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Microbial indicators of soil quality and soybean yield in agricultural production system using cover crops under no-tillage

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Abstract

Cover crops improve the physical, chemical and biological quality of the soil and boost crop yield. However, the magnitude of the effects on the microbial activity in tropical soils of Cerrado is still little explored. This study aimed to evaluate the soil microbial attributes and soybean yield after cultivation of cover crops in a sandy clay loam oxisol under no-tillage system, in a region with weather dominated by a bimodal rainfall pattern (Aw type). The experiment was designed in randomized blocks, using a split-plot scheme, with different cover crops in the plots and different soil sampling times in the subplots. The cover crops treatments consisted of the previous cultivation of *Crotalaria spectabilis* (*C. spectabilis*), *Crotalaria ochroleuca* (*C. ochroleuca*), *Cajanus cajan* (pigeon pea); *Urochloa ruziziensis* (brachiaria) and *Pennisetum glaucum* (millet) as monocrop and the intercropping of millet + *C. spectabilis* and millet + *C. ochroleuca*. Soil samplings for microbial and chemical attributes evaluations were performed before soybean sowing and after soybean harvest, which occurred at 12 and 18 months after sowing the cover crops. Dry mass (DM) productions of cover crops at full vegetative development stage and soybean yield were also estimated. All the cover crops used in the present study, except the millet + *C. spectabilis* intercrop produced more than 6 Mg ha^{-1}, with is considered the minimum amount of dry mass indicated as adequate for conservation systems under tropical conditions. Soil cultivated with brachiaria and millet showed higher microbial biomass at 18 months than at 12 months. Similarly, soil cultivated with *C. ochroleuca*, brachiaria, pigeon pea, millet + *C. ochroleuca* and millet + *C. spectabilis* showed higher microbial N at 18 months than at 12 months. Eighteen months after sowing of cover crops, millet maintained the highest soil microbial biomass (161.24 µg C g soil^{-1}) and the millet + *C. spectabilis* intercrop exhibited the highest soil microbial N contents (30.02 µg N g soil^{-1}) across treatments. *Crotalaria ochroleuca,* brachiaria, millet + *C. ochroleuca* and monoculture millet increased soybean yield cultivated in succession, after one single cycle of crop rotation.

Keywords: *Glycine max* L.; soil quality; no-tillage system; microbial biomass.

Abbreviations: dry mass_DM; P_phosphorus; K^+_potassium; Ca^{2+} _calcium; Mg^{2+} _magnesium; Al^+_aluminium; H+Al_potencial acidity; soil organic carbon_SOC; V_base saturation; m_aluminium saturation; *Crotalaria spectabilis*_*C. spectabilis*; *Crotalaria ochroleuca*_*C. ochroleuca*; *Cajanus cajan*_pigeon pea; *Urochloa ruziziensis*_brachiaria; *Pennisetum glaucum*_millet; Carbon microbial biomass_C_{mic}, Nitrogen microbial biomass_N_{mic}, Basal soil respiration_BSR; metabolic quotient_qCO_2; component 1_PC1; component 2_PC2.

Introduction

Agricultural grain production systems are increasingly common in the MATOPIBA region, the last agricultural frontier in Brazil, with increasing yield averages (Araújo et al., 2019). In these systems, the maintenance of the soil quality is essential to ensure the sustainability of crops. Among the attributes that describe soil quality, microbial indicators are among those that express changes in use and management more quickly. In the soil, microorganisms are involved in processes such as organic matter decomposition, humus production, nutrient cycling, biological nitrogen fixation, production of complex compounds that contribute to soil aggregation, xenobiotic decomposition, and biological pest and diseases control (Mbuthia et al., 2015). Therefore, monitoring soil microbial attributes allows the adequate assessment of soil quality.

The use and management of soil promote significant changes in microbial biomass, since it is influenced by both human activity and plant development (Kaschuk et al., 2010, Kim et al., 2020, Liu et al., 2023). The adoption of conservationist soil management systems that prioritize the contribution of organic matter to the soil, with the use of cover crops improves soil microbial indicators and therefore, represent a promising alternative for the agriculture sustainability and crop yield (Gattullo et al., 2020). In addition to soil management and plant phenological stage, rainfall also affects soil microbiological attributes, which implies that soil quality is driven by a combination of environmental, climatic, physiological, and edaphic factors.

Soil management with conservation systems, without or with minimum soil disturbance and with the use of cover crops, promoted a short-term increase in soil microbial quality and soybean (*Glycine max* L.) yield in the Cerrado of Piauí (Pires et al., 2020). Similarly, Mbuthia et al., (2015) observed an improvement in microbiological attributes and soil nutrient cycling in soil under no-tillage with cover crops. The authors also observed an increase in cotton yield compared to the conventional tillage system. However, the transition from Native Cerrado to agricultural land uses modifies the microbiological attributes of the soil, which can last for several years after the anthropogenic intervention. Thus, even with the adoption of conservation systems, it may not be possible to restore the soil microbial biomass observed in the soil under native vegetation (Cunha et al., 2021).

