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**COVER CROPS IN SOYBEAN CROP ROTATION SYSTEMS: NUTRIENT  
CYCLING AND SOIL QUALITY**

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**ANDRESSA SELESTINA DALLA CÔRT**

**COVER CROPS IN SOYBEAN CROP ROTATION SYSTEMS: NUTRIENT  
CYCLING AND SOIL QUALITY**

Thesis presented to São Paulo State University, College of Agricultural Sciences, to obtain Doctor of Philosophy degree in Agronomy (Energy in Agriculture).

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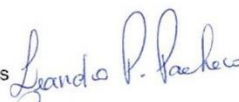




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To my family and everyone I love!





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Persistence is the success of those who dedicate themselves.

The author.



## ABSTRACT

The interactions promoted by the species diversity between crops contribute an efficient agriculture in the nutrients use, soil carbon and microbial biomass management, with cover crops in no-tillage as a fundamental factor for these processes. The objective of this work was to evaluate the potential of annual and cover crops grown in the second crop to promote nutrient cycling, improve soil chemical indicators and increase the yield of soybean sown in succession under long-term in no-tillage in the Cerrado of Mato Grosso. The treatments consisted of MC: monocropped; CS1: *Crotalaria spectabilis* (Roth); CS2: *Pennisetum glaucum* (L.) (millet); CS3: *Urochloa ruziziensis* (Germain & Evrard); CS4: *Cajanus cajan* (L.) Millsp. (pigeon pea); and MIX: all four crops intercropped (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Intercropping cover crops (MIX) and single *U. ruziziensis* (CS3) increased all evaluated soil chemical attributes through nutrient cycling and SDM production under long-term NTS. MIX, *U. ruziziensis* (CS3), and *C. cajan* (CS4) were associated with increases in soybean yield after the stabilization phase of the system, i.e., in the sixth and seventh year of NTS implementation. *Crotalaria spectabilis* is an important cover crop to increase soybean yield by N fixation in the establishment phase of no-tillage system. Shoot nutrient accumulation and release are important indicators of the nutrient cycling process and the potential to increase soybean yield in succession under NTS. The species diversity in MIX favored soybean yield and carbon sequestration, as evidenced by improvements in resistance to soil penetration, carbon from biomass and soil microbial biomass. *U. ruziziensis* was important for enhancing the activity of enzymes that degrade organic residues, particularly  $\beta$ -glucosidase activity, in no-tillage soybean production in the Cerrado, with a positive influence on carbon increase in the system. The monocropped (MC) is not indicated for use in the Cerrado because it is not able to improve the physical and biochemical characteristics of the soil and the soybean yield.

**Keywords:** species diversity; nutrient use; soil microbiota; carbon stocks; soybean grain yield.



## RESUMO

As interações promovidas pela diversidade florística entre as culturas contribuem para uma agricultura eficiente no uso de nutrientes, carbono do solo e manejo da biomassa microbiana, tendo as plantas de cobertura em plantio direto como fator fundamental para esses processos. O objetivo deste trabalho foi avaliar o potencial de culturas anuais e de cobertura cultivadas na segunda safra para promover a ciclagem de nutrientes, melhorar os indicadores químicos do solo e aumentar a produtividade da soja semeada em sucessão sob longo prazo em plantio direto no Cerrado do Mato Grosso. Os tratamentos consistiram em MC: monocultivo; CS1: *Crotalaria spectabilis* (Roth); CS2: *Pennisetum glaucum* (L.) (milheto); CS3: *Urochloa ruziziensis* (Germain & Evrard); CS4: *Cajanus cajan* (L.) Millsp. (feijão guandu); MIX: *C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*. O consórcio de culturas de cobertura (MIX) e *U. ruziziensis* (CS3) aumentou todos os atributos químicos do solo avaliados através da ciclagem de nutrientes e produção de SDM sob SPD de longo prazo. MIX, *U. ruziziensis* (CS3) e *C. cajan* (CS4) foram associados a aumentos na produtividade da soja após a fase de estabilização do sistema, ou seja, no sexto e sétimo ano de implantação do SPD. *Crotalaria spectabilis* é uma planta de cobertura importante para aumentar a produtividade da soja pela fixação de N na fase de estabelecimento do sistema plantio direto. O acúmulo e a liberação de nutrientes na parte aérea são importantes indicadores do processo de ciclagem de nutrientes e do potencial de aumentar a produtividade da soja em sucessão sob SPD. A diversidade de espécies no MIX favoreceu a produtividade da soja e o sequestro de carbono, evidenciado por melhorias na resistência à penetração do solo, carbono da biomassa e biomassa microbiana do solo. *U. ruziziensis* foi importante por aumentar a atividade de enzimas que degradam resíduos orgânicos, principalmente a atividade da  $\beta$ -glicosidase, na produção de soja em plantio direto no Cerrado, com influência positiva no aumento de carbono no sistema. O monocultivo (MC) não é indicado para uso no Cerrado por não ser capaz de melhorar as características físicas e bioquímicas do solo e a produtividade da soja.

**Palavras-chave:** diversidade de espécies; uso de nutrientes; microbiota do solo; estoques de carbono; produtividade de soja.





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## ABBREVIATIONS LIST

AcP	Acid Phosphatase
ADFS	Air dry fine soil
ASI	Aggregate stability index
ARYL	Arylsulphatase
BG	$\beta$ -glucosidase
BNF	Biological nitrogen fixation
CM	Cover crop management
C/N	Carbon/Nitrogen ratio
CEC	Cation exchange capability
CLI	Carbon lability index
CMI	Carbon management index
FDA	Fluorescein diacetate hydrolysis
Gmean	Geometric mean of enzyme activity
MaP	Macroporosity
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MiP	Microporosity
NT	Nitrogen total
NTS	No-tillage system
POC	Particulate organic carbon
PON	Particulate organic nitrogen
PR	Penetration resistance
SQ	Soil quality
SBR	Soil basal respiration
PAR	Photosynthetically active radiation
SDM	Shoot dry mass
SOM	Soil organic matter
SPCS	Soil particulate carbon stocks
SPNS	Soil particulate nitrogen stocks
TOC	Total organic carbon
TSCS	Total soil carbon stocks

TSNS	Total soil nitrogen stocks
URE	Urease
Yld5	Soybean yield in the first five years of the experiment
Yld6_7	Soybean yield in the 6 <sup>th</sup> and 7 <sup>th</sup> years of the experiment



## SYMBOLS LIST

C	Carbon
Ca	Calcium
cm	Centimeter
CO <sub>2</sub>	Carbon dioxide
ha	Hectare
k	Constant of shoot dry mass and nutrient release
K	Potassium
KCl	Potassium chloride
Kg	Kilograms
Kg ha <sup>-1</sup>	Kilograms per hectare
K <sub>2</sub> O	Potassium oxide
MAP	Monoammonium Phosphate
Mg	Magnesium
Mg	Megagram
m	Meter
ml	Milliliter
mm	Millimeter
N	Nitrogen
NH <sub>4</sub> <sup>+</sup>	Ammonium
NO <sub>3</sub> <sup>-</sup>	Nitrate
P	Phosphorus
P <sub>2</sub> O <sub>5</sub>	Diphosphorus pentoxide
Q	Quotient dry mass amounts of shoots and nutrients existing at time t
Q <sub>0</sub>	Dry mass fraction of shoots and nutrients potentially released
qCO <sub>2</sub>	Metabolic quotient
qMIC	Microbial quotient
R <sup>2</sup>	Shrinkage coeficiente
S	Sulfur
T <sub>1/2</sub>	Half-life time
%	Percentage



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## GENERAL INTRODUCTION

Brazil is the world's largest soybean producer, with an estimated production of 122 million tons for the 2021/22 crop. The Cerrado is primarily responsible for the current development of Brazilian agribusiness through grain production. The Midwest is the main soybean-producing region in Brazil, and the state of Mato Grosso is the leader in national production, with 39,4 million tons. Thus, agribusiness in this region is essential for economic growth both locally and nationwide. The current challenge for this production model is to develop technologies that promote advances in environmental quality and efficiency in the use of water and energy resources through low-carbon agriculture (USDA, 2021; CONAB, 2021).

In this line, no-tillage system (NTS) has emerged as production system management alternatives that minimize the negative impacts of conventional cultivation managements and promote sustainability on agriculture. These benefits are particularly important for second crop production systems because water deficit in tropical edaphoclimatic conditions of the Cerrado compared with the first season are less favorable for crop establishment, plant production, nutrient cycling, and soil microbiota development (PACHECO et al., 2017). These conditions require the implementation of adapted crops that enable rapid establishment and development to ensure greater water efficiency after the late summer rains (CASTRO et al., 2017).

By diversifying cover crops in the second crop, NTS can significantly increase phytomass production and nutrient cycling. Decomposition of the cover crop phytomass gradually releases nutrients required by the successor crop, reducing the need for fertilization. Moreover, the producer benefits from increased income through the sale of grain in the second crop and pasture availability in integrated agricultural production systems, further promoting the adoption of these systems. However, to have maximum efficiency in the use of nutrients and system resources, it is necessary that there is synchronization between the release and use by the successor crop. This process is dependent on the composition of the inserted cultures due to their carbon/nitrogen (C/N) ratio and lignocellulosic compounds that affect the decomposition rate of different materials and the release of nutrients (PEREIRA et al., 2016; CHIEZA et al., 2017; FERRARI NETO et al., 2017; TEIXEIRA et al., 2012; CECCON et al., 2008).

Second crop species diversification in NTS also contributes to improvements in the chemical, physical and biological properties of the soil. The associated increases in microbial abundance improve soil quality through the incorporation of carbon as organic matter, which supplies energy to the microbiota and contributes to soil decompaction. Phytomass decomposition by the microbiota also increases nutrient cycling (PACHECO et al., 2009; TORRES et al., 2008). This diversity of im-sown species initiates the process of improving the soil quality that will bring significant results over the years of implantation of the system. Such improvements in soil are possible to be evaluated through indicators of soil quality that show the real state in which the system is. Among these indicators, soil organic matter is present, which is an essential element, a source of nutrients and energy for the soil microbiota, and fundamental for the development processes of agricultural systems (CARLOS et al., 2021; ALMEIDA et al., 2020; DE-POLLI & PIMENTEL, 2005).

These improvements in soil quality accumulate with long-term NTS implementation and can be evaluated using indicators of soil quality. Soil quality indicators are sensitive parameters that indicate the impact of the adopted management strategy on soil health. One of the most important of these indicators is soil organic matter, which is an essential source of nutrients and energy for the soil microbiota and is fundamental for the development processes of agricultural systems. In contrast to soil chemical properties, which are easily and quickly corrected, a long period of observation is required to observe changes in soil biological properties (CARLOS et al., 2021; DALLA CÔRT et al., 2021; ALMEIDA et al., 2020; CRUSCIOL et al., 2015; DE-POLLI & PIMENTEL, 2005).

Accordingly, long-term field studies are essential to make recommendations for second crop annual and cover crops based on their potential benefits for nutrient cycling and biophysiochemical indicators of soil quality. So, we hypothesized that systems with single cover crops or with species diversity (MIX) to improve the physical, chemical, and biological quality in the soil-plant system with increased soybean yield and contribute to the decision of choosing of crops in the second crop that can serve specific systems. The objective of this work was to evaluate the potential of single cover crops or intercropped grown in the second crop to promote nutrient cycling, improve soil chemical indicators and increase soybean yield sown in succession.

## CHAPTER 1

### LONG-TERM CROP DIVERSITY IMPROVES SOIL CHEMICAL ATTRIBUTES, NUTRIENT CYCLING AND SOYBEAN YIELD

#### ABSTRACT

Crop diversity, particularly cover crops under no-tillage, increases agricultural efficiency in the use of nutrients and the soil carbon management. The objective of the present study was to evaluate the impact of single cover crops and intercropping (MIX) under long-term no-tillage on shoot dry mass (SDM) production, nutrient cycling, soil chemical attributes, and soybean yield in the Cerrado of Mato Grosso. The treatments consisted of MC: monocropped; CS1: *Crotalaria spectabilis*; CS2: *Pennisetum glaucum* (millet); CS3: *Urochloa ruziziensis*; CS4: *Cajanus cajan* (pigeon pea); and MIX: all four crops intercropped (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). The shoot dry mass of the cover crop was evaluated at 0, 90, 105, 120, 150, 180, and 210 days after cover crop management (CM) (desiccation) in the crop years from 2014/15 to 2020/21. The chemical properties of the soil were evaluated after the 2019/20 soybean crop harvest. SDM accumulation in MIX was approximately 22% higher than that in CS2 and CS3 and 60% higher than that in MC. Soybean yields were 20% higher in MIX, CS3, and CS4 than in MC, corresponding to an increase of approximately 14 bags of soybean. MIX and CS3 improved soil chemical attributes through nutrient cycling and SDM production under long-term no-tillage. MIX, CS3, and CS4 promoted increases in soybean yield after the stabilization phase of the system. The accumulation and release of nutrients by the shoots was an important indicator of nutrient cycling under no-tillage and consequent increases in the yield of soybean in succession.

**Keywords:** species diversity; nutrient use; soil quality; *Urochloa ruziziensis*; *Cajanus cajan*.

## 1.1 INTRODUCTION

Production costs associated with agricultural inputs have increased considerably in the last two harvests (CONAB, 2022). This scenario is expected to continue for crops in Brazil, emphasizing the need for changes in agricultural production management models to target energy efficiency through nutrient cycling and reduced use of mineral fertilizers and natural resources (OBOUR et al., 2021). Sustainable production models such as no-tillage system (NTS) prioritize the synergy of crops in rotation or succession. In particular, the diversification of species in the second harvest improves nutrient use efficiency by increasing organic matter via carbon (C) sequestration (CRUSCIOL et al., 2021a; BAPTISTELLA et al., 2020).

Careful selection of the cover crops in the second harvest is required. In the Brazilian Cerrado, there are three cultivation periods: crop occurs between October and February, when water availability is favorable for crop development; the second crop occurs between February and June, when water availability decreases, with periods of water stress; and the off-season, which occurs between July and September and is characterized by a period of drought. Species diversification is essential in the second crop period, which is best suited to drought-tolerant species with the genetic potential for high shoot dry mass (SDM) production and nutrient cycling (DALLA CÔRT et al., 2021; PACHECO et al., 2018).

Incorporating crop diversity into management practices can be difficult in the Cerrado and elsewhere in Brazil due to the dominance of the soybean-corn model, which provides high short-term profitability to the producer. Several studies have demonstrated that singly planting *Urochloa ruziziensis*, *Pennisetum glaucum*, *Cajanus cajan*, or *Crotalaria spectabilis* is an efficient second crop option that can improve the biophysiochemical attributes of the soil (DALLA CÔRT et al., 2021; VOLF et al., 2021; BOSSOLANI et al., 2020; FERREIRA et al., 2020; FRANZLUEBBERS & GASTAL 2019; SÃO MIGUEL et al., 2018; PACHECO et al., 2017). However, analyses of long-term effects are necessary to determine whether intercropping a mixture of cover crops in the same area can provide superior results compared with each species individually. In addition to promoting nutrient cycling, crop diversification may protect the soil against the effects of climate change by promoting atmospheric carbon sequestration via incorporation into soil organic matter (SOM), enhancing microbiological diversity,



soil aggregation, and promoting water infiltration and retention in the soil with possible increases in grain yield (OBOUR et al., 2021; FRANZLUEBBERS & GASTAL 2019; PACHECO et al., 2017).

The floristic diversity brought by a mixture of cover crops under NTS represents an innovative consortium model that prioritizes the extraction of nutrients located in deep layers of the soil, which are sometimes inaccessible to genetically improved crops. Cover crops in MIX translocate nutrients extracted from the soil to the photosynthetic apparatus to produce energy sources and carbon skeletons, which are subsequently transformed into SDM. In addition, the variety of root systems allows exploration of a greater volume of soil, formation of biopores, and the release of a diversity of mucilages and exudates, creating a specific rhizosphere for each species (BAPTISTELLA et al., 2020; BERTOLLO et al., 2020). Consequently, the high quality of plant materials provided by mixed cover crops favors the formation of a diverse edaphic fauna that will transform this highly labile material into stable SOM molecules, which will be protected within soil aggregates and serve as reservoirs of nutrients for successor crops. These properties of mixed cover crops suggest that it may be possible to increase soil nutrient availability by managing single or intercropped crops to increase the efficiency of the use of mineral fertilizers and other inputs.

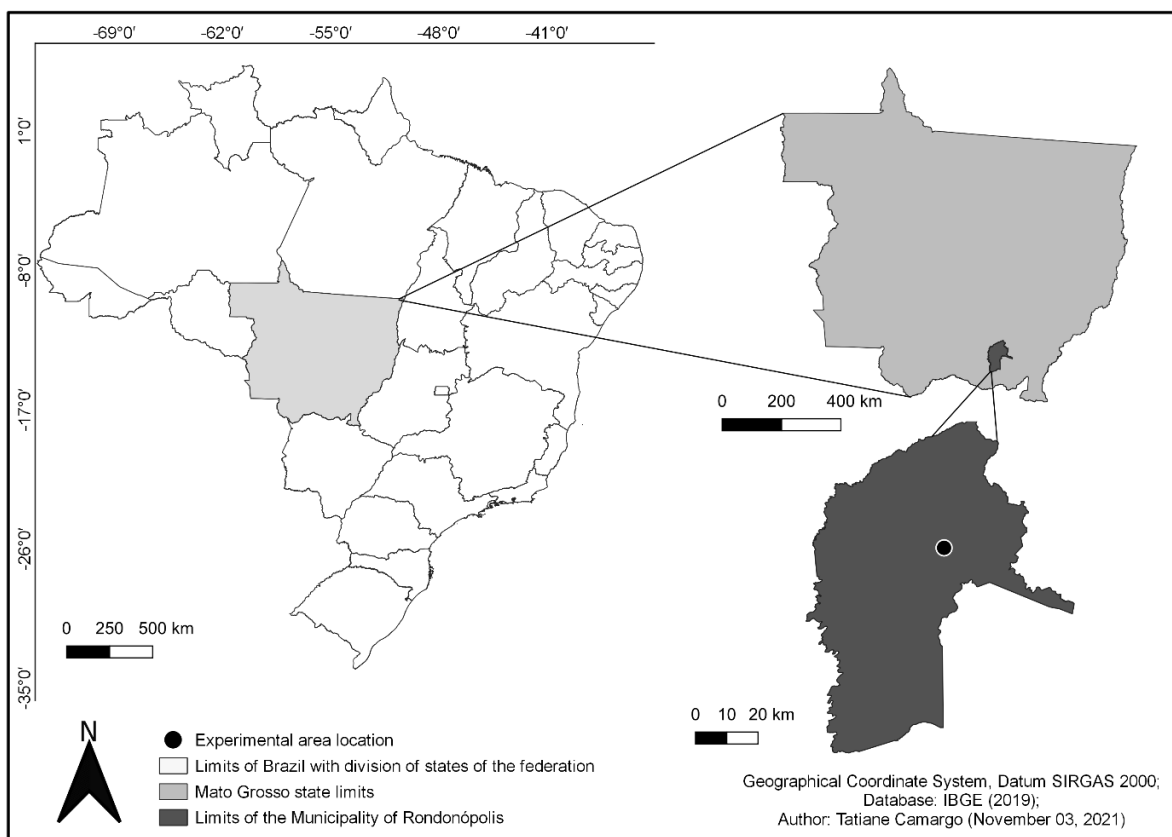
There is a need to study if MIX of cover crops has evaluated nutrient cycling and possible changes in soil fertility and long-term soybean yield. Although the system may not express initial significant increments because it needs construction for its stabilization, long-term production is expected to increase soybean yield. So, we hypothesized that the diversity of cover crops in second crop improve the soil quality, fertility, and soybean yield in long term in no-tillage system. To provide a baseline for future research, the objective of this work was to evaluate the impact of single and intercropped cover crops under long-term no-tillage on SDM production, nutrient cycling, soil chemical attributes, and the soybean yield in succession.

