UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

ALINE ROMA TOMAZ

# POOLS OF SOIL ORGANIC MATTER AND DECOMPOSITION OF PLANT RESIDUES IN A CACTUS-SORGHUM INTERCROPPING SYSTEM WITH MULCHING IRRIGATED WITH RECLAIMED WATER IN THE AGRESTE REGION OF PERNAMBUCO

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Pools of soil organic matter and decomposition of plant residues in a cactussorghum intercropping system with mulching irrigated with reclaimed water in the Agreste region of Pernambuco

> Tese apresentada ao Programa de Pósgraduação em Ciência do Solo da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do título de Doutor em Ciência do Solo.

> Orientador: Prof. Dr. Ademir de Oliveira Ferreira

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Aprovada em 24 de Fevereiro de 2025

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À minha família, em especial à minha mãe, dedico este trabalho.

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### **RESUMO GERAL**

Em condições de clima semiárido os teores de matéria orgânica do solo (MOS) são baixos devido a menor produção de biomassa vegetal, as quais são influenciadas principalmente pelos índices pluviométricos desta região. Logo, a adoção de práticas conservacionistas, como o uso de cobertura morta, diversidade de cultivos e irrigação com água de reuso, podem garantir a sustentabilidade do sistema. Assim, este trabalho teve como objetivo avaliar a dinâmica da MOS em nível de compartimentos em sistemas de consórcio palma (Opuntia stricta) e sorgo (Sorghum sudanense) irrigados com água de reuso de esgoto tratado e com diferentes taxas de cobertura no semiárido, bem como a dinâmica do processo de decomposição da cobertura do solo. O experimento foi conduzido na unidade de reuso de Mutuca, localizado em Pesqueira-PE e consistiu-seem blocos casualizados com 4 repetições, sendo um arranjo fatorial 4x2 em parcelas subdividas com 4 lâminas de irrigação, 0, 80, 100 e 120% da ETc do sorgo e ausência e presença de cobertura morta (8 Mg ha<sup>-1</sup>). Além disso, foram realizadas coletas de solo sob vegetação de Caatinga nativa. As coletas de solo foram realizadas nas profundidades de 0-0,10, 0,10-0,20 e 0,20-0,40 m. Foram determinados os teores de COS e N e dos seguintes reservatórios da MOS: C extraído em água quente (C-AQ), C oxidado em permanganato de potássio (C-OXP), C orgânico particulado (COP), associado aos minerais (COAM) e as substâncias húmicas (SH) (ácidos húmicos (AH), ácidos fúlvicos (AF) e humina (HU)). A decomposição do resíduo vegetal foi avaliada utilizando o método do litter bags por 165 dias. No resíduo remanescente, foram determinados os teores de C e N. Além disso, as coletas de solo em 0-0.10 m e 0.10-0.20 m aos 0, 10, 25, 65 e 165 dias foram realizadas para determinar os teores de NO3<sup>-</sup>, NH4<sup>+</sup> trocáveis, P disponível, C da biomassa microbiana (C-mic) e a respiração basal (C-CO<sub>2</sub>), obtendo-se assim o quociente microbiano (qmic-C) e o quociente metabólico (q-CO<sub>2</sub>). Também foi realizada a Espectroscopia de fluorescência induzida por laser nas amostras de solo e calculado o índice de aromatização (ALIFS) da MOS. Observou-se que a associação entre água de reuso e cobertura morta contribui para o incremento nos estoques de COS e dos diferentes reservatórios da MOS. Os maiores estoques de COS foram observados nos tratamentos com 8 Mg ha<sup>-1</sup> de cobertura morta, associados às lâminas de 80 e 100%. Para os reservatórios, verificou-se o mesmo comportamento, com maiores estoques COP, C-OXP e C-AQ nessas lâminas com cobertura morta. Nas condições deste estudo, os maiores estoques COS estão na fração estável da MOS, especialmente na fração COAM e nas SH, destacandose a fração HU. Em relação a decomposição, os tratamentos irrigados apresentaram as maiores taxas de decomposição e a maior velocidade de decomposição do material vegetal ocorreu após 10 dias sob a lâmina de 80% (16%) e após 25 dias para a lâmina de 100% (16%). Após 165 dias foram decompostos 45, 48, 47 e 49% para as lâminas de 0, 80, 100 e 120%, respectivamente, e o resíduo teve meia-vida de 175 dias. Foram observados maiores teores de NH4<sup>+</sup> e NO3<sup>-</sup> no solo a 0-0,10 m. As lâminas de 80 e 100% apresentaram menores índices de aromatização e maiores teores de COS. Dessa forma, a adoção de água de reuso para irrigação de consórcio de palma e sorgo é uma alternativa de manejo sustentável, que contribui para o incremento dos estoques de COS e dos reservatórios da MOS, para a saúde do solo e desenvolvimento de sua comunidade microbiana, permitindo o desenvolvimento de uma agricultura com baixa emissão de CO<sub>2</sub> sob as condições do clima semiárido Brasileiro.

Palavras-chave: Aromatização. Estoque de carbono. Taxa de decomposição.