The introduction of cover crops in the management system, their effect on the accumulation of soil organic matter (SOM) and on the improvement of microbiological attributes must be quantified regionally and for each production system as it depends on the relief, temperature, humidity conditions, and on the prevailing management system and soil characteristics (Blanco-Canqui, 2022). Thus, any change in the soil management system can directly influence its structure and biological activity and, consequently, the soil fertility, with effects on agroecosystems. Therefore, it is worth highlighting the importance of soil conservation practices in the tropics, where the effects on microbial biomass are much greater than reported for temperate regions (Hungria et al., 2009).

It is hypothesized that the use of cover crops in agricultural systems with crop rotation in the Brazilian Cerrado improves soil microbial quality and soybean yield. Thus, this aimed to evaluate soil microbial attributes and soybean grain yield in a typical Oxisol with cover crops in a crop rotation system under no-tillage.

Results and Discussion

Biomass production of cover crops and soil chemical attributes

Average dry mass (DM) of the cover crops was 7.6 Mg ha^{-1}. Intercropping millet + *C. spectabilis* produced the least amount of DM (5.6 Mg ha^{-1}) and differed from all other cultivated plants that showed higher production (Figure 1). The amount of 6 Mg ha^{-1} DM is the minimum value required to properly making possible the no-tillage system, maintaining the soil covered (Darolt, 1998). Similar phytomass values highlighted in this study were also observed by Pires et al. (2020) in the same region, except for millet + *C. spectabilis* intercropping, in which the authors observed DM values of 8.2 Mg ha^{-1}.

Carneiro et al. (2008) verified phytomass production for millet, pigeon pea and *C. spectabilis* of 16, 39, 17 and 9 Mg ha^{-1}, respectively, in an Oxisol of central Brazil. Except for *C. spectabilis*, the DM values observed for millet and pigeon pea were very different from those found in this study. This is justified by the sowing time, fertilization, and the adequate rainfall for vegetative development in their study. High biomass production is an important requirement for the adoption of a species in conservation systems when using straw for soil protection in the fall-winter period (Jat et al., 2020; Tyler, 2020).

There was a significant effect of cover crops on soil P and K concentrations (Table 1). Highest P concentration was observed with the use of *C. spectabilis* (Table 1). On the other hand, the lowest soil P concentrations occurred with brachiaria and *C. ochroleuca*, indicating that these crops cycled a small amount of P, decreasing its content in the soil. These results are related to the rusticity of these crops, which can grow even in low fertility soils. The potential of cover crops to recycle nutrients depends, among other characteristics, on the potential for phytomass production and the capacity to absorb nutrients from the soil and accumulate them in the plant (Mortensen et al., 2021).

There was an increase in the soil K$^+$ concentration for all the treatments (Table 1) compared to the initial values before the cover crops cultivation (Table 5). The high cycling capacity of K$^+$ by the cover crops used in this study explains this finding. Among the treatments, the highest K$^+$ contents were observed with *C. spectabilis*, brachiaria and *C. ochroleuca*, and the lowest with pigeon pea, millet, millet + *C. spectabilis* and millet + *C. ochroleuca*.

Forage grasses are reported to have great potential for absorption and accumulation of K$^+$ that returns to the soil after desiccation (Brito et al., 2023). Despite that, in this study, two legume species stood out regarding the ability of recycling K$^+$, which opens new perspectives for their use as cover crops in the Cerrado of Piauí, improving soil fertility. Another important aspect when choosing cover crop species in rotation with grain production systems is the synchrony of K$^+$ release by the straw with the demand of the successor crop, mainly soybean, because K$^+$ is one of the most exported nutrients by this crop (21.6 kg K_2O per Mg of grain produced) (Pires et al., 2022). Cycling of K$^+$ by cover crops reduces the use of fertilizers for the successor crop, thereby reducing costs of production (Werle et al., 2008).

Contents of Ca^{2+} did not differ among treatments and were lower than observed before the implementation of the experiment (Table 1). The values are close to the minimum limit of 1.5 cmol$_c$ dm^{-3} for Cerrado soils, as recommended by Sousa and Lobato (2004). Possibly, the concentrations of Ca^{2+} in the soil were not high enough to supply the demand of cover crops, maintaining sufficient levels in the soil. Mg^{2+} contents after cover crops cultivation remained like the initial levels (Table 1), indicating that the amount of Mg^{2+} in the soil was sufficient to meet the demand of cover crops and maintain its levels in the soil. Mg^{2+} content of 0.5 cmol$_c$ dm^{-3} is considered the minimum limit and therefore, observed levels for the treatments tested are considered high (Sousa and Lobato, 2004). Our results show that the use of cover crops is a feasible strategy for the cycling of important macronutrients and the improvement of soil fertility, especially P and K$^+$.