## 1.2 MATERIAL AND METHODS

### 1.2.1 Experimental site

The experiment was carried out in the 2013/14 to 2020/21 seasons to evaluate the evolution of the systems over 7 years of implementation. The experiment was conducted at the Experimental Station of the Federal University of Rondonópolis-MT (UFR) ( $16^{\circ}27'41.75''\text{S}$   $54^{\circ}34'52.55''\text{W}$ , altitude of 292 m) (Figure 1). The soil in the area is classified as dystrophic Red Latosol (SANTOS 2018) with flat relief. Before the present study, soil sampling was performed for chemical analysis, the results of which are shown in Table 1.

**Figure 1 - Graphic representation of experiment location conducted in Rondonópolis-MT in the crop years from 2013/14 to 2020/21**



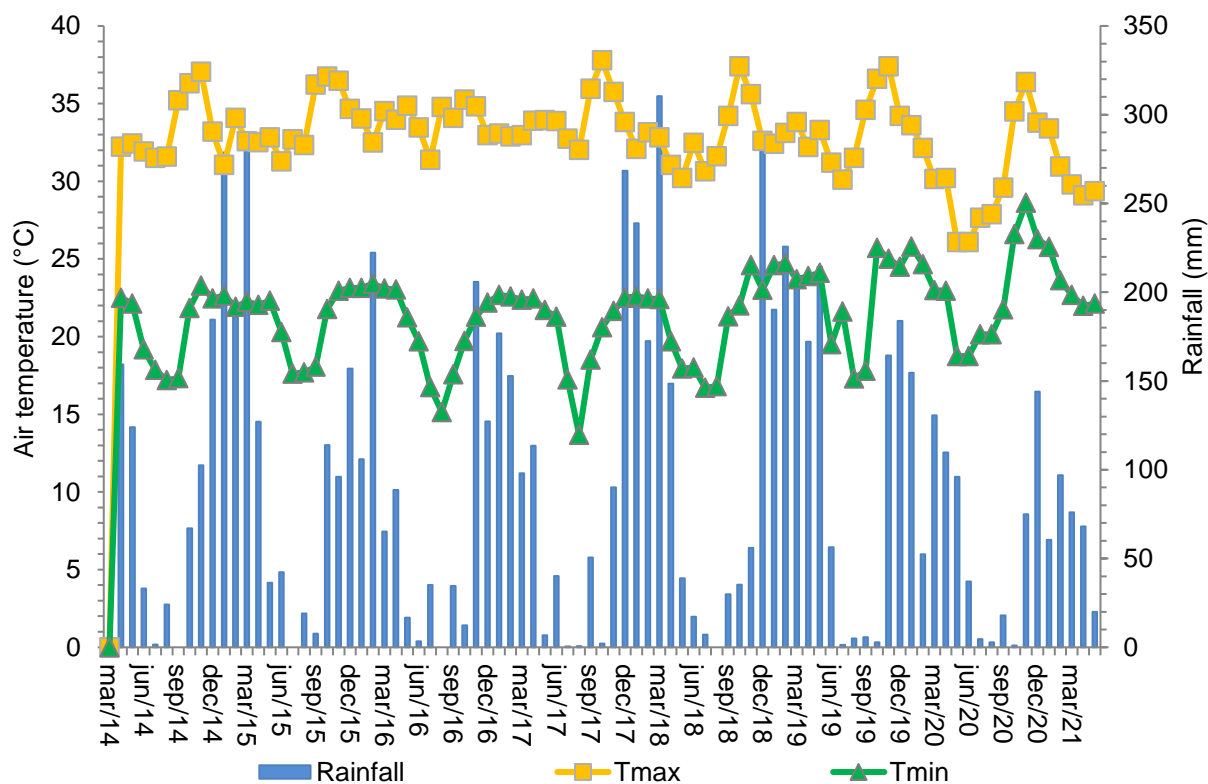
**Table 1 - Chemical and textural characterization of the dystrophic Oxisol at the area opening for the experiment in 2013**

Soil depth	pH	P	K	Ca	Mg	H+Al	T	V	MO	Sand	Silt	Clay
(m)	CaCl <sub>2</sub>	mg dm <sup>-3</sup>						%		g kg <sup>-1</sup>		
0.00-0.10	4.1	5.4	55	0.5	0.2	6.8	7.6	11	17.6	450	125	425
0.10-0.20	4.0	1.4	49	0.2	0.2	7.2	7.6	5.6	19.9	500	100	400
0.20-0.30	4.1	0.2	31	0.3	0.1	6.2	6.7	7.2	13.7	500	100	400

P: available phosphorus (Mehlich 1); exchangeable K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>; T: cation exchange capability at pH 7.0; V: base saturation; OM: Organic matter.

The local climate is tropical, with hot and humid conditions, and is Aw according to the Köppen classification (SOUZA et al., 2013). Figure 2 provides meteorological data since the implementation of the experiment.

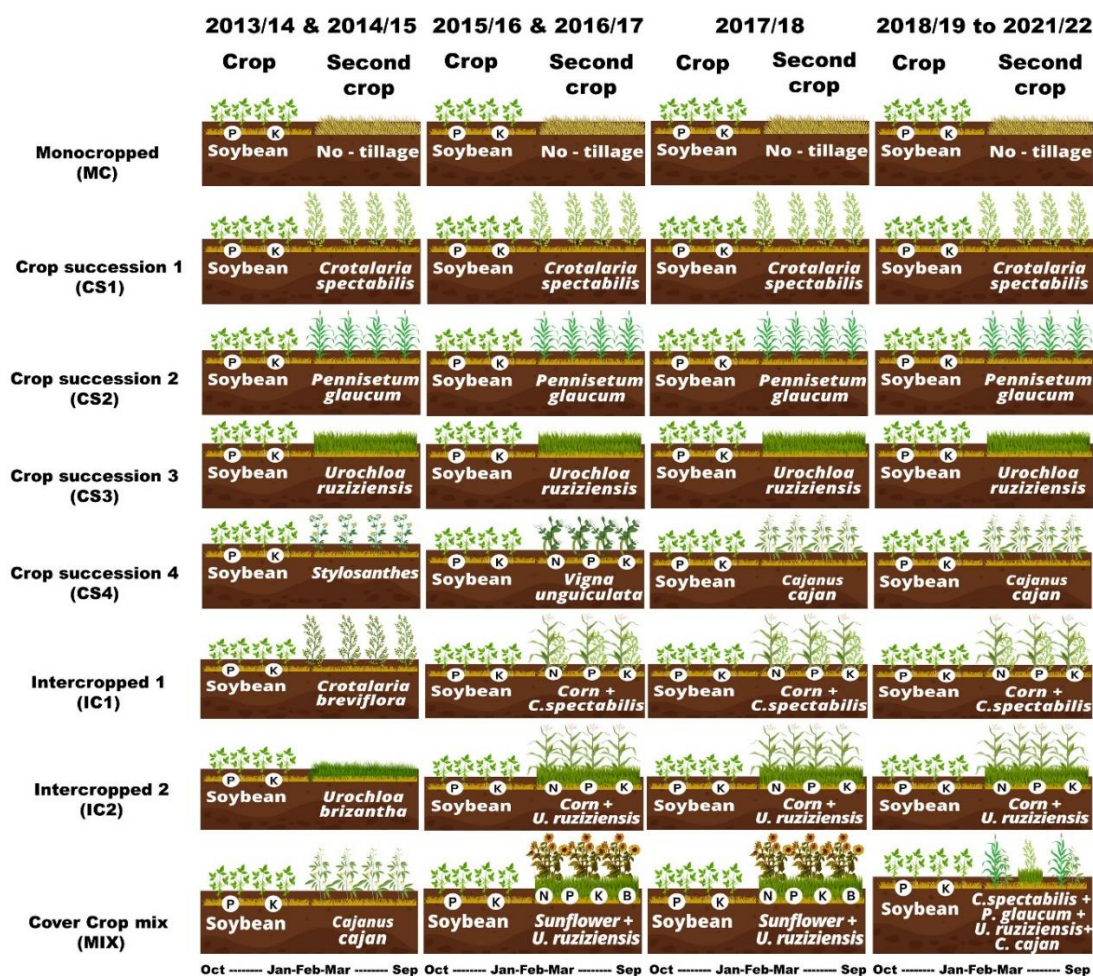
**Figure 2 - Accumulated monthly precipitation and average minimum and maximum air temperatures during the experiment at UFMT in Rondonópolis-MT**



### 1.2.2 Crop systems and experimental design

The experiment was carried out in a randomized block design with six production systems and four replications, resulting in a total of 24 plots, each with dimensions of 7.40 m x 9.50 m. In the second crop, cover crops were used; in the first crop season, soybean was sown as a commercial crop. The treatments consisted of MC: monocropped; CS1: *Crotalaria spectabilis*; CS2: *Pennisetum glaucum* (millet); CS3: *Urochloa ruziziensis*; CS4: *Cajanus cajan* (pigeon pea); and MIX: all four crops intercropped (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Figure 3 presents the treatments used since the implementation of the experiment through the 2020/21 harvest. Some systems were modified after a few years due to the adaptation of the species to the conditions of the Cerrado, but these changes were considered improvements to the trial's scope.

**Figure 3 - Crop systems characterization was implemented in the second crop in all crop years in Rondonópolis-MT.**



N: nitrogen; P: phosphorus; K: potassium; B: boron representing the fertilization used on soybean and in some cover crops.

In the second crop each year, all crops were sown between February and March with a spacing of 0.45 m. In the treatment with sunflower+*U. ruziziensis* from 2015 to 2017, the grass was sown between the rows. With the change to MIX from 2018 to 2021, *P. glaucum* and *C. spectabilis* were sown in the same row, and *U. ruziziensis* and *C. cajan* were sown in the next row; thus, these crops were interspersed in the plots. In all production systems, soybean was sown between September and October under no-tillage after desiccation of the cover crops in June. Soybean cultivars were selected according to technical recommendations for the region and market availability. Soybean was grown with a row spacing of 0.45 m and harvested in February of the following year.

### 1.2.3 Fertilization

For sunflower, fertilization followed the recommendations of Souza and Lobato (2004). However, after the implementation of MIX with sunflower, fertilizers were not used. Fertilizers were not applied in plots with single cover crops. In the first five years of the study, soybean was fertilized to correct soil chemical properties. Specifically, 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> was applied as monoammonium phosphate (MAP, with 10% N and 52% P<sub>2</sub>O<sub>5</sub>) in the sowing furrow, and 100 kg ha<sup>-1</sup> of K<sub>2</sub>O was applied as potassium chloride (KCl, with 60% K<sub>2</sub>O). Half of the KCl was spread pre-sowing, and the remaining half was applied when the soybean was at the V4 phenological stage. To reflect the stabilization phase of the NTS, in the last two agricultural years (2019/20 and 2020/21), the amounts of potassium and phosphate fertilizers were reduced by half to 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as MAP and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O as KCl as maintenance fertilization. The soybean seeds were inoculated at a dose of 150 mL of inoculant for every 50 kg of seeds with liquid inoculant Nitragin Cell Tech® HC, which contained 3x10<sup>9</sup> colony-forming units of *Bradyrhizobium japonicum* per milliliter.

### 1.2.4 Evaluations of SDM, nutrients, and soil properties

The shoot dry mass (SDM) of the cover crops was evaluated at 0, 90, 105, 120, 150, 180, and 210 days after cover crop management (CM). Day 0 was defined as the point of maximum accumulation of SDM and nutrients, when all cover crops were desiccated (normally in June). Therefore, SDM was collected at the second harvest in

all years. The second management desiccation was carried out at 90 days of CM (September/October) for the beginning of the crop and soybean sowing. The SDM was evaluated according to the methodology described by Crusciol et al. (2005). In brief, the SDM of cover crops within an iron square measuring 50 x 50 cm was randomly collected at two sampling points per plot. The collected SDM was dried in an oven at 60°C until a constant mass to obtain the SDM and nutrients. Finally, the samples were ground in a Wiley mill and passed through a 2-mm mesh sieve before determining the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) following the methodologies proposed by Malavolta et al. (1997).

To describe the release of SDM and nutrients from the cover crops after desiccation, the data collected at 0, 90, 105, 120, 150, 180, and 210 days after CM were fit to the exponentially decreasing mathematical model (Equation 1) described by Wieder & Lang (1982):

$$PL = P_o \exp(-kt) \quad (1)$$

Where: PL is the amount of SDM and nutrients existing at time t in days ( $\text{kg ha}^{-1}$ ),  $P_o$  is the fraction of SDM and nutrients potentially released ( $\text{kg ha}^{-1}$ ) and k is the constant for SDM and nutrient release ( $\text{g g}^{-1}$ ).

The value of k was used to calculate the half-life ( $T_{1/2}$  life) of the SDM and the remaining nutrients, which represents the time required for half of the SDM to decompose and half of the nutrients to be released, using the formula  $T_{1/2} \text{ life} = 0.693/k$ , as proposed by Paul & Clark (1989).

Soybean grain yield was evaluated by sampling in two 2-m lines and expressed in  $\text{kg ha}^{-1}$ , corrected for 13% moisture. For soybean yield and all shoot variables, the historical series was constructed using the averages of SDM and nutrient accumulation and release and from the 2014/15 to 2020/21 seasons to allow comparisons between crop systems under NTS over time.

Soil sampling for the analysis of chemical properties was carried out after harvesting the 2019/20 soybean crop by collecting four subsamples in the useful area of each plot (5 m x 5 m). Samples were collected with a probe-type tract at depths of 0.00-0.05, 0.05-0.10, 0.10-0.20 and 0.20-0.30 m. The soil was packed in plastic bags

and taken to a greenhouse, where the bags were opened to obtain air-dried fine earth (ADFE). After drying, the samples were sieved (2-mm mesh) for complete fertility analysis according to the methodology proposed by Raij et al. (2001).

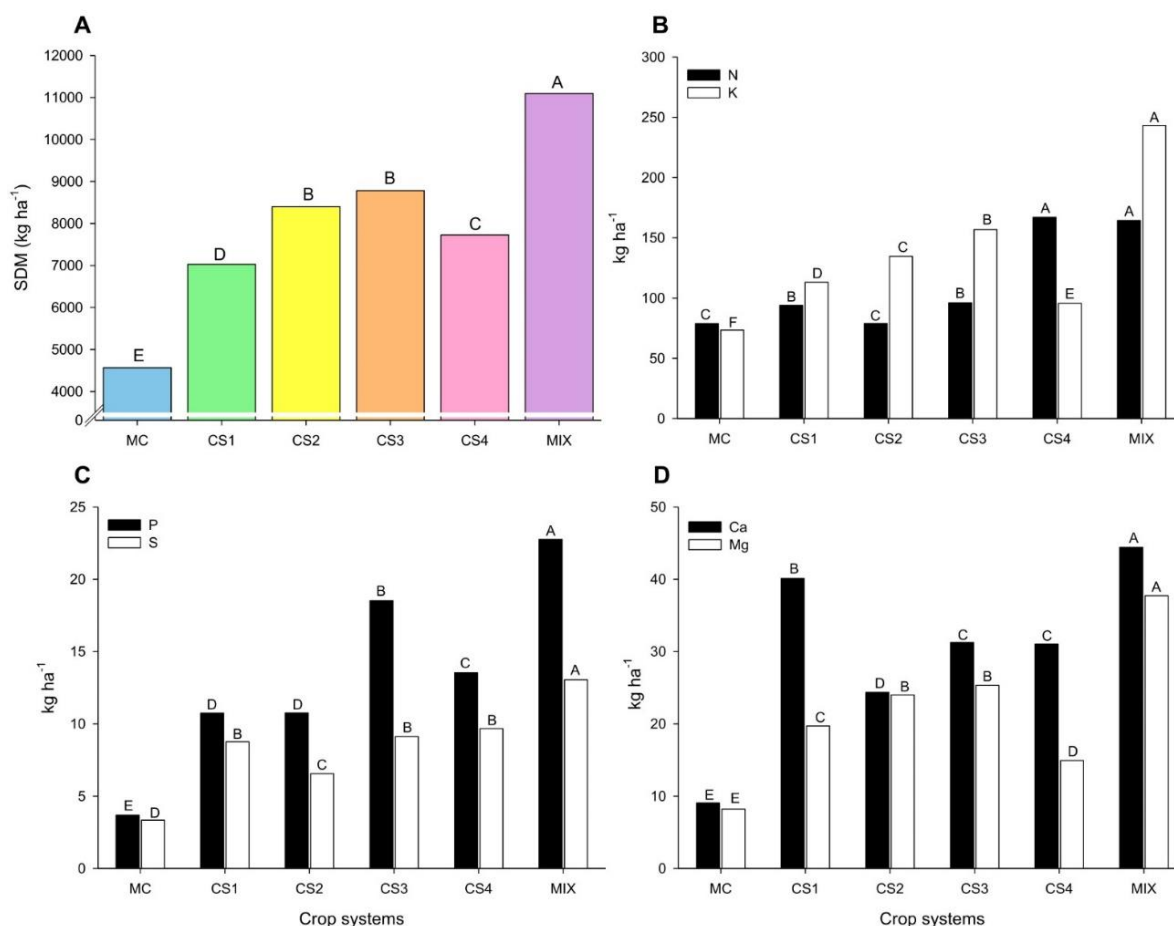
#### *1.2.5 Statistical analysis*

The results were submitted to analysis of variance, and when significant, the qualitative data were submitted to the Fisher LSD test at  $p \leq 0.10$  using SISVAR 5.6 software (FERREIRA, 2008). Quantitative data were fitted into decreasing exponential equations using the SIGMA PLOT 10.0 software (Systat Software Inc., San Jose, CA, USA). A multivariate analysis of shoot data was also performed as a function of soil depths using Principal Component Analysis (PCA) and Pearson's correlation using R software (R CORE TEAM, 2020).

