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**SOIL ORGANIC MATTER DYNAMICS IN LIVESTOCK-FORESTRY
INTEGRATION SYSTEMS IN THE SEMI-ARID REGION OF CEARÁ, BRAZIL**

Rio Largo - AL

2025

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Thesis presented to the Graduate Program in Agronomy:
Plant Production, CECA/UFAL, to obtain the degree of
Doctor of Agronomy.

Advisor: Prof. Dr. Stoécio Malta Ferreira Maia

Co-advisor: Prof. Dr. Marcelo Cavalcante

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To my niece Maria Eduarda, for being
my guiding light throughout this journey.

I dedicate.

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“If today's no good, you'll have tomorrow. if tomorrow's no good, you'll have the next day. if that's no good, there will be another. there's always a tomorrow.”

- Only Yesterday (1991)

Soil organic matter dynamics in livestock-forestry integration systems in the semi-arid region of Ceará, Brazil

ABSTRACT

Although considered a natural solution to mitigate climate change, carbon sequestration in the Brazilian semi-arid region presents challenges. Due to its unique climatic and environmental characteristics, carbon sequestration (C) is more complex, which raises questions about the sustainability of agricultural development in the region. This study investigated the effects of integrated livestock-forest (ILF) systems with different spacings and crops on C storage, microbial activities, and soil quality in the semi-arid region of Ceará. The systems studied included agroforestry systems with sorghum (So), forage cactus (Fc), massai grass (Mg), and buffel grass (Bg), with spacings of 7 m (S7), 14 m (S14), and 28 m (S28) between strips of native vegetation (STN). After 6 years, soil samples were collected up to a depth of 0.5 m. The results showed that the conversion of Caatinga to ILF systems, particularly those with massai and buffel grass, has the potential to increase total soil organic carbon (SOC) and nitrogen (TN) stocks, the fractions of minerals-associated organic matter (MAOM) and particulate organic matter (POM), microbial biomass carbon (MBC), enzymatic activities, and the microbial quotient, resulting in positive impacts on soil quality. Moreover, the S28 and S14 spacings were the most promising for increasing SOC, POM, and MAOM fractions. While S7 showed a greater contribution to the maintenance of soil biomass and microbial activity. These results highlight the role of integrated systems in the maintenance and recovery of SOC, contributing to the adoption of more sustainable and efficient agricultural practices in the Brazilian semi-arid region.

Keywords: soil carbon, Caatinga, nature-based solution.

Dinâmica da matéria orgânica do solo em sistemas de integração pecuária-floresta na região semiárida do Ceará, Brasil

RESUMO

Embora considerado uma solução natural para mitigar mudanças climáticas, o sequestro de carbono no semiárido brasileiro apresenta desafios. Devido as suas características climáticas e ambientais únicas, o sequestro de carbono (C) é mais complexo, o que levanta questionamentos sobre a sustentabilidade do desenvolvimento agrícola na região. Este estudo investigou os efeitos de sistemas de integração pecuária-floresta (IPF) com diferentes espaçamentos e culturas no armazenamento de C, nas atividades microbianas e na qualidade do solo na região semiárida do Ceará. Os sistemas estudados incluíram sistemas de IPF com sorgo (So), palma forrageira (Pf), capim massai (Cm) e capim buffel (Cm), em espaçamentos de 7 m (E7), 14 m (E14) e 28 m (E28) entre faixas de vegetação nativa (FxVN). Após 6 anos, amostras de solo foram coletadas até 0,5 m de profundidade. Os resultados mostraram que a conversão de Caatinga para sistemas IPF, com destaque para os sistemas com capim massai e buffel, têm potencial para aumentar os estoques totais de carbono orgânico do solo (COS) e nitrogênio (NT), as frações da matéria orgânica associada aos minerais (MOAM) e da matéria orgânica particulada (MOP), carbono da biomassa microbiana (CBM), atividades enzimáticas e quociente microbiano, resultando em impactos positivos na qualidade do solo. Além disso, os espaçamentos E28 e E14 foram os mais promissores para aumentar o COS, as frações MOP e MOAM. Enquanto o E7 apresentou maior contribuição para a manutenção da biomassa e atividade microbiana do solo. Estes resultados destacam o papel de sistemas integrados na manutenção e recuperação do COS, contribuindo para adoção de práticas agrícolas mais sustentáveis e eficientes no semiárido brasileiro.

Palavras-chaves: carbono do solo, Caatinga, solução baseada na natureza.

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1 GENERAL INTRODUCTION

The global carbon balance has been drastically altered by anthropogenic activities, especially since the beginning of the industrial era (SÁ et al., 2017). Despite the need to control and reduce greenhouse gas (GHG) emissions to limit global warming to 2°C, anthropogenic emissions continue to rise (MAIA et al., 2022). The IPCC (Intergovernmental Panel on Climate Change) in its sixth assessment report presented scenarios projecting increases in global temperature between 1.4 and 4.4 °C by the end of the century, if actions are not taken to reduce GHG emissions (IPCC 2021). Therefore, global GHG mitigation goals will be essential to limit emissions, including the fulfillment of the Nationally Determined Contributions (NDCs) for the Paris Agreement (UNFCCC, 2015), the implementation of the Sustainable Development Goals (SDGs) (UN, 2024), and the restoration of ecosystems (UN, 2019). The United Nations General Assembly declared 2021-2030 the United Nations Decade on Ecosystem Restoration and states that restoring 350 million hectares of degraded land between 2021 and 2030 could remove up to 26 gigatons of additional GHGs from the atmosphere. (UN, 2019).

Brazil has implemented several important national and international initiatives aimed at the recovery of degraded areas and the reduction of GHG emissions, such as the Low Carbon Agriculture Plan (ABC Plan) (BRASIL, 2012) and the Nationally Determined Contributions (BRASIL, 2020). These initiatives promote practices and measures for more sustainable agriculture and can offset GHG emissions by 0.3 to 1.17 Pg C per year, which represents 2.7 to 10.4% of global GHG emissions (SÁ et al., 2017). This compensation largely occurs through carbon (C) sequestration in the soil (BASSO et al., 2022), which is the largest terrestrial C reservoir, storing at least three times more C than the atmosphere (CRISPY et al., 2022). Thus, a relatively small increase in soil C stocks can play a significant role in removing atmospheric concentrations of carbon dioxide (CO₂) (MINASNY et al, 2017).

Strategies to increase soil C involve reducing losses and increasing C inputs, for example, cover crops (ZHU et al., 2024), crop rotation (DAL FERRO et al. 2020), no-till farming (SMITH and CHALK, 2021), as well as integrated agricultural systems, that is, the combination of trees, agricultural crops, and pastures in the same area. Studies estimate that integrated systems currently store 6930 Tg C (CHAPMAN et al., 2020) and that global expansion could sequester up to 284 Tg C per year (GRISCOM et al., 2017).

In Brazil, the expansion of areas with integrated systems has been remarkable and their implementation receives government support, as these systems are included in the ABC plan (BRASIL, 2012) and are essential for Brazil to meet its targets agreed upon in the Paris

Agreement, the NDCs. However, the adoption and studies of integrated systems in the Brazilian semi-arid region are still scarce. In this region, the characteristics of low soil fertility, high rates of SOM decomposition, high soil erosion, and sporadic precipitation that limit agricultural production make it a highly fragile ecosystem vulnerable to the impacts of climate change (SILVA et al., 2011). Given this scenario, the adoption of integrated systems could help achieve the development of a more efficient and sustainable agriculture in the region. Moreover, integrated systems could contribute to the recovery of degraded pastures, which already represent more than 13 million hectares (LAPIG, 2023).

Integrated agricultural systems are increasingly seen as a method to advance GHG mitigation initiatives and the recovery of degraded areas (ZEPPETELLO et al., 2022) and bring significant benefits to the dynamics of soil organic matter (SOM) (BIELUCZYK et al., 2020; TADINI et al., 2021; DAMIAN et al., 2023). SOM is a key component of terrestrial ecosystems, with its origin related to the accumulation of organic compounds due to the conversion of atmospheric CO₂ into carbohydrates through photosynthesis. The benefits of SOM are related to soil quality and fertility, as they contribute to nutrient storage, aggregation, and soil erosion control (GIANINI et al., 2023). It is also a primary source of carbon (C) and energy for microorganisms and soil biota, which are essential organisms in soil functioning (Martínez-García et al., 2018). In this way, when seeking to improve the spatial and temporal arrangements of integrated systems for different edaphoclimatic conditions, it becomes essential to understand the dynamics of SOM in these systems.

Given this context, this study investigated the effects of livestock-forest integration systems (ILF) with different crops and spatial arrangements on SOM dynamics and soil quality in an area in the semi-arid region of Ceará. The specific objectives of the study consisted of: i) to evaluate the total C stock in ILF systems; ii) evaluating the effects of ILF systems on the fractions and origin of SOM; iii) assessing the microbiological properties of the soil in ILF systems; and iv) relating which soil parameters have the greatest potential as indicators of soil quality.

CHAPTER I

2 LITERATURE REVIEW

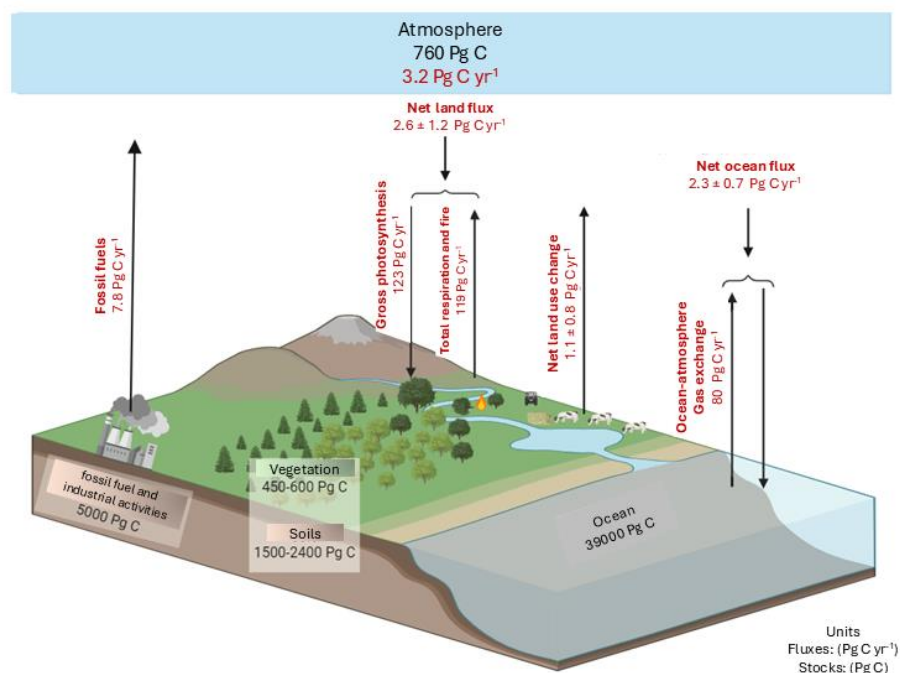
2.1 GLOBAL CARBON BALANCE

2.1.1 Carbon and Global Changes

C plays a central role in life and climate systems on Earth. It is the basic source of chemical energy in living organisms, and in the form of CO₂, it is an important greenhouse gas (GHG) in the atmosphere that helps regulate the Earth's climate. (DEVRIES 2022). The balance of C on the Earth's globe is controlled by three reservoirs: the ocean, the terrestrial, and the atmospheric. Figure 1 schematically shows the interrelationships that occur between these three reservoirs.

The ocean is the largest carbon reservoir and is estimated to contain 39000 Pg C (1 Pg = Pentagram = 10⁹ t = 1 billion metric tons), which is approximately 90% of the carbon contained in combined terrestrial, oceanic, and atmospheric reservoirs. The terrestrial reservoir is composed of the geological, the soil, and the biota. The geological (fossil fuels and other industrial activities) is estimated at 5000 Pg C. The carbon stocks held in biota and soils are estimated at 450–650 Pg C and 1500–2400 Pg C, respectively. The atmosphere is the smallest reservoir, estimated at 760 Pg C (Crispy et al., 2022).

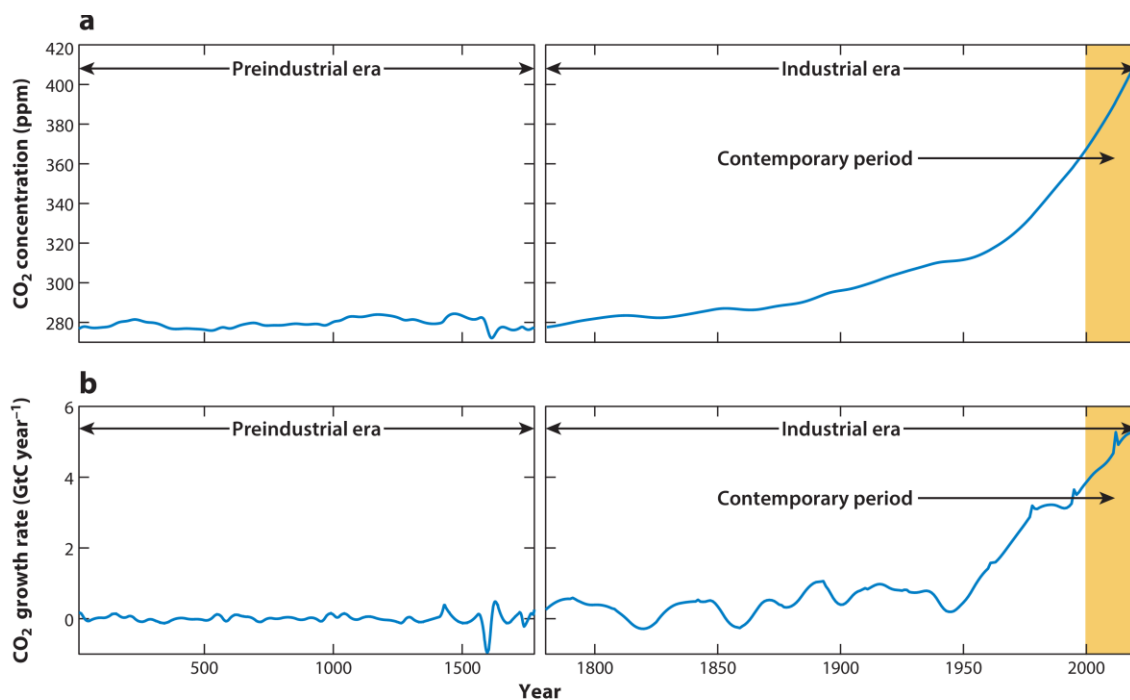
Figure 1 - Global carbon pools and fluxes to the atmosphere (Adapted from IPCC, 2013).



Source: Author (2025).

Although the atmosphere is the smallest C reservoir in the global cycle, it is growing at a rate of 3.2 Pg C per year, due to interventions in terrestrial reservoirs, which added 700 ± 75 Pg C to the atmosphere between 1750 and 2019. Of this total, $41\% \pm 11\%$ remained in the atmosphere (FRIEDLINGSTEIN et al., 2021; CRIYSP et al., 2022). Figure 2 (DEVRIES, 2022) shows that before the start of the industrial revolution (~ 1780), the carbon flows into and out of the atmosphere were in balance. However, with the onset of the industrial revolution, the atmospheric concentration of CO₂ has been steadily increasing, mainly due to the combustion of fossil fuels, which has driven economic development over the past 250 years. According to Flach et al. (1997), during the 1950s and 1960s, the annual CO₂ emissions from the burning of fossil fuels exceeded for the first time the CO₂ emissions from the conversion of forests and natural pastures into cultivated areas.

Figure 2 - (a) Atmospheric CO₂ concentrations during the pre-industrial era (0 AD to 1780) (left) and during the industrial era (1780 to present) (right). (b) The time rate of change of atmospheric CO₂, highlighting (left) a preindustrial steady state prior to 1780 AD and (right) the positive growth rate of atmospheric CO₂ over the industrial era, reaching a value of $4.76 \text{ GtC year}^{-1}$ in the contemporary period (2001–2020, yellow) (Adapted from Devries, 2022).



Source: Devries (2022).

The increase in CO₂ emissions and other GHGs (CH₄, N₂O, CFCs (chlorofluorocarbons)) accelerates global climate changes, resulting in extreme weather events

that can harm human development in the future (GAO et al., 2019). The term global warming refers to the acceleration of the natural greenhouse effect due to changes resulting from anthropogenic actions. Two main characteristics determine the impact of different GHGs on the climate: the period they remain in the atmosphere (also known as "lifetime") and their ability to absorb energy (their "radiative efficiency") (IEA, 2021). In this way, the GWP, the English acronym for Global Warming Potential, was developed to allow comparisons of the impacts of different gases on global warming. Specifically, it is a measure resulting from the amount of energy that the emissions of 1 ton of gas will absorb over a certain period, in relation to the emissions of 1 ton of carbon dioxide (CO₂). In this way, the higher the GWP, the more a given gas warms the Earth compared to CO₂ (VALERRO, 2019). The GWP of CH₄ and N₂O are respectively 29.8 and 273 (IPCC, 2021).

The agriculture, forestry, and other land use sectors significantly contribute to the CO₂ balance. (NYAWIRA et al., 2024). Globally, the sector was responsible for 13 Gt CO₂eq. per year in 2019, which represented about 22% of net anthropogenic GHG emissions, with approximately half coming from agriculture and the other half from forestry and other land use activities (IPCC, 2022).

2.1.2 Soil organic matter and soil carbon sequestration

The organic matter in soils, composed of dead and decomposing plant tissues, as well as the living community of microbial decomposers and their residues, contains three times more C than the biota, and double what exists in the atmosphere (GLEIXNER, 2013). In this context, soil organic carbon (SOC) constitutes the largest carbon reservoir in terrestrial ecosystems, thus playing a significant role in atmospheric CO₂ levels (GAMA et al., 2023). The dynamic equilibrium of SOC is the balance between the additions and losses of C. Vegetative residues above and below the soil, including woody material such as dead roots, add C to the soil, thus renewing parts of the SOC reservoir. Simultaneously, microbes decompose SOC, resulting in the release of CO₂ into the atmosphere. If the C balance is positive, the soil absorbs C and C sequestration is achieved (DON et al., 2023). In this sense, depending on the management practices adopted, the soil can be a source or a sink of atmospheric CO₂, directly contributing to the greenhouse effect.

The management systems that use soil preparation for plant production are the main source of C losses. This is because during this process, the soil aggregates break down, allowing the decomposition of SOM that was protected from the action of microorganisms, and the increase in soil microbial activity results in a greater flow of C mineralization (ZHANG et al.,

2024). Numerous studies show that the conversion of natural vegetation into cultivated areas, associated with long-term soil preparation practices, causes significant losses in soil C content (JHA et al., 2020; SONG et al., 2022; CHOUDHARY and MEENA, 2022).

On the other hand, SOM becomes a carbon sink when the additions of C are greater than the losses due to oxidation. Minasny et al. (2017) state that increasing global SOM stocks by 0.4% per year could offset global GHG emissions from anthropogenic sources, a figure that emphasizes the potential of soil management as a strategy for mitigating climate change.

The sequestration of SOC can be accelerated through positive changes in land use and agricultural practices, such as cover cropping, minimum tillage, crop rotation, direct planting, and integrated production systems (SMITH and CHALK, 2021; ZHU et al., 2024; LUSTOSA FILHO et al., 2024). These management practices also contribute to soil quality, promoting microbial diversity, stabilization of organic matter, and soil fertility, and consequently, a more productive system (WU et al., 2023). Meta-analysis studies have indicated that SOC stocks can increase by 16% in systems with cover crops compared to systems without cover crops (Jian et al., 2020), and that the practice of no-till farming can increase SOC stocks by about 0.4 Mg C ha⁻¹ year⁻¹ compared to conventional soil tillage. (WEST and POST, 2002; NICOLOSO and RICE, 2021).

2.2 IMPACT OF INTEGRATED AGRICULTURAL SYSTEMS ON SOIL ORGANIC MATTER

2.2.1 Integrated Agricultural Systems: Concepts and Types

The demand for food, bioenergy, and forest products in contrast to the need to reduce deforestation and mitigate GHG emissions requires solutions that encourage socioeconomic development without compromising the sustainability of natural resources (VILELA et al., 2019). In this sense, there are intense calls for the worldwide dissemination of the concept of sustainable agriculture, consisting of agricultural systems that conserve soil, water, and biodiversity, do not degrade the environment, and are economically viable and socially acceptable (BALBINO et al., 2012).

The intensification of land use, in an efficient and rational manner, in already anthropized areas is one of the alternatives for the sustainable development of agriculture (SÁ et al., 2017), with the adoption of integrated agricultural systems being one of the main strategies to intensify production and mitigate the effects of climate change (Brewer and GAUDIN, 2020). Integrated agricultural systems combine agricultural, livestock, and/or forestry activities in the same area, in intercropping, succession, or rotation, and seek the

synergistic effects among the components of the agroecosystem, increasing nutrient cycling, diversifying rural production, and thereby reducing the economic risks for the farmer (BALBINO et al., 2011). According to these authors, the integration systems strategy encompasses four modalities:

- i) Integrated crop livestock (ICL) - system that integrates the crop and livestock component in rotation, consortium, or succession, in the same area, in the same agricultural year or over several years, in sequence or interspersed.
- ii) Integrated crop livestock forest (ICLF) – system that integrates the components of crops, livestock, and forest, in rotation, consortium, or succession, in the same area. The crop component can be used in the initial phase of implementing the forest component or in cycles during the system's development.
- iii) Integrated livestock forest (ILF) - system that integrates livestock and forest components in a consortium.
- iv) Integrated crop forest (ICF) – system that integrates the components of forest and crop, through the intercropping of tree species with agricultural crops (annual or perennial). The crop component can be used in the initial phase of implementing the forest component or in cycles during the system's development.

2.2.2 *Integrated systems as a nature-based solution for carbon sequestration*

Terrestrial carbon sequestration is at the heart of nature-based solutions (NBS) to mitigate climate change (YANG et al., 2022; NYARKO et al., 2024). The NBS are understood as "actions to protect, conserve, restore, sustainably use, and manage natural or modified terrestrial, freshwater, coastal, and marine ecosystems that effectively and adaptively address social, economic, and environmental challenges, while providing human well-being, ecosystem services, resilience, and benefits for biodiversity" (UNITED NATIONS, 2022).

Studies in Brazil have already shown positive effects of adopting integrated systems on C sequestration (MAIA et al., 2007; CÁ et al., 2022; DAMIAN et al., 2023), as well as on the chemical properties (LIEBERG et al., 2017; DAMIAN et al., 2021) and microbiological properties of the soil (CAMELO et al., 2023; DAMIAN et al., 2021). In this way, integrated agricultural systems have been considered one of the main strategies for carbon accumulation in the soil (BREWER and GAUDIN, 2020), where simulations indicated that the adoption of these systems with full utilization of the synergistic interactions between components can reduce carbon losses by 25% by 2050 (SÁ et al., 2017).

Lira Junior et al. (2020) observed that a 5-year silvopastoral system (*Urochloa decumbens* and *Gliricidia sepium*) improved the quality of SOM and led to an increase in soil C and N levels by about 37% and 82%, respectively, compared to *Urochloa decumbens* in monoculture. Increases ranging from 1.0 to 4.31 Mg C ha⁻¹ year⁻¹ were observed by Freitas et al. (2022) after seven years of introducing ICLF systems in a transition area between the Cerrado and Caatinga biomes in Brazil. The authors stated that these results were favored by the presence of the forest component, which facilitated the entry of plant debris and roots. Silva et al. (2021) found that in the monoculture of *Brachiaria decumbens*, carbon stocks were higher than in the other systems evaluated (*Brachiaria decumbens* + corn and agroforestry systems) after four years of implementation.

In Brazil, the implementation of integrated systems is part of the national program, the ABC plan, which aims to promote the adoption of sustainable production technologies. The proposals outlined in the ABC plan, especially the adoption of integrated systems, are essential for Brazil to meet the target of a 43% reduction in GHG emissions by 2030, as agreed in 2015 during the 21st Conference of the Parties (COP 21) in Paris (BRASIL, 2012; UNFCCC, 2015). Thus, considering that the synergistic interactions between the components are influenced by the spatial and temporal arrangements of the systems (FREITAS et al., 2022), and that the magnitude of these changes indicates whether the system is gaining C or not, it becomes essential to understand the impact of integrated agricultural systems under different edaphoclimatic conditions. This is of particular importance in semi-arid regions, due to their high vulnerability to the impacts of global climate change.

2.2.3 Carbon stocks in integrated systems in non-semiarid areas

The Brazilian semi-arid region encompasses a total area of approximately one million km², which represents 12% of the national territory, continuously including parts of the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, and northern Minas Gerais (MEDEIROS et al., 2012; ALTHOFF et al., 2018). The climate of the region is classified by Köppen as type BSh, representing a hot and dry climate with average annual rainfall of 800 mm, average annual temperatures of 27 °C, and a dry season ranging from 5 to 9 months per year. (ALTHOFF et al., 2018).

In the Brazilian semi-arid region, the predominance of conventional agricultural practices combined with the region's environmental variables, such as low precipitation and high temperatures, has significantly reduced the levels of SOM, with a direct impact on carbon and nitrogen (N) stocks. Medeiros et al. (2021) found SOC losses ranging from 12 to 27% under

pastures compared to the Caatinga. Santana et al. (2019) found SOC stock losses ranging from 10% to 55% in conventional agricultural systems, as well as a 36% loss of N. Similarly, Medeiros et al. (2020) found that in the conversion of Caatinga to conventional annual agricultural systems, SOC stocks reduced by around 26 and 13%.

Given this, the adoption of agroecosystems, such as integrated systems, which maximize carbon inputs and minimize management-induced losses, can improve SOC storage in semi-arid environments. In this sense, maximizing the potential for SOC accumulation under the management of integrated systems will require knowledge about the interactions between the components of the systems, soil microbial ecology, and SOM dynamics (BREWER and GAUDIN, 2020), enabling the identification of which arrangements and interactions are more sustainable for the region.

Few studies evaluating the dynamics of SOM in the Brazilian semi-arid region under integrated agricultural systems have been conducted. However, results from studies in the Brazilian semi-arid region show positive responses in C stocks and soil microbiology when agroforestry systems are adopted. Maia et al. (2007) evaluated C stocks in agroforestry and conventional systems compared to native vegetation in the semi-arid region of Ceará and found reductions in C stocks of 39.8% in the conventional system and only 6.8% in the silvopastoral system for the 0-40 cm depth layer, concluding that the silvopastoral system can be recommended as a sustainable alternative soil management system in the semi-arid region of Brazil. Similarly, Tonucci et al. (2023) concluded that integrated agricultural systems in the Caatinga biome are sustainable alternatives for land use in the semi-arid region, when they evaluated agroforestry systems with sorghum, millet, beans, massai grass, and woody forest vegetation in the semi-arid region of Ceará and observed gains of 50% and 26% in soil C stocks, compared to native vegetation (Caatinga), in the 0-100 cm layer.

In the Pernambuco semi-arid region, intercropping systems of forage cacti (*Nopalea cochenillifera* Salm Dyck) with shrub legumes: gliricidia (*Gliricidia sepium*) and leucaena (*Leucaena leucocephala*) have favored the soil microbiota, with results showing higher microbial biomass and microbial quotient compared to the monoculture of forage cactus (CAMELO et al., 2020). Miguel et al. (2020) studied the influence of agroforestry systems on the biological attributes of soil in the semi-arid region of Piauí and found that agroforestry systems improved the biological quality of the soil and may be more sustainable than slash-and-burn agricultural systems in the Caatinga in the long term.

2.3 EVALUATION OF SOIL ORGANIC MATTER

Measuring the total soil C does not provide information about the likely persistence of SOM. The persistence of SOM for C sequestration and SOM accumulation is provided by functionally distinct SOM components (SMITH et al., 2024). The active compartment of organic matter consists of labile fractions (easily decomposable) with a half-life of only a few days or years. The passive compartment of SOM consists of more stable materials, remaining in the soil for hundreds or thousands of years. The slow compartment of SOM not only has intermediate properties between the active and passive, but also, probably, includes fractions of organic particles of the same size, which are rich in lignin and other slowly decomposable and chemically resistant components (BRADY and WEIL, 2016).

Granulometric fractionation, which divides SOC into mineral-associated organic carbon (MAOC) and particulate organic carbon (POC), is a simple and efficient method that provides more information about the quality of SOC than just the quantity. (ZHANG et al., 2024). The POC reservoir is characterized by the carbon mass in particulate organic matter (POM), which is a reservoir of organic carbon bound to sand particles in the soil (53–2000 μm), being a less humified but more active fraction. The minerals-associated organic matter (MAOM) is a fraction smaller than 53 μm and is bound to mineral surfaces. POM has a faster rate of turnover of years to decades, while MAOM is on average longer lived than POM with estimated turnover rates of decades to centuries (COTRUFO and LAVALLEE, 2022). However, how much C can be stored in soil and for how long is a matter of debate (MANZONI and COTRUFO, 2024). Lehmann et al. (2020) highlight that the persistence of the added C depends on the balance of stabilization and destabilization processes. Cotrufo and Lavalle (2024) complement by stating that the persistence of SOM will depend on the inhibition of microbial activity, the degree of its limitation and carbon use efficiency, and microbial access constraints, climate, and the soil's geochemical characteristics.

The measurement of the natural abundance of ^{13}C has been used to assess the origin of organic material added to the soil. The basic principle of this approach is because vegetation has distinct photosynthetic pathways. (SHANG and TIESSEN, 2000). Plants that use RuBisCO (C3 metabolism) for CO_2 fixation exhibit isotopic composition ($\delta^{13}\text{C}$) ranging from -24 to -34 ‰, while plants that use PEPcase (C4 metabolism) show higher values than C3, ranging from -6 to -19 ‰. (Smith and Epstein, 1971). In this way, the isotopic composition of SOM reflects the original vegetation and the effects as plant species with contrasting $\delta^{13}\text{C}$ signals are introduced.

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CHAPTER II

3 CHANGES IN CARBON STOCKS AND QUALITY OF THE SOIL ORGANIC MATTER UNDER DIFFERENT ARRANGEMENTS OF INTEGRATED LIVESTOCK-FOREST SYSTEMS IN THE SEMI-ARID REGION OF BRAZIL

ABSTRACT

Integrated production systems can have a positive impact on the carbon footprint, mitigating the effects of climate and environmental change. In this study, the quantity and quality of the soil organic matter (SOM) was evaluated in integrated livestock-forest (ILF) systems in the semi-arid region of the state of Ceará, Brazil. The study was carried out in an area that included four ILF systems: ILF with sorghum (So), forage cactus (Fc), massai grass (Mg), and buffel grass (Bg), each at a spacing of 7 m (S7), 14 m (S14) and 28 m (S28) between strips of native trees (Caatinga) (SNT). These systems were compared with an area of native vegetation (NV). Soil samples were collected to a depth of 50 cm to evaluate changes in the composition of the SOM (C, N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and organic matter fractions) in the different arrangements of the ILF systems. Our results showed that over a period of six years, converting the Caatinga into ILF systems was sufficient to increase C and N stocks in the top layer (0-10 cm) of the soil when considering only the SOC of the livestock components (Bg, Mg, Fc, So). The systems including Mg and Bg at spacings S28 and S14 were the most promising for increasing the SOC, and the POM and MAOM fractions, and presented the highest $\delta^{13}\text{C}$ values. In contrast, the Fc systems promoted the greatest reductions in SOC, with the greatest loss occurring at spacing S28. The weighted results of the livestock and forestry components show that the ILF systems were even more promising, with a reduction in the loss of C stocks even in the deepest layers (0-30 and 0-50 cm). The adoption of certain ILF systems in the Caatinga therefore show potential for maintaining or even recovering SOC, with the possibility of contributing to soil quality and mitigating climate change.

Keywords: integrated production systems; soil carbon; Caatinga; soil quality; dryland.

