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**CARBON BALANCE IN SOYBEAN AND INTERCROPPED MAIZE PRODUCTION
SYSTEMS IN A LONG-TERM EXPERIMENT**

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SYSTEMS IN A LONG-TERM EXPERIMENT**

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To my beloved family, whose unwavering support and encouragement have been my rock throughout this academic endeavor. Their love, understanding, and patience have been my constant pillars of strength,
I dedicate

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"But how could you live and have no story to tell?"

Dostoevsky, F. **White Nights**. Translated by Ronald Meyer. 1st ed. New York: Penguin Classics, 2016. 128 P.

ABSTRACT

Human CO₂ emissions play a pivotal role in global warming. Since soil serves as a significant reservoir of carbon (C), depending on how it is managed, it can either release or sequester atmospheric C. Agricultural production systems, like no-till and intercropping, offer essential alternatives from both environmental and agronomic perspectives. This study examined the long-term impact (15 years) of no-till and soil cover on C dynamics in the soil and atmosphere. The treatments involved different cropping systems, with various plants intercropped with maize in a soybean-maize succession: (1) maize without intercropping (fallow); (2) Ruzigrass (intercropped with *Urochloa ruziziensis*); (3) Sunn hemp (intercropped with *Crotalaria spectabilis*); and (4) Sunn hemp+ruzigrass. Intercropped systems, particularly those with ruzigrass, exhibited an increase in dry matter, nitrogen, and C input both above and below the ground. Due to the substantial input, ruzigrass in intercropping achieved a positive C balance ($\sim 0.7 \text{ Mg ha}^{-1}$), whereas when alone (2) and combined with sunn hemp (4), the balance was less negative. Ruzigrass intercropped system (2) resulted in an increase of around 30% in C stock (0-1 m depth) compared to fallow. Grain yield and relative emissions (kg of C-CO₂ per kg of grains) were notably enhanced with the introduction of ruzigrass into the systems. The results emphasize the importance of intercropping with ruzigrass in tropical conditions, showcasing how cover crops are valuable amidst global climate changes and the imperative to increase food production.

Keywords: carbon dioxide emissions; carbon sequestration; conservationist systems.

RESUMO

Emissões antrópicas de CO₂ desempenham um papel fundamental no aquecimento global. O solo atua como um reservatório significativo de carbono (C) e a depender de como é manejado, pode emitir ou sequestrar C atmosférico. Sistemas conservacionistas de produção agrícola, incluindo semeadura direta e introdução de culturas de cobertura, oferecem alternativas essenciais tanto na perspectiva ambiental quanto agronômica. Este estudo examinou o efeito de longa duração (15 anos) de semeadura direta e diferentes culturas de cobertura na dinâmica do C no solo e na atmosfera. Os tratamentos envolveram diferentes sistemas de cultivo, com diferentes culturas de cobertura em consórcio como o milho em sistemas de sucessão soja-milho: (1) milho sem consórcio (pousio); (2) *Urochloa ruziziensis* (consorciado com braquiária); (3) *Crotalaria spectabilis* (milho consorciado com crotalária); e (4) Crotalária + braquiária. Sistemas consorciados, especialmente aqueles consorciados com braquiária, mostraram um aumento na matéria seca, nitrogênio e aporte de C tanto na parte aérea quanto nas raízes. Devido ao maior aporte de C, o sistema com braquiária (2) alcançou um saldo de C positivo ($\sim 0,7 \text{ Mg ha}^{-1}$), enquanto crotalária + braquiária (4), o saldo foi menos negativo. O sistema consorciado com braquiária (2) resultou em um aumento de aproximadamente 30% no estoque de C (0-1 m de profundidade) em comparação ao pousio. A produtividade de grãos e as emissões relativas (kg de C-CO₂ por kg de grãos) melhoraram significativamente com a introdução de braquiária nos sistemas. Os resultados destacam a importância do consórcio com braquiária em condições tropicais, demonstrando como as culturas de cobertura são valiosas diante das mudanças climáticas globais e da necessidade premente de aumentar a produção de alimentos.

Palavras-chave: emissões de dióxido de carbono; sequestro de carbono; sistemas conservacionistas.

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ABBREVIATIONS AND ACRONYMS LIST

Al	Aluminum
BS	Base saturation
C	Carbon
C	Calcium
CC	Cover crops
CE	Carbon stocks
CEC	Cation exchange capacity
CO ₂	Carbon dioxide
Cu	Copper
DAS	Days after sowing
DAS	Days after sowing
F	Fallow
Fe	Iron
GHG	Greenhouse gases
GY	Grain yield
IPCC	The Intergovernmental Panel on Climate Change
IRGA	Infra red gas analyzer
K	Potassium
LOM	Living organic matter
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NLOM	Non-living organic matter
NTS	No-tillage system
P	Phosphorus
RE	Relative emissions
RG	Ruzigrass
S	Sulfur
SCCI	Soil carbon conservation index
SH	Sunn hemp
SOC	Soil organic carbon
SOM	Soil organic matter
Zn	Zinc

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1 INTRODUCTION

Global climate change, resulting from greenhouse gas (GHG) emissions, including CO₂, is manifested through extreme heatwaves, storms, hurricanes, and widespread drought (Lee *et al.*, 2023; Zapparoli *et al.*, 2018). Agriculture contributes to 20-25% of global CO₂ emissions (Masson-Delmotte *et al.*, 2021). The soil is crucial for mitigating CO₂ emissions (Abbas *et al.*, 2020) as it is the Earth's second-largest carbon (C) reservoir with a stock of 2,500 Pg (1 Pg = 10⁹ tons) (Lal; Negassa; Lorenz, 2015). Conservationist agricultural practices, in alignment with UN Sustainable Development Goals 13 and 15, target reduced soil GHG emissions, increased atmospheric C sequestration, biodiversity, and improved ecosystem health, fostering global sustainability.

The soybean/maize succession is prevalent in Brazil, particularly in no-tillage systems (NTS). Together, they contribute to 86% of the country's grain production, covering 78% of the cultivated area. Soybean is exclusively grown in the summer, while about 79% of maize is produced in the autumn-winter season (Conab, 2023). NTS, acknowledged for enhancing soil aggregate stability, play a crucial role in protecting C by reducing CO₂ emissions from soil organic matter (SOM) decomposition (Conceição; Dieckow; Bayer, 2013; Neto *et al.*, 2021). Thus, conservationist practices like NTS with cover crops (CC), significantly increase C incorporation in Cerrado ecosystems (Tivet *et al.*, 2013). The increase in soil C storage depends on the balance between cumulative C-CO₂ emissions and C inputs from plant residues and the formation of stable compounds in the soil (Rigon; Calonego, 2020). C addition to soil under NTS compared to conventional tillage, contributed up to 30 Mg ha⁻¹ of C in the 0-1 m depth (Corazza *et al.*, 1999). Intercropping involving grass (Rosolem; Li; Garcia, 2016) and/or legumes (Rigon; Calonego, 2020) elevate C input, sequestration, and yield (Das *et al.*, 2014; Rosolem; Li; Garcia, 2016), what is desired considering the rapidly increasing world population, necessitating greater food production (Lutz; Sanderson; Scherbov, 2008).

Increasing yield per unit area holds significant potential for reducing the emission of C-CO₂ per kg of grain produced. However, in Central Brazil's grain-producing regions, maintaining crop residue and soil cover is challenging due to scarce fall-winter rainfall and high temperatures hastening residue degradation (Assunção *et al.*, 2019). Additionally, there is a shortage of information on the C balance benefits of

intercropping, encompassing inputs from crop residues and roots, as well as C-CO₂ outputs. Moreover, there is insufficient data concerning the introduction of other species into intercropping systems, particularly legumes in both simple intercropping with maize and triple intercropping systems (maize/forage grass/legume).

We posited the following hypotheses: I - Intercropped systems involving *Crotalaria spectabilis* and/or *Urochloa ruziziensis* augment soil C stocks through increased C input both above and below ground; II – Intercropped systems would yield higher, leading to reduced kg of C-CO₂ per kg of grains; and, III - The final C balance in intercropped systems is either neutral or positive, with conservationist systems facilitating agricultural production while maintaining neutral net C emissions to the environment. This study aimed to assess the C balance and soil C stock in a long-term experiment involving soybean cultivation during the spring-summer season and maize in succession during the autumn-winter season. The intercropping configurations included *Urochloa ruziziensis*, *Crotalaria spectabilis*, *U. ruziziensis*+*C. spectabilis*, and a non-intercropped system.

2 LITERATURE REVIEW

2.1 National agriculture

The cultivation of soybean (*Glycine max* [L.] Merrill) stands as one of the foremost global commodities, holding significant economic and social importance (Boerema *et al.*, 2016). It serves as a raw material for vegetable oil production and plays a fundamental role in animal nutrition (Eskandari; Cober; Rajcan, 2013; Rennó *et al.*, 2015). Soybean exhibits average oil and protein contents of 22% and 39%, respectively, in grains (Hackenhaar *et al.*, 2019).

In the 2022/2023 growing season in Brazil, soybean production reached 155 million tons, covering an area of 44 million hectares, with an average yield of 3.5 Mg ha⁻¹ (Conab, 2023). Furthermore, soybean oil production proves to be compelling from both technical and financial perspectives, surpassing the profitability of other vegetable oil sources such as sunflower, castor, or peanut (Roscoe; Richetti; Maranhão, 2007). This underscores the significance of soybean cultivation not only in meeting nutritional demands but also in contributing to the economic sustainability of agricultural practices on a global scale.

The cultivation of maize (*Zea mays* L.), analogous to soybean, holds an irreplaceable position in sustaining humanity's diverse needs. Predominantly recognized for its pivotal role in the production of animal feed (Reis *et al.*, 2016), maize extends its influence as a versatile raw material contributing to various food products within the human diet (De Sousa *et al.*, 2019). Moreover, the recent inclusion of maize in ethanol production further amplifies its significance in the realm of bioenergy (Scully *et al.*, 2021).

In the global agricultural landscape, maize commands the second-largest cultivated area (Santpoort, 2020). In Brazil, approximately 79% of maize is cultivated during the autumn-winter season, contributing to a production of 102 million tons, covering an area of 17 million hectares, with an average yield of 6 Mg ha⁻¹ in the 2022/2023 growing season (Conab, 2023). This dual role as a major contributor to both animal and human sustenance, coupled with its emerging role in renewable energy, underscores the multifaceted importance of maize in contemporary agricultural practices.

In global terms, for the year 2022, worldwide soybean parameters encompassed an area of 133.8 million hectares, and an aggregate production of 348 million tons. Conversely, for maize on a global scale, the corresponding metrics were an area spanning 203.5 million hectares, and a total production of 1.16 billion tons. In the case of Brazil, the soybean crop exhibited an area of 40.9 million hectares and yielding a total production of 130.7 million tons. This performance accounted for approximately 38% of the global soybean production. As for maize, the reached area of 210.4 million hectares, resulting in a total production of 109.4 million tons. This represented around 9% of the global maize production (FAO, 2022).

Agriculture holds paramount importance from social, economic, and environmental perspectives globally. However, it stands as a significant contributor to anthropogenic GHG emissions, accounting for approximately 20-25% of emissions worldwide (Masson-Delmotte *et al.*, 2021). This crucial sector is profoundly affected by global climate change, manifesting through phenomena such as heatwaves, intense droughts, and storms (Lee *et al.*, 2023; Zapparoli *et al.*, 2018). Furthermore, the intensification of global warming leads to additional effects, including ocean acidification, hurricanes, atmospheric pollution, and ozone layer depletion (Zapparoli *et al.*, 2018). Conservationist systems, characterized by the integration of CC, possess the capacity to enhance both agronomic and environmental aspects of productive systems.