Soil microbial attributes

There was an effect of cover crops, sampling time and interaction between these factors for all microbial indicators evaluated (Table 2). Cultivation of exclusive brachiaria and millet affected C_{mic} at different times, with an increase in microbial density from 12 to 18 months (Table 3). Amorim et al. (2020) also observed high C_{mic} values with the incorporation of brachiaria straw into the soil, which was justified by the type of residue and incorporation time that positively affected this microbial indicator. In a no-tillage system in the same region of the present study (Pires et al., 2020), higher C_{mic} values were also observed with the use of brachiaria and millet approximately 18 months after the cultivation of these cover crops, compared to other species used in the study, such as crotalarias and spontaneous vegetation. The great amount of C_{mic} in the soil is related to functions such as decomposition, accumulation of organic matter and nutrient cycling (Araújo and Monteiro, 2007). It is also noteworthy that the treatment with brachiaria was one of those that presented the highest K^+ cycling in this study (Table 1).

For most cover crops used in this study, N_{mic} was around 50% higher at 18 months, than at 12 months (Table 3), except for millet that did not exhibit difference for this attribute at the times evaluated and the *C. spectabilis* that presented a reduction of 25% from 12 to 18 months. It is noteworthy that in the second sampling period, the highest N_{mic} contents occurred in the treatments with pigeon pea and millet + *C spectabilis*. For the intercropping, the increase in N_{mic} was 4 times greater at 18 months, than at 12 months.

Microbial N is an important reserve of organic nitrogen with mineralization potential (Moreira, 2006). Therefore, the higher the N content of the microbial biomass, the faster its cycling in the soil. This is an important tool for crop nutrition as N is one of the most limiting nutrients and any difference in mineralization, immobilization and nitrification rates can have a significant effect on plant production and sustainability of agricultural production systems (Gennaro et al., 2014).

Basal soil respiration (BSR) differed across times of evaluation for *C. ochroleuca* and brachiaria. In both treatments, there was a reduction in BSR at 18 months, compared to 12 months (Table 3). Microbial respiration is influenced by temperature, humidity, and nutrient availability (Araújo and Monteiro, 2007). The highest BSR in *C. ochroleuca* and brachiaria at 12 months coincided with the highest rainfall in this period (Figure 3). Soil moisture is a relevant factor on microbial activity, regulating it in various ways: as a component of protoplasm, gas exchange and acting in the transport and dissolution of soil nutrients (Alexander, 1978). Higher values of BSR (C-CO_2 release) imply greater biological activity, which is directly related to the availability of soil carbon and/or microbial biomass (Allen et al., 2011). Thus, a high respiration rate can be interpreted as a desirable characteristic when considering that the decomposition of organic waste will make nutrients available (Bakhshandeh et al., 2019).

Differences in qCO_2 also occurred for *C. ochroleuca* and brachiaria, with higher values at 12 months compared to 18 months. For the other cover crops, there were no differences between the periods evaluated. High qCO_2 values indicate energy directed towards cell maintenance rather than growth and reproduction, so that a proportion of carbon from biomass will be lost as CO_2 (Araújo and Monteiro, 2007).

For brachiaria, an increase in C_{mic} and decrease in qCO_2 at 18 months was evident, indicating a higher use of energy for growth and reproduction of the microbial community, in relation to the first sampling period. The reduction in qCO_2 values at 18 months indicates that the microbial biomass is being more efficient, as less CO_2 per unit of microbial biomass is lost. As a given microbial biomass becomes more efficient, less carbon is lost as CO_2 by respiration and a significant C fraction is incorporated into the microbial tissue (Puttaso et al., 2011; Blagodatskaya et al., 2014).

Soybean development and yield

Among the cover crops evaluated, there was a significant difference for soybean leaf area and yield (Table 4). The largest soybean leaf area occurred with the intercropping of millet + *C. ochroleuca* and millet + *C. spectabilis*, which differed from the other cover crops.

Average soybean grain yield across treatments in this study was 1.15 Mg ha^{-1}, which is way below average soybean yield in the region (IBGE 2022). It is important to emphasize that the low yield of soybeans is attributed to low rainfall during crop growth (Figure 3). The occurrence of dry spells during soybean growth may have been harmful to the absorption of water and nutrients by the soybean, compromising yield. Despite that, soybean yield was higher in *C. ochroleuca*, brachiaria, millet + *C. ochroleuca* and millet, than in the other treatments, possibly because of less evapotranspiration and better use of water.

It is known that the potential yield of a crop is determined by genetic and environmental factors. Water availability affects the growth and development of the soybean crop, especially during the reproductive period, a phase of high physiological activity (Sentelhas et al., 2015; Gavili et al., 2019). Moreover, this study refers to a no-tillage system in the first years of implementation and therefore, there was no sufficient time for soil stabilization especially regarding treatment effects on SOC (Table 1) and other soil attributes that could boost the resilience of plants to water shortage. Pacheco et al. (2011) also observed that the soybean crop showed little variation in yield in areas with cover crops newly incorporated into the no-tillage system.

