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SOIL FERTILITY, MICROBIAL ACTIVITY, AND CARBON STOCKS IN A CULTIVATION CHRONOSEQUENCE IN THE BRAZILIAN CERRADO

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Thesis submitted to the Graduate Program in Agricultural Sciences - PPGCA, Campus Professora Cinobelina Elvas - CPCE, Federal University of Piauí - UFPI, in the field of Soil Science, as a requirement for obtaining the degree of Doctor in Agricultural Sciences.

Advisor: Prof. Dr. Ronny Sobreira Barbosa
Co-advisor: Prof. Dr. Yuri Jacques Agra Bezerra da Silva

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ABSTRACT

The increasing demand for food has driven the conversion of native areas into agricultural land, a process that has significantly impacted the Cerrado (Brazilian savanna) in recent years, with important implications for soil carbon (C) balance. In Cerrado soils, typically acidic, nutrient-poor, and predominantly sandy, the development of cropping systems that promote C accumulation is crucial for agricultural sustainability. In this context, this study evaluated a cultivation chronosequence (native vegetation vs. 12, 18, and 24 years of cultivation) to identify temporal changes in soil fertility, microbial activity, and C stocks within the 0.0-50 cm profile of an Oxisol (Latossolo Amarelo Distrófico) under the Cerrado biome in the state of Piauí, Brazil. The following variables were analyzed: soil organic carbon (SOC), microbial biomass carbon (MBC), basal soil respiration (C-CO_2), microbial quotient (*q*Mic), metabolic quotient (*q*CO_2), soil acidity (pH), and the stocks of C, Ca, Mg, K, P, S, Fe, Mn, Zn, B, and Cu, as well as CO_2 sequestration across five soil depths (0-10, 10-20, 20-30, 30-40, and 40-50 cm). No significant differences were observed among areas for MBC, *q*Mic, and *q*CO_2. However, SOC, C-CO_2, and C stocks increased with cultivation time. Improvements relative to the native area were observed down to 50 cm depth for base sum, base saturation, and the stocks of C, Ca, and S. In contrast, increases in cation exchange capacity (CEC) and Mg and K stocks were limited to the upper 30 cm. Reductions in exchangeable Al and changes in soil pH extended to 20 cm, whereas increases in P and micronutrient stocks (Cu, Mn, Zn, and B) were restricted to the surface layer (0-10 cm). CO_2 sequestration rates were 0.96, -0.06, and 1.69 Mg ha^{-1} yr^{-1} for the areas cultivated for 12, 18, and 24 years, respectively. Overall, cultivation time enhanced microbial activity (resulting in CO_2 sequestration), improved chemical fertility, and increased C and nutrient stocks down to 50 cm depth. On average across the soil profile (0-50 cm), after 24 years of cultivation, base saturation increased by 429% and base sum by 660%, highlighting the importance of locally adapted soil management systems. Our findings indicate that regional environmental and climatic conditions directly influence the effectiveness of agricultural practices and their outcomes.

Keywords: Nutrient leaching, Carbon sequestration, Sandy soils, Cultivation time, Native vegetation.

RESUMO

A crescente demanda por alimentos tem impulsionado a conversão de áreas nativas em terras agricultáveis, processo que tem impactado significativamente o bioma Cerrado (Savana brasileira) nos últimos anos, trazendo implicações importantes para o equilíbrio de carbono (C) no solo. Em solos de Cerrado, tipicamente ácidos, pobres em nutrientes e normalmente arenosos, o desenvolvimento de sistemas de cultivo que promovam o acúmulo de C é crucial para a sustentabilidade agrícola. Neste sentido, este estudo avaliou uma cronossequência de cultivo (vegetação nativa vs. 12, 18 e 24 anos de cultivo) para identificar mudanças temporais na fertilidade, atividade microbiana e estoques de C na profundidade de 50 cm de um Latossolo Amarelo Distrófico (Oxisol) sob o bioma Cerrado no estado do Piauí, Brasil. Foram analisados o carbono orgânico total (SOC), carbono da biomassa microbiana (MBC), respiração basal do solo (C-CO_2), quociente microbiano (*q*Mic) e metabólico (*q*CO_2), acidez do solo (pH), estoques de C, Ca, Mg, K, P, S, C, Fe, Mn, Zn, B e Cu, além do sequestro de CO_2 em cinco profundidades (0,0-10; 10-20; 20-30; 30-40 e 40-50 cm). Não foram observadas diferenças significativas entre as áreas para MBC, *q*Mic e *q*CO_2, contudo houve incremento nos teores de SOC, C-CO_2 e nos estoques de C com o decorrer do tempo de cultivo. Ocorreram melhoras em relação a área nativa até 50 cm de profundidade na soma de bases, saturação por bases e estoques de C, Ca e S. Para a capacidade de troca catiônica e estoques de Mg e K, a melhora restringiu-se aos 30 cm de profundidade. A redução de Al e as modificações no pH chegaram aos 20 cm, enquanto o aumento nos estoques de P e micronutrientes (Cu, Mn, Zn e B) ficaram limitados à camada superficial (0,0-10 cm). As taxas de sequestro de CO_2 foram de 0,96, -0,06 e 1,69 Mg ha^{-1} ano^{-1} para as áreas cultivadas a 12, 18 e 24 anos, respectivamente. De maneira geral, o tempo de cultivo incrementou a atividade microbiana (proporcionando sequestro de CO_2), a fertilidade química e os estoques de C e nutrientes até 50 cm de profundidade. Na média do perfil (0,0-50 cm), após 24 anos de cultivo, a saturação por bases aumentou 429% e a soma de bases 660%, indicando a importância dos estudos de sistemas de manejo locais. Nossos resultados indicam que características ambientais e climáticas regionais influenciam diretamente a eficácia das práticas agrícolas implementadas e também seus resultados.

Palavras-chave: Lixiviação de nutrientes, Sequestro de carbono, Solos arenosos, Tempo de cultivo, Vegetação nativa.

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1 GENERAL INTRODUCTION

The increasing global demand for food, fiber, and energy has intensified the pressure on agricultural systems, requiring continuous productivity gains and the expansion of agricultural frontiers (Liang *et al*., 2023; Searchinger *et al*., 2018; Powlson *et al*., 2016). In this scenario, Brazil has established itself as one of the key players in global agriculture, driven by the incorporation of technologies adapted to tropical conditions and high production efficiency (FAO, 2025; Contini *et al*., 2010). In the 2024/2025 harvest, the country cultivated approximately 87.7 million hectares of grains, reaching a production of around 352 million tons, which reinforces the strategic relevance of the national agricultural sector (CONAB, 2026).

Soybean stands out as the primary crop of Brazilian agribusiness. Between 2000 and 2025, the cultivated area grew by more than 300%, while production increased by over 440% (CONAB, 2026), reflecting both territorial expansion and advances in productivity associated with the use of improved cultivars, mechanization, and modern management practices. This performance has positioned Brazil among the world's largest producers and exporters of soybean (Valdes; Gillespie; Dohlman, 2023), with agribusiness accounting for approximately 30% of the national Gross Domestic Product (GDP) (CEPEA, 2026).

This agricultural expansion in Brazil has occurred mostly over the Cerrado biome (FAO, 2025), especially in the MATOPIBA region, which encompasses areas of the states of Maranhão, Tocantins, Piauí, and Bahia (Lima *et al*., 2019). This region is the country's current agricultural frontier, mainly due to its topography favorable to mechanization, land availability, and production potential, factors that have driven its rapid agricultural occupation (FAO, 2025; Valdes; Gillespie; Dohlman, 2023; Pires *et al*., 2016). The advancement of agriculture in these areas was facilitated by credit policies, infrastructure investments, and the development of technologies adapted to local edaphoclimatic conditions (Contini *et al*., 2010). Consequently, the Cerrado has become the most heavily impacted Brazilian biome by agricultural expansion in recent decades (FAO, 2025; Silva *et al*., 2013; Oliveira *et al*., 2014).

The Cerrado occupies about 204.7 million hectares, corresponding to approximately 24% of the Brazilian territory. It harbors high biodiversity and plays a strategic role in water regulation by concentrating important underground aquifers (Faleiro *et al*., 2008; IBGE, 2004). In the state of Piauí, the biome covers approximately 12 million hectares, representing about 46% of the state's area, which highlights its environmental and agricultural relevance (CONAB, 2026; Leite *et al*., 2010).

Despite its high production potential, Cerrado soils present natural limitations, such as high acidity, low nutrient and organic matter content, and, frequently, a sandy texture (Curi *et al*., 2017; Donagemma *et al*., 2016). Although these soils respond positively to liming and fertilization practices, allowing for high productivity levels (Queiroz, 2009; Maia *et al*., 2022), the conversion of natural areas to agricultural use promotes significant changes in the chemical, physical, and biological soil attributes. These changes are particularly critical in sandy soils, which have lower water and nutrient retention capacity and greater susceptibility to degradation (Vezzani; Mielniczuk, 2009; Ratke *et al*., 2020).

In this context, soil quality is a central element for assessing the sustainability of agricultural systems, integrating physical, chemical, and biological indicators sensitive to management-imposed changes (Doran; Parkin, 1994; Doran; Zeiss, 2000). Indicators such as Carbon stocks, organic matter, and microbial activity are especially relevant in sandy soils, where organic matter plays a fundamental role in structural stability and cation exchange capacity (Yost; Hartemink, 2019; Liang *et al*., 2023).

Although significant progress has been made, gaps remain in the understanding of changes in soil attributes in subsurface layers (Tiefenbacher *et al*., 2021), particularly in agricultural frontier regions. Recent evidence suggests that shifts in Carbon stocks and biological activity can occur in deeper soil layers, depending on the duration of cultivation and the management system adopted (Liang *et al*., 2023; Maia *et al*., 2022; Huang *et al*., 2022). Therefore, this study aimed to evaluate changes in Carbon stocks, biological activity, and nutrient content within the 0.0-50 cm depth of an Oxisol (Latossolo Amarelo Distrófico) in the Cerrado of Piauí across a cultivation chronosequence.

2 EXPERIMENTAL STRATEGY

2.1 Study area location and soil pit excavation

Sampling was conducted at the Chapada do Céu Farm (Figure 1), located in the municipality of Sebastião Leal, Piauí (PI), Brazil (07°40’44” S, 44°04’16” W), at an altitude of 445 meters above sea level. The site is situated within the transition zone between the Cerrado and Caatinga biomes and at the boundary between semi-arid and tropical climates, exhibiting transitional climatic characteristics. Potential evapotranspiration exceeds rainfall for most of the year, with an average temperature of 26.7 °C and a mean annual rainfall of 1,015 mm; in certain years, precipitation levels fall below 800 mm (Climate-Data, 2024).

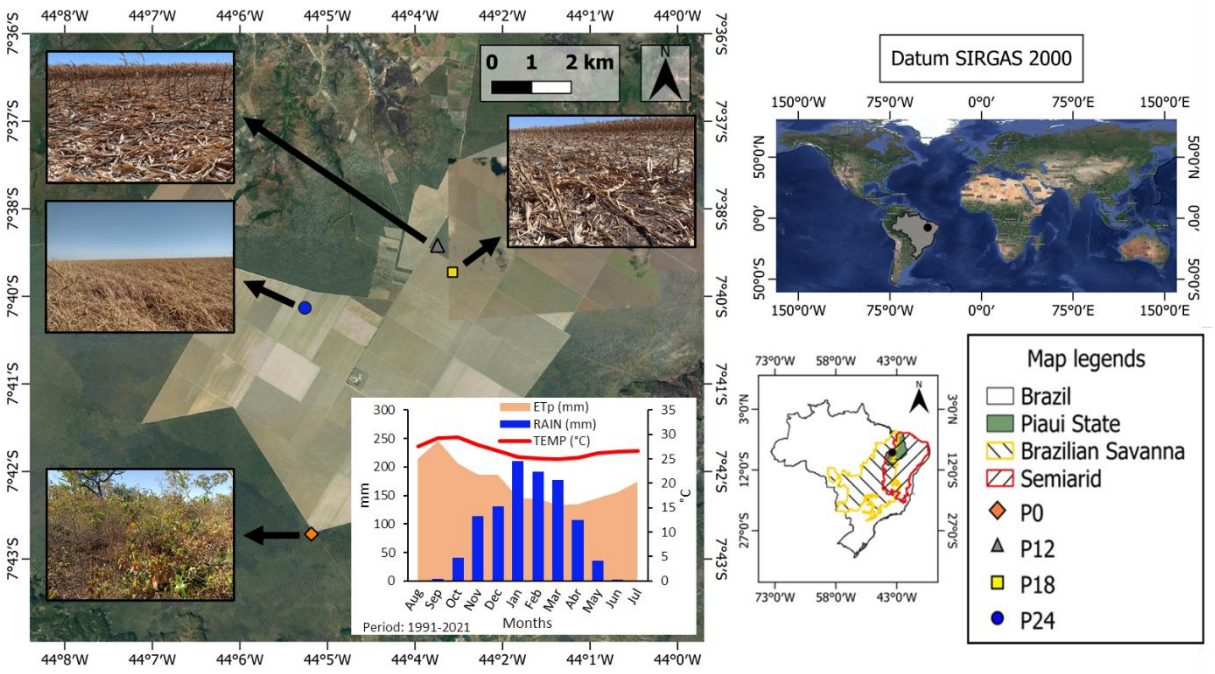


Figure 1 - Location and soil cover conditions of soil samples from native forest (P0) and cultivated areas under 12 (P12), 18 (P18), and 24 (P24) years of agricultural use, and historical averages of rainfall (RAIN), temperature (TEMP), and evapotranspiration (ETp) in the studied region.

The four sites selected for this study are characterized by flat topography and soils classified as Latossolo Amarelo Distrófico típico according to the Brazilian Soil Classification System (Santos *et al.*, 2025), which is equivalent to the Oxisol order in the United States Soil Taxonomy (Soil Survey Staff, 2022). All sites presented a sandy clay loam texture (Figure 1). These soils are derived from sedimentary rocks of the Piauí Formation, Balsas Group

(Pfaltzgraff; Torres; Brandão, 2010). The areas are distinguished by their duration of commodity crop production: 12 (P12), 18 (P18), and 24 years (P24), in addition to a native vegetation area (P0) used as a reference. Sampling was conducted between August 17 and 19, 2022.

In each selected field, sampling points were located approximately 100 m from the edges to minimize the effects of soil compaction caused by agricultural machinery in the borders. Soil pits were then excavated using a backhoe loader, reaching dimensions of at least two meters in depth and three meters in length (Figure 2).



Figure 2 - Trench digging process using a loader.

2.2 Soil morphological description and sampling

Initially, morphological descriptions of the soil profiles were carried out according to Santos *et al*. (2015), evaluating attributes such as color, texture, structure, consistency, and horizon transitions. Subsequently, disturbed soil samples were collected from each horizon for soil classification and both physical and chemical analyses (Figure 3).

Disturbed and undisturbed soil samples were collected in three replicates at the following depths: 0.0–0.1 m, 0.1–0.2 m, 0.2–0.3 m, 0.3–0.4 m, and 0.4–0.5 m. Undisturbed samples were placed in insulated polystyrene boxes and transported to the laboratory, where they were stored under refrigeration immediately after collection until analysis of soil basal respiration ($C–CO_2$) and microbial biomass carbon (MBC). After completion of the biological analyses, the disturbed samples were air-dried and passed through a 2.0 mm sieve prior to chemical analyses (Appendix 1). Undisturbed samples were collected using a volumetric ring

of known volume (Figure 4). Soil penetration resistance was determined down to 0.5 m depth using a penetrometer.



Figure 3 - Description, demarcation of horizons, and collection of samples for soil classification.

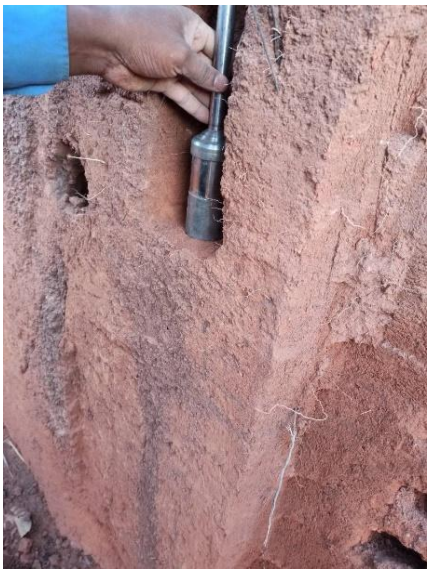


Figure 4 - Undisturbed samples collected using the volumetric ring method.

3 CHAPTER 1

THEORETICAL BACKGROUND

3.1 Brazilian agriculture and the Cerrado biome

Brazilian agriculture has demonstrated high productive performance over the past decades, consolidating itself as one of the pillars of the national economy. In the 2024/2025 growing season, the area cultivated with grains reached approximately 87.7 million hectares, with an estimated production of 352 million tons, highlighting the remarkable productive capacity of the sector (CONAB, 2026). In this context, soybean stands out as the country's main agricultural crop, recording between 2000 and 2025 an increase of 338.92% in cultivated area and 446.19% in production (CONAB, 2026). This growth is primarily associated with productivity gains driven by technological advances, genetic improvement, and the adoption of modern management practices.

Concurrently, agribusiness has significantly expanded its economic contribution, with an approximate 950% increase in gross domestic product between 2000 and 2025, currently accounting for about 30% of Brazil's gross domestic product (CEPEA, 2026). The strategic importance of soybean for the national economy is further evidenced by Brazil's position in the international market, as national production represented approximately 41% of global production in the 2022/2023 season (Valdes; Gillespie; Dohlman, 2023).

Soybean cultivation has expanded rapidly in recent years, particularly in the region known as MATOPIBA, which comprises the states of Maranhão, Tocantins, Piauí, and Bahia (CONAB, 2026; Lima *et al*., 2019). Located within the Cerrado biome, this region represents Brazil's main contemporary agricultural frontier, exhibiting high potential for productive growth and playing a strategic role in national economic development (Valdes; Gillespie; Dohlman, 2023). Agricultural expansion in MATOPIBA has attracted producers from different regions of the country, driven mainly by lower land prices and topographic conditions favorable to large-scale mechanization (Pires *et al*., 2016). More broadly, the advancement of Brazilian agriculture in this and other territories is associated with pioneering entrepreneurs, the feasibility of agricultural mechanization, the implementation of public policies for rural credit and minimum price guarantees, and the development and adaptation of agricultural technologies to local edaphoclimatic conditions (Contini *et al*., 2010).

However, the rapid expansion of agriculture in Brazil has resulted in a substantial reduction of natural areas within the Cerrado biome, particularly in its northeastern portion, where land occupation has been largely oriented toward grain production for export markets (Brannstrom *et al*., 2008). In addition to soybean expansion, other anthropogenic activities have contributed to landscape transformation in this biome, such as the introduction of exotic species, including eucalyptus plantations, and the clearing of native vegetation for charcoal production (Oliveira *et al*., 2014). As a consequence of this process, the Cerrado has become the Brazilian biome most impacted by agricultural expansion in recent decades (Valdes; Gillespie; Dohlman, 2023; Lima *et al*., 2019; Silva *et al*., 2013), driven by increases in production and productivity made possible mainly by intensive mineral fertilizer use (Lu; Tian, 2017) and the adoption of more efficient soil management systems (Queiroz, 2009).

The Cerrado biome is recognized as the most biodiverse tropical savanna in the world, characterized by high ecological complexity and a wide diversity of plant and animal species (Faleiro *et al*., 2008). It presents a marked seasonal climate with a well-defined dry season and vegetation adapted to these conditions, predominantly composed of species with deep root systems and twisted morphology, which favor survival under water-limited environments (Curi *et al*., 2017). The biome harbors approximately one-third of Brazil's biodiversity and about 5% of the world's flora and fauna, underscoring its global ecological relevance (Faleiro *et al*., 2008).

In terms of territorial extent, the Cerrado occupies approximately 204.7 million hectares in central Brazil, encompassing areas of the states of Bahia, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Piauí, São Paulo, and Tocantins, in addition to the Federal District. This corresponds to about 24% of Brazilian territory, making the Cerrado the second largest biome in South America (IBGE, 2004). In the state of Piauí, the biome covers approximately 12 million hectares, representing 46% of the state area, 36.9% of the northeastern Cerrado, and 5.9% of the Brazilian Cerrado (Leite *et al*., 2010). Beyond its remarkable biodiversity, the Cerrado plays a strategic role in hydrological regulation, as its subsoil hosts important aquifers responsible for supplying several major river basins in the country (Silva *et al*., 2013).

3.2 Cerrado soils and agricultural potential

Despite their significant agricultural importance, Cerrado soils present inherent limitations associated with low natural fertility, high acidity, low nutrient and organic matter

contents, and, in many cases, sandy texture (Curi *et al*., 2017). However, these soils respond efficiently to acidity correction practices and mineral fertilization, enabling high productivity levels when properly managed (Queiroz, 2009; Donagemma *et al*., 2016; Lu; Tian, 2017; Maia *et al*., 2022). Due to its vast territorial extent, the Cerrado has become one of the most relevant regions for Brazilian agriculture, contributing substantially to the production of grains, fibers, and other crops (Lustosa Filho *et al*., 2021). Nevertheless, the rapid expansion of mechanized agriculture has intensified the reduction of natural areas within the biome, where recent land occupation has been largely concentrated on large-scale grain production oriented toward export markets (Brannstrom *et al*., 2008).