### 1.3 RESULTS

SDM accumulation in MIX was approximately 22% higher than that in CS2 and CS3 and 60% higher than that in MC (Figure 4A). Similar patterns of differences in accumulation among the treatments were observed for all nutrients analyzed (Figure 4B, C, and D). Among the single treatments, N was highest in CS4, P was highest in CS3, and Ca was highest in CS1 in the second harvest. When comparing the efficiency of nutrient accumulation between MIX and MC, an increase in MIX of 43% for N, 22% for P and 38% for K was observed. This would represent  $107 \text{ kg ha}^{-1}$  of urea,  $57 \text{ kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$  and  $224 \text{ kg ha}^{-1}$  of  $\text{K}_2\text{O}$  that would no longer be applied in the system due to the cycling of these nutrients, generating savings for the producer.

**Figure 4 - Seven-year average (2014 to 2020) of shoot dry mass production (A), and accumulated amounts of N, K (B), P, S (C), Ca, and Mg (D) at cover crop management (CM) at the second crop**

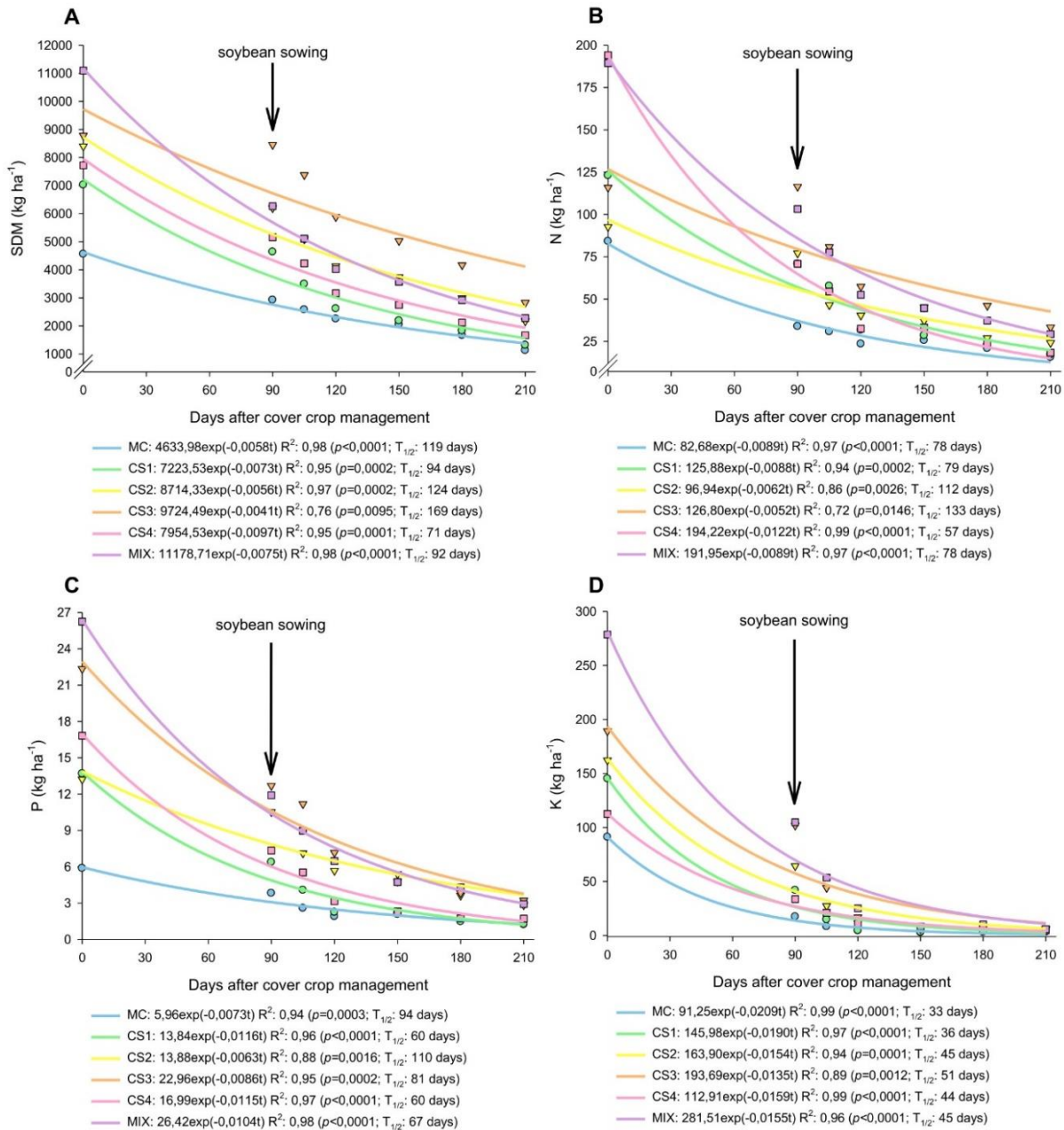


MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

The SDM decomposition rate was highest in CS4, with a  $T_{1/2}$  of 71 days, and MIX and CS1, with an average of 93 days. The rate of nutrient release into the soil was highest in MIX (N, P, K, Ca, Mg and S), CS3 (P and Mg), CS4 (N and S), and CS1 (Ca). Soybean yield was highest in MIX, CS3, and CS4, with increases of 20% compared with the control treatment (MC), equivalent to approximately 14 bags of soybean (Figures 5 and 6).

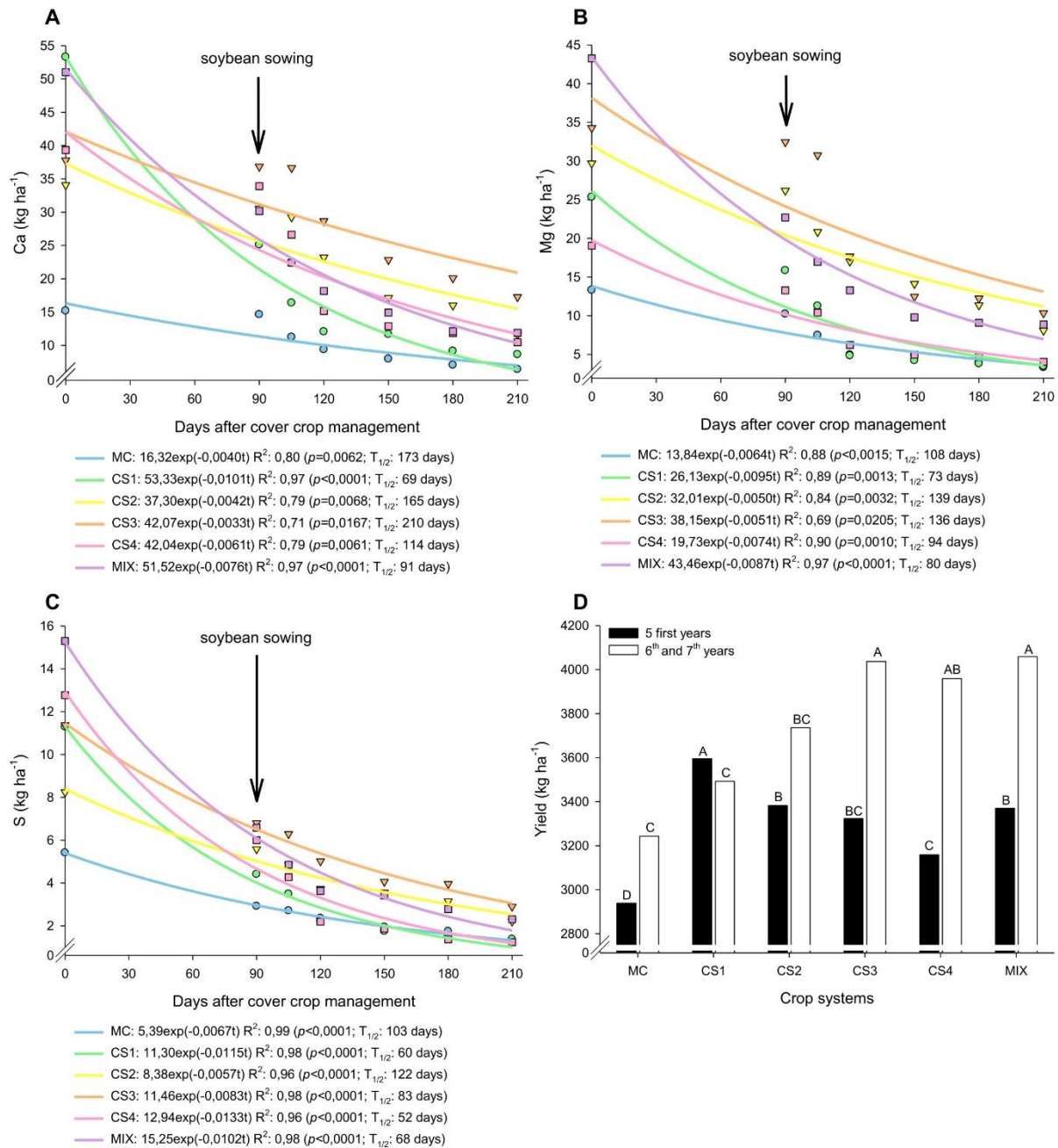


**Figure 5 - Seven-year average (2014/15 to 2020/21) of shoot dry mass decomposition (A) and N (B), P (C), and K (D) release after cover crop management**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*).  $T_{1/2}$ : half-life time.

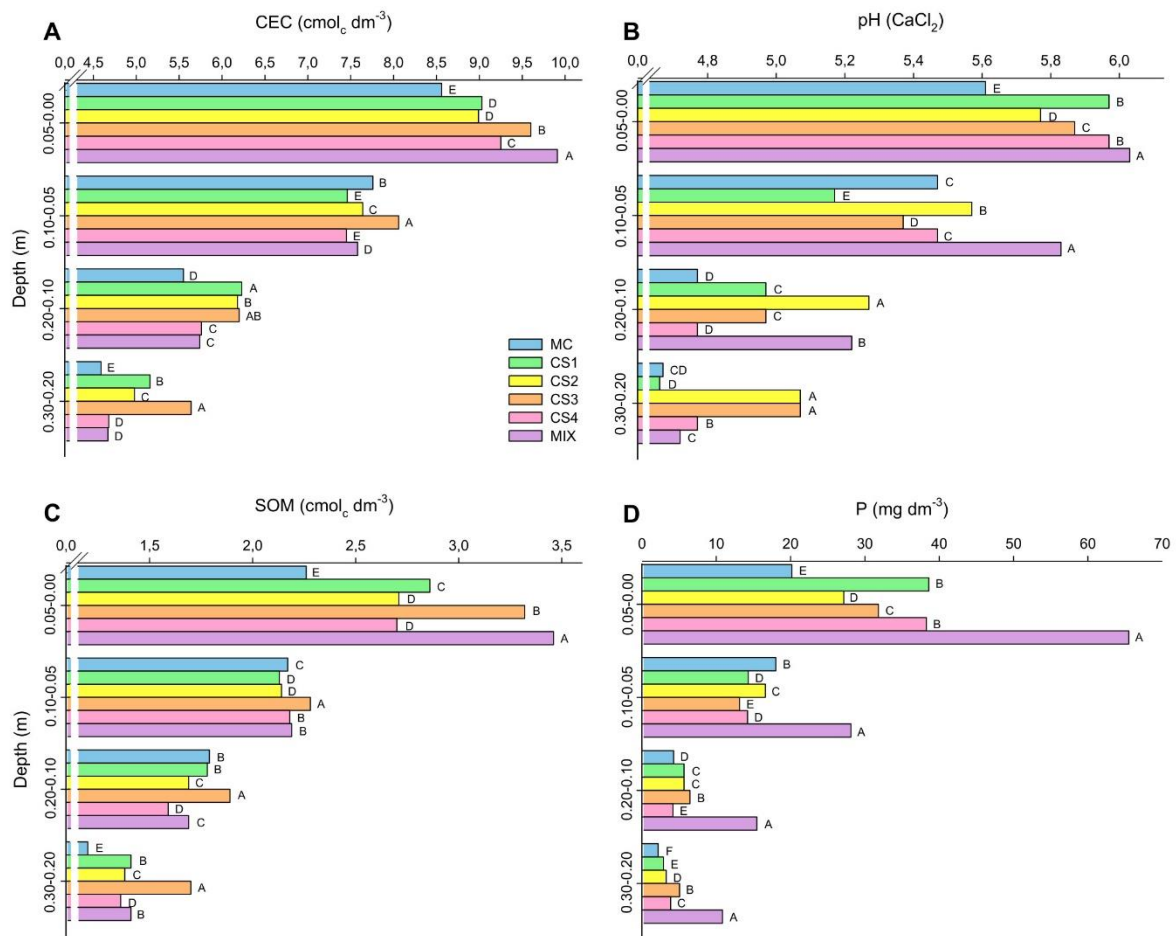
**Figure 6 - Seven-year average (2014/15 to 2020/21) of Ca (A) Mg (B) and S (C) release after cover crop management and soybean grain yield averages in the first five years and the 6<sup>th</sup> and 7<sup>th</sup> years**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). T<sub>1/2</sub>: half-life time. For soybean yield, the different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

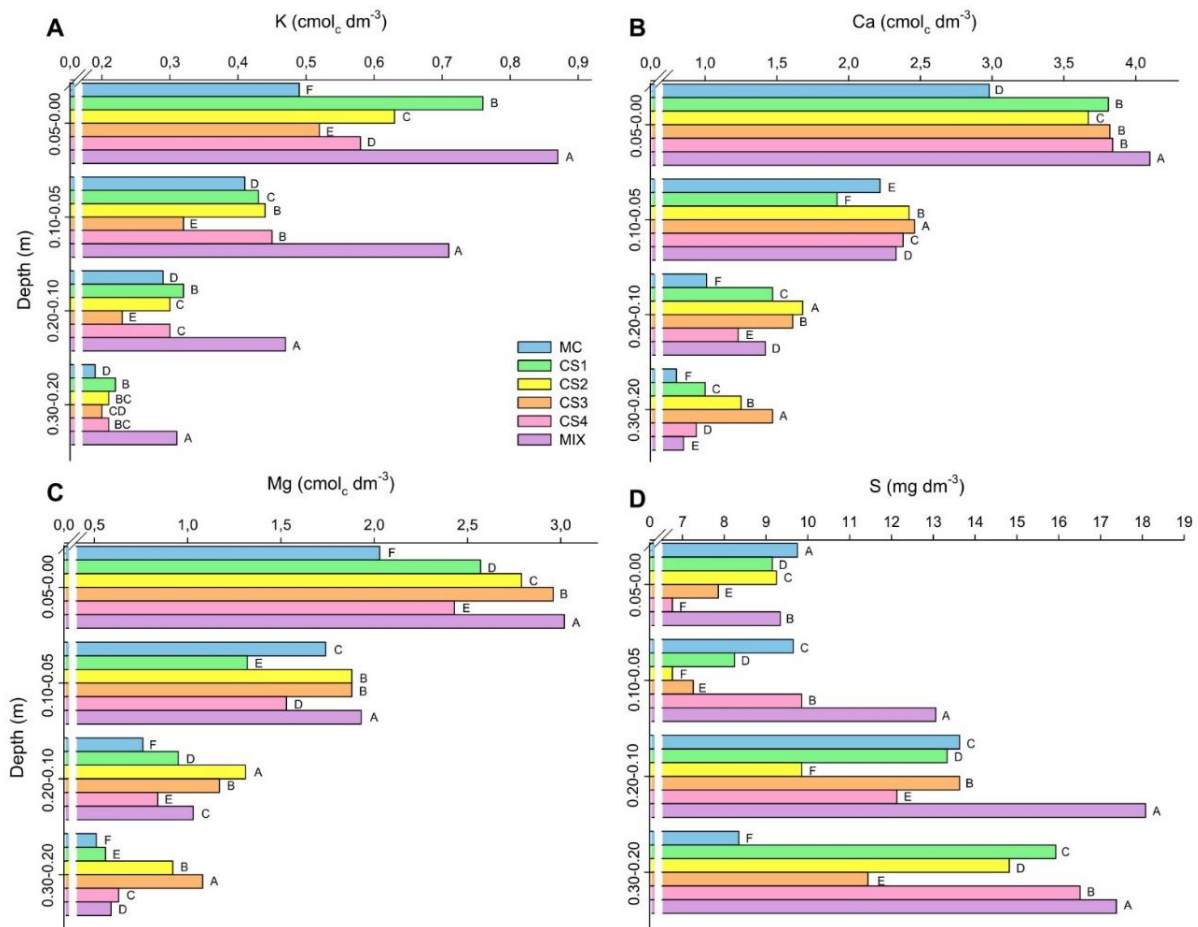
In respect to soil chemical attributes, MIX produced superior results in terms of CEC, superficial SOM, pH up to the 0.10-0.20 m layer, and P, K, Ca, Mg and S contents (Figures 7 and 8). CS3 excelled in CEC and SOM in all layers, in pH up to a depth of 0.20-0.30 m, and in Ca and Mg contents up to a depth of 0.20-0.30 m.