2.2 Organic matter-driven soil improvement with cover crops

Several advantages of incorporating CC in crop systems, particularly in tropical conditions, have been identified. These benefits encompass soil physical characteristics (Calonego; Rosolem, 2010), biological aspects (Torbert; Reeves; Mulvaney, 1996), and chemical attributes, highlighting enhanced nutrient cycling (Pariz *et al.*, 2016). Additional advantages include weed suppression (Teasdale; Beste; Potts, 1991), control of plant diseases (Acharya *et al.*, 2017; Larkin; Griffin; Honeycutt, 2010), and a substantial production of crop residues for soil coverage (Rosolem; Li; Garcia, 2016). Results have demonstrated that intercropping with legumes or grasses improves C content in the soil (Rigon; Calonego, 2020; Rosolem; Li; Garcia, 2016). The increase in soil C resulting from the action and presence of CC is attributed to the

greater input of plant material, both aboveground and primarily belowground (Bretas *et al.*, 2021; Rosolem; Li; Garcia, 2016; Sokol; Bradford, 2019).

The incorporation of CC into agricultural systems has a significant impact on soil dynamics, particularly in relation to SOM and pH regulation (Vanzolini *et al.*, 2017). CC contribute to an increase in SOM, acting as a weak acid in the soil (Nyakatawa; Reddy; Sistani, 2001). In acidic tropical soils, this elevated SOM content buffers pH, leading to a rise in pH levels and a reduction in soil acidity by decreasing H^+ concentrations (Vanzolini *et al.*, 2017). This process involves enhanced occupation of soil colloid sites by metallic cations and various transformative processes, such as organic nitrogen (N) mineralization and decarboxylation of organic acids (Sharma; Irmak; Padhi, 2018). Conversely, in alkaline soils, less common in national contexts, the addition of SOM may reduce pH through the mineralization of SOM, generating CO_2 , which forms carbonic acid and releases bicarbonates, resulting in the release of H^+ (Elert; Pardo; Rodriguez-Navarro, 2015).

SOM, prevalent in soils with CC, significantly affects cation exchange capacity (CEC), constituting a substantial portion in both mineral and organic soils (Wulanningtyas *et al.*, 2021). In tropical soils, total organic C outweighs the importance of clay in CEC (Bruun; Elberling; Christensen, 2010; Wattel-Koekkoek *et al.*, 2001). Limestone application in tropical soils corrects acidity and enhances cultivation, but 20 to 30% of applied (calcium) Ca and magnesium (Mg) complex with SOM, limiting immediate plant availability (Pocknee; Sumner, 1997; Wattel-Koekkoek *et al.*, 2001). The considerable quantity of SOM is crucial for national soil fertility, correlating directly with soil CEC and fertility, especially in areas employing practices like NTS and CC adoption (Pocknee; Sumner, 1997).

SOM influences metal interactions through cationic adsorption and chelation mechanisms (Clarholm; Skyllberg; Rosling, 2015; Karlsson; Persson; Skyllberg, 2006). Functional groups in SOM, ranked by metal affinity, include alcoholic, amino, azo, N ring, carboxylate, ether e carbonyl. Cation chelation is most likely with iron (Fe^{3+}) and least with manganese (Mn^{2+}) (Karlsson; Persson; Skyllberg, 2006). Agricultural practices promoting SOM improve micronutrient availability and reduce aluminum (Al) exchangeability, while the pKa of organometallic complexes influences Al^{3+} complexation (Hernandez-Soriano; Jimenez-Lopez, 2012).

In tropical soils, dominated by kaolinite (1:1 phyllosilicate) and Fe and Al sesquioxides, aggregation processes are primarily influenced by physical and

chemical factors such as wetting-drying cycles, the association of SOM with multivalent cations, and pH (Ghimire; Machado; Bista, 2017; Mccauley; Jones; Jacobsen, 2009). The pH, in this context, is linked to the charge of clay components, specifically in 1:1 clays and oxi-hydroxides. Notably, the zero-point charge of kaolinite is 4.0, and for hydroxides, it is 7.8 (Hernandez-Soriano; Jimenez-Lopez, 2012; Huang; Wu; Yu, 2009). Considering the pH range of weathered soils from 4 to 6, kaolinites facilitate aggregation more efficiently (Mccauley; Jones; Jacobsen, 2009).

The SOM can also retain a significant amount of water, especially in 2:1 clays, due to mineral expansibility. The hydrophobicity increases with SOM polymerization, reducing its water retention capacity (Huntington, 2006). Exposure to intense wetting and drying cycles favors mineralization, diminishing water retention. Soil texture is crucial, with clayey soils exhibiting high capacity, while sandy soils rely more on SOM. Soil management plays a critical role in efficient water storage (Ankenbauer; Loheide, 2017).

Essential elements for plants can originate from various sources, including mineral fertilization and the organic fraction of soils. Elements like N, phosphorus (P), and sulfur (S) are integral components of organic structures, while calcium (Ca), magnesium (Mg), and potassium (K) exist as cations on the surface of organo-mineral complexes (Lehmann; Kleber, 2015). Additionally, elements such as Mn, copper (Cu), Fe, and zinc (Zn) can be complexed or chelated within the organic fraction of soils (Mukherjee; Zimmerman, 2013). In this way, CC can influence various aspects in crop systems, which can impact the quantity and quality of SOM and, consequently, the dynamics of GHG emissions from the soil (Paul, 2016).

2.3 Greenhouse gas emissions

The greenhouse effect serves as a pivotal determinant for life on Earth, playing a relevant role in maintaining its habitability. Without this natural phenomenon, the heat derived from solar radiation would dissipate into space, significantly altering the thermal conditions of our planet. In the absence of the greenhouse effect, the Earth's temperature, solely influenced by solar radiation, would plummet to approximately -19 °C. However, owing to the greenhouse effect's essential contribution, the global average temperature has surged to a life-sustaining 14 °C (Kweku *et al.*, 2018).

The process of heating in the Earth's atmosphere involves the absorption and re-emission of radiation by GHG. When sunlight reaches the Earth, it consists of various wavelengths, including visible light. These shorter wavelengths can penetrate the atmosphere and reach the Earth's surface (Tian *et al.*, 2016). The Earth then absorbs this incoming solar radiation and emits it back into the atmosphere as infrared radiation, which has longer wavelengths. CO₂, CH₄, and water vapor, are particularly effective at absorbing and re-emitting infrared radiation (Kweku *et al.*, 2018).

When infrared radiation is emitted from the Earth's surface, GHG in the atmosphere absorb some of this energy. The absorbed energy causes the molecules of these gases to vibrate and become excited (Ramanathan; Feng, 2009). Following this absorption, GHG re-emit infrared radiation in various directions, including back towards the Earth's surface. This process effectively traps heat within the atmosphere, contributing to the warming of the planet (Kweku *et al.*, 2018; Ramanathan; Feng, 2009).

Nevertheless, the delicate balance of the natural greenhouse effect has been disrupted by contemporary anthropogenic activities, resulting in a remarkable escalation of GHG emissions to unprecedented levels (Lee *et al.*, 2023). This perturbation has led to a consequential increase in global temperatures and has become a driving force behind the ongoing propulsion of climate changes on a global scale (Igłński; Babiak, 2017).

The primary anthropogenic GHGs include CO₂, CH₄, and N₂O (Zapparoli *et al.*, 2018). Among these, CO₂ takes precedence as the most significant GHG due to its highest emission levels. CH₄ and N₂O also play pivotal roles, with a global warming potential assigned to them being 82,5 and 273 times greater than that of CO₂ over a 100-year period (Lee *et al.*, 2023).

Studies indicate that prior to the industrial revolution, up to the year 1750, the concentrations of CO₂, CH₄, and N₂O were approximately 280 ppm, 700 ppb, and 270 ppb, respectively. Presently, these concentrations have risen to 414 ppm, 1877 ppb, and 331 ppb (Yoro; Daramola, 2020). These gases contribute to the greenhouse effect in proportions of 54%, 18%, and 7% for CO₂, CH₄, and N₂O, respectively (Lee *et al.*, 2023).

The emission of CO₂ from agricultural production systems arises from various sources, including the expansion of agricultural areas (Cerri *et al.*, 2009), the combustion of fossil fuels (El-Fadel; Massoud, 2001), soil erosion (Lal, 2018), and the

decomposition of SOM (Conceição; Dieckow; Bayer, 2013). The latter is induced by soil movement, involving the disintegration and increased exposure of SOM to microbial activity (Rigon; Calonego, 2020).

2.4 Agricultural production systems and greenhouse gas emissions

Agricultural production systems, despite being significant GHG emitters, also play a crucial role in GHG mitigation through C and N sequestration, with soil serving as a vital reservoir for these elements (Lal; Negassa; Lorenz, 2015). The soil, recognized as a substantial storage site for C and N, emerges as a formidable tool in addressing the current surge in GHG emissions (Abbas *et al.*, 2020).

Approximately 1,505 Pg of soil organic carbon (SOC) are estimated to be stored within the first meter of the terrestrial environment, out of the total 2,200 Pg of C (Batjes, 1996). For N, the soil reserve is estimated at approximately 133-140 Pg in the top meter (Abbas *et al.*, 2020). Adjustments in agricultural production systems and soil management can significantly contribute to GHG emission mitigation by reducing the flux of these gases from the soil to the atmosphere and enhancing the return of C from the atmosphere to the soil in the form of SOC.

These adaptations in agricultural systems align with various Sustainable Development Goals (SDGs) proposed by the United Nations (UN), including SDG 13 (climate action), SDG 15 (life on land), and SDG 2 (zero hunger), each with established targets set for 2030 within the 2030 Agenda. The pursuit of agricultural systems with a lower relative emission of GHGs, indicating reduced emissions per unit of food produced, becomes imperative in achieving these sustainable development objectives.

This recognition of the substantial C stocks within the soil underscores the importance of sustainable land management practices. By optimizing agricultural systems and soil management, it becomes possible to foster C sequestration in soils, which aligns with broader environmental objectives, including mitigating climate change and promoting soil health. Therefore, exploring and implementing strategies that enhance C sequestration in the soil represent crucial steps towards achieving a sustainable and resilient balance in terrestrial ecosystems.

2.5 Soil organic matter

SOM is pivotal for the health and sustainability of global agricultural ecosystems, impacting soil fertility, crop quality, and resilience to adverse conditions. Beyond its chemical composition, it plays a crucial role in promoting sustainable farming practices, reducing the reliance on chemical inputs, and contributing to environmental conservation (Lehmann; Kleber, 2015; Mccauley; Jones; Jacobsen, 2009). This significance goes beyond immediate concerns, linking SOM to long-term soil health and the pursuit of sustainable agricultural systems for present and future generations (Senesi; Loffredo, 2018). Recognizing and enhancing the role of SOM in soil management practices is imperative for achieving long-term ecological balance and ensuring food security (Lal, 2020).

The SOM comprises derivatives of plant and animal materials incorporated into or disposed on the soil surface, in various stages of decomposition, excluding the aerial parts of plants (Senesi; Loffredo, 2018). Within the soil's organic fraction, constituting a maximum of 5% of the overall soil composition, 65% represents stable SOM (humified), while 35% is decomposable, susceptible to decomposition (such as litter, roots, and organisms) (Stolt; Lindbo, 2010). The deposition of organic material onto the soil occurs through both biotic and abiotic processes. Biotically, organisms like annelids, ants, and microorganisms play a role. Abiotically, organic material is deposited by rainfall, which, besides dissolving CO_2 into H_2CO_3^- , washes organic material from vegetation to the soil (Senesi; Loffredo, 2018; Stolt; Lindbo, 2010). In this process, low-molecular-weight metabolites, such as sugars, disaccharides, and proteins, are transported (Tisdall, 2020).