Soil constitutes a fundamental component of the Earth system, playing a central role in several biogeochemical processes essential for ecosystem maintenance and agricultural production (Dai *et al*., 2019). Under natural conditions, over thousands of years, soils develop a relatively stable structure determined by their particle-size distribution and chemical composition, as well as by the intense activity of biological agents (Vezzani; Mielniczuk, 2009). However, the conversion of these natural environments to agricultural use imposes alterations to this stability and may trigger degradation processes when management is inadequate, particularly in sandy soils, which are more vulnerable to changes imposed by intensive use (Ratke *et al*., 2020). Despite these limitations, continuous global population growth has increased food demand, placing pressure on production systems to expand agricultural frontiers. Consequently, soils previously considered marginal due to their low productive capacity have been progressively incorporated into agricultural activities.

Studies conducted on sandy soils in the Cerrado region of Piauí have reported low pH values, reduced exchangeable base contents, and low cation exchange capacity, with soil organic matter being the main component responsible for cation exchange capacity in these edaphic environments (Souza *et al*., 2019). Additionally, physical characteristics associated with high sand content, such as low aggregate stability, increase susceptibility to water and wind erosion when management is inadequate (Ratke *et al*., 2020). In this context, the adoption of management practices that promote increased base saturation and improved nutrient availability to plants is essential to maintain agricultural productivity in these soils (Bonfim-Silva *et al*., 2014; Taiz *et al*., 2017), particularly in recently cultivated areas (Oliveira *et al*., 2024). Therefore, advancing the understanding of the functioning and dynamics of sandy soils in the Cerrado is essential to sustaining productivity gains in Brazilian agriculture, particularly given the recent territorial expansion, which has predominantly taken place on these soil types.

Sandy soils are generally characterized by sand contents around 700 g kg^{-1} (Centeno *et al*., 2017; Santos *et al*., 2025) and, although they represent approximately 31% of the Earth's surface, they have historically received less attention in scientific research compared to clayey soils (Yost; Hartemink, 2019; Huang; Hartemink, 2020). In Brazil, sandy soils correspond to approximately 8% of agricultural areas and occupy about 15% of the total area of the Cerrado biome, highlighting their considerable territorial representativeness (Lumbreras, 2015).

These soils are characterized by high infiltration rates and hydraulic conductivity, associated with low water and nutrient retention capacity, as well as greater susceptibility to drought and wind erosion, particularly under inadequate management (Yost; Hartemink, 2019). Given these limitations, the adoption of conservation management practices is essential to maintain the productive sustainability of these systems (Donagemma *et al*., 2016). Despite their wide distribution and increasing incorporation into agricultural activities, knowledge gaps remain regarding the behavior and response of sandy soils to agricultural use, particularly in subsurface layers, reinforcing the need for studies evaluating soil attributes throughout profile (Yost; Hartemink, 2019).

3.3 Soil quality and its indicators

The concept of soil quality (SQ) emerged in the early 1990s, initially associated with agricultural suitability, but later evolved into a broader approach encompassing the soil's capacity to perform ecological functions, sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran; Parkin, 1994; Conceição *et al*., 2005). The assessment of SQ is based on the use of physical, chemical, and biological indicators that are sensitive to changes imposed by different soil management systems (Doran; Zeiss, 2000). However, selecting the most appropriate properties for evaluation remains challenging, as ideal values vary according to soil type, climatic conditions, and the specific edaphoclimatic context. In this sense, SQ is grounded in the premise that different management systems may degrade, maintain, or improve soil ecosystem functions, directly influencing the sustainability of production systems (Franzluebbers, 2002).

Several indicators can be used to assess soil quality; however, for a soil attribute to be considered a reliable indicator, it must be highly sensitive to management-induced changes, capable of distinguishing among land-use systems, and directly related to soil ecosystem functions (Doran; Zeiss, 2000). Soil organic matter (SOM) is widely recognized as one of the main SQ indicators, especially in sandy soils, where it plays a fundamental role in water and

nutrient retention, aggregate stability, and cation exchange capacity (Vezzani; Mielniczuk, 2009; Yost; Hartemink, 2019). Soil carbon (C) stock is another key indicator for assessing land-use sustainability due to its positive effects on nutrient dynamics, soil structure, and atmospheric CO_2 sequestration (Sousa *et al*., 2023).

SOM represents the largest terrestrial reservoir of carbon, storing approximately twice as much C as the atmosphere and about three times more than plant biomass, highlighting its central role in global biogeochemical cycles (Swift, 2001; Bernoux *et al*., 2009). Maintaining SOM contents close to natural levels is therefore essential for long-term agricultural sustainability, particularly in sandy soils, which are highly dependent on this component to sustain their physical, chemical, and biological functions.

However, the conversion of natural ecosystems to agricultural use often results in significant changes in SOM stocks and quality, potentially leading to substantial C losses depending on the management system adopted (Lal, 2018; Medeiros *et al*., 2020; Tiefenbacher *et al*., 2021; Medeiros; Cesário; Maia, 2023; Briedis *et al*., 2023; Liang *et al*., 2023). These losses contribute to climate change and reduce soil fertility and balance (De Polli; Pimentel, 2005; Figueiredo *et al*., 2018). Conversely, appropriate management systems can increase C stocks, enhancing CO_2 sequestration and contributing to climate change mitigation, while simultaneously improving soil quality (Ferreira *et al*., 2014; Jiang *et al*., 2018; Lal, 2018; Freitas *et al*., 2022; Medeiros; Cesário; Maia, 2023).

Understanding the quality of soil organic matter is fundamental for sustainable long-term management and for evaluating the impacts of management systems on the environment. Soil microbial biomass carbon (MBC) is an important indicator due to its high sensitivity to management practices (Wiesmeier *et al*., 2019; Ferreira *et al*., 2014). It functions as a dynamic nutrient reservoir for plants (Berthrong; Buckley; Drinkwater, 2013), since nutrient cycling is closely associated with microbial activity (Tiefenbacher *et al*., 2021). Additionally, soil basal respiration (C–CO_2), expressed as carbon release in the form of CO_2, represents the biological oxidation of organic matter by aerobic microorganisms and provides an estimate of the intensity of biological activity responsible for decomposition processes (Braga *et al*., 2014).

The combination of MBC and C–CO_2 measurements allows the calculation of the metabolic quotient (qCO_2), which expresses the amount of CO_2 released per unit of microbial biomass (Braga *et al*., 2014; Wiesmeier *et al*., 2019). The qCO_2 reflects the efficiency of microbial biomass in utilizing C for biosynthesis and is considered an important indicator of soil biological activity and substrate quality. As microbial biomass becomes more efficient, less C is lost as CO_2 through respiration and more C is incorporated into microbial tissues (Braga *et*

al., 2014). High microbial activity may indicate rapid transformation of organic residues into plant-available nutrients through nutrient cycling. Variations in qCO_2 may precede detectable changes in soil properties, reflecting both improvement and degradation processes in soil quality (Berthrong; Buckley; Drinkwater, 2013; Wiesmeier *et al*., 2019). Furthermore, the ratio between MBC and soil organic carbon (SOC), known as the microbial quotient (qMic), expresses the proportion of C immobilized in microbial biomass and enables evaluation of the efficiency of organic matter use by soil microorganisms (Franchini *et al*., 2007).

Traditionally, studies investigating the effects of management systems on C dynamics and biological activity have focused on surface soil layers (Tiefenbacher *et al*., 2021), mainly due to their greater sensitivity to anthropogenic interventions and the difficulty of promoting changes at greater depths (Martínez *et al*., 2016). However, recent evidence indicates that management-induced changes are not restricted to surface layers and may reach depths of 0.5 m (Maia *et al*., 2022), 0.6 m (Huang *et al*., 2022), and even 0.8 m (Singh; Ghimire; Acharya, 2024). The main factors influencing these changes appear to be the management system adopted (Maia *et al*., 2022), soil type and climatic conditions (Liang *et al*., 2023), duration of system implementation (Sá; Lal, 2009), and plant biomass production (Singh; Ghimire; Acharya, 2024).

Soil acidity (pH) is another important SQ indicator, as it represents a major limitation to crop productivity (Agegnehu *et al*., 2021; Oliveira *et al*., 2024). High acidity levels favor plant toxicity, primarily due to increased availability of aluminum (Al^{3+}) and hydrogen (H^{+}), and interfere with the availability and uptake of essential nutrients such as calcium, magnesium, and phosphorus (Donagemma *et al*., 2016; Taiz *et al*., 2017; Agegnehu *et al*., 2021; Silva *et al*., 2021; Enesi *et al*., 2023).

High exchangeable aluminum contents may restrict root development to superficial soil layers, limiting water and nutrient uptake at depth (Raij, 2013). In general, the pH range considered optimal for most cultivated species is between 6.0 and 7.0 (Bonfim-Silva *et al*., 2014; De Freitas *et al*., 2017; Taiz *et al*., 2017). However, a large proportion of Brazilian agricultural soils present moderate to high acidity, resulting in reduced crop productivity when corrective practices are not properly implemented (Zancanaro, 2006). Therefore, practices aimed at pH correction are essential for maintaining productivity in Cerrado soils, particularly in recently incorporated agricultural areas (Oliveira *et al*., 2024).

Soil acidity correction is predominantly carried out through liming, which increases soil pH and improves nutrient availability (Bonfim-Silva *et al*., 2014; Taiz *et al*., 2017). Liming also increases base saturation (V) due to the addition of calcium and magnesium to the soil

exchange complex (Oliveira *et al*., 2024). Base saturation is one of the main indicators of soil fertility and an important SQ indicator, as it expresses the proportion of exchangeable bases (calcium, magnesium, potassium, and sodium) relative to the soil cation exchange capacity (CEC). Soils with low V values generally present limitations to crop development and agricultural productivity (Taiz *et al*., 2017).

Studies indicate that even when liming is applied superficially, increases in V may occur in subsurface layers, reaching depths of up to 0.60 m (Caires; Banzatto; Fonseca, 2000; Melinski *et al*., 2019). However, in recently cultivated areas, lime rates or time since application may not be sufficient to raise subsurface V to levels comparable to those observed at the surface, particularly in the absence of lime incorporation (Soratto; Crusciol, 2008). This highlights the need for further studies on the dynamics of acidity correction and fertility management in sandy soils. Such research is particularly relevant in regions such as the Cerrado of Piauí, which not only has high agricultural expansion potential but is also characterized by a predominance of soils derived from sedimentary rocks, especially sandstones of the Piauí Formation, Balsas Group (Pfaltzgraff; Torres; Brandão, 2010), which are highly susceptible to subsurface chemical limitations.

3.4 Soil management practices and the importance of studies in new agricultural frontiers

Growing concern over the sustainability of economic activities linked to natural resource use, particularly agriculture and livestock production, has intensified in recent years. In this context, the adoption of cropping systems capable of promoting soil carbon (C) accumulation has become increasingly important for sustaining agricultural productivity while mitigating environmental impacts (Powlson *et al*., 2016; Searchinger *et al*., 2018; Zhu *et al*., 2022; Freitas *et al*., 2022; Maia *et al*., 2022). Understanding how farmers in specific regions achieve increases in soil C stocks, as well as identifying the associated changes in soil attributes under different management systems, is essential for supporting public policies aimed at simultaneously ensuring food security and environmental conservation (Althoff *et al*., 2018; Tiefenbacher *et al*., 2021).

This issue is particularly relevant in tropical environments such as the Cerrado, where high temperatures and elevated soil moisture during the rainy season accelerate soil organic matter (SOM) mineralization, while climatic restrictions during the dry season limit biomass production by cover crops (Silva *et al*., 2013). In regions with even more restrictive conditions for agriculture, such as the Brazilian semiarid zone—characterized by low rainfall and naturally

low organic matter content—SOM accumulation becomes even more challenging, especially under rainfed systems (Althoff *et al*., 2018). Under such conditions, management systems based on little or no soil disturbance, combined with conservation practices, play a key role in maintaining and increasing SOM stocks (Powlson *et al*., 2016; Yost; Hartemink, 2019; Brevilieri *et al*., 2024).

Soil tillage remains a central practice for creating favorable conditions for crop establishment and root development. Conventional tillage consists of disturbing surface soil layers to incorporate amendments and fertilizers, increase pore space, improve permeability, and enhance air and water storage (Gabriel Filho *et al*., 2000). Although this practice may also contribute to weed, pest, and soil-borne pathogen control, it often increases the susceptibility of soil to erosion and nutrient leaching by exposing the soil surface (Xiong *et al*., 2020). In addition, repeated tillage operations may induce subsurface compaction, restricting rainfall infiltration and increasing surface runoff, thereby intensifying soil particle transport (Andognini *et al*., 2020; Xiong *et al*., 2020).

In contrast, minimum tillage is characterized by reduced soil disturbance, although its intensity depends on crop requirements and site-specific conditions (He *et al*., 2021; Ramalho; Cavichioli, 2023). In soybean production systems within the studied region, farmers generally perform only occasional tillage operations aimed at alleviating soil compaction. Green manure use is also common and, combined with reduced soil mobilization, contributes to lower water losses and improved physical soil properties, including bulk density and penetration resistance (Oliveira *et al*., 2017; Telles *et al*., 2020).

Tillage directly influences soil C dynamics and structure because aggregate disruption exposes physically protected organic carbon to microbial decomposition. Consequently, conventional tillage is recognized as one of the main drivers of soil C stock depletion (Al-Kaisi; Yin, 2005). Conversely, conservation-based systems involving minimal soil disturbance and adequate crop residue management contribute to preserving aggregate-associated organic matter and reducing its degradation (Leite *et al*., 2010). Such strategies are therefore essential for mitigating climate change impacts and maintaining food production under increasing global demand (Tiefenbacher *et al*., 2021).

Agricultural commodity production in the Cerrado region of Piauí is largely conducted under minimum tillage systems. In some situations, this practice is adopted to alleviate soil compaction; in others, it facilitates fertilizer incorporation or cover crop establishment. This management particularity highlights the need to investigate its long-term effects on soil quality. Moreover, modern agriculture increasingly relies on high fertilizer inputs to sustain

productivity (Lu; Tian, 2017; Tiefenbacher *et al*., 2021). Understanding nutrient behavior under these systems and how management influences nutrient distribution throughout the soil profile is therefore fundamental for improving nutrient use efficiency, reducing production costs, and minimizing environmental impacts (Hou, 2023; Valdes; Gillespie; Dohlman, 2023).

Accordingly, long-term studies integrating chemical and biological soil attributes throughout the profile are needed, particularly in sandy soils of the Cerrado region of Piauí and under cultivation chronosequences. Such investigations are essential for understanding the impacts of agricultural use on soil quality, supporting sustainable management strategies, and guiding public policies that reconcile agricultural expansion with natural resource conservation. This need is particularly relevant in regions dominated by soils derived from sedimentary rocks, especially sandstones of the Piauí Formation, belonging to the Balsas Group (Pfaltzgraff; Torres; Brandão, 2010).

The underlying hypothesis of this study is that prolonged agricultural use of naturally low-fertility soils, such as those of the Cerrado region of Piauí, may promote significant improvements in the soil system, reflected in carbon accumulation, increased microbial activity, and enhanced nutrient contents over cultivation time, including in subsurface layers. These increases are expected to be primarily associated with the continuous addition of inputs by farmers and the downward movement of nutrients within the soil profile. Thus, the objective of this study was to identify the changes promoted by cultivation time on carbon stocks, biological activity, and nutrient contents within the 0–50 cm layer of a Oxisol in the Cerrado region of the state of Piauí, Brazil.

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4 CHAPTER 2

CARBON STOCK AND SOIL BIOLOGICAL ACTIVITY UNDER DIFFERENT CULTIVATION TIMES IN THE CERRADO REGION OF PIAUÍ

ABSTRACT

The increasing demand for food has driven the conversion of native areas into agricultural land, a transformation with significant implications for soil carbon (C) balance due to current cultivation systems. Developing and implementing cropping systems that promote C accumulation is crucial for maintaining agricultural sustainability. In this context, this study aimed to understand land management in the state of Piauí, located within the Brazilian Cerrado, investigating biological indicators of soil quality in a cultivation chronosequence on a Oxisol. The chrono sequence comprised four areas: one native area and three cultivated areas for 12, 18, and 24 years. The biological quality indicators included soil organic carbon (SOC), microbial biomass carbon (MBC), basal soil respiration (C-CO_2), microbial quotient (*q*Mic), and metabolic quotient (*q*CO_2), as well as C stock and CO_2 sequestration across the 0.0-10, 10-20, 20-30, 30-40, and 40-50 cm soil layers. The results showed no significant differences among areas for MBC, qMic, and *q*CO_2, but the SOC stock, C-CO_2, and C stock were increased with longer cultivation periods. CO_2 sequestration rates were 0.96, -0.06, and 1.69 Mg ha^{-1} $year^{-1}$ in the areas cultivated for 12, 18, and 24 years, respectively. Overall, the duration of cultivation enhanced microbial activity and C stock, contributing to CO_2 sequestration compared to the native area. Our findings evidence that research focusing on region-specific management systems are essential to ensure the long-term agriculture sustainability, since each region has distinct environmental and climatic conditions that directly influence the outcomes of implemented agricultural practices.

Keywords: Basal respiration, Carbon sequestration, Carbon stock, Microbial activity, Microbial biomass, Minimum tillage.

4.1 Introduction

The conversion of native vegetation into agricultural production systems places considerable pressure on natural ecosystems (Lal, 2018; Searchinger *et al*., 2018; Liang *et al*.,

2023; Nguyen *et al*., 2023), significantly influencing carbon (C) dynamics (Lal, 2018), which in turn affect carbon stocks (Bayer *et al*., 2000) and alter the quality of soil organic matter (SOM) (Freitas *et al*., 2022). Carbon stock is a key indicator for assessing land-use sustainability (Sousa *et al*., 2023), enhancing nutrient dynamics, soil structure, and atmospheric CO_2 reservoir.

The SOM quality understanding is essential for evaluating the environmental impacts of specific management systems. This is particularly important because soil constitutes the largest terrestrial reservoir of carbon, containing approximately twice the amount of carbon found in the atmosphere (Wiesmeier *et al*., 2019) and three times that stored in plant biomass (Bernoux *et al*., 2009). However, depending on the agricultural management system adopted, soil organic carbon (SOC) levels may remain stable, decrease, or increase beyond those observed under native vegetation, potentially contributing to atmospheric CO_2 reduction (Ferreira *et al*., 2014; Jiang *et al*., 2018; Lal, 2018; Freitas *et al*., 2022; Medeiros; Cesário; Maia, 2023).

Integrating SOC content and carbon stocks with biological quality indicators are necessary to investigate the management systems on the SOM decomposition. In addition, soil microbial biomass carbon (MBC) is a key example of such indicators, as it is highly sensitive to management practices (Ferreira *et al*., 2014; Wiesmeier *et al*., 2019). According to Berthrong, Buckley e Drinkwater (2013), MBC serves as an important reservoir of plant-available nutrients, and its cycling is strongly dependent on microbial activity (Tiefenbacher *et al*., 2021). Additionally, basal respiration (C-CO_2), which reflects the biological oxidation of organic matter into CO_2 by aerobic microorganisms, provides an estimate of the biological activity responsible for this decomposition process.

The combination of MBC and C-CO_2 measurements provides the amount of CO_2 evolved per unit of microbial biomass, known as the metabolic quotient (qCO_2). The qCO_2 reflects the efficiency of the microbial biomass in utilizing available carbon for biosynthesis, thus serving as another key indicator of biological activity and substrate quality (Wiesmeier *et al*., 2019). As microbial biomass becomes more efficient in utilizing resources in its environment, less carbon is lost as CO_2 through respiration, allowing an incorporation into microbial tissues.

High microbial activity may indicate a rapid transformation of organic residues into plant-available nutrients through nutrient cycling. Changes in qCO_2 can precede shifts in organic matter dynamics and, consequently, in soil properties, serving as early indicators of soil improvement or degradation (Berthrong; Buckley; Drinkwater, 2013; Wiesmeier *et al*., 2019).

It is also possibly relating the MBC to SOC levels, known as microbial quotient (*q*Mic) that represents the proportion of carbon immobilized in the microbial biomass and reflects the efficiency of MBC in utilizing SOC (Franchini *et al*., 2007).

Research aiming to understand the effects of management systems on carbon dynamics and biological activity have typically focused on surface soil layers (Tiefenbacher *et al*., 2021). However, some studies indicates that the changes are limited not only to upper layers but also to deeper soil horizons (Huang *et al*., 2022; Singh; Ghimire; Acharya, 2024).

In Brazilian Northeastern, the Cerrado of Piauí plays a significant role in agricultural production, particularly soybean (*Glycine max*) (Oliveira *et al*., 2024; Projeto MapBiomas, 2024). The region is characterized by a tropical climate and predominantly sandy soils, which pose challenges for increasing SOC and carbon stocks (Lal, 2018; Sousa *et al*., 2023). Also, the commodity production in the Piauí Cerrado is predominantly conducted under a minimum tillage system, adopted to alleviate soil compaction and incorporate fertilizers or cover crops. Despite previous studies in the region, these specific conditions underscore the need to investigate the long-term effects of minimum tillage systems, particularly within the 0-50 cm soil depth, in comparison to native forest environments.

We hypothesize that prolonged land use may result in significant benefits in terms of carbon accumulation and increased soil microbial activity in Cerrado biome. In this context, this study aimed to evaluate the influence of the time of land use for agricultural production on carbon stocks and biological activity in the Cerrado of Piauí, in the 0-50 cm layer.