The systematic management of crop rotation with vegetative cover over the years provides a beneficial effect on the yield of the subsequent crop (Garbelini et al., 2022). In the same region of this study, highest soybean yields were obtained in succession to *C. ochroleuca* and intercropping of millet + *C. spectabilis*, which was attributed to highest nutrient cycling by these cover crops (Pires et al., 2020). Similarly, Balbinot Júnior et al. (2017) found that previous cultivation of brachiaria increased the soybean yield, compared to fallow. On the other hand, Pacheco et al. (2011) and Valicheski et al. (2012) reported no influence of cover crops on the yield of soybean cultivated in succession. Contrasting results found in this study and in literature suggest that long-term studies in stabilized no-tillage systems are necessary to elucidate whether the initial effect of cover crops is maintained over the years.

Principal component analysis

The principal component analysis explained 72% of the data variation, with 41% in component 1 (PC1) and 31% in component 2 (PC2) (Figure 2). Plant dry mass production (DM) of cover crops correlated positively with soybean yield (GP), except for the intercropping millet + *C. spectabilis*, as this was the one that produced the least amount of DM (Figure 1).

Table 1. Soil chemical attributes (average of the 0.0-0.10, 0.10-0.20 and 0.20-0.40 m soil depths) after the use of cover crops in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil, 2016.

Cover crops	pH	SOC	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	CEC	BS	m
		g kg $^{-1}$	-----mg dm^{-3}----		----------------cmol$_c$dm^{-3}-------------					%	
C. spectabilis	5.7^{ns}	19.85^{ns}	44.67 a	78.09 a	1.85^{ns}	1.11^{ns}	0.15^{ns}	2.59^{ns}	5.73^{ns}	52^{ns}	10^{ns}
C. ochroleuca	5.5	20.38	19.13 c	70.54 a	1.56	0.91	0.14	3.08	5.70	43	11
Pigeon pea	5.6	20.29	27.21b	63.10 b	1.54	0.95	0.15	2.67	5.29	49	11
Brachiaria	5.7	19.19	19.33 c	72.02 a	1.58	0.97	0.09	2.37	5.10	51	7
Millet	5.6	19.56	30.63 b	61.37 b	1.58	0.98	0.16	2.58	5.28	48	13
millet + *C. spectabilis*	5.7	19.90	32.88 b	63.78 b	1.73	0.99	0.10	2.40	5.24	51	8
millet + *C. ochroleuca*	5.5	19.27	26.63 b	59.44 b	1.67	1.07	0.10	2.66	5.53	49	6
F test	ns	*	*	*	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.51	7.62	45.14	17.68	34.79	36.27	10.50	27.33	12.38	29.00	10.80

pH in H_2O; SOC: soil organic carbon; P: phosphorus, K^+: potassium, Ca^{2+}: calcium, Mg^{2+}: magnesium, Al^{3+}: aluminum, H+Al: potential acidity, CEC: cation exchange capacity, BS: base saturation, m: aluminum saturation. *: Significant at 5% probability; ^{ns}: non-significant; Means followed by the same lowercase letter in the column for each attribute, belong to the same group according to the Scott-Knott test ($p \leq 0.05$).

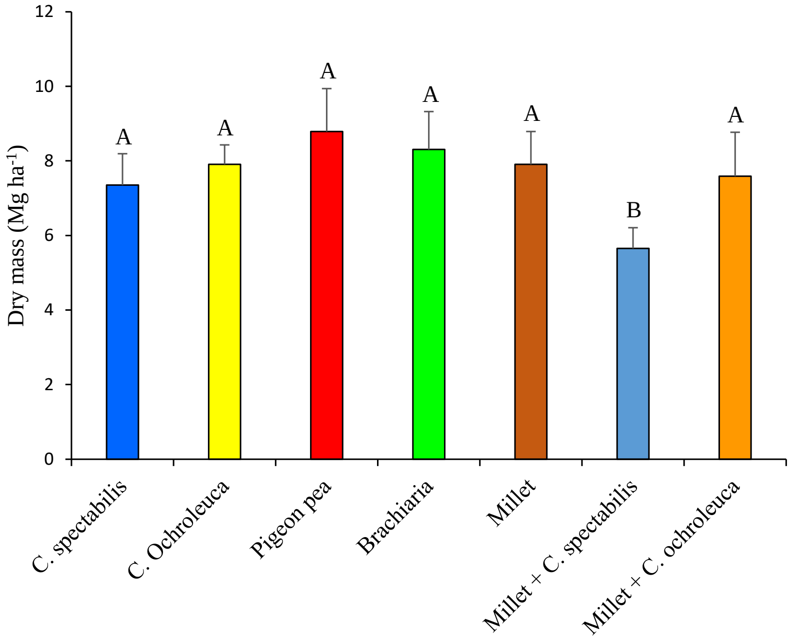

Figure 1. Dry mass production (DM) of cover crops used in the study in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil, 2016. Means followed by the same letter on the bars belong to the same group according to the Scott–Knott test ($p \leq 0.05$). Error bars indicate standard errors (n= 4).

Table 2. Analysis of variance for soil microbial indicators affected by cover crops cultivation and sampling time in the 0.00 to 0.10 m soil depth.