The organic matter in the soil is distributed across two primary compartments: living organic matter (LOM) and non-living organic matter (NLOM) (Stolt; Lindbo, 2010). LOM, constituting approximately 2% of the total SOM, is characterized by C immobilized in the soil, with 5 to 10% attributed to roots, 15 to 30% to soil fauna, and 60 to 80% to microorganisms (Stolt; Lindbo, 2010). Within LOM, organisms are categorized into microflora, microfauna, mesofauna, and macrofauna. Microflora encompasses microorganisms such as bacteria, fungi, actinomycetes, and algae (Lal, 2020; Magdoff; Weil, 2004). Microfauna primarily includes protozoa. Mesofauna comprises collembolans and mites, while macrofauna encompasses annelids, termites, isopterans, and coleopterans (Kononova, 2013).

All soil fauna categories, namely microfauna, mesofauna, and macrofauna, exhibit varying degrees of functions including the reduction of deposited (input) material size within the system, separation of material components, mixing of organic and inorganic fractions, formation and maintenance of soil pores, and regulation and dispersion of microflora (Frouz, 2018). Microflora, in turn, function as decomposer agents and serve as a labile C reservoir. It is noteworthy that a stable and well-managed ecosystem will harbor higher stocks of C, microbial biomass, basal respiration, and CO₂ and C/N flux (Frouz, 2018; Kononova, 2013). Conversely, such an ecosystem will display a lower metabolic quotient (CO₂ flux/microbial biomass) (Stolt; Lindbo, 2010).

Approximately 98% of the total C in SOM is attributed to NLOM. NLOM is commonly categorized into macroragrangeous matter (3 to 20%) and humus (Stolt; Lindbo, 2010). Within the humus fraction, 70% comprises humic substances, while the remaining 30% constitutes non-humic substances. In the literature, macroragrangeous matter is also referred to as particulate organic matter and LOM (Senesi; Loffredo, 2018). LOM, in turn, is subdivided into free LOM, essentially comprising litter, and occluded LOM, representing a more advanced stage of transformation with slower cycling. Non-humic substances may include carbohydrates, lignin, lipids, organic acids, polyphenols, nucleic acids, pigments, and proteins (Kononova, 2013).

2.6 Land use and soil carbon stocks

The reduction in C loss occurs with an increase in soil aggregate stability, thereby enhancing the physical protection of C in the soil. This, in turn, augments the potential for nutrient storage, water retention, and the accumulation of C in the soil (Conceição; Dieckow; Bayer, 2013).

C losses in agricultural systems stem from soil movement, leading to the disintegration and increased exposure of organic matter to microbial degradation (Rheinheimer *et al.*, 1998). In regions characterized by tropical and subtropical climates, the degradation of SOM is rapid, primarily due to elevated temperatures and heightened microbial activity (Mielniczuk *et al.*, 2003). This degradation is further exacerbated under suboptimal management conditions (Assunção *et al.*, 2019).

The enhancement of SOM stabilization is contingent upon the practiced soil tillage method (Castro Filho *et al.*, 2002; Castro *et al.*, 2015), management techniques

(Cerri *et al.*, 2009), crop species selection within the production system (Lal, 2018), and the presence of CC (Bernoux, 2006). Conservation-oriented soil management systems exhibit a greater capacity for maintaining SOM stability (Tivet *et al.*, 2013). Findings indicate that the most substantial C addition to the soil occurs in NTS compared to conventional tillage, with reported contributions of 22 Mg ha⁻¹ (Corazza *et al.*, 1999).

Similarly, the elevation of N fixation in the soil is an outcome of the employed land management practices, with the utilization of sustainable cultivation systems capable of increasing the soil N stock (Bayer *et al.*, 2000). Results demonstrate that the adoption of NTS has led to an augmentation of soil N stocks compared to conventional systems (Alvarez *et al.*, 2014). N is crucial for the augmentation of soil C stock, playing an essential role in the straw mineralization process and its conversion into stable SOC (Castro *et al.*, 2015; Rigon; Calonego, 2020; Rigon; Franzluebbers; Calonego, 2020). The use of intercropping species with the main crop enhances the potential for C and N accumulation in the soil (Rigon; Calonego, 2020; Rigon; Franzluebbers; Calonego, 2020).

The enhancement of soil C stock is contingent upon cumulative CO₂ emissions being less than the inputs of C via plant residues. In addition to striving for an increase in C input, it is imperative to facilitate the conversion of C into stable SOM (Calonego; Rosolem, 2010; Rigon; Franzluebbers; Calonego, 2020). The chemical composition of the plant material introduced into the production system plays a crucial role in the formation of stable organic material, where the C/N ratio significantly influences SOC dynamics (Li *et al.*, 2020; Rigon; Calonego, 2020). Decomposition of plant material is accelerated in readily decomposable materials, i.e., those with a lower C/N ratio (Xavier *et al.*, 2019). Consequently, the introduction of species with a higher N content in their composition ensures greater structural stability of SOC, considering that N is essential for soil microorganisms in the formation of humified material within the system (Rigon; Calonego, 2020; Xavier *et al.*, 2019).

The cultivation of *Crotalaria juncea* as a CC has been shown to increase SOC stock by approximately 10%, contributing approximately 6 t C ha⁻¹ (Rigon; Calonego, 2020). Furthermore, in the same study, there was an observed N input increase of about 11% compared to the treatment without CC (fallow). Similarly, an increase in soil C and N content was noted when *C. juncea* was used as a CC, resulting in a C/N ratio

of approximately 10, promoting long-term soil C enhancement (Rosolem; Li; Garcia, 2016).

Greater C stock was observed when using hairy vetch and rye as CC in NTS, both in a long-term study and in conventional tillage (Olson; Ebelhar; Lang, 2014). The combination of leguminous CC and NTS led to a $1.15 \text{ Mg ha}^{-1} \text{ year}^{-1}$ increase compared to conventional tillage without CC (Veloso *et al.*, 2018). The use of pigeon pea and velvet bean as CC increased the soil C accumulation rate by $0.38\text{--}0.59 \text{ Mg ha}^{-1} \text{ year}^{-1}$ (Amado *et al.*, 2006).

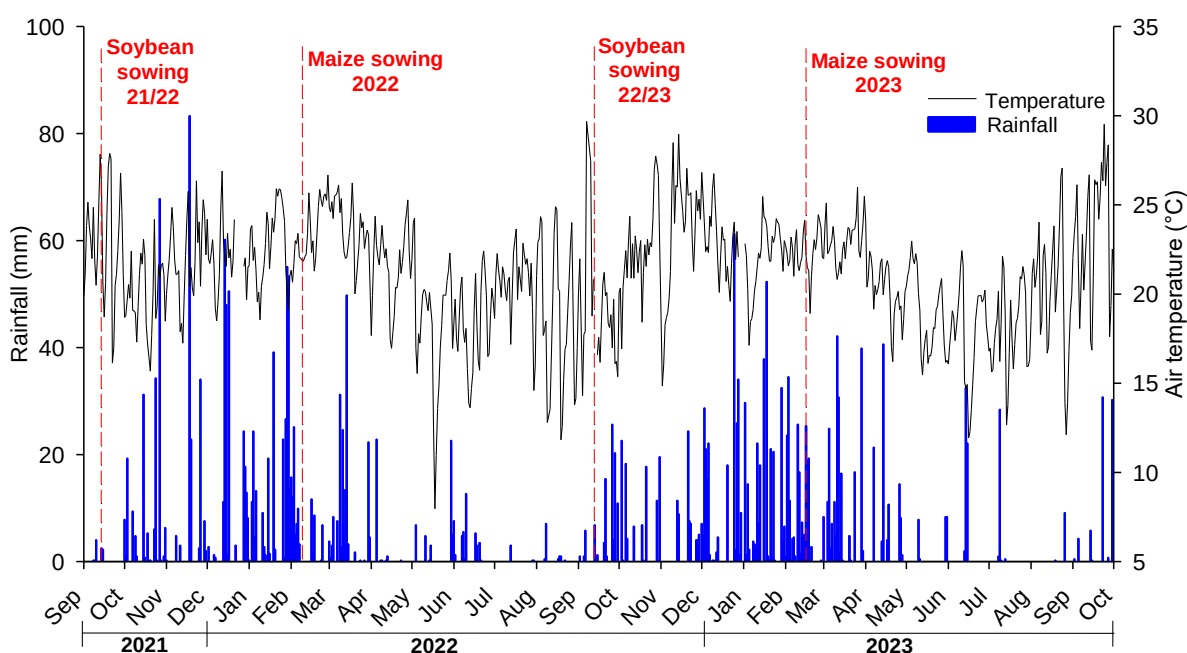
In an area with NTS and *U. ruziziensis* as a CC, elevated levels of C and N were observed, particularly in the upper soil layers (Souza *et al.*, 2018). The presence of *C. spectabilis*, *U. ruziziensis*, *U. brizantha* cv. Marandu, and *Pennisetum glaucum* L. as CC increased particulate SOC and labile N content in a four-cycle experiment (Ensinas *et al.*, 2016).

3 MATERIAL AND METHODS

3.1 Site description and experimental design

This study is part of a long-term field experiment documented on the GLTEN Metadata Portal ([GLTEN](#)), in Botucatu, São Paulo State, Brazil (22° 83' 3" S, 48° 42' 64" W, at an elevation of 765 m above sea level). The soil is identified as a Typic Haplorthox (Staff, 2014) or Oxisol (Jahn *et al.*, 2006). Before implementing the experiment (2006), chemical attributes were evaluated in the 0–20 m soil depth (Raij *et al.*, 2001). The obtained results were as follows: SOM content: 18.2 g dm⁻³; pH (CaCl₂): 4.2; P content (resin): 3.6 mg dm⁻³; K, Ca, Mg, and CEC: 0.8, 11.6, 5.7, and 75.0 mmolc dm⁻³, respectively; along with a base saturation (BS) of 24.2%. In accordance with the Köppen-Geiger classification, the climate in the region is of the Cwa type (Alvares *et al.*, 2013), featuring a hot and rainy season in summer and a dry season in winter, with an average annual precipitation of 1,314 mm. Monthly values of rainfall and mean air temperature during the experiment are depicted in Figure 1.

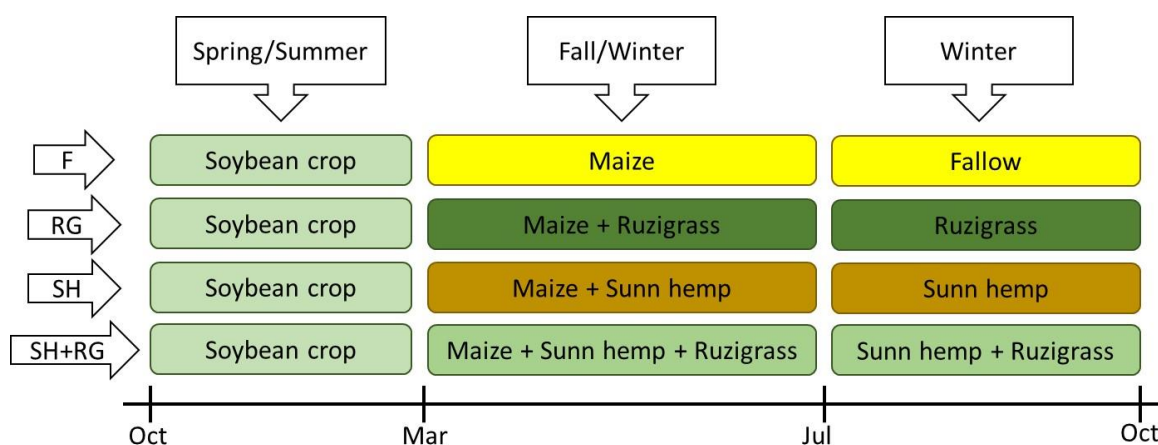
Figure 1 - Climatological data (precipitation and average air temperature) during the period from September 9, 2021, to October 10, 2023



The experiment was structured in a randomized complete block design with four replications, and each plot measured 69.3 m² (6.3 m × 11 m). The applied treatments

involved different species intercropped with maize: (I) Fallow (F, no intercropping); (II) Ruzigrass (RG, maize intercropped with *Urochloa ruziziensis*); (III) Sunn hemp (SH, maize intercropped with *Crotalaria spectabilis*); and (IV) Sunn hemp+ruzigrass (SH+RG). Crop production systems are composed of the soybean-maize succession, with soybeans sowed in mid-October, constituting the spring/summer season crop. Maize and CC (ruzigrass and sunn hemp) are sown together in mid-March, immediately following the soybean harvest, forming the autumn/winter season crop. During the off-season, from mid-July to September, representing winter, CC continue to develop until the next growing season (Figure 2).