4.2. Material and Methods

4.2.1 Location, climate, and characterization of experimental area

The study was conducted at four sites within Chapada do Céu farm (07°40’44” S; 44°04’16” W), located in the city of Sebastião Leal, southwestern region of Piauí state, northeastern Brazil (Figure 5). The sites included a native forest area (P0) and cultivated areas under agricultural use for 12 (P12), 18 (P18) and 24 (P24) years, the entire farm area is situated at an elevation of 445 meters, lies at the ecological boundary between the Cerrado and Caatinga biomes and marks the transition zone between semi-arid and tropical climates. This transitional condition is characterized by potential evapotranspiration exceeding precipitation for most of the year, with an average annual temperature of 26.7 °C, mean annual rainfall of 1015 mm, and, in some years, total precipitation falling below 800 mm (Climate-Data, 2024).

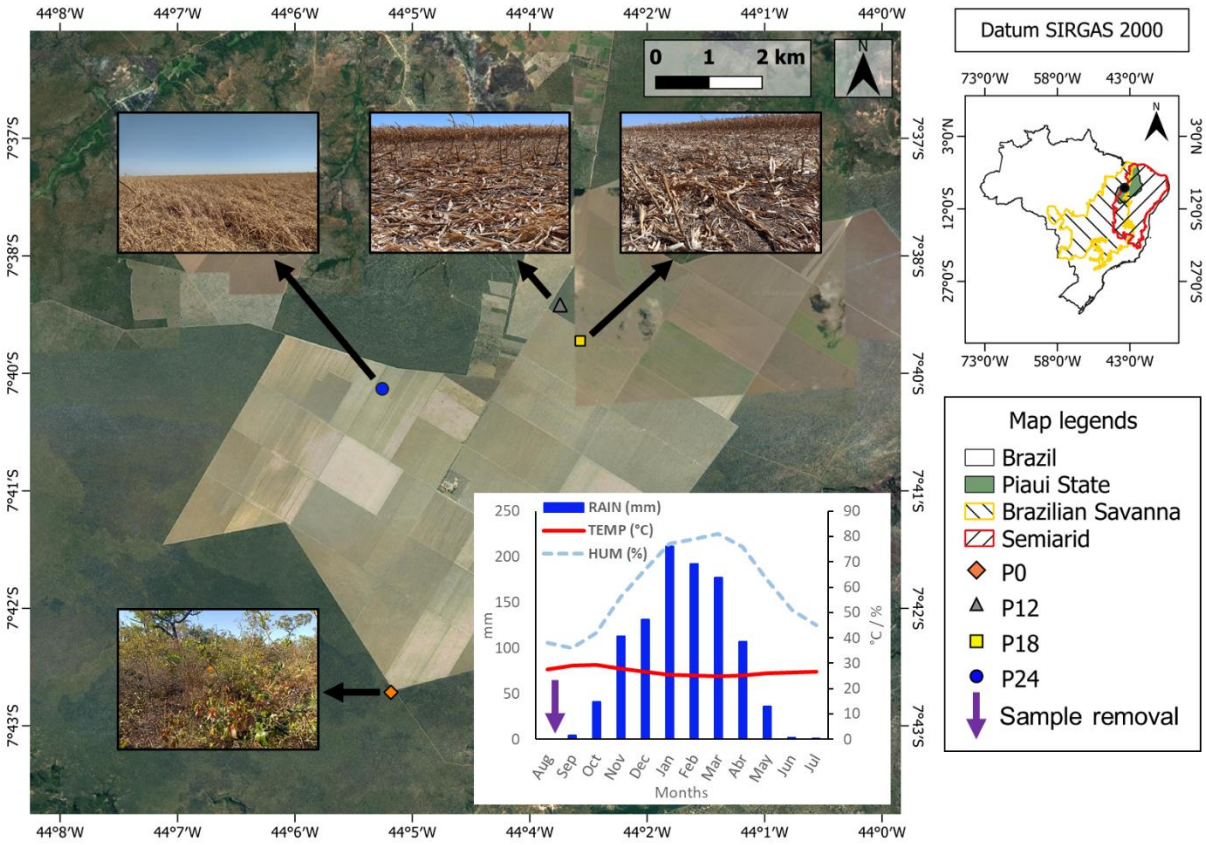


Figure 5 - Location and soil cover conditions of soil samples from native forest (P0) and cultivated areas under 12 (P12), 18 (P18), and 24 (P24) years of agricultural use, and historical averages of rainfall (RAIN), temperature (TEMP), and relative humidity (HUM) in the studied region.

The four sampling sites present flat terrain, characterized as Yellow Latosol according to official Brazilian soil classification system (Santos *et al*., 2018), which corresponds to the *Oxisols* order in the U.S. Soil Taxonomy system (Soil Survey Staff, 2022). These soils are derived from sedimentary rocks of the Piauí Formation, Balsas Group (Pfaltzgraff; Torres; Brandão, 2010). The sites differ in terms of duration of agricultural use for commodity production, with 200 hectares each (P12, P18 and P24), except the P0 that remains undisturbed.

The adopted minimum tillage technique in the study area involves minimal soil disturbance, with tillage performed only when strictly necessary for alleviating compaction or incorporating soil amendments. As a result, most crop cycles are established without soil turning. Since the 2008/09 growing season, pearl millet (*Pennisetum glaucum*) has been used as a cover crop to protect the soil by adding surface mulch, thereby maintaining moisture and reducing thermal amplitude. These practices have contributed to stable yields even under low rainfall conditions (Figure 6).

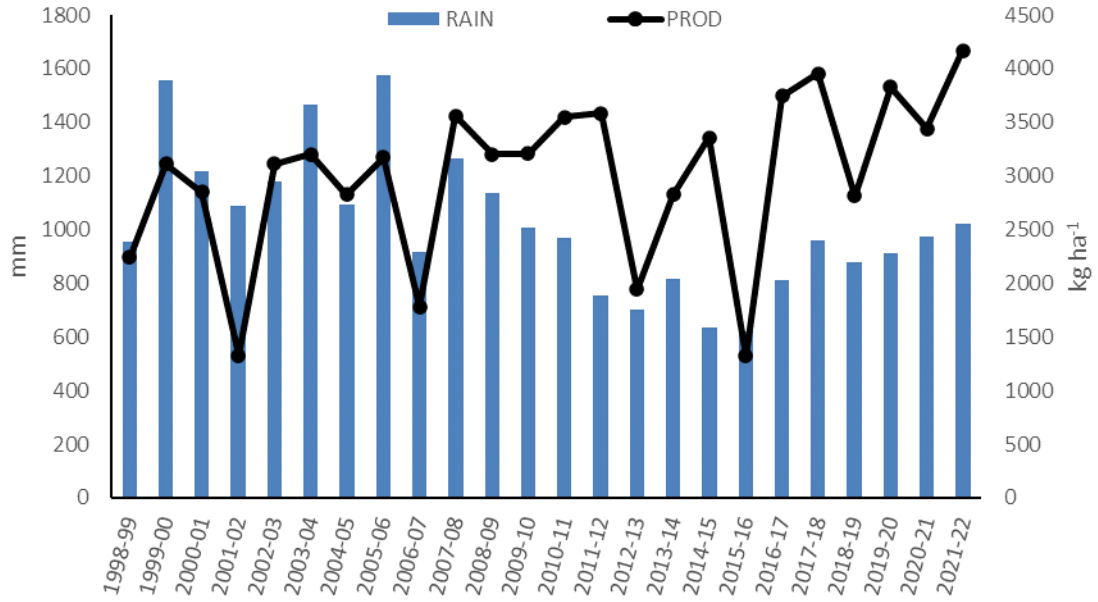


Figure 6 - Annual average soybean (*Glycine max*) yields (PROD) and mean seasonal rainfall (RAIN) at Chapada do Céu Farm, Sebastião Leal, Piauí, Brazil.

4.2.2 Land use history

Due to the rainfall distribution pattern in the Piauí Cerrado, farmers in the region typically carry out sowing between from October to December of one year and harvesting between February and May of the following year. Therefore, we defined as agricultural season beginning in August and ending in July of the subsequent year. This approach was adopted to align the cropping cycles with the regional rainfall regime and more accurately reflect local farming practices (Figure 5).

The agricultural year is divided into two annual cropping cycles, referred to as the first and second seasons. The first season (main crop) is typically used for soybean cultivation, while the second season (off-season) uses the final of rainy period by growing crops with lower water requirements or producing dry biomass solely for soil protection. Herein, cultivation intended exclusively for soil cover occurred twice in each of the cultivated areas (P12, P18, and P24).

Although soybean was cultivated during the first season in most years, other crops were grown in certain years. In the P24 area, cotton (*Gossypium hirsutum*) was grown during the 2007/08 season, but was not introduced in the P12 and P18 areas. Additionally, maize (*Zea mays*) was planted in P24 only during the 2015/16 season, when nitrogen fertilization was reduced to 21 kg ha^{-1} due to drought conditions. In contrast, maize was cultivated twice in both P12 and P18. Differences were also observed among the areas during the second season: in

P24, maize was not cultivated; in P18, it was grown once; and in P12, it was cultivated twice (Figure 7).

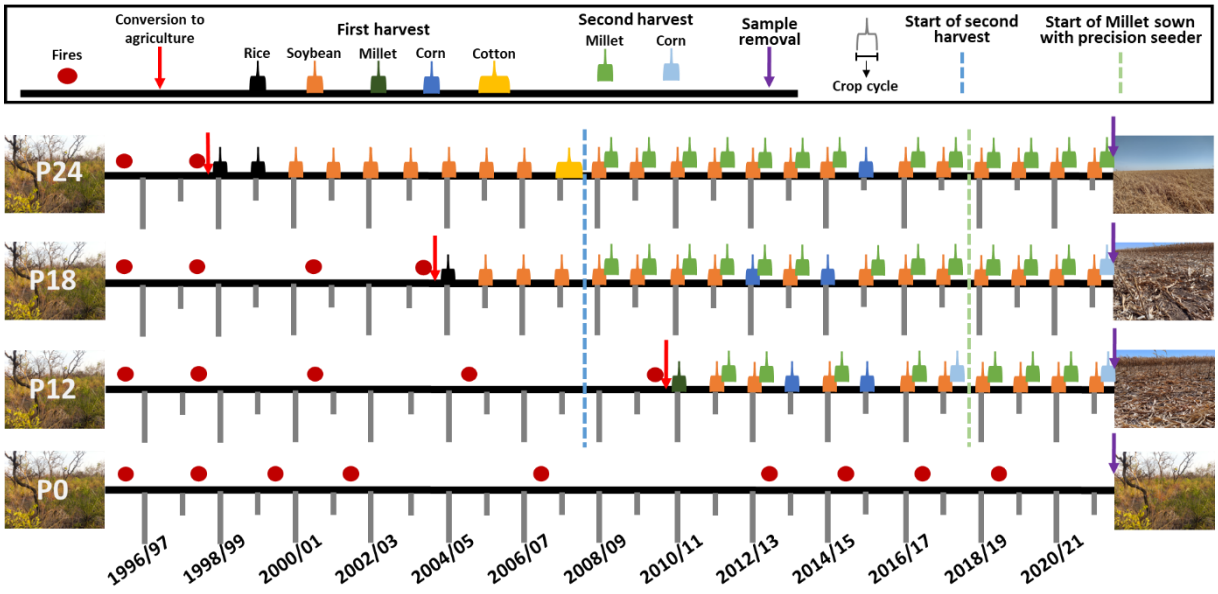


Figure 7 - History of burning events and crops cultivated during the first and second seasons in the native vegetation area (P0) and in areas under cultivation for 12 (P12), 18 (P18), and 24 (P24) years.

The area with no apparent human intervention (native vegetation; P0) did not receive any external nutrient inputs. In contrast, the cultivated areas (P12, P18, and P24) were amended with high doses of limestone and fertilizers (Table 1). These amendments aimed to reduce toxic aluminum levels and to create favorable soil conditions for soybean (*Glycine max*) production (Oliveira *et al*., 2024), the region's most economically important crop (Projeto MapBiomas, 2024).

Table 1 - Total amount of limestone, gypsum, macro and micronutrients applied in native vegetation area (P0) and areas cultivated for 12 (P12), 18 (P18), and 24 (P24) years.

Study areas	kg ha^{-1}					g ha^{-1}				
	Limestone*	Gypsum**	N	P_2O_5	K_2O	B	Cu	Mn	Zn	Fe
P0	-	-	-	-	-	-	-	-	-	-
P12	13300	4500	314	912	1056	3050	636	3403	3710	722
P18	18000	5500	357	1389	1696	4091	848	4156	9571	1898
P24	16500	8000	281	2058	2142	7437	1573	6637	18648	4580

*Dolomitic limestone with an average of 26,95% CaO and 14,66% MgO; **Average of 22,39% CaO and 17,75% S

At the time of converting native areas to agricultural land, known as initial soil preparation, soil amendments and fertilizers were incorporated into the soil. In the cultivated areas (P12, P18 and P24), the initial preparation was carried out in two stages. The first occurred immediately after deforestation, applying 6.8, 3.5 and 3.0 Mg ha^{-1} of limestone in P12, P18 and P24, respectively (Appendix 2). These inputs were incorporated using a 28-inch disc harrow, passed three times, followed by leveling with a 22-inch leveling harrow, applied twice. In the subsequent cropping season, an additional 3.5, 3.5, and 2.0 Mg ha^{-1} of limestone was applied in these areas, incorporated with the 28-inch disc harrow passed twice, followed by subsoiling to a depth of 35 cm (twice), and final leveling with the 22-inch leveling harrow, also applied twice.

Area P12 received the highest limestone application rate during the initial soil preparation, whereas P18 and P24 received split applications to avoid potential negative impacts on the rice crop (*Oryza sativa*). This approach was feasible in P12, as pearl millet (*Pennisetum glaucum*) was grown instead of rice, with the sole purpose of producing straw for soil cover (Figure 7).

Following the initial tillage, all other fertilizers were broadcast on the soil surface without incorporation, including limestone and agricultural gypsum, except for initial fertilization applied in the planting furrow until the 2012/13 growing season. From the 2013/14 season onward, all fertilization practices were carried out entirely on the surface, with no incorporation whatsoever (Figure 8). For all cases, fertilization was based on soil analysis and technical recommendations.

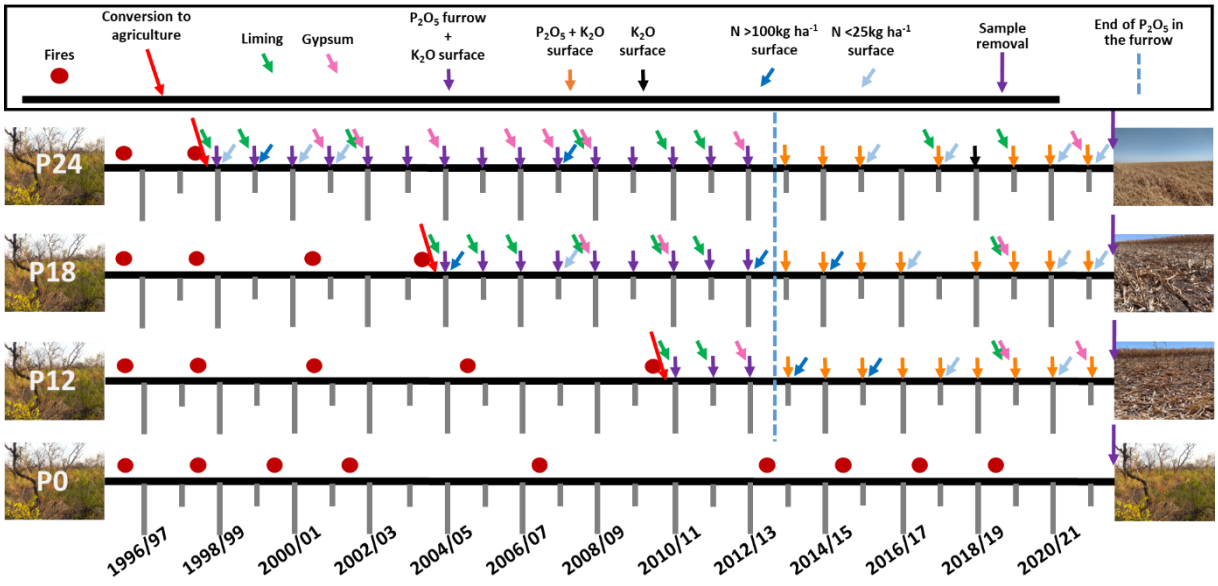


Figure 8 - History of burning events and fertilization in the native forest area (P0) and in areas under cultivation for 12 (P12), 18 (P18), and 24 (P24) years.

Starting from the 2018/19 growing season, pearl millet cultivation began to be carried out using precision seeders; and before sowing was performed a broadcasting, and the seeds were incorporated into the soil using a leveling harrow to a depth of approximately 5.0 cm (Figure 7). In the same season, subsoiling was conducted in the P24 area to a depth of 25 cm to alleviate soil compaction. In the following season (2019/20), the same operation was performed in the P18 and P12 areas, representing the last soil disturbance prior to the sampling date.

Inoculants (*Bradyrhizobium* sp.) were employed in soybean cultivation during all seasons, following the specific recommendations of manufacturers. In certain years, nitrogen (N) fertilization was also applied due to the use of phosphorus sources containing N (Complementary Table 1), applied in-furrow (prior to the 2013/14 season), with low cost. From the 2013/14 season onward, monoammonium phosphate (MAP: 10-50-00) became the primary phosphorus source.

4.2.3 Soil sampling and area physical characterization

In each P0, P12, P18 and P24 area, sampling was conducted approximately 100 meters from field edges to minimize the potential effects of soil compaction caused by agricultural machinery. Soil samples were collected in August 2022, right after the second harvest (Figures 7 and 8), from trenches (one per study site), each measuring 1.0 x 1.0 x 1.0 meters. Within each trench, both disturbed and undisturbed soil samples were taken in triplicate, 50 cm apart from each other, from the following depth intervals: 0-10, 10-20, 20-30, 30-40 and 40-50 cm.

The disturbed soil samples were divided into two portions. One portion was placed in plastic bags, transported to the laboratory and the samples were air-dried, gently crushed, and sieved through a 2.0 mm mesh to obtain the air-dried fine soil fraction, which was subsequently used for particle size analysis (Appendix 3). The second portion was placed in plastic bags equipped with air vents and transported in insulated boxes containing ice to the laboratory, and employed to measure the SOC and biological activity indicators in the soil. The samples were homogenized, sieved through a 2.0 mm mesh, and stored in a cold chamber until analysis, which was performed within a maximum period of 30 days, as recommended by Jenkinson and Powlson (1976).

Particle size analysis was carried out according to Gee and Or (2002). Soil bulk density (BD) was determined using the core method, based on the difference between the weight of the saturated soil and the oven-dried soil at 105°C, as described by Teixeira *et al*. (2017). The results are presented in Table 2.

Table 2 - Particle size distribution, textural classification and bulk density (BD) in native forest (P0) and cultivated areas under 12 (P12), 18 (P18) and 24 (P24) years of management.

Study areas	Layer	Sand	Silt	Clay	Texture*	BD
	cm	---------- g kg^{-1} ----------				g cm^{-3}
P0	0-20	748	34	218	Sandy clay loam	1.38
P12		730	34	236	Sandy clay loam	1.41
P18		702	28	270	Sandy clay loam	1.52
P24		627	43	330	Sandy clay loam	1.41
P0	20-50	741	32	228	Sandy clay loam	1.50
P12		682	31	287	Sandy clay loam	1.40
P18		648	24	328	Sandy clay loam	1.51
P24		555	36	409	Sandy clay	1.49

* Classified as Santos *et al.* (2018).

4.2.4 Determination of soil organic carbon and biological indicators

The soil organic carbon (SOC) was determined by wet oxidation using potassium dichromate ($K_2Cr_2O_7$), with subsequent colorimetric reading at 650 nm (Silva *et al.*, 1999). Among the soil biological indicators, microbial biomass carbon (MBC) was assessed using the irradiation-extraction method, employing a microwave oven to eliminate microorganisms and release cellular components (Mendonça; Matos, 2005). MBC was calculated as the difference in SOC between irradiated and non-irradiated samples, with analyses performed in triplicate and utilizing a Kc of 0.33.

Soil basal respiration ($C\text{-}CO_2$) was estimated based on the amount of $C\text{-}CO_2$ released over a seven-day incubation period (Jenkinson; Powlson, 1976). The incubation procedure involved placing 20 g of soil samples into sealed jars containing 10 mL of 1.0 mol L^{-1} NaOH solution. Following incubation, the NaOH was titrated with 0.5 mol L^{-1} HCl using phenolphthalein as an indicator.

Based on these results, the metabolic quotient (qCO_2) was calculated as the ratio between basal respiration ($C\text{-}CO_2$) and microbial biomass carbon (MBC), while the microbial quotient (qMic) was determined as the ratio between MBC and SOC (Anderson; Domsch, 1993).