**Figure 7 - Soil chemical attributes and phosphorus in soil layers under cover crops after soybean harvest (crop season 2019/20)**



Cation exchange capability: CEC; Soil organic matter: SOM. MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

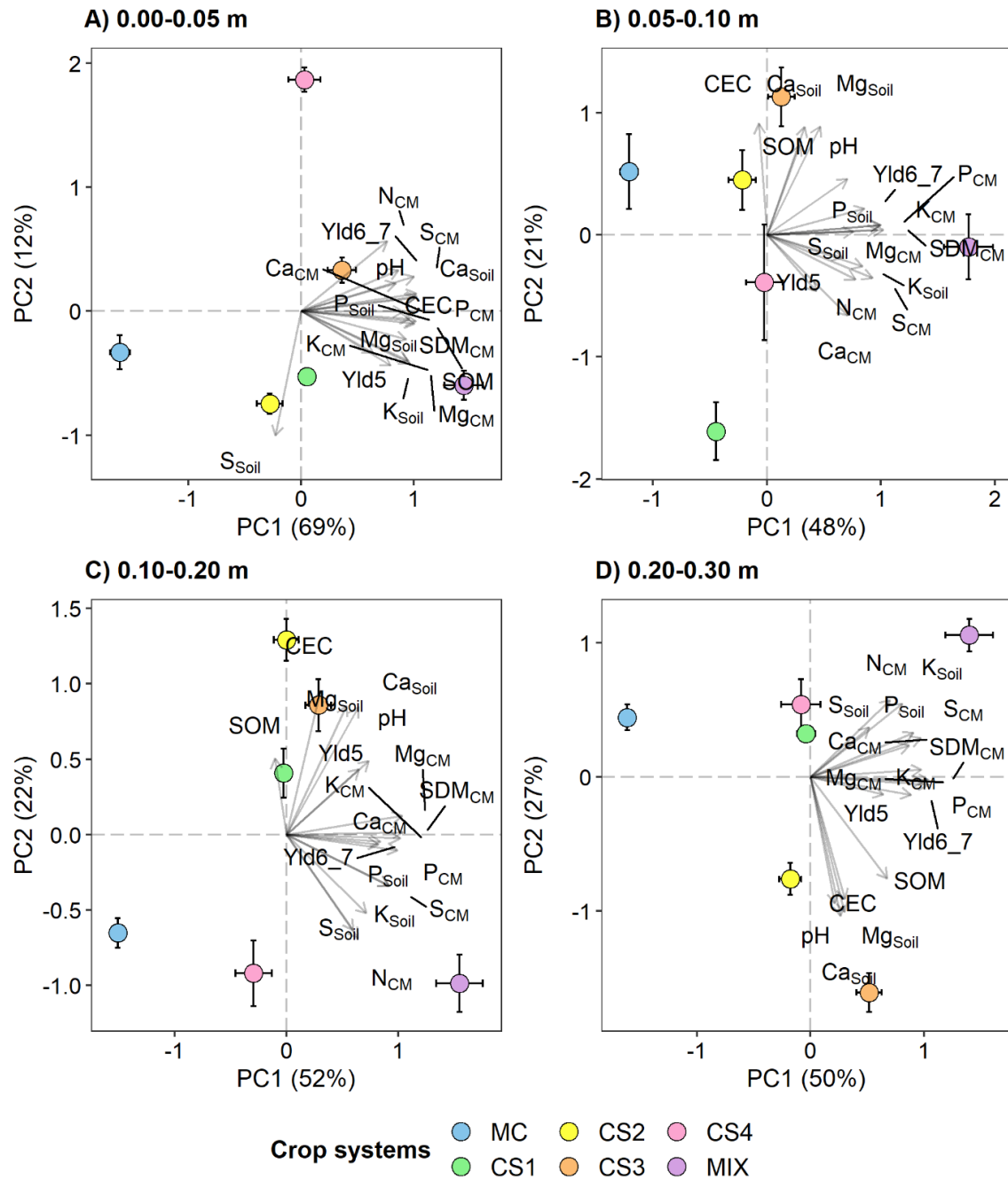
**Figure 8 - Soil nutrients, K (A), Ca (B), Mg (C), and S(D) in soil layers under cover crops after soybean harvest (crop season 2019/20)**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

Principal component analysis (PCA) for soil fertility as a function of shoot variables showed that PC1 explained 48–69% of the variation in the dataset, while PC2 explained 12–27% (Figure 9). These analyses revealed correlations of MIX and CS3 with soybean yield, shoot nutrients, and soil fertility variables at the four evaluated depths. In addition, Pearson's correlation analysis (Figure 10) demonstrated that nutrient accumulation in the aerial parts of the cover crops was highly positively correlated with soybean yield in the last two harvests (Yld6\_7).

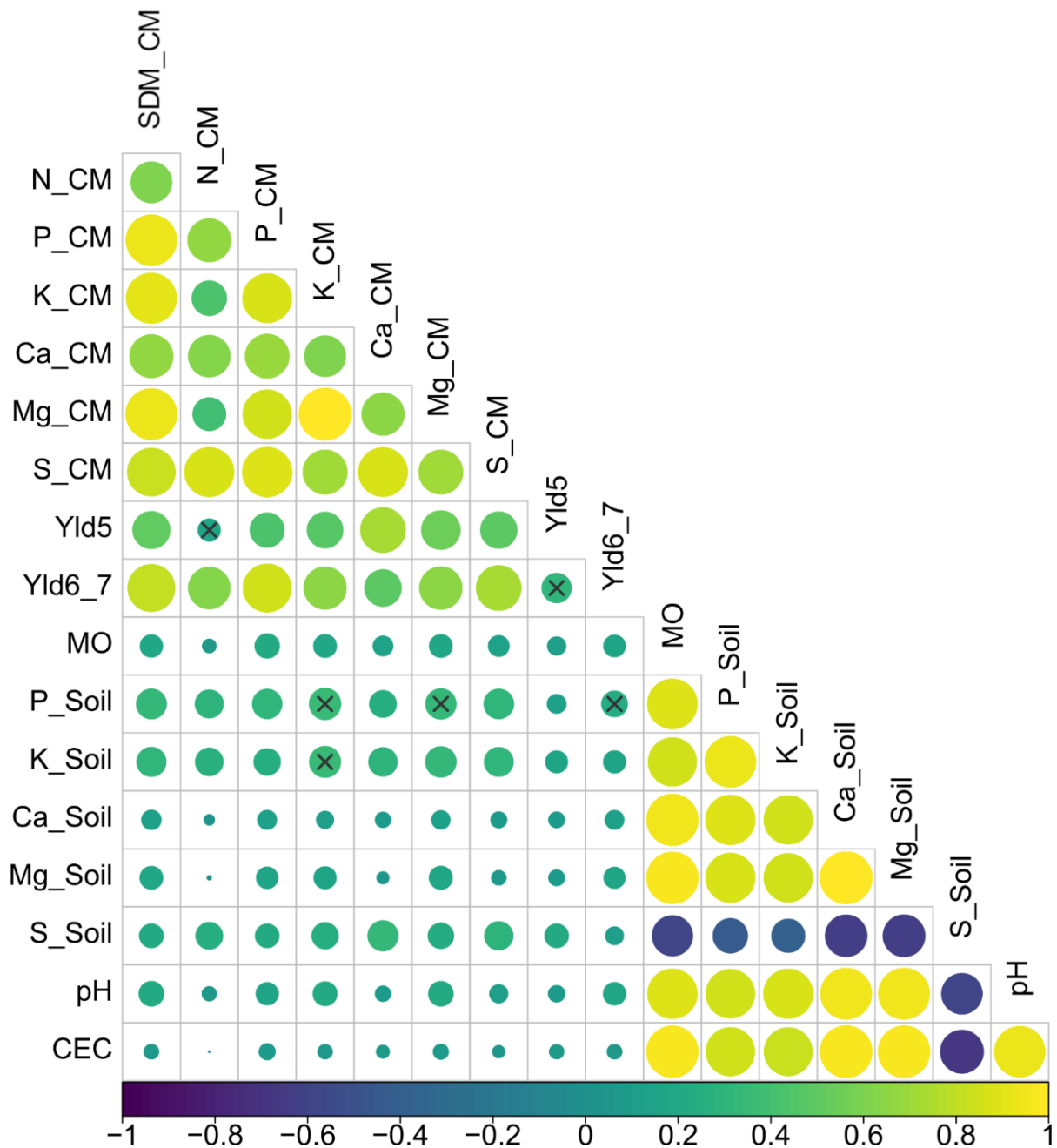
**Figure 9 - Principal Component Analysis of shoot dry mass production, nutrient accumulation at cover crop management (CM), soybean yield in the first five years of the experiment and the last two years (6<sup>th</sup> and 7<sup>th</sup>) in function of the soil layers**



Variables at the cover crop management (CM): Shoot dry mass: SDM<sub>CM</sub>; Nitrogen: N<sub>CM</sub>; Phosphorus: P<sub>CM</sub>; Potassium: K<sub>CM</sub>; Calcium: Ca<sub>CM</sub>; Magnesium: Mg<sub>CM</sub>; Sulfur: S<sub>CM</sub>; Soybean yield in the first five years of the experiment: Yld5, and 6<sup>th</sup> and 7<sup>th</sup> years: Yld6\_7; Soil organic matter: SOM; pH; Cation exchange capability: CEC; Nitrogen: N<sub>soil</sub>; Phosphorus: P<sub>soil</sub>; Potassium: K<sub>soil</sub>; Calcium: Ca<sub>soil</sub>; Magnesium: Mg<sub>soil</sub>; Sulfur: S<sub>soil</sub>. MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*).



**Figure 10 - Heatmap correlation (Pearson) based on shoot dry mass production, nutrient accumulation at cover crop management (CM), soybean yield in the first five years of the experiment and the last two years (6<sup>th</sup> and 7<sup>th</sup>) and chemical soil attributes**



Variables at the cover crop management (CM): Shoot dry mass: SDM\_CM; Nitrogen: N\_CM; Phosphorus: P\_CM; Potassium: K\_CM; Calcium: Ca\_CM; Magnesium: Mg\_CM; Sulfur: S\_CM; Soybean yield in the first five years of the experiment: Yld5, and 6<sup>th</sup> and 7<sup>th</sup> years: Yld6\_7; Soil organic matter: SOM; pH; Cation exchange capability: CEC; Nitrogen: N\_soil; Phosphorus: P\_soil; Potassium: K\_soil; Calcium: Ca\_soil; Magnesium: Mg\_soil; Sulfur: S\_soil.

## 1.4 DISCUSSION

The greater accumulation of SDM and nutrients in MIX emphasizes the importance of a diversity of crops with different phenological cycles in production systems. Each cover crop has a specific function in the system that depends on its morphophysiological characteristics. *P. glaucum* has a short cycle and contributed to nutrient accumulation in the system in the initial phase of the second crop. As water availability decreased during the second season and *P. glaucum* entered senescence, the perennial species *C. spectabilis*, *U. ruziziensis*, and *C. cajan* were able to continue vegetative growth and nutrient accumulation in SDM, which resulted in a greater quantity and quality of organic residues for soybean sown in succession.

Oliveira Jr et al. (2014) found that *U. ruziziensis* and millet have allelopathic potential and, in this study resulted in interactions between species in the MIX system may be resulted in the exudation of more diverse secondary metabolites and increased carboxylic and humic acids by the grasses. According Brandani & Santos (2016) and Moreira & Siqueira (2006), the high lignin content of grasses favors the increase microbial biomass in the soil due to the addition of SOM and proteins that reduces resistance to root growth along the soil profile. These inputs are positively reflected in the ability of this system to absorb and allocate nutrients in the organic fraction of SDM under NTS.

N content in SDM was high when *C. cajan* was cultivated as a single crop (CS4), demonstrating the importance of this legume for production under NTS in terms of N cycling, including biological fixation of atmospheric nitrogen ( $N_2$ ). *C. cajan* also increased N cycling when multiple cover crops were intercropped in the MIX system, possibly by metabolites release that increase the N fixation. These observations are supported by Arantes et al. (2020), Ferreira et al. (2017), and Carvalho et al. (2022), who found that *Crotalaria* sp. and *C. cajan* increased the community of diazotrophic microorganisms responsible for the conversion of  $N_2$  into inorganic forms that can be assimilated by plants ( $NO_3^-/NH_4^+$ ) and improved the lignin:N ratio, which was associated with an increase in soil POC.

In the system in which *Urochloa ruziziensis* was grown as a single cover crop (CS3), P cycling was predominant due to the root abundance of P and probable root exudation of enzymes and organic acids (OAs) capable of solubilizing poorly labile

fractions of P. According to Baptistella et al. (2021), Teutscherova et al. (2019) and Louw-Gaume et al. (2017), *U. ruziziensis* specialized to grow in environments with low available P by developing mechanisms such as greater root system development with intense colonization of mycorrhizal fungi and exudation of OAs and acid phosphatases. Almeida et al. (2020) and Louw-Gaume et al. (2017) found that *U. ruziziensis* leaches several OAs, mainly citric, oxalic, and malic acids, from the shoots or the root surface that can solubilize P and increase its availability to the grass. These organic acids make P available to plants due to their high affinity for specific minerals: oxalate releases P associated with Ca, and citrate and malate release P bound to Fe and Al oxides. Since OAs are highly energetic molecules produced by *U. ruziziensis* and are used in microbial metabolism, a cycle of the stimulation of the production and decomposition of OAs is created. Thus, the solubilization of P by OAs increases the bioavailability of P in the soil solution or bound to SOM such as phytates (Almeida et al., 2018). This P is protected from losses to the environment but is generally not available to plants.

Another important finding is the accumulation of Ca in the shoots of *C. spectabilis* (CS1). This accumulation can be attributed to the root morphology (i.e., pivot) and root physiology of the plant, which favor the absorption of Ca from the soil during the second crop. These results confirm those of Araújo et al. (2021), who found that *C. spectabilis* increased soil pH of 5.0 for 5.3 after management and thus enhanced soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  levels. These changes increased the accumulation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in plant tissues, demonstrating that this cover crop can cycle nutrients, increase CEC and base saturation, and reduce Al content and potential acidity.

The intermediate half-life of SDM decomposition and nutrient release in MIX reflects the diversity of plants in this system (Figure 5A), which varied in their decomposition rates. Importantly, half of this SDM was still present under the soil at the time of soybean sowing. The presence of *U. ruziziensis*, whether as a single cover crop (CS3) or intercropped (MIX), was particularly important for increasing the quantity of SDM on the soil surface under NTS, as a long residue half-life of 210 days was observed in CS3. Pacheco et al. (2017) reported superior development of *U. ruziziensis* when intercropped with corn compared with oversowing with soybean; the accumulated SDM reached  $18,000 \text{ kg ha}^{-1}$ , confirming the quantitative importance of *U. ruziziensis* for soil protection in the Cerrado. Rosolem et al. (2012) also observed



high persistence of SDM from *U. ruziziensis* on the soil surface and found that its root system had decomposed by only 10% at 45 days after desiccation.

The results of the present study confirm that cover crop intercropping (MIX) provides adequate quantitative maintenance of phytomass on the soil surface under NTS and more efficient nutrient cycling during the second crop. MIX was among the systems with the highest rates of nutrient release to the soil, primarily due to the inclusion of two legumes (*C. spectabilis* and *C. cajan*) that favor organic residues enriched with N. Single *C. spectabilis* (CS1) and *C. cajan* (CS4) also showed high rates of nutrient release (N, S, and Ca). According to Teodoro et al. (2011), the deep and differentiated root systems of these two legumes increase Ca and P accumulation in the Cerrado by exuding citric acid and other organic compounds that solubilize Ca-bound P and P adsorbed to Fe oxides and hydroxides. After legume cultivation, Rosa et al. (2017) observed an increase in C content in the fulvic acid fraction, which includes low molecular weight compounds, and a higher density of carboxylic groups such as organic acids and sugars, demonstrating the lability of legume tissues. Rosa et al. (2017) also noted that increasing the fulvic character of humic substances via cover crop insertion promotes humic acid and humin content and, in turn, SOM stabilization with the continued insertion of plant material in the system.

Soybean grain yield in the last two agricultural years (Yld6\_7) was the most appropriate indicator of the effects of nutrient cycling promoted by cover crops. Thus, there was an initial phase of “system construction” in the first five years of the NTS. The crop diversity provided by MIX was important for obtaining high soybean yields, demonstrating that interactive nutrient cycling occurred between the crop species over time and provided a more suitable environment for the development of soybean in succession. The positive results of soybean yield in succession to single *U. ruziziensis* (CS3) highlight the importance of this species as a quantitative and qualitative promoter (P cycling) of phytomass under NTS, as reported by Baptistella et al. (2021), Almeida et al. (2020) and Almeida et al. (2018). The effect of diversity over the seasons was clear in CS4, in which *Stylosanthes* (2014) was followed by *V. unguiculata* (2015 and 2016) and *C. cajan* (2017 to 2020). Bennet et al. (2012) stated that crop diversity generally increases crop yield by 10%. Osterholz et al. (2018) emphasized that the use of diversified legumes contributes to reducing the risk of N loss through gross

ammonification, that is, the gross flux of  $\text{NH}_4^+$  produced by microbial mineralization of organic N compounds, during grain crop growth.

With respect to soil chemical attributes (Figures 7 and 8), MIX efficiently increased SOM content and CEC, mainly in the superficial layers of the soil. These increases can be attributed to the quantitative effect of the SDM of *U. ruziziensis* intercropped with the other cover crops, as similar effects were observed for the single grass (CS3) up to a depth of 0.20-0.30 m. *U. ruziziensis* has an abundant, fasciculated root system, which intensifies the contribution of organic residues to the soil, as corroborated by Carvalho et al. (2022), Rosolem et al. (2019), and Rosolem et al. (2012). In addition, but promoting the accumulation of C and N in the soil, *U. ruziziensis* increases microbial synthesis of SOM fractions and C stabilization in the soil to reduce greenhouse gases through C sequestration (Eberhardt et al., 2021; Nishigaki et al., 2020; Yao et al., 2019; Schmatz et al., 2016). The increases in SOM and CEC in deeper soil layers in CS3 are attributable to the lack of interspecific competition. The diversity of cultures in MIX also provided a better soil pH buffering effect in the layers up to 0.10-0.20 m. Weakly acidic functional groups in SOM such as carboxylic and phenolic acids form a buffer system through protonation and deprotonation, as observed by Xu et al. (2012), Latifah et al. (2017) and Shi et al. (2019). This increased buffering can reduce limestone use over time and prevent drastic changes in soil composition that can negatively affect crops and microbiota.

The results for soil nutrient contents confirmed that shoot residues in MIX promoted the cycling of nutrients, particularly P, K, Ca, Mg, and S. However, it is necessary to explain that the MIX was not fertilized, but the sunflower, previously implanted, was fertilized for three years with doses of  $100 \text{ kg ha}^{-1}$  of MAP, KCl and urea and there may still have been some residual effect of the fertilizations in this area treatment. The interaction among cover crop species proved to be a decisive factor in the nutrient cycling process, with each playing specific roles but together promoting a synergy of factors that resulted in greater efficiency in the use of nutrients. This synergy was greatest for cycling of P, K, and S. S is easily leached to depths below 0.30 m, as demonstrated by Bossolani et al. (2021b), Crusciol et al. (2019), and da Costa et al. (2018), and MIX enhanced S cycling through absorption by the roots at greater depths, allocation in organic residues and subsequent release to the soil. The effects of *U. ruziziensis* on Ca and Mg cycling at greater soil depths were also evident in CS3, besides Ca and P also stimulate the root growth of soybean in succession (Crusciol et

al., 2021b; Bossolani et al., 2021a; Bossolani et al., 2021b; dos Reis et al., 2018). These effects justify the high grain yields of soybean in succession to *U. ruziziensis*, even when the latter was cropped singly.