# Pools of soil organic matter and decomposition of plant residues in a cactus-sorghum intercropping system with mulching irrigated with reclaimed water in the Agreste region of Pernambuco

#### **GENERAL ABSTRACT**

In semiarid climate conditions, soil organic matter (SOM) contents is low due to the lower plant biomass input, which are influenced by the rainfall indices of this region. The agricultural systems adopted in this region, i.e., intensive soil preparation and monoculture, have caused the decline of soil carbon stocks (SOC). Thus, this study aimed to evaluate the dynamics of SOM and it's pools in intercropping systems of palm (Opuntia stricta) and sorghum (Sorghum sudanense) irrigated with sewage reused water and with different mulch rates in the semiarid region, as well as the dynamics of the soil mulch decomposition process. The experiment was conducted at the Mutuca farm reuse unit, located in Pesqueira-PE and consisted of randomized blocks at 4 replications, in a 4x2 factorial arrangement in split plots with four irrigation levels, 0, 80, 100, and 120% of the ETc of sorghum and two levels of crop residue inputs, absence (0 Mg ha<sup>-1</sup>) and presence of mulch (8 Mg ha<sup>-1</sup>). In addition, soil sampling were carried out in the native Caantiga forest non-disturbed (reference treatment). Soil sampling were performed at 0-0.10, 0.10-0.20, and 0.20-0.40 m depth. The SOC and N contents of the soil and the following MOS reservoirs were determined: C extracted in hot water (HWEO-C), C oxidized in potassium permanganate (POX-C), particulate organic C (POC), associated with minerals (MAOC) and humic substances (HS) (humic acids (HA), fulvic acids (FA) and humin (HU)). The decomposition of the plant residue was evaluated using the litter bag method during 165 days. In the remaining residue, the C and N contents were determined. Additionally, soil samples were collected at 0-0.10 m and 0.10-0.20 m depth and 0, 10, 25, 65, and 165 days were performed to determine the contents of exchangeable  $NO_3^-$ ,  $NH_4^+$ , available P, microbial biomass C (C-mic) and basal respiration (C-CO<sub>2</sub>), thus obtaining the microbial quotient (qmic-C) and the metabolic quotient (q-CO<sub>2</sub>). Laser-induced fluorescence spectroscopy was also performed on the soil samples and the aromatization index (ALIFS) of SOM was evaluated. The association between reused water and mulch contributes to the increase in SOM stocks and the different SOM reservoirs. The highest SOM stocks were observed in the treatments with 8 Mg ha<sup>-1</sup> of mulch, associated with 80% and 100% depth. For the reservoirs, the same behavior was observed, triggering higher POC, POX-C, and HWEO-C stocks in these mulched layers. Under the study conditions, the highest SOC stocks are in the stable fraction of SOM, especially in the MAOC and HS fractions, emphasizing the HU fraction. Regarding decomposition, the irrigated treatments presented the highest decomposition rates, highlighting the plant material decomposition that occurred after 10 days under the 80% irrigation level (16%) and after 25 days for the 100% depth (16%). After 165 days, 45, 48, 47, and 49% were decomposed for the 0, 80, 100, and 120% depths, respectively, and the residue had a half-life of 175 days. Higher NH4<sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents were exhibited in the soil at 0-0.10 m depth. The 80 and 100% irrigation level presented lower aromatization rates and higher SOC contents. Consequently, the adoption of reused water for irrigation of palm and sorghum consortium was a sustainable management alternative, which contributes to the increase of SOC stocks and SOM pools, thereby allowing the soil health and development of the soil microbial community, providing the development of agriculture with low CO<sub>2</sub> emissions under the conditions of the Brazilian semiarid climate.

Keywords: Aromatization. Carbon stock. Decomposition rate.

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# **1. GENERAL INTRODUCTION**

Soil represents the principal carbon (C) reservoirs in terrestrial ecosystems, with relationship with atmosphere and vegetation. This organic matter predominantly originates from plant, animal, and microbial residues at various stages of decomposition. Soil organic carbon (SOC) is distributed across distinct organic matter pools, which are generally categorized as labile and recalcitrant. The labile pool is particularly responsive to management practices, whereas the recalcitrant pool comprises the predominant fraction of soil organic matter (SOM). SOM is critical for maintaining soil quality and health, as it contributes to enhancing of C and N stocks, and promoting the aggregation of primary soil fractions (sand, silt, and clay), ensuring its stability, increasing cation exchange capacity (CEC) and serving as a nutrient source for plants and microorganisms, retaining in addition, the soil moisture to regulate the temperature and nutrients to support plant growth.

In conventional intensive tillage systems under tropical climate, a decline in soil C stocks and a decrease in soil quality have been well-documented, particularly in the semiarid region of Brazil, which is covered by the Caatinga biome. This region is characterized by high temperatures and evapotranspiration rate, coupled with low and unevenly distributed rainfall, as well as limited biomass production from plant species. These conditions lead to a significant loss of soil carbon, which can exceed 50%, causing the soil to function as a source of  $CO_2$  to the atmosphere.

In this sense, adopting techniques that provide higher soil moisture and stimulate crop development and biomass production, such as agricultural water reuse, is essential to guarantee the sustainability of production systems. Furthermore, considering that SOM is a key attribute for maintaining soil quality, conservation practices may be adopted to mitigate soil degradation and greenhouse gas emissions, such as the use of mulch and crop diversification, including intercropping cactus and forage sorghum. The reused water is a viable alternative for irrigation systems in semiarid regions where there is a lack of water availability. Besides being a source of nutrients, and reducing fertilizer acquisition costs, it also provides organic matter, potentially increasing SOM reservoirs, especially in a long-term condition.