RESUMO

Os sistemas integrados de produção podem ter um impacto positivo na pegada de carbono, mitigando as alterações climáticas e ambientais. Nesse estudo foi avaliado a quantidade e qualidade da matéria orgânica do solo (MOS) em sistemas de integração pecuária-floresta (IPF) no semiárido do estado do Ceará, Brasil. O estudo foi realizado em uma área com quatro

sistemas de IPF: IPF com sorgo (So), palma forrageira (Pf), capim massai (Cm), e capim buffel (Cb), todos em espaçamentos de 7 m (E7), 14 m (E14) e 28 m (E28) entre faixas de árvores nativas (Caatinga) (FxVN). Esses sistemas foram comparados com uma área de vegetação nativa (VN). Amostras de solo foram coletadas até a profundidade de 50 cm para avaliar as alterações na composição da MOS (C, N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ e das frações da matéria orgânica) nos diferentes arranjos dos sistemas de IPF. Nossos resultados mostraram que no período de seis anos, a conversão da Caatinga em sistemas de IPF foi suficiente apenas para aumentar os estoques de C e N do solo na camada mais superficial (0-10 cm), quando considerado apenas o carbono orgânico do solo (COS) dos componentes pecuários (Cb, Cm, Pf, So). Os sistemas com Cm e Cb nos espaçamentos E28 e E14 foram os mais promissores para aumentar o COS, as frações MOP e MOAM e apresentou os maiores valores de $\delta^{13}\text{C}$. Em contraste, os sistemas com Pf promoveram as maiores reduções no COS, com a maior perda ocorrendo no espaçamento E28. Os resultados ponderados dos componentes pecuários e florestal indicam que os sistemas de IPF foram ainda mais promissores, com a redução das perdas dos estoques de C até nas camadas mais profundas (0-30 e 0-50 cm). A adoção de determinados sistemas de IPF na Caatinga, portanto, apresenta potencial para manutenção ou mesmo recuperação do COS, com possibilidade de contribuir para a qualidade do solo e mitigação das mudanças climáticas.

Palavras chaves: sistemas integrados de produção; carbono do solo; Caatinga; qualidade do solo; terras secas.

3.1 INTRODUCTION

One of the most important global challenges today is to reduce atmospheric greenhouse gas (GHG) emissions to prevent the advancement of climate and environmental change. In the sixth assessment report of the Intergovernmental Panel on Climate Change (IPCC), the agriculture, forestry and other land use sector was responsible for approximately 22% (13 Gt CO_2 -eq.) of the total net anthropogenic GHG emissions (IPCC, 2022). However, the agricultural sector cannot be considered simply as a source of GHG, since it is one of the sectors with the greatest potential for mitigation, largely due to the sequestration of soil organic carbon (SOC) (SUKHOVEEVA et al. 2023). Yet, despite SOC accumulation being considered a natural-based solution to address climate issues on a global scale (MINASNY et al., 2017), it is widely recognized that the conversion of native vegetation into conventional cropping systems results in a long-term loss of SOC (MEDEIROS et al., 2023), especially in semi-arid regions, such as that of Brazil.

In the semi-arid region of Brazil, native vegetation (Caatinga) has been undergoing deforestation to extract firewood and timber, and to establish crops and pastures (MEDEIROS et al., 2023). Agricultural activity covers a total area of 34.3 million ha, of which 68% is currently under livestock farming and 31% under other types of land use (MAPBIOMAS, 2023). However, 56% of these pasture areas show signs of degradation (LAPIG, 2023), while in the agricultural areas, it is estimated that 47% of annual crops are still cultivated under a conventional system (IBGE, 2017). As a consequence of such land-use dynamics, combined with the soil and climate conditions of the region (short and irregular periods of rain, high evapotranspiration and high temperatures), large areas of the semi-arid region have significantly reduced levels of soil organic matter (SOM), which has a direct impact on soil carbon (C) and nitrogen (N) stocks (SANTANA et al., 2019).

It is in this context that integrated farming systems have emerged, characterized by spatial and temporal combinations of pasture, crops and/or trees (e.g. integrated crop-livestock – ICL, integrated crop-livestock-forest – ICLF, integrated livestock-forest – ILF, integrated crop-forest – ICF) that seek a synergistic effect between components. Integrated systems have gained great prominence in recent years and have been adopted in tropical and subtropical areas of Brazil (POLIDORO et al. 2020), as well as in countries with a temperate climate (FRANZLUEBBERS and MARTINS, 2022). Among integrated systems, ILF offers benefits in terms of improving animal welfare (BROOM et al., 2013), increasing productivity and yield (BRUNETTI et al., 2022), and C fixation in the soil (LANDHOLM et al., 2019), resulting in a more productive and sustainable system.

SOM dynamics in response to management is often documented to infer the contribution of integrated systems to soil quality (VALANI et al. 2021). It is known that SOM is not homogeneous, containing components of different formation and residence times. Due to this heterogeneity, the physical fractionation of SOM has been widely used to study and predict its formation and renewal more accurately, and divides SOM into particulate organic matter (POM), a labile fraction that has a higher recycling rate of the organic constituents, and mineral-associated organic matter (MAOM), which consists of compounds leached from plant material or transformed by organisms, and protected from decomposition by chemical bonding to mineral surfaces (COTRUFO and LAVALLEE, 2022).

Studies show that different arrangements of integrated systems improve the quantity and quality of SOM (BIELUCZYK et al. 2020), increase the amount of chemically stable organic material in deeper layers of the soil (TADINI et al. 2021), and contribute to reducing C emissions in agricultural soils (SÁ et al. 2017). On the other hand, few studies have investigated

the isotopic composition of SOM (e.g. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in integrated systems as a tool for assessing the origin of the SOM (OLIVEIRA et al., 2018) and then identify the most promising species for accumulating C in the soil.

According to Damian et al. (2023), the contribution of integrated systems to SOC sequestration largely depends on the quantity and quality of the biomass provided by the arboreal and non-arboreal components of the system. However, Bieluczyk et al. (2023) points out that the arrangement of the trees can determine whether integration with pasture and/or agricultural crops will result in synergy or competition. Due to the complexity of managing integrated agricultural systems, Freitas et al. (2022) highlighted the need for studies to better understand the feasibility of adopting a system; this varies based on the characteristics of the region, the environmental and social conditions, and the different arrangements to be used.

However, in the semi-arid region of Brazil, there are few agricultural areas with integrated systems, or studies that understand the C dynamics of these systems. There are even fewer studies that associate the effect of the spacing and arrangement of the forestry component in ILF systems on SOC stocks. As such, it is of great importance to quantify SOC stocks in different ILF systems in the semi-arid region of Brazil, and then understand how adopting this strategy can minimize C losses and the release of GHG in the largest seasonally dry forest biome in South America, the Caatinga, which covers 75% of the semi-arid region of Brazil (CAVALCANTE and SAMPAIO, 2022). The hypothesis of this study is that the spacing between the forest component may influence the effects of ILF systems on C accumulation in the soil. Therefore, the aims of this study were: i) to evaluate how ILF systems with different spacings and forage crops affect carbon stocks in the soil and soil organic matter (SOM) fractions; and ii) use isotopic SOM fractions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to detect the origin, recycling and accumulation of SOM in different integrated agricultural systems.

3.2 MATERIAL AND METHODS

3.2.1 *Description of the study area*

The study was conducted at the Teaching, Research and Extension Unit on the Limoeiro do Norte Campus of the Federal Institute of Science and Technology of Ceará (IFCE), 05°10'53" S and 38°00'43" W, at an altitude of 146 m. The soil is classified as a Cambisol (FAO, 2015). According to the Köppen classification the local climate is type BSh (KOTTEK et al., 2006), with an average annual temperature of 29°C and annual precipitation of 719 mm. The chemical and physical characterization of the study area is shown in Table 1.

Table 1 - Characterization of the chemical and physical attributes of the soil in areas of native vegetation (NV), and integrated livestock-forest systems with sorghum (So), forage cactus (Fc), massai grass (Mg), buffel grass (Bg) and a strip of native trees (SNT).

System	Spacing	Sand	Silt	Clay	pH	Total CEC	BS
		(g kg ⁻¹)			(H ₂ O)	(cmol _c kg ⁻¹)	(%)
NV		474.36	227.20	298.44	7.40	11.15	91.45
SNT		472.39	174.16	353.45	6.98	8.90	89.30
So	28	508.24	220.84	270.92	6.43	9.26	75.90
Fc	28	486.89	195.66	317.45	5.55	10.16	57.40
Mg	28	447.37	274.54	278.10	6.65	10.46	72.85
Bg	28	468.66	230.54	300.80	6.78	9.34	80.23
So	14	445.79	117.76	361.74	5.98	7.84	78.75
Fc	14	485.64	187.69	326.67	5.83	7.95	74.70
Mg	14	456.93	225.50	317.57	6.83	8.43	93.08
Bg	14	406.55	247.49	345.97	6.93	8.69	89.00
So	7	474.76	172.92	352.33	6.88	9.40	77.45
Fc	7	529.93	89.22	380.85	5.88	10.29	63.43
Mg	7	435.49	213.95	350.56	6.83	8.13	92.05
Bg	7	424.48	239.28	336.24	6.25	7.81	79.65

Source: Author (2025). pH H₂O: potential of hydrogen in water (H₂O); Total CEC: Total cation exchange capacity; BS: base saturation.

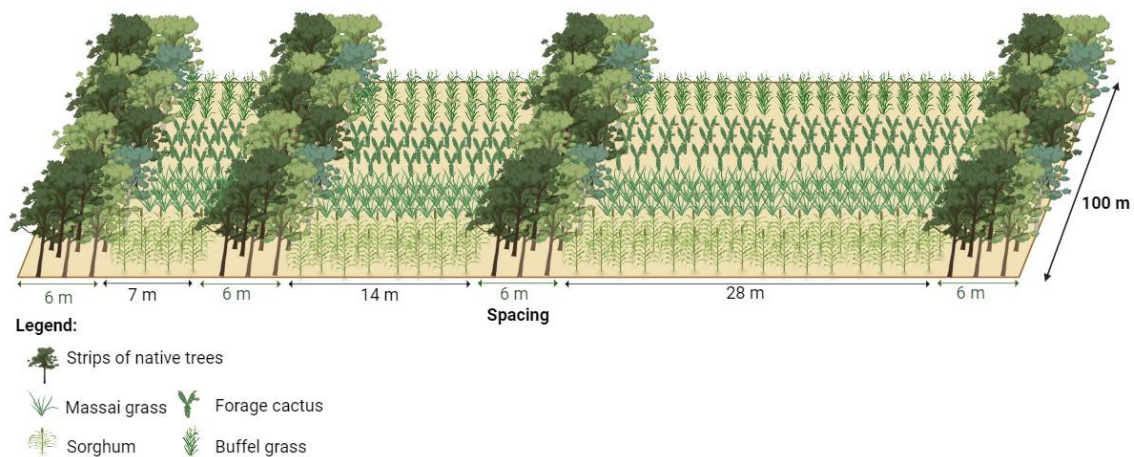
The area was deforested during the dry period of 2015, using a tractor with a front blade, and removing 100% of the vegetation present in the cultivable area (understory), leaving only strips of native trees, six meters wide. All the residual material was directed towards the edges. The area had previously consisted of arboreal caatinga in a stage of secondary succession. Phosphate fertilization was carried out annually, applying Mono Ammonium-Phosphate (MAP) fertilizer, 44% P₂O₅ – 10% N, at a dose of 200 kg ha⁻¹ year⁻¹ due to the low levels of phosphorus verified by a chemical analysis of the soil. Weed control was both mechanical and chemical.

The area consisted of four ILF systems arranged in three different spacings (7, 14 and 28 m) between 6-metre strips of trees from the Caatinga (Figure 1), corresponding to a forest cover of 46.15%, 30.00% and 17.64%, respectively. The livestock components were planted during the rainy season of 2016: *Pennisetum ciliare* (L.) Link (buffel grass - Bg), *Megathyrsus maximus* (Jacq.) Simon and Jacobs (massai grass - Mg), *Opuntia stricta* (Haw.) (forage cactus - Fc) and *Sorghum bicolor* (L.) Moench. (sorghum - So), corresponding to the non-grazed livestock system. The sorghum was planted and harvested annually, under conventional management practices, tilling the soil every year. The massai grass and buffel grass were

allowed to grow freely during the rainy season; at the end of the dry season, the grass was cut mechanically to an average height of 0.05 m above the ground, with the aim of standardizing the pastures for the next growing season. The forage cactus was cut every two years, and the residue removed from the area.

The treatments consisted of ILF systems with sorghum (ILFSo), forage cactus (ILFFc), massai grass (ILFMg) and buffel grass (ILFBg), at a spacing of 7 m (S7), 14 m (S14) or 28 m (S28) between the strips of native trees (SNT - Caatinga), giving a total of 13 treatments (Figure 1) plus the area of native vegetation used as reference (NV - area of Caatinga), which was located near the ILF area (i.e. under similar environmental conditions such climate, elevation and soil granulometry). The native vegetation was phytosociological in structure, with the following identified tree species: *Cordia goeldiana*, *Mimosa caesalpiniaefolia*, *Cenostigma pyramidale*, *Commiphora leptophloeos* and *Mimosa tenuiflora*.

Figure 1 - Representation of the area with ILF systems at different spacings between strips of native trees (Caatinga).



Source: Author (2025).

3.2.2 Soil sampling and laboratory analysis

Soil sampling was carried out in January 2022 during the dry season. Disturbed samples were collected from five trenches (replications) in each area at four different depths: 0–10, 10–20, 20–30 and 30–50 cm, giving a total of 280 samples (4 depths $\times$ 5 replications $\times$ 14 treatments). Undisturbed soil samples to determine the soil density were collected in three replications using Kopeck rings (5 $\times$ 5 cm, height $\times$ diameter), giving a total of 168 samples. The rings were oven-dried in the laboratory at 105°C for 48 h and the soil density calculated

based on the weight of the oven-dried samples and the total volume of the rings (TEIXEIRA ET al., 2017). The values of soil density were shown in the supplementary material (Table S1).

The disturbed samples were air-dried and sieved (<2 mm), and the roots and plant debris were removed. The particle size fractions of the soil (clay, silt and sand) were determined using the pipette method (TEIXEIRA et al., 2017). Subsamples of the disturbed samples were ground and sieved (0.25 mm mesh) before determining the soil organic carbon (SOC), total nitrogen (TN) and stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ atoms by continuous flow mass spectrometry in a Carlo Erba CHN 1110 elemental analyzer coupled to a Delta Plus isotope ratio mass spectrometer.

The soil organic matter was physically fractionated into particulate organic matter (POM) and mineral-associated organic matter (MAOM) following the particle size method proposed by Cambardella and Elliott (1992). Briefly, 30 ml of sodium hexametaphosphate solution (5 g l^{-1}) was added to 10 g of soil (< 2 mm) and dispersed in a horizontal shaker for 16 h (140 rpm). The dispersed solution was then passed through a 0.053 mm mesh while gently adding a jet of distilled water. The coarse fraction (> 0.053 mm - POM) retained on the sieve was stored in a container, dried in an oven (60°C) and ground (< 0.25 mm); the C was determined by dry combustion (TOC-V Shimadzu, coupled to the SSM-5000A Shimadzu solid sample module). The carbon in the MAOM fraction was determined as the difference between the SOC and the C-POM (Mendonça and Matos, 2017).

3.2.3 Calculation of the stocks of soil carbon and nitrogen

The stocks of SOC and TN were calculated by multiplying the content (C or N), soil density, and thickness of the soil layer. To minimize any discrepancies in soil density caused by the management practices, the calculated stocks were corrected based on the equivalent soil mass, following the method proposed by Sisti et al. (2004), as per Equation 1.

$$C_s = \sum C_{Ti} + \{[MT_n - (\sum MT_i - \sum MS_i)] CT_n\} \quad (1)$$

where C_s is the stock of SOC or TN (Mg C ha^{-1}) in the soil to a depth equal to a similar mass of soil in the reference profile; $\sum C_{Ti}$ is the sum of the total carbon content (Mg ha^{-1}) from layer 1 (surface) to layer n-1 (penultimate) in the soil profile under treatment; MT_n is the mass of soil (Mg ha^{-1}) in the last layer of the soil profile under treatment; $\sum MT_i$ is the sum of the mass of soil (Mg ha^{-1}) from layer 1 (surface) to layer n (last) in the profile under treatment; $\sum MS_i$ is the sum of the mass of soil (Mg ha^{-1}) from layer 1 (surface) to layer n (last layer) in the profile of the reference soil (NV); CT_n is the concentration of C or N (Mg Mg soil^{-1}) in the last layer of the soil profile under treatment.

The stocks were evaluated in two ways. In the first, only the data of the livestock component (Bg, Mg, So and Fc) were considered. In the second, the stocks of both components (livestock and forestry) were considered, corresponding to the ILFBg, ILFMg, ILFFc and ILFSo treatments. In other words, the C and N stocks were calculated based on the weighted average of the stocks in the forestry component (SNT) and livestock component (Bg, Mg, Fc and So), considering the percentage of forest cover in the SNT at each spacing, which were equal to 46.15%, 30.00% and 17.64% at a spacing of 7, 14 and 28 m, respectively.

3.2.4 Carbon Management Index

The carbon management index (CMI) was calculated as per the methodology proposed by Blair et al. (1995) and adapted by Diekow et al. (2005) – Equation 2, where CPI is the carbon pool index and LI is the carbon lability index.

$$CMI = CPI \times LI \times 100 \quad (2)$$

The CPI and LI were calculated as per Equations 3 and 4, respectively. Where L is the lability of carbon, calculated according to Equation 5.

$$CPI = \frac{TOC \text{ of the ILF or NTS systems}}{TOC \text{ of the NV}} \quad (3)$$

$$LI = \frac{L \text{ of the ILF or NTS systems}}{L \text{ of the NV}} \quad (4)$$

$$L = \frac{\text{labile C}}{\text{non-labile C}} \quad (5)$$

where labile and non-labile C are represented by the C-POM and C-MAOM stocks, respectively. NV was considered the reference area (CMI = 100%).

3.2.5 Statistical analysis

The data were subjected to presuppositions of normality (Shapiro-Wilks test), homoscedasticity (Bartlett test), nonadditivity (Tukey test), and independence of residuals (Durbin-Watson test) for ANOVA to be valid. When necessary, any outliers were removed. ANOVA was carried out for all treatments to evaluate the effects of the integrated systems (ILFFc, ILFSo, ILFMg, ILFBg), the system components (Fc, So, Mg, Bg and SNT) and spacings between the SNT (S7, S14 and S28) on the properties of the soil. When significant, Tukey's test ($p < 0.05$) was used to analyze the effects of the ILF systems and spacings. The ILF systems were also separately compared with the reference area (Caatinga) using Dunnett's test ($p < 0.05$). The SNT was compared with the reference area using Student's test ($p < 0.05$). The statistical analysis was carried out using the R software (R Core Team, 2021).

3.3 RESULTS

3.3.1 Carbon and Nitrogen content

The SOC content decreased with depth in each of the ILF systems (Table 2). Furthermore, although some of the losses were not significant, SOC levels were lower in the ILF components compared to NV, with reductions that varied between 2% and 38%, depending on the depth, crop and spacing (Table 2). On average, the smallest SOC losses occurred in the following order: Bg<Mg<So<Fc. In NV, the SOC content throughout the soil profile varied from 13.2 to 4.3 g kg⁻¹, and in SNT, from 14.6 to 4.4 g kg⁻¹. In the livestock components, the levels ranged from 12.0 to 3.4 g kg⁻¹, 11.7 to 3.4 g kg⁻¹, 9.6 to 3.2 g kg⁻¹ and 10.4 to 2.7 g kg⁻¹ for Bg, Mg, Fc and So, respectively.

In SNT, the SOC content in the 0-10 and 10-20 cm layers was higher than in NV by 10% and 16%, respectively, and did not differ at the deepest layer (30-50 cm). In the 20-30 cm layer there was a loss of SOC, which was 8% less than in NV (Table 2).

The N content in the ILFs was lower than in NV. The N losses varied according to the spacing of each system (Table 2). In general, except in systems with Mg, the smallest N losses in the top layer (0-10 cm) occurred at S14. At the deeper layers, the smallest loss also occurred at S14, except for the system with Bg, where the greatest loss occurred in the 10-20 cm layer. In the 20-30 cm layer, the smallest N loss occurred at S28 and S14, for Mg and Fc, respectively. In the systems with So, the different spacings had no effect on the N content in any of the layers.

Table 2 - Carbon and nitrogen content (g kg⁻¹) at depths of 0-10, 10-20, 20-30 and 30-50 cm under native vegetation (NV) and strips of native trees (SNT), and in the components of the ILF systems with buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT of Caatinga (7, 14 and 28 m).

Systems	C (g kg ⁻¹)			N (g kg ⁻¹)		
	7m	14m	28m	7m	14m	28m
<i>0-10 cm</i>						
Bg	9.04 (±0.76) a*	12.01 (±0.41) b ^{ns}	11.19 (±0.79) b*	0.88 (±0.09) a*	1.16 (±0.03) b ^{ns}	0.88 (±0.05) a*
Mg	10.43 (±0.76) a*	11.69 (±1.00) a ^{ns}	11.25 (±1.24) a*	1.02 (±0.05) a*	1.13 (±0.08) a ^{ns}	1.05 (±0.11) a*
Fc	8.78 (±0.70) a*	9.54 (±0.67) a*	6.62 (±1.37) a*	0.77 (±0.05) a*	0.91 (±0.04) b*	0.82 (±0.14) ab*
So	9.29 (±0.21) ab*	8.12 (±0.31) a*	10.44 (±1.40) b*	0.87 (±0.01) a*	0.88 (±0.12) a*	0.84 (±0.06) a*
SNT		14.63 (±0.89) *			1.19 (±0.1) ^{ns}	
NV		13.18 (±1.21)			1.22 (±0.1)	
<i>10-20 cm</i>						
Bg	6.06 (±0.19) a ^{ns}	5.49 (±0.36) a*	5.43 (±0.28) a*	0.62 (±0.02) a*	0.63 (±0.04) a*	0.54 (±0.04) b*

Mg	6.09 (± 0.50) a ^{ns}	6.12 (± 0.55) a ^{ns}	6.74 (± 0.74) a ^{ns}	0.66 (± 0.04) a ^{ns}	0.64 (± 0.04) a [*]	0.63 (± 0.06) a [*]
Fc	6.00 (± 0.24) a [*]	5.88 (± 0.59) a [*]	4.92 (± 0.54) b [*]	0.55 (± 0.03) a [*]	0.61 (± 0.02) a [*]	0.54 (± 0.04) a [*]
So	6.11 (± 0.57) ab ^{ns}	5.69 (± 0.42) a [*]	6.52 (± 0.36) b ^{ns}	0.62 (± 0.06) a [*]	0.62 (± 0.03) a [*]	0.63 (± 0.05) a [*]
SNT		8.01 (± 0.52) [*]			0.76 (± 0.1) ^{ns}	
NV		6.87 (± 0.30)			0.74 (± 0.1)	
<i>20-30 cm</i>						
Bg	5.15 (± 0.27) a [*]	5.45 (± 0.39) a ^{ns}	4.41 (± 0.38) b [*]	0.59 (± 0.03) a ^{ns}	0.55 (± 0.05) a [*]	0.56 (± 0.07) a [*]
Mg	4.88 (± 0.42) a [*]	4.29 (± 0.60) b [*]	5.22 (± 0.43) a [*]	0.56 (± 0.03) a [*]	0.53 (± 0.05) a [*]	0.62 (± 0.02) b ^{ns}
Fc	5.08 (± 0.55) a [*]	4.68 (± 0.24) a [*]	3.87 (± 0.24) b [*]	0.50 (± 0.05) ab [*]	0.52 (± 0.02) a [*]	0.46 (± 0.02) b [*]
So	4.64 (± 0.29) a [*]	4.27 (± 0.49) a [*]	4.46 (± 0.15) a [*]	0.56 (± 0.03) a [*]	0.52 (± 0.03) a [*]	0.52 (± 0.03) a [*]
SNT		5.54 (± 0.35) [*]			0.61 (± 0.0) ^{ns}	
NV		6.04 (± 0.16)			0.63 (± 0.0)	
<i>30-50 cm</i>						
Bg	3.56 (± 0.25) ab [*]	3.39 (± 0.11) a [*]	3.97 (± 0.10) b ^{ns}	0.49 (± 0.01) a ^{ns}	0.47 (± 0.02) a ^{ns}	0.46 (± 0.07) a [*]
Mg	3.87 (± 0.27) a ^{ns}	3.38 (± 0.92) a [*]	3.75 (± 0.34) a ^{ns}	0.49 (± 0.01) a ^{ns}	0.53 (± 0.02) a ^{ns}	0.47 (± 0.02) a ^{ns}
Fc	3.62 (± 0.18) a [*]	3.59 (± 0.29) a [*]	3.21 (± 0.14) a [*]	0.47 (± 0.01) a ^{ns}	0.47 (± 0.01) a ^{ns}	0.41 (± 0.04) a [*]
So	4.04 (± 0.44) a ^{ns}	2.69 (± 0.19) b [*]	3.27 (± 0.11) c [*]	0.42 (± 0.04) a [*]	0.42 (± 0.02) a [*]	0.40 (± 0.03) a [*]
SNT		4.37 (± 0.47) ^{ns}			0.56 (± 0.0) [*]	
NV		4.30 (± 0.44)			0.52 (± 0.0)	

Source: Author (2025). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. values in parentheses represent the standard deviation from the mean.

3.3.2 Natural abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the soil

The conversion of native vegetation into integrated systems resulted in significant changes in the $\delta^{13}\text{C}$ isotopic signature (Table 3). The $\delta^{13}\text{C}$ isotopic signal in NV and SNT was statistically similar at all depths, ranging from -25.5 to -25.1 ‰, values characteristic of C3 plants.

In the ILF systems, $\delta^{13}\text{C}$ values were very close to those of NV, ranging from -24.1 to -25.3 ‰ (Table 3). In this case, after six years, there was a small contribution to the composition of the soil organic matter from the C4 species (massai grass, sorghum and buffel grass) and the forage cactus (CAM species) introduced into the ILF systems. In the system with Bg at a spacing of 28 m there were increases of 0.84, 0.86, 0.94 and 1.02 ‰ in the abundance of $\delta^{13}\text{C}$ at depths of 0-10, 10-20, 20-30 and 30-50 cm, respectively, while in the system with Mg, $\delta^{13}\text{C}$ increased by 1.02 and 0.89 ‰ at depths of 0-10 and 30-50 cm, respectively ($p < 0.001$). At spacing S14, increases in $\delta^{13}\text{C}$ occurred in the systems with Fc (0.75 ‰; 0-10 cm), Mg (0.83 ‰; 10-20 cm and 0.93 ‰; 30-50 cm) and Bg (0.76 ‰; 10-20 cm) ($p < 0.001$).

The isotopic signal of $\delta^{15}\text{N}$ in NV ranged from 10.8 to 11.2 ‰, with the highest values in the 0-10 and 10-20 cm layers. The SNT presented an abundance of $\delta^{15}\text{N}$, similar to that of NV, with an average value of 11.28 ‰ at all depths. In the integrated systems, these values varied from 10.5 to 12.1 ‰. In general, there was an increase in the abundance of $\delta^{15}\text{N}$ at a depth of 30-50 cm in each of the integrated systems, this increase being significant ($p < 0.05$) in the systems with Fc at spacings S7 and S14, and So at S7 (Table 3).

Table 3 - Natural abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) at depths of 0-10, 10-20, 20-30 and 30-50 cm under native vegetation (NV) and in the components of the ILF systems: strips of native trees (SNT), buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT (7, 14 and 28 m).

Systems	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
	7m	14m	28m	7m	14m	28m
<i>0-10 cm</i>						
Bg	-24.76 (± 0.6) a ^{ns}	-24.70 (± 0.9) a ^{ns}	-24.52 (± 0.5) a [*]	10.81 (± 0.7) a ^{ns}	11.17 (± 0.6) a ^{ns}	10.86 (± 0.5) a ^{ns}
Mg	-24.76 (± 0.3) a ^{ns}	-24.92 (± 0.7) a ^{ns}	-24.34 (± 0.3) a [*]	11.50 (± 0.6) a ^{ns}	11.48 (± 1.0) a ^{ns}	11.08 (± 0.7) a ^{ns}
Fc	-24.96 (± 0.5) a ^{ns}	-24.61 (± 0.6) a [*]	-24.78 (± 0.7) a ^{ns}	11.68 (± 0.7) a ^{ns}	12.06 (± 0.6) a ^{ns}	11.35 (± 0.8) a ^{ns}
So	-25.07 (± 0.6) a ^{ns}	-25.04 (± 0.6) a ^{ns}	-25.29 (± 0.4) a ^{ns}	11.74 (± 0.4) a ^{ns}	11.37 (± 0.6) a ^{ns}	11.34 (± 0.3) a ^{ns}
SNT		-25.54 (± 0.8) ^{ns}			11.28 (± 1.1) ^{ns}	
NV		-25.36 (± 1.1)			11.18 (± 0.7)	
<i>10-20 cm</i>						
Bg	-24.51 (± 0.1) a ^{ns}	-24.32 (± 0.5) a [*]	-24.22 (± 0.2) a [*]	10.68 (± 0.9) a ^{ns}	11.38 (± 0.6) a ^{ns}	11.37 (± 0.7) a ^{ns}
Mg	-24.83 (± 0.4) a ^{ns}	-24.26 (± 0.4) a [*]	-24.68 (± 0.3) a ^{ns}	11.60 (± 0.6) a ^{ns}	11.44 (± 0.9) a ^{ns}	10.52 (± 0.9) a ^{ns}
Fc	-24.73 (± 0.5) a ^{ns}	-24.71 (± 0.4) a ^{ns}	-24.63 (± 0.3) a ^{ns}	11.73 (± 0.5) a ^{ns}	11.66 (± 0.5) a ^{ns}	11.02 (± 0.3) a ^{ns}
So	-25.16 (± 0.6) a ^{ns}	-24.93 (± 0.5) a ^{ns}	-25.00 (± 0.2) a ^{ns}	11.64 (± 0.4) a ^{ns}	11.20 (± 0.5) a ^{ns}	11.20 (± 0.7) a ^{ns}
SNT		-25.18 (± 0.7) ^{ns}			11.40 (± 1.2) ^{ns}	
NV		-25.08 (± 0.5)			11.06 (± 1.0)	
<i>20-30 cm</i>						
Bg	-24.79 (± 0.4) a ^{ns}	-24.54 (± 0.2) a ^{ns}	-24.15 (± 0.3) a [*]	10.58 (± 1.2) a ^{ns}	10.70 (± 1.1) a ^{ns}	10.95 (± 0.5) a ^{ns}
Mg	-24.61 (± 0.4) a ^{ns}	-24.66 (± 0.6) a ^{ns}	-24.36 (± 0.4) a ^{ns}	11.23 (± 0.2) a ^{ns}	11.53 (± 0.8) a ^{ns}	11.01 (± 0.6) a ^{ns}
Fc	-25.13 (± 0.5) a ^{ns}	-24.87 (± 0.6) a ^{ns}	-24.62 (± 0.4) a ^{ns}	11.75 (± 0.8) a ^{ns}	11.88 (± 0.6) a ^{ns}	11.38 (± 0.7) a ^{ns}
So	-25.15 (± 0.6) a ^{ns}	-25.09 (± 0.6) a ^{ns}	-25.03 (± 0.4) a ^{ns}	10.95 (± 0.3) a ^{ns}	11.15 (± 0.6) a ^{ns}	10.90 (± 0.5) a ^{ns}
SNT		-25.51 (± 0.7) ^{ns}			11.36 (± 0.5) ^{ns}	
NV		-25.09 (± 0.3)			10.97 (± 0.5)	
<i>30-50 cm</i>						
Bg	-24.36 (± 0.3) a ^{ns}	-24.59 (± 0.3) a ^{ns}	-24.05 (± 0.2) a [*]	10.87 (± 0.7) a ^{ns}	10.94 (± 0.6) a ^{ns}	10.75 (± 0.8) a ^{ns}
Mg	-24.64 (± 0.1) a ^{ns}	-24.15 (± 0.2) a [*]	-24.19 (± 0.4) a [*]	11.42 (± 0.6) a ^{ns}	11.51 (± 1.1) a ^{ns}	10.79 (± 0.4) a ^{ns}
Fc	-24.88 (± 0.5) a ^{ns}	-24.72 (± 0.4) a ^{ns}	-24.40 (± 0.6) a ^{ns}	12.06 (± 0.5) a [*]	12.06 (± 0.7) a ^{ns}	11.35 (± 0.9) a ^{ns}
So	-24.96 (± 0.6) a ^{ns}	-24.74 (± 0.5) a ^{ns}	-24.81 (± 0.4) a ^{ns}	11.70 (± 0.5) a [*]	11.62 (± 0.7) a ^{ns}	11.40 (± 0.8) a ^{ns}

SNT	-25.41 (± 0.7) ^{ns}	11.09 (± 0.8) ^{ns}
NV	-25.08 (± 0.9)	10.81 (± 0.9)

Source: Author (2025). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. values in parentheses represent the standard deviation from the mean.