PCA confirmed the effect of nutrient cycling, with high similarity in the data for shoot nutrients, soil fertility variables, and soybean yield detected between PC1 and PC2. The high similarity of these variables between MIX and single *U. ruziziensis* (CS3) confirms the benefits of these systems for nutrient cycling and increasing soybean yield in succession discussed above. Pearson's correlation analyses demonstrated that nutrient accumulation in the aerial part of cover crops is an important indicator of the potential of cover crop systems to promote nutrient cycling and increase soybean yield under NTS.

## 1.5 CONCLUSIONS

1. Intercropping cover crops (MIX) and single *U. ruziziensis* (CS3) increased all evaluated soil chemical attributes through nutrient cycling and SDM production under long-term NTS.
2. MIX, *U. ruziziensis* (CS3), and *C. cajan* (CS4) were associated with increases in soybean yield after the stabilization phase of the system, i.e., in the sixth and seventh years of NTS implementation.
3. *Crotalaria spectabilis* is an important cover crop to increase soybean yield by N fixation in the establishment phase of no-tillage system.
4. Shoot nutrient accumulation and release are important indicators of the nutrient cycling process and the potential to increase soybean yield in succession under NTS.

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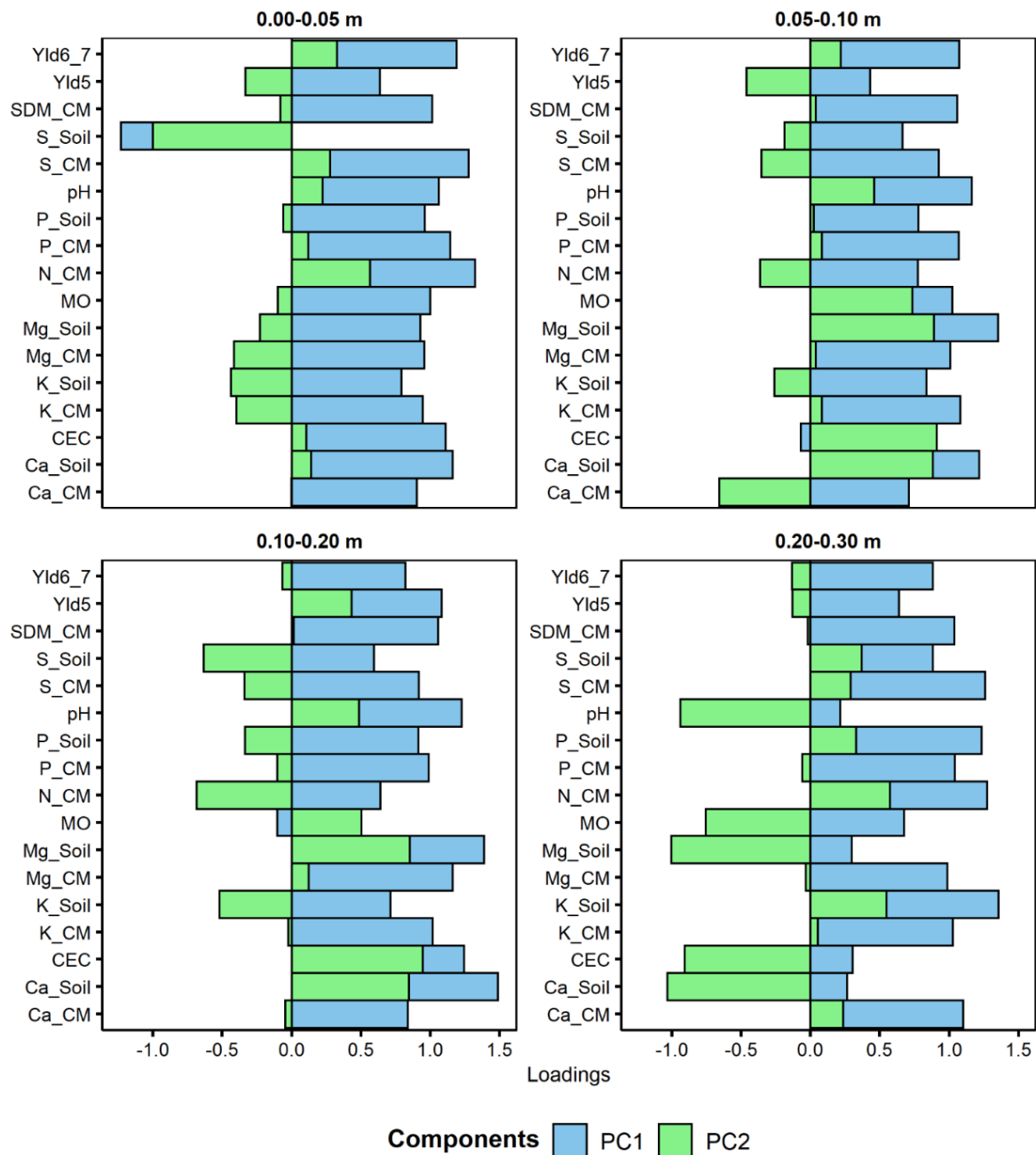
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## SUPPLEMENTARY MATERIAL

**Figure 1 - Loadings demonstrate the importance of each variable within PC1 and PC2 at soil depths**



Variables at cover crop management (CM): Shoot dry mass: SDM\_CM; Nitrogen: N\_CM; Phosphorus: P\_CM; Potassium: K\_CM; Calcium: Ca\_CM; Magnesium: Mg\_CM; Sulfur: S\_CM), soybean yield in the first five years of the experiment (Yld5) and the last two years (6<sup>th</sup> and 7<sup>th</sup>) (Yld\_67) and chemical soil attributes (Soil organic matter: SOM; pH; Cation exchange capability: CEC; Nitrogen: N\_soil; Phosphorus: P\_soil; Potassium: K\_soil; Calcium: Ca\_soil; Magnesium: Mg\_soil; Sulfur: S\_soil).

**Table 1 - Statistical analysis for soybean grain yield, shoot dry mass, and nutrients accumulation at cover crop management and soil chemical attributes protected by Fisher's LSD test at  $p \leq 0.10$ .**

<i>F</i> probability	Yld_5	Yld6_7	SDM	N	P	K	Ca	Mg	S	SOM	pH	CEC
<b>Cover crop management</b>	0.0002	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-
<b>Soil depth (m)</b>												
0.00-0.05	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.05-0.10	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.10-0.20	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.20-0.30	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Soybean grain yield on 5 first years (Yld\_5); soybean grain yield on 6<sup>o</sup> and 7<sup>o</sup> last years (Yld6\_7) shoot dry mass (SDM); nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), soil organic matter (SOM), cation exchange capability (CEC).

## CHAPTER 2

### COVER CROPS DIVERSITY ON THE MICROBIOLOGICAL, BIOCHEMICAL AND PHYSICAL ATTRIBUTES OF SOIL UNDER LONG-TERM NO-TILLAGE

#### ABSTRACT

The evolution of the use of no-tillage in grain production systems requires the development of techniques that can promote floristic diversity and plant interactions with soil physical and biochemical attributes. Accordingly, the objective of the present study was to evaluate the potential of cover crops to improve the microbiological, biochemical and physical attributes of soil and increase soybean yield under long-term no-tillage in the Cerrado of Mato Grosso. The experiment was installed in Rondonópolis-MT beginning with the 2013/14 harvest, and data from the 2017/18 to 2020/21 crop seasons are presented in this work. The treatments consisted of MC: monocropped; CS1: *Crotalaria spectabilis*; CS2: *Pennisetum glaucum* (millet); CS3: *Urochloa ruziziensis*; CS4: *Cajanus cajan* (pigeon pea); and MIX: all four crops intercropped (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). The total C stock was highest in CS3, and the stock of particulate C was highest in CS2, CS3 and MIX. MIX, CS3 and CS4 showed lower penetration resistance at all depths. Analyses of soil enzymatic activity and microbial attributes demonstrated that microbial biomass C (MBC) and microbial biomass N (MBN) were 60% and 67% higher in MIX than in MC, respectively. Soybean grain yield was highest in CS3 and MIX, with an increase of approximately 10.4 bags compared with MC. MIX, CS3 and CS2 enriched total C and C stocks in the soil and enhanced enzymatic activity. The species diversity in MIX favored soybean yield and carbon sequestration, as evidenced by improvements in resistance to soil penetration, carbon from biomass and soil microbial biomass. *U. ruziziensis* was important for enhancing the activity of enzymes that degrade organic residues, particularly  $\beta$ -glucosidase activity, in no-tillage soybean production in the Cerrado, with a positive influence on carbon increase in the system. The monocropped (MC) is not indicated for use in the Cerrado because it is not able to improve the physical and biochemical characteristics of the soil and the soybean yield.

**Keywords:** soil microbiota; enzyme activity; nutrient cycling; carbon stocks; soil decompaction.

## 1.1 INTRODUCTION

The increasing demand for inputs in multiple agribusiness sectors alongside the growing demand for grain emphasizes the need to increase the efficiency of biomass and energy flows in these systems (CONAB, 2022). No-tillage systems (NTSs) are an alternative form of sustainable management that emerged decades ago and are constantly evolving to optimize production systems and available resources (MOTTER & ALMEIDA, 2015). Studies of plant-microbiota interactions in production systems are essential to increase NTS efficiency. These interactions are most effective in diversified environments with minimal disturbance that maintain biogeochemical cycles, as these conditions promote efficiency in the use of bioenergetic resources and CO<sub>2</sub> sequestration (CARVALHO et al., 2021; CRUSCIOL et al., 2021).

In the Brazilian Cerrado, the most common production model is soybean followed by corn as the second crop, but the diversification of intercropped species in the second crop is becoming an important tool for increasing the efficiency of nutrient cycling and promoting the growth of the microbial community. Species diversity promotes microbiological genetic diversity, as edaphic microorganisms have a high affinity for plant materials inserted into the system (LI et al., 2020; WILPISZESKI et al. 2019). A greater diversity of plant and microbial species also provides biophysiochemical benefits by increasing productive potential and the contributions of carbon (C) and nitrogen (N) to the system to promote a regenerative agriculture (NONG et al., 2020).

Cover crops diversity in no-tillage system has been important technologies to recover soil characteristics in search of sustainable development in agriculture and improve soil physics properties such as soil agregation, density, aeration, water infiltration (LAL, 2020; ZHOU et al., 2020). The different root systems of these cultures, when decomposing, form channels in the soil, called biopores that increase soil gas exchange and the successor cultures take advantage of this to more easily access available water and nutrients. Crop diversity also increases soil SOM, favoring the aggregation of soil particles, along with root exudation and cementing substances from soil microbiota to reduce the damage to soil structure (ZHOU et al., 2020). Soil aggregation protects SOM and soil C from erosion losses or microbiota attacks,

increasing C sequestration and crop yield (ABDALLA et al., 2022; LAL, 2020; SILVA et al., 2018).

Given the importance of C sequestration in production environments, it is crucial to multiply microbial biomass (MB), one of the main soil-transforming agents. MB is a highly labile part of soil organic matter (SOM) and accumulates C in the form of microbial SOM stabilized from dead cells of microorganisms, called microbial necromass. As a result, the level of stable C molecules in the soil is increased in soils with high MB (BATISTA et al., 2022; JANSSEN & HOFMOCKEL, 2020). When organic material is limited or of low quality, MB can also degrade labile C to maintain physiological functions, which is known as soil basal respiration (SBR).

Promoting the contribution of enzymatic activity to the bioavailability of nutrients allocated in organic compartments can improve nutrient use efficiency and reduce costs for the producer. Ensuring a high content of diversified and quality organic material in the soil is fundamental for these relationships, as edaphic fauna participate in exchanges with the environment. These exchanges include the release of enzymes, organic acids and secondary metabolites in the rhizosphere, which enrich the system and the crops sown in it (WANG et al., 2021; VISHWAKARMA et al., 2021). We hypothesized that MIX can improve the physical, chemical and microbiological attributes of soil and increase soybean yield in the long term. Thus, the objective of this work was to evaluate the contribution of cover crops to improving biophysiochemical attributes and soybean yield under long-term no-tillage in the Cerrado of Mato Grosso.

## 1.2 MATERIAL AND METHODS

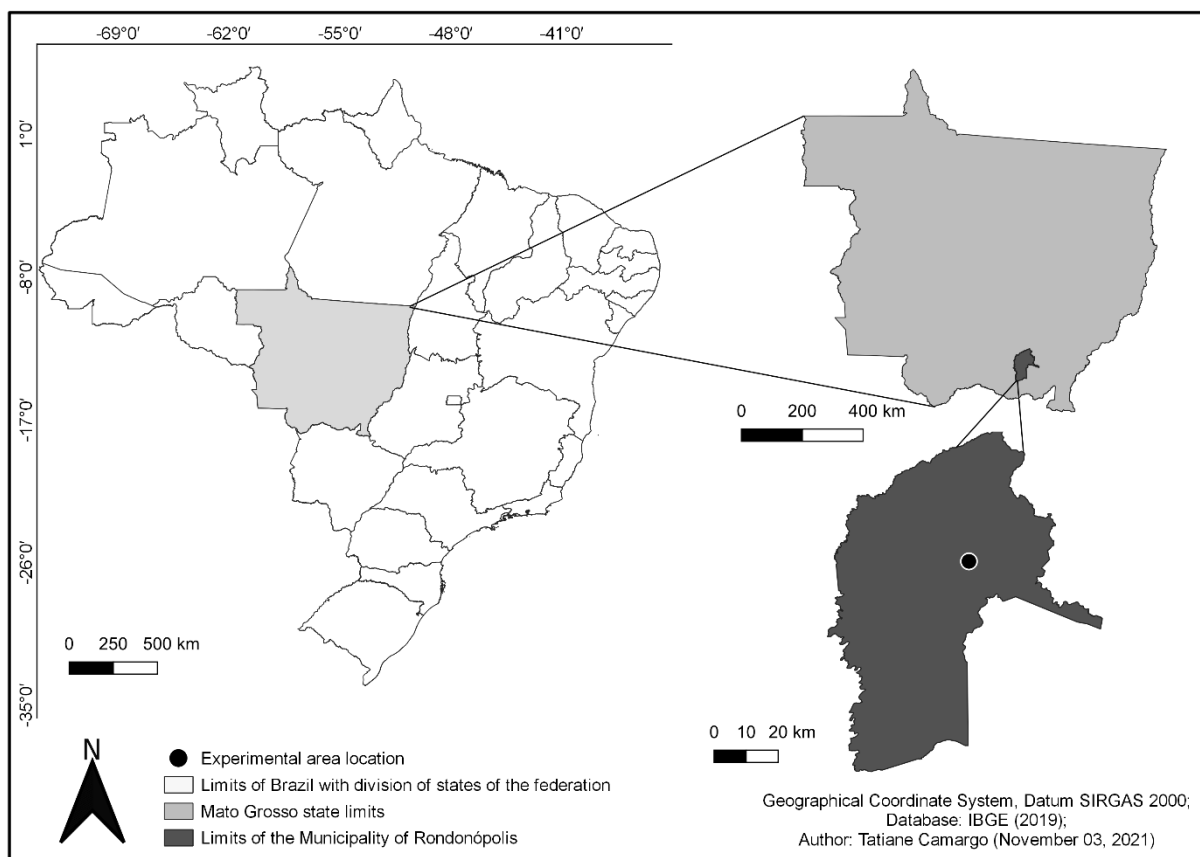
### 1.2.1 *Experimental site*

The experiment was beginning with the 2013/14 harvest, and data from the 2017/18 to 2020/21 crop seasons are presented in this work. The experiment was conducted at the Experimental Station of the Federal University of Rondonópolis-MT (UFR) (16°27'41.75"S 54°34'52.55"W, altitude of 292 m) (Figure 1). The soil in the area is classified as dystrophic Red Latosol (SANTOS 2018) with flat relief. Before the



present study, soil sampling was performed for chemical analysis, the results of which are shown in Table 1.

**Figure 1 - Graphic representation of experiment location conducted in Rondonópolis-MT in the crop years from 2013/14 to 2020/21**



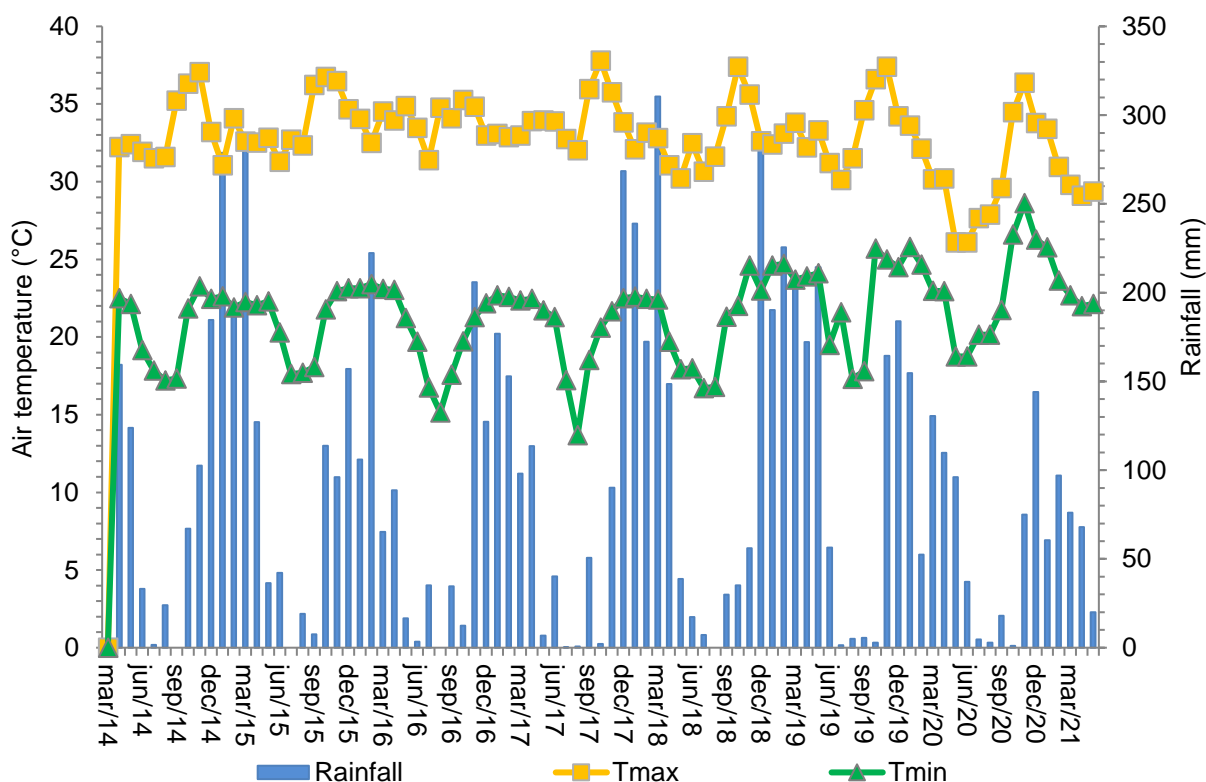
**Table 1 - Chemical and textural characterization of the dystrophic Oxisol at the area opening for the experiment in 2013**

Soil depth	pH	P	K	Ca	Mg	H+Al	T	V	MO	Sand	Silt	Clay
(m)	CaCl <sub>2</sub>	mg dm <sup>-3</sup>						%		g kg <sup>-1</sup>		
0.00-0.10	4.1	5.4	55	0.5	0.2	6.8	7.6	11	17.6	450	125	425
0.10-0.20	4.0	1.4	49	0.2	0.2	7.2	7.6	5.6	19.9	500	100	400
0.20-0.30	4.1	0.2	31	0.3	0.1	6.2	6.7	7.2	13.7	500	100	400

P: available phosphorus (Mehlich 1); exchangeable K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>; T: cation exchange capability at pH 7.0; V: base saturation; OM: Organic matter.