Soil carbon stocks (C Stock) were calculated by multiplying the SOC concentrations by the BD and the corresponding thickness of each soil layer evaluated (Bayer *et al.*, 2000). To account for differences in bulk density among treatments, C Stock were corrected to an equivalent soil mass (Ellert; Bettany, 1995). In this method, the native forest (reference) bulk

density was used to determine the equivalent soil depth for each cultivated treatment, ensuring that comparisons were based on the same soil mass rather than fixed depth according to the following equation:

$$Equivalent\ soil\ layer = \frac{Average\ bulk\ density\ (native\ forest)}{Average\ bulk\ density\ (cultivated\ area)}\ x\ layer$$

The carbon stock in the 0.0-50 cm depth interval was used to estimate CO_2 emissions or sequestration (Carvalho *et al*., 2009), using the native forest area as a reference (Ribeiro *et al*., 2023), according to the following equation:

$$Emission/Sequestration\ CO_2 = \frac{C\ Stock\ (Cultivated\ area) - C\ Stock\ (native\ forest)}{system\ usage\ time}$$

To convert the carbon stock into CO_2 equivalents, a conversion factor of 3.67 was applied (molar mass of CO_2 divided by the molar mass of C).

4.2.5 Statistical analyses

Although the study did not involve experimental plots, the sampling structure comprised four land-use periods (0, 12, 18, and 24 years) and five soil depths (0-10, 10-20, 20-30, 30-40, and 40-50 cm), each with three replicates. Given the observational nature of the data, no formal statistical design was assumed. Instead of traditional analysis of variance, comparisons among land-use durations and soil depths were performed using 95% confidence intervals. For each variable, mean values and their corresponding 95% confidence intervals were calculated to assess differences among areas and soil layers. Overlapping confidence intervals were interpreted as evidence of no statistical difference, whereas non-overlapping intervals indicated significant differences among groups. All statistical procedures, including the calculation of means, confidence intervals, and data distribution checks were conducted using RStudio (Build 443) and R (version 4.3.1).

4.3 Results

4.3.1 Soil organic carbon, microbial biomass carbon and soil basal respiration

The SOC values (g C kg^{-1} soil) ranged from 3.73 in the 40–50 cm layer of P18 to 16.6 in the 0–10 cm layer of P24 (Table 3). Notably, up to a depth of 30 cm, the SOC content in the area with the longest cultivation period (P24) was 21% higher than that observed in the native

forest (P0). In the 30-40 cm and 40-50 cm layers, no significant differences were detected among the studied areas.

Table 3 - Means ± standard deviation for soil organic carbon (SOC), expressed in g C kg^{-1}; soil carbon stocks (C stock), expressed in Mg ha^{-1}; soil basal respiration (C-CO_2) expressed in mg CO_2 g^{-1} soil day^{-1} across four areas and five soil layers.

Layer	Study areas				
cm	P0	P12	P18	P24	Average
SOC (g C kg^{-1} soil)	CV% (Study area):13.30	CV% (Layer): 8.08			
0-10	13.72 ± 2.15	15.18 ± 0.83	14.95 ± 0.70	16.62 ± 0.33	15.12
10-20	7.58 ± 0.52	8.88 ± 0.72	8.33 ± 1.13	10.00 ± 0.72	8.70
20-30	5.71 ± 0.18	6.32 ± 0.20	5.40 ± 0.35	7.65 ± 0.20	6.27
30-40	5.84 ± 0.66	4.89 ± 0.82	4.75 ± 0.51	5.64 ± 0.31	5.28
40-50	4.31 ± 0.87	4.24 ± 0.21	3.73 ± 0.06	5.16 ± 0.26	4.36
Average	7.43	7.90	7.43	9.01	
C stock (Mg ha^{-1})	CV% (Study area):15.82	CV% (Layer): 10.74			
0-10	17.46 ± 2.73	19.32 ± 1.06	23.00 ± 0.89	21.14 ± 0.42	20.23
10-20	11.30 ± 0.78	13.23 ± 1.07	12.60 ± 1.68	14.90 ± 1.07	13.01
20-30	8.44 ± 0.26	9.35 ± 0.30	8.50 ± 0.52	11.31 ± 0.30	9.40
30-40	8.97 ± 1.01	7.50 ± 1.26	6.90 ± 0.79	8.66 ± 0.48	8.01
40-50	6.16 ± 1.24	6.06 ± 0.30	5.60 ± 0.08	7.38 ± 0.37	6.30
Total	52.32	55.46	52.04	63.40	
C-CO_2 (mg CO_2 g^{-1} soil day^{-1})	CV% (Study area):60.14	CV% (Layer): 24.98			
0-10	13.60 ± 1.54	18.10 ± 3.59	23.40 ± 3.42	34.30 ± 9.74	22.35
10-20	10.00 ± 1.79	10.60 ± 7.24	11.60 ± 3.08	23.30 ± 9.83	13.88
20-30	10.50 ± 1.26	13.30 ± 4.98	14.60 ± 0.75	15.10 ± 6.27	13.38
30-40	14.00 ± 3.31	6.00 ± 2.58	10.10 ± 1.97	13.80 ± 3.21	10.98
40-50	10.10 ± 6.38	3.70 ± 2.67	8.80 ± 2.55	10.90 ± 5.32	8.38
Average	11.60	10.30	13.70	19.50	

P0: native forest area; P12, P18 and P24 refer to areas under cultivation for 12, 18 and 24 years, respectively.

Comparing the soil layers within the same soil management, a decreasing trend in SOC content from the surface to the subsurface was observed (Table 3). Approximately 37% of the SOC within the 0-50 cm profile is concentrated in the surface layer (0-10 cm), while only 10% is present in the deepest layer (40-50 cm). These findings indicate that the SOC content in deeper layers should not be overlooked, as according to Lal, Negassa e Lorenz (2015) approximately 50% of the SOC stored up to a depth of 1.0 m is located within the 30-100 cm layer.

Microbial biomass carbon (MBC) values ranged from 40.9 mg C kg^{-1} soil in the deepest layer of P0 to 331.9 mg C kg^{-1} soil in the surface layer of P18 (Table 4). The cultivated areas consistently exhibited higher MBC values than the native forest across all layers, with increases of 121% (P12), 214% (P18), and 152% (P24) within the 0-50 cm profile.

Soil basal respiration (C-CO_2, mg CO_2 kg^{-1} soil day^{-1}) ranged from 3.7 in the 40–50 cm layer of P12 to 34.3 in the 0-10 cm layer of P24 (Table 3). After 24 years of cultivation, C-CO_2 increased by 152% in the 0-10 cm layer, 133% in the 10-20 cm layer, and 44% in the 20-30 cm layer. On average, C-CO_2 was 67% higher in P24 compared to the native forest (P0). It is important to note that the time of year can influence these results, and lower C-CO_2 values indicate greater stability (Dalla Costa *et al*., 2014]. In the 0-10 cm and 10-20 cm layers, C-CO_2 values in P24 were higher than those observed in the other areas, followed by P18, P12, and P0, respectively, in both layers. Below 20 cm depth, no significant differences were detected among the studied areas.

4.3.2 Microbial quotient, metabolic quotient, and carbon stock and sequestration

The microbial quotient (*q*Mic) ranged from 0.75% in the surface layer of P0 to 3.36% in the 40-50 cm layer of P18 (Table 4). It followed a pattern similar to that of MBC, in which all cultivated areas exhibited higher values than the native forest. However, unlike MBC, *q*Mic values were higher in the deepest layer (40-50 cm) and lower in the surface layer (Table 4).

Table 4 - Means ± standard deviation for microbial biomass carbon (MBC), expressed in mg C kg^{-1} soil; microbial quotient (*q*Mic), expressed in MBC SOC^{-1}; metabolic quotient (*q*CO_2), expressed in mg C-CO_2 mg^{-1} MBC dia^{-1}; CO_2 sequestration rates (Seq. CO_2), expressed in Mg ha^{-1} $year^{-1}$ across four areas and five soil layers.

Layer	Study areas				Average
cm	P0	P12	P18	P24	
MBC (mg C kg^{-1} soil)	CV% (Study area):111.94	CV% (Layer): 39.80			
0-10	106.30 ± 49.89	140.90 ± 137.79	331.90 ± 170.90	311.40 ± 91.00	222.60
10-20	63.10 ± 49.67	125.00 ± 78.96	216.50 ± 116.86	120.50 ± 45.40	131.30
20-30	52.00 ± 26.42	136.40 ± 90.79	175.00 ± 89.19	123.90 ± 11.97	121.80
30-40	49.30 ± 14.90	165.40 ± 112.84	128.40 ± 111.55	118.80 ± 41.07	115.50
40-50	40.90 ± 10.23	121.60 ± 126.49	125.10 ± 11.08	110.20 ± 16.11	99.50
Average	62.30	137.90	195.40	157.00	
qMic (MBC SOC^{-1})	CV% (Study area):117.07	CV% (Layer): 42.18			
0-10	0.70 ± 1.54	0.90 ± 3.59	2.30 ± 3.42	1.90 ± 9.74	1.40
10-20	0.90 ± 1.79	1.40 ± 7.24	2.80 ± 3.08	1.20 ± 9.83	1.60

20-30	0.90	±	1.26	2.10	±	4.98	3.20	±	0.75	1.60	±	6.27	2.00
30-40	0.90	±	3.31	3.20	±	2.58	2.70	±	1.97	2.10	±	3.21	2.20
40-50	1.00	±	6.38	2.80	±	2.67	3.40	±	2.55	2.10	±	5.32	2.30
Average	0.90			2.10			2.90			1.80			
qCO_2 (mg C-CO_2 mg^{-1} MBC day^{-1})				CV% (Study area):113.95					CV% (Layer): 56.16				
0-10	0.15	±	0.07	0.23	±	0.16	0.10	±	0.08	0.11	±	0.03	0.15
10-20	0.25	±	0.18	0.17	±	0.20	0.07	±	0.04	0.23	±	0.17	0.18
20-30	0.24	±	0.11	0.16	±	0.14	0.10	±	0.05	0.12	±	0.04	0.15
30-40	0.30	±	0.10	0.06	±	0.06	0.13	±	0.10	0.14	±	0.09	0.16
40-50	0.23	±	0.14	0.05	±	0.03	0.07	±	0.02	0.10	±	0.04	0.11
Average	0.23			0.13			0.09			0.14			
Seq. CO_2 (Mg ha^{-1} $year^{-1}$)													
0-50				0.96			-0.06			1.69			

P0: native forest area; P12, P18 and P24 refer to areas under cultivation for 12, 18 and 24 years, respectively.

The metabolic quotient (qCO_2), expressed in mg CO_2 mg^{-1} MBC day^{-1}, ranged from 0.05 in the 40-50 cm layer of P12 to 0.3 in the 30-40 cm layer of the native forest (P0) (Table 4). All cultivated areas present lower qCO_2 values than the native forest across all layers, except for P12 in the surface layer.

Soil C stocks (Mg ha^{-1}) ranged from 5.3 in the deepest layer of P18 to 21.14 in the surface layer of P24, showing a consistent decline with increasing soil depth across all study sites (Table 3). In the surface layer, C stocks were superior to P24 and inferior to P0 in the 0-10 and 10-20 cm layers. In the 20-30 cm layer, P18 showed the lowest values, P24 remained superior, and P0 and P12 were similar. No significant differences in C stock were observed among the areas below 30 cm depth.

The C stock in the 0.0-50 cm soil depth was used to estimate the annual C emission or sequestration in the cultivated areas (P12, P18 and P24) relative to the native forest (P0). The results indicate that the areas cultivated for 12 and 24 years sequestered 0.96 and 1.69 Mg ha^{-1} $year^{-1}$, respectively.

4.4 Discussion

4.4.1 SOC and microbial activity in soil

Conservation management systems that involve little or no disturbance are essential for maintaining, or even increasing, soil organic carbon (SOC) levels (Powlson *et al*., 2016). This

is because continuous plant cover creates favorable conditions for sustaining soil biological activity (Sá; Lal, 2009). However, in the Brazilian Cerrado region, the dry season inhibits biomass production (Figueiredo *et al*., 2018) and creates favorable conditions for fires, especially during El Niño years (Rocha; Nascimento, 2021).

Contrary to common belief, native areas in the study region are more prone to fires than anthropogenic ones (Rocha; Nascimento, 2021), likely due to the fire prevention monitoring carried out by farmers. The region has between 1.2 and 4.75 fire outbreaks per km², making it one of the most affected by fire in the entire Brazilian Cerrado Biome (Rocha; Nascimento, 2022). This reinforces the importance of local management studies for the long-term sustainability of agriculture (Chatterjee *et al*., 2018), as such management practices can increase SOC levels not only at the surface but also in the subsurface layers (Maia *et al*., 2022; Huang *et al*., 2022).

In the present study, changes in SOC resulting from long-term cultivation were more pronounced within the top 30 cm of the soil profile, highlighting the limited extent of alterations in deeper soil layers (Figure 9). Some studies have shown that even conventional tillage does not significantly affect SOC in deeper layers (Martínez *et al*., 2016). However, other research indicates that changes are not confined to surface horizons and may extend to depths of 50 cm (Maia *et al*., 2022), 60 cm (Huang *et al*., 2022), or even 80 cm (Singh; Ghimire; Acharya, 2024). Nevertheless, there are locations where no differences have been observed between cultivated and native areas (Figueiredo *et al*., 2018), while in others, reductions in SOC levels have been reported (Ferreira *et al*., 2014). This underscores the importance of locally adapted conservation management strategies for sustainable agriculture and long-term SOC accumulation (Chatterjee *et al*., 2018).

Sá and Lal (2009), investigating SOC in the 0.0-40 cm layer also in a Oxisol, reported findings comparable to those of the present study. After ten years of cultivation without soil disturbance, SOC levels matched those of the native forest area, and surpassed them after more than 20 years of no-tillage cultivation up to the 30-40 cm soil layer (Table 3). Furthermore, occasional subsoiling in Oxisols does not appear to reduce SOC levels when compared to undisturbed areas (Brevilieri *et al*., 2024).

These findings reinforce the importance of conservation agriculture systems, characterized by minimal or no soil disturbance, in promoting SOC accumulation. However, most studies focus exclusively on the upper 30 cm of the soil profile, often neglecting deeper roots (Tiefenbacher *et al*., 2021). Although surface layers are indeed more responsive to

management-induced changes (Ferreira *et al.*, 2014), deeper soil horizons should not be overlooked in long-term studies.

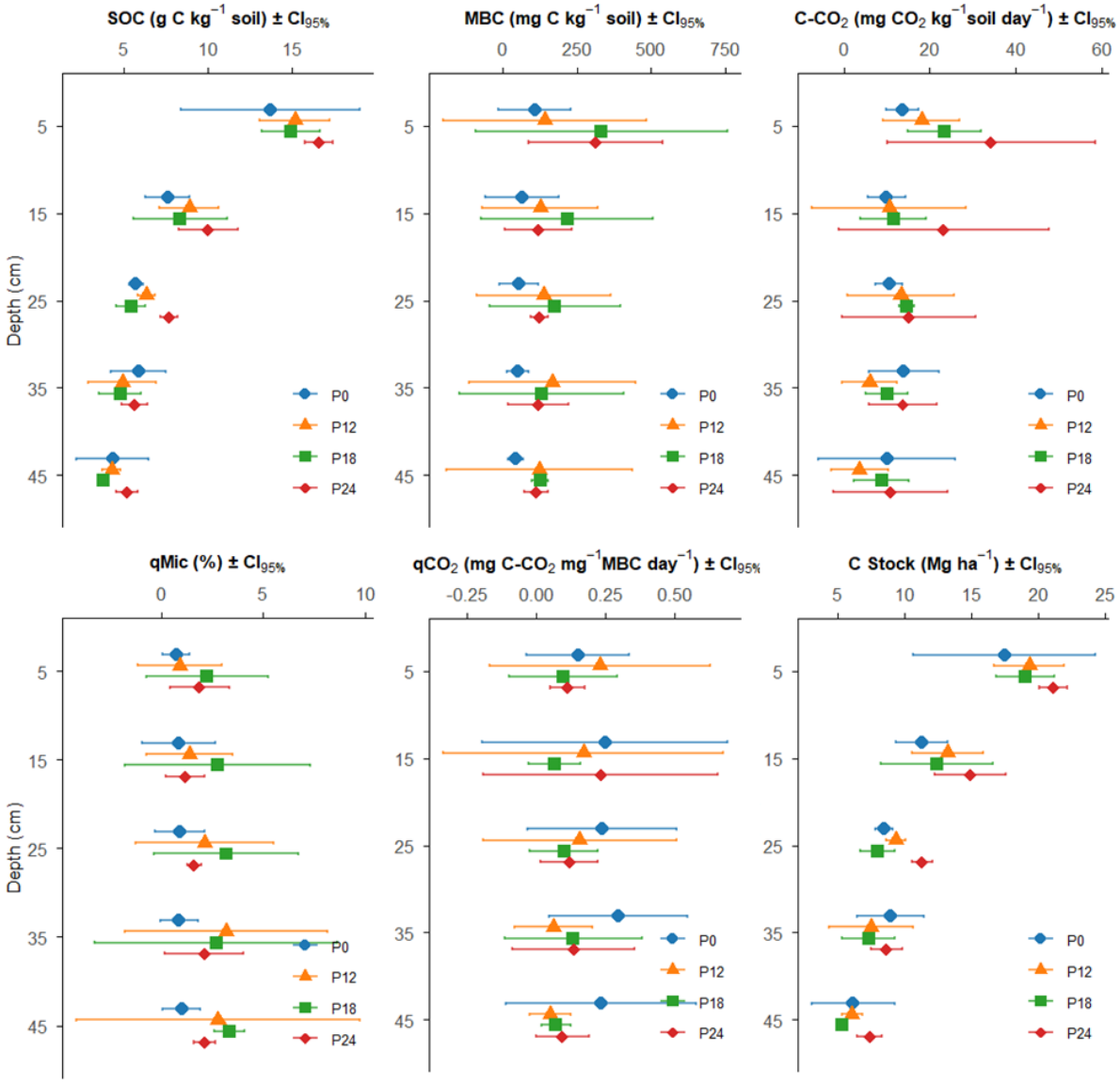


Figure 9 - Means ± 95% confidence interval for soil organic carbon (SOC), microbial biomass carbon (MBC), soil basal respiration (C-CO_2), microbial quotient (*q*Mic), metabolic quotient (*q*CO_2), and carbon stock (C stock) at the 0-50 cm soil depth from native vegetation area (P0) and areas cultivated for 12 (P12), 18 (P18) and 24 (P24) years.

Numerous studies have shown that alterations in SOC levels after conversion of native areas to agricultural use are influenced by climate, soil type (Liang *et al.*, 2023), management system (Lal; Negassa; Lorenz, 2015; Fu *et al.*, 2021; Maia *et al.*, 2022), plant biomass production (Singh; Ghimire; Acharya, 2024), and the duration of system implementation (Sá; Lal, 2009). These findings highlight the importance of detailed long-term cultivation studies

aimed at improving the understanding of systems that can enhance SOC levels and the underlying mechanisms driving this increase.

One of the factors that may inhibit sufficient biomass production to raise SOC levels is the prolonged dry season characteristic of the Piauí region, as well as in other similar regions (Figueiredo *et al*., 2018). Such conditions make it impossible to maintain continuous soil cover, thereby preventing the maintenance of favorable conditions for biological activity and the accumulation of SOC through a sustained carbon input (Sá; Lal, 2009; Martínez *et al*., 2016).

Several indicators can be used to assess the condition and activity of the soil microbial biomass. Microbial biomass carbon (MBC), for instance, is a highly sensitive indicator of soil biological quality, responding markedly to changes in soil management practices (Berthrong; Buckley; Drinkwater, 2013; Ferreira *et al*., 2014; Wiesmeier *et al*., 2019; Tiefenbacher *et al*., 2021). The present study indicates that the adopted management system has maintained MBC levels comparable to those found under native vegetation (Table 4). Some authors have reported that approximately 20 years of no-till cultivation are required to restore MBC contents to levels similar to those of native vegetation (Sá; Lal, 2009; Figueiredo *et al*., 2018). In contrast, pasture systems tend to increase MBC levels (Carneiro *et al*., 2008), whereas conventional tillage generally results in reductions (Figueiredo *et al*., 2018).

The substantial input of organic matter from the vigorous root development of grasses (Carneiro *et al*., 2008) or other cover crops (Singh *et al*., 2024) may explain the maintenance of MBC even in recently converted agricultural areas. This is further supported by the absence of fire in these cultivated areas, in contrast to the high incidence of fires in native areas (Rocha; Nascimento, 2022). Another contributing factor was likely the application of fertilizers, which promotes enhanced plant growth (Tiefenbacher *et al*., 2021).

The microbial respiration (C-CO_2) comprises another biological quality indicator that provides an estimate of biological activity through the oxidation of organic matter to CO_2 by soil microorganisms (Braga *et al*., 2014). Herein, C-CO_2 levels remained stable throughout the soil profile in the native forest area (P0). In contrast, in the cultivated areas (P12, P18 and P24), surface layers exhibited increasing C-CO_2 levels at 0-20 cm soil layer with longer cultivation duration (Table 3), a pattern also reported by Sá and Lal (2009). However, our findings indicate that the C-CO_2 increase was restricted to the upper 30 cm of the soil, with values below this depth resembling those observed in the native forest area (Figure 9), suggesting greater stability in deeper soil layers.

The microbial quotient (*q*Mic) is an indicator of biological quality that reflect system stability. It also serves to illustrate the efficiency of MBC utilizes SOC (Franchini *et al*., 2007).

Higher values of *q*Mic indicate a greater incorporation of SOC into the microbial biomass, which is associated with increased nutrient cycling and greater availability of organic carbon to soil microorganisms. Furthermore, *q*Mic values below 1.0% may suggest limiting conditions for microbial activity in the soil (Jakelaitis *et al*., 2008).