3.3.3 Carbon and nitrogen stocks

When evaluating the crops adopted in the ILF systems, there was an overall reduction in the soil C and N stocks at depths of 0-30 and 0-50 cm (Figure 2). The most pronounced impacts were seen on the SOC stock in the systems with Fc, with losses that varied from 7.65 to 10.41 Mg C ha⁻¹ and 8.91 to 13.15 Mg C ha⁻¹ for the 0-30 and 0-50 cm layers, respectively ($p < 0.001$). The same occurred with the N stocks, where the losses ranged from 0.82 to 1.04 Mg N ha⁻¹ (0-30 cm) and 0.96 to 1.31 Mg N ha⁻¹ (0-50 cm) under Fc. C and N losses in the system with So varied from 6.30 to 9.87 Mg C ha⁻¹ and 0.68 to 0.80 Mg N ha⁻¹ (0-30 cm), and from 7.72 to 13.60 Mg C ha⁻¹ and 0.89 to 1.13 Mg N ha⁻¹ (0-50 cm) ($p < 0.001$).

On the other hand, the systems with Mg and Bg resulted in the smallest losses in C and N stocks in the 0-30 and 0-50 cm layers (Figure 2b-c). When converting NV to the system with Mg, the reductions ranged from 2.77 to 5.59 Mg C ha⁻¹ and 0.22 to 0.40 Mg N ha⁻¹ (0-30 cm), and from 3.85 to 6.75 Mg C ha⁻¹ and 0.19 to 0.48 Mg N ha⁻¹ (0-50 cm) ($p < 0.001$). In the system with Bg, the losses ranged from 3.61 to 6.58 Mg C ha⁻¹ and 0.29 to 0.78 Mg N ha⁻¹ (0-30 cm), and from 5.81 to 8.14 Mg ha⁻¹ and 0.40 to 0.90 Mg N ha⁻¹ (0-50 cm). Although the systems with Bg and Mg showed a loss of C and N stocks in the 0-30 and 0-50 cm layers, the adoption of these systems afforded C and N stocks in the 0-10 cm layer similar to those in NV depending on the spacing. In the 0-10 cm layer, the C and N stocks in NV were 17.85 Mg C ha⁻¹ and 1.20 Mg N ha⁻¹. In the systems with Mg and Bg, the stocks ranged from 15.58 to 18.93 Mg C ha⁻¹ and 1.13 to 1.91 Mg N ha⁻¹, and from 15.28 to 17.39 Mg C ha⁻¹ and 0.59 to 1.28 Mg N ha⁻¹, respectively (Figure 2a).

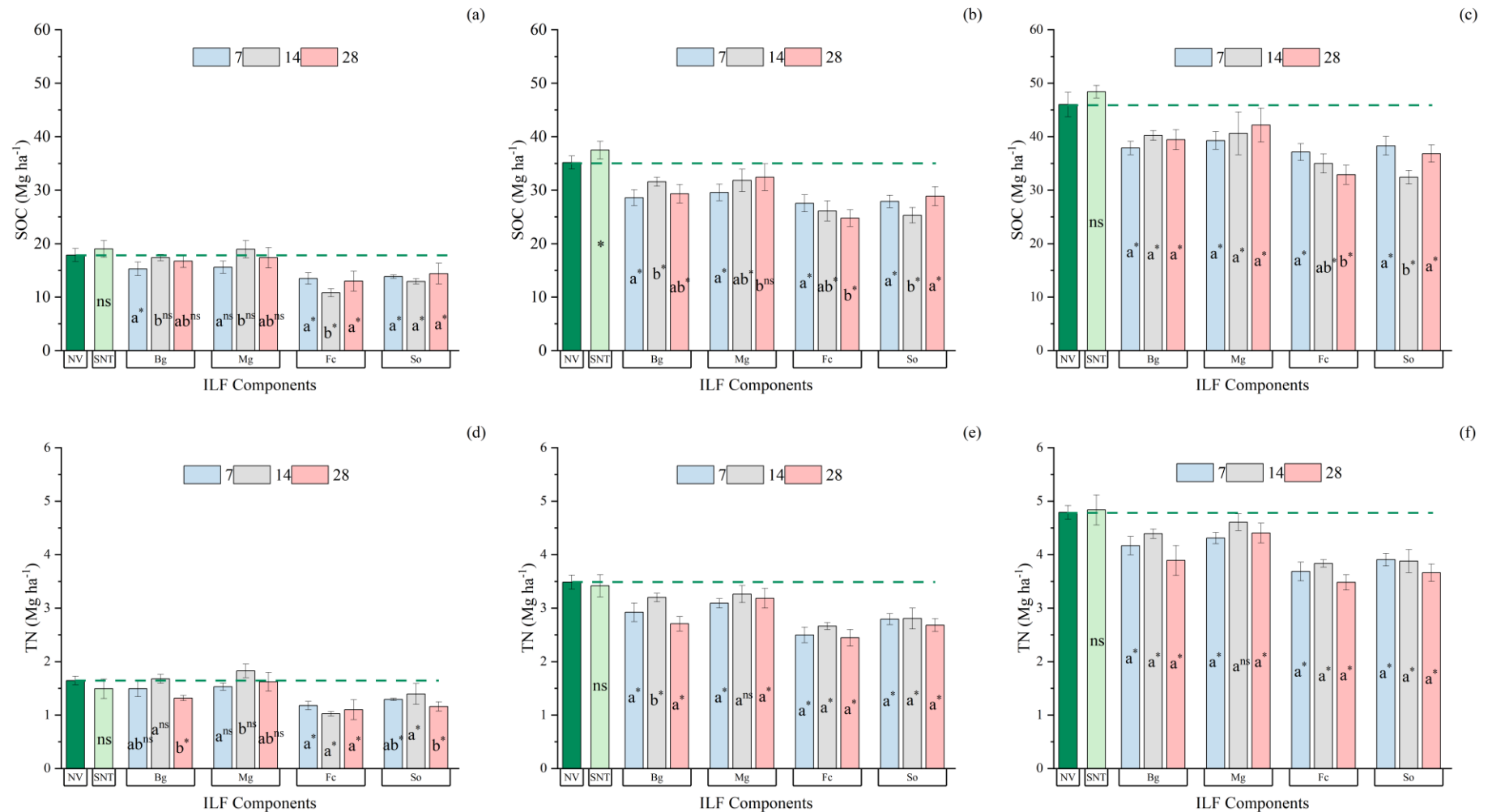
By comparing the different spacings within the same crop, it was possible to see which spacing resulted in the smallest loss of SOC. At the 0-30 cm layer, the adoption of the greatest spacing in the system with Fc resulted in a greater loss of C, since at S28 the loss was 10.41 Mg C ha⁻¹, while at S7 it was 7.65 Mg C ha⁻¹ ($p < 0.001$). On the other hand, the most significant reduction in SOC stocks in the systems with massai grass (Mg) was seen at S7 (5.55 Mg C ha⁻¹), where C losses were around 50% greater compared to S28 (2.77 Mg C ha⁻¹). In the system with Bg, the carbon loss at S7 was 6.48 Mg C ha⁻¹, 45% higher than at S14 (3.61 Mg C ha⁻¹).

In the system with So, the effect of spacing was less pronounced, with the greatest loss of C occurring at S14 (9.87 Mg C ha⁻¹).

In the 0-50 cm layer, changes in SOC stocks did not occur to the same extent as in the 0-30 cm layer, and differences between the spacings were only found in the systems with Fc and So, where under Fc, the SOC stock went down from 37.11 Mg C ha⁻¹ at S7 to 32.88 Mg C ha⁻¹ at S28, while under So, the most significant reduction was seen when the values decreased from 38.50 Mg C ha⁻¹ at S7 to 32.88 Mg C ha⁻¹ at S14.

The C stocks in SNT were greater than in NV, with increases of 1.16, 2.33 and 2.39 Mg C ha⁻¹ in the 0-10, 0-30 and 0-50 cm layers, respectively (Figure 2a-c). For the TN stock, SNT did not differ from NV, with values of 1.54 Mg N ha⁻¹ (0-10 cm), 3.42 Mg N ha⁻¹ (0-30 cm) and 4.84 Mg N ha⁻¹ (0-50cm). There was also no difference in N stocks in the 0-30 and 0-50 cm layers as a function of the spacing between the integrated systems, except under Bg in the 0-30 cm layer, where the stock was 9% and 18% greater at S14 than at S7 (2.92 Mg N ha⁻¹) and S28 (2.71 Mg N ha⁻¹), respectively ($p < 0.05$) (Figure 2e-f).

Figure 2 - Carbon (a, b, c) and nitrogen (d, e, f) stocks (Mg ha^{-1}) in the 0-10, 0-30 and 0-50 cm layers under native vegetation (NV), and in the components of the ILF systems: strips of native trees (SNT), buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT (7 m, 14 m and 28 m). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the components of the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. Error bars represent the standard deviation from the mean.

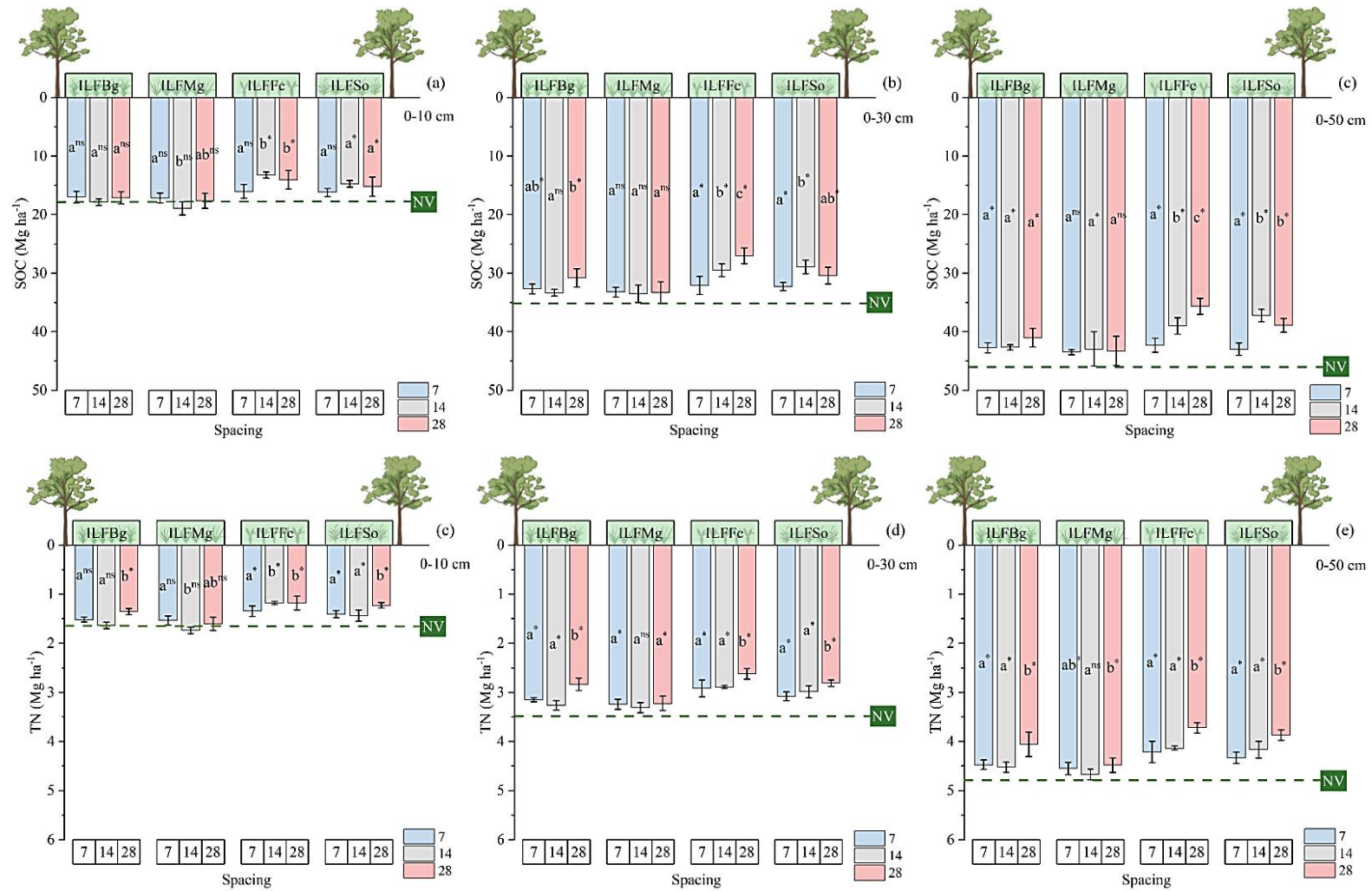


Analyzing the components of the ILF systems showed similar SOC stocks to those in NV, but only in the 0-10 cm layer in the Bg and Mg systems (Figure 2a). However, based on the weighted average obtained from the values for the SOC stocks in the livestock (Bg, Mg, Fc and So) and forestry (SNT) components, the ILF systems were able to increase C stocks at the deeper layers, so that in the ILF system with massai grass (ILFMg), the C stocks showed levels equal to those of NV in the 0-30 cm layer at all spacings, with an average of $33.35 \text{ Mg C ha}^{-1}$, while in the system with buffel grass, the spacing S14 did not differ from NV. (Figure 3). In the ILF system with massai grass (ILFMg), the same was seen in the 0-50 cm layer at spacing S7 ($43.48 \text{ Mg C ha}^{-1}$) and S28 ($43.30 \text{ Mg C ha}^{-1}$); whereas in the areas of ILF with forage cactus (ILFFc) and sorghum (ILFSo), the C stock did not differ from that of NV in the top layer of the soil at S7.

In general, the 7-metre spacing had the smallest C losses in the ILF systems, with values of 0.70 to $3.71 \text{ Mg C ha}^{-1}$. At S14, there were losses of 1.64 to $8.80 \text{ Mg C ha}^{-1}$; however, in the 0-10 cm layer, there was an increase in C stock of 0.02 and $1.10 \text{ Mg C ha}^{-1}$ in the ILFBg and ILFMg systems, respectively. At S28, C losses in the ILF systems ranged from 0.19 to $10.35 \text{ Mg C ha}^{-1}$ (Figure 4).

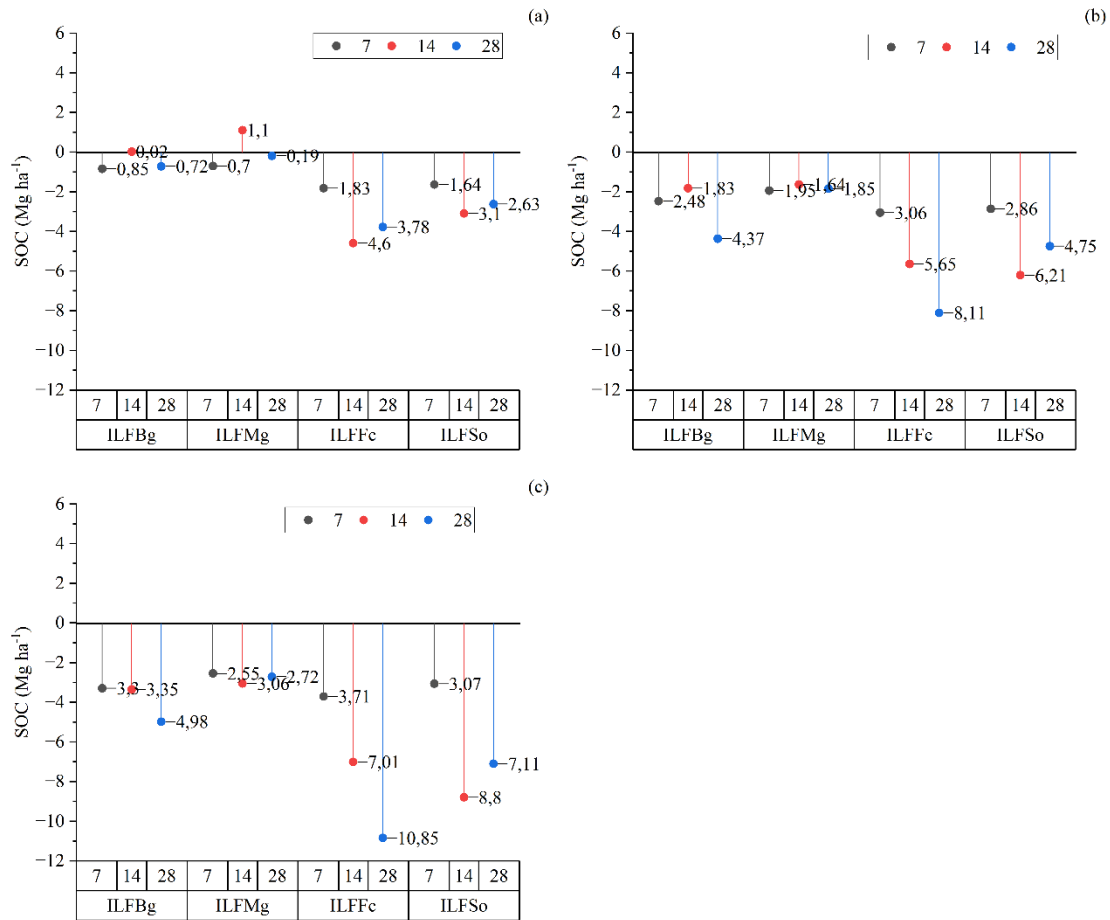
The N stock in the ILFBg, ILFMg, ILFFc and ILFSo systems showed similar behavior to that seen when evaluating the components of the ILF systems. In general, spacings S7 and S14 afforded the highest N values in each of these systems (Figure 3).

Figure 3 - Carbon and nitrogen stocks (Mg ha^{-1}) in the 0-10, 0-30 and 0-50 cm layers under native vegetation (NV), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSO) at different spacings between the strips of trees (7, 14 and 28 m). Mean values followed by the same letter in the same ILF system do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV). Error bars represent the standard deviation from the mean.



Source: Author (2025).

Figure 4 - Losses in carbon stock (Mg ha^{-1}) in the 0-10 (a), 0-30 (b) and 0-50 cm (c) layers under native vegetation (NV), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSo) at different spacings between the SNT of Caatinga (7, 14 and 28 m).



Source: Author (2025).

3.3.4 Soil organic matter fractions

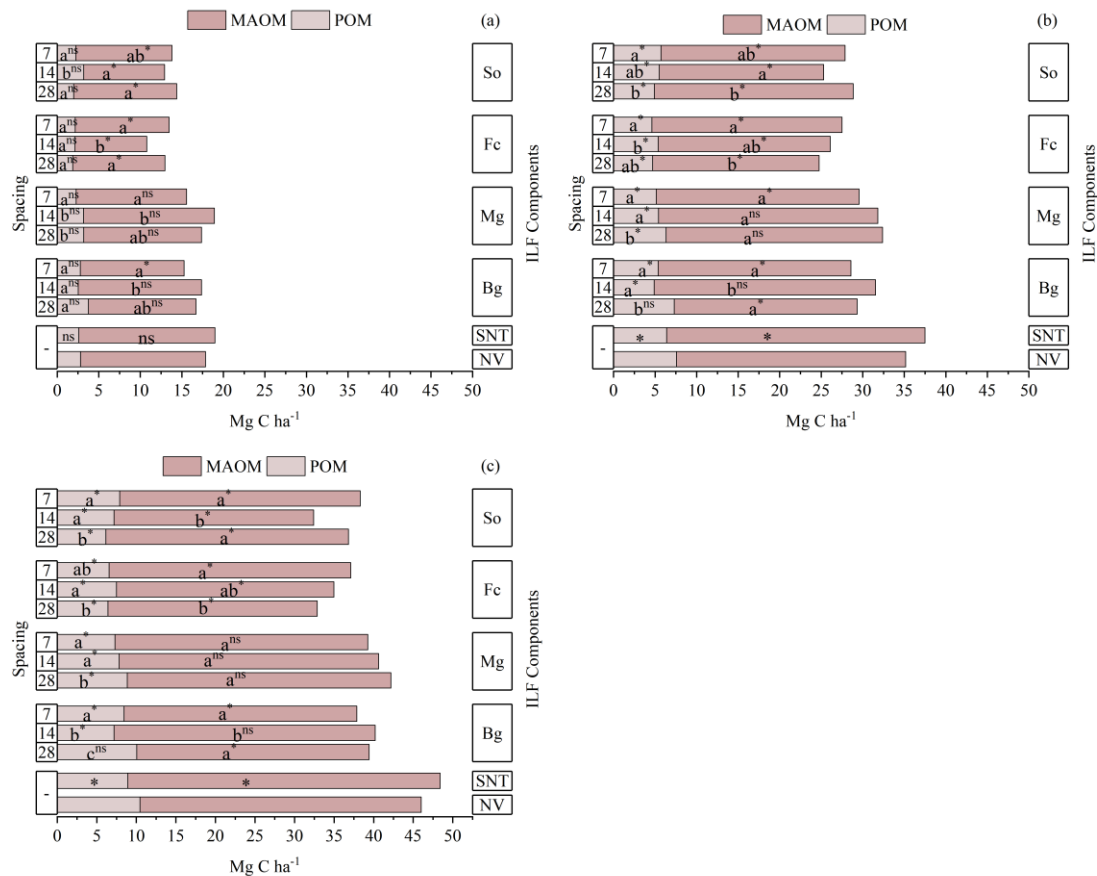
Table S2 presents the carbon content (g kg^{-1}) in the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions. The adoption of ILF systems resulted in changes in C stocks in the different SOM fractions (Figure 5a-c). In the 0-10 cm layer, POM stocks ($>53 \mu\text{m}$) under the ILF systems did not differ from those in NV (2.82 Mg ha^{-1}), with values ranging from 1.86 to 3.77 Mg ha^{-1} (Figure 5a). In the 0-30 and 0-50 cm layers, the ILF systems presented lower POM levels at each spacing compared to the native vegetation, with the exception of Bg at S28 (7.31 Mg ha^{-1} ; 0- 30 cm and 10.05 Mg ha^{-1} ; 0-50 cm), with stocks similar to those of NV (7.56 Mg ha^{-1} ; 0-30 cm and 10.46 Mg ha^{-1} ; 0-50 cm) (Figure 5b-c).

The different spacings determined the intensity of the change in SOM quality in the different ILF systems. Under So, the lowest POM values were seen at S28 in each of the soil layers. In the other systems with grasses (Mg and Bg), the largest spacing (S28) afforded the highest POM values. In the system with Fc, the different spacings had no effect on the POM fraction in the top layer of the soil (0-10), while in the 0-30 and 0-50 cm layers, the lowest POM values were seen at S7 (4.63 Mg ha⁻¹) and S28 (6.41 Mg ha⁻¹), respectively.

In the system with Bg, S14 (14.85 Mg ha⁻¹; 0-10 cm, 26.66 Mg ha⁻¹; 0-30 cm, and 33.03 Mg ha⁻¹; 0-50 cm) afforded the smallest losses in the MAOM fraction in relation to NV (15.03 Mg ha⁻¹; 0-10 cm, 27.58 Mg ha⁻¹; 0-30 cm, and 35.56 Mg ha⁻¹; 0-50 cm). In the system with Mg, only S7 in the 0-30 cm layer reduced the MAOM stock (12%) in relation to NV. The MAOM fraction was greater at S7 under Fc. In the 0-30 and 0-50 cm layers specifically, MAOM values in Fc at S7 were approximately 14% and 15% higher, respectively, in relation to S28 (20.04 Mg ha⁻¹; 0-30 and 26.46 Mg ha⁻¹; 0-50 cm). The MAOM decreased in the ILF system with So, where the lowest MAOM values were recorded at S14 (9.72 Mg ha⁻¹; 0-10 cm, 19.84 Mg ha⁻¹; 0-30 cm, and 25.24 Mg ha⁻¹; 0-50 cm). With regard to So, it is important to note that although the POM fraction was lower at S28, this spacing favored formation of the MAOM fraction, with an MAOM stock 28%, 21% and 22% greater than at S14 in the 0-10, 0-30 and 0-50 cm layers, respectively.

In SNT, the C stock in both SOM fractions in the top layer of soil was similar to that in NV. As the depth increased, the MAOM fraction was 11% and 12% greater compared to NV in the 0-30 and 0-50 cm layers, respectively. In SNT, the loss of SOC occurred in the POM fraction, which was smaller by around 15% in relation to NV, where stocks were 7.59 and 10.46 Mg ha⁻¹ in the 0-30 and 0-50 cm layers, respectively. (Figure 5b-c).

Figure 5 - Carbon stocks (Mg ha⁻¹) in the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions in the 0-10 (a), 0-30 (b) and 0-50 cm (c) layers under native vegetation (NV), and in the components of the ILF systems: strips of native trees (SNT), buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT (7, 14 and 28 m). Mean values followed by the same letter in the same component of the ILF system and fraction (POM and MAOM) do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant ^{ns}, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV.



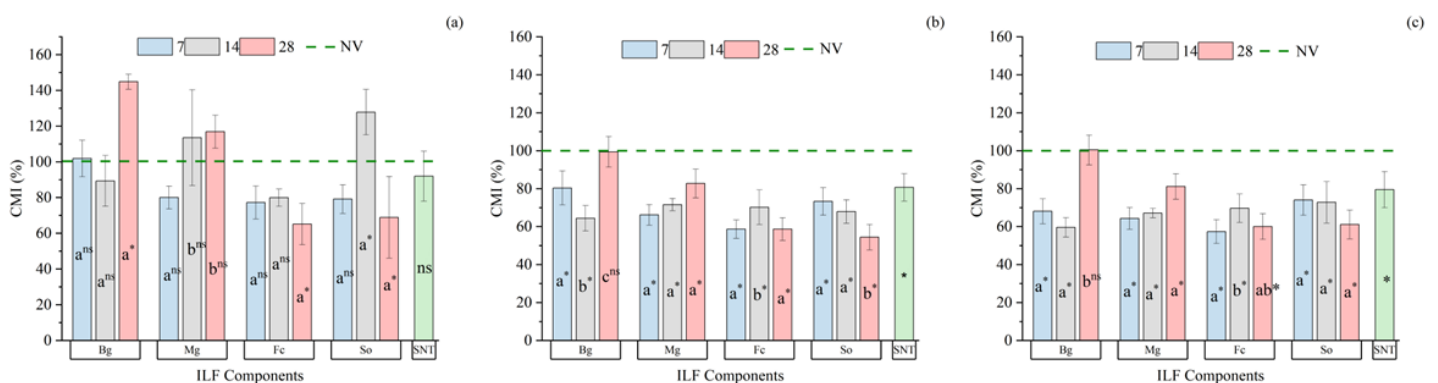
Source: Author (2025).

The effects of converting Caatinga into ILF systems on the CMI are shown in Figure 6. In the 0-10 cm layer, the highest CMI values were seen for the ILF systems with grasses, with average values of 112%, 103.5% and 91.9% for Bg, Mg and So, respectively (Figure 6a). For Fc, the average CMI value was 74.1%. Comparing the different spacings, the systems with Bg at spacing S14 (144.9%) and So at spacing S28 (127.8%) resulted in CMI values that were higher than those in NV. In the system with Mg, spacings S14 (113.5%) and S28 (116.9%) resulted in CMI values similar to those of NV.

In the 0-30 cm layer in the system with Bg, the CMI at S7 and S14 was 68% and 59%, values that were well below those of NV (100%) (Figure 6b). Due to the increase in lability (L) at S28 (Table S3), the CMI under Bg at S28 reached the same level as in NV. In the system with Mg, all of the spacings resulted in a CMI value that was lower than in NV, with S28 affording the highest value (81%). In the integrated systems, the lowest CMI values were found under Fc, with 57% (S7), 70% (S14) and 60% (S28). CMI values in the system with So were similar at S7 (73%) and S14 (74%), and lower at S28 (61%). In the 0-50 cm layer, the CMI showed similar behavior to that seen for the 0-30 cm layer (Figure 6c).

For SNT, the CMI in the 0-10 cm layer was similar to that in NV, with a value of 92% (Figure 6a). For the 0-30 cm layer, despite there being an increase in the C and MAOM stocks in relation to NV, the 16% loss in the POM fraction resulted in a CMI value lower than that of NV, of 79% (Figure 6b).

Figure 6 - Carbon management index (CMI) in the 0-10 (a), 0-30 (b) and 0-50 cm (c) layers under native vegetation (NV), and in the components of the ILF systems: strips of native trees (SNT), buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT (7, 14 and 28 m). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant ns, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. Error bars represent the standard deviation from the mean.



Source: Author (2025).

3.4 DISCUSSION

In general, the C and N content of the soil decreased significantly with depth in each of the systems under evaluation. This can be attributed to the surface layer of the soil being more biologically active (NGABA et al., 2020), as it receives the greatest input of organic material

(PEREIRA et al., 2023), coupled with root production and decomposition, which is normally more intense at the surface (DAMIAN et al., 2023).

The results of this study suggest that, over six years, integrated livestock-forest systems in the semi-arid region of Brazil have altered the dynamics of the SOC. There are studies evaluating the relationships between integrated agricultural systems and soil carbon sequestration in semi-arid regions around the world (AUKEMA et al., 2023) and in Brazil (Maia et al., 2007; TONUCCI et al., 2023). However, the time taken to adopt a system is a factor that must be taken into account, as more significant changes tend to occur in the medium and long term, as seen by Bieluczyk et al. (2020), who found gains in C and N in ILP systems in the south of Brazil nine years after they were implemented.

Crop characteristics also have a variable effect on the storage and deposition of C in the soil by the plants (SUKHOVEEVA et al., 2023), whether through the quantity and quality of the deposited litter (PEREIRA et al., 2023) or through variations in the deposition of roots and exudates (NGABA et al., 2020). Studies have shown that buffel grass and massai grass can produce around 10.55 g plant⁻¹ and 23.07 g plant⁻¹ of dry roots, respectively (PATIDAR et al., 2023), while for sorghum, the estimates were 11.62 g plant⁻¹ (NGIDI et al., 2024). Regarding the shoots, dry biomass production of forage sorghum and forage cactus in the semi-arid region of Brazil was 8.18 Mg ha⁻¹ and 12.5 Mg ha⁻¹, respectively (PINHEIRO et al., 2024; QUEIROZ et al., 2015), while buffel grass and massai grass respectively produced around 14.85 and 8.7 Mg ha⁻¹ (OSMAN et al., 2008; OLIVEIRA et al., 2019).

The results showed that losses in SOC stocks were more pronounced in the system with Fc. The low deposition of organic residue by plants with a crassulacean acid metabolism (CAMELO et al., 2021), despite the high production of shoot biomass (COELHO et al., 2023), probably leaves the soil more exposed to solar radiation, accelerating the process of SOM decomposition, and resulting in the rapid loss of SOC. As a result, under forage cactus, C is mainly input from the roots (NOVARA et al., 2014). However, the shallowness of the cactus root system, with its horizontal distribution in the soil (COELHO et al., 2023), results in smaller contributions to soil C at depth.