Figure 2 - Configuration and chronological order of production systems. F (Fallow); RG (Ruzigrass); SH (Sunn hemp); SH+RG (Sunn hemp+ruzigrass); Soybean (*Glycine max*); Maize (*Zea mays*); sunn hemp (*Crotalaria spectabilis*) and; ruzigrass (*Urochloa ruziziensis*)



At the beginning of the experiment in 2006, liming application was determined based on soil chemical analysis at 0.0–0.2 m depth, with the aim of raising the BS to 70% (Raij, 1996). Lime was reapplied whenever the BS in the Fallow system (F) dropped below 50%, thus, additional applications took place in 2011, 2015, 2018, and 2022. The applied doses were 4.0, 4.7, 3.8, 4.5, and 3.4 Mg ha⁻¹. The lime (CaMg(CO₃)₂) contained 166 g kg⁻¹ Ca and 105 g kg⁻¹ Mg. In 2022, in conjunction with liming, the application of phosphogypsum was also carried out (Caires; Guimarães, 2018), resulting in a rate of 2.27 Mg ha⁻¹. Phosphogypsum contained 200 g kg⁻¹ Ca, 150 g kg⁻¹ S, < 1g kg⁻¹ P, and < 1g kg⁻¹ fluorine. Throughout the experiment period (2006–2017), various crops were cultivated, and specific details regarding these

previous crops are outlined in Table 1. In 2017, all systems were modified following the same model adopted in this study. The chemical and physical characteristics of the soil (F system) during the execution of this study (2021) are presented in Table 2.

Table 1 – Crop rotations in systems Fallow, Ruzigrass, Sunn hemp and Sunn hemp+ruzigrass from 2006 to 2023

	Fallow	Ruzigrass	Sunn hemp	Sunn hemp+ruzigrass
----- 2006/2007 -----				
Crop season	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Off-season	Fallow	<i>Urochloa ruziziensis</i>	<i>Pennisetum glaucum</i>	<i>Avena sativa</i>
----- 2007/2008 -----				
Crop season	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>Cajanus cajan</i>	<i>Phaseolus vulgaris</i>
----- 2008/2009 -----				
Crop season	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>Crotalaria juncea</i>	<i>Ricinus communis</i>
----- 2009/2010 -----				
Crop season	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>Pennisetum glaucum</i>	<i>Sorghum bicolor</i>
----- 2010/2011 -----				
Crop season	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>Lupinus albus</i>	<i>Crambe abyssinica</i>
----- 2011/2012 -----				
Crop season	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>
Off-season	Fallow	<i>U. brizantha</i>	<i>Pennisetum glaucum</i>	<i>Triticum aestivum</i>
----- 2012/2013 -----				
Crop season	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>
Off-season	Fallow	<i>U. brizantha</i>	<i>Crotalaria juncea</i>	<i>Triticum aestivum</i>
----- 2013/2014 -----				
Crop season	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>

Off-season	-	-	-	-
----- 2014/2015 -----				
Crop season	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>	<i>Zea mays</i>
Off-season	Fallow	<i>U. brizantha</i>	<i>Avena sativa</i>	<i>X tritico-secale</i>
----- 2015/2016 -----				
Crop season	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>
Off-season	Fallow	<i>U. brizantha</i>	<i>Pennisetum glaucum</i>	<i>Brassica napus</i>
----- 2016/2017 -----				
Crop season	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>	<i>Oriza sativa</i>
Off-season	Fallow	<i>U. brizantha</i>	<i>Crotalaria juncea</i>	<i>Triticum aestivum</i>
----- 2017/2018 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>
----- 2018/2019 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>
----- 2019/2020 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>
----- 2020/2021 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>
----- 2021/2022 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>

First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>
----- 2022/2023 -----				
First crop	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>	<i>Glycine max</i>
Second crop	<i>Zea mays</i>	<i>Zea mays</i> <i>U. ruziziensis</i>	<i>Zea mays</i> <i>C. spectabilis</i>	<i>Zea mays</i> <i>C. spectabilis</i> + <i>U. ruziziensis</i>
Off-season	Fallow	<i>U. ruziziensis</i>	<i>C. spectabilis</i>	<i>C. spectabilis</i> + <i>U. ruziziensis</i>

Source: Author, 2024.

Table 2 – Chemical, physical and granulometric properties of the soil in October 2021

Soil attribute	Soil depth (m)						
	0-0.05	0.05-0.1	0.1-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1
pH ⁵	6,3	5,7	4,7	4,2	4,1	4,0	4,0
Al ¹	0,0	0,0	6,2	10,5	15,9	21,1	20,3
Ca ^{1, 6}	193,6	64,2	24,0	14,3	9,4	8,4	8,2
Mg ^{1, 6}	42,1	18,5	7,1	4,6	4,0	4,6	3,8
K ^{1, 6}	2,2	1,0	0,5	0,5	0,4	0,3	0,2
P ^{2, 6}	106,5	51,0	29,9	6,3	4,3	4,3	4,3
Sand ³	415,0	456,0	478,0	378,0	330,0	403,0	402,0
Silt ³	119,0	105,0	72,0	81,0	96,0	84,0	83,0
Clay ³	466,0	439,0	450,0	541,0	574,0	513,0	515,0
Bulk density ^{4, 8}	1,3	1,4	1,4	1,3	1,3	1,3	1,2

Source: Author, 2024.

¹mmolc dm⁻³, ²mg dm⁻³, ³g kg⁻¹, ⁴Mg m⁻³, ⁵CaCl₂ 0.01 mmol L⁻¹ (Quaggio; Raij, 2001), ⁶Extraction of P and Ca²⁺, Mg²⁺, K⁺ and Al³⁺ was carried out with cation and anion exchange resin (Van Raij *et al.*, 2001), ⁷determination of clay, silt and sand was by pipet method (Gee; Bauder, 1986), and ⁸determination of bulk density was carried out by volumetric ring.

3.2 Crop sowing and establishment

In the first growing season, 2021/2022, soybean (NEO 580 IPRO) was sown on October 14, 2021, and harvested on March 7, 2022 (Figure 3). On March 8, 2022, maize (P3707VYH) was sown and subsequently harvested on July 21, 2022 (Figure 4). For soybean, a seeding rate of 13 plants m⁻¹ was used, and during the crop cycle, the fertilization rates were 20 kg ha⁻¹ of N, 65 kg ha⁻¹ of P₂O₅, and 50 kg ha⁻¹ of K₂O.

For maize, the sowing rate was 3 plants m^{-1} , with fertilization of 112 kg ha^{-1} of N, 78 kg ha^{-1} of P_2O_5 , and 45 kg ha^{-1} of K_2O . Concurrently with maize sowing, ruzigrass was sowed at a rate of 4.5 kg of seeds ha^{-1} , and sunnhemp at 18 kg of seeds ha^{-1} . The CC were sown simultaneously with maize, with the CC seeds being mixed along with the fertilizer. In the following growing season, soybean (NEO 580 IPRO) was sown on October 9, 2022, and harvested on March 18, 2023. Maize (P3707VYH) was sown on March 19, 2023, and harvested on August 18, 2023. For soybean, a planting rate of 12 plants m^{-1} was utilized, and during the crop cycle, the fertilization rates were 20 kg ha^{-1} of N, 65 kg ha^{-1} of P_2O_5 , and 50 kg ha^{-1} of K_2O . For maize, the planting rate was 3 plants m^{-1} , with: 120 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5 , and 100 kg ha^{-1} of K_2O . All crops were sown with a spacing of 0.5 m between rows. The sowing of CC for the 2022/2023 season was the same as that used in the 2021/2022 season. Phytosanitary treatments were carried out following the crops requirements.

Figure 3 - Soybean sown in no-tillage in the experiment in the 2021/2022 and 2022/2023 seasons



Figure 4 - Maize sown in no-tillage in the experiment in the 2021/2022 and 2022/2023 seasons



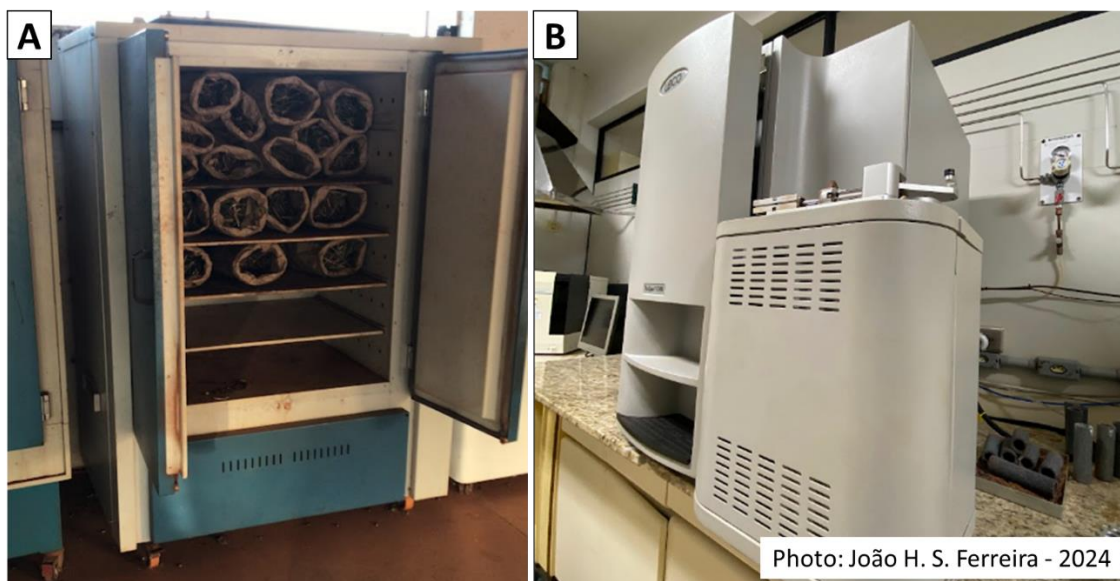
3.3 Evaluations performed during the experiment

3.3.1 C and N input by plants

Dry matter (DM) samplings from the aboveground and belowground of grain-producing crops (soybean and maize) and CC were conducted during both the seasons. Sampling occurred at the maximum DM accumulation stage in all crops, specifically at R4 in soybean (Araújo *et al.*, 2021), R1 in maize (Martins *et al.*, 2017), excluding the grains, and post-harvest during pre-desiccation for CC. During the period of maize crop collections, CC were also collected, albeit at slightly different moments.