The use of mulch reduces soil degradation due to preventing the impacts of water and wind erosion, and the management based on permanent soil mulch effectively increases SOM levels and improves soil physical, chemical, and biological properties. These technologies, combined with intercropping physiologically distinct species, enhance the productivity of regional agricultural systems, contributing to increased C stocks in different SOM reservoirs and mitigating greenhouse gas emissions. Moreover, it is necessary to understand how irrigation with reused water affects the decomposition process of plant residues used as mulch under semiarid conditions, in addition to ensuring that local populations and farmers have access to these tools, which can enhance the productivity of subsistence crops and reduce costs by minimizing the use of expensive synthetic inputs.

In this context, studying SOM dynamics associated with conservation practices in the Agreste region of Pernambuco State, Brazil is essential to understanding how these systems can mitigate the greenhouse gas emissions, thereby increasing SOM levels in different reservoirs. Additionally, assessing the feasibility of these practices for regional farmers is crucial, considering that land abandonment in the Caatinga biome is recurrent due to poor natural resource management, particularly through inadequate conventional practices such as burning, intensive tillage, and monocropping.

# 1.1. Hypotheses

- The adoption of an intercropping system based on forage cactus (*Opuntia stricta*) and sorghum (*Sorghum sudanense*), irrigated with reused water in the Brazilian Semiarid region is expected to increase C contents in both labile and stable SOM pools reach levels compared to native vegetation.
- Reused water increases the decomposition rate of soil mulching, especially at irrigation levels of 80 and 100% of sorghum ETc.
- 3. The association of reused water irrigation with soil mulching has a greater potential to enhance C stocks across different SOM pools than the implementation of these practices individually.
- 4. The high soil moisture at 120% of sorghum ETc would intensify soil organic matter aromatization index, potentially inhibiting microbial biomass and nutrient turnover.

# **1.2. Objectives**

# 1.2.1. General objective

Evaluate the dynamics of SOM at the reservoir level in forage cactus and sorghum intercropping systems irrigated with reused water in the Agreste region of Pernambuco, alongside the temporal decomposition process of mulch.

# 1.2.2. Specific objectives

- 1. Characterize the labile and stable SOM pools in the forage cactus and sorghum intercropping system.
- 2. Evaluate the effect of reused water combined with mulch on SOC stocks.
- 3. Determine the decomposition rate of mulch under semiarid conditions.
- 4. Assess the effect of reused water on SOC stocks in SOM reservoirs.
- 5. Evaluate the influence of reused water on soil microbial activity.
- 6. Assess the effect of irrigation with reused water on the SOM aromatization index.
- 7. Evaluate the contribution of irrigation with reused water to decomposition and nutrient availability in the soil.

# 2. CHAPTER I: LITERATURE REVIEW

#### 2.1. Soil Organic Matter

Soils are the primary reservoir of organic carbon (C) in most terrestrial ecosystems, containing approximately 1550 Pg C globally, which is about twice the amount of C in the atmosphere (760 Pg C) and three times the amount in biomass (560 Pg C) (LAL, 2004; LAL, 2008). The main entry route of C into the soil is soil organic matter (SOM), composed of plant, animal, and microbial derivatives in a protoplasm form or entire alive. It is characterized as a complex and heterogeneous mixture of several organic compounds that are capable to performing multiple functions in different environments (BALDOCK; BROOS, 2012; ESCALONA; PETROV; OOSTENBRINK, 2021; MASOOM et al., 2016).

SOM is the primary source of C in the soil, and its reservoirs can be separated into labile and stable forms, in which are influenced by soil management practices. Conventional systems based on intensive tillage led to decreased plant productivity and can result in a rapid decline in SOM levels. Conversely, increased crop residue inputs or the addition of external sources can contribute to an increase in SOM levels (HAYNES SWIFT; STEPHEN, 1991).

As a key attribute of the C cycle, SOM is the primary energy source for microorganisms involved in its cycling through various metabolic processes, such as enzyme production for decomposition, and production of organic acids, among others (GUHRA; STOLZE; TOTSCHE, 2022; ZHAO et al., 2021). The molecules that compose SOM in the soil are partially used for energy acquisition, catabolism, and for building microbial biomass, an anabolic process (KÄSTNER; MILTNER, 2018; LIANG; SCHIMEL; JASTROW, 2017).

Recent studies exhibit that the contribution of dead microbial biomass (i.e., microbial necromass) to C storage is highly significant (LORENZ et al., 2021; WANG et al., 2021) and varies according to land use. In agricultural soils under temperate climates, the contribution of necromass from the biota accounts for over 50% of the total organic carbon. In pastures, this contribution is 61.8%. (LIANG et al., 2019). In this context, SOM performs several functions in the soil, being directly related to nutrient cycling and the availability of these nutrients for plant nutrition, aggregate formation in the soil, improving its physical quality (GUHRA et al., 2022), water retention, aeration, temperature regulation, thereby benefiting the microbial community by improving its habitat. When combined with conservation agricultural practices, the SOM, when well-managed reduce CO<sub>2</sub> emissions, directly contributing to global warming mitigation (BONGIORNO et al., 2019).