In this study, higher *q*Mic values were found in the deeper soil layers, while values below 1.0% were observed throughout the soil profile of the native forest area (Table 4). Ferreira *et al*. (2014), studying different management systems in the Brazilian semi-arid region, also reported higher *q*Mic values in deeper layers, although they found values below 1.0% only in the surface layers of the native vegetation area. Two likely factors limiting microbial growth in this study are low water availability (Figueiredo *et al*., 2018) and the frequent fires characteristic of the native vegetation in the region (Rocha; Nascimento, 2022).

Situations of high microbial biomass efficiency are associated with less CO_2 lost to the atmosphere and greater C incorporation into MBC (Wiesmeier *et al*., 2019). This can be measured through metabolic quotient (qCO_2). A lower qCO_2 indicates lower CO_2 loss and greater MBC efficiency in utilizing available C for biosynthesis, suggesting greater C accumulation in the microbial biomass and, consequently, in the soil of these cultivated areas compared to native forests.

4.4.2 Increase of C stock and C sequestration due to cultivation

The carbon accumulation was observed in the cultivated areas, as indicated by the qCO_2 levels (Table 4). These areas showed higher values compared to the native forest area (P0), particularly in the surface soil layer, which appears to be more sensitive to such changes (Briedis *et al*., 2023; Medeiros; Cesário; Maia, 2023). The greater C stocks are likely due to the higher concentration of roots (Martínez *et al*., 2016) and the deposition of plant residues (Sá; Lal, 2009; Singh; Ghimire; Acharya, 2024). Similarly, other long-term studies have also reported increases in carbon stocks following the conversion of native areas to agricultural land (Ferreira *et al*., 2014; Maia *et al*., 2022). Typically, the increase is associated with conservation agriculture practices (Chatterjee *et al*., 2018), which are more effective at accumulating stable carbon fractions in the soil, reaching levels comparable to those observed in native forest areas (Figueiredo *et al*., 2018).

Other studies have reported reductions in soil carbon stocks (Medeiros; Cesário; Maia, 2023; Figueiredo *et al*., 2018), indicating average losses of approximately 13% in the 0.0-5.0 cm layer (Searchinger *et al*., 2018) and 33% in the 0.0-10 cm layer (Briedis *et al*., 2023), mostly

associated with intensive soil tillage (Liang *et al*., 2023). In contrast, occasional soil scarification appears to have no significant long-term impact on carbon stocks (Brevilieri *et al*., 2024), as observed in the present study. Consistently, these variations are strongly influenced by biome, climate, soil type, and management practices (Fu *et al*., 2021; Maia *et al*., 2022; Liang *et al*., 2023), underscoring the importance of locally adapted conservation management strategies (Chatterjee *et al*., 2018).

Our results are aligned with those that reported carbon sequestration rates for the Brazilian Cerrado region range from 0.4 to 1.7 Mg ha^{-1} yr^{-1} (Bernoux *et al*., 2009). Under conservation-oriented management practices alone, carbon sequestration rates between 0.25 and 1.0 Mg ha^{-1} yr^{-1} can be achieved (Lal, 2018), depending on site-specific conditions and climate. The low input of biomass remains the principal factor contributing to long-term carbon losses in soils (Searchinger *et al*., 2018). Due to the prolonged dry season characteristic of these regions, average biomass production in agricultural areas of the Cerrado and Caatinga biomes is approximately 3.12 and 2.16 Mg ha^{-1} yr^{-1}, respectively (Bustamante *et al*., 2015).

Adopting strategies to enhance biomass production may be key to increasing soil carbon stocks and, consequently, sequestering atmospheric CO_2 (Bayer *et al*., 2000). Practices such as implementing multiple cropping systems per year (Jiang *et al*., 2018; Tiefenbacher *et al*., 2021), avoiding soil disturbance (Bayer *et al*., 2000; Lal, 2018), and incorporating organic residues (Huang *et al*., 2022) have been widely cited in the literature as effective when applied over the long term (Maia *et al*., 2022). Increases in root production within the 0.0-20 cm soil layer, and the resulting improvements in MBC and soil C stocks (Tiefenbacher *et al*., 2021), appear to be crucial factors for carbon sequestration in the Brazilian Cerrado region. Additionally, extending the cultivation period is also associated with greater carbon sequestration (Maia *et al*., 2022). To achieve these gains, the use of cover crops with vigorous and deep root systems is essential, as they promote nutrient cycling at greater soil depths, enhance soil health and fertility, and contribute to increased carbon stocks through the sequestration and immobilization of carbon in these layers (Tiefenbacher *et al*., 2021; Singh; Ghimire; Acharya, 2024).

Constant occurrence of wildfires in areas of native vegetation constitutes an important trait the seem to strongly influence C stocks in the region (Santos; Pereira; Rocha, 2014). The study area is among the most severely affected by fires within the entire Cerrado biome in Brazil (Rocha; Nascimento, 2022). As reported by Projeto MapBiomas (2024), the native areas experience wildfires approximately every two to three years. However, following the conversion of these native areas to agricultural land, no further fire events were recorded (Figure 3). According to some authors (Rocha; Nascimento, 2022), savanna vegetation areas

tend to exhibit a higher incidence of fire outbreaks compared to anthropized landscapes, which is consistent with the findings of this study. Furthermore, climatic phenomena such as El Niño tend to intensify drought conditions and, consequently, the frequency of wildfires (Rocha; Nascimento, 2021).

In summary, our data suggest that carbon sequestration in the studied areas is likely result of combined effects like absence of annual wildfires (Santos; Pereira; Rocha, 2014), application of chemical fertilizers (Jiang *et al*., 2018; Brevilieri *et al*., 2024), cultivation of cover crops (Singh; Ghimire; Acharya, 2024), and accumulation of crop residues or litter due to reduced or absent soil disturbance (Bayer *et al*., 2000; Ferreira *et al*., 2014; Chatterjee *et al*., 2018; Poulton *et al*., 2018). In addition to soil carbon accumulation, the conversion of native areas to agricultural land aims primarily at food production to meet growing demand (Searchinger *et al*., 2018). It is worth noting that making agriculture both sustainable and efficient remains an extremely complex challenge (Mallinger *et al*., 2024). In this study, since the adoption of a second annual cropping season, production stability has improved, with satisfactory yields even in years of low rainfall, along with a continuous increase in average productivity over time (Figure 2). This reinforces the notion that while modest increases in SOC may not necessarily lead to immediate yield gains, they can nonetheless provide important ancillary benefits to soil health and function (Poulton *et al*., 2018).

4.5 Conclusion

This long-term evaluation of agricultural areas in the Cerrado of Piauí under minimum tillage, with the use of cover crops, fertilization, and the absence of wildfire recurrence, demonstrates clear improvements in soil carbon dynamics and biological functioning. Over more than two decades of continuous cultivation, SOC concentrations and carbon stocks in the 0–30 cm layer surpassed those of native vegetation.

Microbial biomass, microbial quotient, and the metabolic quotient also improved with cultivation time, demonstrating a more efficient use of carbon substrates and a more stable microbial environment. These changes resulted in a net sequestration of 1.69 Mg C ha^{-1} yr^{-1} after 24 years of cultivation.

The study further shows that the 20-50 cm layer contains a large share of total carbon (40%), microbial biomass (49%), microbial activity (47%), and carbon stock (42%) within the 0-50 cm profile, emphasizing that subsurface layers contribute significantly to carbon storage and microbial functioning and should not be overlooked.

Our study reinforces that conservation-oriented management adapted to the environmental conditions of northeastern Brazil can maintain productivity while enhancing soil quality and carbon sequestration. Continued research is necessary to advance the understanding of carbon stabilization mechanisms and the key soil organisms involved in these processes across both surface and subsurface layers.

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5 CHAPTER 3

IMPACT OF LAND CONVERSION AND CULTIVATION TIME ON SOIL FERTILITY IN A 24-YEAR CHRONOSEQUENCE IN THE BRAZILIAN SAVANNA

ABSTRACT

The Brazilian Cerrado has been significantly impacted by agricultural expansion. However, its soils are typically acidic, nutrient-poor and sandy, requiring continuous management to sustain productivity. This study evaluated a cultivation chronosequence (native vegetation vs. 12, 18, and 24 years of cultivation) to identify temporal changes in soil fertility within the upper 0.5 m of an Oxisol under Cerrado vegetation in Piauí, Brazil. We assessed soil acidity, organic matter (SOM), and nutrient stocks (Ca, Mg, K, P, S, C, Fe, Mn, Zn, B, and Cu) across five depths. Significant increases in SOM, base sum (BS), base saturation (V), and stocks of C, Ca, and S occurred down to 50 cm. After 24 years, V increased from 6.63% to 37.02% in the profile, with BS increments exceeding 700% in the surface and nearly 200% in the 0.4-0.5 m layer. Cation exchange capacity and stocks of Mg and K improved down to 30 cm, while Al reduction reached 20 cm and P increases were limited to the top 10 cm. Soil pH modifications reached 20 cm, whereas increases in K, Cu, Mn, Zn, and B were concentrated in the surface (0.0-0.1 m). Management of amendments and fertilization appeared to have influenced the rate of vertical movement of Al, Ca, Mg, P and S. Overall, longer cultivation periods significantly enhanced chemical fertility throughout the 0.0-0.5 m profile.

Key words: Cerrado, Nutrient leaching, Sandy soils, Soil carbon stocks.

5.1 Introduction

The growing global demand for food has created pressure to convert native areas into agricultural land in countries that still possess native vegetation (Lal, 2018; Searchinger *et al*., 2018; Liang *et al*., 2023). However, the conversion of these natural ecosystems for agricultural use can influence soil carbon dynamics (Lal, 2018; Medeiros *et al*., 2020) and cause changes in organic matter quality (Sá *et al*., 2014). In most cases, the conversion of native areas tends to decrease soil carbon stocks (Lal, 2018; Medeiros *et al*., 2020; Tiefenbacher *et al*., 2021;

Medeiros; Cesário; Maia, 2023; Briedis *et al*., 2023; Liang *et al*., 2023), consequently, cultivation systems that promote C accumulation should be studied and implemented, aiming for long-term agricultural sustainability (Powlson *et al*., 2016; Searchinger *et al*., 2018; Zhu *et al*., 2022; Maia *et al*., 2022). Further, improved soil management practices are crucial to addressing challenges related to climate change (Tiefenbacher *et al*., 2021).

In Brazil, the Cerrado biome (Brazilian Savanna) has become an important agricultural frontier, offering favorable conditions for high-yield crops like soybean (*Glycine max*) and corn (*Zea mays*) (Projeto MapBiomas, 2024). It is characterized by xeromorphic vegetation with deep root systems, an adaptation to approximately six months of drought (Curi, *et al*., 2017), poor soils, and frequent natural fires (Rocha; Nascimento, 2022; Santos; Pereira; Rocha, 2014). This vegetation includes trees and shrubs with twisting branches, thick bark, and shiny leaves, as well as an extensive layer of grasses below (Curi, *et al*., 2017). The predominant climate is seasonal tropical, with two well-defined seasons: a dry period, generally from May to September, and a rainy period, from October to April (Curi, *et al*., 2017). The Cerrado is one of the biomes most affected by human occupation and agricultural expansion in recent years (Valdes; Gillespie; Dohlman, 2023; Lima *et al*., 2019), driven primarily by relatively low land prices and a topography favorable for mechanization (Pires *et al*., 2016).

However, Cerrado soils are typically acidic, with a low phytoavailability of nutrients, sandy (commonly more than 50% sand), and subjected to dry climate conditions, which make cultivation difficult without proper amendments (Donagemma *et al*., 2016), fertilization (Lu; Tian, 2017; Enesi *et al*., 2023), and adequate crop and soil management practices (Maia *et al*., 2022). Soil acidity (pH) is a major constraint on crop productivity in these soils (Agegnehu *et al*., 2021; Oliveira *et al*., 2024), as it directly affects the availability and uptake of essential nutrients by plants (Taiz *et al*., 2017; Agegnehu *et al*., 2021; Enesi *et al*., 2023). The main reason for low productivity in acidic soils is aluminum toxicity combined with low availability of calcium, magnesium, potassium, and phosphorus (Enesi *et al*., 2023). This highlights the need for liming and periodic fertilization to sustain agricultural land use. Modern agriculture has relied on intensive application of fertilizers to achieve high yields (Lu; Tian, 2017; Tiefenbacher *et al*., 2021). Therefore, understanding nutrient dynamics in these soils and how management practices influence nutrients distribution within the soil profile is essential to maintain or increase soil's productivity while reducing fertilizer use (Hou, 2023), thereby lowering production costs (Valdes; Gillespie; Dohlman, 2023).

Our hypothesis is that soil fertility will be increased over time with cultivation, even in subsurface layers, after habitat conversion from native forest to agricultural land in soils with a

low nutrient content, such as those found in Cerrado biome in Brazil, due to the continuous application of nutrients and the subsequent downward migration of these elements. Additionally, we hypothesized that the effect will be more evident in deeper soil layers with time after habitat conversion. Based on that, the main objective of this study was to evaluate alterations in soil chemical indicators of soil fertility as a function of the cultivation time within the 0.0-0.5 m layer of a Yellow Latosol (Oxisol) under Cerrado vegetation in northeastern Brazil. For that, four different fields (from native to 12, 18 and 24 years after cultivation) were selected.

5.2 Materials and Methods

5.2.1 Study area location and climate

The study was conducted at the *Chapada do Céu* farm (07°40’44” S; 44°04’16” W; 445 m altitude), located in the state of Piauí, northeastern Brazil, within the Cerrado biome. Soil samples were collected along a chronosequence consisting of four distinct areas: one uncultivated (native) area and three areas under cultivation for 12, 18, and 24 years (Figure 10).

The native vegetation consists of xeromorphic species highly adapted to nutrient-poor soils under frequent natural fires (Santos; Pereira; Rocha, 2014; Rocha; Nascimento, 2022). This vegetation is characterized by gnarled trees reaching up to 20 m, thick-barked shrubs, and a high diversity of herbaceous species with deep root systems (Curi *et al*., 2017), representing the native Cerrado with no apparent human intervention. The region has an average annual temperature of 26.7 °C and an average annual rainfall of 1015 mm (Climate-Data, 2025) on the edge of the semi-arid climate. Monthly rainfall and temperature indices for Sebastião Leal, the municipality where the study site is located, are shown in Figure 10.

Due to the rainfall distribution, local farmers typically sow soybean, the region's main crop of economic interest (Projeto MapBiomas, 2024), or other crops such as corn and cotton (*Gossypium hirsutum L.*) between October and December, with harvesting occurring between February and July of the following year. Thus, the agricultural year begins in August and ends in July of the subsequent year. Figure 11 presents the results of the *in-situ* morphological evaluation of the four studied soil profiles.

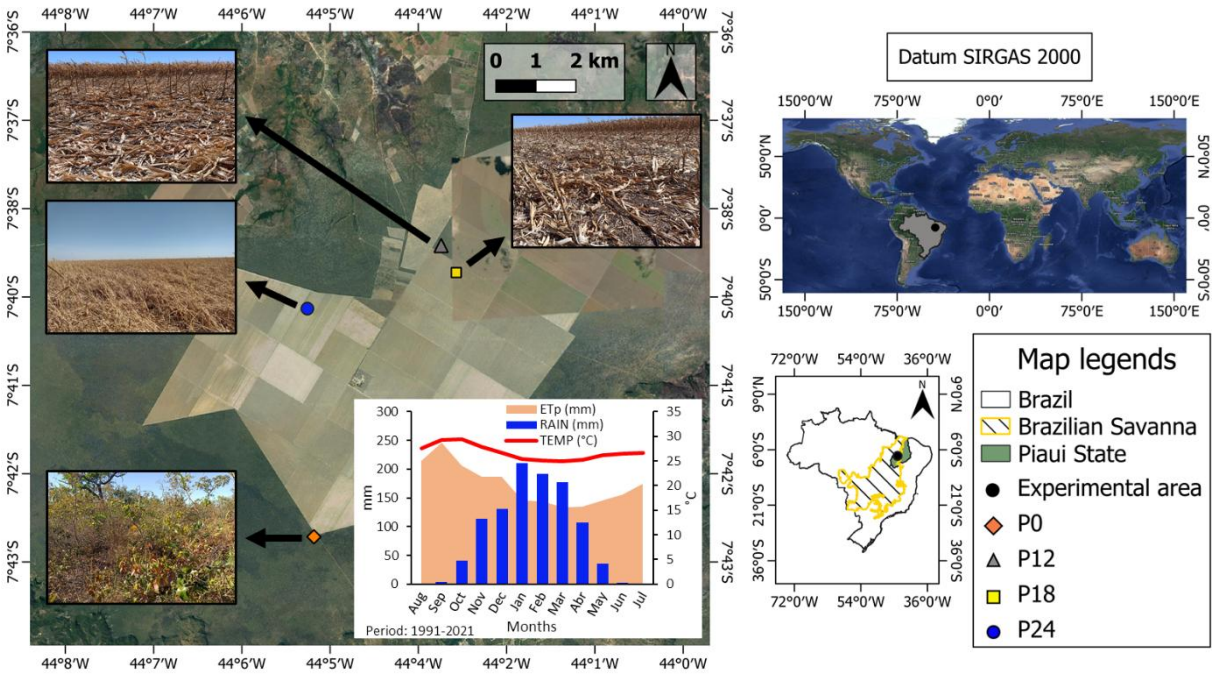
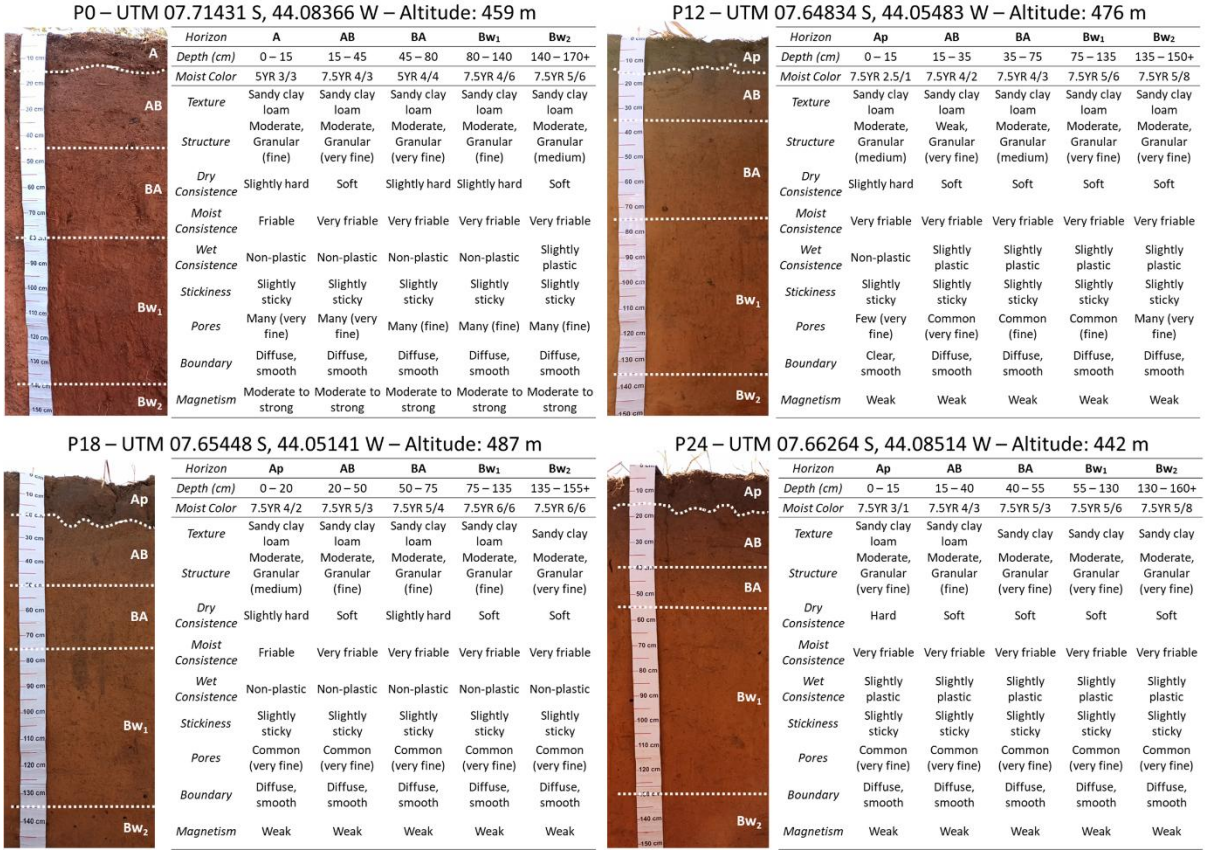


Figure 10 - Location and soil cover conditions from native vegetation (P0) and cultivated areas under 12 (P12), 18 (P18), and 24 (P24) years of habitat conversion from native forest to agricultural land, and historical averages of evapotranspiration (ETp), rainfall (RAIN) and temperature (TEMP) in the studied region.