The local climate is tropical, with hot and humid conditions, and is Aw according to the Köppen classification (SOUZA et al., 2013). Figure 2 provides meteorological data since the implementation of the experiment.

**Figure 2 - Accumulated monthly precipitation and average minimum and maximum air temperatures during the experiment at UFMT in Rondonópolis-MT**

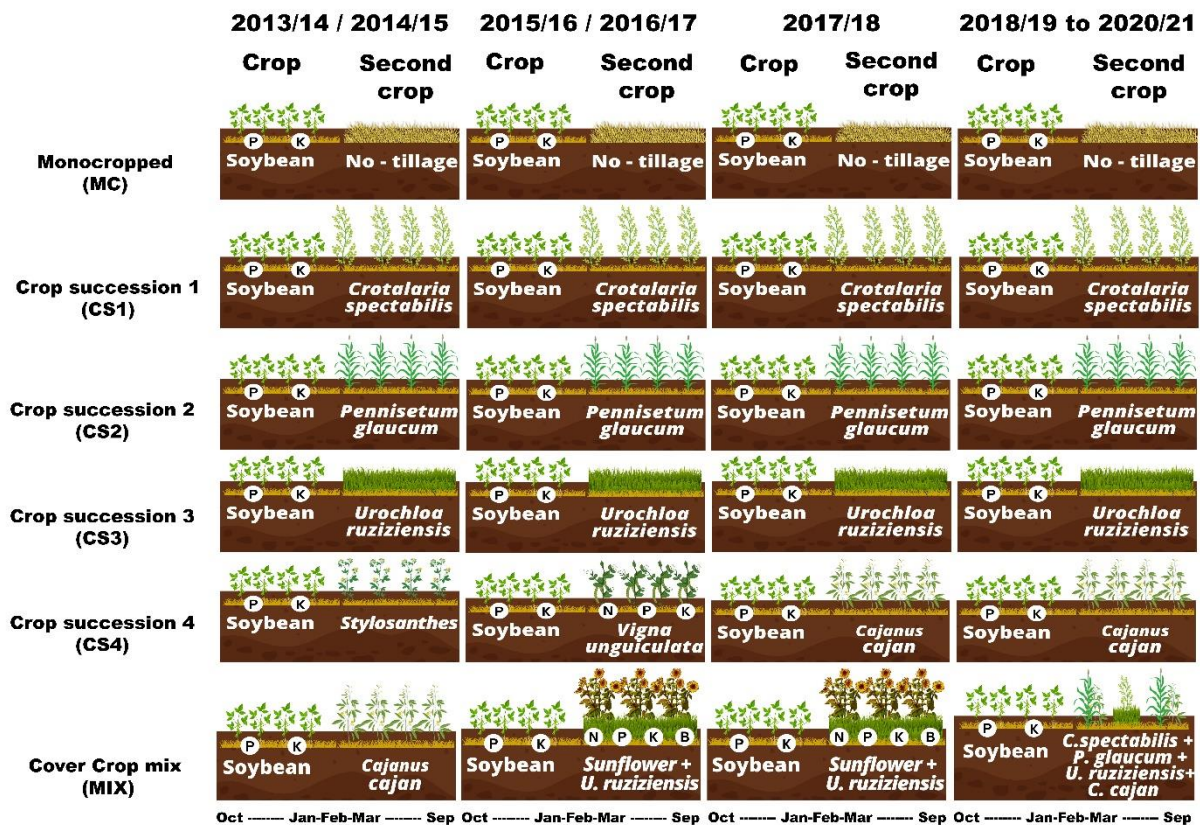


### 1.2.2 Crop systems and experimental design

The experiment was carried out in a randomized block design with six production systems and four replications, resulting in a total of 24 plots, each with dimensions of 7.40 m x 9.50 m. In the second crop, cover crops were used; in the first crop season, soybean was sown as a commercial crop. The treatments consisted of MC: monocropped; CS1: *Crotalaria spectabilis*; CS2: *Pennisetum glaucum* (millet); CS3: *Urochloa ruziziensis*; CS4: *Cajanus cajan* (pigeon pea); and MIX: all four crops intercropped (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Figure 3 presents the treatments used since the implementation of the experiment through the 2020/21 harvest. Some systems were modified after a few years due to the adaptation of the

species to the conditions of the Cerrado, but these changes were considered improvements to the trial's scope.

**Figure 3 - Crop systems characterization was implemented in the second crop in all agricultural years in Rondonópolis-MT.**



N, nitrogen; P, phosphorus; K, potassium; B: boron representing the fertilization used on soybean and in some cover crops.

In the second crop each year, all crops were sown between February and March with a spacing of 0.45 m. In the treatment with sunflower+*U. ruziziensis* from 2015 to 2017, the grass was sown between the rows. With the change to MIX from 2018 to 2021, *P. glaucum* and *C. spectabilis* were sown in the same row, and *U. ruziziensis* and *C. cajan* were sown in the next row; thus, these crops were interspersed in the plots. In all production systems, soybean was sown between September and October under no-tillage after desiccation of the cover crops in June. Soybean cultivars were selected according to technical recommendations for the region and market availability. Soybean was grown with a row spacing of 0.45 m and harvested in February of the following year.

### 1.2.3 Fertilization

For sunflower, fertilization followed the recommendations of Souza and Lobato (2004). However, after the implementation of MIX with sunflower, fertilizers were not used. Fertilizers were not applied in plots with single cover crops. In the first five years of the study, soybean was fertilized to correct soil chemical properties. Specifically, 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> was applied as monoammonium phosphate (MAP, with 10% N and 52% P<sub>2</sub>O<sub>5</sub>) in the sowing furrow, and 100 kg ha<sup>-1</sup> of K<sub>2</sub>O was applied as potassium chloride (KCl, with 60% K<sub>2</sub>O). Half of the KCl was spread pre-sowing, and the remaining half was applied when the soybean was at the V4 phenological stage. To reflect the stabilization phase of the NTS, in the last two agricultural years (2019/20 and 2020/21), the amounts of potassium and phosphate fertilizers were reduced by half to 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as MAP and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O as KCl as maintenance fertilization. The soybean seeds were inoculated at a dose of 150 mL of inoculant for every 50 kg of seeds with liquid inoculant Nitragin Cell Tech® HC, which contained 3x10<sup>9</sup> colony-forming units of *Bradyrhizobium japonicum* per milliliter.

### 1.2.4 Evaluations of soil attributes

To determine total organic carbon (TOC) and total nitrogen (TN), two subsamples were collected from the useful area (5 m x 5 m) of each plot at depths of 0.00-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40 m. To facilitate the removal of the soil, the first two depths were sampled with galvanized augers with a diameter of 3 inches, and the last two depths were sampled with galvanized augers with a diameter of 2.5 inches. The soil was packed in plastic bags and taken to a greenhouse, where the bags were opened to obtain air-dried fine soil (ADFS). The soil was subsequently ground in a ball mill before determining total C and N using an elemental analyzer (LECO® Model TruSpec™ CHNS), which is considered the world standard for soil organic matter (SOM) determination (SWIFT, 1996).

The physical fractionation of organic matter was determined only at depths of 0.00-0.10 and 0.10-0.20 m according to the method of Cambardella and Elliot (1992) with modifications. Soil (20 g) was added to a snap-cap flask containing 80 mL of sodium hexametaphosphate solution (7.5 g L<sup>-1</sup>) and horizontally agitated at 150 oscillations min<sup>-1</sup>. The supernatant was washed in a 53-µm sieve with the aid of a

washing machine, deposited in a beaker, weighed and dried in an oven at 65°C for 24 hours. The samples were then weighed again to obtain the difference in mass and ground in a porcelain mortar to determine particulate organic carbon (POC) and particulate organic nitrogen (PON) using an automatic elemental analyzer (LECO®, TruSpec™ CHNS model).

TOC, TN, POC and PON stocks were calculated in equivalent soil masses considering a density of 1.3 kg dm<sup>-3</sup> for all treatments, due to all the densities found being close to this measure, being taken as an average. The equivalent mass method takes into account the treatments soil mass in relation to the soil with the highest mass, which is taken as a reference (ELLERT & BETTANY, 1995). STOC40 and STN40 were calculated as the sum of the 0.00-0.40 m layers (0.00-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40 m), and STOC20, STN20, SPOC20 and SPON20 were calculated as the sum of the 0.00-0.20 m layers (0.00-0.05, 0.05-0.10, 0.10-0.20 m). The carbon management index (CMI) and carbon lability index (CLI) and their components in the 0.00-0.20 m depth layer were also calculated (BLAIR et al. 1995); the monocropped (MC) treatment was considered as a reference (CMI=100). To calculate the indexes, POC was considered the labile carbon fraction, and the difference between TOC and POC was considered the non-labile carbon fraction. The following equations were used:

$$\text{Carbon lability (CL)} = \frac{\text{POC (labile carbon)}}{\text{TOC (non-labile carbon)}} \quad (1)$$

$$\text{Stock carbon index (SCI)} = \frac{\text{TOC of crop systems}}{\text{TOC of MC}} \quad (2)$$

$$\text{Carbon lability index (CLI)} = \frac{\text{CL of crop systems}}{\text{CL of MC}} \quad (3)$$

$$\text{Carbon management index (CMI)} = \text{SCI} * \text{CLI} * 100 \quad (4)$$

For the physical evaluations of the soil, macroporosity (MaP) and microporosity (MiP) were analyzed according to the methodology reported by Embrapa (1997). Trenches measuring 30 x 30 cm were dug, and soil samples were collected from the 0.00-0.05 m, 0.05-0.10 m and 0.10-0.20 m layers using volumetric rings. The aggregate stability index (ASI) was measured according to the method described by Kemper and Chepil (1965) with modifications proposed by Carpenedo and Mielniczuck (1990) and Silva and Mielniczuck (1997). Undisturbed soil monoliths measuring 10 x 10 x 10 cm were collected and divided into two blocks at depths of 0.00-0.05 m and 0.05-0.10 m to quantify the soil volume by aggregate size classes by dry sieving of the natural soil samples. The samples were subsequently subjected to a turbulent flow of water by means of a mechanical vibrating agitator, and the wet soil was sieved with successive mesh sizes of 2.00, 1.00, 0.50, 0.25 and 0.105 mm. The aggregates retained on each sieve were subsequently dried and weighed to determine the index. Penetration resistance (PR) (STOLF et al, 1983) was measured with the aid of an electronic soil compaction meter (model PLG1020, Penetrolog) at four points opposite the collection sites of the volumetric rings and monoliths at depths of 0.00-0.10, 0.10-0.20 and 0.20-0.40 m. Soil moisture was determined gravimetrically in the same layers and standardized to a soil density of 1.3 kg dm<sup>-3</sup> for all treatments.

To determine microbial biomass C (MBC) and microbial biomass N (MBN), two subsamples were collected at a depth of 0.00-0.10 m between the soybean sowing lines in the useful area (5 m x 5 m) of each plot and refrigerated at 10°C until analysis. MBC and MBN were determined by the fumigation-extraction method (BROOKES et al., 1985) with a soil:extractor ratio of 1:2.5 according to Tate et al. (1988) and correction factors of 0.33 and 0.54 for C and N, respectively (SPARLING AND WEST, 1988; BROOKES et al., 1985).

Soil basal respiration (SBR) was determined using the method proposed by Jenkinson and Powlson (1976) in a 2-L glass bottle that was hermetically sealed to prevent CO<sub>2</sub> from entering or escaping for 24 hours. The flask was then opened, and the indicator was added. Titration was carried out with hydrochloric acid solution under magnetic stirring. The metabolic ( $q\text{CO}_2$ ) and microbial ( $q\text{MIC}$ ) quotients were calculated as follows:

$$q_{CO2} = \frac{\text{soil basal respiration (SBR)}}{\text{micobial biomass C (MBC)}} \quad (5)$$

$$q_{MIC} = \frac{\text{micobial biomass C (MBC)}}{\text{total organic carbon (TOC)}} \quad (6)$$

The samples used to determine microbial biomass were also used to determine soil enzymatic activity. Fluorescein diacetate hydrolysis (FDA) and acid phosphatase (AcP) were assessed according to the methodology of Dick (1996), urease (URE) according to Tabatabai and Bremner (1972), arylsulfatase (ARYL) according to Tabatabai (1994), and  $\beta$ -glucosidase (BG) according to Eivazi and Tabatabai (1988). The geometric mean of enzymatic activity (GMean) was also calculated.

Soybean grain yield was evaluated in the 2017/18 to 2020/21 harvests by harvesting the grain in 2-m lengths of two rows and expressed in kg ha<sup>-1</sup> (standardized at 13% moisture).

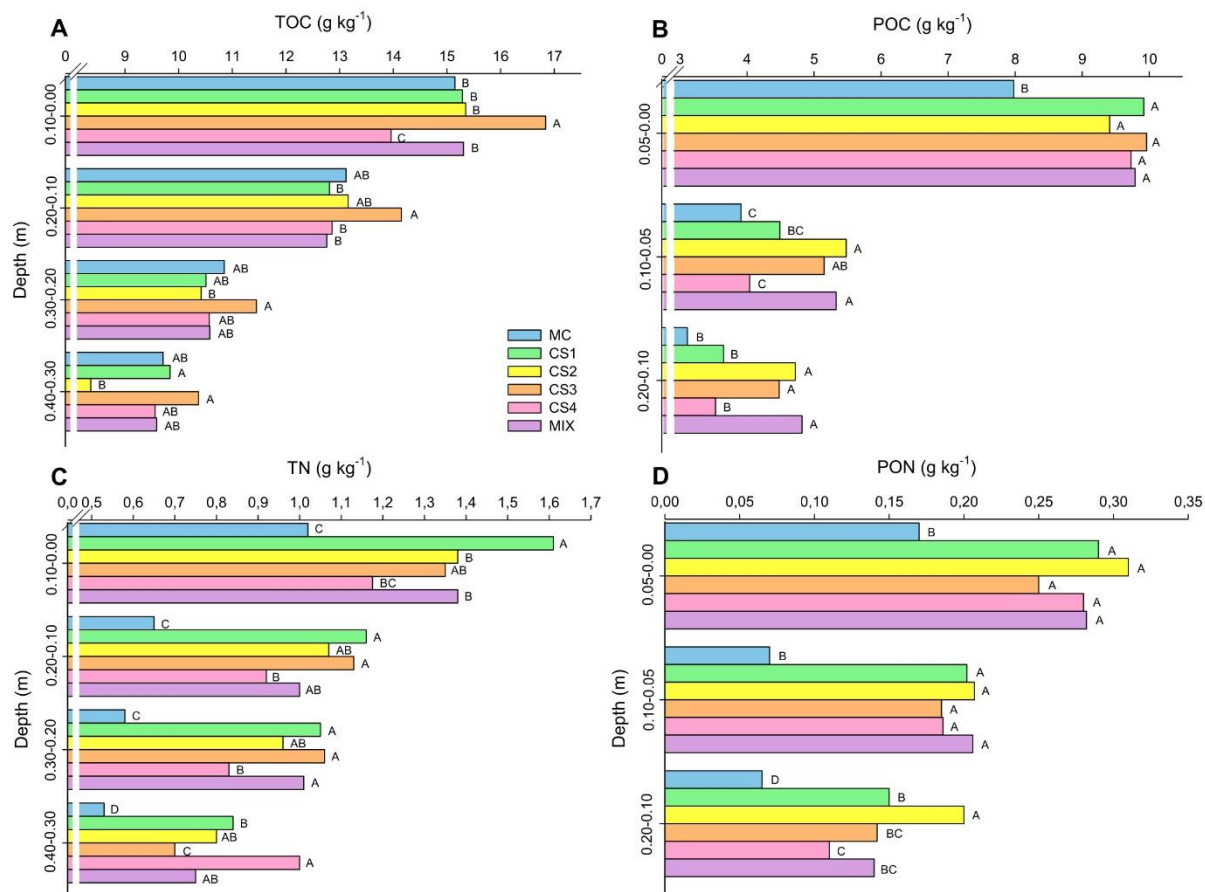
#### 1.2.5 Statistical analysis

The results were submitted to analysis of variance, and when significant, the qualitative data were submitted to the Fisher LSD test at  $p \leq 0.10$  using SISVAR 5.6 software (FERREIRA, 2008). Multivariate analysis of environmental and microbial attributes was performed using principal component analysis (PCA) and Pearson's correlation in R software (R CORE TEAM, 2020). However, before assessing Pearson's correlation, multiple regression was performed to measure the degree of parsimony of the model.

### 1.3 RESULTS

The analyses of soil biochemical attributes showed that TOC was highest in CS3 at all four evaluated depths (up to 0.40 m). POC accumulation was greater in all layers in CS2, CS3 and MIX. TN and PON accumulation differed greatly between MC and the other production systems. TN was highest in the first layer in CS1 and the last layer in CS4, and PON was highest in the last layer in CS2 (Figure 4).