The aboveground sampling was conducted over a meter in each plot. Belowground sampling was performed using a probe-type auger, during the same period as the aboveground sampling, down to a soil depth of 1 m. Both above and belowground samplings were carried out at three points per plot. Aboveground samples were placed in paper bags and dried in a forced circulation oven at 65 °C for 72 hours. Belowground samples were washed with water to remove soil, and the roots were placed in paper bags, following the same procedure as for aboveground samples. Subsequently, the DM mass of the samples was determined. These samples were ground, and sub-samples were subjected to the automatic elemental analyzer LECO-TruSpec® CHNS (Figure 5) for the determination of total C and N contents, consequently allowing for the determination of cumulative C and N.

Figure 5 - Drying of aboveground samples in a muffle furnace (A) and LECO-TruSpec® CHNS (B)



3.3.2 Soil CO₂-C flux

The measurement of soil CO₂-C flux was conducted using a portable analyzer, the Infrared Gas Analyzer (IRGA), specifically the LI-COR model LI-8100A. This equipment employs absorption spectroscopy in the infrared electromagnetic spectrum to monitor CO₂-C concentration. The analyzer's configuration involved a 120-second reading cycle, with 15 seconds allocated for pre-purification, 15 seconds for post-purification, and 90 seconds for the actual reading. The resulting CO₂-C flux data were reported in $\mu\text{mol m}^{-2} \text{s}^{-1}$ and subsequently converted to $\text{g m}^{-2} \text{h}^{-1}$. The equipment is equipped with a camera, which was affixed to PVC cylinders during assessments. These cylinders, matching the camera's diameter (20 cm) with height of 15 cm, were installed in the experiment and secured at a depth of 3 cm in the soil, remaining 12 cm above the ground (Figure 6). Two PVC cylinders were positioned per plot during the sowing of soybeans and maize, remaining in area until the harvest of both crops. Simultaneously with the determination of CO₂-C flux, readings of soil temperature and soil moisture were conducted using the Time Domain Reflectometry (TDR) 5TM® equipment from Decagon Devices. These measurements were obtained at two distinct points within each sample unit-specifically, one within the crop rows and another in the inter-row spaces.

Figure 6 - Measurements of CO₂-C emission fluxes in soybean and maize



The assessments of CO₂-C flux were conducted at 1, 3, 5, 8, 12, 17, 23, 30, 45, 60, 90, and 120 days after the sowing (DAS) of soybean and maize in both seasons. The assessments of CO₂-C emission were conducted between 8 and 10 a.m. This evaluation schedule aligns with the methodology proposed by La Scala Jr et al. (2009) and Teixeira et al. (2010). The cumulative C-CO₂ emissions were estimated using the area under the emission curves method throughout the evaluation period. The trapezoidal method was employed for this calculation, utilizing the "pracma" and "tidyverse" packages in RStudio. The cumulative emission data were then converted into Mg ha⁻¹ of C-CO₂.

3.3.3 Soil N and C stocks

Evaluations of the total-N and C contents in the soil were carried out before sowing of soybean crop, in October of 2021, and October of 2022. Soil samples were taken at depths of 0-0.5, 0.5-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40, 0.40-0.60, 0.60-0.80, 0.80-1.0 m at three points per plot, forming a composite sample. Subsequently,

the samples were air-dried, crushed, sieved through a 2 mm mesh sieve and ground in a ball mill for determination of total-N and C by LECO-TruSpec® CHNS. The N and C stocks (CE) in the soil profile were calculated as a function of the total-N and C content in each layer and the soil density determined by the volumetric ring method. Utilizing the annual data on emitted C-CO₂, C input through plant, and CE, it was possible to compute the soil C protection index (SCPI), expressed in kg C-CO₂ per kg C (plant + soil) (Costa *et al.*, 2008). Subsequently, based on the data regarding the input of C from plant and the differences in CE, the rates of incorporation of C from residues into soil organic C (SOC) were calculated (Bayer *et al.*, 2006).

3.3.4 Grain yield and relative CO₂ emission

The determination of grain yield (GY) in crop-producing plants (soybean and maize) involved collecting plants (soybean) and ears (maize) from three functional rows within the plot during both seasons studied. Subsequently, threshing was performed, and yield was quantified in Mg ha⁻¹, corrected to 13% moisture. Based on the results pertaining to cumulative C-CO₂ emissions and crop yield, the quantity of C-CO₂ emitted due to grain productivity was determined, expressed in kg C-CO₂ per kg of grains.

3.3.5 C balance

Building on the results of C-CO₂ emissions during the crop cycles and the values of C input from plants (both above and belowground), the C balance was calculated using the equation:

$$C\ balance = C\ input - C\ output$$

Where: C balance represents the C balance in Mg ha⁻¹; C input denotes C inputs (Mg ha⁻¹) via plants of soybean, maize, ruzigrass, and sunnhemp; and C output refers to C-CO₂ losses (Mg ha⁻¹) from the soil to the atmosphere.

3.4 Data analysis

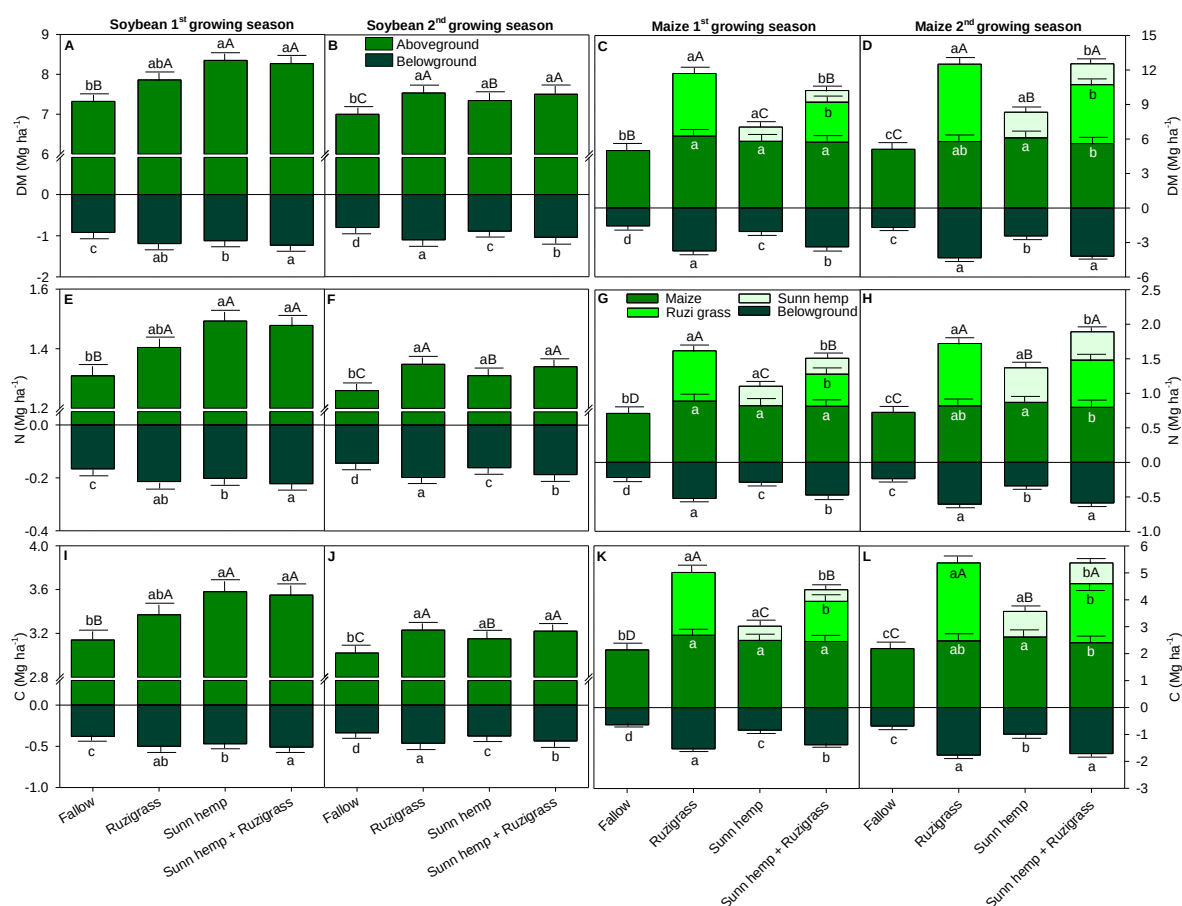
The data underwent normality testing using the Shapiro-Wilk test and homoscedasticity testing using the Levene test. Subsequently, analysis of variance was conducted ($p \leq 0.05$). In cases where significant differences were detected, means were compared using the LSD test. The comparison of C:N ratios was carried out among various species, without extending this analysis to different treatments.

4 RESULTS

4.1 Dry matter, N, and C inputs during the soybean and maize seasons

Different crop production systems resulted significant differences ($p \leq 0.05$) in DM, N, and C soybean inputs (Figure 7). Intercropped systems (RG, SH, and SH+RG) increased DM production compared to F, which was also observed for N and C inputs.

Figure 7 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass, respectively in each graph) on above and belowground inputs of dry matter (DM, A, B, C and D), N (E, H, G and H C), and C (I, J, K and L) of soybean and maize, 2021/2022 and 2022/2023 growing seasons



Different lowercase letters indicate significant differences among plants of the same species across treatments ($p \leq 0.05$, by LSD test), and different uppercase letters indicate differences in the overall total among treatments ($p \leq 0.05$, by LSD test).

Error bars represent the standard error of the mean.

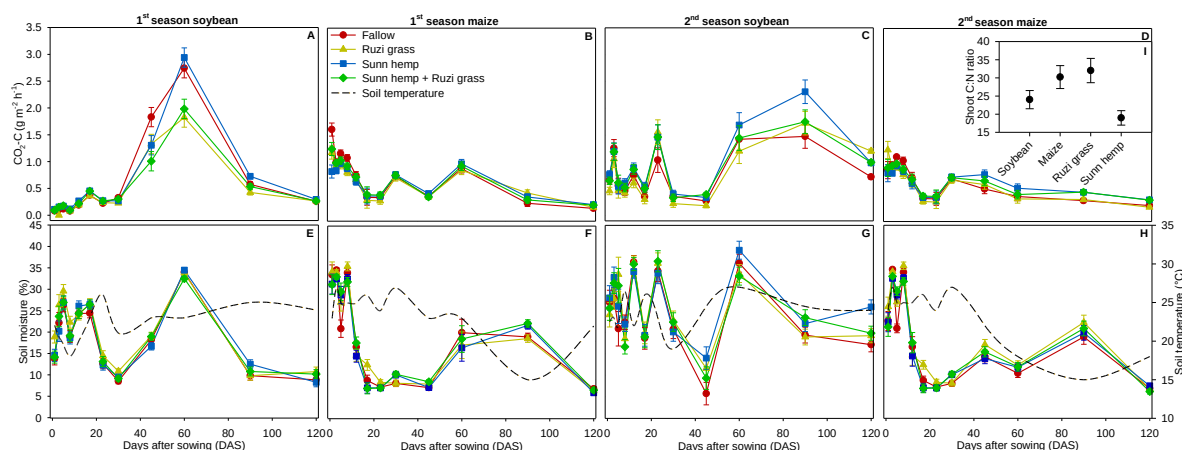
In both studied growing seasons, intercropped systems achieved higher above and belowground DM compared to F. It is noteworthy that the RG had higher inputs for both above and belowground, as well as SH+RG, emphasizing the importance of *U. ruziziensis* intercropping for crop residue production of soybean production systems. The average C input in intercropped systems (above and belowground) was 3.39 Mg ha⁻¹, whereas F obtained 3.08 Mg ha⁻¹ (Figure 7 E and D), representing approximately a 9.2% difference.