P0 – UTM 07.71431 S, 44.08366 W – Altitude: 459 m

Horizon	A	AB	BA	Bw_1	Bw_2
Depth (cm)	0 – 15	15 – 45	45 – 80	80 – 140	140 – 170+
Moist Color	5YR 3/3	7.5YR 4/3	5YR 4/4	7.5YR 4/6	7.5YR 5/6
Texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Structure	Moderate, Granular (fine)	Moderate, Granular (very fine)	Moderate, Granular (very fine)	Moderate, Granular (fine)	Moderate, Granular (medium)
Dry Consistence	Slightly hard	Soft	Slightly hard	Slightly hard	Soft
Moist Consistence	Friable	Very friable	Very friable	Very friable	Very friable
Wet Consistence	Non-plastic	Non-plastic	Non-plastic	Non-plastic	Slightly plastic
Stickiness	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky
Pores	Many (very fine)	Many (very fine)	Many (fine)	Many (fine)	Many (fine)
Boundary	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth
Magnetism	Moderate to strong	Moderate to strong	Moderate to strong	Moderate to strong	Moderate to strong

P12 – UTM 07.64834 S, 44.05483 W – Altitude: 476 m

Horizon	Ap	AB	BA	Bw_1	Bw_2
Depth (cm)	0 – 15	15 – 35	35 – 75	75 – 135	135 – 150+
Moist Color	7.5YR 2.5/1	7.5YR 4/2	7.5YR 4/3	7.5YR 5/6	7.5YR 5/8
Texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Structure	Moderate, Granular (medium)	Weak, Granular (very fine)	Moderate, Granular (medium)	Moderate, Granular (very fine)	Moderate, Granular (very fine)
Dry Consistence	Slightly hard	Soft	Soft	Soft	Soft
Moist Consistence	Very friable	Very friable	Very friable	Very friable	Very friable
Wet Consistence	Non-plastic	Slightly plastic	Slightly plastic	Slightly plastic	Slightly plastic
Stickiness	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky
Pores	Few (very fine)	Common (very fine)	Common (fine)	Common (fine)	Many (very fine)
Boundary	Clear, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth
Magnetism	Weak	Weak	Weak	Weak	Weak

P18 – UTM 07.65448 S, 44.05141 W – Altitude: 487 m

Horizon	Ap	AB	BA	Bw_1	Bw_2
Depth (cm)	0 – 20	20 – 50	50 – 75	75 – 135	135 – 155+
Moist Color	7.5YR 4/2	7.5YR 5/3	7.5YR 5/4	7.5YR 6/6	7.5YR 6/6
Texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay
Structure	Moderate, Granular (medium)	Moderate, Granular (fine)	Moderate, Granular (fine)	Moderate, Granular (fine)	Moderate, Granular (very fine)
Dry Consistence	Slightly hard	Soft	Slightly hard	Soft	Soft
Moist Consistence	Friable	Very friable	Very friable	Very friable	Very friable
Wet Consistence	Non-plastic	Non-plastic	Non-plastic	Non-plastic	Non-plastic
Stickiness	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky
Pores	Common (very fine)	Common (very fine)	Common (very fine)	Common (very fine)	Common (very fine)
Boundary	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth
Magnetism	Weak	Weak	Weak	Weak	Weak

P24 – UTM 07.66264 S, 44.08514 W – Altitude: 442 m

Horizon	Ap	AB	BA	Bw_1	Bw_2
Depth (cm)	0 – 15	15 – 40	40 – 55	55 – 130	130 – 160+
Moist Color	7.5YR 3/1	7.5YR 4/3	7.5YR 5/3	7.5YR 5/6	7.5YR 5/8
Texture	Sandy clay loam	Sandy clay loam	Sandy clay	Sandy clay	Sandy clay
Structure	Moderate, Granular (very fine)	Moderate, Granular (fine)	Moderate, Granular (very fine)	Moderate, Granular (very fine)	Moderate, Granular (very fine)
Dry Consistence	Hard	Soft	Soft	Soft	Soft
Moist Consistence	Very friable	Very friable	Very friable	Very friable	Very friable
Wet Consistence	Slightly plastic	Slightly plastic	Slightly plastic	Slightly plastic	Slightly plastic
Stickiness	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky	Slightly sticky
Pores	Common (very fine)	Common (very fine)	Common (very fine)	Common (very fine)	Common (very fine)
Boundary	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth	Diffuse, smooth
Magnetism	Weak	Weak	Weak	Weak	Weak

Figure 11 - *In situ* evaluation of the soil profiles from native vegetation (P0) and cultivated areas under 12 (P12), 18 (P18), and 24 (P24) years.

5.2.2 Study areas characterization and land-use history

The four study areas are located on flat terrain and characterized by a sandy clay loam texture in the top 20 cm. The average granulometric composition (sand, silt, and clay) and physical properties, soil bulk density (BD), particle density (PD), soil penetration resistance (SPR), macroporosity (MA), microporosity (MI), and total porosity (TP) for this layer are summarized in Table 5. These soils are derived from sedimentary rocks of the Piauí Formation, Balsas Group (Pfaltzgraff; Torres; Brandão, 2010). At the time of sampling, they were classified as *Latossolo Amarelo Distrófico* according to the Brazilian Soil Classification System (Santos *et al*., 2018), which corresponds to the Oxisol order in the US Soil Taxonomy (Soil Survey Staff, 2022).

Table 5 - Means of granulometric properties (Sand, Silt and Clay), soil bulk density (BD), particle density (PD), soil penetration resistance (SPR), macroporosity (MA), microporosity (MI) and total porosity (TP) for native vegetation (P0) and areas cultivated for 12 (P12), 18 (P18), and 24 years (P24) under 0-20 cm layer.

Study areas	Sand	Silt	Clay	Texture*	BD	PD	SPR	MA	MI	TP
	g kg^{-1}	g kg^{-1}	g kg^{-1}		g cm^{-3}	g cm^{-3}	MPa	cm^3 cm^{-3}	cm^3 cm^{-3}	cm^3 cm^{-3}
P0	748	34	218	Sandy clay loam	1.38	2.53	0.73	0.25	0.20	0.45
P12	730	34	236	Sandy clay loam	1.41	2.46	1.34	0.19	0.24	0.43
P18	702	28	270	Sandy clay loam	1.52	2.51	2.10	0.13	0.26	0.39
P24	627	43	330	Sandy clay loam	1.41	2.54	1.38	0.17	0.27	0.44

* Classification according Santos *et al*. (2018).

The cultivated areas (P12, P18, and P24) were managed under a minimum tillage system, with soil disturbance limited to essential decompression (Brevilieri *et al*., 2024) via scarification (25-35 cm depth) or the incorporation of soil amendments using a leveling harrow (5-15 cm depth). In most seasons, planting was performed without prior tillage. Agriculture in the region follows two annual cycles, the first season (planted between October and December) focuses on maximizing economic return, while the second (planted between February and April) utilizes the remaining rainfall for less water-demanding crops or biomass production for soil protection and nutrient cycling (Carvalho; Nascente, 2014). In this study, cover cropping was implemented twice in each cultivated area. Since the 2008/2009 season, the second season has been established in all cultivated areas, predominantly with millet (*Pennisetum glaucum*).

The native vegetation remained undisturbed by human intervention and received no nutrient inputs. In contrast, the cultivated areas received significant fertilization (Table 6), based on annual soil analyses and technical recommendations (Sousa; Lobato, 2004).

Table 6 - Average annual macro and micronutrient inputs for native vegetation (P0) and cultivated areas for 12 (P12), 18 (P18) and 24 (P24) years.

Study areas	kg ha^{-1} year^{-1}						g ha^{-1} year^{-1}				
	Ca	Mg	K	P	S	N	Fe	Mn	Zn	B	Cu
P0	-	-	-	-	-	-	-	-	-	-	-
P12	84.17	97.99	73.03	33.19	66.58	26.14	60.13	283.57	309.21	254.19	53.02
P18	68.60	88.41	78.20	33.69	54.25	19.86	105.42	230.88	531.70	227.26	47.11
P24	74.76	60.78	74.09	37.45	59.18	11.70	190.81	276.56	776.98	309.89	65.56

Upon the conversion of native areas to agricultural land, intense soil disturbance occurred, following the addition of correctives. This soil disturbance was necessary for the incorporation of these correctives into the arable layer (0.0-0.2 m). This is a typical management practice for farmers in the region, which aims to create a suitable environment for soybean production (Oliveira *et al*., 2024), the region's main crop of economic interest (Projeto MapBiomas, 2024). This intense soil disturbance was divided into two stages. The first occurred immediately following deforestation, with limestone being incorporated using a plowing harrow (± 18 cm) for three passes, followed by two passes with a leveling harrow (± 10 cm). The second stage occurred in the subsequent year, again with the addition of limestone incorporated with a plowing harrow for two passes, followed by scarification at 0.35 m for two passes and a leveling harrow for two passes.

Management practices across the cultivated areas were largely similar, however, the area cultivated for 12 years received the full recommended dose of limestone during the initial soil preparation (6.8 Mg ha^{-1}) (Sousa; Lobato, 2004), whereas the 18 and 24-year-old areas received split applications between the initial preparation (3.5 and 3.0 Mg ha^{-1}, respectively) and the subsequent year (3.5 and 2.0 Mg ha^{-1}, respectively). Total lime applications reached 13.30, 18.00, and 16.50 Mg ha^{-1} for the areas cultivated for 12, 18, and 24 years, respectively, with gypsum applications of 4.50, 5.50, and 8.00 Mg ha^{-1}, respectively. Dolomite limestone was used to both neutralize toxic aluminum (Al) for plants and to provide them with calcium (Ca) and magnesium (Mg) (Sousa; Lobato, 2004). Gypsum supplied the necessary sulfur (S) and helped correct the subsurface soil (Oliveira *et al*., 2024; Raij, 2008).

During the sampling season, all cultivated areas received a formulated fertilizer for B and Zn correction. However, the specific product used in the 12-year-old field contained S as a trace component of its formulation, resulting in an application of 0.34 kg ha^{-1} of S. Conversely, the remaining cultivated areas utilized a different fertilizer brand for B and Zn correction which did not contain S, meaning the 0.34 kg ha^{-1} of S application was exclusive to the 12-year-old field. Following initial preparation, most fertilizers and soil amendments were surface-applied without incorporation. Phosphate fertilization was applied in the planting furrow until the 2012/13 season, thereafter, all fertilization was surface-applied (Figure 12A).

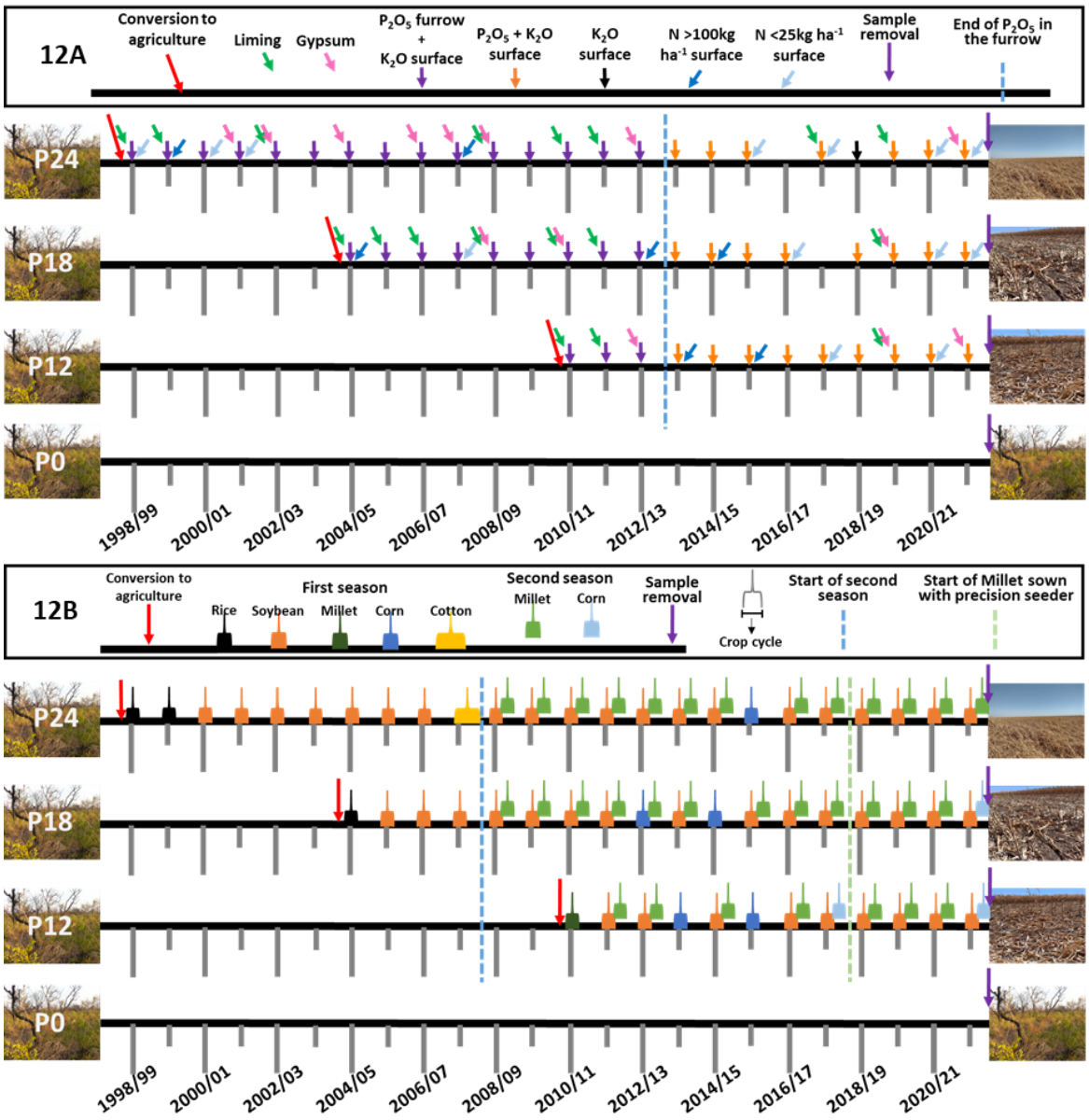


Figure 12 - Fertilization history per season (12A) and history of crops cultivated during the first and second seasons (12B) in the native vegetation (P0) and in areas under cultivation for 12 (P12), 18 (P18), and 24 (P24) years.

Regarding crop management, all areas were farmed without soil disturbance for the main crop after the initial preparation, and after the 2017/2018 season, the second season was also managed under no-till (Figure 12B). The differences for the first season were that the area cultivated for 24 years was planted with cotton (*Gossypium hirsutum*) once (during the 2007/08 season), whereas the other areas were not. Maize (*Zea mays*) was also planted only once in this area (during the 2015/16 season), while it was planted twice in the areas cultivated for 12 and 18 years. During the second growing season, further differences were observed: maize was not planted in the 24-year-old field, whereas it was planted once in the 18-year-old and twice in the 12-year-old fields (Figure 12B). The native vegetation shows no relevant human intervention.

5.2.3 Soil sampling and laboratory analysis

Samples were collected from four soil pits (one per study area), measuring approximately 3.0 × 3.0 × 1.5 m. Within each pit, three replicates were collected (at 50 cm intervals) as both disturbed and undisturbed samples at depths of 0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, and 0.4-0.5 m. Sampling occurred in August 2022. To avoid edge effects and machinery compaction, pits were located approximately 100 m within each plot. Due to the commercial nature of the fields, sampling was concentrated in these pits rather than distributed across the entire area to ensure minimal interference with farm operations. Samples were stored in plastic bags, air-dried, and sieved (< 2 mm) for analysis.

Soil pH was measured in water (1:2.5 ratio) (Teixeira *et al*., 2017). Total organic carbon (TOC) was determined by the modified Walkley-Black method, using potassium dichromate (K_2Cr_2O7) oxidation and quantified via colorimetry at 650 nm. The conversion of TOC to soil organic matter (SOM) was performed by multiplying the TOC by 1.724 (Machado; Bernardi; Santos, 2003). Available Potassium (K), Sodium (Na), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), and Phosphorus (P) were extracted with Mehlich^{-1} (0.0125 mol L^{-1} H_2SO_4 and 0.050 mol L^{-1} HCl), while Calcium (Ca), Magnesium (Mg), and Aluminum (Al) were extracted with 1 mol L^{-1} KCl. K and Na were determined by flame photometry, P by spectrophotometry at 660 nm, Ca, Mg, Cu, Fe, Mn, and Zn by atomic absorption, and Al by titration (Teixeira *et al*., 2017). Potential acidity (H+Al) was extracted with a 0.5 mol L^{-1} calcium acetate solution and determined by titration. Sulfur (S) was determined by spectrophotometry at 420 nm after extraction and filtration (Teixeira *et al*., 2017), and boron (B) was extracted with hot water and, after centrifugation, determined by spectrophotometry at 540 nm (Tedesco *et al*., 1995).

Following the analyses, the bases sum (BS), potential cation exchange capacity (CEC), and base saturation (V) were calculated (Teixeira *et al*., 2017). The contents of Ca, Mg, and K were converted to mg dm^{-3} using their respective atomic masses and valences. Subsequently, the stocks of Ca, Mg, K, P, S, C, Fe, Mn, Zn, B and Cu were calculated by multiplying the concentration of each element by the soil bulk density and the corresponding thickness of each evaluated soil layer (Bayer *et al*., 2000).

Particle size fractions were determined by slow agitation (16 h) using 0.1 mol L^{-1} NaOH as a chemical dispersant. The sand fraction was separated by a 0.053 mm sieve, and the clay and silt fractions by sedimentation based on Stokes' Law (Teixeira *et al*., 2017). Undisturbed soil samples were used to quantify soil porosity, performed using a Richards extractor, and soil bulk density, performed using the volumetric cylinder method (Teixeira *et al*., 2017). Particle density was calculated as the ratio between total porosity and soil bulk density (Teixeira *et al*., 2017), and soil penetration resistance was measured with an IAA/Planalsucar impact penetrometer with a 30° cone angle.

5.2.4 Experimental design and statistical analysis

This study employed an observational sampling approach. The structure consisted of four land-use times (0, 12, 18, and 24 years) and five soil depths (0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, and 0.4-0.5 m), with three replicates per depth per area. Given the non-experimental, observational nature of the chronosequence, we prioritized the use of 95% confidence intervals (CI) for comparisons rather than traditional analysis of variance. Differences were considered significant when confidence intervals did not overlap, whereas overlapping intervals were interpreted as indicating no clear difference. Analyses were performed using R (version 4.3.1) and RStudio (Build 443).

5.4 Results

Available sodium stocks were zero in all soil layers and treatments, so this variable was excluded from the results and discussion. Soil organic matter (SOM), base sum (BS), and base saturation (V) exhibited significant differences among areas throughout the entire evaluated profile (0.0-0.5 m) (Appendix 4). In contrast, differences in soil pH (water) and cation exchange capacity (CEC) were primarily concentrated in the upper layers (up to 30 cm), while

exchangeable Aluminum (Al^{3+}) and for potential acidity (H+Al) showed differences restricted to the 20 cm and 10 cm depths, respectively (Figure 13).

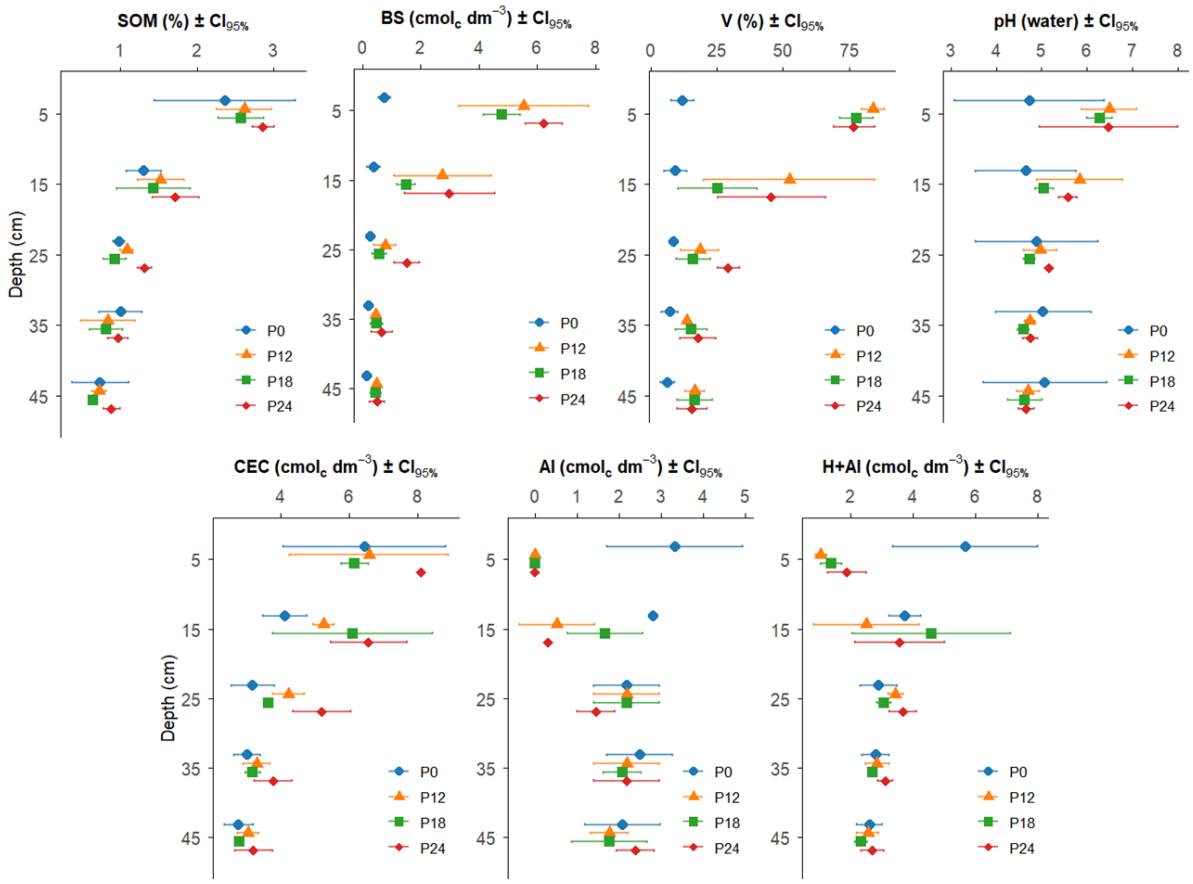


Figure 13 - Means ± 95% confidence interval for Soil Organic Matter (SOM, %), Bases Sum (BS, $cmol_c\ dm^{-3}$), Base saturation (V, %), pH (pH, water), Cation Exchange Capacity (CEC, $cmol_c\ dm^{-3}$), Aluminum (Al, $cmol_c\ dm^{-3}$) and Potential acidity (H+Al, $cmol_c\ dm^{-3}$) in native vegetation (P0) and cultivated areas for 12 (P12), 18 (P18), and 24 (P24) years at the 0-50 cm soil depth.