Source of variation	Microbial indicators					
	C_{mic}	N_{mic}	C:N Ratio	BSR	$q\mathrm{CO}_2$	
Cover crops (CC)	*	*	*	*	ns	
Sampling time (ST)	ns	*	*	*	ns	
CC * ST	*	*	*	*	*	
CV (%)	55.3	32.2	28.8	41.1	113.2	

C_{mic} and N_{mic}: carbon and nitrogen of the microbial biomass, respectively; C:N Ratio: carbon and nitrogen ratio; BSR: basal soil respiration; $q\mathrm{CO}_2$: metabolic quotient. *: Significant at 5% probability; ^{ns}: non-significant.

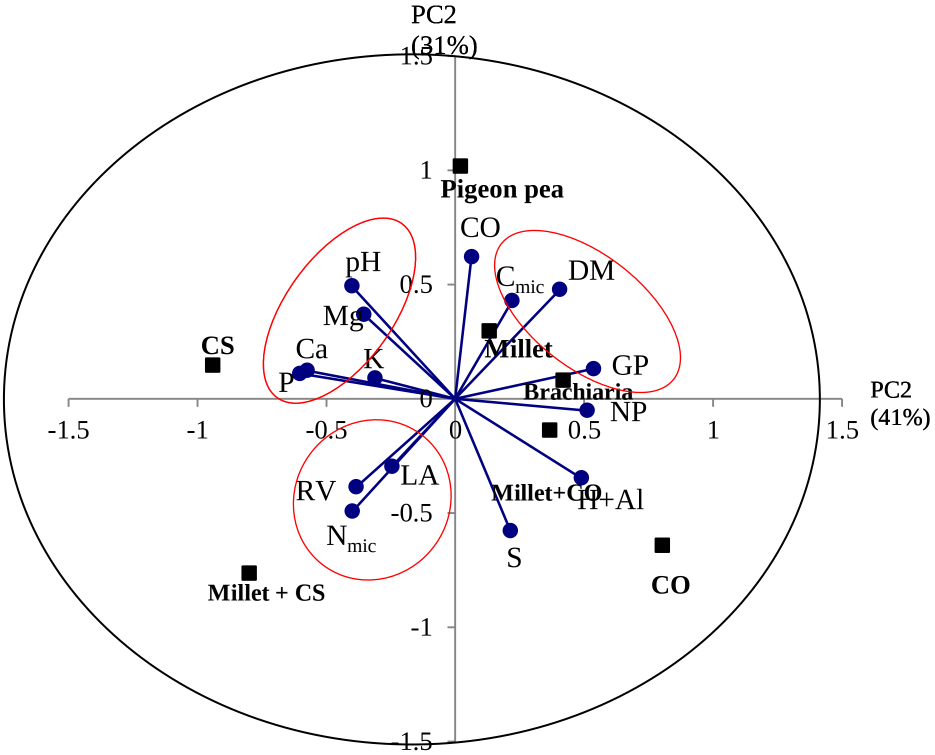

Figure 2. Principal component analysis (PCA), for soil microbial and chemical attributes, agronomic traits and soybean yield after cover crops cultivation in the municipality of Baixa Grande do Ribeiro, Piauí, Brasil, 2016. CS: *C. spectabilis*; Pigeon pea: *Cajanus cajan*; CO: *C. ochroleuca*; Millet+CS: millet+*C. spectabilis*; Millet+CO: Millet+*C. ochroleuca*. DM: dry mass of cover crops; GP: Grain productivity; NP: number of pods of soybean; LA: Leaf area of soybean; RV: Root volume.

Table 3. Microbial indicators of a typical Oxisol in the 0-0.10m layer with sampling times at 12 and 18 months after sowing of cover crops in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil, 2016.

Cover crops	12 months (December/15)					18 months (July/16)				
	C_{mic}	N_{mic}	C:N Ratio	BSR	qCO_2	C_{mic}	N_{mic}	C:N Ratio	BSR	qCO_2
	µg g^{-1}soil			mg CO_2 kg soil h^{-1}	mg C-CO_2 g^{-1} C-BMS h^{-1}	µg C g soil^{-1}	µg N g soil^{-1}		mg CO_2 kg soil h^{-1}	mg C-CO_2 g^{-1} C-BMS h^{-1}
C. ochroleuca	103.64Aa	7.78Ba	13.32Ab	4.42Aa	0.04Aa	97.30Ab	16.62Ac	6.44Bc	0.78Ba	0.01Ba
Brachiaria	68.18Bb	9.72Ba	7.01Ab	2.77 Ab	0.05Aa	111.45Ab	18.13Ac	6.34Ac	0.7Ba	0.01Ba
C. spectabilis	128.18Aa	12.31Aa	10.64Ab	0.68Ac	0.01Ab	96.00Ab	9.20Bd	12.7A1b	0.98Aa	0.01Aa
Pigeonpea	76.36Aa	9.72Ba	8.36Ab	1.05Ac	0.01Ab	82.97Ab	23.36Ab	3.73Ac	1.40Aa	0.02Aa
Millet + *C. ochroleuca*	136.36Aa	6.48Ba	21.92Aa	0.67Ac	0.01Ab	106.88Ab	13.96Ac	7.78Bc	1.22Aa	0.01Aa
Millet	84.55Bb	7.13Aa	12.71Bb	0.73Ac	0.01Ab	161.24Aa	7.19Ad	24.25Aa	1.24Aa	0.01Aa
Millet + *C. spectabilis*	49.09Ab	7.13Ba	7.54Ab	0.93Ac	0.02Ab	64.79Ab	30.02Aa	2.10Ac	1.10Aa	0.02Aa