# 2.2. Soil Organic Matter Reservoirs

The SOM pools in the soil can be separated into labile or active and stable forms. The labile C is the pool that is directly available for microbial activity and is considered the primary energy source for then (BONGIORNO et al., 2019). The stable pool, on the other hand, includes humic substances derived from the humification of plant, animal, and microbial residues, comprising humic acids (HA), fulvic acids (FA), and humins (H) (ESCALONA; PETROV; OOSTENBRINK, 2021; KÖGEL-KNABNER, 2002).

According to Bongiorno et al. (2019), labile C holds significant potential as an indicator of soil functions, particularly in relation to nutrient cycling. This is determined by factors such as soil nutrient levels and C mineralization, soil aggregation as assessed by stable water aggregates, C sequestration, which is observed through variations in SOC, and the provision of habitat for biodiversity, evaluated using biological indicators like microbial biomass and the abundance of faunal groups. The labile fractions of SOM can be distinguished based on the methodology of extraction, through chemical, physical, or biological fractionation. Chemical fractionation can be used to obtain dissolved organic C (DOC), which is C extracted in water that passes through a 0.45  $\mu$ m mesh filter, hydrophilic DOC, the most bio-disposable portion of DOC, with these two fractions making up a small part of SOM (ELCOSSY et al., 2020; INAGAKI et al., 2021).

In addition, labile C can also be extracted in hot water, a fraction that is present in higher concentrations in soil than DOC, and oxidized with permanganate, being termed the active fraction (ELCOSSY et al., 2020; INAGAKI et al., 2021). About 45% to 60% of hot water-soluble C includes carbohydrates and amides derived from soil microorganisms, enzymes, exudates, and root lysates, while permanganate-oxidized C also contains compounds such as lignin and complex polysaccharides (GHANI; DEXTER; PERROTT, 2003; HAYNES and BEARE, 1997) and has been suggested as an indicator of soil quality. The hot water-soluble fraction is primarily found in the soil solution or weakly bound to minerals and is sensitive to seasonal variations (BONGIORNO et al., 2019).

Regarding physical fractionation, particulate organic carbon (POC) (CAMBARDELLA; ELLIOTT, 1992) is mainly characterized by partially decomposed organic residues, along with microbial biomass and fresh residues. This pool is highly sensitive and serves as an indicator of short-term changes in SOC stock (ROCCI et al., 2021). Also, there is C associated with minerals through chemical bonds between SOM and mineral surfaces,

occlusion in micropores, which protects this fraction from microbial decomposition (LAVALLEE; SOONG; COTRUFO, 2020). For the biological pool, microbial biomass C is determined by irradiating and measuring the CO<sub>2</sub> produced constantly from microbial respiration.

For the stable SOM pool, extraction is performed using an alkaline solution by solvating organic compounds through the ionization of carboxyl and phenolic groups. When the solution is reacidified, HA precipitates, and the supernatant corresponds to FA (ESCALONA; PETROV; OOSTENBRINK, 2021). The stability of these fractions has been attributed to the presence of cationic bridges through external and internal sphere complexes (GALICIA-ANDRÉS et al., 2021), along with physical protection in soil aggregates. The material that does not dissolve is the insoluble fraction known as humin (H).

Due to their recalcitrance, humic substances provide a continuous energy source for specific microorganisms involved in their decomposition, such as certain groups of fungi and actinomycetes. Furthermore, they contribute to increasing the soil's cation exchange capacity (CEC), water retention, soil aggregation, heavy metal complexation, and other functions (WEI et al., 2020). Raiesi (2021) observed changes in the recalcitrant fraction of SOM in long-term rainfed wheat cultivation (i.e., more than 50 years), with a 29% reduction in non-labile fractions, 40% in FA fractions, and 43% in H. The HA fraction remained unchanged over time, thereby indicating that the adopted management practices influence the quantity and quality of specific SOM pools.

#### 2.3. Land Use Changes and Carbon Stocks

Considering the global climate change and growing CO<sub>2</sub> emission rates into the atmosphere, proposals have been investigated to promote land-use changes through the adoption of conservation practices that enhance the synthesis and accumulation of SOM. This allows for a positive soil C balance, as the soil functions as a sink, sequestering C in different SOM pools (BHATTACHARYYA et al., 2022; LAL, 2004). This occurs because SOM stores more C than vegetation and the atmosphere combined (LAL, 2004; LEHMANN; KLEBER, 2015; OSANAI et al., 2015). According to Deb and Mandal (2021), factors such as low microbial oxidation, high clay content, and soil aggregation contribute to C sequestration.

From an agricultural perspective, soil becomes a source of  $CO_2$  to the atmosphere when losses due to oxidation exceed C immobilization through plant residues (SÁ et al., 2008; SÁ; LAL, 2009). When soil cultivation replaces native ecosystems, the temporal dynamic equilibrium (dC/dt = 0) is disrupted, leading to a reduction in SOM levels (CERRI et al., 2008).