On the other hand, when cultivating grasses, there is greater shoot production and deposition and, above all, constant rhizodeposition and the uniform distribution of root exudates in the soil. As such, the conversion of NV into ILF systems with massai grass and buffel grass was sufficient to increase SOC stocks in the 0-10 cm surface layer and resulted in the smallest SOC losses in the 0-30 and 0-50 cm layers. According to Bayer et al. (2011), on average, around 21% and 12% of the C biomass of the roots and shoots, respectively, are converted into SOC.

Silva et al. (2013) reported that grasses can contribute up to 50% of the C supplied to the soil. Similarly, Manna et al. (2005) reported a transfer of 45% C to the soil by sorghum. In this respect, the results and trends found in the systems with massai grass and buffel grass show that SOC accumulation can increase over the years, even at the deepest layers.

The isotopic signal data helps to confirm these results, since, in the ILF systems with massai grass and buffel grass (-24.5 ‰), $\delta^{13}\text{C}$ was generally isotopically heavier than in the other ILF systems (-24.8 ‰; Fc and -25.0 ‰; So), showing a significant deposition of C from the roots of these systems. This gradual increase in $\delta^{13}\text{C}$ has previously been seen in areas of native vegetation that were converted into systems with grasses (Loss et al., 2016) and forage cactus (CAMELO et al., 2021). Isotopic determinations of the natural abundance of $\delta^{13}\text{C}$ in SOM have been used to assess the origin of the SOM. The use of $\delta^{13}\text{C}$ allows the sources of SOC derived from old and new vegetation to be identified, as long as the two types of vegetation have distinct photosynthetic pathways. Plants that use RuBisCO (C3 metabolism) for CO_2 fixation have an isotopic composition ($\delta^{13}\text{C}$) ranging from -24 to -34 ‰, while plants that use PEPcase (C4 metabolism) range from -6 to -19 ‰ (SMITH and EPSTEIN, 1971).

Furthermore, the observed increase in SOC accumulation under the Bg and Mg systems can be explained by the increase in C stocks in the SOM fractions. In the POM fraction, the beneficial effect was seen mainly in the top layer of the soil (0-10 cm), while in the MAOM fraction, the increase extended to the deeper layers (0-30 and 0-50 cm). The formation of POM in the surface layers is favored by the input of residue above the ground, while in the subsoil, C input via the roots and exudates tends to form compounds of microbial origin that favor the formation of MAOM (PIMENTEL et al., 2024).

The POM varied between 25% and 13% of the total SOC in the ILF systems, while MAOM was between 86% and 75%. This is in line with other studies, which show that in most soils, POM contributes between 10% and 25% of the total SOC (Curtin et al., 2019). Although POM is considered a highly sensitive fraction to changes in management, increases in the POM play a crucial role in SOC sequestration, since the more labile C fraction helps in the formation of the more stable SOM fraction. Silva et al. (2020) found that the MAOM fraction in an ILF system with *Urochloa brisantha* and Eucalyptus was greater when compared to conventional pasture. According to the authors, the presence of tree species creates a microclimate that favors the activity of microorganisms that help in the process of transforming organic residue and in the formation of more-stable SOM fractions.

As a result of the increase in POM, CMI values increased under the grass systems, mainly at spacings of 14 and 28 meters, since the CMI is highly influenced by the most labile

SOM fraction (RAMESH et al., 2015). The CMI provides a sensitive measure of the rate of change in the soil C dynamics of a system relative to a more stable soil (BLAIR et al. 1995). This is because the greater the SOC reservoir and C lability, the greater the availability of C and energy for soil microbial activity, this being a crucial point for soil quality (RAMESH et al., 2015). These results are consistent with previous studies conducted in silvopastoral systems (MAIA et al., 2007 and DAMIAN et al., 2023), which also found a CMI greater than 100% in the top layers of the soil.

In the system with sorghum, C losses were more significant compared to the other grasses (Bg and Mg), despite sorghum showing high biomass production and a substantial capacity for C storage in agricultural soils (NGIDI et al., 2024). For better understanding, we must consider the management adopted in the areas of sorghum, where unlike the systems with Bg or Mg, sorghum (So) was planted and harvested annually using the conventional system, i.e. the soil was tilled annually during preparation. This practice of constantly tilling the soil promotes the breakdown of soil aggregates, especially macroaggregates, compromising the physical protection of the SOM, and facilitating aeration and microbial activity, which in turn accelerates oxidation of the SOM (MEDEIROS et al., 2023). The potential for SOC recovery under ILF systems therefore depends on efficient management strategies, such as less tillage.

As for the effect of spacing on the components of the ILF systems, the results showed that in the massai grass and buffel grass, SOC levels increased with the distance between the strips of trees, showing the lowest SOC losses at a spacing of 28 meters. This is possible, since proximity to the forest component can limit forage growth, mainly because under shade, the photosynthetic processes of grasses are altered and there is a reduction in CO₂ assimilation, resulting in the reduced production of stems and leaves (SANTIAGO-HERNÁNDEZ et al., 2016). Based on the results of work carried out in the present study area, the average total forage biomass in the buffel grass and massai grass was lower at S7 ($\approx 2700 \text{ kg ha}^{-1} \text{ year}^{-1}$) compared to S14 ($\approx 3600 \text{ kg ha}^{-1} \text{ year}^{-1}$) and S28 ($\approx 3900 \text{ kg ha}^{-1} \text{ year}^{-1}$) (SANTOS NETO et al., 2023a). This was a consequence of the lower incidence of photosynthetically active radiation on the surface of the leaves, which caused a reduction in tiller population density, reflecting in lower total forage biomass production at S7. The same was seen for the forage cactus, where at spacings S14 and S28, total biomass production was approximately 50% greater (SANTOS NETO et al., 2023b).

Our results corroborate earlier studies carried out with integrated systems in Brazil. An area under a 22-year-old silvopastoral system in Minas Gerais showed that increased shading reduced forage production by approximately 44%, while an increase in forage biomass

production associated with the greater deposition of signal grass litter in points furthest from the trees, reflected in significant increases in the SOC stock (PACIULLO et al., 2021; CÁ et al., 2022). Similarly, the biomass of cultivated grasses (PEZZOPANE et al., 2017), as well as the volume of fine roots (BIELUCZYK et al., 2023) decreased close to eucalyptus trees in an ICLF system, which consequently affected the SOC stock (BIELUCZYK et al., 2020) in six-year-old integrated agricultural systems in the southeast of Brazil.

Based on results for the forage cactus in the same study area, the lowest biomass production also occurred at S7 and was associated with the limited arrival of solar radiation in the environment close to the arboreal component, affecting CO₂ assimilation (SANTOS NETO et al., 2023b). However, unlike the systems with Mg and Bg, in the system with forage cactus, the increased production of total biomass for increases in the distance between the strips of trees did not reflect in an increase in SOC, the low deposition of organic residue by the cactus leaving the soil more exposed. Whereas in this case, where the influence of tree shade was greater (S7), the soil was less exposed to high temperatures, which may have contributed to a lower rate of SOM decomposition.

In general, the different spacings in the ILF systems did not result in significant variations in the soil N stocks in the 0-30 or 0-50 cm layers, although changes were seen in the top layer. In the 0-10 cm layer, the S7 and S14 spacings resulted in the largest N stocks in most of the ILF systems. This is probably due to the forest component in this ILF system comprising mainly leguminous species, while the addition of material with a low C/N ratio (tree litter) resulted in its rapid decomposition and mineralization, thereby increasing the nutrient content of the soil, especially N. Another reason for the increase in N stocks is the addition of N via symbiosis between leguminous plants and N-fixing bacteria (CAMELO et al., 2021). Cá et al. (2022) attributed the introduction of N into an ILF system to the increase in litter deposition from leguminous plants. The reduction in spacing between the strips of trees may therefore have contributed to more litter being deposited by the tree species between the SNT, which resulted in the largest stocks of N in the ILF systems at spacings of 7 and 14 meters over the six-year period.

At our study site, the fact that the residual material from the suppressed Caatinga was directed to the edges of the SNT probably contributed to the gains of 7% (0-30 cm) and 5% (0-50 cm) in SOC stocks seen in the SNT compared to NV. Similar results were found by Tonucci et al. (2023), who attributed the gain in SOC in ILF systems mainly to the input of suppressed Caatinga biomass. Increases in the SOC in integrated systems are associated with the forest component, mainly because the forest helps to reduce temperatures and increase humidity, with

a positive effect on SOM and soil-nutrient dynamics (TONUCCI et al., 2023). Furthermore, Upson et al. (2016) pointed out that each tree in an ILF system can intercept more solar radiation and water than an equivalent tree in a forest, which may contribute to a greater input of SOC.

In addition to the SOC stocks in each component of the ILF systems, we evaluated the effect of the ILF systems on the stock of SOC, taking into account the C data obtained for the livestock and forestry components together. As such, when analyzing the C stocks in all the components of the ILF systems, i.e. the weighted average of the SOC data in the SNT and in each crop, the ILF systems proved to be even more promising, with the loss in C stocks decreasing even at the deepest layers (Figure 3a-c). Furthermore, SOC losses in the ILF systems (SNT and Bg/Mg/Fc/So) began to occur in the following order: S7<S14<S28. In the 0-50 cm layer, the average losses were 3.16 (S7), 5.56 (S14) and 6.29 (S28) Mg C ha⁻¹, corresponding to 7%, 12% and 14%, respectively. As part of the NV is preserved in strips in the ILF systems in this study, the SOC is also preserved. As a result, the impact on soil C in relation to the total deforestation of NV is minimized. Therefore, the higher SOC stocks at the 7-metre spacing are related to the larger area of NV, given the percentage of forest cover in the SNT, which was 46.7% at S7, compared to 30% at S14 and 18% at S28.

Although in general, the 7-metre spacing presented the smallest SOC losses, it must be pointed out that there was no significant statistical difference between the spacings in the ILF systems with buffel grass at the layers under evaluation (0-10 and 0-50 cm), while the massai grass showed better results at a spacing of 14 meters in the 0-10 cm layer, with no statistical difference in the 0-30 and 0-50 cm layers. In other words, these results show that it is possible to adopt certain ILF systems at greater spacings without promoting additional soil C losses, which is important, as it means the possibility of expanding the commercially exploited area, which is particularly relevant in the semi-arid region of Brazil, a region characterized by small and medium-sized producers.

Furthermore, these ILF systems can make a bigger difference in SOC over time. The results shown in this study, although already promising, only cover a short period of six years. Because of this, we encourage more research to look at how C builds up in integrated systems in the semi-arid region. This should include long-term studies, when changes in SOC buildup may become more evident.

3.5 CONCLUSIONS

Conversion of the Caatinga into ILF systems altered the accumulation of carbon and nitrogen in the soil. The integrated systems were affected by the different spacings between the

strips of trees in the ILF systems in the Caatinga, as well as by the livestock component. In general, adopting ILF systems in areas of NV was found to reduce the SOC in the 0-30 and 0-50 cm layers. However, in spite of these losses, the results in the top layer indicate a significant potential for ILF systems with massai grass and buffel grass for recovering the SOC stocks and SOM fractions, especially when greater spacings are used between the forest component.

Six years after implementation, the C4 plants (massai grass and buffel grass) were a source of SOM in the ILF systems, with slight increases in $\delta^{13}\text{C}$, while the increased spacing between the SNT provided the soil with a greater contribution of both labile SOM (POM) and more-stable SOM (MAOM). In this respect, the use of short grasses in integrated systems can maximize C inputs to the soil in the semi-arid region of Brazil, helping to establish more-sustainable practices in the region. On the other hand, for the ILF systems with forage cactus and sorghum the six-year period was not enough to promote C accumulation in the soil. More time will therefore be needed to evaluate the contribution of these system components to the sequestration of soil C. In the system with sorghum, the different spacings had little effect on SOM dynamics, largely due to the constant tilling in these areas.

Considering the C data obtained in the strips of livestock and forest components together, the smallest C losses began to occur at S7, but with few statistical differences in relation to S14 and S28. As such, our results show that, from the point of view of soil C, the 14- and 28-metre spacings are just as interesting as S7, albeit with a greater productive area, making ILF arrangements more viable for the region.

Finally, the results of this study can encourage the adoption of integrated production systems in the Caatinga as a management strategy to increase C sequestration in soils in the semi-arid region, thereby contributing to Brazil's efforts to fulfil international commitments to reduce greenhouse gas emissions (NDC of Brazil).

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SUPPLEMENTARY MATERIAL

Table S1 - Soil density (g cm^{-3}) at depths of 0-10, 10-20, 20-30 and 30-50 cm under native vegetation (NV) and strips of native trees (SNT), and in the components of the ILF systems with buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT of Caatinga (7, 14 and 28 m).

Systems	Soil density (g cm^{-3})					
	7m	14m	28m	7m	14m	28m
	<i>0-10 cm</i>			<i>10-20 cm</i>		
Bg	1.69 (± 0.13) a*	1.45 (± 0.09) b ^{ns}	1.49 (± 0.26) ab ^{ns}	1.34 (± 0.16) a ^{ns}	1.28 (± 0.21) a ^{ns}	1.36 (± 0.09) a ^{ns}
Mg	1.49 (± 0.11) a ^{ns}	1.62 (± 0.06) a ^{ns}	1.54 (± 0.09) a ^{ns}	1.30 (± 0.05) a ^{ns}	1.37 (± 0.15) a ^{ns}	1.33 (± 0.14) a ^{ns}
Fc	1.53 (± 0.07) ab ^{ns}	1.70 (± 0.15) a*	1.35 (± 0.09) b ^{ns}	1.44 (± 0.07) a ^{ns}	1.43 (± 0.19) a ^{ns}	1.29 (± 0.08) a ^{ns}
So	1.49 (± 0.16) a ^{ns}	1.59 (± 0.09) a ^{ns}	1.38 (± 0.07) a ^s	1.37 (± 0.04) a ^{ns}	1.35 (± 0.05) a ^{ns}	1.27 (± 0.02) a ^{ns}
SNT		1.30 (± 0.04) ^{ns}			1.34 (± 0.08) ^{ns}	
NV		1.35 (± 0.02)			1.33 (± 0.05)	
	<i>20-30 cm</i>			<i>30-50 cm</i>		
Bg	1.20 (± 0.07) a ^{ns}	1.35 (± 0.14) a ^{ns}	1.36 (± 0.30) a ^{ns}	1.13 (± 0.06) a ^{ns}	1.27 (± 0.16) a ^{ns}	1.30 (± 0.19) a ^{ns}
Mg	1.17 (± 0.03) a ^{ns}	1.35 (± 0.16) a ^{ns}	1.38 (± 0.19) a ^{ns}	1.14 (± 0.08) a ^{ns}	1.35 (± 0.20) a ^{ns}	1.30 (± 0.29) a ^{ns}
Fc	1.38 (± 0.05) a ^{ns}	1.32 (± 0.08) a ^{ns}	1.41 (± 0.07) a ^{ns}	1.29 (± 0.09) a ^{ns}	1.40 (± 0.08) a ^{ns}	1.39 (± 0.11) a ^{ns}
So	1.28 (± 0.0) a ^{ns}	1.30 (± 0.13) a ^{ns}	1.16 (± 0.07) a ^{ns}	1.24 (± 0.06) a ^{ns}	1.24 (± 0.05) a ^{ns}	1.29 (± 0.27) a ^{ns}
SNT		1.30 (± 0.18) ^{ns}			1.28 (± 0.22) a ^{ns}	
NV		1.36 (± 0.19)			1.26 (± 0.06)	

Source: Author (2025). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. Values in parentheses represent the standard deviation from the mean.

Table S2 - Carbon content (g kg^{-1}) in the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions at depths of 0-10, 10-20, 20-30 and 30-50 cm under native vegetation (NV) and strips of native trees (SNT), and in the components of the ILF systems with buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT of Caatinga (7, 14 and 28 m).

Systems	POM (g kg^{-1})			MAOM (g kg^{-1})		
	7m	14m	28m	7m	14m	28m
	<i>0-10 cm</i>					
Bg	1.75 (± 0.39) a ^{ns}	1.62 (± 0.37) a ^{ns}	2.41 (± 0.30) b ^{ns}	7.28 (± 0.91) a*	10.39 (± 0.61) b ^{ns}	8.79 (± 0.98) a*
Mg	1.60 (± 0.15) a ^{ns}	1.86 (± 0.25) ab ^{ns}	2.25 (± 0.42) b ^{ns}	8.83 (± 0.89) a*	9.83 (± 1.14) a ^{ns}	8.99 (± 1.37) a*
Fc	1.42 (± 0.10) a ^{ns}	2.09 (± 0.47) b ^{ns}	1.38 (± 0.24) a ^{ns}	7.37 (± 0.69) a*	7.45 (± 0.65) a*	8.24 (± 1.39) a*
So	1.44 (± 0.16) a ^{ns}	1.85 (± 0.47) a ^{ns}	1.55 (± 0.36) a ^{ns}	7.85 (± 0.18) a*	6.28 (± 0.58) b*	8.89 (± 1.33) a*
SNT		2.24 (± 0.60) ^{ns}			12.39 (± 1.71) ^{ns}	
NV		1.87 (± 0.48)			11.31 (± 1.08)	
	<i>10-20 cm</i>					

Bg	1.28 (± 0.20) a ^{ns}	0.93 (± 0.18) a *	1.59 (± 0.32) a ^{ns}	4.78 (± 0.32) a ^{ns}	4.56 (± 0.36) ab ^{ns}	3.84 (± 0.59) b *
Mg	1.20 (± 0.46) a ^{ns}	0.96 (± 0.10) a ^{ns}	1.27 (± 0.28) a ^{ns}	4.89 (± 0.68) a ^{ns}	5.16 (± 0.54) a ^{ns}	5.47 (± 0.75) a ^{ns}
Fc	0.98 (± 0.08) a ^{ns}	1.14 (± 0.37) a ^{ns}	1.09 (± 0.08) a ^{ns}	5.02 (± 0.26) a ^{ns}	4.74 (± 0.39) a ^{ns}	3.83 (± 0.59) b *
So	1.34 (± 0.17) a ^{ns}	1.08 (± 0.29) a ^{ns}	0.92 (± 0.19) a *	4.77 (± 0.68) a ^{ns}	4.61 (± 0.22) a ^{ns}	5.60 (± 0.29) b ^{ns}
SNT		1.57 (± 0.52) ^{ns}			6.44 (± 0.52) *	
NV		1.47 (± 0.31)			5.41 (± 0.42)	
<i>20-30 cm</i>						
Bg	1.06 (± 0.24) a *	0.87 (± 0.25) a *	1.42 (± 0.28) a ^{ns}	4.09 (± 0.43) a ^{ns}	4.58 (± 0.38) a ^{ns}	2.99 (± 0.51) b *
Mg	1.28 (± 0.13) a *	1.03 (± 0.28) a *	1.26 (± 0.37) a *	3.60 (± 0.31) ab ^{ns}	3.25 (± 0.51) a *	3.96 (± 0.31) b ^{ns}
Fc	1.18 (± 0.46) a *	0.98 (± 0.08) a *	0.99 (± 0.13) a *	3.90 (± 0.59) a ^{ns}	3.70 (± 0.25) a ^{ns}	2.89 (± 0.31) b *
So	1.18 (± 0.42) a *	0.90 (± 0.11) a *	1.27 (± 0.14) a *	2.99 (± 0.30) a ^{ns}	3.38 (± 0.47) a *	3.20 (± 0.23) a *
SNT		1.14 (± 0.26) *			4.40 (± 0.33) ^{ns}	
NV		1.87 (± 0.62)			4.17 (± 0.69)	
<i>30-50 cm</i>						
Bg	1.16 (± 0.25) a ^{ns}	0.90 (± 0.16) a ^{ns}	1.01 (± 0.23) a ^{ns}	2.40 (± 0.31) a *	2.49 (± 0.20) a *	2.96 (± 0.24) a ^{ns}
Mg	0.86 (± 0.17) a ^{ns}	1.07 (± 0.29) a ^{ns}	0.90 (± 0.30) a ^{ns}	3.01 (± 0.26) a ^{ns}	2.31 (± 1.05) b *	2.85 (± 0.33) ab ^{ns}
Fc	0.79 (± 0.17) a ^{ns}	0.85 (± 0.14) a ^{ns}	0.67 (± 0.08) a *	2.83 (± 0.23) a ^{ns}	2.74 (± 0.28) a ^{ns}	2.54 (± 0.13) a ^{ns}
So	0.77 (± 0.09) a ^{ns}	0.70 (± 0.16) a *	0.55 (± 0.08) a *	3.27 (± 0.49) a ^{ns}	1.99 (± 0.33) b *	2.72 (± 0.13) a ^{ns}
SNT		0.93 (± 0.21) ^{ns}			3.43 (± 0.37) ^{ns}	
NV		1.06 (± 0.19)			3.24 (± 0.43)	

Source: Author (2025). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. Values in parentheses represent the standard deviation from the mean.

Table. S3 - Carbon pool index (CPI), carbon lability index (LI), lability (L) and carbon management index (CMI) at depths of 0-10, 10-20, 20-30 and 30-50 cm under native vegetation (NV) and strips of native trees (SNT), and in the components of the ILF systems with buffel grass (Bg), massai grass (Mg), forage cactus (Fc) and sorghum (So) at different spacings between the SNT of Caatinga (7, 14 and 28 m).

Systems	CPI			LI		
	7m	14m	28m	7m	14m	28m
<i>0-30 cm</i>						
Bg	0.81 (± 0.04) a	0.90 (± 0.02) A	0.83 (± 0.05) ab	0.84 (± 0.09) a	0.66 (± 0.06) b	1.21 (± 0.13) b
Mg	0.84 (± 0.04) a	0.90 (± 0.06) ab	0.92 (± 0.07) b	0.77 (± 0.09) a	0.75 (± 0.07) a	0.89 (± 0.12) a
Fc	0.78 (± 0.04) a	0.74 (± 0.05) b	0.70 (± 0.04) b	0.74 (± 0.10) a	0.94 (± 0.13) b	0.86 (± 0.12) ab
So	0.79 (± 0.03) ab	0.72 (± 0.04) a	0.82 (± 0.05) b	0.94 (± 0.12) a	1.01 (± 0.14) a	0.74 (± 0.06) b
SNT	1.02 (± 0.05)			0.75 (± 0.10)		
<i>0-50 cm</i>						
Bg	0.82 (± 0.03) a	0.87 (± 0.02) a	0.86 (± 0.04) a	0.98 (± 0.12) a	0.74 (± 0.08) b	1.16 (± 0.10) c
Mg	0.85 (± 0.04) a	0.88 (± 0.09) a	0.92 (± 0.07) a	0.77 (± 0.07) a	0.82 (± 0.09) a	0.91 (± 0.12) a
Fc	0.81 (± 0.03) a	0.76 (± 0.04) ab	0.71 (± 0.04) b	0.73 (± 0.07) a	0.92 (± 0.12) b	0.83 (± 0.11) ab

So	0.83 (± 0.04) a	0.70 (± 0.03) b	0.80 (± 0.04) a	0.88 (± 0.10) a	0.96 (± 0.07) a	0.68 (± 0.06) b
SNT	1.05 (± 0.03)			0.77 (± 0.06)		
Systems	L			CMI (%)		
	7m	14m	28m	7m	14m	28m
	<i>0-30 cm</i>					
Bg	0.23 (± 0.02) a ^{ns}	0.18 (± 0.02) b [*]	0.33 (± 0.04) c [*]	68 (± 6.6) a [*]	60 (± 5.1) a [*]	100 (± 7.8) b ^{ns}
Mg	0.21 (± 0.03) a [*]	0.21 (± 0.02) a [*]	0.25 (± 0.03) a ^{ns}	64 (± 5.7) a [*]	67 (± 2.5) a [*]	81 (± 6.7) a [*]
Fc	0.20 (± 0.03) a [*]	0.26 (± 0.03) b ^{ns}	0.24 (± 0.03) ab ^{ns}	57 (± 6.3) a [*]	70 (± 7.4) b [*]	60 (± 6.8) ab [*]
So	0.26 (± 0.03) a ^{ns}	0.28 (± 0.04) a ^{ns}	0.21 (± 0.02) b [*]	74 (± 8.1) a [*]	73 (± 10.9) a [*]	61 (± 7.6) b [*]
SNT	0.21 (± 0.03) *			79 (± 9.5) *		
NV	0.28 (± 0.04)			100		
	<i>0-50 cm</i>					
Bg	0.29 (± 0.04) a ^{ns}	0.22 (± 0.02) b ^{ns}	0.34 (± 0.03) c ^{ns}	80 (± 9.0) a [*]	64 (± 6.7) b [*]	99 (± 8.1) c ^{ns}
Mg	0.23 (± 0.02) a ^{ns}	0.24 (± 0.03) a ^{ns}	0.27 (± 0.03) a ^{ns}	66 (± 5.4) a [*]	72 (± 3.2) a [*]	83 (± 7.6) b [*]
Fc	0.22 (± 0.02) a ^{ns}	0.27 (± 0.04) b ^{ns}	0.24 (± 0.03) ab ^{ns}	59 (± 4.8) a [*]	70 (± 9.1) b [*]	59 (± 6.0) a [*]
So	0.26 (± 0.03) a ^{ns}	0.28 (± 0.02) a ^{ns}	0.20 (± 0.02) b ^{ns}	73 (± 7.2) a [*]	68 (± 6.1) a [*]	54 (± 6.7) b [*]
SNT	0.23 (± 0.02) *			81 (± 7.2) *		
NV	0.30 (± 0.03)			100		

Source: Author (2025). Mean values followed by the same letter in the same component of the ILF system do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$) – compares the ILF systems with the reference area (NV), and by t-test ($p < 0.05$) – compares SNT with NV. Values in parentheses represent the standard deviation from the mean.

CHAPTER III

4 SOIL MICROBIOLOGICAL PROPERTIES IN INTEGRATED LIVESTOCK-FOREST SYSTEMS UNDER DIFFERENT SPACINGS AND FORAGE CROPS IN THE SEMI-ARID REGION OF BRAZIL

ABSTRACT

In the semi-arid region of Brazil, one of the biggest challenges has been to increase, in a sustainable way, the productive capacity of the soil. In this context, the adoption of integrated agricultural systems is considered a promising strategy for sustainable agricultural intensification. In this study, the microbiological properties of the soil were evaluated in integrated livestock-forest systems (ILF) in the semi-arid region of the state of Ceará, Brazil. The study was carried out in an area that included four ILF systems: ILF with sorghum (ILFSo), forage cactus (ILFFc), massai grass (ILFMg), and buffel grass (ILFBg), each at a spacing of 7 m (S7), 14 m (S14) or 28 m (S28) between strips of native Caatinga trees (NTS). These systems were compared with an area of native vegetation (NV). Soil samples were analyzed for microbial biomass carbon (MBC), basal respiration (BR), activity of the soil enzymes β -glucosidase and easily extractable glomalin (EEG), and soil organic carbon (SOC). The results showed that the ILFMg and ILFBg systems were the most promising for maintaining soil microbial biomass and activity, and SOC. The ILFFc and ILFSo systems promoted improvements in MBC, but were not sufficient to promote the accumulation of SOC. The spacing of 7 m between SNT gave the highest values for MBC, the microbial quotient ($qMIC$) and SOC, and the lowest for the metabolic quotient (qCO_2). ILF systems with grasses increased average EEG levels in the 10-20 cm layer, and increased β -glucosidase activity in the 0-10 and 10-20 cm layers. In conclusion, ILF systems with grasses stood out in terms of soil microbiological activity and SOC accumulation in areas of the Caatinga.

Keywords: Semi-arid region, enzyme activity, microbial carbon, integrated agricultural systems.

RESUMO

Na região semiárida do Brasil, um dos maiores desafios tem sido aumentar, de forma sustentável, a capacidade produtiva do solo. Neste contexto, a adoção de sistemas agrícolas integrados é considerada uma estratégia promissora para a intensificação agrícola sustentável.

Neste estudo, foi avaliado as propriedades microbiológicas do solo em sistemas de integração pecuária-floresta (IPF) no semiárido do estado do Ceará, Brasil. O estudo foi realizado em uma área com quatro sistemas de IPF: IPF com sorgo (IPFSo), palma forrageira (IPFPf), capim massai (IPFCm), capim buffel (IPFCb), todos com espaçamentos de 7 m (E7), 14 m (E14) e 28 m (E28) entre faixas de árvores nativas da Caatinga (FxVN). Esses sistemas foram comparados com uma área de vegetação nativa (VN). Amostras de solo foram analisadas quanto ao carbono da biomassa microbiana (CBM), respiração basal (RB), atividades das enzimas do solo β -glicosidase e glomalina facilmente extraível (GFE), e carbono orgânico do solo (COS). Os resultados mostraram que os sistemas de IPFCm e IPFCb foram os sistemas mais promissores para a manutenção da biomassa e atividade microbiana do solo, assim como do COS. Os sistemas IPFPf e IPFSo promoveram melhorias no CBM, porém não foram suficientes para promover o acúmulo de COS. O espaçamento de 7 m entre FxVN apresentou os maiores valores de CBM, quociente microbiano (qMIC) e COS e os menores de quociente metabólico (qCO₂). Os sistemas de IPF com gramíneas aumentaram os teores médios de GFE na camada de 10-20 cm e aumentaram as atividades da β -glicosidase nas camadas de 0-10 e 10-20 cm. Em conclusão, os sistemas de IPF com gramíneas se destacaram quanto as atividades microbiológicas do solo e de acúmulo de COS em áreas de Caatinga.

Palavras-chaves: Caatinga, atividades enzimáticas, carbono microbiano, sistemas integrados.

4.1 INTRODUCTION

The semi-arid region of Brazil covers around 12% of the country, which corresponds to approximately one million km² (ALTHOFF et al., 2018) and is home to around 28 million people (INSA, 2023). The region presents conditions of high temperature and low rainfall and is covered by the dry forest known as Caatinga, an exclusively Brazilian biome (Vilela et al., 2019). Current agricultural activity is still mainly based on conventional agricultural practices, semi-extensive livestock farming, and the exploitation of timber and firewood as a source of energy (LOURENÇO et al. 2022). The indiscriminate use of forest resources (TAVARES et al., 2019), the establishment of subsistence crops, and overgrazing (SANTOS et al., 2022), combined with the environmental conditions of the region, has led to soil degradation in the area. A high level of soil degradation generates desertification in arid and semi-arid environments (ABDELRAHMAN, 2023), and it is estimated that in the semi-arid region of Brazil, 16% of the area is susceptible to desertification (ALVALÁ et al., 2019).

With the need to recover degraded areas and the search for more conservationist systems, the adoption of sustainable agriculture has intensified in recent years (ZANDONA et

al., 2019). In this context, integrated production systems are seen as strategies for integrating agricultural, livestock and forestry systems in the same area, and are recognized for their potential to achieve synergy between the components (BIELUCZYK et al. 2023). The integration of trees and pasture, known as the integrated livestock-forest system (ILF), contributes to animal welfare (LOPES et al., 2016) and to increased productivity and yield in the area (ALMEIDA et al. 2021). Furthermore, the introduction of ILF systems can affect the dynamics of soil organic carbon (SOC), which in turn affects the quality and quantity of soil organic matter (SOM), soil aggregation, the microbial population, and enzyme activity (SARTO et al., 2020a).

For example, intercropping brachiaria with shrubby legumes increased soil C five years after implementing a silvopastoral system in a region with a subtropical climate (LIRA JUNIOR et al., 2020). Abreu et al. (2020) showed that the presence of eucalyptus in a four-year silvopastoral system in the Brazilian Cerrado increased C stocks and improved microbial activity in the soil. Guillot et al. (2019) reported that the forest component can promote changes in the microbiota and SOM input, as an increase in microbial activity was seen near the rows of trees. Therefore, when developing strategies to mitigate soil degradation and climate change, it is crucial to consider the soil microbial community, as this represents part of the living component of SOM and plays an essential role in soil functionality, as well as in nutrient availability, and the decomposition, mineralization and stabilization of the SOM (SIX et al., 2006).