C_{mic} and N_{mic}: carbon and nitrogen of the microbial biomass, respectively; C:N Ratio: carbon to nitrogen ratio; BSR: basal soil respiration; qCO_2: metabolic quotient. Means followed by capital letters in the lines compare the same cover crop treatment at different times. Means followed by lowercase letters in the column (cover crops) belong to the same group according the Scott–Knott test ($p \leq 0.05$).

Table 4. Average values for leaf area (LA), number of pods (NP), root volume (RV), stem diameter (SD), aerial dry mass (ADM) and grain productivity (GP) of soybean cultivated in succession to cover crops in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil, 2016.

Cover crops	LA	NP	RV	SD	ADM	GP
	(cm^2 plant^{-1})	(plants^{-1})	(cm^3)	(mm)	(kg ha^{-1})	(Mg ha^{-1})
C. ochroleuca	1312 b	19.79 ^{ns}	4.40 ^{ns}	5.31 ^{ns}	352^{ns}	1.24 a
Brachiaria	1213 b	20.04	4.65	4.93	322	1.29 a
C. spectabilis	1407 b	17.21	4.88	5.01	382	1.06 b
Pigeonpea	1343 b	16.92	4.15	5.05	379	1.11 b
Millet+ *C. ochroleuca*	1654a	21.08	4,58	5.04	381	1.18 a
Millet	1200 b	19.17	4.25	5.12	332	1.25 a
Millet+ *C. spectabilis*	1636 a	15.71	4.85	4.98	404	0.94 b
Average	1396	18.55	4.53	5.06	364.9	1.15
CV (%)	14.20	23.83	41.17	4.02	15.64	11.52

^{Ns}: Non-significant; Means followed by the same letter in the column belong to the same group according the Scott–Knott test ($p \leq 0.05$).

The millet, *C. ochroleuca*, brachiaria and the intercropping of millet + *C. ochroleuca* showed a high correlation with soybean yield (Figure 2), as also confirmed in Table 2, indicating that the cultivation of these cover crops can increase soybean yield even in periods of water stress, what happened during soybean development. In addition, it should be noted that the use of millet and brachiaria as cover crops provided an increase in soil C_{mic} at 18 months (Table 3) and the correlation of C_{mic} with soybean yield in PCA is evident. Pires et al. (2020) also found positive correlation of C_{mic} with soybean yield. This reinforces the hypothesis that the increase in microbial biomass, in addition to improved soil quality, boosts crop yield, possibly due to greater nutrient cycling.

The results of the present study indicate that soil conservationist management practices with the use of cover crops without soil disturbing, increase microbial quality and soybean yield, therefore, being a recommended practice to maintain the quality of Cerrado soils under intensive agriculture, thus allowing for greater sustainability of agricultural systems.

Materials and Methods

Experiment location and area history

The experiment was carried out at Tropical Farm, in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil (08°42'54.2'' S and 45°01'41.4'' W and 495 m altitude). The climate is Aw according to Köppen-Geiger climate classification - tropical with a rainy season in the summer (from November to April), and a dry season in the winter (from May to October). Average temperature in the coldest month is above 18ºC. Total yearly rainfall is above 750 mm, reaching 1800 mm in some years (Alvares et al., 2013). The Figure 3 displays data of air temperature and precipitation in the experimental area.

The soil of the experimental area is an Oxisol (Soil Survey Staff, 2014), with a sandy clay loam texture (255 g kg^{-1} clay), deep and well drained, in flat relief, cultivated mainly with soybean. Cerrado was the original vegetation of the area, with phytophysiognomy of savanna woodlands (Cerradão; Furley, 2002). In 1990, the experimental area was deforested and cultivated with cashew (*Anacardium occidentale* L.). In 2011, cashew plants were eliminated, and soil was tilled with a plow and leveling harrow for the establishment of brachiaria (*Urochloa brizantha*), which remained in the area until 2013. In the 2013/2014 cropping season, soybean (FTS Paragominas RR®) was sown on the brachiaria straw. Before soybean sowing, the soil was tilled with a leveling harrow for the incorporation of 2,000 kg ha^{-1} dolomitic limestone (neutralizing power of 85%). At soybean sowing, fertilization was performed with 173 kg ha^{-1} K_2O as KCl, 22.5 kg ha^{-1} of sulfur (Sulfogran90®), and 50 kg ha^{-1} of P_2O_5 as single superphosphate (Sousa and Lobato, 2004).

