes more C to maintain a lower microbial population. The greater the abundance and diversity of roots, the greater the exudation of organic compounds that will serve as a source of C and energy for soil microorganisms (Chávez et al. 2011; Dhakal and Islam 2018).

The total C and N stocks also increased in the soil, mainly under Pigeon Pea intercropping, compared to single grasses (Table 1). These results demonstrate that grasses, when intercropped with legumes, promote the C and N improvements in the soil. Similar results were found by Frasier et al. (2016) and Laroca et al. (2018), who observed increases of C and N stocks in an experiment using grass-legume intercropping. Frasier et al. (2016) assigned the increase of total N stock to the better residue quality (lower C/N ratio) provided by leguminous plants. Almeida et al. (2016) also documented improvements in microbial biomass C and N under grass-legume intercropping systems, which may result in higher soybean yields.

Soybean under N-fixing bacteria inoculation does not rely to N fertilizer because it reduces restricts carbohydrates to nodule metabolism (Denison and Harter 1995). However, some studies have shown that N addition via leguminous crops may positively influence the soybean yields (Nascente and Stone 2018; Tanaka et al. 1992). The higher-N input to the soil-plant system, derived from the biological N fixation, may contribute to improvements of soybean yield due to the N source, that is gradually released through mineralization (Cicek et al. 2014; Pacheco et al. 2017). The biological N fixation can contribute a considerable N fraction in the soil, releasing to the soybean in succession, reflecting in increases in grain yield (Pacheco et al. 2017). Likewise, soybean is positively influenced by N fertilization in pasture grasses in advance (Costa et al. 2021).

The β -glucosidase activity was also higher under grass-legume intercropping (Fig. 1A). This enzyme acts on the hydrolysis of organic compounds and it is important in the C life cycle (Bowles et al. 2014). It has a direct relationship with the N stock in the soil (Stieven et al. 2014), which was also higher under grass-legume intercropping (Table 2). The higher FDA activity found in grass-legume intercropping treatments (Fig. 1B) corroborate the results of Ferreira et al. (2017), which found higher FDA activity in agroecological production systems with legumes compared to conventional systems using single grasses.

The acid phosphatase and urease activity were not affected by treatments (Figs. 1D and 1E). Laroca et al. (2018) found similar results where the acid phosphatase activity was not influenced by legumes or grasses, because the activity of this enzyme is more related to phosphate fertilization, which was the same in all treatments. Ye et al. (2017) reported a reduction of the acid phosphatase activity, and associated this result with the excess available phosphorous in the soil. Lanna et al. (2010) documented greater urease activity related to higher N content in the soil. Despite not differing significantly (Fig. 1E), same effect was found in this study, where the urease activity was correlated ($r = 0.51^*$) to total N stocks (Table 3).

Regarding the arylsulfatase activity, studies conducted in Brazil indicate that the quality of residues added to the soil are more determinant in the activity of this enzyme than the quantity of residues (Balota et al. 2011), and the input of more labile organic residues helps the activity of this enzyme (Lisboa et al. 2012). In addition, the higher contents of total organic C stocks (Table 1) can provide high levels of sulfur in the form of sulfate esters which, in turn, are substrates for this enzyme (Balota et al. 2014).

In summary, legume intercrops increased microbial activity and biomass and total C and N stocks, and $qMIC$, and decreased basal respiration and qCO_2 , compared to single grasses in pasture phase. However, the adoption of ICLS under no-till using single grasses in pasture phase is already an important way to improve soil quality (Sarto et al. 2020) and grain yield, even in the short-term

(Carneiro et al. 2009). In the Brazilian Cerrado region, soil C improvements can reach 26 g kg^{-1} under ICLS compared to *U. decumbens* as cover crop (Gazolla et al. 2015). Alves et al. (2011), evaluating microbial properties, documented a microbial biomass C 60% higher under ICLS compared to the native vegetation of Cerrado region. Therefore, grass-legume intercropping in the pasture phase of ICLS is an additional tool to maximize soil quality improvements and soybean yields even in the short-term.

5. Conclusions

Our results highlights the importance of grass-legume intercropping at the pasture stage in ICLS in the Brazilian Cerrado region. The grass-legume intercropping, mainly with Cowpea, increases the microbial biomass carbon and nitrogen and microbial quotient. On the other hand, single grasses cause stress in the microbiota by the increase of the basal respiration and metabolic quotient. In general, an increase in the total enzymatic activity of soil is observed when using grass-legume intercropping at the pasture stage, mainly with Pigeon pea. The grass-legume intercropping is an effective strategy to increase the total organic carbon and nitrogen stocks in the soil, which reflect in an increase in soybean grain yield.