Soil microorganisms are generally quantified by estimating their C content, known as microbial biomass carbon (MBC), while microbial activity is quantified using soil microbial respiration, as this represents the oxidation of organic matter by aerobic soil organisms (SOUZA et al., 2021). Microbial communities also act as the primary source of soil enzymes, and govern nutrient and SOM transformations (SEKARAN et al., 2021). For example, β -glucosidase is one of the most common enzymes produced by microbiota that is found in the soil and contributes to the final step of cellulose degradation (TABATABAI, 1994). Glomalin, a glycoprotein produced in soils by arbuscular mycorrhizal fungi (AMF), acts as an efficient indicator of the performance of AMF, which are responsible for the formation of stable soil aggregates due to the production of adhesive expolysaccharides (LUNA et al., 2016).

Since, as a general rule, changes in land use in the Caatinga have adversely altered microbiological diversity and activity in the soil, contributing to C losses and increasing greenhouse gas emissions (LOURENÇO et al., 2022), the adoption of ILF systems in these areas can induce a positive change in soil quality (BATISTA et al., 2018), since the introduction

of tree species can promote microbial diversity (CARSON et al., 2010). However, although the Caatinga is one of the most sensitive areas to climate change (IPCC, 2019), it is also one of the least studied semi-arid regions in the world (LOURENÇO et al., 2022). Understanding the changes in soil microbiology induced by different ILF systems in the Caatinga biome is therefore essential for measuring the contribution of these systems to maintaining soil health and sustainable development in the region. Studies that evaluate the effects of ILF systems on soil microbiota in the semi-arid region of Brazil are scarce; furthermore, these effects may not be uniform since they can be affected by the distance from the forest component. The aims of this study therefore were i) to determine the MBC in ILF systems under different crops and spatial arrangements in an area in the semi-arid region of Ceará, ii) evaluate the effects of using ILF systems on soil microbial activity, and iii) determine the enzyme activity of β -glucosidase and glomalin in integrated systems.

4.2 MATERIAL AND METHODS

4.2.1 Description of the study site

The study was conducted at the Teaching, Research and Extension Unit on the Limoeiro do Norte Campus of the Federal Institute of Science and Technology of Ceará (IFCE), 05°10'53" S and 38°00'43" W at an altitude of 146 m. The soil is classified as a Cambisol (FAO, 2015). According to the Köppen classification, the local climate is type BSh (KOTTEK et al., 2006), with an average annual temperature of 29°C and annual precipitation of 719 mm. The chemical and physical characteristics of the study area are shown in Table 1.

Table 1 - Characterization of the chemical and physical attributes of the soil in areas of native vegetation (NV), and integrated livestock-forest systems with sorghum (ILFSo), forage cactus (ILFFc), massai grass (ILFMg), buffel grass (ILFBg) and strips of native trees (NTS).

Systems	Spacing	Sand	Silt	Clay	pH	Total CEC	BS
		(g kg ⁻¹)			(H ₂ O)	(cmolc kg ⁻¹)	(%)
NV		474.36	227.20	298.44	7.40	11.15	91.45
SNT		472.39	174.16	353.45	6.98	8.90	89.30
ILFSo	28	508.24	220.84	270.92	6.43	9.26	75.90
ILFFc	28	486.89	195.66	317.45	5.55	10.16	57.40
ILFMg	28	447.37	274.54	278.10	6.65	10.46	72.85
ILFBg	28	468.66	230.54	300.80	6.78	9.34	80.23
ILFSo	14	445.79	117.76	361.74	5.98	7.84	78.75
ILFFc	14	485.64	187.69	326.67	5.83	7.95	74.70

ILFMg	14	456.93	225.50	317.57	6.83	8.43	93.08
ILFBg	14	406.55	247.49	345.97	6.93	8.69	89.00
ILFSo	7	474.76	172.92	352.33	6.88	9.40	77.45
ILFFc	7	529.93	89.22	380.85	5.88	10.29	63.43
ILFMg	7	435.49	213.95	350.56	6.83	8.13	92.05
ILFBg	7	424.48	239.28	336.24	6.25	7.81	79.65

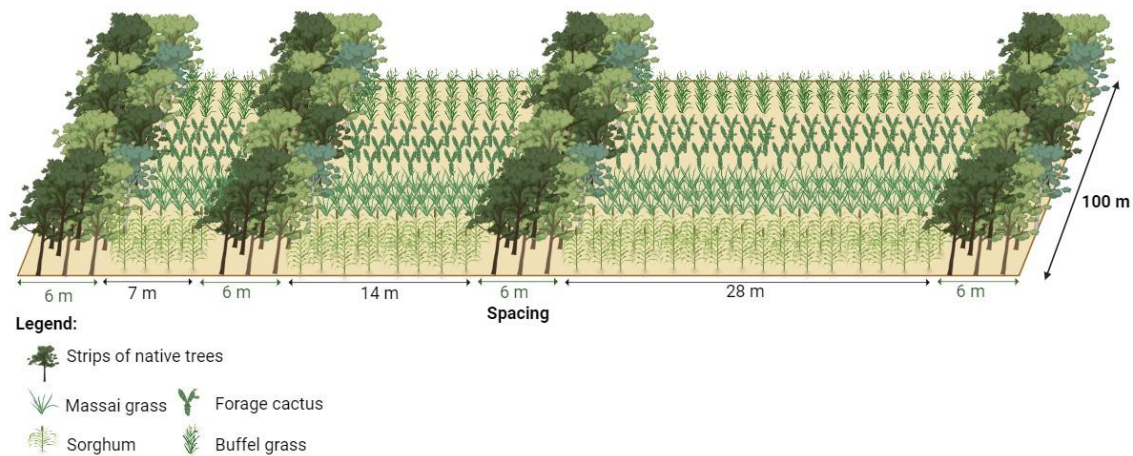
Source: Author (2025). pH H₂O: potential of hydrogen in water (H₂O); Total CEC: Total cation exchange capacity; BS: base saturation.

The area was deforested during the dry period of 2015, using a tractor with a front blade, and removing 100% of the vegetation present in the cultivable area (understory), leaving only strips of native trees, six meters wide. All the residual material was directed towards the edges. The area had previously consisted of arboreal caatinga in a stage of secondary succession (SANTOS NETO et al. 2021). Phosphate fertilization was carried out annually, applying Mono Ammonium-Phosphate (MAP) fertilizer, 44% P₂O₅ – 10% N, at a dose of 200 kg ha⁻¹ year⁻¹ due to the low levels of phosphorus verified by a chemical analysis of the soil. Weed control was both mechanical and chemical.

The area consisted of four ILF systems arranged in three different spacings (7, 14 and 28 m) between 6-metre strips of Caatinga vegetation (Figure 1). The pasture components were planted during the rainy season of 2016: *Pennisetum ciliare* (buffel grass - Bg), *Megathyrus maximus* (massai grass - Mg), *Opuntia stricta* (forage cactus - Fc) and *Sorghum bicolor* (sorghum - So), corresponding to the non-grazed livestock system. The sorghum was planted and harvested annually, under conventional management practices, tilling the soil every year. The massai grass and buffel grass were allowed to grow freely during the rainy season; at the end of the dry season, the grass was cut mechanically to an average height of 0.05 m above ground, with the aim of standardizing each pasture for the next growing season. The forage cactus was cut every two years, and the residue removed from the area.

The treatments consisted of ILF systems with sorghum (ILFSo), forage cactus (ILFFc), massai grass (ILFMg) and buffel grass (ILFBg), at a spacing of 7 m (S7), 14 m (S14) or 28 m (S28) between the strips of native trees (NTS - Caatinga), giving a total of 13 treatments (Figure 1) plus the area of native vegetation used as reference (NV - area of Caatinga). The native vegetation was phytosociological in structure, with the following identified tree species: *Cordia goeldiana*, *Mimosa caesalpiniaefolia*, *Cenostigma pyramidale*, *Commiphora leptophloeos* and *Mimosa tenuiflora* (SANTOS NETO et al., 2021).

Figure 1 - Representation of the area with ILF systems with different spacings between the strips of native trees (Caatinga).



Source: Author (2025).

4.2.2 Soil sampling and laboratory analysis

Soil sampling was carried out in January 2022 during the dry season. Disturbed samples were collected from five trenches (replications) in each area at two different depths: 0–10 and 10–20 cm, giving a total of 140 samples (2 depths $\times$ 5 replications $\times$ 14 treatments). The samples were stored in a cold chamber in the laboratory at 5°C to 10°C for microbiological analysis.

The MBC was determined using the irradiation-extraction method (ISLAM and WEIL (1998). The extracts were analyzed for organic carbon content (TOC-V Shimadzu), and the microbial biomass was determined from the difference between the values obtained from the irradiated and non-irradiated samples. Basal soil respiration (BR) was determined using the method of Curl and Rodriguez-Kabana (1972) and Stotzky (1965) and was estimated from the amount of CO₂ released from the soil during the 50-day incubation period. The released carbon was calculated from the emission rates for each period (mg C g⁻¹ soil day⁻¹), which were on days 2, 4, 7, 16, 22, 29, 36, 43 and 50 (Mendonça and Matos, 2017). This procedure was carried out in three replications for each layer of soil.

The metabolic quotient (qCO₂), represented by the ratio between basal respiration and the microbial biomass, expressed in mg CO₂ mg MBC⁻¹ day⁻¹, and the microbial coefficient (qMIC), the ratio between microbial biomass carbon and the total soil organic carbon, expressed as a %, were both calculated using the MBC and BR data (ANDERSON, 2003). The soil organic carbon (SOC) was determined by the continuous flow mass spectrometry method using a Carlo Erba CHN 1110 elemental analyzer. The stocks of MBC and SOC were calculated by

multiplying the MBC or SOC content by the density of the soil and the thickness of the soil layer.

β -glucosidase activity was determined using the method described by Tabatabai (1994). This method is based on the colorimetric p-nitrophenol formed after the addition of colorless substrates specific to the enzyme under evaluation. EEG extraction was carried out using the method of Wright and Upadhyaya (1998) and Bradford (1976), with the reading taken by spectrophotometer at 595 nm.

4.2.3 Statistical analysis

All the statistical analyses were carried out using the R Studio 4.1.2 software (R Core Team, 2021) at a p -value ≤ 0.05 . The data were subjected to tests of normality and homogeneity of variance. When necessary, any outliers were removed. ANOVA was carried out on all the treatments to evaluate the effects of the integrated systems (ILFFc, ILFSO, ILFMg, ILFBg) and spacings between the SNT (S7, S14 and S28) on the microbiological properties of the soil (MBC, BR, qCO₂ and qMIC). When significant, Tukey's test ($p < 0.05$) was used to analyze the effects of the ILF systems and spacings. The ILF systems were also compared individually to the reference area (Caatinga) using Dunnett's test ($p < 0.05$), while the SNT were compared with the reference area using Student's test ($p < 0.05$). The β -glucosidase and EEG data showed heterogeneous variance, and in this case non-parametric ANOVA and the Friedman comparison test were used ($p < 0.05$). The cumulative C-CO₂ emission data (considering the measurements from each day of incubation) were submitted to simple linear regression analysis, and Tukey's test was used to compare the cumulative C-CO₂ emissions between the spacings in each of the ILF systems.

4.3 RESULTS

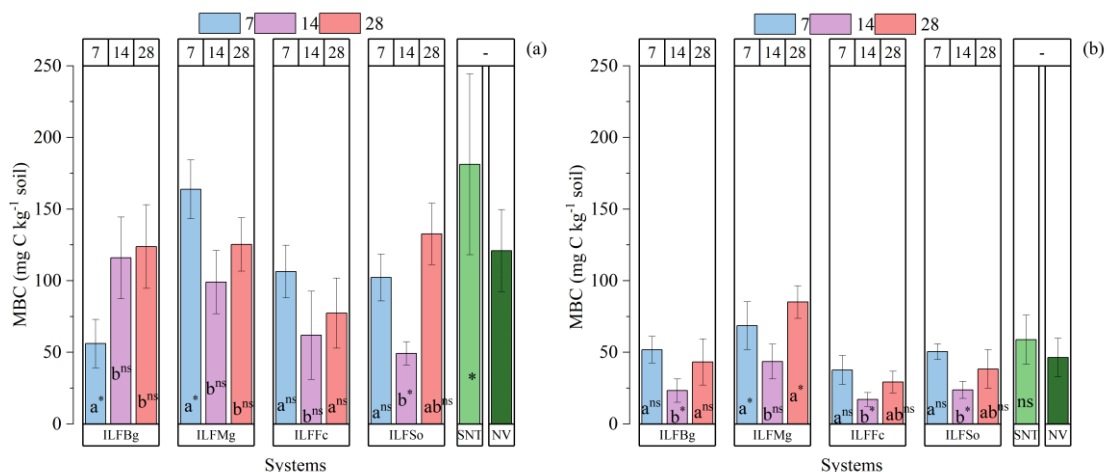
4.3.1 Microbial biomass carbon

The conversion from NV into ILF systems had a significant effect on the soil MBC (Figure 2) and BR (Figure 3). On average, in the surface layer (0-10 cm), the MBC in the ILF systems with grasses was similar to that of the NV (120.9 mg C kg⁻¹ soil), with values of 98.6, 129.3 and 94.7 mg C kg⁻¹ soil for ILFBg, ILFMg and ILFSO, respectively (Figure 2a). At a depth of 10-20 cm, the ILFMg system (65.7 mg C kg⁻¹ soil) gave an increase of approximately 42% in MBC compared to the NV (Figure 2b). In the NTS, MBC values were 181.2 (0-10 cm)

and 58.7 mg C kg⁻¹ soil (10-20 cm). These values were, respectively, 49% and 27% higher than those obtained in the NV (Figure 2a-b).

The different spacings of the ILF systems had a marked effect on the MBC. At a depth of 0-10 cm, the MBC increased significantly at the largest spacings in ILFBg, with increases of 59.8 and 67.7 mg C kg⁻¹ for S14 and S28, respectively, compared to S7 (59.8 mg C kg⁻¹). The opposite was seen in ILFMg, where the S14 and S28 spacings reduced the MBC by 64.7 and 38.6 mg C kg⁻¹, respectively, compared to S7 (163.8 mg C kg⁻¹) (Figure 2a). At a depth of 10-20 cm, the highest MBC value was obtained at S7 (51.8 mg C kg⁻¹) in the ILFBg system, while in ILFMg the highest value was seen at S28 (85.1 mg C kg⁻¹) (Figure 2b). In the ILFFc system, spacings S7 (106.37 mg C kg⁻¹) and S28 (77.40 mg C kg⁻¹) showed similar MBC values to that of NV. In ILFFc, S14 reduced the MBC by 44.4 and 15.48 mg C kg⁻¹ compared to S7 and S28, respectively, in the top layer. At a depth of 10-20 cm, the MBC was greater at S7 (37.67 mg C kg⁻¹), albeit not differing statistically from S28 (29.23 mg C kg⁻¹). At a depth of 0-10 cm in ILFSO, MBC was greater at S28 (132.6 mg C kg⁻¹) and at a depth of 10-20 cm at S7 (50.5 mg C kg⁻¹), however, spacings S7 and S28 did not differ from each other at either depth.

Figure 2 - Microbial biomass carbon (MBC) in the 0-10 (a) and 10-20 cm (b) layers under native vegetation (NV), a strip of native vegetation (SNT), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSO) at different spacings between the SNT of Caatinga (7 m, 14 m and 28 m). Mean values followed by the same letter in the same ILF system and layer of soil do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$), compares the ILF systems with the reference area (NV), and SNT with NV by t-test ($p < 0.05$). Error bars represent the standard deviation from the mean.



Source: Author (2025).

In the integrated system with sorghum (ILFSo), the smallest MBC stock was seen at S14, with a reduction of 50% in relation to NV at both depths (Table 2). Similarly, in the ILFFc system, MBC stock was considerably reduced at S14, with losses of 56% and 67% in the 0-10 and 10-20 cm layers, respectively, compared to NV. In ILFBg, in the 0-10 cm layer, the carbon stocks at S28 and S14 were similar to those in NV, while S7 reduced the MBC stock by 56%. Unlike the ILFBg system, S7 in ILFMg afforded the greatest MBC stock, 50% greater than in NV.

The ILFBg and ILFMg systems provided the same SOC stocks in the 0-10 cm layer as in NV, the only exception being S7 in the system with buffel grass (Table 2). While for the ILFSo and ILFFc systems there was a reduction in SOC stocks at all the adopted spacings. In the 10-20 cm layer, the change in SOC stocks did not occur to the same extent as in the 0-10 cm layer. and varied with the spacing.

Table 2 - Stocks of microbial biomass carbon (MBC) and soil organic carbon (SOC) in the 0-10 and 10-20 cm layers under native vegetation (NV), a strip of native vegetation (NTS), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSo) at different spacings between the SNT of Caatinga (7 m, 14 m and 28 m). Mean values followed by the same letter in the same ILF system and layer of soil do not differ by Tukey's test ($p < 0.05$). *, ^{ns} significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$), compares the ILF systems with the reference area (NV), and SNT with NV by t-test ($p < 0.05$). Values in parentheses represent the standard deviation from the mean.

Systems/ Spacing		Stock (Mg ha ⁻¹)			
		MBC	SOC	MBC	SOC
		0-10 cm		10-20 cm	
ILFBg	7	0.09 (± 0.04) a*	15.3 (± 1.3) a*	0.07 (± 0.02) a ^{ns}	8,1 (± 0.3) a ^{ns}
	14	0.17 (± 0.03) b ^{ns}	19.0 (± 1.6) b ^{ns}	0.03 (± 0.02) b*	7,0 (± 0.5) b*
	28	0.18 (± 0.04) b ^{ns}	16.7 (± 1.2) ab ^{ns}	0.06 (± 0.02) a ^{ns}	7,4 (± 1.2) ab ^{ns}
ILFMg	7	0.24 (± 0.03) a*	15.6 (± 1.1) a ^{ns}	0.09 (± 0.02) a ^{ns}	7,9 (± 0.7) a*
	14	0.16 (± 0.03) b ^{ns}	18.9 (± 1.6) b ^{ns}	0.06 (± 0.02) b ^{ns}	8,4 (± 0.8) ab ^{ns}
	28	0.19 (± 0.00) b ^{ns}	17.4 (± 1.9) ab ^{ns}	0.11 (± 0.02) a*	8,9 (± 1.0) b ^{ns}
ILFFc	7	0.16 (± 0.03) a ^{ns}	13.5 (± 1.1) a*	0.06 (± 0.01) a ^{ns}	8,6 (± 0.3) a ^{ns}
	14	0.07 (± 0.03) b*	10.8 (± 0.8) b*	0.02 (± 0.01) b*	6,4 (± 0.7) b*
	28	0.10 (± 0.03) b ^{ns}	13.0 (± 1.9) a*	0.04 (± 0.01) ab ^{ns}	6,4 (± 0.7) b*
ILFSo	7	0.15 (± 0.03) a ^{ns}	13.8 (± 0.3) a*	0.08 (± 0.02) a ^{ns}	9,2 (± 0.9) a ^{ns}
	14	0.08 (± 0.04) b*	12.9 (± 0.5) a*	0.03 (± 0.02) b*	7,7 (± 0.6) b*
	28	0.18 (± 0.08) a ^{ns}	14.4 (± 1.9) a*	0.05 (± 0.02) b*	8,3 (± 0.5) b ^{ns}

SNT	0,24 ($\pm 0,04$) *	17.3 (± 4.3) ^{ns}	0.08 (± 0.01) ^{ns}	10.8 (± 0.7) *
NV	0,16 ($\pm 0,03$)	17.9 (± 1.2)	0.06 (± 0.01)	9.1 (± 0.4)

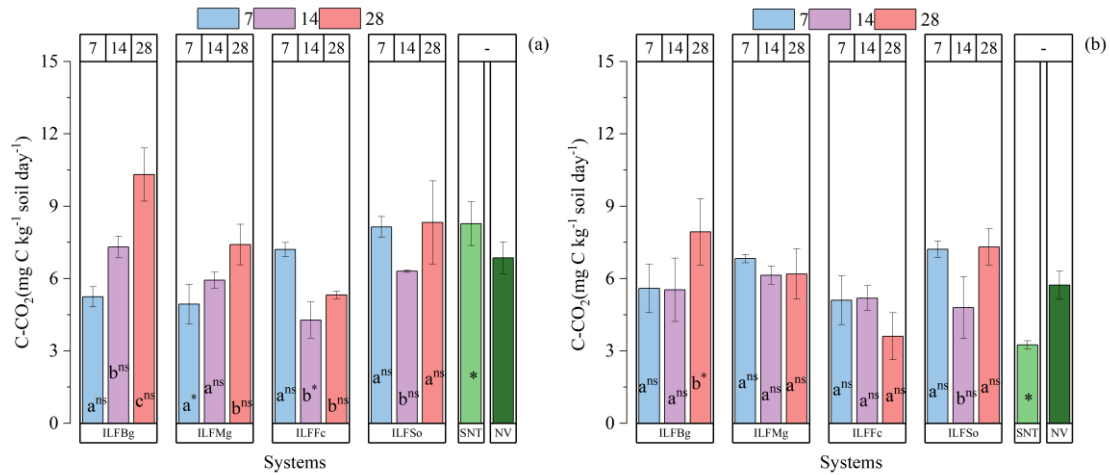
Source: Author (2025). Mean values followed by the same letter in the same ILF system and layer of soil do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$), compares the ILF systems with the reference area (NV), and SNT with NV by t-test ($p < 0.05$). Values in parentheses represent the standard deviation from the mean.

4.3.2 Basal soil respiration

In general, no significant differences in BR were found between the ILF systems with grasses and the NV at depths of 0-10 and 10-20 cm (Figure 3a-b). The average BR rates in these systems were 7.61, 6.09, 7.59 and 6.85 mg C kg⁻¹ soil day⁻¹ (0-10 cm) and 6.35, 6.39, 6.44 and 5.73 mg C kg⁻¹ soil day⁻¹ (10 -20 cm), for ILFBg, ILFMg, ILFSO and NV, respectively. In the system with forage cactus (ILFFc), the BR was lower (5.60 mg C kg⁻¹ soil day⁻¹) in relation to NV (5.85 mg C kg⁻¹ soil day⁻¹) at a depth of 0-10 cm. In the NTS, the BR was 8.38 mg C kg⁻¹ soil day⁻¹, 21% higher than in NV, while at a depth of 10-20 cm, BR was 3.25 mg C kg⁻¹ soil day⁻¹, 43% lower than in NV.

Regarding the effect of spacing in the ILF systems, S28 afforded the highest BR in the 0-10 cm layer in the ILFBg and ILFMg systems, with values of 10.31 and 7.41 mg C kg⁻¹ soil day⁻¹. These values were higher than those obtained at S7 in ILFBg and ILFMg, of 5.54 and 4.94 mg C kg⁻¹ soil day⁻¹. In ILFSO, the highest BR occurred at S28 at both depths, however neither differed from S7. In contrast, S7 resulted in the highest BR in the 0-10 cm layer in the ILFFc system (7.20 mg C kg⁻¹ soil day⁻¹). In ILFFc, the BR did not differ at the different spacings in the 10-20 layer.

Figure 3 - Basal soil respiration (C-CO₂) in the 0-10 (a) and 10-20 cm (b) layers under native vegetation (NV), a strip of native vegetation (NTS), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSO) at different spacings between the SNT of Caatinga (7 m, 14 m and 28 m). Mean values followed by the same letter in the same ILF system and layer of soil do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant, respectively, by Dunnett's test ($p \leq 0.05$), compares the ILF systems with the reference area (NV), and SNT with NV by t-test ($p < 0.05$). Error bars represent the standard deviation from the mean.



Source: Author (2025).

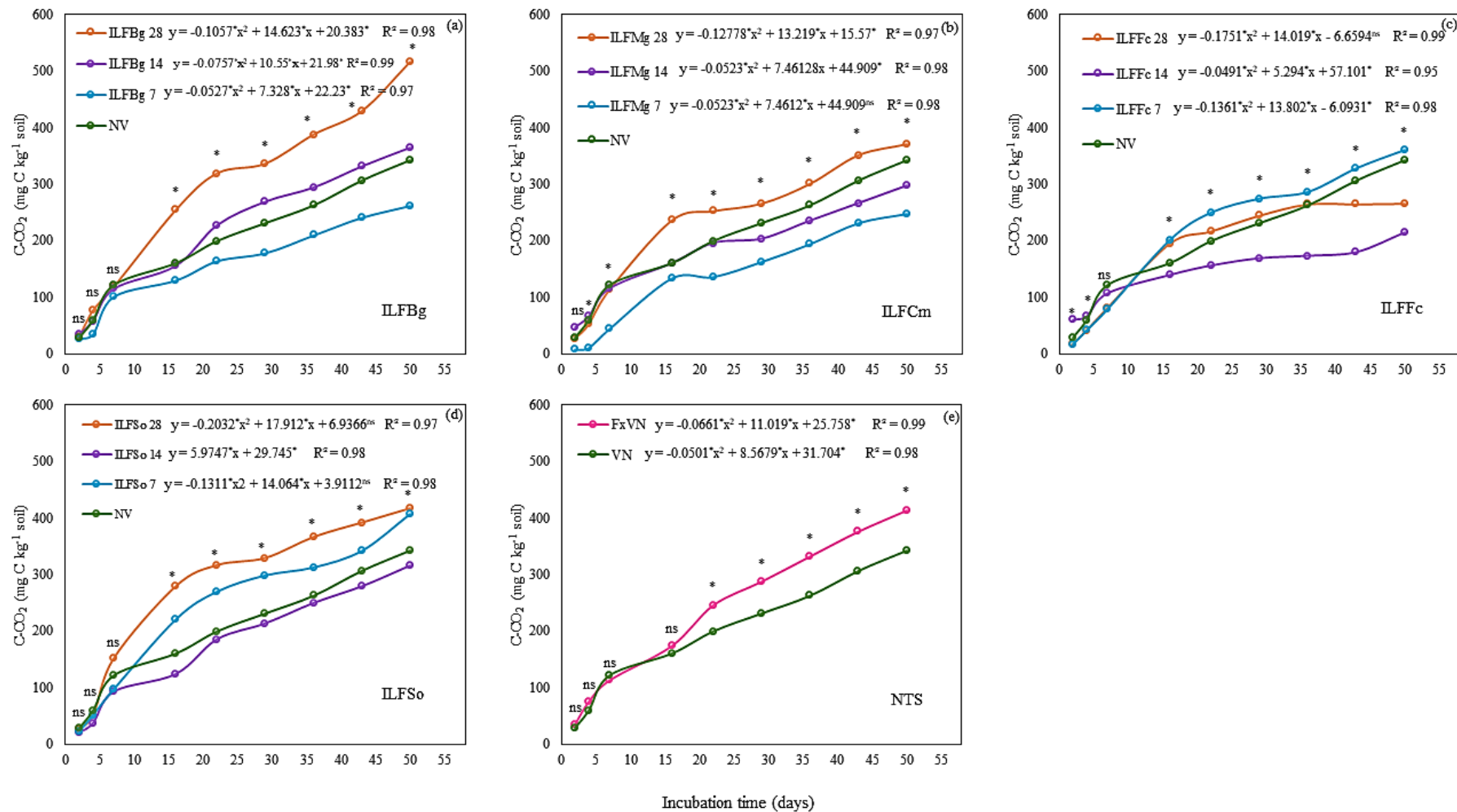
There were progressive cumulative C-CO₂ emissions from the soil throughout the 50 days of incubation (Figure 4). The results of the regression analysis showed that the quadratic model presented a high level of fit for describing the cumulative C-CO₂ curves in each of the ILF systems, since the coefficients of determination (R^2) varied between 0.95 (ILFFc - S14) and 0.99 (ILFBc - S14).

Based on ANOVA, from day 2 to day 7, cumulative C-CO₂ emissions in NV and at the different spacings in the ILFMg and ILFSO systems were similar (Figure 4a-d). From day 16 onwards, emissions were significantly higher at S28 until the end of incubation, with a total cumulative C-CO₂ after 50 days incubation of 515.52 and 416.21 mg C kg⁻¹ soil for ILFBg and ILFSO, respectively. In these systems, the lowest cumulative C-CO₂ emissions were seen at S7 in the ILFBg system (262.18 mg C kg⁻¹) and at S14 in the ILFSO system (315.22 mg C kg⁻¹).

In the ILFMg system at S7, emissions were significantly lower compared to the other spacings and to NV from day 2 (7.68 mg C kg⁻¹) to day 50 (246.82 mg C kg⁻¹) (Figure 4b). From day 2 to day 7, the highest emissions occurred at S14. However, from day 16 onwards, the highest emissions were at S28, with significant increases of 37% and 50% on day 50 compared to S14 and S7.

In the ILFFc system, cumulative C-CO₂ emissions were higher at S14 during the first days of incubation compared to S7, S28 and NV (Figure 4c). However, from day 16 until the end of incubation, the emissions at S14 decreased significantly by around 34%. On day 50, the greatest emissions were seen at S7, with a value of 360.24 mg C kg⁻¹.

Figure 4 - Cumulative C-CO₂ emissions under native vegetation (NV). and ILF systems with buffel grass (ILFBg) (a), massai grass (ILFMg) (b), forage cactus (ILFFc) (c) and sorghum (ILFSO) (d) at different spacings between the SNT of Caatinga (7 m, 14 m and 28 m) and the strip of native vegetation (NTS) (e), in the 0-10 cm layer after 50 days incubation. Error bars represent the standard deviation from the mean. *, ns significant and non-significant, respectively, compares the spacings in the ILF and NV systems using Tukey's test ($p \leq 0.05$). *, ns in the equation, compare the different incubation times in each ILF system using regression analysis ($p \leq 0.05$).



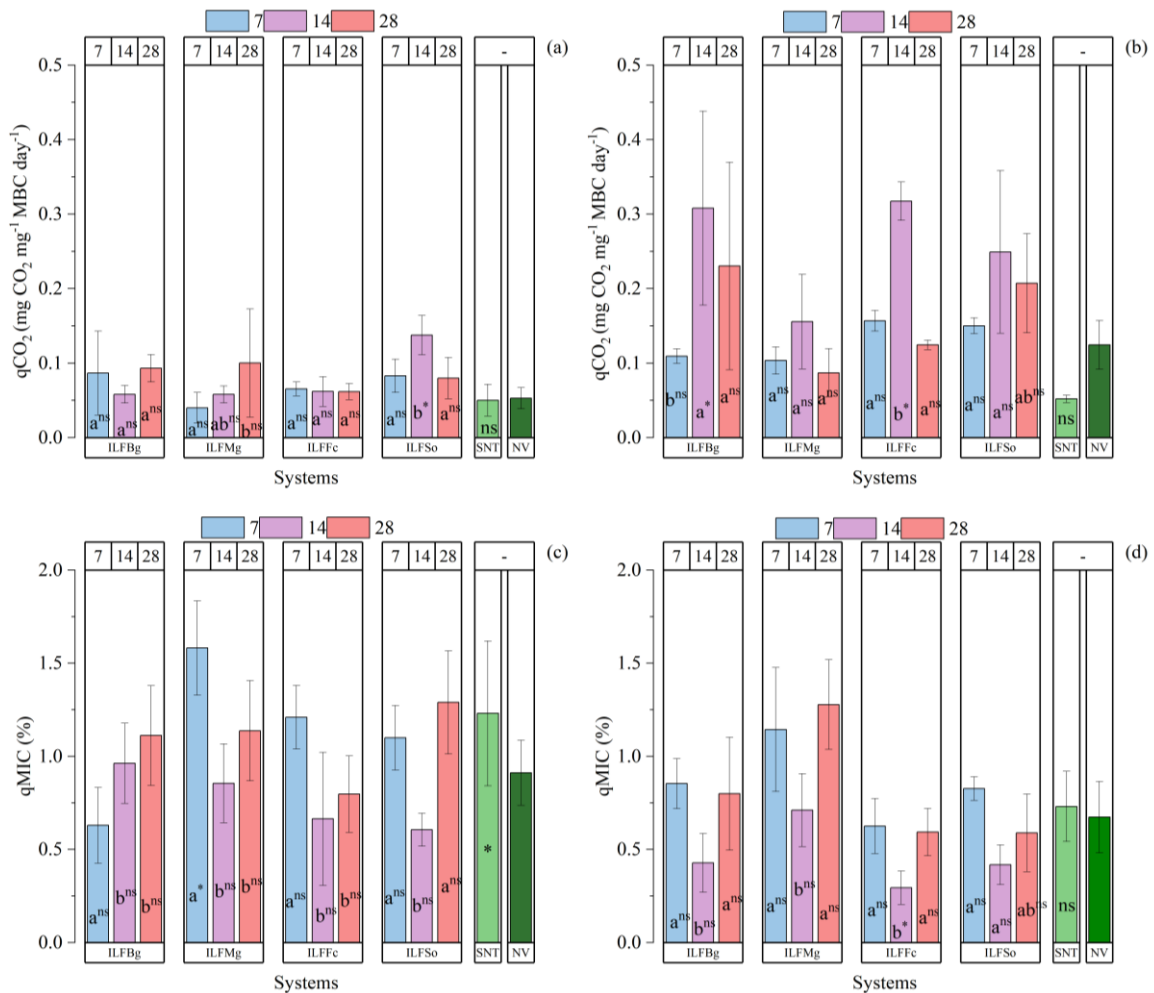
4.3.3 Metabolic and microbial quotient

In general, in the 0-10 cm layer, qCO_2 was similar between NV ($0.05 \text{ mg}^{-1} \text{ MBC day}^{-1}$), the different ILF systems, and SNT (Figure 5a). However, the ILFSo system at S14 showed a higher qCO_2 in relation to NV and to the other spacings, with a value of $0.14 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC day}^{-1}$ (Figure 5a). In the ILFMg system, S28 showed a higher qCO_2 ($0.10 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC day}^{-1}$) in relation to S7 and S14, but none differed from NV.