Before installation of the experiment, soil samples were collected in the 0.00-0.10, 0.10-0.20 and 0.20-0.40 m soil layers for the determination of chemical composition and soil texture (Table 5).

Field experiment description

In January 2015, a field experiment was installed in a randomized block design with split plots and four replicates. The plots (12 x 12 m) were composed by cover crops species, and the subplots, the periods of soil sampling for microbial analysis, at 12 and 18 months after the cover crops sowing. Five cover crop treatments were cultivated as monocrop: 1) *Crotalaria spectabilis* (*C. spectabilis*), 2) *Crotalaria ochroleuca* (*C. ochroleuca*), 3) *Cajanus cajan* (pigeon pea); 4) *Urochloa ruziziensis* (brachiaria) and 5) *Pennisetum glaucum* (millet); and two cover crop treatments cultivated as intercropping: 6) millet + *C. spectabilis* and 7) millet + *C. ochroleuca*.

Seeds of cover species were spread in the area and incorporated with a disc harrow. The quantities of seeds used were: 15 kg ha^{-1} of *C. spectabilis*; 10 kg ha^{-1} of *C. ochroleuca*; 45 kg ha^{-1} of pigeon pea; 3.5 kg ha^{-1} of brachiaria; 35 kg ha^{-1} of millet; 9+5 kg ha^{-1} for the millet + *C. spectabilis* intercropping, and 4+8 kg ha^{-1} for the millet + *C. ochroleuca* intercropping, respectively. In the full development stage, the shoot of the cover crops was sampled to determine the dry mass (DM). Plants from 0.5 m^2 in each plot were cut close to the ground, sent to the laboratory, dried in an air circulation oven at 65°C, until reaching constant mass. Dry mass results were expressed in Mg ha^{-1}. After samples collection, the cover crops were desiccated with potassium glyphosate (2.0 L ha^{-1}) and flumioxazine® base (0.1 L ha^{-1}) to facilitate the sowing of soybean and to avoid the formation of a seed bank.

Soybean cultivation and yield evaluation

Sowing of soybean (Monsoy M8644®), with an early cycle (110 days) was carried out on January 23, 2016, two weeks after desiccation of the cover crops. Soybean rows were spaced at 0.45 m, with a population of 289,000 plants ha^{-1},

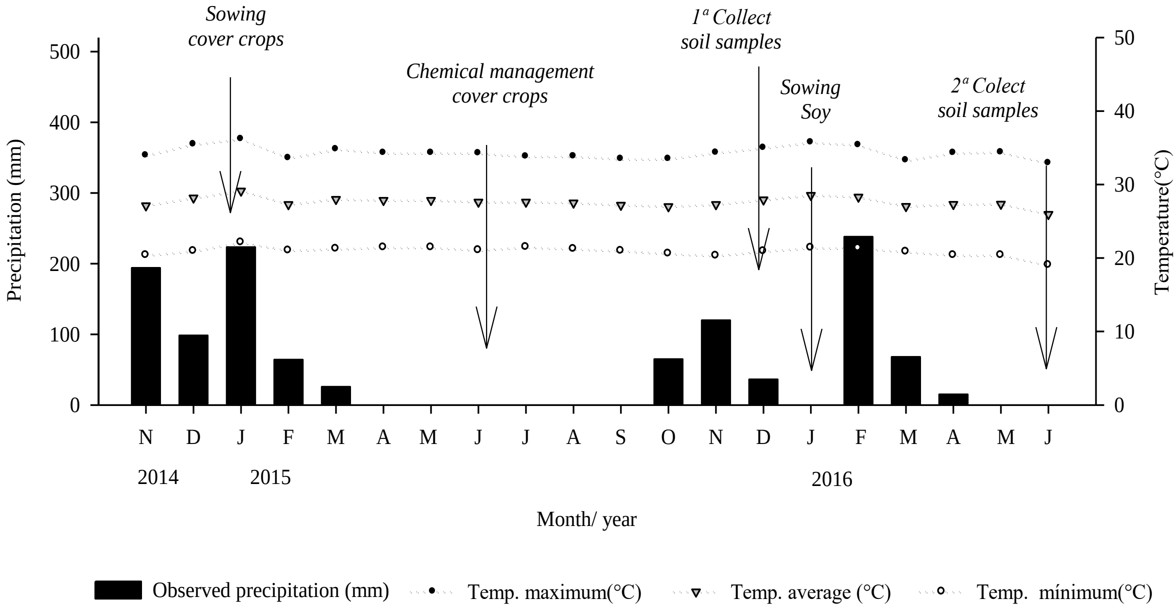

Figure 3. Precipitation accumulated, monthly rainfall and recorded temperatures in the experimental area during the study period in the municipality of Baixa Grande do Ribeiro, Piauí, Brazil, 2016.

Table 5. Chemical and granulometric soil composition before the implementation of the experiment, in Baixa Grande do Ribeiro, Piauí, Brazil.