Declarations

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Figures

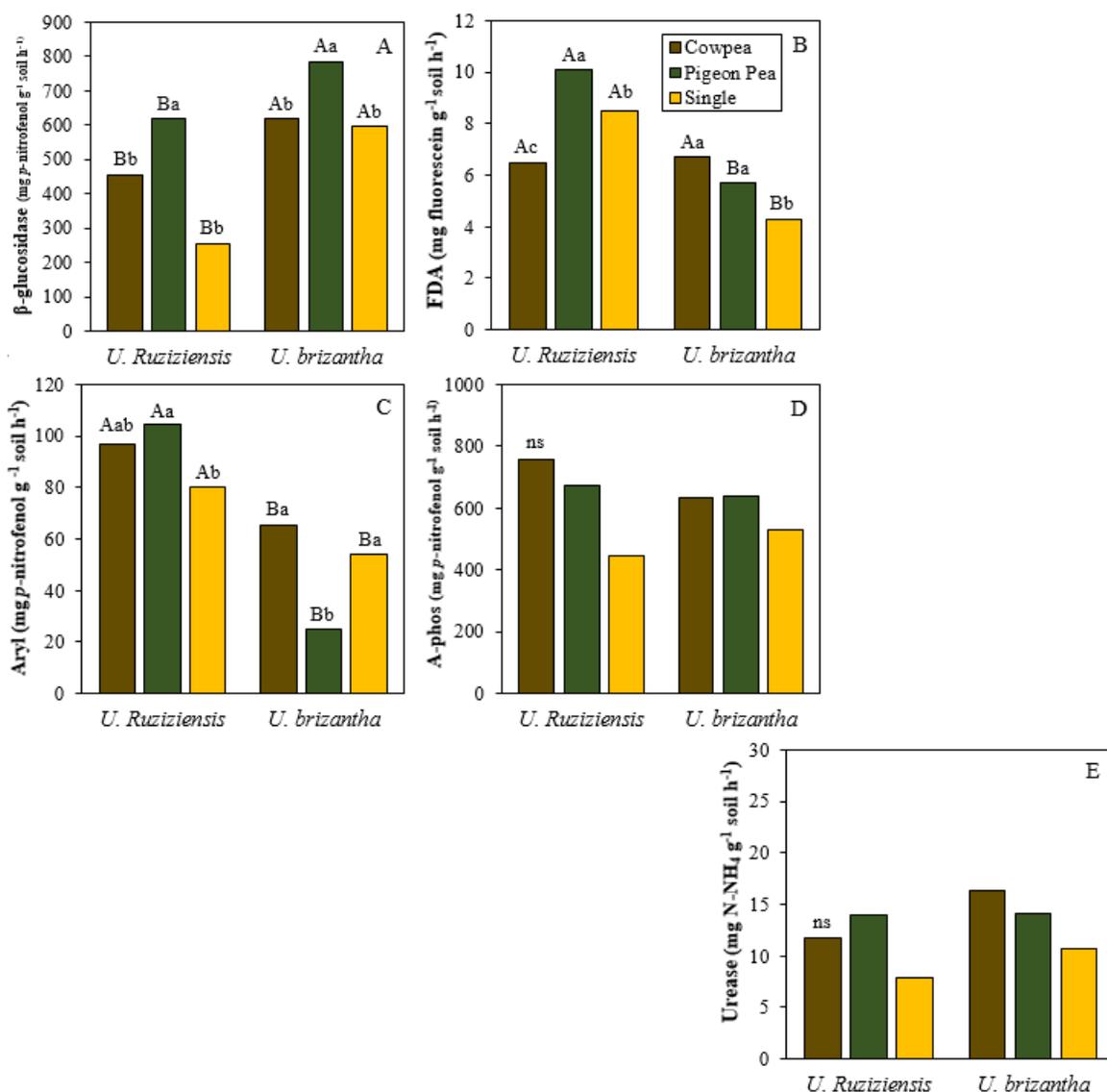


Figure 1

β -glucosidase (A), fluorescein diacetate (FDA) (B), arylsulfatase (Aryl) (C), acid phosphatase (A-phos) (D), and urease (E) activity two years after the adoption (March 2018) of an integrated crop-livestock system under no-tillage and different grasses and legumes intercrops at the pasture stage. Tukey's test ($p < 0.05$) = Uppercase letters indicate differences between grasses. Lowercase letters indicate differences among intercropping. ns = not significant.