The $qMIC$ was also similar between the ILF and NV systems (0.91%), except for the ILFMg system at S7, which had a higher $qMIC$ (1.58%) (Figure 5c). In ILFBg, the highest $qMIC$ occurred at spacings S14 and S28 (0.96% and 1.11%) compared to S7 (0.63%). In the 10-20 cm layer, the conversion of NV into ILF systems, and the adoption of different spacings between the SNT in these systems, had no effect on the qCO_2 or $qMIC$, with the exception of the ILFBg system ($0.31 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC day}^{-1}$) and ILFFc ($0.32 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC day}^{-1}$) at S14, where the qCO_2 was higher than in NV (Figure 5b and d).

Figure 5 - Metabolic quotient (qCO_2) (a) and microbial quotient ($qMIC$) (b) in the 0-10 cm layer under native vegetation (NV), a strip of native vegetation (NTS), and ILF systems with buffel grass (ILFBg), massai grass (ILFMg), forage cactus (ILFFc) and sorghum (ILFSo) at different spacings between the SNT of Caatinga (7 m, 14 m and 28 m). Mean values followed by the same letter in the same ILF system and layer of soil do not differ by Tukey's test ($p < 0.05$). *, ns significant and non-significant,

respectively, by Dunnett's test ($p \leq 0.05$), compares the ILF systems with the reference area (NV), and SNT with NV by t-test ($p < 0.05$). Error bars represent the standard deviation from the mean.



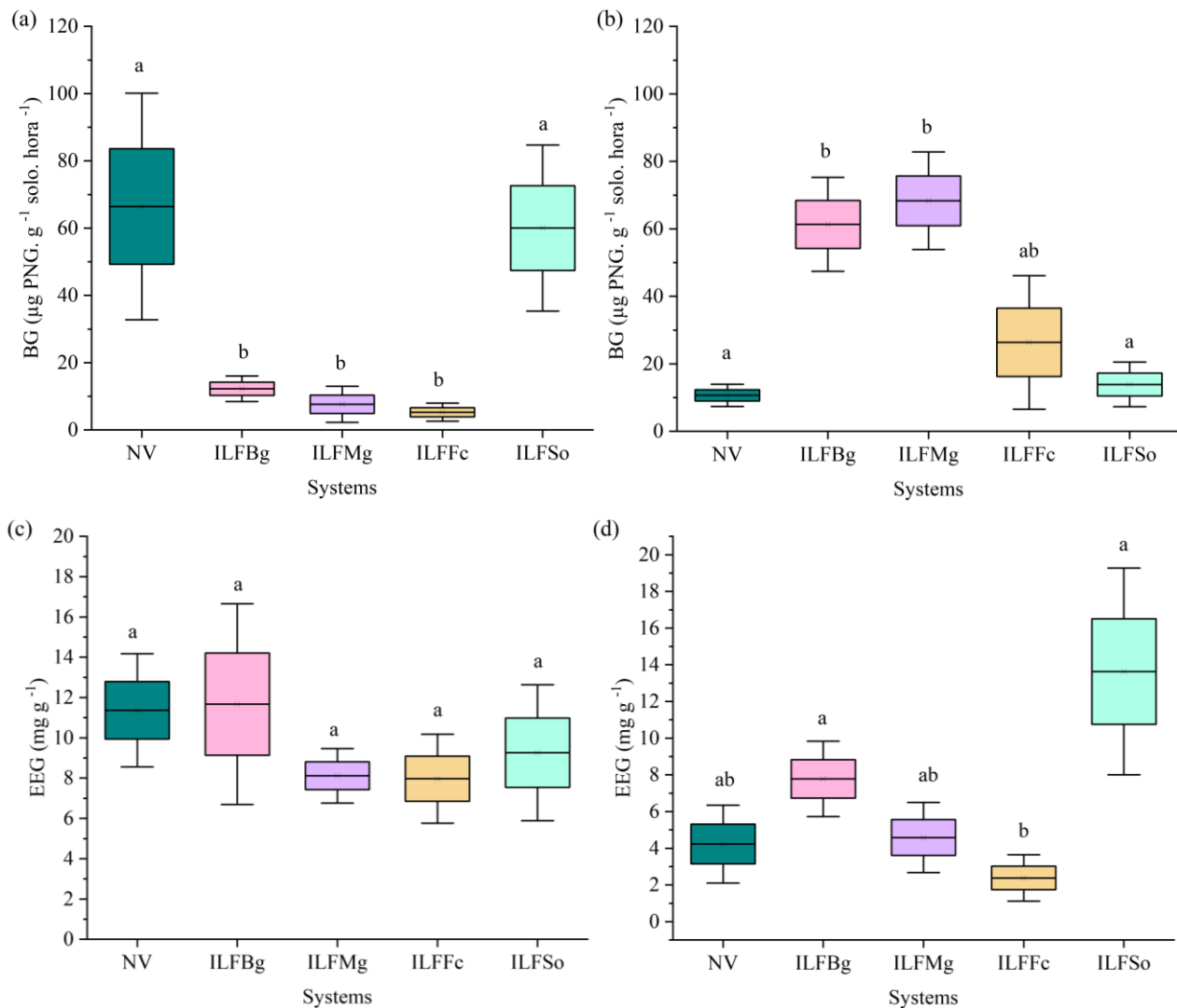
Source: Author (2025).

4.3.4 Enzyme activity

The effects of the different ILF systems on the enzyme activity of β -glucosidase and EEG are shown in figure 6. In the 0-10 cm layer, the highest levels for β -glucosidase were seen in ILFSO and NV, with average values of 59.40 and 59.08 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$, respectively. The lowest values for β -glucosidase were recorded in ILFBg (12.25 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$), ILFMg (7.61 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$) and ILFFc (5.26 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$) (Figure 6a). In the 10-20 cm layer, the lowest β -glucosidase activity occurred in NV (10.65 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$), while intermediate values were seen in ILFFc (26.35 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$) and ILFSO (13.89 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$), with the highest values in ILFMg (68.33 $\mu\text{g PNG g}^{-1} \text{ soil hr}^{-1}$) and ILFBg (61.87 $\mu\text{g PNG}$) (Figure 6b). When evaluating the effect of the ILF systems on EEG activity, each of the systems show similar activity in the top layer, with average values ranging from

7.97 to 11.67 mg g⁻¹ (Figure 6c). In the 10-20 cm layer, the greatest EEG activity was seen in the ILFSo (11.64 mg g⁻¹) and ILFBg 97.68 (mg g⁻¹) systems. In ILFMg and NV, the average activity was 4.59 and 4.23 mg g⁻¹, respectively. While the lowest value for EEG was found in ILFFc (2.38 mg g⁻¹).

Figure 6 - Boxplots of the enzymes β -glucosidase (BG) and easily extractable glomalin (EEG) in different ILF systems in the 0-10 cm (a and c) and 10-20 cm layers (b and d). NV: native vegetation; ILFBg, ILFMg, ILFFc, ILFSo: ILF systems with massai grass, buffel grass, cactus and sorghum, respectively. In each boxplot, the line inside the box indicates the median, the top and bottom lines of the box are the 1st and 3rd quartiles, respectively, while the bars indicate the maximum and minimum values. 'x' indicates the mean. Different letters indicate significant differences according to the Friedman test ($p < 0.05$).



Source: Author (2025).

4.4 DISCUSSION

In Brazil, integrated agricultural systems have increased substantially in recent years, especially in the Cerrado and Atlantic Forest biomes (OLIVEIRA et al., 2024; DAMIAN et al., 2021), where there is a marked predominance of the use of eucalyptus as a forest component, which is adopted in various arrangements, such as in single rows, double rows and strips, among others (SARTO et al., 2020b; OLIVEIRA et al., 2022). On the other hand, in the semi-arid region of Brazil, other arrangements have been tested. Initially, agroforestry systems (AFS), which come in a variety of designs, often involving thinning of the native vegetation while maintaining varying percentages of the forest component distributed randomly between the agricultural or livestock components (WRI BRASIL, 2018). More recently, systems with regular bands of arboreal components have emerged, the majority composed of leguminous species, such as gliricidia and leucena (LIRA JUNIOR et al., 2020; CAMELO et al., 2023). The aim of the present study was to evaluate a further option for integrated systems, using strips of native vegetation at different spacings to exploit the agricultural or livestock component. In other words, we sought to take advantage of the ecological benefits provided by the trees, but in arrangements that would facilitate crop management and offer the possibility of expanding its adoption. In this context, it is essential to evaluate the environmental sustainability of these systems; in this case, understanding the soil-management-microbiology interactions, which affect the accumulation and dynamics of the SOM.

Overall, the results found in this study show the benefits of ILF systems for the biological properties of the soil, as evidenced by maintenance of the MBC, low values for the metabolic quotient (qCO_2), and glomalin results. These results are consistent with earlier research that reported the positive effect of integrated systems on microbial biomass carbon and biological activity under temperate (SEKARAN et al., 2021), tropical (ABREU et al., 2020), subtropical (LIRA JUNIOR et al., 2020) and semi-arid (CAMELO et al., 2021) conditions.

The values for MBC, which reflect the density of soil microbial populations under Caatinga and ILF systems were similar. Microbial communities are known to have a direct relationship with the input of C to the soil, since they are the main regulators of SOM and nutrient availability (SARTO et al., 2020b). In the present case, we found that over a six-year period, maintenance of the microbial population in the systems with Massai and buffel grass coincided with an increase in SOC stocks in the 0-10 cm layer, depending on the spacing. Omer et al. (2023) states that perennial grasses not only increase the contribution of root biomass but also conserve root-derived carbon and microbial activity due to the reduced number of tillage operations. Furthermore, the fasciculate root system of grasses, which results in greater

exudation of organic compounds, leads to an increase in MBC due to the increase in C and energy sources for microbial biomass (BRORING et al., 2023).

It was also found that ILF systems with grasses showed greater microbial activity, evidenced by the release of C-CO₂. This can be attributed to the greater availability of soil carbon and microbial biomass, and the metabolic capacity of soil microorganisms (ANDERSON, 2003; SANTOS et al., 2022). However, a high rate of basal respiration may suggest the soil microbiota are under stress (BRORING et al., 2023), which is why it is always important to consider the values of the metabolic quotient (qCO₂), which represents the amount of C released via CO₂ per unit of microbial biomass (ANDERSON, 2003). It was found that in NV and in most of the ILF arrangements there was no significant difference in qCO₂ values, especially in the top layer of soil.

Unlike the systems with massai and buffel grass, the system with sorghum was subjected yearly to conventional soil preparation. However, although soil turning in the ILF with sorghum had a negative effect on the levels of SOC, there was no effect on soil biomass or microbial activity. These results contradict Han et al. (2024), who state that conventional tillage can break down macroaggregates, accelerating the loss of soil organic C and reducing the activity and composition of the soil microbiota, whereas direct planting or reduced tillage can help restore soil nutrients, restructure the microbial community, and benefit the aggregate stability of the soil. In the present case, the high biomass input from the sorghum (NGIDI et al., 2024) probably helped to compensate for the negative effect of disturbing the soil.

Similarly, the system with forage cactus resulted in C losses, but was positive in terms of recovering MBC. This shows that the impact of the initial disturbance caused when implementing the system, together with the change in biomass input that was probably reflected in the loss of SOC, is easing after six years. Integrated systems are expected to promote a greater input of C to the soil (LIRA JUNIOR et al., 2020), especially in more-labile forms that can be easily used as a food source by the soil microbiota (PAUSCH and KUZUYAKOV, 2018). Results obtained in the same area showed that adoption of the ILFBg and ILFMg systems had the potential, after six years, to increase labile C inputs to the soil, while for the ILFFc and ILFSO systems, a longer period would be needed to evaluate the contribution of these systems to managing the SOM.

Studies have confirmed that integrated systems show great potential to increase C accumulation in agricultural soils, including in semi-arid regions. Damian et al. (2021), in a study of 3-year ILF systems in subtropical areas, found a 63% increase in MBC under ILF compared to a conventional system of pasture management. Under similar environmental

conditions to those in the present study, Camelo et al. (2021) found that agroforestry systems that integrate forage cactus and tree legumes have the potential to improve microbial activity in the soil. Similarly, agroforestry systems with grasses and shrubby legumes offer an improvement in microbial biomass and soil organic matter in arid and semi-arid regions (OMER et al., 2023).

It is known that the forestry component in integrated systems can promote microbial diversity compared to single crops (SARTO et al., 2020b). However, the effects of tree-integrated farming systems are rather difficult to generalize (Sarto et al., 2020b), as they depend not only on the components chosen for the systems, but also on the design and type of management (PRASAD et al., 2023). In fact, the increase in microbial activity in the ILFBg, ILFMg, ILFSO and ILFFc systems depended on the spacing between the NTS.

Specifically, the arrangement of trees and crops directly affects the density of the trees in the system, resulting in varying contributions to the soil carbon (PRASAD et al., 2015). The larger stock of SOC in high-density stands is attributed to the greater accumulation of litter on the ground, which can stimulate decomposition of the SOM that later converts to more stable C in the soil (PRASAD et al. 2015). In addition, the greater accumulation of plant residue above and below the ground under the tree canopy provides better microenvironmental conditions, conserving soil moisture and reducing the air and ground surface temperatures, resulting in an environment that is conducive to soil microbial activity (Guillot et al. (2019) and that favors decomposition of the SOM (CHEN et al., 2018). This may explain the higher MBC values found at S7 in most ILF systems at both depths under evaluation. Results obtained by Santos Neto et al. (2021) in the same area as the present study, confirmed that the presence of trees in integrated systems resulted in a change in the microclimate for each of the adopted spacings, promoting environments with higher relative humidity and lower average temperatures.

Another reason for the highest MBC values to occur at S7 is the reduction in spacing between the strips of trees possibly having contributed to the deposition of more litter from the trees, which mainly comprised leguminous species. Grass species generally have a higher C:N ratio in their residue, resulting in a slower rate of decomposition compared to the high N content in legumes (LIRA JUNIOR et al., 2020). The integration of species that offer high vegetation cover and a high C:N ratio (grasses) with species with a low C:N ratio (legumes) therefore maintains a continuous supply of energy to the microbial communities by preserving the nutrient balance in the soil, while the greater quantity and better quality of the plant residue provided by the system promotes microbial growth (THAPA et al., 2021).

The 28-metre spacing also resulted in MBC values similar to that in NV. Based on results from other studies carried out in the same area as the present study, the average total forage biomass under buffel and massai grass was higher at S28 (3900 kg ha⁻¹ yr⁻¹), compared to S7 (2700 kg ha⁻¹ yr⁻¹) and S14 (3600 kg ha⁻¹ yr⁻¹) (SANTOS NETO et al., 2023a). The same was seen for forage cactus, where at spacing S28 total biomass production was approximately 50% higher (SANTOS NETO et al., 2023b). Crop biomass production is known to be a key factor for the soil microbiota, especially in agroecosystems in semi-arid regions (THAPA et al., 2021).

For qMIC, it is known that values below 1% may occur due to ecological or management factors that limit the microbial activity of the soil biomass (ANDERSON, 2003). On the other hand, higher qMIC values mean that organic C is more easily accessible to the soil microbiota. Considering the different spacings between the NTS, qMIC values were less than 1% at S14 in all the ILF systems. At S7 and S28, the values were greater than 1%, except in ILFBg-S7 and ILFFc-S28, which leads to the conclusion that in general, the adoption of spacings S7 and S28 in ILF systems provided the soil microbiota with better conditions, since higher qMIC values indicate more C incorporated into microbial cells (CAMELO et al., 2021).

Another point is that the accumulated CO₂ emissions were highest at S28 over the 50 days of evaluation in almost all the ILF systems. The only exception was the ILFFc system, where the highest emissions occurred at S7. Generally, the greater release of CO₂ is due to the increase in biological activity, which is directly related to the labile carbon in the soil; this, in turn, is directly affected by the quantity and quality of the litter deposited on the ground. In this respect, the higher biomass production seen at S28 probably contributed to microbial growth, renewal and activity in the soil.

As there is an inverse relationship between MBC and qCO₂, the reduction in qCO₂ at higher MBC values suggests less energy expended in using the available organic C. In this case, spacing S7 reduced qCO₂ values in most of the ILF arrangements under study, indicating a balanced and favorable environment for the microbiota to function. This confirms that these systems can result in greater metabolic efficiency, since the lower qCO₂ values observed may indicate that the microbial biomass is losing less C in the form of CO₂ and incorporating more C into the microbial tissue (Anderson, 2003). In addition, greater metabolic efficiency is also expected to result in an increase in total soil carbon, and indeed, the ILFBg and ILFMg systems already show promise in recovering soil C in the 0-10 cm layer. Spacing S14, however, showed high levels of qCO₂ in the 10-20 cm layer, suggesting that the microbial population was under

metabolic stress and required a greater amount of C as an energy source for maintenance (BRORING et al., 2023).

In relation to enzymes, plant roots release organic substances (sugars, amino acids and organic acids), which act as sources of energy for microorganisms and stimulate the production of various hydrolyzing enzymes (DAMIAN et al., 2021; SEKARAN et al., 2021). Depending on the plant species in the system and the depth of the soil, the ILF systems increased the production of enzymes involved in the C cycle and soil microbial activity, such as β -glucosidase and the glycoprotein glomalin. β -Glucosidase is involved in the final stage of cellulose degradation, which is the main source of carbohydrates available to organic matter (LUKASZEWICZ and OLSZOWSKA, 2003). The degradation of cellulose and the release of glucose, the main source of carbon and energy absorbed by soil microorganisms, means this enzyme is crucial to the C cycle (AGNIHOTRI et al., 2021). In the present study, the ILFSo system showed greater BG activity in the 0-10 cm layer, compared to the other ILF systems, while the ILFMg and ILFBg systems were responsible for increasing BG activity in the subsurface layer. This is consistent with other studies which have found that ILF systems with grasses afford an increase in BG activity both on the surface (SARTO et al., 2020b; DAMIAN et al., 2021) and in the subsurface layer (SARTO et al., 2020a).

Well-managed grazing systems, such as integrated production systems, are known to increase the input of organic residue both above and below the ground. This increased input has a positive effect on the organic C and N pools in the soil, on microbial biomass (THAPA et al., 2021) and on SOM renewal (DAMIAN et al., 2021), which may have led to higher qMIC values in the ILF systems evaluated here. Increased cycling of SOM also promotes greater activity of the β -glucosidase enzyme, as the microorganisms have a greater source of SOM (SARTO et al., 2020b). Studies have already shown the positive correlation between the enzyme β -glucosidase and soil microbial biomass (ABDALLA et al., 2009; MARTINEZ-SALGADO et al., 2010).

The conversion of Caatinga into ILF systems also led to changes in the activity of the glycoprotein glomalin. This enzyme is present in the wall of the hyphae and spores of arbuscular mycorrhizal fungi (AMF) and is considered an essential component of the SOC pool, acting as a soil conditioner, improving fertility, aggregation, aeration, water retention capacity, level of nutrients and plant productivity (MATOS et al., 2022). All the ILF systems under evaluation afforded EEG levels in the top layer similar to those in NV. In the 10-20 cm layer, EEG levels were higher in the ILFBg, ILFMg and ILFSo systems, and in this case the average levels were even higher than in NV, although there were no statistical differences. This suggests that in ILF systems with grasses, due to the high production of root biomass, constant rhizodeposition, and

uniform distribution of root exudates at depth by the grasses (BAPTISTELLA et al., 2020), there may be a significant deposition of hyphae and spores, resulting in more EEG released at depth. Consequently, increasing the glomalin content may be a strategy for improving soil C storage in deeper layers (WANG et al., 2018; MATOS et al., 2022).

In addition, glomalin is estimated to contain approximately 37% C, and is responsible for 4%-5% of the total C in the soil (RILLIG et al., 2001). Glomalin levels in the soil are therefore closely related to SOM. Furthermore, as AMF are major producers of glomalin, an increase in glomalin levels indicates greater mycorrhizal fungi activity in the soil (MATOS et al., 2022). AMF hyphae and spores make up around 20%-30% of the soil microbial biomass, and the contribution of AMF to BR can reach up to 25% (ZHANG et al., 2016). As such, higher glomalin levels may indicate higher microbial density and greater activity (WANG et al., 2018). Sousa et al. (2013) investigated the relationship between agroforestry systems and the AMF community in a semi-arid region of the State of Paraíba in the northeast of Brazil, and found that the presence of trees increased sporulation, mycorrhizal colonization and the production of infective AMF propagules in agroforestry systems with buffel grass and forage cactus. Similarly, under subtropical conditions, combining grass with eucalyptus favored the AMF community (ZANDONA et al., 2019).

4.5 CONCLUSIONS

The data obtained herein suggest that the models of integrated livestock-forest systems under evaluation can have beneficial effects on soil microbial biomass and activity, and on SOC stocks. Especially, the ILF systems with massai and buffel grass, which had the highest values for MBC, SOC and qMIC and the lowest values for qCO₂, while the systems with forage cactus had the lowest values for MBC and SOC. There was an increase in MBC and soil microbial activity in the ILF system with sorghum, but this was not enough to increase the stock of SOC.

The effect on biomass and microbial activity in the ILFBg, ILFMg, ILFSO and ILFFc systems depended on the spacing between the NTS. We found higher values for MBC, qMIC and SOC at spacings of 7 and 28 m. Similarly, spacing S7 reduced qCO₂ values in most of the ILF systems, while spacing S14 showed higher qCO₂ levels in the 10-20 cm layer.

Enzyme activity was sensitive to the conversion from NV into ILF, and positive responses varied with the depth of the soil. β -glucosidase activity was higher in the ILF systems with grasses (buffel grass, massai grass and sorghum) compared to NV. In the top layer, glomalin levels in the ILF systems were similar to those of NV, while in the 10-20 cm layer, average EEG levels in the ILFBg, ILFMg and ILFSO systems were higher than in NV.

Finally, our results lead to the conclusion that the integrated systems with buffel grass and massai grass were the most promising in terms of maintaining the biomass and microbial activity of the soil, reflecting in increases in SOC stocks, especially when the 7-metre spacing was used. For the system with sorghum, the use of conventional tillage may have had a negative impact on the results; therefore, no-tillage, or even direct planting (which also advocates crop rotation) are highly recommended options for the semi-arid region of Brazil.

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CHAPTER IV

5 ASSESSMENT OF SOIL QUALITY VIA MULTIVARIATE ANALYSES IN INTEGRATED LIVESTOCK-FOREST SYSTEMS WITH VARYING SPACINGS AND FORAGE CROPS IN BRAZIL'S SEMI-ARID REGION

ABSTRACT

Integrated systems contribute to agricultural sustainability by promoting soil quality. This study evaluated the effect of integrated livestock-forest systems (ILF) with different spacings and crops on soil quality in the Brazilian semi-arid region. The studied systems included four ILF systems: ILF with sorghum (So), forage cactus (Fc), massai grass (Mg), and buffel grass (Bg), at spacings of 7 m (S7), 14 m (S14), and 28 m (S28) between strips of native trees (Caatinga) (SNT). These systems were compared with an area of native vegetation (NV). The evaluations included physical properties: soil texture, soil bulk density (BD), macroaggregates (MA), mesoaggregates (ME), and microaggregates (MI); chemical properties: pH, P, K⁺, Na⁺, Ca⁺², Mg⁺², soil organic carbon (SOC), total nitrogen (TN), isotope $\delta^{13}\text{C}$, particulate organic matter (POM), and mineral-associated organic matter (MAOM); and biological properties: microbial biomass carbon (MBC) and soil basal respiration (C-CO₂). The systems were evaluated based on multivariate PCA analyses and the multivariate stability index (multi-trait stability index - MTSI). The results indicate that ILF systems with grasses, especially with Mg and Bg, have positive impacts on soil quality, minimizing losses of SOC, MAOM, TN, and MBC after the conversion from NV. In these systems, the gains of TN, POM, and MBC were up to 11%, 34%, and 35%, respectively. SOC was the variable most correlated with other soil attributes. The MTSI analysis identified the Mg S28 and Bg S28 systems as having the best average performance and stability across multiple soil characteristics. The use of MTSI was effective in classifying agricultural systems.

Keywords: multi-trait stability index, integrated production systems, soil organic matter, agroforestry, Dryland.

RESUMO

Os sistemas integrados contribuem para a sustentabilidade agrícola, promovendo a qualidade do solo. Este estudo avaliou o efeito de sistemas de integração pecuária-floresta (IPF) com diferentes espaçamentos e culturas, na qualidade do solo no semiárido brasileiro. Os sistemas

estudados incluíram integração pecuária-floresta com sorgo (So), palma forrageira (Pf), capim massai (Cm) e capim buffel (Cb), em espaçamentos de 7 m (E7), 14 m (E14) e 28 m (E28) entre faixas de vegetação nativa (FxVN). Esses sistemas foram comparados com uma área de vegetação nativa (VN). As avaliações incluíram propriedades físicas: textura do solo, densidade do solo (DS), macroagregados (MA), mesoagregados (ME), microagregados (MI); químicas: pH, P, K⁺, Na⁺, Ca⁺², Mg⁺², carbono orgânico do solo (COS), nitrogênio total (NT), isótopo estável de $\delta^{13}\text{C}$, matéria orgânica particulada (MOP) e matéria orgânica associada aos minerais (MOAM); e biológicas: carbono da biomassa microbiana (CBM) e respiração basal do solo (C-CO₂). Os sistemas foram avaliados com base nas análises multivariadas de PCA e índice de estabilidade multivariada (multi-trait stability index - MTSI). Os resultados indicam que os sistemas de IPF com gramíneas, especialmente com capim massai e buffel, têm impactos positivos na qualidade do solo, minimizando perdas de COS, MOAM, NT e CBM após a conversão da VN. Nestes sistemas, os ganhos de NT, MOP e CBM foram de até 11%, 34% e 35%, respectivamente. O COS, MOAM e NT foram as variáveis mais sensíveis para indicar mudanças no solo, com o SOC sendo a variável mais correlacionada com outros atributos do solo. A análise MTSI identificou os sistemas Mg E28 e Bg E28 como os de melhor desempenho médio e estabilidade de múltiplas características do solo. O uso do MTSI foi eficaz na classificação de sistemas agropecuários.

Palavras chaves: índice de estabilidade multivariada, sistemas integrados de produção, matéria orgânica do solo, terras secas.

5.1 INTRODUCTION

Over the past few decades, anthropogenic activities have degraded approximately 25% of global soil (SRIVASTAVA et al. 2019). Given this scenario, the reduction of soil degradation and its effects on the environment have been an important theme in global environmental policies (MATOS et al. 2022). In this context, in recent years, Brazil's environmental legislation and policy have been modified to make agriculture more sustainable, exemplified by the low-carbon agriculture plan (ABC Plan), created in 2010 with the goal of promoting the expansion of the planted area with integration systems (crop-livestock, livestock-forest, crop-forest, crop-livestock-forest integration, and agroforestry systems) by 4 million hectares by 2020. This plan was renewed and renamed the ABC+ Plan, with the new goal of increasing the area by 10.1 million hectares by 2030. (BRASIL 2023). Moreover, the Nationally Determined Contributions (NDC), updated in 2024, established that Brazil must reduce its greenhouse gas emissions by

59% to 67% by 2035, compared to 2005 emissions. For this, the expansion of integrated agricultural systems is among the actions and measures that support the achievement of the target set in the NDC (BRASIL 2024).

Changes in policies and environmental legislation have driven changes in land use in Brazil, which directly reflect on soil quality, defined as the ability of soil to sustain the production of food and fibers, being crucial for maintaining ecosystem services, including crop yield, carbon (C) and nutrient storage, water movement and storage, and a diverse microbial ecosystem. (DORAN and ZEISS 2000). Soil quality can be inferred through the measurement of a series of indicators that are sensitive to variations in management and climate (VALANI et al. 2022). These indicators are related to the physical, chemical, and biological properties of the soil, which interact in a complex manner to determine the effectiveness of soil functioning (BÜNEMANN et al. 2018).

For example, soil organic matter (SOM) has a direct impact on water retention and release, productivity, overall sustainability of agricultural and natural ecosystems, and soil nutrient cycling (OMER et al. 2023). In turn, Tian et al. (2017) stated that, to better understand nutrient cycling in soil management, it is important to understand the availability of carbon (C) and nitrogen (N) in the system. Moreover, the microbial community is often correlated with soil aggregation (SARTO et al. 2020). Therefore, monitoring soil quality indicators is essential to understanding soil functionality and assisting in decision-making regarding management practices and systems that improve soil quality.

Miguel et al. (2020) studied the influence of agroforestry systems on the biological attributes of soil in the semi-arid region of Brazil and found that agroforestry systems improved the biological quality of the soil and may be more sustainable than slash-and-burn agricultural systems in the Caatinga in the long term. Maia et al. (2007) and Nogueira (2009) observed that, when compared to the Caatinga, agroforestry and silvopastoral systems can maintain soil quality, increase system productivity, and accumulate carbon at rates ranging from 29% to 38.7% after 12 years of implementation. Moreover, carbon accumulation rates between 0.60 and 0.90 Mg ha⁻¹ year⁻¹ have been observed in other Brazilian biomes when integrated systems are implemented in areas of monoculture of annual crops where no soil conservation practices were used or in degraded pasture areas (CARVALHO et al., 2014; OLIVEIRA et al., 2022). Similarly, Damian et al. (2021) observed that the adoption of agroforestry and silvopastoral integration systems provided an improvement of approximately 98% and 82% in soil chemical properties related to fertility (P, Ca, and CEC), respectively, when compared to the conventional system.

In this sense, integrated agricultural systems have been evaluated as an alternative for sustainable and economical production, with the ability to promote soil quality restoration (ZANDONA et al. 2019). However, although there are studies on how integrated agricultural systems can affect soil quality (SEKARAN et al. 2021; VALANI et al. 2022), there are gaps in the literature that need to be filled, as pointed out by Valani et al. (2020). After reviewing 92 articles, the authors observed the lack of comparison within integrated systems, as less than 10% of the reviewed articles studied two different systems, and none of them evaluated more than three integrated systems. Moreover, only 20% of the reviewed articles evaluated integrated systems that have a forest component.

Regarding the Brazilian semi-arid region, it is estimated that 16% of the areas are susceptible to desertification (ALVALÁ et al. 2019), largely due to the degradation process that these soils have been undergoing over the years, as a result of the intensification of land use associated with conventional management practices, which have contributed to soil C losses (MEDEIROS et al. 2023). In this way, the adoption of integrated agricultural systems in the Brazilian semi-arid region emerges as an alternative for maintaining soil quality and recovering degraded soils, since these systems are associated with the formation of SOM and nutrient cycling (BIELUCZYK et al. 2020; TONUCCI et al. 2023), as well as in soil aggregation and microbial activity (SARTO et al. 2020).