SOM content increased with cultivation time across the entire soil profile. Notably, the 24-year-old area consistently maintained higher SOM levels than the native vegetation down to a depth of 30 cm. CEC followed a vertical distribution pattern similar to SOM, with cultivated areas exhibiting higher values than P0, particularly within the 0.1-0.3 m range (Figure 13). As expected for Oxisols, SOM and consequently CEC values decreased with depth across all areas (Sá; Lal, 2009; Mandal *et al*., 2022), with the surface layer always registering the highest values and the 0.4-0.5 m layer the lowest for both.

SB and V were significantly higher in the cultivated areas than in the native vegetation across all soil layers, reflecting the cumulative input of exchangeable bases from agricultural management. Both variables clearly distinguished the managed systems from the natural

ecosystem. No significant differences were observed among cultivated areas for V in the superficial layers (0.0-0.2 m). For SB, however, the 24-year-old field was superior to the 18-year-old field in the 0.0-0.1 m layer, while the 12-year-old field remained statistically similar to both. In the 0.2-0.3 m layer, 24-year-old field was again superior to 18-year-old for both variables, with 12-year-old field once more exhibiting values similar to both areas (Figure 13). In all treatments, SB and V tended to decrease with increasing soil depth, except for V under native vegetation, which showed no distinct differences among layers.

Cultivated areas consistently exhibited higher pH and lower Al^{3+} levels than the native vegetation down to 20 cm depth. In the native area, Al^{3+} content decreased with depth, whereas cultivated areas displayed an inverse pattern, with Al^{3+} levels increasing in deeper layers. Regarding potential acidity (H+Al), differences were most pronounced at the surface (0.0-0.1 m), where native vegetation exhibited significantly higher values than all cultivated areas. Within the managed systems, the surface layer showed the highest pH and the lowest H+Al compared to the subsoil (Figure 13). While pH remained constant across the profile in the native vegetation, it showed a decreasing trend with depth in the cultivated areas, reflecting the stratification of liming effects. Conversely, native vegetation showed a decreasing trend for H+Al with depth, despite its stable pH values.

Calcium (Ca Stock) and Sulfur (S Stock) stocks differed among areas throughout the entire soil profile (0.0-0.5 m), following patterns similar to those observed for SOM, SB, and V. In contrast, Magnesium (Mg Stock) and Potassium (K Stock) stocks showed significant differences only down to 30 cm depth (Figure 14).

Both Ca Stock and Mg Stock exhibited a clear decreasing trend with soil depth across all areas. Cultivated fields consistently presented higher Ca Stock than the native vegetation across all soil layers, whereas higher Mg Stock in cultivated areas was restricted to the upper 30 cm. No significant differences were observed among cultivation times for Ca Stock and Mg Stock in the superficial layer (0.0-0.1 m) or down to 30cm depth. However, similar to the results for SB and V, the numerical values for Ca Stock and Mg Stock in the 12-year-old field were higher than those in the 18-year-old field in the 0.1-0.2 m and 0.2-0.3 m layers (Figure 14), suggesting that variations in liming history influenced these results (Oliveira *et al*., 2024).

S Stock displayed a contrasting vertical distribution compared to Ca Stock and Mg Stock. In cultivated areas, S Stock tended to increase with soil depth, whereas no clear vertical differentiation was observed under native vegetation. In the 0.1-0.2 m layer, all areas showed similar S Stock. However, below this depth, cultivated areas consistently exhibited higher S

Stock than the native vegetation, highlighting the cumulative effect of long-term fertilization on sulfur accumulation in deeper soil layers (Figure 14).

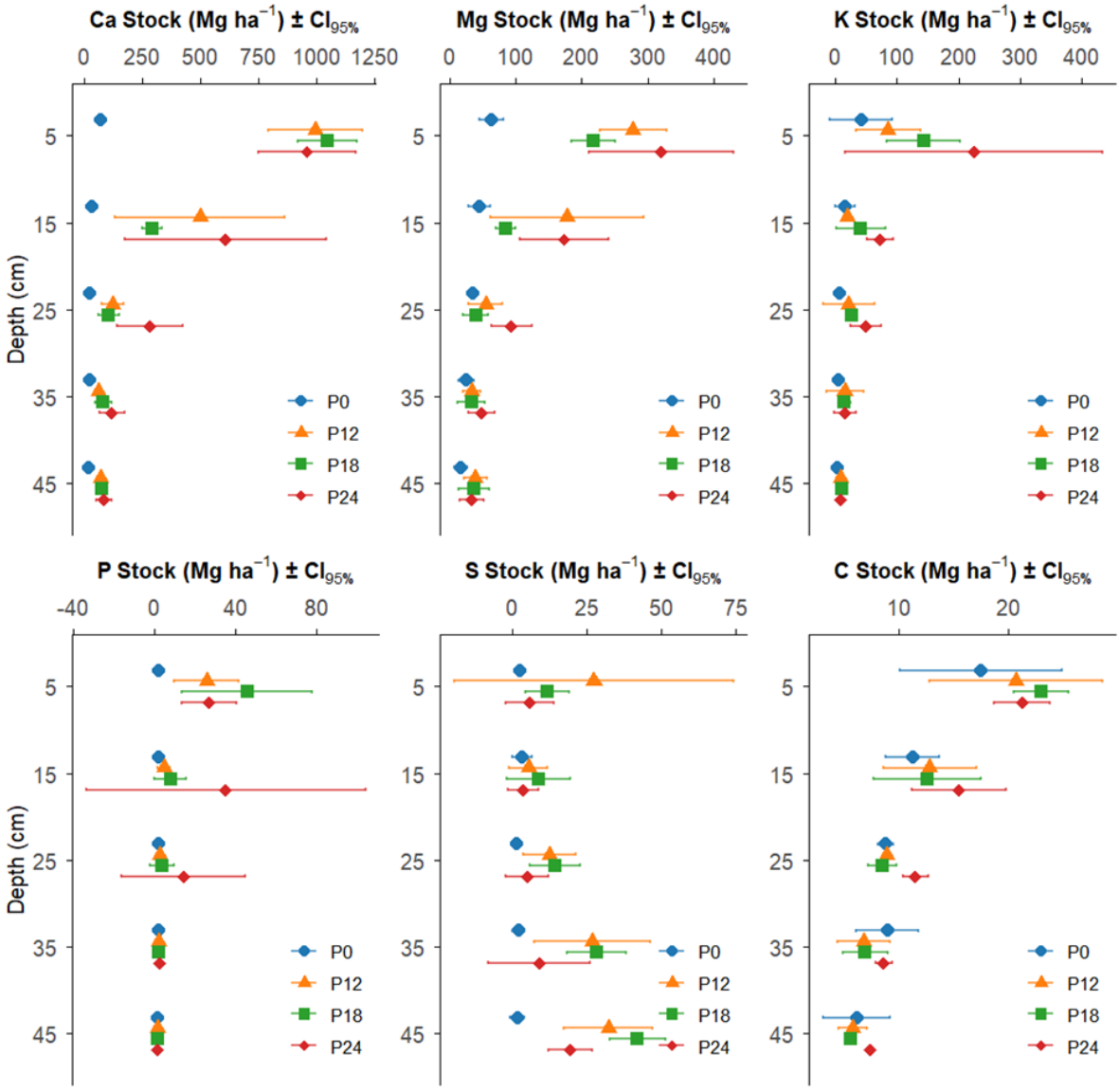


Figure 14 - Means ± 95% confidence interval for Calcium stock (Ca Stock, Mg ha^{-1}), Magnesium stock (Mg Stock, Mg ha^{-1}), Potassium stock (K Stock, Mg ha^{-1}), Phosphorus stock (P Stock, Mg ha^{-1}), Sulfur stock (S Stock, Mg ha^{-1}) and Carbon stock (C Stock, Mg ha^{-1}) in native vegetation (P0) and cultivated areas for 12 (P12), 18 (P18), and 24 (P24) years at the 0-50 cm soil depth.

K Stock tended to increase with cultivation time in the upper layers, with significant differences among areas observed down to 30 cm. Below 20 cm, K Stock was statistically similar across all treatments. In cultivated fields, K Stock decreased with increasing soil depth, a common pattern in tropical soils (Mandal *et al.*, 2022) probably due to the concentration of

fertilization and recycling in the surface, whereas the native vegetation showed no clear vertical differentiation (Figure 14).

Carbon (C Stock) and Phosphorus (P Stock) stocks exhibited patterns similar to those observed for SOM and CEC, with higher values associated with increasing cultivation time down to 30 cm depth (Figure 14). C Stock consistently decreased with increasing soil depth across all study areas. For P Stock, this decreasing trend with depth was evident only in cultivated areas (Mandal *et al*., 2022), while the native vegetation showed no clear differences among soil layers. In the surface layer (0.0-0.1 m), like the Ca Stock and Mg Stock, cultivated areas presented higher P Stock than the native vegetation (Perin; Ceretta; Klamt, 2003), but no consistent differences were observed among cultivation times. Notably, an increase in P Stock was observed in the 0.1-0.2 m layer with increasing cultivation time. Furthermore, the 24-year-old field exhibited higher means of P stocks in the 0.2-0.3 m layer compared to the other areas. This result underscores the cumulative effect of 15 years of furrow fertilization in this area, compared to 9 years in the 18-year-old field and only 3 years in the 12-year-old, reflecting the buildup of P over decades of continuous agricultural management.

A general tendency toward increasing stocks for most micronutrients (Mn, Zn, B, and Cu) was observed in the superficial layer (0.0-0.1 m) with increasing cultivation time (Figure 15). This trend highlights the cumulative effect of long-term fertilization in this layer without soil incorporation, as well as the low mobility of these nutrients in the soil (Santos *et al*., 2021). Below the surface layer, no significant differences in micronutrient levels were observed with increasing cultivation time.

In the topsoil (0.0-0.1 m), the 24-year-old field exhibited significantly lower Iron stocks (Fe Stock) than the native vegetation, but significantly higher stocks for Manganese (Mn Stock) and Zinc (Zn Stock). While the mean values for Mn Stock increased numerically with cultivation time in this layer, all managed areas were statistically similar due to overlapping confidence intervals. For Zn Stock in this layer, 12-year-old field and native vegetation were similar, while the 18-year-old field showed intermediate values that overlapped both native vegetation and areas cultivated for 12 and 24 years. Regarding Boron (B Stock) and Copper (Cu Stock) stocks, no significant differences were detected among the study areas in the surface layer, reflecting high spatial variability in these nutrient stocks (Figure 15).

Regarding vertical distribution, Mn Stock tended to decrease with increasing profile depth across all study areas. However, this vertical stratification was statistically significant only for Zn Stock in the 12-year-old and 24-year-old fields, and for B Stock specifically in the

18-year-old field. Furthermore, Fe Stock exhibited a clear decreasing trend as cultivation time increased in the soil layers below 30 cm depth.

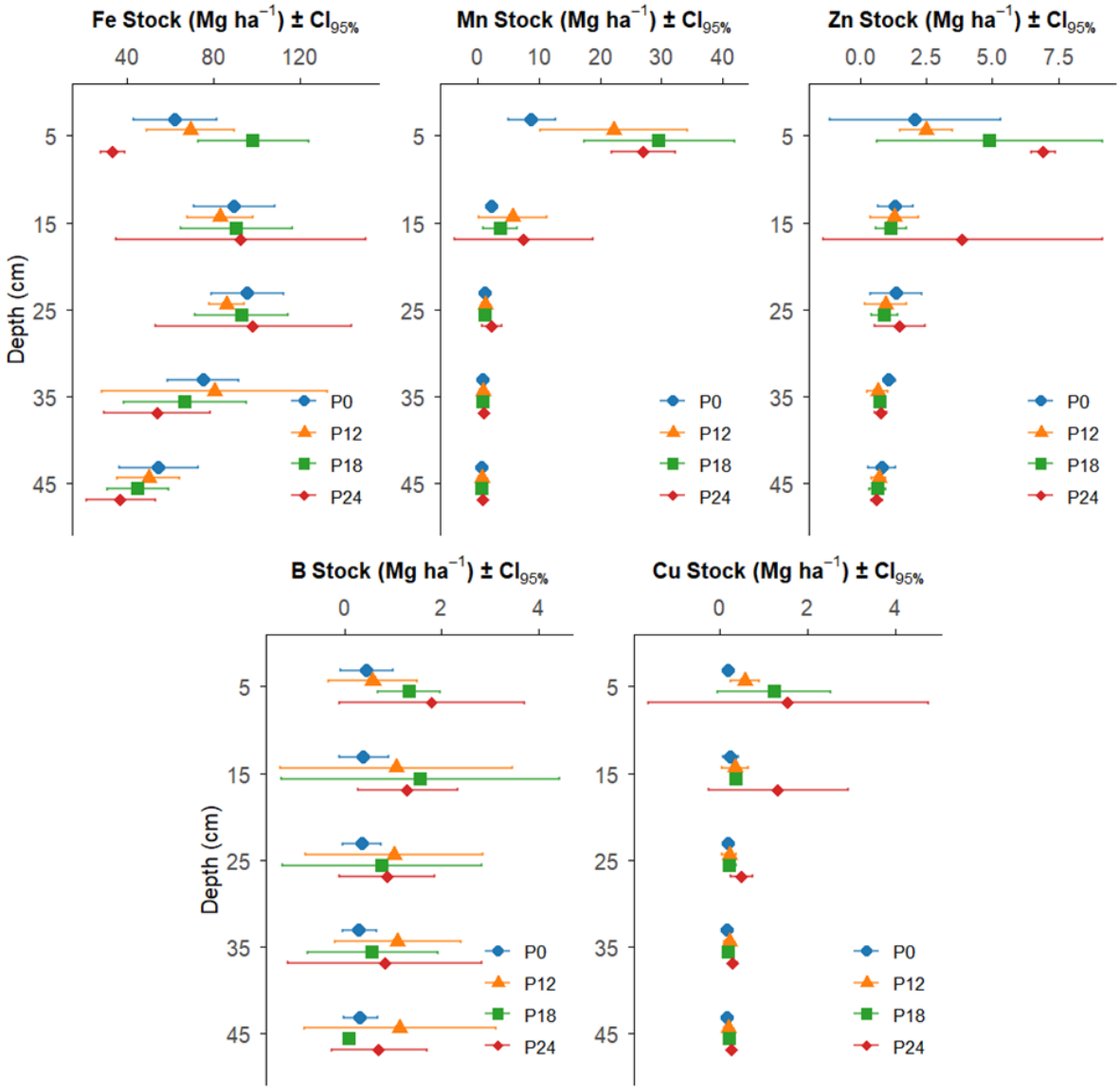


Figure 15 - Means ± 95% confidence interval for Iron stock (Fe Stock, Mg ha^{-1}), Manganese stock (Mn Stock, Mg ha^{-1}), Zinc stock (Zn Stock, Mg ha^{-1}), Boron stock (B Stock, Mg ha^{-1}) and Copper stock (Cu Stock, Mg ha^{-1}) in native vegetation (P0) and cultivated areas for 12 (P12), 18 (P18), and 24 (P24) years at the 0-50 cm soil depth.

5.5 Discussion

Our results support the hypothesis that the fertility of Cerrado soils increases with cultivation time. They also partially support the idea that this improvement extends deeper into the profile as cultivation time increases (Figures 13, 14 and 15). The nutrient dynamics shown here are the result of a complex interaction between agricultural management (including soil

management, amendments, and fertilization), cultivation time (ranging from native forest to agricultural land), and a humid climate (with an annual rainfall of around 1000 mm), which strongly promotes nutrient leaching in sandy soils characteristic of the Cerrado biome. The evolution of soil chemical properties from the native system (uncultivated Brazilian Cerrado) to agricultural systems cultivated for up to 24 years shows clear trends based on both soil depth (mainly up to 30 cm) and cultivation time.

The observed increase in SOM with cultivation time in the upper to 20 cm, and particularly down to 30 cm after 24 years of cultivation (Figure 13), should have been facilitated by liming and gypsum applications. These amendments supplied Ca^{+2} and Mg^{+2} to the soil which, at high concentrations in the first soil layers, were able to chemically replace Al^{+3} from exchange sites, reducing Al toxicity and enhancing soil fertility (Perin; Ceretta; Klamt, 2003). In this respect, the observed decrease in Al^{+3} in the first 20 cm of soil depth (12, 18, and 24 years under cultivation) and 30 cm of soil depth (only after 24 years), should have allowed plants to develop their root systems (Tiefenbacher *et al*., 2021) and accumulate C compounds, through root exudates and necromass after root decomposition (Figueiredo *et al*., 2018), thereby enhancing the SOM content (Sá; Lal, 2009; Martínez *et al*., 2016; Singh; Ghimire; Acharya, 2024).

The dry season, which occurs in native Cerrado soils in Piauí, inhibits biomass production (Figueiredo *et al*., 2018), and these acidic soils (pH < 5) where Al toxicity is common, limit plant root growth and microbial activity (Tiefenbacher *et al*., 2021). Furthermore, these soils have a reduced capacity to retain water and nutrients due to their sandy texture (Yost; Hartemink, 2019) (Table 5), explaining their relatively low and easily oxidizable organic matter content (Souza *et al*., 2019). In these sandy soils, where the mineral fraction contributes little to nutrient retention, SOM plays a fundamental role as the primary source of cation exchange sites. Thus, the higher SOM content in soils that have been cultivated for a longer period explains part of the observed increase in CEC (Sá; Lal, 2009; Maia *et al*., 2022; Huang *et al*., 2022), most markedly after 24 years of cultivation, which shows the highest CEC values up to 30 cm. The 12-year-old and 18-year-old agricultural fields also showed increasing CEC values, though more moderately than the 24-year-old field. The lack of significant differences found between cultivated fields and the native soils below 30 cm soil depth suggests that a gradual improvement in soil quality with increasing cultivation time occurred in these soils found in Cerrado (Chatterjee *et al*., 2018) and, a limited effect on the first centimeters of the soil (Briedis *et al*., 2023; Medeiros; Cesário; Maia, 2023).

The uncultivated soil had a low pH (< 5) and a high potential acidity (H+Al) (Souza *et al*., 2019), typical of tropical soils where high precipitation promotes the leaching of base cations. In cultivated soils, the pH increases over time, reaching up to 6.5 in the top 10 cm, although this improvement was limited to the top 20 cm. Liming with a mixture of calcium and magnesium carbonates not only neutralizes soil acidity but also provides a slow-release source of Ca^{2+} and Mg^{2+} (Donagemma *et al*., 2016; Agegnehu *et al*., 2021; Ejigu *et al*., 2023). This could restrict its effect to the upper layers of the soil profile, but the application of gypsum, with a higher solubility, supplies Ca that could have been transported to deeper soil layers more quickly with rainfall than with liming (Perin; Ceretta; Klamt, 2003; Raij, 2008; Oliveira *et al*., 2024), as gypsum's solubility is higher than calcium and magnesium carbonates, contributing to Al replacement in the soil cation exchange sites.

Interestingly, the initial management seems to have influenced the speed of subsurface correction. The 12-year-old field, which received a higher initial dose of limestone, showed higher pH, Ca and Mg stocks in the 0.1-0.2 m layer than the 18-year-old field, and was also similar to the 24-year-old field for Mg Stock at 0.2-0.3 m layer (Figure 14). Oliver *et al*. (2021), studying sandy soils in Australia, and Mkhonza, Buthelezi-Dube and Muchaonyerwa (2020), studying humic soils in South Africa, found similar results, where higher doses led to greater pH correction in the subsurface layers. This was also observed by Oliveira *et al*. (2024) in an Oxisol near the study area. This suggests that applying larger amounts of limestone at once may be crucial for accelerating subsurface correction without soil disturbance, thereby promoting subsoil amelioration with minimal disruption (Enesi *et al*., 2023).

Additionally, the sequential application of lime and gypsum resulted in a more pronounced decrease in toxic Al in the upper layers (0-20 cm) of all cultivated fields. This effect extended down to 30 cm in the 24-year-old cultivated field, where long-term amendment allowed for greater downward movement within the soil profile. However, it should be highlighted that H+Al was only significantly reduced in the top 10 cm, where soil pH was increased and where the direct contact with limestone was greatest.