Depth	pH	P	K^+	OM	Ca^{2+}	Mg^{2+}	Al^{3+}	CEC	BS	Sand	Silt	Clay
(m)	H_2O	--mg dm^{-3}--		g dm^{-3}	--------$cmol_c dm^{-3}$--------				%	------- g kg^{-1}-------		
0.0–0.10	6.1	30.1	37.2	34.0	2.0	1.8	0.2	5.1	47	690	66	244
0.10–0.20	5.9	21.4	30.4	34.0	1.9	1.5	0.3	4.6	46	656	77	267
0.20–0.40	5.4	10.5	22.1	33.7	0.8	0.2	0.5	3.5	29	-	-	-

pH: hydrogen potential, P: phosphorus, K^+: potassium, OM: Organic matter, Ca^{2+}: calcium, Mg^{2+}: magnesium, Al^{3+}: aluminum, CEC: cation exchange capacity, BS: base saturation.

corresponding to 13 plants per linear meter. Soybean was not fertilized at planting. The control of weeds and pests was carried out following technical recommendations for the crop. Weed control was performed with the application of glyphosate (975 g ha^{-1}) and chlorimuron-ethyl (20 g ha^{-1}), 31 days after soybean sowing, when the plants had the 4° trefoil completely expanded (V5), and the weeds had between 2 and 10 definitive leaves (forbs) or until 4 tillers (grasses). A tractor mounted sprayer was used, equipped with a bar with twenty-four flat jet nozzles 0.5 m spaced, with a 250 L ha^{-1} application flux. After 30 and 50 days of soybean development, the insecticide Deltamethrin was sprayed in order to control *Anticarsia gemmatalis*, *Pseudoplusia includens*, *Euschistus heros* and *Diabrotica speciosa*. The final soybean stand was determined in subplots, by estimating the number of plants sequenced in 1m samples. Fifteen days before harvesting, 10 plants were also sampled in subplots, for determination of leaf area, pod number, stem diameter, and total root volume. Soybean plants were harvested at 115 days after emergence, at stage R8 (Fehr et al., 1971). Yield was evaluated from the harvest and mechanical grinding of plants contained in the two central lines of 12 m in each subplot (eliminating 1 m of the border at the ends). Soybean grains harvested in each subplot were processed, weighed, the moisture was determined and the crop yield (kg ha^{-1}) corrected to 13% moisture was calculated.

Soil chemical and microbial analysis

Soil sampling to determine microbial indicators was carried at a depth of 0.00-0.10 m, before soybean sowing and after its harvest, respectively at 12 and 18 months after the sowing of the cover crops. Soil samples were placed in plastic bags, identified, and stored in a cold chamber at 4ºC. In the laboratory, the samples were air-dried, homogenized, and then sieved in a 2mm mesh sieve. Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were analyzed by the irradiation-extraction method. The basal soil respiration (BSR) was determined through the quantification of CO_2 released after 7 days of incubation under aerobic conditions. The metabolic quotient (qCO_2) was obtained by the ratio between basal respiration and soil microbial biomass, per unit of time, according to Anderson and Domsch (1993).

At the beginning of soybean pod formation (R3), 14 months after sowing of the cover crops, soil samples were collected at the depths of 0.0-0.10, 0.10-0.20 and 0.20-0.40 m for chemical analysis. The samples were air dried, then sieved in a 2mm mesh sieve. Soil pH in water, macronutrients (Ca^{2+}, Mg^{2+}, P and K^+), fertility attributes (Al^{3+}, H+Al, SB, T, V, CEC, m) and soil organic carbon (SOC) were determined, according to Silva (2009).

Statistical analysis

The data were submitted to analysis of variance and, when significant, the means of the cover crops were grouped using the Scott-Knott's test ($p<0.05$) with the aid of the statistical software SISVAR (Ferreira, 2011). The influence of the cover crops on the soil microbial attributes, nutrient cycling and soybean yield were analyzed through multivariate statistics using principal component analysis (PCA) (Kroonenberg et al., 1997). Biplot graphs were prepared considering the first two principal components with the largest variances and eigenvalues greater than 1.0 (Mora-Aguilera et al., 1993). Furthermore, the importance of the variation factors was assessed through the amount of variation explained by each of them in relation to the total variation.

Conclusions

All the cover crops used in the present study produce an amount of minimum dry mass indicated as adequate for conservation systems (above 6 Mg ha^{-1}), except the millet + *C. spectabilis* intercropping. Soil cultivated with brachiaria and millet showed higher microbial biomass at 18 months than at 12 months. Similarly, soil cultivated with *C. ochroleuca*, brachiaria, pigeon pea, millet + *C. ochroleuca* and millet + *C. spectabilis* showed higher microbial N at 18 months than at 12 months. Soybean grain yield was highest after the cultivation of *C. ochroleuca,* brachiaria, millet and millet *+ C. ochroleuca.* Results of this study show the potential of cover crops to improve soil microbial quality and increase soybean yield, even after one single cycle of crop rotation.

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