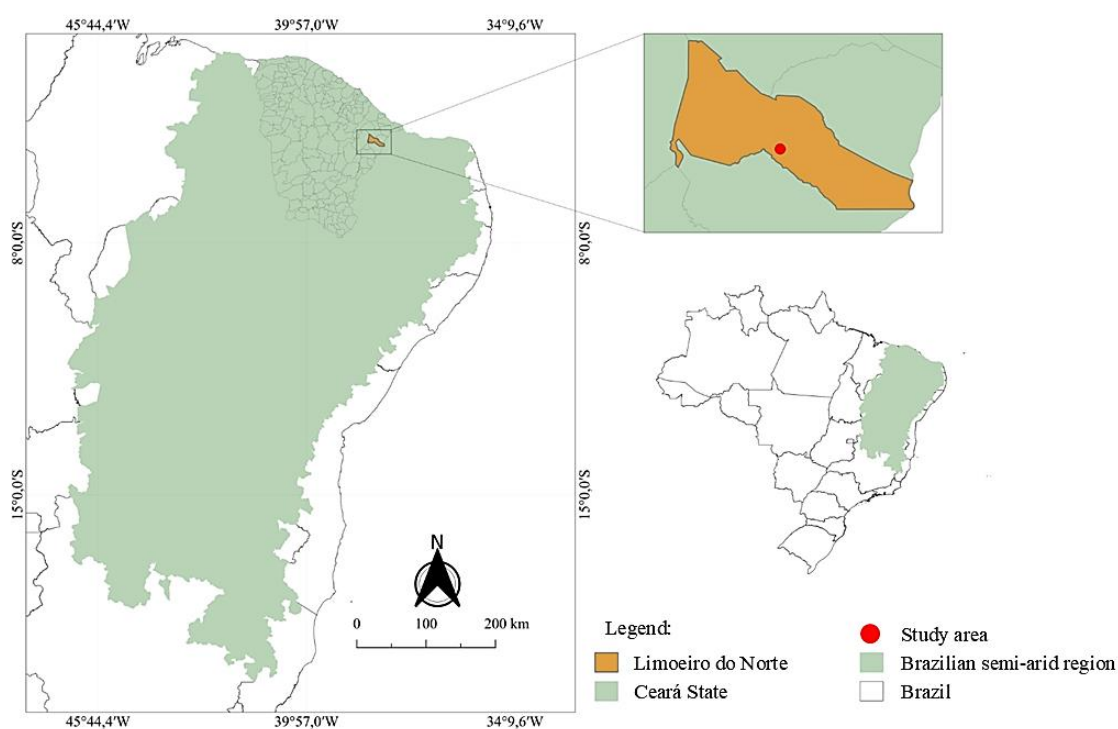
Thus, this study raised the hypothesis that integrated livestock-forest (ILF) systems alter the dynamics of the physical, chemical, and biological properties of the soil, and that such changes would promote improvements in soil quality, varying according to the selection of crops and distance from the forest component. In this context, the objectives of this study were: (i) to evaluate the impact of ILF systems under different spacings and forage crops on soil quality in the semi-arid region of Ceará, Brazil; and (ii) to identify which soil attributes have the greatest potential as indicators of soil quality. Additionally, the multi-trait stability index (MTSI) (OLIVOTO et al. 2019) was used to rank the ILF systems, considering the average performance and stability of each system based on the integrated and weighted analysis of soil attributes. The MTSI index was recently introduced in the field of genetic improvement as an innovative tool for selecting stable and high-yield genotypes in multi-environment trials (METs) based on multiple traits, considering fixed and random effects models (OLIVOTO et al. 2019). Although previous studies (OLIVOTO et al. 2021; HEMAYATI et al. 2024) have focused on the use of MTSI to evaluate the stability of cultivars in breeding programs, our results will provide new perspectives on how MTSI can be used in other areas of knowledge.

5.2 MATERIAL AND METHODS

5.2.1 Description of the study site

The study was conducted at the Teaching, Research and Extension Unit on the Limoeiro do Norte Campus of the Federal Institute of Science and Technology of Ceará (IFCE), 05°10'53" S and 38°00'43" W at an altitude of 146 m (Figure 1). The soil is classified as a Cambisol (FAO, 2015). According to the Köppen classification, the local climate is type BSh (KOTTEK et al. 2006), with an average annual temperature of 29°C and annual precipitation of 719 mm.

Figure 1 - Location of the study area.



Source: Author (2025).

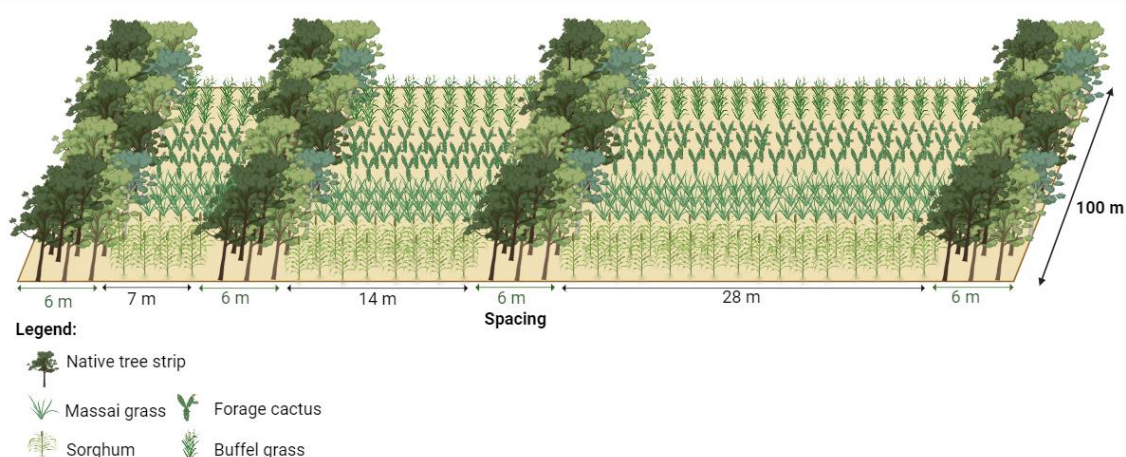
During the dry period of 2015, a tractor with a front blade deforested the area, removing 100% of the vegetation in the cultivable area (understory), leaving only strips of native trees, six meters wide. All the residual material was directed towards the edges. The area had previously consisted of arboreal caatinga in a stage of secondary succession. Phosphate fertilization was carried out annually, applying monoammonium phosphate (MAP) fertilizer, 44% P₂O₅–10% N, at a dose of 200 kg ha⁻¹ year⁻¹ due to the low levels of phosphorus verified by a chemical analysis of the soil. Weed control was both mechanical and chemical.

The area consisted of four ILF systems arranged in three spacings (7, 14, and 28 m) between 6-meter strips of Caatinga vegetation (Figure 2). The pasture components were planted

during the rainy season of 2016: *Pennisetum ciliare* (buffel grass - Bg), *Megathyrsus maximus* (massai grass - Mg), *Opuntia stricta* (forage cactus - Fc) and *Sorghum bicolor* L. (sorghum - So), corresponding to the non-grazed livestock system. Under conventional management practices, we planted and harvested the sorghum annually, tilling the soil each year. The massai grass and buffel grass were allowed to grow freely during the rainy season; at the end of the dry season, the grass was cut mechanically to an average height of 0.05 m above ground, with the aim of standardizing each pasture for the next growing season, and all residual material was removed from the area. The forage cactus was cut every two years, and residual material was removed also from the area.

In this context, the treatments consisted of ILF systems with sorghum (So), forage cactus (Fc), massai grass (Mg) and buffel grass (Bg), at spacing of 7 m (S7), 14 m (S14) or 28 m (S28) between the strips of native trees (SNT - Caatinga), giving a total of 13 treatments (Figure 2) plus the area of native vegetation used as reference (NV - area of Caatinga). The native vegetation was phytosociological in structure, with the following identified tree species: *Cordia goeldiana*, *Mimosa caesalpiniaefolia*, *Cenostigma pyramidale*, *Commiphora leptophloeos*, and *Mimosa tenuiflora*.

Figure 2 - Representation of the area with ILF systems at different spacings between strips of native trees (Caatinga).



Source: Author (2025).

Soil sampling was carried out in January 2022 during the dry season. Disturbed samples were collected from five trenches (replications) in each area at four different depths: 0–10, 10–20, 20–30, and 30–50 cm, giving a total of 280 samples (4 depths × 5 replications × 14 treatments). The samples were stored in a cold chamber in the laboratory at 5°C to 10°C for microbiological analysis.

5.2.2 Soil analysis

The variables of the evaluated physical properties included soil texture (sand, silt, and clay), bulk density (BD), macroaggregates (MA), mesoaggregates (ME), and microaggregates (MI). The granulometric analysis was performed according to the pipette method (TEIXEIRA et al. 2017). The BD was measured using the volumetric ring method, with three repetitions collected per system. In the laboratory, the rings were dried in an oven at 105 °C for 48 hours, and the BD was calculated based on the weight of the oven-dried samples and the total volume of the rings (TEIXEIRA et al. 2017). The water-stable aggregates were evaluated according to the procedure described by Teixeira et al. (2017) and were grouped into three classes: macroaggregates (diameter class > 2.00 mm), mesoaggregates (diameter classes between < 2.00 and > 0.25 mm), and microaggregates (diameter class < 0.25 mm), according to the procedure described by Costa Júnior et al. (2012).

In the chemical characterization of the soil, $H^+ + Al^{+3}$ (calcium acetate extractor), pH in water, P, K^+ , and Na^+ extracted by Mehlich-1, and Ca^{+2} , Mg^{+2} , and Al^{+3} exchangeable using KCl extractor were determined. With the values from the soil chemical analyses, the total cation exchange capacity (total CEC = $K^+ + Ca^{+2} + Mg^{+2} + Na^+ + H^+ + Al^{+3}$) was calculated, according to Teixeira et al. (2017).

Soil organic carbon (SOC), total nitrogen (TN), and the stable isotope $\delta^{13}C$ were determined by the continuous flow mass spectrometry method using a Carlo Erba CHN 1110 elemental analyzer that is coupled to a Delta Plus isotope ratio mass spectrometer.

The soil organic matter was physically fractionated into particulate organic matter (POM) and mineral-associated organic matter (MAOM) following the particle size method proposed by Cambardella and Elliott (1992). The determination of C in the POM fraction was performed by dry combustion in an elemental analyzer (TOC-V Shimadzu, coupled to the SSM-5000A Shimadzu solid sample module). The carbon of the MAOM fraction was determined by the difference between the total soil carbon and the POM (MAOM = SOC – POM) (MENDONÇA and MATOS 2017). The stocks of SOC, NT, POM, and MAOM were calculated by multiplying the content, BD, and soil layer thickness.

For the microbiological variables, the carbon in microbial biomass (MBC) was determined by the irradiation and extraction method, according to Islam and Weil (1998). The carbon contained in the microbial biomass was quantified using a Shimadzu TOC-V analyzer. To determine the basal respiration ($C-CO_2$) of the soil, 50 g of soil were incubated for 50 days, with readings taken after 2, 4, 7, 16, 22, 29, 36, 43, and 50 days. The released carbon was

calculated using the emission rates for each period ($\text{mg C g}^{-1} \text{ soil day}^{-1}$) (MENDONÇA and MATOS 2017).

5.2.3 Statistical analysis

Univariate cluster analysis, correlations, PCA, and multivariate clustering

We applied the univariate Tocher clustering test to the data from the 0-10 and 10-20 cm layers, using Euclidean distance as the intergroup limit, adopting the maximum value of the dissimilarity measure found in the set of smallest distances involving each treatment (REGAZZI and CRUZ 2020). The analyses were conducted with the assistance of the Genes software, version 1990.2022.23. (CRUZ 2013).

The Pearson correlation coefficient was also calculated between the analyzed variables, with the aim of understanding the linearity between the variables.

Next, we standardized the data ($X = 0.0$ and $s = 1.0$) and conducted principal component analysis (PCA), considering only the variables that presented a factor loading value above 0.60. Variables not associated with the principal components (PCs) ($|r| < 0.60$) were removed from the database, and a new analysis was conducted (Araújo et al. 2013). Orthogonal rotation (varimax criterion) was applied in the PCA analysis.

The variables that were studied were put into two groups based on the rules set by Kaiser in 1958. These rules say that the principal components (PCs) must have eigenvalues greater than one ($\lambda > 1.0$), the percentage of total variance for each PC must be greater than 10% (GOVAERTS et al. 2007), and the sum of the variance caused by the PCs must be $\geq 70\%$ (RENCHEER 2002).

Subsequently, the data were subjected to cluster analysis, adopting the hierarchical method of UPGMA group linkage, based on the Euclidean distance with standardized data, to describe the similarity between the management and land use systems. The Mojena criterion (MOJENA 1977) was used to define the optimal number of groups, adopting $k = 1.25$. To test the efficiency of the hierarchical method, the cophenetic correlation coefficient was calculated, which varies from 0 to 1, where the higher the value, the greater the efficiency, with less loss of information. In this analysis, the Mantel test was applied ($P < 0.05$). These analyses were performed with the aid of the MultivariateAnalysis packages, version 0.4.4 (AZEVEDO 2021), from R software, version 3.5.2. (R Core Team 2018).

Multi-trait stability Index – MTSI

MTSI consists of a multivariate method that was developed for the classification of genotypes regarding performance and stability in multi-environment trials. In this research, "genotype (G)" was considered as the ILF systems (S) and "environment (E)" as the soil layers (0-10, 10-20, 20-30, and 30-50 cm), considering that physical, chemical, and biological attributes influence the phenotypic behavior of the studied species (GULL et al. 2019). Initially, to quantify the stability of each treatment, the WAASB index (Weighted Average of Absolute Scores) (equation 1) was calculated, which corresponds to the weighted average of the absolute scores obtained by the singular value decomposition of the BLUP (Best Linear Unbiased Prediction) matrix (OLIVOTO et al. 2019) of the SxE interaction effects in a mixed linear model. The BD and $\delta^{13}\text{C}$ variables did not show a significant SxE interaction ($P > 0.05$) and were excluded from the analyses.

$$\text{WAASB}_i = \sum_{k=1}^p |IPCA_{ik} \times EP_k| / \sum_{k=1}^p EP_k, \quad \text{equation 1}$$

Where WAASB_i is the weighted average of the absolute scores of treatments i (or environment), $IPCA_{ik}$ is the score of treatment i in the k th IPCA, and EP_k is the amount of variance explained by the k th IPCA. The most stable treatment is the one with the lowest WAASB value. In this research, all effects were considered random, using the argument random = "all" from the waasb function ().

The WAASBY index for each treatment for each response variable (rW_{ij}) takes values in the range of 0-100, facilitating the definition of an ideal type, where 100 is assigned to the treatment that simultaneously exhibits the highest performance and stability (ideal type). This index is calculated according to equation 2:

$$\text{WAASBY}_i = \frac{(rY_i \times \theta_Y) + (rW_i \times \theta_S)}{\theta_Y + \theta_S}, \quad \text{equation 2}$$

Where WAASBY_i is the simultaneous ranking index for treatment i that weighs between performance (Y) and stability (W); θ_Y and θ_S are the weights for the response and stability variables (WAASB), assumed to be 50% and 50%, respectively; rY_i and rW_i are the scaled values (0–100) for the response and stability variables (WAASB), respectively, calculated according to equation 3:

$$rY_i = rW_i = \frac{nma - nmi}{oma - omi} \times (O_i - oma) + nma, \quad \text{equation 3}$$

Where nma and nmi are the new rescaled maximum and minimum values; oma and omi are the original maximum and minimum values. O_i is the original value for the response variable or WAASB index of the i th treatment. The values of nma and nmi are chosen according to the variable. In this study, high values were assumed for SOC, TN, POM, MAOM, MA, ME,

pH, P, K, Ca, Mg, CEC, BS, silt, and clay ($nma = 100$ and $nmi = 0$) and low values for microaggregates, Na, H + Al, and sand ($nma = 0$ and $nmi = 100$).

The next step consisted of exploratory factor analysis to group correlated characteristics and calculate factor scores of the treatments. The model used for analysis was that of Equation 4:

$$X = \mu + Lf + \varepsilon, \text{ equation 4}$$

Where $X = px1$ is the matrix of rescaled observations; $\mu = px1$ is the matrix of standardized means; $L = px f$ is the factor loading matrix; $f = px1$ is the matrix of common factor loadings; $\varepsilon = px1$ is the residual matrix; p and f correspond to the numbers of variables and common factors retained, respectively. The eigenvalues and eigenvectors were derived from the correlation matrix, which was subjected to varimax rotation to obtain the final loadings used in the calculation of treatment scores, according to Equation 5:

$$F = Z (A^T R^{-1})^T, \text{ equation 5}$$

Where $F = Txf$ is the matrix with the factor scores, $Z = qxp$ is the matrix with rescaled means, and $A = px f$ is the matrix with the canonical loadings, $R = pxp$ is the correlation matrix between the variables; T , f , and p represent the treatments, response variables, and retained factors, respectively.

The ranking of treatments, based on performance and stability, considering multi-environments and multi-characters, was made possible with the MTSI method, which is based on the Euclidean distance from the treatment to the ideotype, using the scores obtained in the exploratory factor analysis. Using the `mtsi()` function with the argument `index = "waasby,"` the index was calculated according to equation 6:

$$MTSI_i = \left[\sum_{j=1}^f (F_{ij} - F_j)^2 \right]^{0.5}, \text{ equation 6}$$

Where $MTSI_i$ is the multivariate stability index of the i th treatment; F_{ij} is the j th score of the i th treatment; and F_j is the j th score of the ideotype (OLIVOTO et al. 2019). The treatment with the lowest MTSI value is closer to the ideotype and, therefore, shows higher average performance and stability in all environments for all the studied variables. All analyses were performed with the aid of the "metan" package, version v.1.18.0 (OLIVOTO et al. 2019), in the R software. (R Core Team 2018).

5.3 RESULTS

5.3.1 Physical, chemical, and biological properties of soil

Using the Tocher method, we observed the formation of different similarity groups among the treatments related to the physical, chemical, and biological attributes of soil quality. Among these groups, BD stood out as having the highest number, while the ILF systems with forage cactus (Fc) and ILF with sorghum (So), both at the S28 spacing, did not differ from NV and showed the lowest densities (Table 1). Regarding the other variables, in general, the ILF So and Fc systems showed less similarity with the NV, except for the microbiological variables. In MBC and C-CO₂, the spacings of 7 m (S7) and 28 m (S28) belonged to the same group as NV. Regarding the chemical properties, the amounts of SOC, MAOM, TN, and K⁺ were lower in the ILF systems with sorghum and forage cactus compared to NV at all the spacings that were tested. This was especially true for K⁺, where ILF Fc had the lowest averages in both soil layers no matter what the spacing was.

The systems with buffel grass (Bg) and massai grass (Mg) were the ones that least differed from NV. In general, in these systems, the spacings of 14 and 28 m were responsible for the highest averages for SOC, POM, MAOM, NT, MBC, and C-CO₂ in the 0-10 cm layer. Furthermore, for the 28 m spacing, the POM fraction was higher by 34% and 13% in the ILF systems with Bg and Mg, respectively, compared to NV, in the 0-10 cm layer. Similarly, the 14 m spacing provided higher N stocks than NV in Bg and Mg. The MBC in the system with Mg (S7) was also 35% higher compared to NV. Regarding soil aggregates, the ILF systems with grass under S28 spacing stood out for their macroaggregate values (MA). In this case, the systems with Bg and So showed MA values 27% and 33% higher compared to NV.

In the 10-20 cm layer, all ILF systems differed from NV in terms of NT, $\delta^{13}\text{C}$, and MA values (Table 1). Similarly, the POM values in NV showed lower averages compared to the ILF systems, except for the Bg (S28) system, which belonged to the same group as NV, and So (S7), whose POM value was 9% higher than NV. For the SOC and MAOM values, only the ILF systems with Fc (S28) and Bg (S14 and S28) did not belong to the same group as NV, showing the lowest averages. It was found that MBC and soil microbial activity (C-CO₂) were higher in ILF systems with grasses spaced 7 and 28 m apart, meaning that the averages were higher than those in NV.

Table 1 - Averages of the physical, chemical, and biological soil variables of the ILF systems with buffel grass (Bg), massai grass (Mg), sorghum (So), and forage cactus (Fc) in the 0-10 cm and 10-20 cm layers. NV: native vegetation; SNT: strips of native trees; S7, S14, and S18: spacings of 7, 14, and 28 m between SNT.

Systems	BD	MA	ME	MI	pH	Na ⁺²	P	K ⁺	Ca ⁺²	Mg ⁺²	H+Al ⁺³	δ13C	SOC	TN	POM	MAOM	MBC	C-CO ₂
	g cm ⁻³	----- % -----				----- mg dm ⁻³ -----				----- cmol _c dm ⁻³ -----			‰	----- Mg ha ⁻¹ -----				mg kg ⁻¹
0 – 10 cm																		
NV	1.35 f	44.7 b	45.8 d	9.5 d	7.5 a	55.0 c	3.0 b	176 c	9.4 a	2.8 a	0.4 c	-25.4 a	17.85 a	1.65 b	2.82 b	15.0 a	120.9 b	6.85 b
SNT	1.30 g	45.1 b	45.8 d	9.1 d	7.5 a	60.0 c	4.0 b	186 c	8.4 a	1.6 b	0.2 d	-25.5 a	19.01 a	1.49 c	2.61 c	14.7 a	181.2 a	8.28 b
Bg S7	1.69 a	31.9 d	55.9 c	12.2 c	6.9 b	70.0 b	11.0 b	210 b	6.2 b	1.6 b	0.7 c	-24.8 d	15.28 b	1.49 c	2.78 b	12.5 c	56.1 d	5.24 c
Bg S14	1.45 e	25.3 e	61.4 b	13.3 c	7.0 a	86.0 a	12.0 b	270 a	6.6 b	1.8 b	0.7 c	-24.7 d	17.39 a	1.68 a	2.54 c	14.8 a	115.9 b	7.31 b
Bg S28	1.49 d	56.2 a	35.3 f	8.5 e	7.0 a	65.0 b	14.0 b	180 c	7.1 b	1.6 b	1.0 c	-24.5 e	16.71 b	1.32 d	3.77 a	12.9 c	123.8 b	10.31 a
Mg S7	1.49 d	20.9 f	68.6 a	10.4 d	7.0 a	74.0 b	22.0 a	210 b	6.5 b	1.7 b	0.5 c	-24.8 d	15.58 b	1.53 b	2.29 c	13.3 b	163.8 a	4.94 c
Mg S14	1.62 b	31.6 d	58.9 c	9.5 d	6.9 b	95.0 a	14.0 b	290 a	7.0 b	2.2 a	0.2 d	-24.9 c	18.93 a	1.83 a	3.17 b	15.8 a	98.9 c	5.93 c
Mg S28	1.54 d	44.0 b	43.4 e	12.6 d	7.2 a	87.0 a	35.0 a	230 b	7.1 b	1.8 b	2.1 c	-24.3 e	17.37 a	1.62 b	3.20 b	14.2 b	125.3 b	7.41 b
Fc S7	1.53 d	27.2 e	62.9 b	9.9 c	5.8 c	22.0 e	7.0 b	31 e	5.1 c	0.9 c	5.8 a	-24.9 c	13.48 c	1.18 d	2.18 c	11.3 d	106.4 b	7.20 b
Fc S14	1.70 a	36.2 c	47.9 d	15.8 b	5.2 d	21.0 e	9.0 b	56 e	4.6 d	0.5 d	4.0 b	-24.6 d	10.79 d	1.02 e	2.14 c	8.6 e	16.7 e	4.28 c
Fc S28	1.35 f	37.7 c	43.5 d	18.8 a	5.5 c	21.0 e	8.0 b	33 e	4.4 d	0.7 d	6.4 a	-24.8 d	12.99 c	1.10 e	1.86 d	11.1 d	77.4 c	5.32 c
So S7	1.49 d	18.5 f	64.1 b	17.4 a	7.2 a	60.0 c	6.0 b	184 c	6.4 b	1.1 d	2.1 c	-25.1 c	13.83 c	1.29 d	2.23 c	11.6 d	102.2 b	8.14 b
So S14	1.59 c	15.3 g	71.4 a	13.3 c	6.4 b	42.0 d	7.0 b	138 d	6.2 b	1.2 d	1.2 c	-25.0 c	12.93 c	1.39 c	3.21 a	9.7 e	49.3 d	6.30 b
So S28	1.38 f	59.8 a	30.7 f	9.5 d	6.9 b	84.0 a	14.0 b	260.0 a	6.0 c	2.0	1.6 c	-25.3 b	14.39 c	1.16 e	1.99 d	12.4 c	132.6 b	8.32 a
LDI	0.05	3.67	4.64	1.62	0.50	13.0	13.0	38.0	1.0	0.6	1.8	-0.18	2.14	0.15	0.56	1.08	32.6	1.99
10 – 20 cm																		
NV	1.33 d	30.4 b	56.4 b	13.2 c	7.6 a	45.0 c	1.0 b	144.0 b	7.2 a	2.3 a	1.1 c	-25.1 a	9.13 b	0.98 a	1.95 b	7.19 b	1.33 d	46.4 c

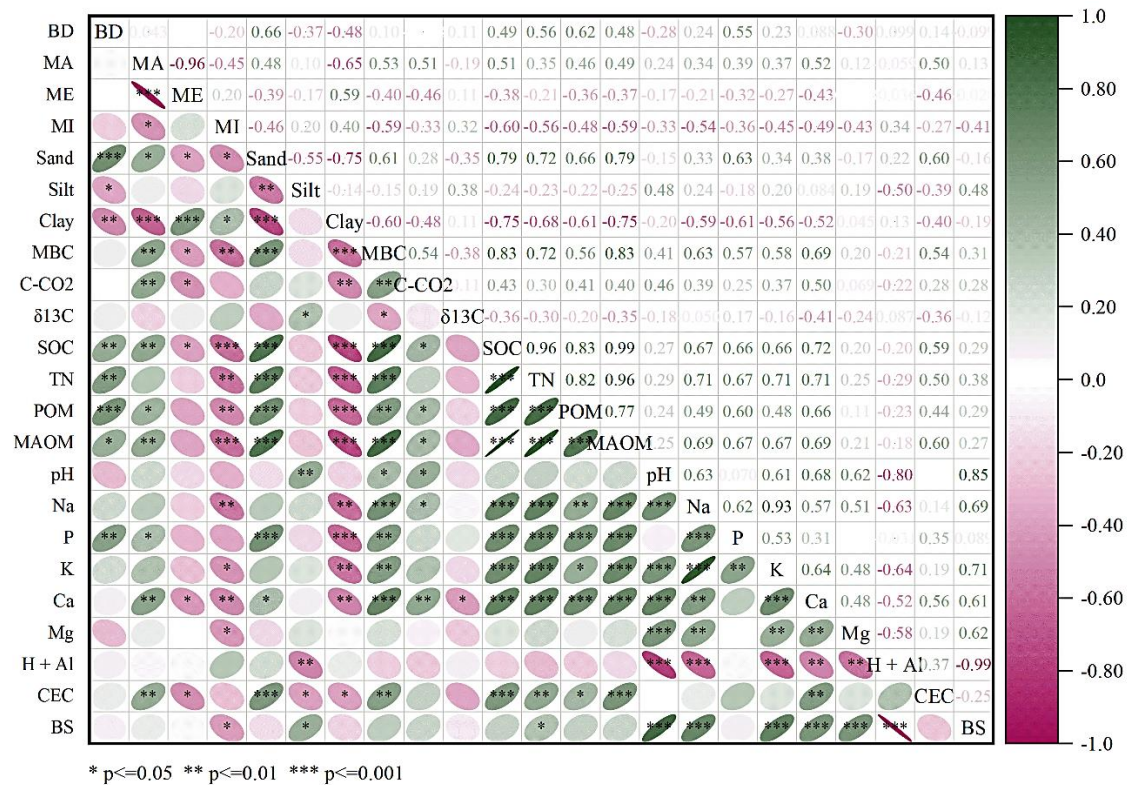
SNT	1.34 c	33.1 b	52.7 c	14.2 b	7.2 b	43.0 c	1.0 b	127.0 b	5.6 b	1.8 b	0.8 d	-25.2 a	10.76 a	1.02 a	2.33 a	8.43 a	1.34 c	58.9 b
Bg S7	1.34 c	14.9 f	75.5 a	9.7 d	6.9 b	52.0 b	2.0 a	15.0 f	4.0 c	1.9 b	1.1 c	-24.5 e	8.13 b	0.84 c	1.60 c	6.53 b	1.34 c	51.8 c
Bg S14	1.28 f	14.9 f	69.4 a	15.6 b	7.2 b	59.0 a	1.0 b	190.0 a	5.2 b	1.9 b	0.2 e	-24.3 f	7.03 c	0.80 d	1.10 f	5.93 c	1.28 f	23.3 d
Bg S28	1.36 b	36.9 a	44.0 d	19.1 a	7.3 a	27.0 e	2.0 a	60.0 d	6.2 a	1.1 c	1.3 c	-24.2 f	7.39 c	0.73 e	1.99 b	5.40 c	1.36 b	43.3 c
Mg S7	1.30 e	35.1 a	53.1 c	11.8 c	7.1 b	49.0 b	2.0 a	148.0 b	6.0 b	1.2 c	0.5 d	-24.8 c	7.94 b	0.87 c	1.33 e	6.61 b	1.30 e	68.5 b
Mg S14	1.37 b	29.4 c	56.7 b	13.9 c	7.0 b	55.0 a	2.0 a	175.0 a	5.0 b	1.7 b	0.6 d	-24.3 f	8.39 b	0.88 b	1.32 e	7.08 b	1.37 b	43.6 c
Mg S28	1.33 d	22.2 e	57.8 b	19.9 a	6.9 b	53.0 a	2.0 a	136.0 b	5.8 b	1.6 b	2.0 b	-24.7 d	8.93 b	0.84 c	1.55 d	7.39 a	1.33 d	85.1 a
Fc S7	1.44 a	16.4 f	69.3 b	14.3 b	6.0 c	28.0 e	1.0 b	62.0 d	4.6 c	1.7 b	4.4 a	-24.7 c	8.62 b	0.78 d	1.35 d	7.27 b	1.44 a	37.7 c
Fc S14	1.43 a	11.9 g	69.4 a	18.7 a	5.9 c	22.0 e	2.0 a	46.0 e	4.5 c	1.6 b	1.8 b	-24.7 c	8.37 b	0.86 c	1.77 c	6.60 b	1.43 a	23.9 d
Fc S28	1.29 e	26.5 d	57.8 b	15.7 b	5.6 d	16.0 f	2.0 a	39.0 e	4.7 c	1.6 b	4.4 a	-24.6 d	6.37 c	0.70 e	1.41 d	4.96 c	1.29 e	29.2 d
So S7	1.37 b	21.5 e	62.6 b	15.9 b	7.1 b	37.0 d	2.0 a	100.0 c	5.7 b	1.7 b	1.7 b	-25.2 a	9.22 a	0.93 b	2.12 a	7.11 b	1.37 b	50.5 c
So S14	1.35 c	13.7 g	69.9 a	16.3 b	6.1 c	28.0 e	2.0 a	82.0 d	4.2 c	1.3 c	1.7 b	-24.9 b	7.67 b	0.84 c	1.32 e	6.35 b	1.35 c	23.7 d
So S28	1.27 f	29.4 c	56.5 b	14.2 b	6.9 b	40.0 c	3.0 a	122.0 c	5.0 b	2.0 a	1.9 b	-25.0 b	8.26 b	0.80 d	1.24 e	7.01 b	1.27 f	38.3 c
IDL	0.01	2.9	8.6	2.2	0.3	6.0	1.0	24.0	1.0	0.3	0.3	-0.12	1.54	0.05	0.21	1.04	0.01	16.5

Source: Author (2025).