**Figure 4 - Total organic carbon (TOC) (A) and N (TN) (C) and particulate organic carbon (POC) (B) and N (PON) (D) on four soil layers evaluated in function of cover crops in no-tillage system**

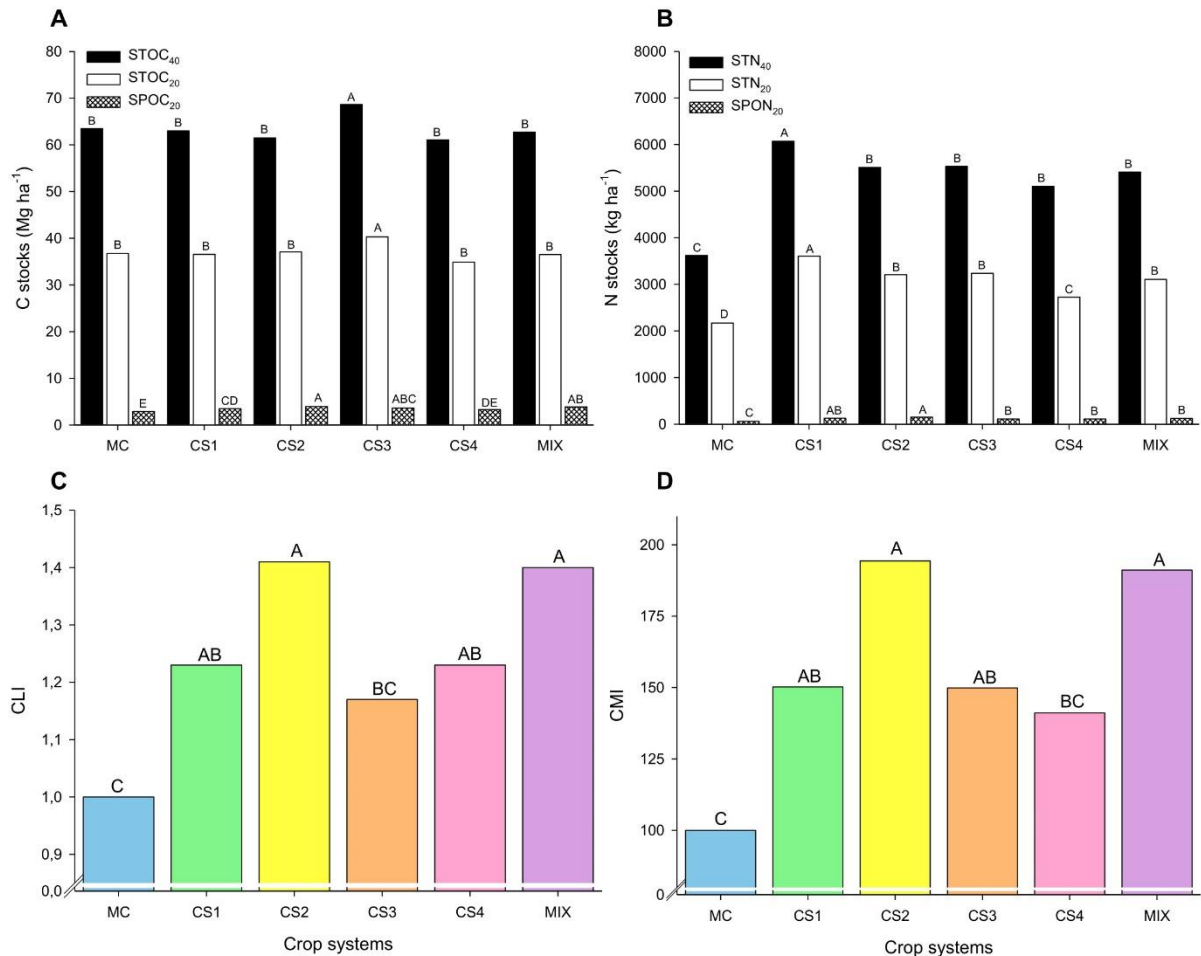


MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

CS3 had the highest total C stock (STOC40 and STOC20), and CS2, CS3 and MIX had the highest particulate C stock (SPON20) (Figure 5). CS1 had the highest total N stock (STOC40 and STOC20), and particulate N was highest in CS2 (SPON20). CLI and CMI were highest in CS2 and MIX.



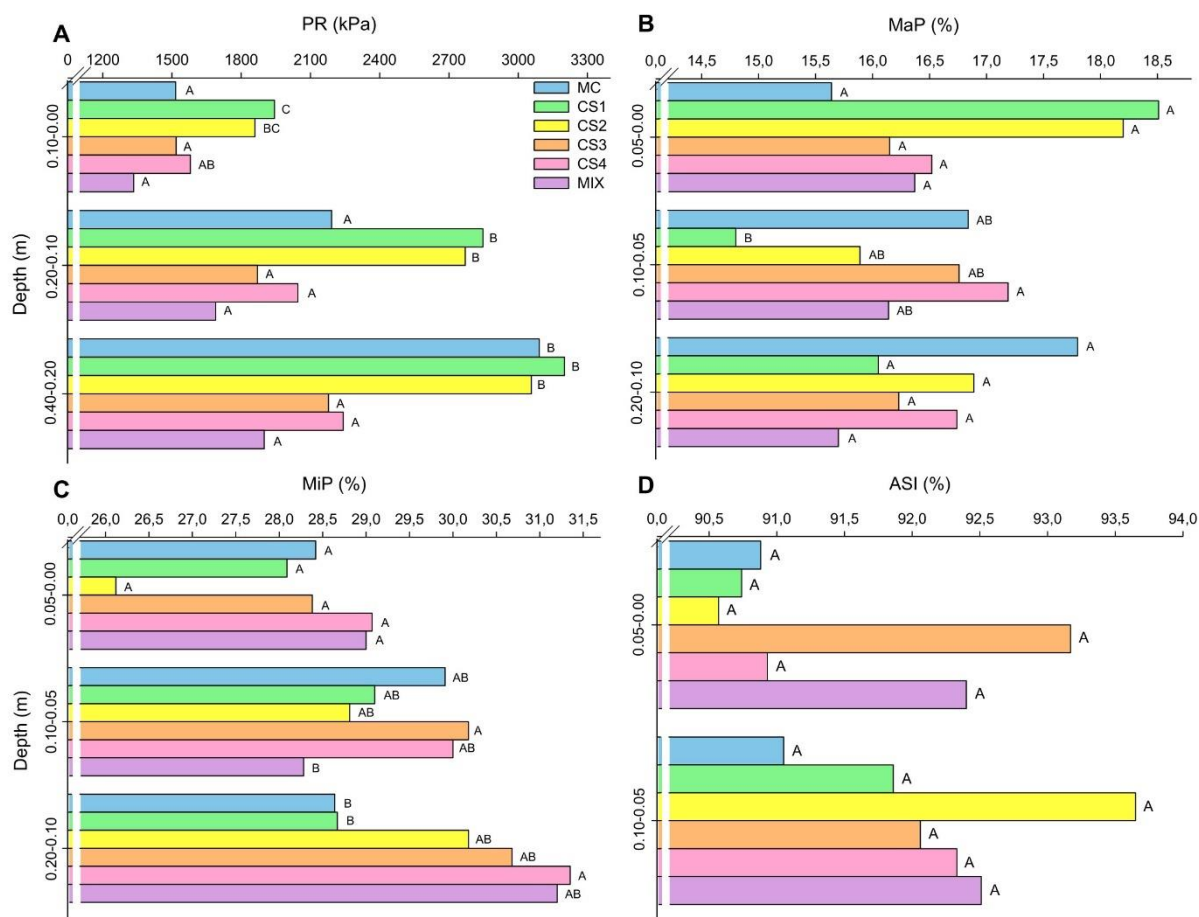
**Figure 5 - Stocks of total organic carbon (STOC<sub>40</sub> and STOC<sub>20</sub>) (A) and total nitrogen (STN<sub>40</sub> and STN<sub>20</sub>) (B) on 0.00-0.40 m and 0.00-0.20 m depths and stocks of particulate organic carbon (SPOC<sub>20</sub>) (A) and particulate organic nitrogen (SPON<sub>20</sub>) (B) at 0.00-0.20 m depth, C lability index (C) and C management index (D) on Red Oxisol of Cerrado in function of cover crops on no-tillage system**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

MIX, CS3 and CS4 showed lower penetration resistance at all depths (Figure 6). The differences in macroporosity (MaP), microporosity (MiP) and the aggregate soil index (ASI) among the treatments were not significant.

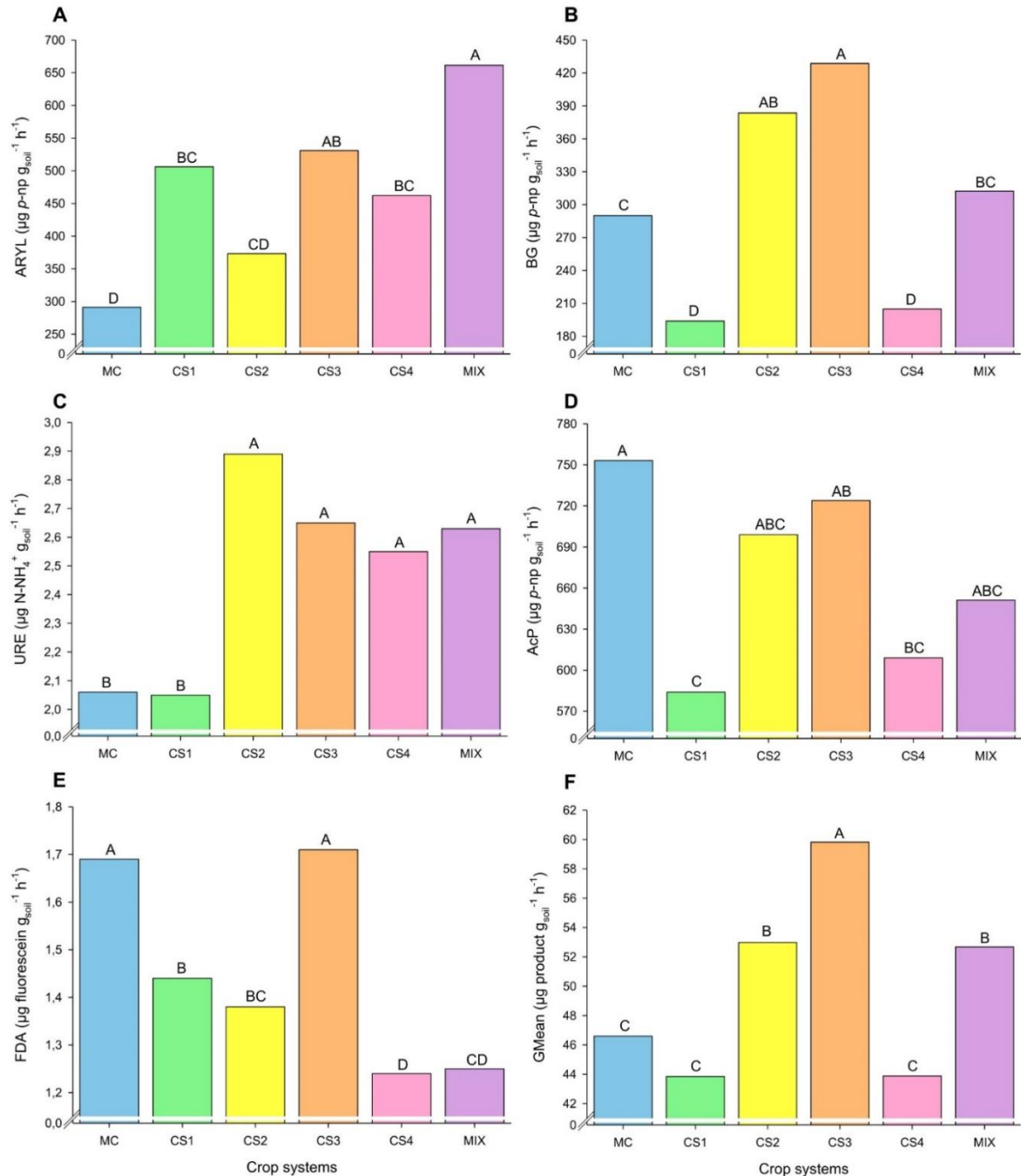
**Figure 6 - Penetration resistance (PR) (A) and microporosity (MaP) (B), microporosity (MiP) (C) and aggregate stability index (ASI) (D) on Red Oxisol of Cerrado in function of soil layers and cover crops on no-tillage system**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

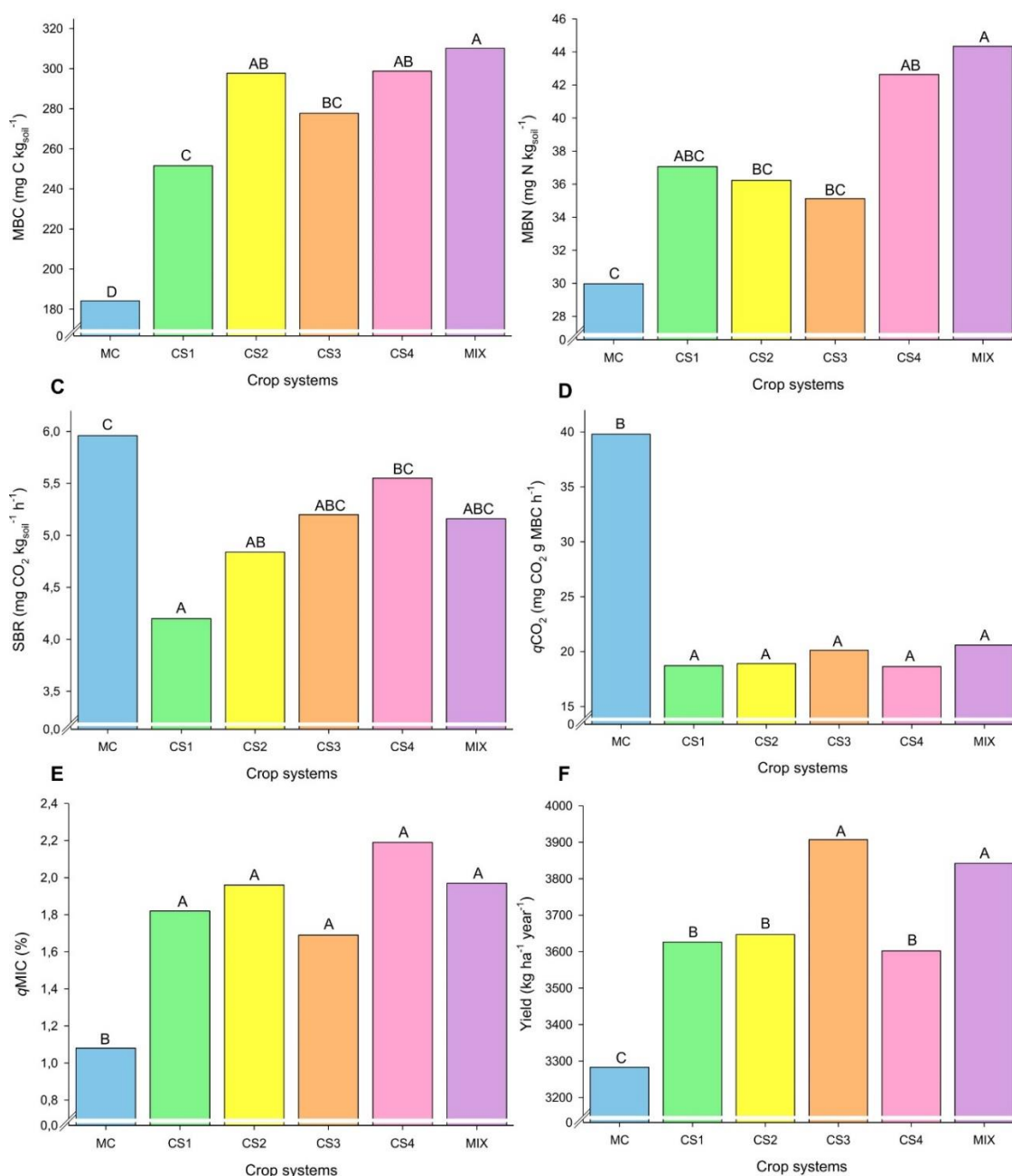
With respect to soil enzymatic activity and microbial attributes, the best treatment was MIX (ARYL, URE, MBC, MBN and  $qMIC$ ), which also exhibited 60% (MBC) and 67% (MBN) increases in the microbial community compared with MC. URE and  $qMIC$  were high in CS2 and CS4, BG, URE, FDA, GMean and  $qMIC$  were high in CS3, and AcP, FDA, SBR and  $qCO_2$  were high in MC (Figures 7 and 8). CS3 and MIX provided the highest soybean grain yield, with an increase of approximately 10.4 bags ( $620 \text{ kg ha}^{-1}$ ) compared with MC (Figure 8 F).