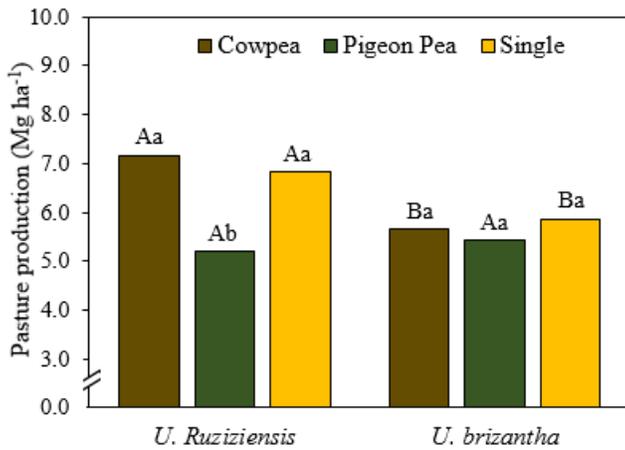


Figure 2

Pasture shoot biomass before the start of grazing in an integrated crop-livestock system under no-tillage and different grass-legume intercropping at the pasture stage on average of 2016 and 2017 growing seasons. Tukey's test ($p < 0.05$) = Uppercase letters indicate differences between grasses. Lowercase letters indicate differences among intercropping.

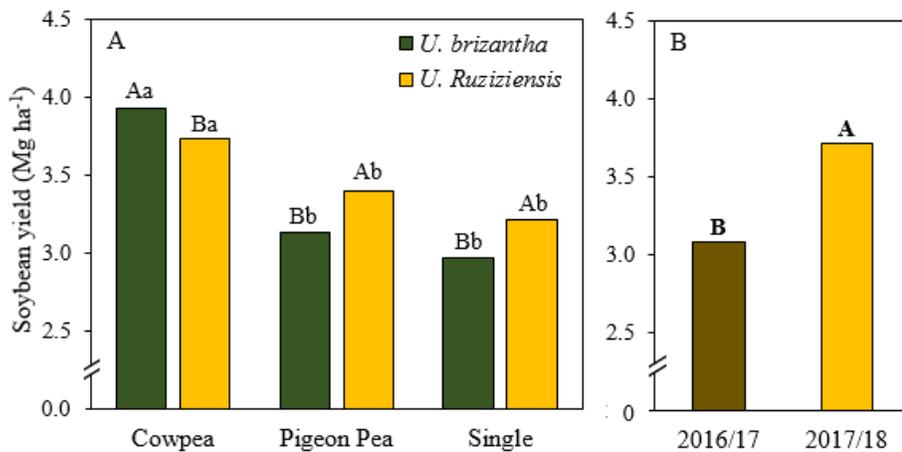


Figure 3

Soybean yield in an integrated crop-livestock system under no-tillage and different grasses and legumes intercrops at the pasture stage (A) in 2016/17 and 2017/18 cropping seasons (B). Tukey's test ($p < 0.05$) = Uppercase letters indicate differences between grasses or cropping seasons. Lowercase letters indicate differences among intercropping.

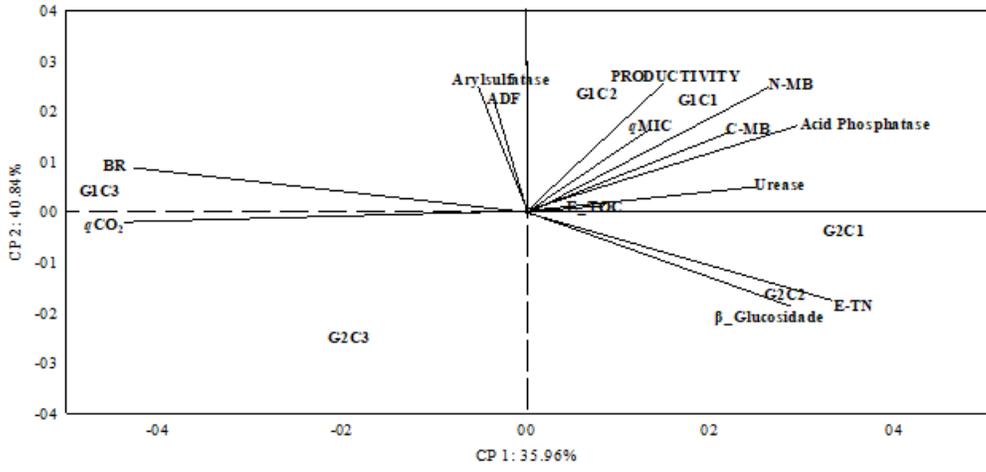


Figure 4

Graphical distribution of soil biochemical properties and soybean yield according to their similarities in an integrated crop-livestock system under no-tillage with intercrops at the pasture stage. G1C1: U. Ruziziensis intercropped with Cowpea; G1C2: U. Ruziziensis intercropped with Pigeon pea; G1C3: U. Ruziziensis single; G2C1: U. brizantha intercropped with Cowpea; G2C2: U. brizantha intercropped with Pigeon pea; G2C3: U. brizantha single.

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