These SOM losses vary depending on soil tillage, cropping systems, and climatic conditions (BAYER et al., 2011; SÁ et al., 2008). The use of plowing and harrowing for soil tillage is the primary factor responsible for these losses (BRUCE et al., 1999; SÁ et al., 2008).

The mechanisms involved in SOM loss include: a) The breakdown of soil aggregates exposes SOM to microbial decomposition; b) The mixing of fresh organic material with soil creates more favorable conditions for decomposition; c) Increased microbial activity, due to greater soil aeration and an increased supply of labile organic matter, results in higher C mineralization (REICOSKY, 1995). The severity of these losses is greater in tropical climates (LAL; LOGAN, 1995). On the other hand, soil acts as a CO<sub>2</sub> sink when the following practices are adopted: a) Minimum mechanical soil disturbance; b) Intensification of crop rotation systems; c) Adoption of practices that enhance crop productivity; d) Establishment of permanent vegetation cover.

Sá et al. (2015) reported that, in tropical regions, a minimum biomass C input of 4.15 Mg C ha<sup>-1</sup> year<sup>-1</sup> (9.46 Mg ha<sup>-1</sup> year<sup>-1</sup> of plant biomass) is required to maintain a dynamic equilibrium level. However, it is necessary to exceed this minimum amount of plant residue to achieve a positive C balance, ensuring greater system stability. De Oliveira Ferreira et al. (2021) found that at least 50–75% more crop residues are required, equivalent to 14.2 to 16.55 Mg ha<sup>-1</sup> year<sup>-1</sup> of plant biomass covering the soil. Notably, it would be possible to achieve the benefits of Conservation Agriculture, including high plant residue accumulation, C stock increases, soil density stabilization, enhanced faunal and microbial biomass C, and improved soil water retention (DE OLIVEIRA FERREIRA et al., 2021; SÁ, 2004).

Under humid tropical climate conditions in Lucas do Rio Verde, Mato Grosso, Sá et al. (2022) determined an annual C input of 8.4 Mg ha<sup>-1</sup> in no-tillage systems (NTS), with a sequestration rate of 2.15 Mg ha<sup>-1</sup> of C (at 0-0.10 m). In these conditions, comparing native vegetation with a SOC stock of 48.0 Mg ha<sup>-1</sup> in the 0–0.20 m layer to NTS with crop rotation, it was observed that in a system with maize (*Zea mays* L.) and *Brachiaria ruziziensis* cv. Ruzis, the SOC was 44.2 Mg ha<sup>-1</sup>. In rotation systems predominated by soybean, sorghum (*Sorghum bicolor* (L.)), *Brachiaria ruziziensis* cv. Ruzis, and maize, the SOC stock was 43.3 Mg ha<sup>-1</sup>. About pearl millet (*Pennisetum typhoides* Burm.), maize, and *Brachiaria ruziziensis* cv. Ruzis, the SOC was 40.7 Mg ha<sup>-1</sup>. These findings indicate that adopting no-till systems with soil mulch and diverse grass-based crops can restore SOC sotcks (SÁ et al., 2015).

The intercropping of forage cactus and forage sorghum can increase biomass production and consequently C inputs to the soil by improving the efficiency of nutrient, water, and solar radiation use (CHIMONYO; MODI; MABHAUDHI, 2018; DINIZ et al., 2017; LIMA et al., 2018), in addition to their production synergy. Although both are forage species, sorghum and forage cactus have different photosynthetic metabolisms. Sorghum, a C4 plant with daytime C assimilation, has high biomass production potential, strong economic returns, drought tolerance, and water-use efficiency compared to other C4 crops. It is also well-adapted to arid and semiarid environments (ROBY et al., 2017). Forage cactus, on the other hand, follows the Crassulacean Acid Metabolism (CAM) pathway, with stomatal closure during the day and nighttime C assimilation. It has a greater adaptive capacity to abiotic factors and, like sorghum, responds positively to irrigation (DINIZ et al., 2017).

# 2.4. Stabilization of organic matter through the humification process

The humification process of SOM consists of transforming labile compounds into more stable components in the soil, with a high degree of aromatization. This process occurs through the chemical stabilization of SOM, which can take place via the incorporation or preservation of compounds resistant to chemical degradation, such as lignin, phenolic and aromatic compounds, and aliphatic components (BAYER et al., 2002; SENESI et al., 2016).

The humification process can be determined in the soil using spectroscopic techniques, such as laser-induced fluorescence spectroscopy (LIFS), and by assessing the humification index. In laser-induced fluorescence spectroscopy, LIFS emission spectra measure C in more complex or rigid structures, such as aromatic groups and quinones, in intact (chemically unprocessed) soil samples. When fluorescence is excited at near-ultraviolet or blue wavelengths, it provides information on more humified structures (GONZALEZ-PEREZ et al., 2006; MARTINS et al., 2011).

Specific spectral regions, such as wavelengths between 525-550 nm, suggest the presence of molecular components possibly characterized by a resistant polycondensation of the aromatic structure with a high degree of conjugation and its ability to carry substituents such as carbonyl and carboxyl groups. The presence of aromatic compounds in the samples indicates an advanced SOM humification index (MARTINS et al., 2011; MILORI et al., 2006; TADINI et al., 2021; TIVET et al., 2013). In contrast, the presence of labile compounds results in lower humification indices, either due to the accumulation of plant residues in the soil, which dilutes humified SOM and physically protects labile SOM in aggregates, or due to conditions that do not favor intense microbial decomposition processes (MARTINS et al., 2011; SENESI et al., 2016).