In maize cropping, the difference ($p \leq 0.05$) between crop production systems becomes more evident (Figure 7). Systems that included ruzigrass as a CC (RG and SH+RG) exhibited higher DM, N, and C inputs. In both growing seasons, RG and SH+RG resulted greater belowground C (Figure 7 E and F), 1.6 Mg ha⁻¹ in both systems, higher than F and SH systems, which was 0.79 Mg ha⁻¹. The total C input in RG and SH+RG averaged 6.6 Mg ha⁻¹, exceeding F by 2.3 times.

4.2 CO₂-C fluxes

Crop systems influenced ($p \leq 0.05$) both CO₂-C fluxes and soil moisture, but not the soil temperature (Figure 8). Soybean cropping (Figure 8 A and C) achieved higher peaks in CO₂-C fluxes than maize (Figure 8 B and D). In soybean cultivation during 2021/2022, the maximum flux was recorded at 60 DAS, averaging 2.36 g m⁻² h⁻¹, while in soybean cultivation during 2022/2023, it was 1.7 g m⁻² h⁻¹. For maize, the maximum emission values were 1.21 and 0.88 g m⁻² h⁻¹, respectively, for the 2021/2022 and 2022/2023 seasons.

Figure 8 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass) on CO₂-C fluxes (A, B, C and D), soil moisture, temperature (E, F, G and H) and shoot C:N ratio (I) of all species used in the study during the 2021/2022 and 2022/2023 seasons



Error bars represent the standard error of the mean.

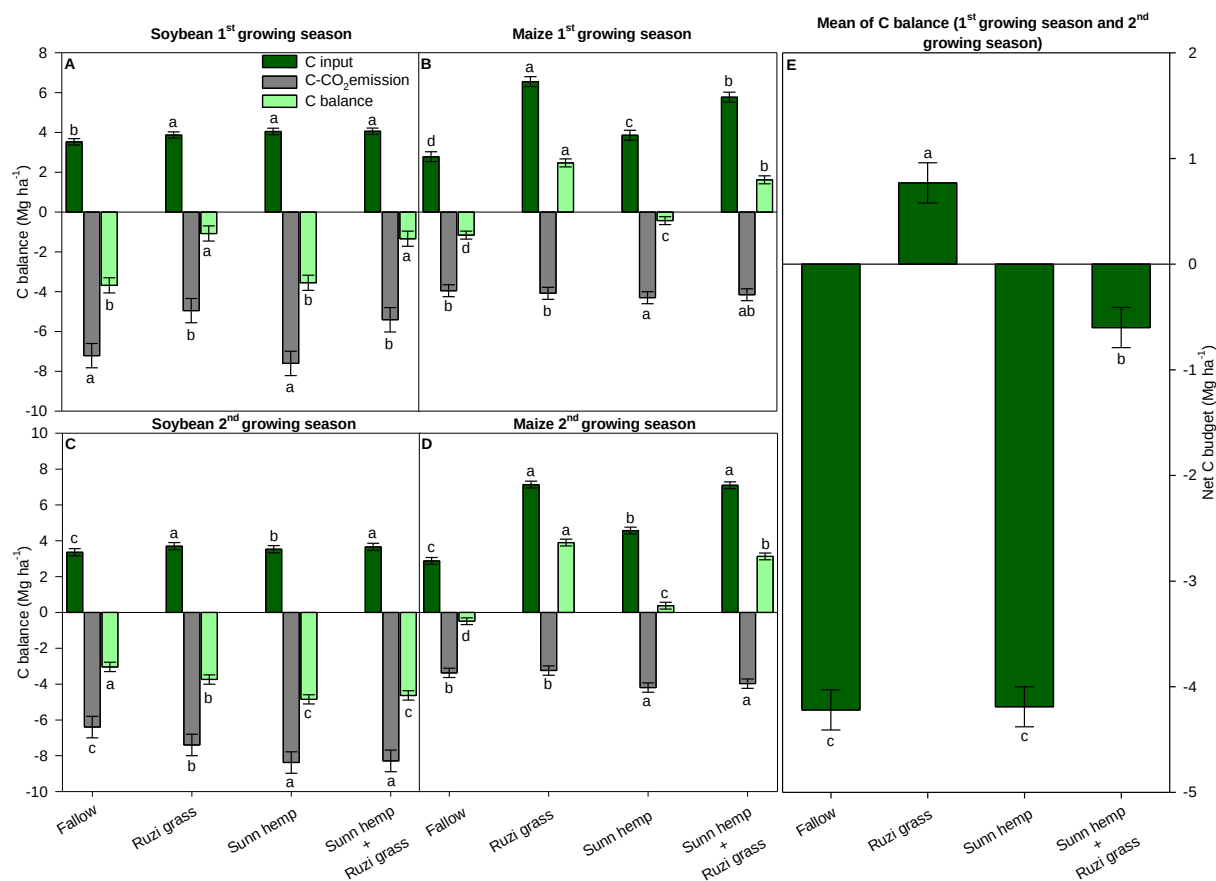
It was observed that the highest values of CO₂-C flux occurred when soil moisture and temperature also increased. This can be confirmed by examining the soil moisture and temperature observations in soybean and maize during 2021/2022, as well as in maize during 2022/2023 (Figure 8 A and E). In these instances, higher values of soil moisture and temperature were followed by elevated CO₂-C flux. Similarly, in the case of maize (Figure 8 B, F, D, and H), it was noted that at 90 DAE, there were declines in both temperature and CO₂-C flux, despite the soil moisture remaining around 20.4 g m⁻² h⁻¹ in both growing seasons. It has been observed that Fabaceae species, specifically soybean and sunn hemp, i.e., species with a lower shoot C:N ratio (Figure 8 I), achieved the highest peaks in CO₂-C flux in both soybean crops (Figure 8 A and C).

4.3 Cumulative C-CO₂ emission and C balance

Cumulative C emissions and C balance differed among the treatments ($p \leq 0.05$), with the highest values of cumulative C emissions observed in soybean crops (Figure 9 A and C). Specifically, in the first and second soybean seasons, the cumulative C-CO₂ emission averages among the treatments were 6.31 and 7.85 Mg

ha⁻¹, while in maize, they were 4.12 and 3.67 Mg ha⁻¹, respectively. Associated with higher emissions in soybean and lower C input by the soybean crop (Figure 9 A and C), the C balances in soybean were all negative, while in maize (Figure 9 B and D), the opposite was observed. In maize, there was higher C input and lower C emission, therefore, treatments such as RG and SH+RG achieved positive C balances. The positive C balance in maize averaged 3.19 and 2.38 Mg ha⁻¹ in RG and SH+RG, respectively. The mean of C balances (Figure 9 E) was positive only for RG, and less positive for SH+RG.

Figure 9 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass) on the C balance (composed of C input [total of above and belowground], cumulative C-CO₂ emission, and C balance) in soybean and maize crops during the 2021/2022 and 2022/2023 seasons, as well as the overall average considering both soybeans and maize

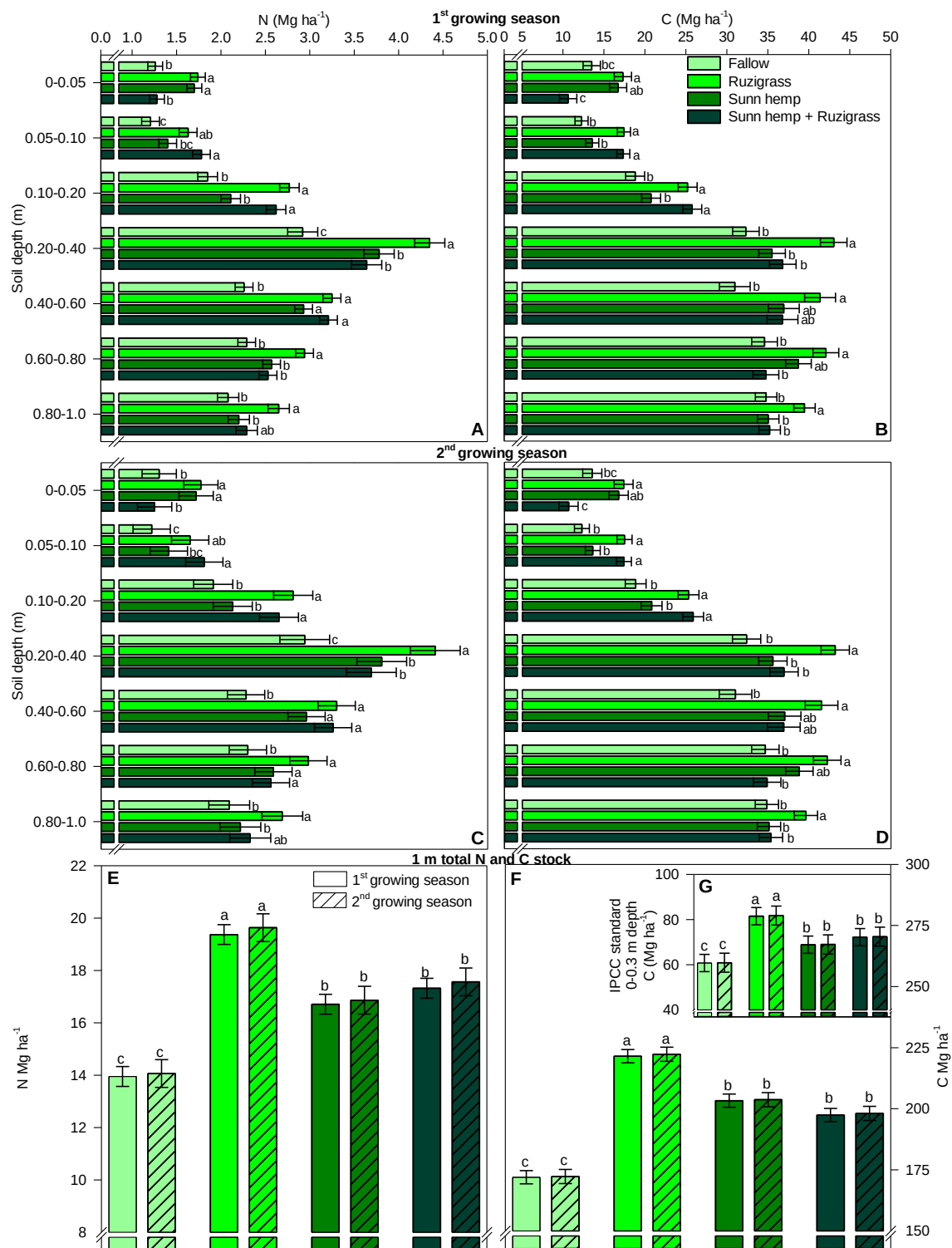


Different letters indicate significant differences among treatments ($p \leq 0.05$, by LSD test). Error bars represent the standard error of the mean.

4.4 Soil N and C stocks

An increase ($p \leq 0.05$) in both N and C stocks were observed in the intercropped systems (Figure 10). The highest values of N and C stocks were observed in the RG system throughout the profile (Figure 10 A, B, C, and D) and in total (Figure 10 E and F). In the 1 m depth stock, RG had an average of 221.9 Mg ha^{-1} of C, while F obtained an average of $172.1 \text{ Mg C ha}^{-1}$, representing a difference of $\sim 50 \text{ Mg ha}^{-1}$ of C.