**Figure 7 - Soil enzymes in function of crop systems with diversity levels. Arylsulfatase (ARYL) (A),  $\beta$ -glucosidase (BG) (B), Acid phosphatase (AcP) (C), Urease (URE) (D), Flurescein diacetate hydrolysis (FDA) (E) and GMean (F) that represents geometric mean of enzyme activity**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

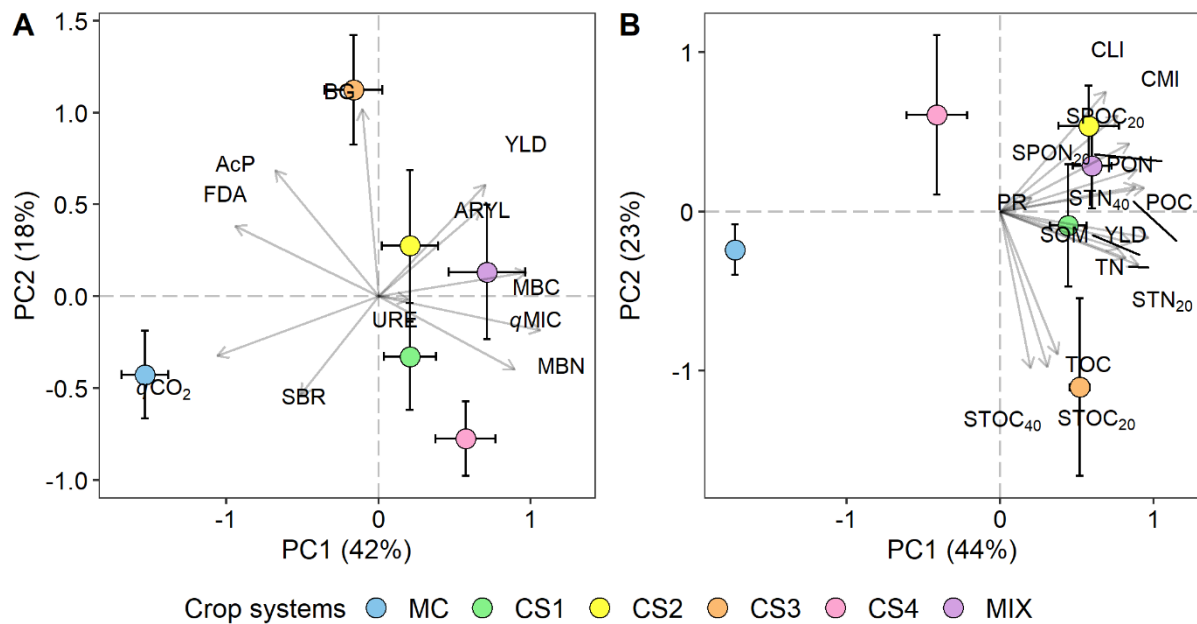
**Figure 8 - Biological and biochemical attributes of the soil quality represented by microbial biomass carbon (MBC) (A), microbial biomass N (MBN) (B), soil basal respiration (SBR) (C), biomass-specific respiration ( $qCO_2$ ) (D), microbial quotient ( $qMIC$ ) (E) and soybean grain yield in the four last years of the experiment (F)**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Different letters in the column indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

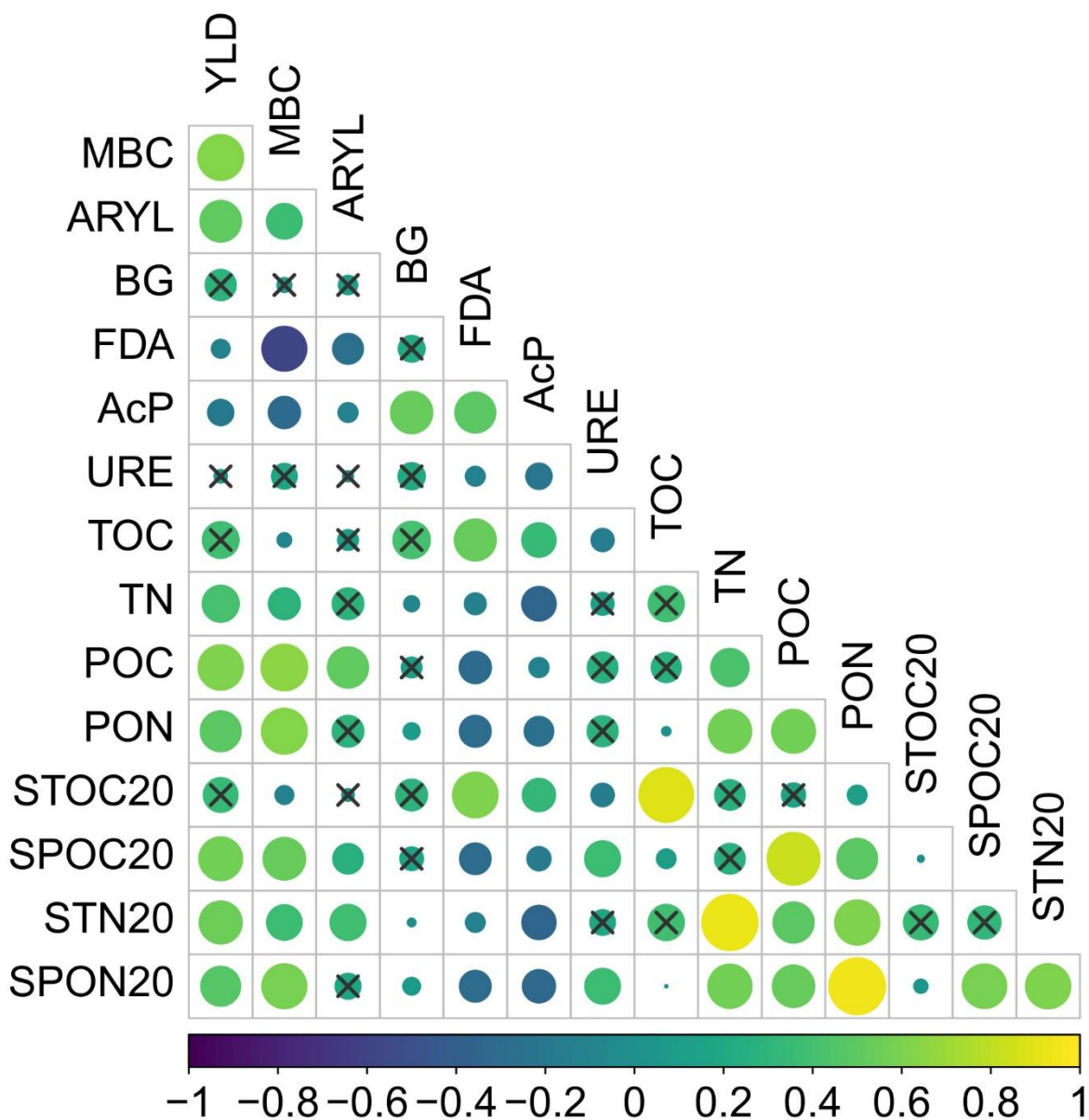
In the PCA of the evaluated attributes, PC1 and PC2 explained 60% and 67% of the data variation, respectively (Figure 9 A and B). MIX, CS1 and CS2 were strongly correlated with all variables except  $q\text{CO}_2$ , which was strongly correlated with MC. Notably, CS3 was strongly correlated with variables related to soil C levels and stocks (TOC, STOC<sub>40</sub> and STOC<sub>20</sub>) and BG enzymatic activity.

**Figure 9 - Principal Component Analysis of Acid phosphatase (AcP), Arylsulfatase (ARYL),  $\beta$ -glucosidase (BG), Urease (URE), Fluorescein diacetate hydrolysis (FDA), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), soil basal respiration (SBR), biomass-specific respiration ( $q\text{CO}_2$ ), microbial quotient ( $q\text{MIC}$ ) (A), total organic carbon (TOC), total nitrogen (TN), particulate organic carbon (POC) and N (PON), stocks of total organic carbon (STOC<sub>40</sub> and STOC<sub>20</sub>) and total nitrogen (STN<sub>40</sub> and STN<sub>20</sub>) on 0.00-0.40 m and 0.00-0.20 m depths and stocks of particulate organic carbon (SPOC<sub>20</sub>), particulate organic nitrogen (SPON<sub>20</sub>) at 0.00-0.20 m depth, C lability index and C management index (B)**



Pearson's correlation analysis identified positive correlations of soybean yield and MBC with most of the variables related to soil C and N contents (totals, particulates, stocks) and with ARYL (Figure 10).

**Figure 10 - Heatmap correlation (Pearson) for biochemical attributes in function of soybean yield**



MC: Monocropped (fallow in no-tillage); CS1: Crop succession 1 (*Crotalaria spectabilis*); CS2: Crop succession 2 (*Pennisetum glaucum*); CS3: Crop succession 3 (*Urochloa ruziziensis*); CS4: Crop succession 4 (*Cajanus cajan*); MIX (*C. spectabilis*+*P. glaucum*+*U. ruziziensis*+*C. cajan*). Significance levels are indicated by: \*  $p \leq 0.10$  \*\*  $p \leq 0.05$ ; \*\*\*  $p \leq 0.0001$ . For soybean yield, different letters indicate significant differences between treatments by Fisher's protected LSD test at  $p \leq 0.10$ .

## 1.4 DISCUSSION

The higher accumulation of TOC, POC and C stocks in CS2, CS3 and MIX emphasize the importance of *U. ruziziensis* and *P. glaucum* as species that quantitatively increase residue input under no-tillage via the production of shoot dry mass (SDM) and roots, which can be further transformed into stable C molecules. Higher BG activity was also observed in these treatments (Figure 7 B) and according to Tenelli et al. (2019), BG activity is an indirect indicator of C mineralization and can be used to quantify the incorporation of labile C into the soil and the release of glucose as an energy source for the microbiota. Even in these soil conditions, it is difficult to increase C contents, these results affirm that the dynamism of these species are determinants of the viability of MIX as a production system to increase the dynamic reserves of labile C to be used by the microbiota and stabilize it, incorporating C into the soil. High production of SDM by *U. ruziziensis* and *P. glaucum* in the Cerrado was also reported by Pacheco et al. (2018) and São Miguel et al. (2018).

The association of *C. spectabilis* (CS1) with greater accumulation of TN and N stocks corroborates the ability of this legume to incorporate N into the system previously described by Souza et al. (2018). However, *C. cajan* (CS4) accumulated more TN in the last layer because its pivot root system can explore the soil in depth and promote the incorporation of nutrients and organic matter. In CS3 and MIX, TN increased in all layers due to the high root volume for incorporation of this nutrient; in addition, the floristic diversity provided by MIX increased the energy supply for the microbial community. With respect to PON, lability of N molecules was observed in all production systems, which, along with the high  $qMIC$ , demonstrated the quality of the plant material inserted (Figure 8 E). However, URE activity was high (Figure 7 C), possibly because the amine groups ( $NH_2$ ) in the cover crops were less accessible at the time of the evaluation. As the availability of plant residues for which the microbiota had affinity increased, so did the demand for enzymes to degrade this material. According to Carlos et al. (2020; 2021), urease degrades structurally simple nitrogen compounds ( $NH_2$ ) and does not have high capacity for degrading stable, complex compounds; they found that soil management under no-tillage increased urease activity by 96% compared with conventional management.

The high CLI and CMI in CS2 (Figure 5 C and D) indicate that the accelerated development of *P. glaucum* made labile material available to the system before the decomposition of the other species. This influence of *P. glaucum* was also evident in MIX. CLI and CMI are indicators of the quality of the management system, and thus these high values indicate that MIX and CS2 improved chemical and microbiological attributes such as stocks, enzymatic activity and microbial biomass (MB). In addition, materials that are easily decomposed provide an easily accessible source of food with low energy cost to the edaphic biota, as these materials are composed of low molecular weight carbon chains rich in organic acids and sugars that can be transformed into energy to increase MB (Batista et al., 2022; Soong et al., 2020).

The contributions of *U. ruziziensis* (CS3) and *C. cajan* (CS4) were fundamental to the lower penetration resistance (PR) observed in MIX (Figure 6 A). According to Herrada et al. (2017) and Guimarães et al. (2019), the infiltration of cover crop roots into the soil forms biochannels through which the soybean crop can later travel, minimizing the effects of natural soil densification. MIX and CS3 also showed higher soybean yield, possibly indicating a more favorable environment for plant development (Figure 8 F). Albrecht et al. (2018) concluded that the use of diverse cover crops stimulates exploration for natural resources and promotes biophysiochemical improvements in the soil in agricultural systems. Calonego et al. (2011) reported that intercropping of corn with *Brachiaria* reduced penetration resistance compared to single. Although trends toward differences in MaP, MiP and ASI were observed in CS2, CS3, CS4 and MIX, these differences were not significant enough to infer changes in these attributes over the course of the experiment. Although a period of 7 years was sufficient to observe differences in biology and soil fertility (Chapter 1), changes in physical attributes, except PR, seem to require a longer time to occur. Castro et al. (2017) reinforce that in weathered soils such as those of the Cerrado, management with forage crops in rotation can contribute to increase SOM and soil aggregation over time.

The greater activity of ARYL in MIX may be related to the diversity of organic residues resulting from the four cover crop species in the consortium. In particular, an increase in S radicals linked to organic molecules of plant residues may have occurred. Supporting this hypothesis, Mus et al. (2019) and Divito & Sadras (2014) reported that the inclusion of two legumes in the consortium increases the demand for S absorption,



as S is a constituent of the catalytic sites and proteins responsible for BNF. Sulfur is one of the most challenging nutrients to manage in the Brazilian Cerrado because it can be leached along the soil profile, and techniques that can favor S allocation in the organic fraction are fundamental for S efficiency in agricultural systems.

AcP activity was highly related to the presence of grasses (CS2 and CS3), including in the fallow treatment (MC), which predominantly contained weeds of the grass family (*Cenchrus echinatus* and *Digitaria horizontalis*). This may reflect the evolutionary ability of grasses to survive in acidic environments with low P availability, as attested by Baptistella et al. (2021), Almeida et al. (2020) Teutscherova et al. (2019), Almeida et al. (2018) and Louw-Gaume et al. (2017). Mndzebele et al. (2020) noted that phosphatase activity is an indicator of P deficiency in the soil; when P concentrations are low, plant roots and microorganisms synthesize phosphatases to solubilize poorly labile P fractions in the soil. According to Carlos et al. (2021) and Tenelli et al. (2019), releasing high amounts of AcP to the soil can make phosphates linked to particulate SOM available through the dephosphorylation of plant substrates, in addition to increasing the availability of other P fractions. As P is the least available nutrient in Cerrado soils, diversified systems that provide high enzymatic activity to increase the availability of P to crops are essential for increasing soil quality in production systems and the soybean yield in succession.

The higher FDA activity in MC and CS3 indicates that these treatments had higher levels of high molecular weight molecules that require enzymes such as proteases, lipases and esterases for decomposition, consistent with the results of Fei et al. (2020). According to Messias et al. (2011), these enzymes not only increase the availability of elements in soil by breaking carbon chains but are also versatile biocatalysts with wide biotechnological applications in industry. Consequently, these enzymes could be future tools for increasing nutrient bioavailability in production systems. The higher GMean values in CS3, CS2 and MIX are consistent with the greater residue production in these systems (Chapter 1). This residue production favored the maintenance of soil temperature and microbial community growth, resulting in a living, balanced system in the soil.

The importance of species diversification for increasing soil microbial biomass (MBC and MBN) was evident in MIX (Figure 8). The high SBR and qMIC also indicate

the presence of an active microbiota due to the quality of the material provided by MIX. In addition, the low  $q\text{CO}_2$  indicates that the microbiota had a sufficient energy supply to avoid physiological stress and to maintain the efficiency of C use (Batista et al., 2020; 2022). This favorable environment for the proliferation of edaphic fauna improved the stability of the production system and resulted in high soybean yield in succession under no-tillage (Figure 8F). By contrast, MC, which had a low supply of residues and high degradation activity, became an environment for the maintenance of cellular activities (catabolism) and not microbial community growth, as also reported by Batista et al. (2022). As a result,  $\text{CO}_2$  was released into the atmosphere rather than fixed as organic materials and SOM, making the fallow system unfeasible for production under no-tillage.

Soybean yields were highest in CS3 and MIX, with increases of 16% compared with fallow (MC). These increased yields indicate that these treatments provided high-quality organic residues for the development of microbial biomass and greater nutrient availability for soybean. In a study of the impact of consortia of functional microorganisms on nutrient acquisition and use efficiency, Wang et al. (2021) found positive responses of soybean growth and yield and promising potential for improving crop performance in the field. Each of the species found in MIX perform functions that improve the biophysical and chemical properties of the soil, as evidenced by the low PR and in the attributes related to the management of carbon and microbial biomass. Amorim et al. (2020), Chamberlain et al. (2020) and Pires et al. (2020) also demonstrated that crop diversity under long-term no-tillage can improve the structure, activity and proliferation of the microbial community to favor nutrient cycling and greater crop yield.

PCA showed that CS3 was correlated with BG and variables related to soil C due to the high production of aerial and root dry mass by *U. ruziziensis* and the greater incorporation of C into the soil system. MIX, which combined the four cover crops, was located centrally in the PCA plot, clearly indicating the balanced effect created by the floristic diversity in this treatment. Carvalho et al. (2021), Nishigaki et al. (2021) and Chachal et al. (2020) noted that plant residues are the main source of C and N and that floristic diversity enhances C protection and stability, thereby increasing C stocks in the soil and reducing  $\text{CO}_2$  emissions to the atmosphere.

In Pearson's correlation analysis, soybean grain yield was positively correlated with soil C and N contents due to the nutritional bank provided by the latter attributes. The high correlation of yield with ARYL may be related to the ability of this enzyme to increase the availability of S, which is a component of the proteins and amino acids necessary for soybean grain formation according to Sfredo & Lantmann (2007). In addition, according to Mus et al. (2019) and Divito & Sadras (2014), S participates in N metabolism and is a component of ferredoxin, an enzyme involved in BNF. The cover crop species enriched the microbiota, which improved the bioavailability of nutrients for successor crops and made the NTS more balanced in the long run.

## 1.5 CONCLUSIONS

1. The species diversity in MIX favored soybean yield and carbon sequestration, as evidenced by improvements in resistance to soil penetration, carbon from biomass and soil microbial biomass.
2. *U. ruzizensis* was important for enhancing the activity of enzymes that degrade organic residues, particularly  $\beta$ -glucosidase activity, in no-tillage soybean production in the Cerrado, with a positive influence on carbon increase in the system.
3. The monocropped (MC) is not indicated for use in the Cerrado because it is not able to improve the physical and biochemical characteristics of the soil and the soybean yield.

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## SUPPLEMENTARY MATERIAL

**Table 1 - Statistical analysis for environmental, microbial and physical attributes protected by Fisher's LSD test at  $p \leq 0.10$**

[illegible]

Total organic carbon: TOC; Particulate organic carbon: POC; Total soil carbon stocks: TSCS; Soil particulate carbon stocks: SPCS; Carbon lability index: CLI; Carbon management index: CMI; Nitrogen total: Nt; Particulate organic nitrogen: PON; Total soil nitrogen stocks: TSNS; Soil particulate nitrogen stocks: SPNS; Microbial biomass carbon: MBC; Microbial biomass nitrogen: MBN; Soil basal respiration: SBR; Biomass-specific respiration:  $q\text{CO}_2$ ; microbial quotient:  $q\text{MIC}$ ; Acid phosphatase: AcP; Arylsulfatase: ARYL;  $\beta$ -glucosidase: BG; Fluorescein diacetate hydrolysis: FDA; Urease: URE; Geometric mean of enzyme activity: GMean; Penetration resistance: PR; Aggregate stability index: ASI; Macroporosity: MaP; Microporosity: MiP; Soybean grain yield: YLD.

## FINAL CONSIDERATIONS

Diversifying production systems and incorporating species with high production of plant material can transform production environments. The results of the present study can be used to guide recommendations for producers in the southern region of Mato Grosso as well as other regions of Brazil. Cover crops such as *U. ruziziensis* in the MIX consortium specialize in extracting nutrients from the deep layers of the soil and returning them to the soil surface. The resulting stable environment promotes the activity of the microbial community and, in turn, the mineralization of nutrients for absorption by the soybean crop in succession. Overall, nutrient use efficiency is improved while reducing the input of mineral fertilizers.

In addition to increasing soybean yield and income, this system benefits producers by reducing the economic costs of inputs. In the long term, soil fertility is also improved, largely due to the accumulation of organic matter particles that enrich the soil and strengthen subsequent crops. In this way, adapted and diversified production systems under no-tillage improve soil quality and nutrient cycling and increase soybean yield for the benefit of rural producers.

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