Furthermore, management practices alter the chemical composition of SOM, including changes in the degree of humification (GONZÁLEZ-PEREZ et al., 2006; TIVET et al., 2013).

In intensive tillage systems, SOM exhibits higher degree of humification, particularly in the surface layer. In natural environments, the degree of humification is lower due to a higher concentration of light SOM or SOM in the early stages of decomposition (MARTINS et al., 2011; MILORI et al., 2006). In conventional systems, changes in total C levels generally occur in fractions composed of polysaccharides, water-soluble C, and, to a lesser extent, in stable mineral-associated fractions (TIVET et al., 2013). In these systems, the relatively higher degree of aromatization suggests that physical protection through aggregation is insufficient to preserve the more labile SOM fraction in the soil (TIVET et al., 2013). Higher fluorescence intensity is associated with higher SOM humification indices and a more significant presence of aromatic substances (MARTINS et al., 2011; TADINI et al., 2021). Therefore, the humification index is an efficient indicator of the chemical recalcitrance and resistance of SOM (SENESI et al., 2018).

# 2.5. Decomposition Rate of Plant Residues

The assessment of the decomposition rate of plant residues is crucial for understanding the impact of agricultural activities on global climate change. In general, the decomposition of residues begins with easily transformable components such as sugars and proteins, and finally the more recalcitrant ones such as cellulose, lignin, and fats (WIEDER; LANG, 1982). It is necessary to consider that decomposition depends on the microbial community and the production of enzymes that act on the transformation of residues. In addition, climatic conditions and management systems directly interfere with the speed of residue decomposition (BRADFORD et al., 2016).

Another predominant factor for residue decomposition is its C/N ratio. The higher the C/N ratio, the slower the decomposition rate, whereas the lower the C/N ratio, the faster the decomposition by microorganisms (FLOSS, 2000). Poaceae, in general, have a high C/N ratio (>25:1), which means their residence time in the soil tends to be longer than that of Fabaceae, for example, which have a lower C/N ratio (<20:1), favoring mineralization (CERETTA et al., 2002). Higher decomposition rates are related to higher initial levels of elements in plants, such as N, while an increase in the proportion of lignin, cellulose, and hemicellulose can reduce microbial attack (LINDSEY et al., 2013).

Canalli et al. (2020) observed that the consortium of Poaceae + Fabaceae showed higher decomposition rates, with values between 0.40 and 0.49% per day. In contrast, the single cultivation of Canola (*Brassica napus*) had a decomposition rate of 0.38% per day, and the

consortium of black oat (*Avena strigosa*) + ryegrass (*Lolium multiflorum*) had a rate of 0.37% per day.

Since climatic factors influence decomposition, it is expected that different biomes will have distinct decomposition rates of SOM. For the Cerrado, Ribeiro et al. (2022), in a croplivestock-forest integration system with eucalyptus, observed that the half-life for labile compounds was ten days, while for recalcitrant compounds, it was 1386 days. In the Caatinga biome under forest management with selective cutting by species, the half-life of litter was 182 days compared to unmanaged Caatinga and management with superficial cutting and selective cutting by diameter (MATOS et al., 2022). Decomposition in the Brazilian Semiarid region is highly affected by climatological variables, especially rainfall (DOS SANTOS LIRA et al., 2020).

Regarding agricultural systems in the Brazilian Semiarid region, data on this topic are scarce, and further assessments are needed to determine how crops and climatic conditions reflect on the dynamics of SOM and its contribution to global climate change, both under rainfed conditions and with the use of irrigation.

### 2.6. Dynamics of SOM in Systems Irrigated with Reused Water

According to the National Water and Sanitation Agency (ANA), Brazil has 8.2 million hectares (Mha) of irrigated land, with 35.5% (2.9 Mha) using fertigation with reused water and 64.5% (5.3 Mha) irrigated with water from natural sources. Projections indicate the addition of 4.2 Mha by 2040, with a 66% increase in water demand. In this context, ANA is regulating sanitation through Federal Law No. 14.026/2020 and discussing the effective use of sanitary effluent in agriculture to reduce the demand for water from natural sources, mitigate contamination issues in receiving bodies, and strengthen agribusiness (ANA, 2021).

The use of reused water is an alternative for irrigation, particularly in regions where water scarcity causes productivity losses and directly affects the population, such as in the Brazilian Semiarid region. Reused water can be defined as the reuse of water, utilized one or more times, to meet human needs and other uses (LAVRADOR FILHO, 1987). The composition of reused water depends on its source, but in general, it consists of 99.9% water and 0.1% solids, of which about 75% corresponds to organic matter.

Notably, the use of reusing sewage-treated water in agriculture brings several benefits, such as providing nutrients for crops, reducing costs on the property, protecting aquatic ecosystems, and increasing SOM levels. Regarding the organic matter in reused water, it is important to consider that it has a low C/N ratio and, consequently, rapid mineralization,

especially under conditions of high moisture and aeration, which favor soil microbial activity. In this context, it is important to understand how the application of reused water through irrigation influences SOM pools. For example, in a soil cultivated with alfalfa irrigated with reused water for 20 years, Adrover, Moyà, and Vadell (2017) reported that water-soluble C varied depending on the soil type, with higher levels in clayey soil with greater aggregate stability.