Figure 10 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass) on soil N stock (A and C), C stock (B and D), total N (E), C (F) stocks and IPCC standard C stock* (IPCC, 2006) in October 2021 and 2022

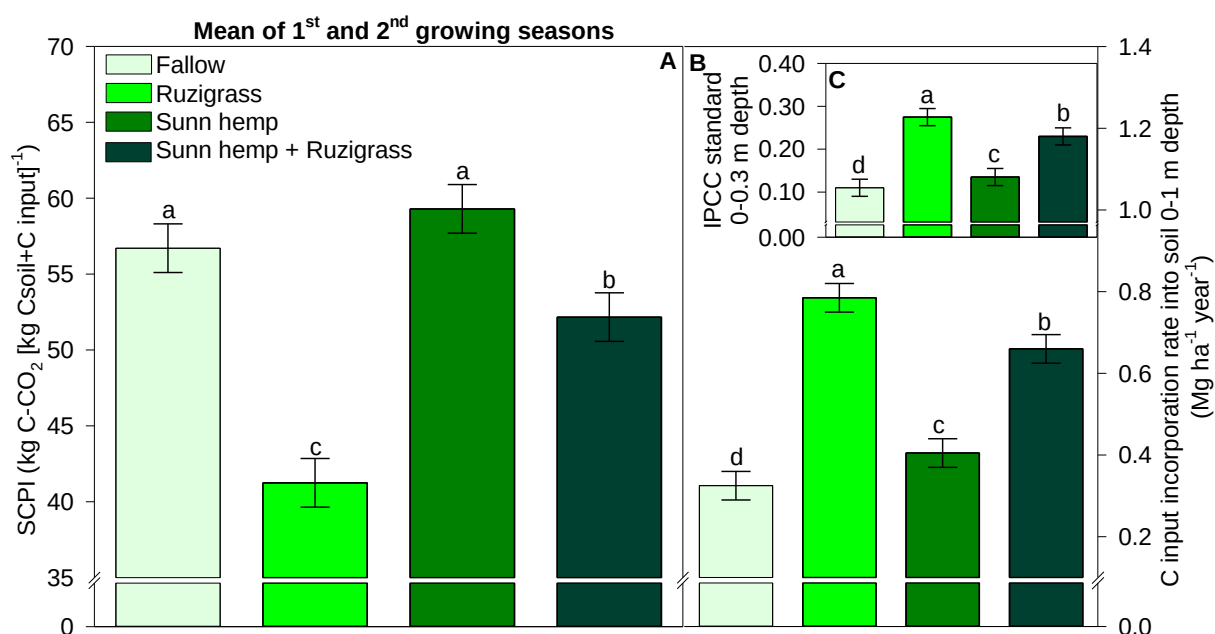


Different letters indicate significant differences among treatments ($p \leq 0.05$, by LSD test). Error bars represent the standard error of the mean.

4.5 Soil C protection index and C input incorporation rate into soil C

Crop systems that achieved higher ($p \leq 0.05$) soil C protection index (SCPI) and a greater incorporation of C into the soil were RG and SH+RG (Figure 11 A and B). It is worth noting that the inclusion of ruzigrass (RG and SH+RG) in the systems provided 0.79 and 0.66, respectively, compared to 0.33 and 0.41 $\text{Mg ha}^{-1} \text{ year}^{-1}$ for F and SH, respectively (Figure 11 B).

Figure 11 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass) on soil C protection index (SCPI, A) and incorporation of C from residues into SOC into 0-1 m depth and 0-0.3 m depth [IPCC standard* (IPCC, 2006)] (respectively B and C)

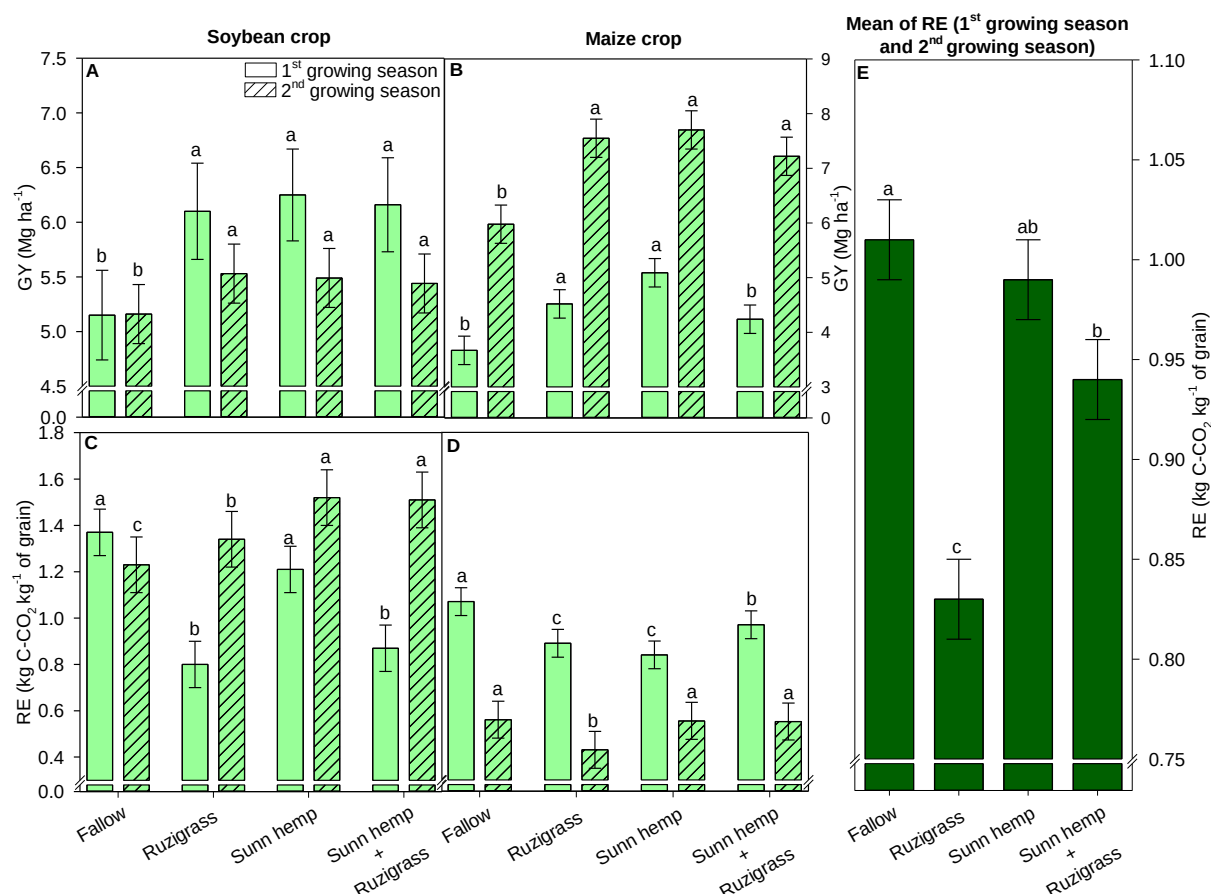


Different letters indicate significant differences among treatments ($p \leq 0.05$, by LSD test). Error bars represent the standard error of the mean.

4.5 Grain yield and relative emission

Intercropping increased GY ($p \leq 0.05$) in both soybean (Figure 12 A) and maize (Figure 12 B) seasons. For soybean, intercropped systems had an average GY of 5.82 Mg ha^{-1} , while F had 5.15 Mg ha^{-1} . The average relative emission (Figure 12 E) was lower in treatments RG and SH+RG, with values of 0.83 and 0.94, respectively, compared to 1.01 and 0.99 $\text{kg C-CO}_2 \text{ kg grain}^{-1}$, respectively for F and SH.

Figure 12 - Effects of different production systems (Fallow, Ruzigrass, Sunn hemp, and Sunn hemp+ruzigrass) on grain yield (GY) and relative C- CO_2 emissions (RE) in 2021/2022, 2022/2023 and mean of both seasons



Different letters indicate significant differences among treatments ($p \leq 0.05$, by LSD test). Error bars represent the standard error of the mean.

5 DISCUSSIONS

5.1 Dry matter, N, and C inputs during the soybean and maize seasons

In every crop season of soybean and maize (Figure 7), higher values ($p \leq 0.05$) of DM, N, and C were found in intercropped systems, especially in maize cultivation, in RG and SH+RG treatments. In no-tillage crop production systems, the correct selection of species is essential. Considering tropical conditions, it is crucial to keep the soil covered for as long as possible with species exhibiting high DM production to mitigate the adverse effects of climate, such as drought or erosion (Das *et al.*, 2014; Rosolem; Li; Garcia, 2016), and improve soil health (Stewart *et al.*, 2018).

Grasses have the advantage of enhancing the system, given that they mostly possess an extensive and vigorous root system capable of penetrating deep into the soil profile, allowing for improved water and nutrient absorption (Silva *et al.*, 2020). Additionally, these roots contribute to an increase in the number of soil macropores, which are essential for plant development in subsequent production cycles (Calonego; Rosolem, 2010). On the other hand, leguminous species, characterized by a taproot system, engage in biological N fixation, thereby contributing N to the system (Torbert; Reeves; Mulvaney, 1996). This N can be utilized by other plants in succession within the system (Abdalla *et al.*, 2019).

Furthermore, systems incorporating *Urochloa spp.* demonstrate a superior capacity to enhance both the chemical and biological attributes of the soil, such as nutrient cycling. The vigorous root system of these plants enables them to access nutrients in soil layers beyond the reach of grain-producing crops (Garcia *et al.*, 2008). Subsequently, these nutrients are redeposited into the soil following plant senescence (Costa *et al.*, 2020; Pariz *et al.*, 2016; Rosolem *et al.*, 2016).

Grasses exhibit higher biomass production compared to Fabaceae, primarily attributed to the C4 photosynthetic mechanism, allowing for better utilization of resources such as CO₂ and nutrients compared to C3 species (Rosolem; Calonego; Foloni, 2005; Rosolem; Li; Garcia, 2016). Regarding aboveground root development, grasses can transfer approximately 30 to 50% of all assimilated C to the root system (Kuzakov, 2010; Paul, 2016), explaining the increased belowground DM, N and C in systems incorporating ruzigrass.

5.2 CO₂-C fluxes, cumulative C-CO₂ and C balance

The CO₂-C emissions are significantly influenced by meteorological conditions (Hiel *et al.*, 2018). Microbial activity, a major driver of CO₂-C emissions, is enhanced under higher soil temperatures and moisture conditions (Zhang *et al.*, 2018). In this study, rainfall patterns were characteristic of the region, with precipitation concentrated during the spring-summer period and a dry winter (Figure 1). During periods of higher precipitation, there was an associated increase in soil moisture (Figure 8 E, F, G, and H), accompanied by a rise in CO₂-C emissions (Figure 8 A, B, C, and D). The differential behavior ($p \leq 0.05$) among various production systems, however, was primarily influenced by the amount and composition of material input into the system, particularly the C:N ratio, which is an essential component in the dynamics of organic material decomposition (Rigon; Calonego, 2020; Xavier *et al.*, 2019).

Different plant species exhibit distinct compositions, and this disparity influences the decomposability of their material (Xavier *et al.*, 2019). In this study, it was observed that during soybean cycles, the highest peaks of CO₂-C and cumulative C-CO₂ occurred. One possible explanation for this is the composition of the residue input into the system during soybean and maize implantation. During no-till soybean seeding, the remaining residue in the soil is derived from the previous growing season's maize (as well as ruzigrass and sunn hemp in intercropped systems). The minimal variation in maize residue among different systems is attributed to the fact that soybean residue composition remains relatively consistent. Upon observing the C:N ratio of all species (Figure 8 D), there is a variation from 19 to 32 among the C:N ratios of the species. Systems without intercropping exhibit lower soil microbial diversity, a factor essential for the intensification of production systems. This is crucial as soil microbes actively participate in various nutrient cycles such as N, P, S, and C (Novelli; Caviglia; Piñeiro, 2017). The elevated CO₂-C levels observed in Fallow during the 2021/2022 season (Figure 8 A) may have resulted from the fast mineralization of soybean residues in succession with fallow, leading to the loss of C (Novelli; Caviglia; Melchiori, 2011).