The increase in base cations (Ca, Mg, and K) reflects the combined application of dolomitic lime and fertilizers across all cultivated fields (Figure 14). The contribution to Mg stocks is particularly relevant in these soils, which are characterized by low Mg contents, as dolomitic limestone serves as an important source of this macronutrient, essential for chlorophyll synthesis and enzyme activation (Taiz *et al*., 2017). The co-application of both Ca and Mg through dolomitic limestone should have helped stabilize base saturation (V) levels, particularly after 24 years of cultivation (Perin; Ceretta; Klamt, 2003). K, being more mobile

than Ca and Mg, increased down to 30 cm with cultivation time (Figure 14), but remains vulnerable to leaching in this sandy, high-rainfall environment, reinforcing the need for continuous fertilization under the edaphoclimatic conditions evaluated in our study (sandy, acidic soils that receive around 1000 mm per year).

In the cultivated fields, higher concentrations of Ca, Mg, K, SB, and V were measured down to 30 cm (Figures 13 and 14), indicating cumulative retention in the soil exchange sites over time (Perin; Ceretta; Klamt, 2003). This was aided by improved CEC (related to the observed increase in SOM) and the regular application of limestone, gypsum, and fertilizers. It should be noted that, on some occasions, the 12-year-old field had higher soil chemical properties to the 18-year-old fields, especially those related to soil amendments (BS, V, pH, Ca and Mg). This could be related to the idea that applying larger quantities of correctives can influence the downward movement of nutrients and increase fertility even in deeper layers (Oliver *et al*., 2021; Oliveira *et al*., 2024). Although the pH correction did not extend beyond 20 cm, the correction of Al reached 30 cm in the 24-year-old cultivated area.

For P Stock all cultivated areas are superior to native vegetation in the superficial layer (Perin; Ceretta; Klamt, 2003). However, P Stock at depth appeared to be more related to specific management history than to time alone. The 24-year-old field, showed higher means down to 30 cm, likely due to the historical use of furrowing shanks for deep fertilizer placement until 2013 (Figure 12A). This allowed a greater amount of P to be added to the deeper layers. Nonetheless, this effect was limited because of P's low mobility in the soil, as it is adsorbed onto the surfaces of Fe and Al oxides under acidic conditions (Mkhonza; Buthelezi-Dube; Muchaonyerwa, 2020; Mandal *et al*., 2022).

On the other hand, S Stock exhibited a completely different vertical pattern, accumulating in deeper layers. This behavior could be explained because S is more labile than P and tends to accumulate in deeper soil layers (Raij, 2008), further enhanced by gypsum applications, a soluble source of Ca and S. The most noticeable S increase occurs in the 12-year field, but the superiority in the surface layer of this area can be explained by the application of a formulated fertilizer containing S during the sampling season, which did not occur in the other areas, given that all cultivated areas received gypsum application in this same season (2.0 Mg ha^{-1}). However, the accumulation of S in the subsurface of cultivated areas for 12 and 18 years, being superior to 24-year-old field, is apparently justified by the gypsum application (1.5 Mg ha^{-1}) that occurred two years before sampling only in those areas, suggesting a downward movement over time, aided by rainfall and the sandy texture that facilitates leaching (Pias *et al*., 2020). This is a point to take into account when designing strategies to amend tropical soils

and fertilization strategies in order to optimize soil pH, decrease Al toxicity, and reduce S losses due to leaching.

Finally, micronutrient stocks (Cu, Mn, Zn, and B) generally increased with cultivation time in the surface layer, linked to SOM accumulation and fertilization. However, different patterns were observed in each case (Figure 15). Cu Stock and B Stock were affected by cultivation time up to 30 cm, while Mn Stock and Zn Stock up to 20 cm soil depth (Figure 15). The availability of these micronutrients is closely linked to SOM content and interactions with Fe and Al oxides (Mandal *et al*., 2022). In sandy soils, their availability fluctuates more due to changes in SOM and pH, underscoring the importance of careful management to prevent deficiencies or toxicity (Santos *et al*., 2021; Mandal *et al*., 2022). The more complex pattern observed for Iron (Fe Stock), which showed no linear relationship with cultivation time, may be attributed to its high sensitivity to pH-dependent solubility and organo-mineral interactions. The increase in soil pH due to liming in cultivated areas promotes the precipitation of Fe into insoluble forms (oxides and hydroxides), reducing its exchangeable fraction compared to the more acidic native vegetation. Furthermore, in these sandy soils, Fe levels are strongly influenced by the parent material mineralogy (Pfaltzgraff; Torres; Brandão, 2010) rather than cumulative fertilization, masking any clear trend related to cultivation time.

To increase agricultural production, a sustainable solution must consider the balanced application of nutrients (Agegnehu *et al*., 2021) at the correct doses and times (Agegnehu *et al*., 2021) for each soil type and climate (Maia *et al*., 2022; Liang *et al*., 2023), reinforcing the importance of studying local management (Chatterjee *et al*., 2018).

5.6 Conclusion

Our study demonstrates that longer cultivation times significantly increased soil chemical fertility across the 0-0.5 m profile in the sandy soils of the Piauí Cerrado. This deep-reaching effect, though not commonly reported in tropical studies, is attributed to the combination of low initial organic matter, sandy texture, and continuous agricultural management. The strategic use of soil amendments was the decisive factor in this improvement, influencing both the vertical extent and the rate of these chemical changes.

Cultivation time promoted marked improvements in soil fertility, characterized by significant increases in soil organic matter (SOM), base sum (SB), base saturation (V), and stocks of C, Ca, and S down to 50 cm depth. Corrective management played a crucial role, affecting the vertical movement of Ca, Mg, K, P and S through the soil profile.

These results highlight the importance of proper soil management and amendment application to enhance and sustain soil fertility, even in the deeper layers of sandy tropical soils. Such practices are fundamental to improving soil functionality and ensuring the long-term sustainability of agricultural production in the Cerrado biome.

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6 FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

Agricultural expansion in the Cerrado has frequently been associated with environmental degradation and soil carbon losses. However, the results of this thesis demonstrate that the prolonged agricultural use of sandy soils in the Piauí Cerrado, under a minimum tillage system with continuous application of soil amendments, fertilizers, and cover crops, and in the absence of recurrent burning, promoted significant improvements in soil quality throughout the 0–50 cm profile. These findings indicate that, contrary to the prevailing perception that tropical sandy soils exhibit low resilience to agricultural use, technically guided conservation management can not only maintain but also enhance chemical and biological attributes over time. Thus, prolonged agricultural use, when combined with systematic acidity correction and strategic fertilization, can convert an originally limiting environment into a productive system with progressive improvements in soil quality.

The evaluated chronosequence showed that cultivation time promoted a consistent increase in carbon stocks down to 50 cm depth, with positive CO_2 sequestration rates reaching 1.69 Mg ha^{-1} yr^{-1} after 24 years of cultivation. Increases in soil organic carbon and basal respiration (C-CO_2) were observed, indicating greater biological activity over time. Although no statistically significant differences were detected for microbial biomass, microbial quotient, and metabolic quotient among the evaluated areas, the results suggest maintenance of microbial functioning associated with increased carbon stocks. Notably, subsurface layers (20–50 cm) accounted for a substantial proportion of carbon stocks, biomass, and microbial activity within the analyzed profile, demonstrating that management effects were not restricted to the soil surface. These findings reinforce the importance of integrated approaches that consider the entire soil profile, particularly in tropical sandy soils.

From a chemical perspective, a marked restructuring of soil fertility was observed throughout the profile. Base saturation increased by 429% and the sum of bases by 660% in the average 0.0–50 cm profile after 24 years of cultivation, reflecting the gradual construction of fertility in an originally dystrophic soil. Improvements extended to different depths depending on the attribute evaluated, indicating that the adopted management influenced the vertical dynamics of nutrients, including in subsurface layers—a pattern still poorly documented for tropical sandy soils.

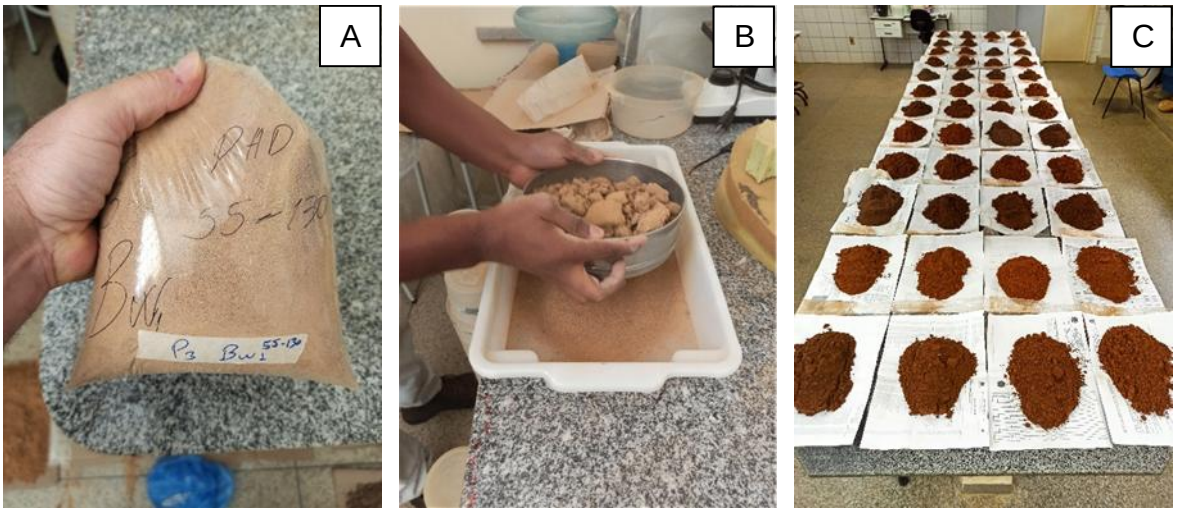
Taken together, this thesis demonstrates that conservation management systems adapted to the edaphoclimatic conditions of the northeastern Cerrado can reconcile agricultural production, soil quality improvement, and carbon sequestration, thereby contributing

simultaneously to food security and climate change mitigation. In this context, agriculture in new frontiers should be understood not only from the perspective of native vegetation conversion, but also in light of the potential for functional soil reconstruction when appropriate and technically guided management practices are adopted.

The results of this thesis open new avenues for scientific investigation. Priority areas include: (i) understanding the mechanisms of carbon stabilization in sandy soils, particularly in subsurface layers; (ii) identifying the microbial groups responsible for carbon cycling and stabilization throughout the soil profile; (iii) evaluating long-term nutrient dynamics, including potential leaching losses and associated environmental impacts; (iv) integrating physical, chemical, and biological attributes into predictive sustainability models; and (v) expanding studies across different climatic and geological contexts within the Cerrado, particularly in soils derived from sedimentary sandstones. Additionally, the expansion of chronosequence-based studies and the consolidation of experimental networks in new agricultural frontiers are recommended in order to strengthen the scientific basis for technical recommendations and public policies aimed at the sustainability of productive systems in tropical sandy soils.

In summary, this thesis contributes to redefining the understanding of the productive and environmental potential of sandy soils in the Piauí Cerrado by demonstrating that cultivation time, when associated with technically grounded conservation management practices, can act as a driver of progressive soil quality improvement rather than necessarily as a degradation agent. The results show that fertility building, carbon stock accumulation, and the strengthening of biological activity are cumulative processes dependent on proper system management, reinforcing the central role of management in achieving long-term sustainability in tropical sandy soils.

APPENDICES



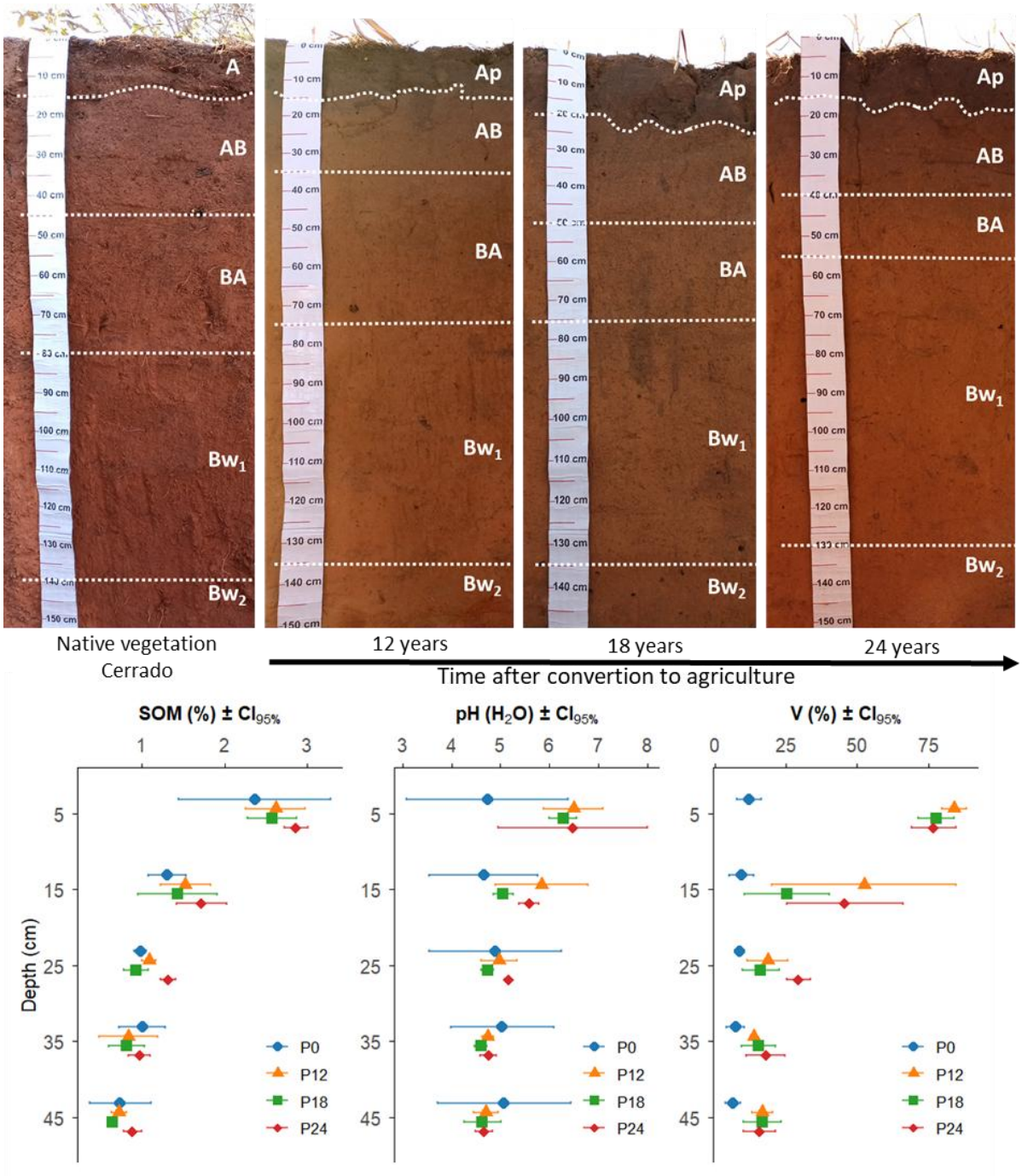
Appendix 1 - Drying (A), sieving (B) and packaging (C) of samples.

Appendix 2 - Limestone, gypsum, macro and micronutrients applied per season in areas cultivated for 12 (P12), 18 (P18) and 24 (P24) years.

Growing season	..... Mg ha^{-1}						..... kg ha^{-1}																					
	Limestone			Gypsum			N			P_2O_5			K_2O			B			Cu			Mn			Zn			F
	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P18	P24	P12	P
1998/1999	X	X	3.0	X	X	0.0	X	X	10.0	X	X	50.0	X	X	40.0	X	X	0.00	X	X	0.00	X	X	0.00	X	X	1.00	X	
1999/2000	X	X	2.0	X	X	0.0	X	X	50.0	X	X	60.0	X	X	60.0	X	X	0.00	X	X	0.00	X	X	0.00	X	X	3.00	X	
2000/2001	X	X	0.0	X	X	0.0	X	X	8.0	X	X	80.0	X	X	80.0	X	X	0.50	X	X	0.16	X	X	0.40	X	X	1.80	X	
2001/2002	X	X	0.0	X	X	0.5	X	X	8.0	X	X	80.0	X	X	80.0	X	X	0.50	X	X	0.16	X	X	0.40	X	X	1.80	X	
2002/2003	X	X	1.5	X	X	0.5	X	X	0.0	X	X	84.0	X	X	97.6	X	X	0.35	X	X	0.11	X	X	0.28	X	X	1.26	X	
2003/2004	X	X	0.0	X	X	0.0	X	X	0.0	X	X	90.0	X	X	99.6	X	X	0.39	X	X	0.12	X	X	0.40	X	X	1.40	X	
2004/2005	X	3.5	0.0	X	0.0	1.0	X	55.5	0.0	X	60.0	99.0	X	54.0	102.6	X	0.01	0.43	X	0.00	0.13	X	0.07	0.49	X	3.10	1.56	X	0.
2005/2006	X	3.5	0.0	X	0.0	0.0	X	0.0	0.0	X	147.8	106.4	X	108.8	100.7	X	0.37	0.36	X	0.11	0.11	X	0.44	0.38	X	1.34	1.31	X	0.
2006/2007	X	2.0	0.0	X	0.0	1.0	X	0.0	0.0	X	106.4	144.4	X	103.6	100.0	X	0.35	0.48	X	0.11	0.15	X	0.28	0.38	X	1.26	1.71	X	0.
2007/2008	X	0.0	0.0	X	0.0	1.0	X	6.5	115.7	X	91.0	86.4	X	89.1	132.2	X	0.41	1.00	X	0.13	0.00	X	0.33	0.00	X	1.46	0.00	X	0.
2008/2009	X	2.5	2.0	X	1.0	1.0	X	0.0	0.0	X	84.4	90.9	X	100.8	100.8	X	0.09	0.09	X	0.03	0.03	X	0.08	0.08	X	0.34	0.34	X	0.
2009/2010	X	0.0	0.0	X	0.0	0.0	X	0.0	0.0	X	81.0	86.4	X	113.6	116.3	X	0.03	0.00	X	0.05	0.00	X	0.14	0.06	X	0.09	0.00	X	0.
2010/2011	6.8	2.0	2.0	0.0	1.0	0.0	0.0	0.0	0.0	63.7	100.7	100.0	25.1	137.7	137.4	0.24	0.44	0.42	0.08	0.19	0.18	0.19	0.47	0.38	0.87	1.47	1.48	0.29	0.
2011/2012	3.5	2.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	119.1	87.0	90.6	158.2	114.3	117.2	0.07	0.04	0.04	0.06	0.04	0.04	0.23	0.12	0.16	0.06	0.08	0.05	0.00	0.
2012/2013	0.0	0.0	0.0	1.0	0.0	1.0	0.0	114.4	0.0	86.4	89.9	91.5	111.6	136.3	114.2	0.39	0.04	0.44	0.14	0.04	0.18	0.39	0.15	0.53	1.32	0.03	1.42	0.43	0.
2013/2014	0.0	0.0	0.0	0.0	0.0	0.0	123.4	0.0	0.0	95.4	110.2	113.9	138.0	113.2	129.0	0.05	0.01	0.01	0.05	0.01	0.01	0.18	0.10	0.10	0.04	0.02	0.02	0.00	0.
2014/2015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	127.0	0.0	99.0	112.5	130.4	120.2	139.2	125.4	0.04	0.06	0.04	0.02	0.03	0.02	0.30	0.40	0.26	0.06	0.08	0.05	0.00	0.
2015/2016	0.0	0.0	0.0	0.0	0.0	0.0	147.3	0.0	21.1	96.2	103.6	101.8	120.1	118.0	124.7	0.10	0.05	0.15	0.03	0.01	0.03	0.40	0.18	0.46	0.08	0.03	0.09	0.00	0.
2016/2017	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.7	0.0	32.5	43.6	0.0	12.8	0.0	0.0	0.05	0.04	0.05	0.03	0.02	0.03	0.38	0.28	0.38	0.07	0.05	0.07	0.00	0.
2017/2018	0.0	0.0	1.5	0.0	0.0	0.0	27.9	0.0	27.2	93.5	0.0	122.5	19.9	0.0	0.0	0.04	0.06	0.04	0.02	0.03	0.02	0.28	0.42	0.30	0.05	0.08	0.06	0.00	0.
2018/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	78.8	0.0	0.0	78.8	82.2	90.5	0.01	0.03	0.04	0.01	0.01	0.02	0.12	0.20	0.32	0.02	0.04	0.06	0.00	0.
2019/2020	3.0	2.0	2.0	1.5	1.5	0.0	0.0	0.0	0.0	79.5	80.0	77.3	79.5	80.0	77.3	0.02	0.02	0.03	0.01	0.01	0.02	0.18	0.20	0.22	0.03	0.04	0.04	0.00	0.
2020/2021	0.0	0.0	0.0	0.0	0.0	0.0	15.1	20.1	14.9	68.0	90.6	67.2	107.2	107.3	85.6	1.00	1.01	1.02	0.01	0.01	0.02	0.24	0.15	0.34	0.05	0.03	0.06	0.00	0.
2021/2022	0.0	0.0	0.0	2.0	2.0	2.0	0.0	24.2	25.8	0.0	0.0	105.9	84.4	97.8	31.4	1.04	1.04	1.04	0.18	0.01	0.02	0.51	0.17	0.33	1.06	0.04	0.07	0.00	0.



Appendix 3 - Separation of sand (A) and sedimentation of clay (B).



Appendix 4 - Soil profiles, means ± 95% confidence interval for Soil Organic Matter (SOM, %), Base Saturation (V, %) and pH in water (pH, H_2O) in the native vegetation and cultivated areas under 12, 18, and 24 years of habitat conversion from native forest to agricultural land in the Cerrado region of Brazil.