In a no-till system with soybean/wheat (*Glycine max* (L.)/*Triticum aestivum*), corn/oat+ common vetch (*Zea mays* L./*Avena sativa* + *Vicia sativa*), and soybean/radish (*Raphanus sativus*) rotations during the spring-summer/autumn-winter seasons, respectively, cultivated for 11 years in clayey soil irrigated with swine wastewater, an increase in microbial biomass was observed in the 0-0.10 m layer (MATOS et al., 2018).

For a clayey Oxisol located in the state of São Paulo, Brazil cultivated with *Urochloa brizantha* cv. and irrigated with reused water for four years, total C contents were not altered over time, nor was POC, despite higher levels in the 0-0.10 m layer. This occurred due to the high mineralization rate and the low C/N ratio of the effluent (COELHO et al., 2020). Besides, Elcossy et al. (2020) reported that hot-water-soluble C, water-soluble C, and biomass C in the 0-0.10 m layer progressively increased with longer land use (greater increments between 20 and 30 years) in soils irrigated with raw effluent in El Gabal El Asfar, Egypt, under vegetable crop cultivation.

It is possible that the use of reused water for irrigation can increase SOC stocks levels and alter SOM pools in long-term cropping systems even under the extreme climate conditions as presented in the Semiarid of the Brazilian northeaster. An increase of 17% to 30% in SOC stocks has observed in soil cultivated with date palms for over 13 years in arid and semiarid regions of Saudi Arabia (AL OMRAN et al., 2012). However, short-term use has not significantly affected SOM pools, necessitating the adoption of other management practices to enhance SOM.

#### 2.7. Use of Mulch in the Intercropping of Palm and Sorghum in the Caatinga Biome

The Caatinga is a Brazilian biome, covering a territorial area of 734,000 km<sup>2</sup> (SILVA et al., 2004). This biome comprises the group of tropical dry forests and constitutes one of the largest semiarid areas in the world, predominantly located in the Northeast region of Brazil. In the entire region exist a large diversity of species and its vegetation is composed by deciduous plants, annual herbs, succulents, and a predominance of shrubs and small trees (RODAL; SAMPAIO, 2002).

The Caatinga biome had high temperatures, with annual averages between  $25^{\circ}$ C and  $30^{\circ}$ C, and annual precipitation ranging from 400 to 800 mm, reaching up to 1000 mm in border zones, with irregular seasonality and intense rainfall. Conversely, potential evapotranspiration ranges from 1500 to 2000 mm annually, resulting in water deficiency and characterizing the biome as semiarid (precipitation/evapotranspiration ratio < 0.5) (GARIGLIO, 2010).

Due to its climatic and vegetation characteristics, the Caatinga biome has one of the lowest SOC stocks (4.8 PgC) compared to other biomes, such as the Atlantic Forest (11.49 PgC), the Cerrado (17.07 PgC), and the Amazon Rainforest (36.10 PgC) (GOMES et al., 2019). This fact, combined with traditional farming practices, has contributed to a decline in carbon, with reductions of over 50% in the SOC stock in this biome and a consequent loss of soil quality (MENEZES et al., 2021). In this context, the adoption of mulching as an agricultural practice has been studied as a measure to mitigate C losses in these systems.

Additionally, intercropping systems has been explored to ensure sustainability under semiarid conditions, such as the use of forage sorghum and forage palm, primarily for animal feed. Mulching helps control water erosion in SemiArid region (LIMA et al., 2020), preserves soil moisture, serves as an energy source for microorganisms, promotes soil aggregation, enhances nutrient availability, stabilizes soil temperature, and controls runoff and surface sealing, among other benefits, including the increase of SOC stock (SILVA et al., 2019).

Jardim et al. (2021) reported that the intercropping of palm + sorghum in the Semiarid region was 47% and 3.5 times more productive in fresh and dry matter input, respectively, compared to palm monoculture. Regarding the influence of this intercropping system on SOM pools, further investigations are needed, both for the labile and recalcitrant pools. In this context, Tavanti et al. (2020) observed that management practices associated with sorghum (*Sorghum bicolor* [L.] Moench) intercropped with Marandu grass (*U. brizantha Marandu*) in Southern Brazil increased the stable SOM pool, associated with minerals, and consequently reduced  $CO_2$  emissions from the soil.

According to Maia et al. (2019), the use of intercropping systems in the Semiarid region has a high potential to increase biomass input. When combined with minimum tillage, this system can maintain the SOC stock and may even increase them in some SOM pools, such as biomass C and humic substances. These systems represent a sustainable alternative for the Semiarid region, which traditionally adopts conventional monoculture systems.