The highest peaks of CO₂-C (Figure 8) and cumulative C-CO₂ (Figure 9) occurred in the SH system in both seasons. Sunn hemp is a species belonging to the Fabaceae family and, in this study, presented the lowest C:N ratio among the other species, i.e., 19. The higher CO₂-C levels can be explained by the greater decomposability of this material compared to the other species. Tissues with a lower

C:N ratio are predisposed to decomposition since the higher N content serves as a substrate for microbial activity, leading to increased CO₂-C emissions (Ding *et al.*, 2006; Liu, 1997). The introduction of sunn hemp into the system may have caused greater decomposition of the residue from other materials added to the system, such as maize (and ruzigrass in the SH+RG system), through a priming effect (Shahbaz *et al.*, 2017). In addition to the C:N ratio, factors such as the quantity of material added to the system, particle size, and soil characteristics, including pH, texture, and aeration (Lal *et al.*, 1991), influence decomposition. Despite the higher potential for decomposition of species with lower C:N ratios, such as soybean and sunn hemp, organic N is essential for the system. It increases the N content, allowing a reduction in the application of N fertilizers (Abdalla *et al.*, 2019; LIU, 1997; Torbert; Reeves; Mulvaney, 1996). Moreover, N is a crucial substrate for the stabilization of SOM (Castro *et al.*, 2015; Rigon; Calonego, 2020; Rigon; Franzluebbbers; Calonego, 2020).

The dissimilarity in CO₂-C emissions between the RG and SH systems may have been influenced by the differing composition of ruzigrass residue. In this study, the ruzigrass species exhibited an average C:N ratio of 32, while maize had a ratio of 30.2. In addition to the higher C:N ratio, grasses have a greater capacity for DM production, characterized by a higher content of recalcitrant materials such as acetylated lignin (Del Río *et al.*, 2007; Rigon; Calonego, 2020). A higher lignin content in the material implies a slower decomposition rate (Tian; Brussaard; Kang, 1995; Valus; Jones, 1973), allowing the soil to remain covered for a more extended period.

The C balance results are a consequence of the amount of C input into the system, where C-CO₂ serves as the C output. A positive balance is therefore derived from a lower input than the C output. Systems incorporating ruzigrass (RG and SH+RG) exhibited, respectively, positive and less negative balances on average across both seasons (Figure 9 E). The association of higher DM production above and belowground is crucial for achieving a positive or closer-to-neutral C balance. This outcome leads to an intensive agriculture approach from an agronomic perspective, environmentally more sound, capable of acting as a C sink rather than a source of emission. On the other hand, F and SH systems showed more negative C balances, a result of low input coupled with elevated C-CO₂ emissions in these systems. It is worth emphasizing that about 50% of all C-CO₂ does not originate from the decomposition of SOM or the mineralization of C inputs. Instead, it arises from the root system's

respiration (Oertel *et al.*, 2016), with different species exhibiting varying degrees of root respiration (Johnson; Barbour; Weyers, 2007; Schlesinger; Andrews, 2000).

5.3 Soil C and N-stocks, soil C protection index and C input incorporation

All intercropped systems exhibited significantly higher ($p \leq 0.05$) N and C stocks in the soil (Figure 10). The influence of no-tillage since the beginning of the experiment allows for a long-term analysis of the effects of tillage and cover crops in the systems. In this context, long-term studies are of paramount importance for evaluating SOM dynamics in tropical conditions. Previous research has demonstrated that experiments with over 10 years of establishment are more suitable for assessing the transformations occurring in SOM (Hu *et al.*, 2023). In shorter time periods, it is not possible to adequately capture the aspects of both quantity and quality of SOM in field experiments.

The increase in N and C stocks is justified by a higher input of DM through roots, which contribute more than aboveground biomass to the formation of stable organic material in the soil (Sokol; Bradford, 2019). Additionally, unlike aboveground biomass, roots are more recalcitrant to decomposition compared to leaves and stems, possessing a higher content of lignin, suberin, polyphenols, and tannins (Lian *et al.*, 2016; Rasse; Rumpel; Dignac, 2005). Consequently, the decomposition of aboveground material is preferential over soil material (Blagodatskaya; Kuzyakov, 2008). In this regard, intercropped systems, especially those including ruzigrass, exhibited higher belowground DM production, thereby facilitating an increase in N and C stocks down to a depth of 1 m. In intensive tropical systems, Tivet *et al.* (2013) concluded that a DM input exceeding 7 Mg ha⁻¹ results in greater C resilience.

The increase in N and C quantities in the soil alone does not necessarily reflect its stabilization, as more labile fractions of SOM are rapidly and easily decomposable in the soil (Blagodatskaya; Kuzyakov, 2008; Hok *et al.*, 2015; Tivet *et al.*, 2013). The C:N ratio of SOM is linked to the degree of its stabilization. In this study, the soil C/N ratios did not change among the treatments, averaging 11.8 between them and across the two seasons. Studies show that the C:N ratio of soil in agroecosystems remains constant, typically around 10 to 12 (Boddey *et al.*, 2003; Rosolem; Li; Garcia, 2016). Thus, the input of DM and C is not the only factor contributing to SOM increase (Hok *et al.*, 2015); however, it remains crucial (Rigon; Calonego, 2020). N is also necessary

for the stabilization of C in the soil (Rosolem; Li; Garcia, 2016). The same authors reported that crops capable of performing biological N fixation, such as soybean and sunn hemp can contribute more than 200 kg ha⁻¹ of N to the system. Therefore, even in the RG system, the input of N by the vigorous root system of ruzigrass and biological N fixation may have contributed to the higher values of C and N stock. The increase in C and N inputs becomes even more important in tropical regions, particularly in no-tillage systems, where DM is decomposed gradually (White; Rice, 2009).

In tropical conditions, the increase in inputs of C and N, specifically through plant residues in various stages of decomposition, enhances SOM protection. Intercropped systems facilitate higher physical SOM protection, where SOM is occluded in aggregates (Bayer *et al.*, 2006), and biochemical protection, as different components from various species form differentially stabilized DM (Bayer *et al.*, 2006; Cotrufo *et al.*, 2019). Grass CC boost C stocks due to their high DM production and drought tolerance, suppressing non-grass species development (Bretas *et al.*, 2021; Cheruiyot *et al.*, 2018). Additionally, Bayer *et al.* (2006) and Meirelles; Henriques (1992) reported higher soil C stock and sequestration in no-tillage compared to native Cerrado vegetation, attributed to the elevated C input from crops versus native Cerrado plants.

The SCPI exhibited superior performance in ruzigrass systems (Figure 11 A). Additionally, C incorporation rates (Figure 11 B and C) were more favorable in ruzigrass systems (RG and SH+RG). These findings demonstrate enhanced C protection within the soil associated with ruzigrass. This implies that the introduction of ruzigrass into the system results in a greater quantity of C retained in the area (Mielniczuk *et al.*, 2003) compared to F and with sunn hemp alone (SH). The higher C incorporation rate in the soil, considering the IPCC standard (Figure 11 C), was 0.27 Mg ha⁻¹ year⁻¹ (RG system). This result is lower than the C incorporation observed in temperate climates in USA, which can reach up to 0.40 Mg ha⁻¹ (Lal *et al.*, 1999; West; Marland, 2002), or in Ireland, where an increase of 0.8 Mg ha⁻¹ year⁻¹ in soil C was observed (Van Groenigen *et al.*, 2011). However, when considering the soil depth of 0-1m, an incorporation rate of 0.79 Mg ha⁻¹ year⁻¹ was found for RG. These outcomes underscore the efficacy of ruzigrass systems in not only maintaining SCPI but also in achieving notable C incorporation rates, particularly when compared to alternative intercropping systems and sole cultivation of sunn hemp.

Accounting for C stock up to 1 m depth in tropical soils allows for a better understanding of C dynamics in agroecosystems. Tropical soils are inherently deep

(Eiten, 1978), and in no-tillage systems with intercropped grasses, such as in this experiment, root development extends to a depth of 1 m. Therefore, differences in both stock and incorporation rate (Figure 10 F and G; and Figure 11 B and C) can be observed with sampling up to 1 m depth, in contrast to the IPCC standard (IPCC, 2006), as conducted in various studies (Powlson *et al.*, 2012; Rawlins *et al.*, 2011; Van Groenigen *et al.*, 2011; West; Marland, 2002). This potential limitation indicates that the IPCC standard may not be adequate for deep tropical soils, potentially leading to an underestimation of the actual C sequestration potential. Addressing this issue entails standardizing the sampling depth for tropical soils to allow deeper sampling, thereby capturing C stored at depths greater than 0.3 m.

5.4 Grain yield and relative emission

Higher GY in intercropped systems results from various benefits of CC in crop systems, especially in tropical conditions. These benefits include an increase in the number of soil macropores (Calonego; Rosolem, 2010), biological N fixation in the case of legumes (Torbert; Reeves; Mulvaney, 1996), nutrient cycling (Costa *et al.*, 2020; Pariz *et al.*, 2016; Rosolem; Li; Garcia, 2016), weed suppression (Teasdale; Beste; Potts, 1991), control of plant diseases (Acharya *et al.*, 2017; Larkin; Griffin; Honeycutt, 2010), and a high production of crop residues for soil coverage (Rosolem; Calonego; Foloni, 2005; Rosolem; Li; Garcia, 2016). With the elevation in GY, even if the system emits more C-CO₂, there will be a lower environmental cost, requiring a lesser amount of C-CO₂ per unit of grains produced. This observation was made in the present study.

Intercropped systems emerge as a compelling approach, showcasing superior performance in terms of C balance, C stock, RE compared to no-intercropping (F). This assertion gains prominence, particularly in tropical agricultural settings, as evidenced by the present study. The strategic incorporation of ruzigrass as a CC within intercropping arrangements, in no-tillage, has been demonstrated to yield noteworthy benefits from both agronomic and environmental standpoints.

Scientific findings consistently affirm that the inclusion of ruzigrass significantly enhances crucial soil parameters. The observed augmentation in soil C content underscores the positive impact of ruzigrass as a CC, promoting C sequestration and contributing to the mitigation of greenhouse gas emissions. These results align with

established literature on the role of CC in bolstering SOC levels and fostering sustainable soil management practices.

Furthermore, the comprehensive evaluation of C balance and stock reveals a commendable synergy within intercropped systems incorporating ruzigrass. The intricate interplay between diverse plant species, particularly the integration of ruzigrass, not only elevates C retention but also fosters a dynamic balance that promotes ecosystem resilience. This is substantiated by rigorous analysis spanning multiple growing seasons, affirming the robustness and consistency of these observed trends. From an agronomic perspective, the positive impact extends to GY, as ruzigrass contributes to improved resource utilization and nutrient cycling. Scientific literature, including studies referenced in this work, attests to the role of CC, especially grass species like ruzigrass, in augmenting nutrient availability and fostering a conducive soil environment for optimal crop growth.

6 CONCLUSIONS

This study highlights the significant impact of intercropping, particularly with ruzigrass, on enduring changes in no-till crop production systems. Key findings underscore the crucial role of intercropping in enhancing C stock, grain yield, and mitigating C-CO₂ emissions. Introducing ruzigrass resulted in substantial increases in dry matter, N, and C inputs, above and belowground, extending to a soil depth of 1 m. Ruzigrass played a pivotal role in achieving a positive C balance, increasing soil C stock, and reducing relative emissions. Notably, isolated sunn hemp intercropping proved less effective for C balance and soil stock compared to systems with ruzigrass. These insights are relevant for soil management practices, particularly in mitigating C-CO₂ emissions in tropical agriculture. The positive influence of intercropping extends across the entire system, promoting enhanced plant development and improved yield. In essence, this study underscores the crucial role of ruzigrass intercropping in shaping sustainable no-till crop production systems under challenging agroecological conditions.

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