5.3.2 Correlation analysis

The Pearson correlation analysis showed significant correlations ($P < 0.05$) of high magnitude, indicating linearity in the relationships between the variables (Figure 3). The highest correlation was observed between SOC x MAOM ($r = 0.99$) and BS x H+Al ($r = -0.99$). Other high-magnitude correlations ($r = > 0.90$ or > -0.90) were obtained between SOC x TN, TN x MAOM, MA x ME, and Na x K ($p < 0.01$). MBC, POM, and Ca showed positive correlations ($r > 0.70$ – 0.90) with SOC, TN, and MAOM. TN also obtained a positive correlation ($r = 0.71$) with Na and K, while BS correlated ($r > 0.71$) with K and pH. Overall, SOC was the variable most strongly correlated with other variables, while $\delta^{13}\text{C}$ was the variable least correlated.

Figure 3 - Pearson correlations between the physical, chemical, and biological soil variables in the studied systems. BD: bulk density; MBC: microbial biomass carbon; C-CO₂: basal soil respiration; $\delta^{13}\text{C}$: natural abundance of $\delta^{13}\text{C}$; SOC: soil organic carbon; TN: total nitrogen; POM: particulate organic matter; MAOM: mineral-associated organic matter; MA: soil macroaggregates; ME: soil mesoaggregates; MI: soil microaggregates; CEC: total cation exchange capacity; BS: base saturation.



Source: Author (2025).

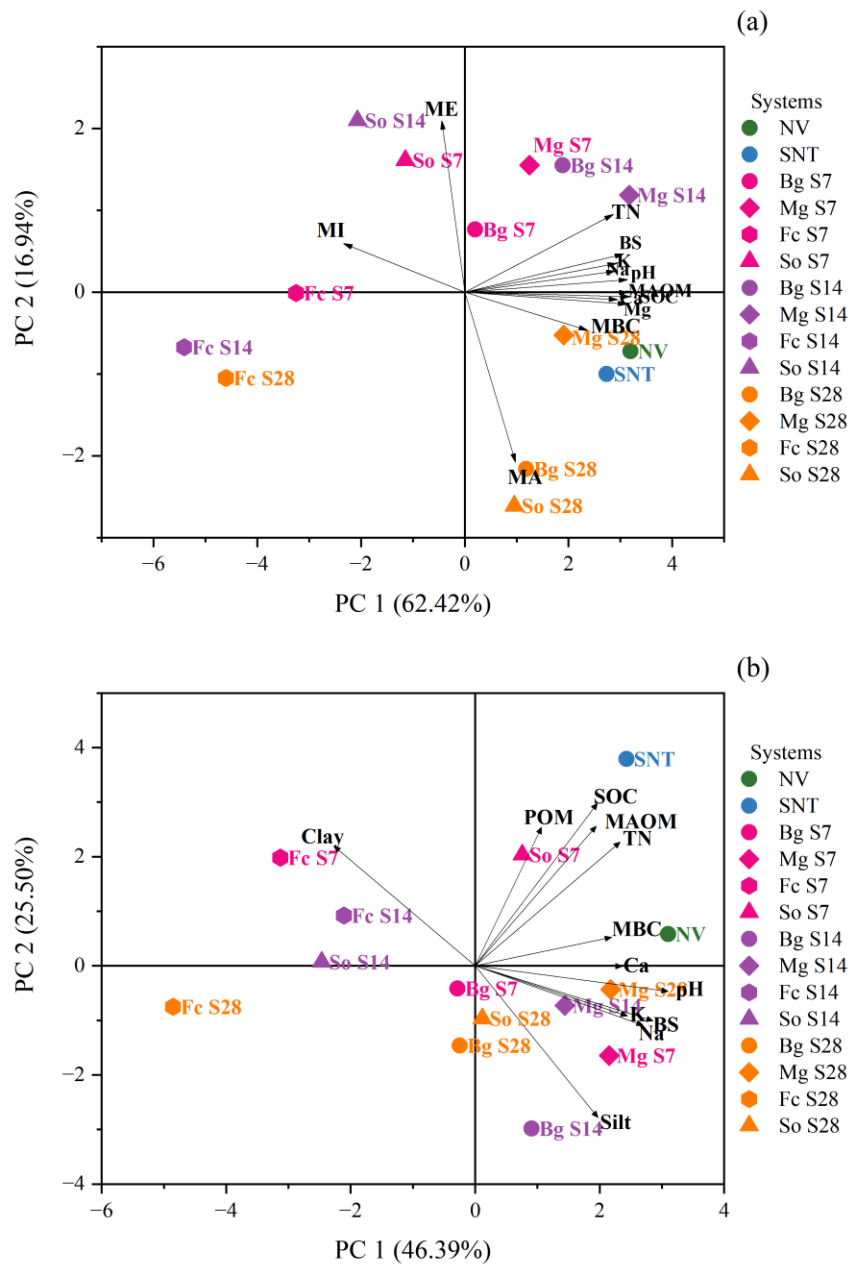
5.3.3 Principal component analysis and UPGMA clustering

Principal component analysis (PCA) explored the relationship of the variables at different SNT spacings in ILF systems, as presented in Figure 4. Regarding the soil layers of 0-10 and 10-20 cm, the total accumulated variation in the first two principal components was 79.36 and 71.89%, respectively.

In the 0-10 cm layer (Figure 4a), PC1, which explains 62.42% of the total variation, was more correlated with the scores of the NV, SNT, and ILF Mg S28 systems, which are related to the highest values of the SOC, MAOM, MBC, Mg, and Ca variables and the lowest values of MI. The systems ILF Mg S7, ILF Mg S14, and ILF Bg S14 also stood out in this component, with higher TN values, Bg S28 and ILF So S28, with higher MA values. On the other hand, the ILF Fc S14 and ILF Fc S28 systems showed the lowest values for the variables related to SOM. The ILF system with sorghum, at spacings S7 and S14, showed the highest averages of ME and MI. The second component (PC2) explained 16.94% of the total data variability and was more influenced by the macroaggregates (MA) and mesoaggregates (ME) of the soil.

In the 10-20 cm layer (Figure 4b), PC1 accounted for 46.39% of the total variation, being more correlated with the NV scores. The ILF So S7 and SNT systems showed the highest values of MAOM, TN, SOC, and POM. The systems with massai grass (Mg) obtained high averages linked to the chemical variables (pH, Ca, K, BS, and Na). In PC2, which explained 25.50% of the total variation, the So S14, Fc S7, and Fc S14 systems showed the highest average values for clay. The Fc S28 system showed lower averages, mainly for the variables related to SOM.

Figure 4 - Biplots of the principal component analysis (PCA 1 and 2) for the physical, chemical, and biological soil variables of the ILF systems with buffel grass (Bg), massai grass (Mg), sorghum (So), and forage cactus (Fc), in the 0-10 cm (a) and 10-20 cm layers. (b). NV: native vegetation; SNT: strips of native trees; S7, S14, and S18: spacings of 7, 14, and 28 m between SNT. MBC: microbial biomass carbon; SOC: soil organic carbon; TN: total nitrogen; POM: particulate organic matter; MAOM: mineral-associated organic matter; MA: soil macroaggregates; ME: soil mesoaggregates; BS: base saturation.

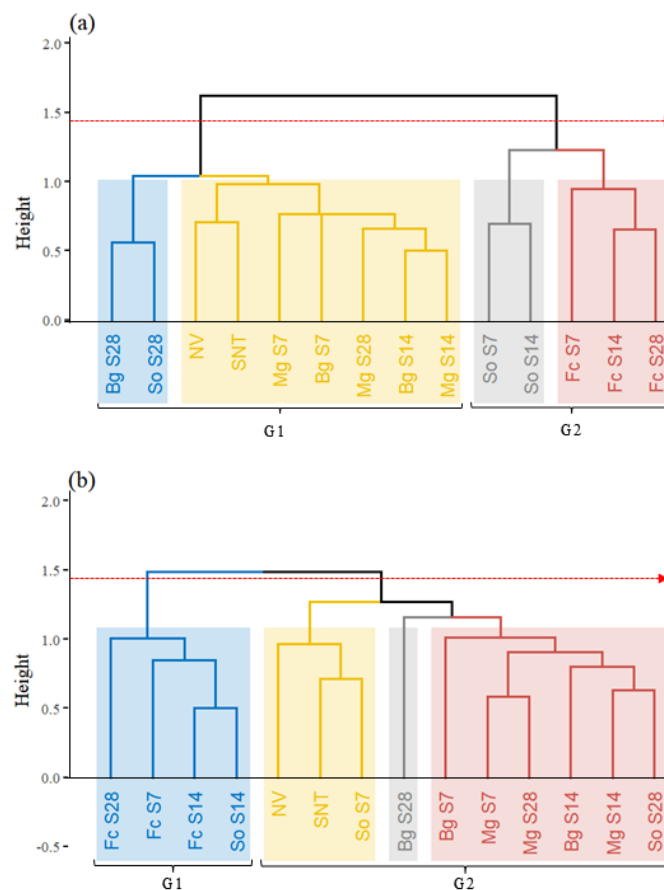


Source: Author (2025).

The cluster analysis for the 0-10 cm layer revealed the formation of two similarity groups (Figure 5a), in which group G1 gathered the NV, SNT treatments, and the ILF systems with Mg (S28, S14, and S28), Bg (S14 and S28), and So (S28). The ILF systems with Fc and So at the spacings S7 and S14 formed cluster G2 (Figure 4a). Most of the analyzed parameters (Figure 3a) showed a correlation with the G1 group, suggesting that this group represents the systems and spacings that significantly improved soil quality in the uppermost layer of the soil.

The hierarchical clustering in the 10-20 cm layer also formed two groups. G1 was formed by the ILF systems with Fc (S7, S14, and S28), and So in the S14 spacing, while group G2 grouped all the other ILF systems plus NV and SNT (Figure 5b) and was associated with most of the analyzed variables (Figure 4b).

Figure 5 - Hierarchical clustering of combinations of ILF systems at different spacings between the forest component in the 0-10 cm (a) and 10-20 cm layers (b). The dotted horizontal line indicates the cutoff points that resulted in two groups. (G1 and G2). Bg: ILF systems with buffel grass; Mg: massai grass; So: sorghum; Fc: forage cactus; NV: native vegetation; SNT: strips of native trees; and S7, S14, and S18: spacings of 7, 14, and 28 m between SNT.



Source: Author (2025).

5.3.4 Multi-trait stability Index

The results of the factor analysis showed that six factors were retained, accounting for 88.5% of the total variation (Table 2). After the varimax rotation, the average communality (h), which is the proportion of common variance present in a given variable, was 0.89 (MA $0.68 \leq h \leq 0.96$ MI), indicating that the largest proportion of variance for each characteristic was influenced by the six factors. The WAASBY values (the higher, the better) for each of the 18 variables were grouped into the six factors (FA) as follows: FA1: pH, Na, Mg, H+Al, and BS; FA2: MA, Ca, CEC, and sand; FA3: SOC, TN, MAOM, and clay; FA4: ME, P, and K; FA5: POM and silt; and FA6: only the MI variable (Table 2).

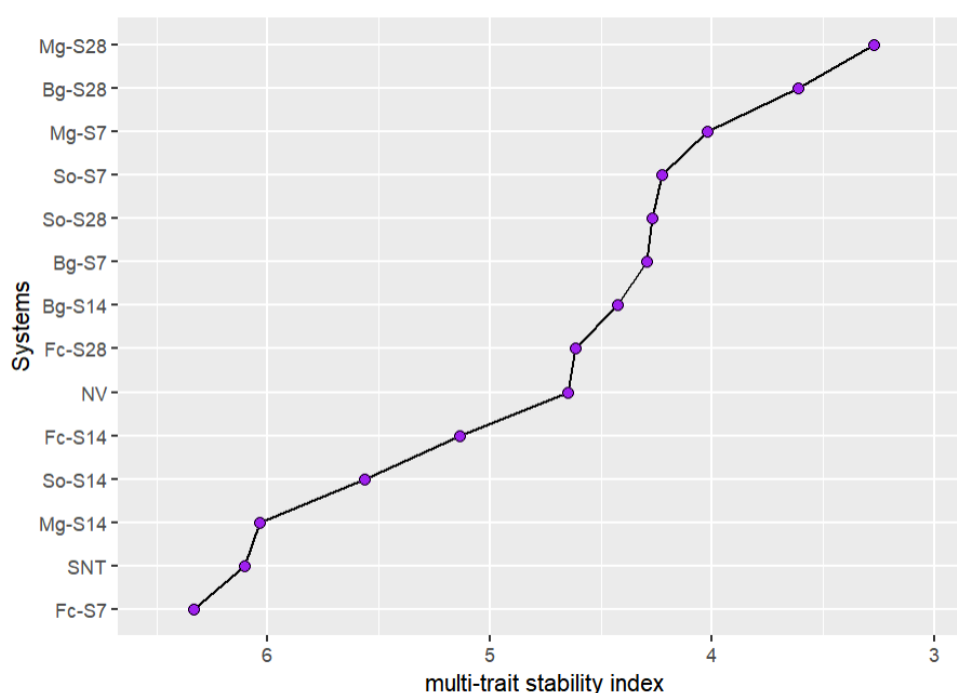
Table 2 - Eigenvalues, explained variance, factorial loadings after varimax rotation, and communalities (h) obtained in the factor analysis.

Variables	FA1‡	FA2§	FA3	FA4	FA5	FA6	h
SOC	-0.122	-0.119	0.909	-0.200	0.002	-0.172	0.925
TN	-0.007	-0.088	0.794	-0.004	0.160	-0.337	0.778
POM	0.041	0.099	-0.014	-0.023	0.907	-0.057	0.837
MAOM	-0.201	-0.027	0.879	-0.175	-0.139	-0.200	0.903
MA	0.073	0.745	0.272	0.171	0.063	0.108	0.679
ME	-0.245	-0.308	-0.495	0.594	-0.355	-0.185	0.914
MI	0.129	0.281	-0.239	-0.014	-0.003	0.898	0.960
pH	-0.619	-0.163	0.175	-0.486	-0.264	-0.288	0.829
Na	0.778	-0.450	-0.335	0.069	0.064	-0.077	0.934
P	0.338	-0.394	-0.021	0.784	-0.042	-0.134	0.904
K	-0.092	-0.223	0.053	-0.921	-0.023	-0.093	0.918
Ca	0.265	-0.756	0.237	0.123	-0.242	-0.294	0.858
Mg	-0.899	-0.039	-0.011	0.216	-0.201	0.236	0.953
H+Al	-0.775	0.181	0.045	-0.283	0.314	-0.238	0.871
CEC	0.383	-0.833	0.208	0.014	-0.167	0.109	0.925
BS	-0.795	0.091	0.091	-0.386	0.202	-0.335	0.950
Sand	-0.096	-0.770	0.010	0.189	0.481	-0.228	0.921
Silt	-0.149	-0.042	0.605	-0.075	0.620	0.326	0.886
Clay	0.001	-0.230	-0.813	-0.205	-0.024	-0.341	0.873
Eigenvalues ¶	5.26	3.81	3.00	1.96	1.73	1.05	-
Variance (Var.; %) ¶	27.700	20.071	15.809	10.314	9.091	5.531	-
Var. Accumulated (%) ¶	27.70	47.77	63.58	73.89	82.99	88.52	-

Source: Author (2025). SOC: soil organic carbon; TN: total nitrogen; POM: particulate organic matter; MAOM: mineral-associated organic matter; MA: soil macroaggregates; ME: soil mesoaggregates; MI: soil microaggregates; CEC: total cations exchange capacity; BS: base saturation. ‡ FA, factor retained; § Bold values indicate the variables grouped within each factor; ¶ The values for all factors are provided in Supplemental Table S1.

The MTSI for the analyzed systems was calculated using the factor scores of these six factors (Table 2). Figure 6 categorized the systems from the highest to the lowest MTSI value. The ILF Mg S28 system achieved the lowest MTSI (3.27), followed by the Bg S28 and Mg S7 systems, with MTSIs of 3.61 and 4.02, respectively (Table 3). This indicates that these systems exhibited the highest average performance and stability, making them more promising according to the evaluated variables.

Figure 6 - Systems ranking for the multi-trait stability index. Bg: ILF systems with buffel grass; Mg: massai grass; So: sorghum; Fc: forage cactus; NV: native vegetation; SNT: strips of native trees; and S7, S14, and S18: spacings of 7, 14, and 28 m between SNT.



Source: Author (2025).

MTSI showed that FA2 (26.83%) was most strongly linked to the Mg S28 system being the farthest from the ideotype (Table 3; Table S2). In other words, Mg S28 obtained the lowest WAASBY values for the variables MA, Ca, CEC, and Sand. On the other hand, FA4 (7.70%) and FA3 (8.97%) had the lowest contribution to the MTSI. This means that the Mg S28 system was the most stable in relation to the variables ME, P, and K (FA4), SOC, TN, MAOM, and clay (FA3). Regarding the ILF Bg S28 system, the smallest contribution to the MTSI was due to FA4 (1.87%), which was related to the variables ME, P, and K. While for the ILF Mg S7 system, FA1 (4.80%; pH, Na, Mg, H+Al, and BS) had the smallest contribution.

The systems with the highest MTSI indices were Fc S7, NTS, and Mg S14 (Figure 6). In these three systems, the greatest contribution to MTSI was related to FA2, FA4, and FA5 (Table 3), which are factors related to the variables MA, Ca, CEC, sand, ME, P, K, POM, and silt (Table 2).

Table 3 - Relative contribution of each factor on the multi-trait stability index (MTSI) of each system. Bold values represent the systems with lower MTSI values.

Systems	FA1	FA2	FA3	FA4	FA5	FA6	MTSI
NV	8.69	20.29	16.58	28.79	11.78	13.88	4.65
SNT	11.02	40.47	18.10	18.50	1.46	10.44	6.11
Bg S7	4.61	28.80	28.86	11.15	2.49	24.09	4.29
Bg S14	5.05	32.49	24.80	12.30	14.44	10.92	4.43
Bg S28	25.62	41.61	15.37	1.87	6.66	8.85	3.61
Mg S7	4.81	30.13	17.58	6.52	16.23	24.73	4.02
Mg S14	5.07	45.00	15.24	0.46	23.89	10.34	6.04
Mg S28	12.95	26.83	8.97	7.70	23.71	19.84	3.27
Fc S7	15.76	24.60	22.49	5.19	24.52	7.45	6.33
Fc S14	24.70	30.70	22.77	2.02	14.12	5.69	5.13
Fc S28	23.05	26.14	20.96	1.98	15.95	11.92	4.61
So S7	4.54	25.64	49.88	6.22	9.48	4.25	4.23
So S14	4.53	26.71	18.82	2.28	27.37	20.28	5.56
So S28	4.02	15.90	28.11	14.18	25.14	12.65	4.27

Source: Author (2025).

5.4 DISCUSSION

In the Brazilian semi-arid region, the increase in soil degradation due to anthropogenic activities and the advances of climate change represent a major obstacle to agricultural sustainability (LIMA et al. 2024). This environmental vulnerability in the Brazilian semi-arid region is exacerbated by the difficulty of finding production systems that are technically and financially viable, increase agricultural and livestock productivity, and maintain soil quality. For this reason, studies aimed at implementing management strategies that promote soil health and increase production sustainably in the region are essential. The present study aimed to evaluate the physical, chemical, and biological properties of the soil in different livestock-forest integration systems in the Brazilian semi-arid region, with the goal of expanding their adoption.

Overall, the results indicated that NV had the lowest BD, likely due to low soil disturbance and higher C stock in natural vegetation areas (Table 1). On the other hand, Batey (2009) observed that the highest BD values in all ILF systems indicated a certain degree of soil

compaction in areas with integrated systems, suggesting that machine traffic and soil tillage during system implementation may be the main cause of soil compaction.

The lower averages for MA and higher for MI and ME in the ILF systems with spacings of 7 and 14 m (S7 and S14) (Table 1), as well as the negative correlation between these variables (Figure 3), suggest that the soil tillage during the experiment's implementation also caused the reduction of MA. With that, there was an increase in MI, but mainly in ME. However, it is important to highlight that the S28 spacing increased the MA values in the ILF systems with grasses. This contribution is also apparent in the PCA biplot (0-10 cm) (Figure 4a), as the vector related to MA is positioned close to the Bg and So systems under a spacing of 28 m (S28) and correlated negatively with MI (Figure 3). Salton et al. (2008) suggested that grasses may play a beneficial role in the formation of macroaggregates through the union of smaller aggregates. This effect confirms the concept of soil aggregation hierarchy proposed by Tisdall and Oades (1982), in which microaggregates are joined to form macroaggregates by transient binding agents (polysaccharides derived from microorganisms and plants) and temporary binding agents (roots and fungal hyphae).

The PCA results also helped highlight the relationships between the physical, chemical, and biological soil variables and the ILF systems. For example, in the 0-10 cm layer, the first component (PC1) was mainly associated with the increase of SOC, MAOM, and MBC in the ILF system with massai grass at a spacing of 28 m (S28). The results are the same as those from the MTSI method. This is shown by the fact that ILF Mg S28 was chosen because it had the lowest value for this index. It was also the ILF system with the best overall performance and stability when looking at the characteristics of the studied layers. This discovery demonstrates the efficient use of the MTSI index in the integrated assessment of multiple soil characteristics, as well as in the subsequent comparison and selection of various agricultural systems. When analyzing our results for the contribution of each factor in the MTSI, we observed that the variables ME, P, and K (FA4) and SOC, TN, MAOM, and Clay (FA3) in the different environments (soil layers) evaluated were the ones that most contributed to the stability and performance of the ILF Mg S28 and Bg S28 systems (Table 3). In this way, these arrangements emerged as the systems with the greatest potential to recover the SOC lost due to conventional management practices (deforestation, plowing, and harrowing). Furthermore, since SOC showed the strongest correlation with other variables (Figure 3), these systems can significantly contribute to maintaining soil quality and even aiding in the recovery of degraded areas.

The importance of SOC as a key indicator of soil quality has also been previously reported in the literature for different types of agricultural practices, including intensive

agriculture, conservation agriculture, and organic agriculture in different agroecosystems (PANWAR et al. 2022; PONNUSAMY et al. 2024). The role of SOC is known to affect numerous edaphic functions, including microbial biodiversity, enzymatic activities, nutrient cycling and release, structural improvement, water retention, and others (PONNUSAMY et al. 2024). Consequently, in the present study, SOC was significantly and positively correlated with soil nutrients (TN, P, K, Ca), biomass and microbial activity (MBC and C-CO₂), MA, POM, and MAOM.

Regarding PC2 (0-10 cm), which represents 16.94% of the total experimental variance, it was characterized by the increase of ME and MI in the ILF areas with sorghum (S7 and S14), which may indicate the breakdown of macroaggregates, probably related to the management adopted in the sorghum areas, where, unlike the other systems, soil tillage (plowing and harrowing) was carried out annually. This practice of constant soil tillage promotes the breakdown of aggregates, especially macroaggregates (MEDEIROS et al. 2023). These results show that, although sorghum exhibits high biomass production and, consequently, can enhance various properties in agricultural soils (NGIDI et al. 2024), conventional cultivation has clearly limited this potential. This means that the ability of ILF systems to improve soil quality depends on efficient management tactics, such as minimum tillage.

It is important to mention that the cluster analysis, based on the variables used in the PCA, grouped the ILF systems with massai and buffel grass (S7, S14, and S28), sorghum (S28), NV, and SNT in the 0-10 cm layer. In other words, although the ILF Mg system (S28) has proven to be the most promising system in maintaining soil quality, overall, all ILF systems with grasses would have this potential. Furthermore, these are systems that would significantly contribute to various ecosystem services, such as water quality and human health, in mitigating the impacts of climate change (LIMA et al. 2024). These results are consistent with previous research that reported a positive effect of integrated systems with grasses on soil carbon sequestration (TONUCCI et al. 2023), on MBC, on biological activities (SEKARAN et al. 2021), and on soil aggregation (FULTZ et al. 2013) in arid and semi-arid regions.

Omer et al. (2023) stated that perennial grasses not only increase the contribution of root biomass and conserve carbon derived from roots but also favor microbial activities due to the reduced number of soil preparation operations. This effect is enhanced by the fibrous root system of the grasses, which results in a greater exudation of organic compounds. Broring et al. (2023) indicate that such exudation leads to an increase in MBC due to the greater supply of nutrients and energy available for microbial biomass.

Differently, the ILF system with forage cactus was characterized by the decrease of MBC, SOC, TN, POM, and MAOM, mainly at the S14 and S28 spacings, in relation to NV (Figure 3). Moreover, the Fc S7 system showed the highest MTSI index, indicating lower average performance and stability. Some aspects may have contributed to these results, such as the low leaf area index and organic residue deposition by CAM metabolism plants, meaning the soil is more exposed to solar radiation, accelerating the SOM decomposition process, resulting in rapid SOC loss (CAMELO et al. 2021); and the superficiality of the cactus root system, with horizontal distribution in the soil, resulting in lower contributions to soil C at depth (COELHO et al. 2023). However, studies show that different management practices can result in C gains in forage palm systems, such as the one conducted by Camelo et al. (2021), who found gains in soil C and N levels by adopting a higher plant population density and more lenient harvesting.

Another important result refers to the higher SOC stocks in the systems with massai and buffel at the 14 and 28 m spacings. Such a result is probably associated with the fact that, under shade, the photosynthetic processes of grasses are altered and there is a reduction in CO₂ assimilation, reducing the production of stems and leaves (SANTIAGO-HERNÁNDEZ et al. 2016). Consequently, the deposition of aerial and root biomass is lower. What is observed in the study area is that the average total forage biomass in buffel and massai grass was higher in S28 (3900 kg DM ha⁻¹ year⁻¹), compared to S7 (2700 kg DM ha⁻¹ year⁻¹) and S14 (3600 kg DM ha⁻¹ year⁻¹) (SANTOS NETO et al. 2023). According to Patidar et al. (2023), buffel and massai grasses can produce about 10.55 g plant⁻¹ and 23.07 g plant⁻¹ of dry roots per year, respectively. From this perspective, the use of grass in integrated systems can promote SOM storage, mainly due to the increased production and deposition of aerial biomass and the constant rhizodeposition and uniform distribution of root exudates in the soil by these crops (BAPTISTELLA et al. 2021). Regarding the contribution of roots to SOC, in an experiment with pastures on the Loess Plateau of China, Yang et al. (2023) found that rhizodeposition increased SOC by 2.65 g kg⁻¹ per year, while under root decomposition, SOC increased by 0.74 g kg⁻¹ in the first 90 days.

The input of residues above the soil also favors the formation of the more labile fraction (POM) of SOM (PIMENTEL et al. 2024). It is known that the increase in POM plays a crucial role in SOC sequestration, as the more labile fraction contributes to the formation of the more stable fraction of SOM. Studies have shown that soil microorganisms mediate the transformation of C from the POM fraction, stabilizing the long-term pool as SOM (COTRUFO et al. 2019). The results also confirmed that soil TN is closely associated with C cycling

(BIELUCZYK et al. 2020), mainly due to its influence on soil biological activity (ACHARYS et al. 2024), favoring the transformation of labile C into more stable forms (BIELUCZYK et al. 2020).

Finally, another aspect concerns the strong influence of SOC on the biological properties of the soil, as it is the main substrate and energy source for microorganisms (OMER et al. 2023), as demonstrated by the strong correlation between SOC and MBC (Figure 3). However, MBC may not be suitable on its own as an indicator to assess soil biological quality, as it does not provide an estimate of activity, only reflecting the total living mass (SANTOS et al. 2022). In this context, soil basal respiration (C-CO₂) helps differentiate management systems in terms of soil microbial activities. However, MBC may not be suitable on its own as an indicator to assess soil biological quality, as it does not provide an estimate of activity, only reflecting the total living mass (Santos et al. 2022). In this context, soil basal respiration (C-CO₂) helps differentiate management systems in terms of soil microbial activities. In this study, it was observed that the correlation between MBC x C-CO₂ and SOC x C-CO₂, although low, was significant (Figure 3). It was also observed that the highest C-CO₂ releases occurred in ILF systems with grasses (Table 1). However, it is important to emphasize, nonetheless, that a high basal respiration rate may suggest the presence of some stress in the soil microbiota (BRORING et al. 2023).

5.5 CONCLUSIONS

The obtained results show that ILF systems with grasses using strips of native vegetation as the forest component result in positive contributions to soil quality, with a highlight on the systems with massai and buffel grass. In these systems, the losses of SOC, MAOM, NT, and MBC resulting from the conversion of NV into ILF systems were minimized. Furthermore, converting NV into ILF with buffel grass and massai grass resulted in increases in the POM fraction of up to 33%. On the other hand, the forage cactus was the system that differed the most from the other systems, with significant losses of SOC, MAOM, NT, and MBC compared to NV. However, we recommend further research, including long-term experiments, to confirm the persistence of these gains/losses over time. Multivariate analyses demonstrated that ME, SOC, MAOM, NT, K, and MBC were the most sensitive variables to indicate changes in the soil and therefore have greater potential as indicators of soil quality. Furthermore, SOC was the variable most strongly correlated with the other evaluated physical, chemical, and biological soil attributes.

The MTSI analysis revealed that the Mg S28 system and the Bg S28 system were the closest to the ideotype. This was primarily due to the stability of the SOC, MAOM, TN, clay, ME, P, and K variables in the various soil layers, which corroborated the results from the PCA analysis. This result suggests that the use of MTSI is effective in classifying different agricultural systems based on the evaluation of the average performance and stability of multiple soil characteristics in various environments, as hypothesized in this work. In this case, this study provides new insights on how MTSI, as long as the data meet the method's criteria, can be used in other areas, beyond genetic improvement.

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SUPPLEMENTARY MATERIAL

Table S1. Principal component analysis of the correlation matrix with the WAASBY values for 13 oat traits.

	Eigenvalues	Variance	Variance (%)
PC1	5.26	27.700	27.70
PC2	3.81	20.071	47.77
PC3	3	15.809	63.58
PC4	1.96	10.314	73.89
PC5	1.73	9.091	82.99
PC6	1.05	5.531	88.52
PC7	0.76	3.995	92.51
PC8	0.63	3.307	95.82
PC9	0.37	1.955	97.77
PC10	0.17	0.881	98.65
PC11	0.11	0.595	99.25
PC12	0.09	0.505	99.75
PC13	0.04	0.246	100.00
PC14	1.47E-16	0.000	100.00
PC15	1.42E-17	0.000	100.00
PC16	-4.18E-17	0.000	100.00
PC17	-1.11E-16	0.000	100.00
PC18	-2.04E-16	0.000	100.00
PC19	-4.88E-16	0.000	100.00

Source: Author (2025).

Table S2: Scores for the 14 systems and multi-trait stability index (MTSI) estimated in the first six factors. Bold values represent the systems with lower MTSI values.

	FA1	FA2	FA3	FA4	FA5	FA6	MTSI
NV	-1.86	-4.63	2.59	-1.92	2.07	1.10	4.65
SNT	-1.45	-1.88	2.15	-1.11	3.15	0.90	6.11
Bg S7	-2.38	-4.25	1.82	2.11	3.54	1.74	4.29
Bg S14	-3.26	-3.70	1.99	-0.04	1.95	0.67	4.43
Bg S28	-1.02	-3.91	3.29	1.00	2.86	0.24	3.61
Mg S7	-2.37	-4.17	2.83	0.57	1.92	-2.51	4.02
Mg S14	-3.35	-1.79	2.66	1.18	0.67	0.78	6.04
Mg S28	-1.83	-4.81	3.69	0.56	1.58	1.09	3.27
Fc S7	-0.57	-3.33	1.20	0.40	-0.11	0.67	6.33
Fc S14	-0.14	-3.50	1.91	0.91	1.81	0.24	5.13
Fc S28	-0.44	-4.12	2.21	0.93	1.70	0.84	4.61
So S7	-2.41	-5.30	1.73	-0.19	0.98	0.81	4.23
So S14	-2.34	-4.30	-0.47	0.53	2.41	0.04	5.56
So S28	-2.38	-4.38	2.66	0.93	0.86	1.45	4.27

Source: Author (2025).