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#### 3. CAPÍTULO II: REUTILIZAÇÃO DE ÁGUA DE ESGOTO: UMA PRÁTICA SUSTENTÁVEL PARA MELHORAR A MATÉRIA ORGÂNICA DO SOLO NO AMBIENTE TROPICAL SEMIÁRIDO

#### Resumo

A decomposição da matéria orgânica do solo (MOS) em ambiente tropical semiárido é pouco compreendida às condições climáticas específicas deste ambiente. O objetivo deste trabalho foi avaliar a taxa de decomposição de resíduos vegetais, a atividade e biomassa microbiana e o índice de aromatização da matéria orgânica do solo sob cultivo consorciado de palma forrageira e sorgo, com cobertura morta e irrigado com água de esgoto doméstico tratado. O experimento consistiu em blocos casualizados com quatro repetições, sendo os tratamentos quatro lâminas de irrigação de 0, 80, 100 e 120% da evapotranspiração da cultura (ETc) do sorgo. A decomposição dos resíduos vegetais foi avaliada usando o método de "litter bag" por 165 dias. Os teores de C e N do resíduo remanescente também foram determinados. Foram coletadas amostras de solo em 0-0,10 e 0,10-0,20 m de profundidade em 0, 10, 25, 65 e 165 dias para determinar os conteúdos de NO3<sup>-</sup> trocável, NH4<sup>+</sup>, o conteúdo de fósforo disponível (P), a biomassa microbiana (C-mic) e emissões de C-CO<sub>2</sub> (respiração basal do solo), obtendo assim o quociente microbiano (qmic-C) e o quociente metabólico (qCO<sub>2</sub>). Além disso, foi realizada a Espectroscopia de Fluorescência Induzida por Laser (LIFS) nas amostras de solo e o índice de aromatização (ALIFS) da MOS foi avaliado. A maior taxa de decomposição ocorreu no solo irrigados após 10 dias com a lâmina de 80% (16%) e após 25 dias com lâmina de 100% (16%). Após 165 dias, as taxas de decomposição foram de 45, 48, 47 e 49% sob lâminas de 0, 80, 100 e 120%, respectivamente. A meia-vida do resíduo foi de 175 dias. O solo apresentou os maiores teores de NH4<sup>+</sup> e NO3<sup>-</sup> na profundidade de 0-0.10 m; no entanto, houve uma diminuição de nitrato em 65 dias, indicando perturbações na atividade microbiana devido ao excesso de água. As lâminas de 80 e 100% exibiram menores índices de aromatização, maiores quantidades de carbono orgânico do solo (COS) e P disponível. Os espectros de fluorescência apresentaram maior intensidade em 525-550 nm, indicando a presença de compostos mais recalcitrantes. Além disso, a irrigação com lâminas de 80 e 100% de água aumentou a decomposição do resíduo vegetal, e apresentou menores valores de ALIFS, indicando a manutenção dos teores de C lábil e recalcitrante mais próximos daqueles encontrados em solos sob vegetação nativa. Por outro lado, o excesso de umidade estimulou a decomposição de compostos lábeis e, consequentemente, a aromatização da MOS. Portanto, a reutilização de água de esgoto doméstico para irrigação de consórcios de palma forrageira e sorgo foi uma alternativa para o manejo agrícola sustentável, particularmente em regiões que enfrentam escassez hídrica.

**Palavras-chave**: Taxa de decomposição. Meia-vida do resíduo. Estresse abiótico. Espectroscopia de fluorescência.

# **3. CHAPTER II: REUSING SEWAGE WATER: A SUSTAINABLE PRACTICE TO IMPROVE SOIL ORGANIC MATTER IN THE SEMIARID TROPICAL ENVIRONMENT\***

#### Abstract

The decomposition of soil organic matter (SOM) in a semiarid tropical environment is poorly understood due to limited water resources. We aimed to assess the rate of plant residue decomposition, microbial activity, biomass, and the aromatization index of organic matter in soils under intercropped forage cactus and sorghum, covered with grasses and irrigated with sewage-treated water. The treatments consisted of randomized blocks with four replicates. The soil received sewage reuse water under irrigation depth of 0, 80, 100, and 120 % of sorghum's crop evapotranspiration (ETc). The plant residue decomposition was evaluated using the litter bag method for 165 days. The C and N contents of the remaining residue were also determined. Soils at 0-0.10 and 0.10-0.20 m depth were sampled in 0, 10, 25, 65, and 165 days to determine the contents of exchangeable NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, available phosphorus (P) content, microbial biomass (C-mic) and C-CO<sub>2</sub> emissions from basal soil respiration, thereby obtaining the microbial quotient (qmic-C) and the metabolic quotient (qCO<sub>2</sub>). Furthermore, Laser-induced Fluorescence Spectroscopy (LIFS) was performed on the soil samples, and SOM's aromatization index (ALIFS) was evaluated. The highest decomposition rate occurred in irrigated soils after 10 days with 80% water depth (16%) and after 25 days with 100% water depth (16%). After 165 days, the decomposition rates were 45, 48, 47, and 49% under 0, 80, 100 and 120% water depth, respectively. The half-life of the residues was 175 days. The soil had the highest contents of NH4<sup>+</sup> and NO<sub>3</sub><sup>-</sup> at 0-0.10 m depth; however, there was a decrease in nitrate at 65 days, indicating disruptions in microbial activity due to excess water. The 80% and 100% water depth exhibited lower aromatization indexes, higher amounts of soil organic carbon (SOC), and available P. The fluorescence spectra had greater intensity at 525-550 nm, indicating the presence of more recalcitrant compounds. Additionally, the irrigation at 80 and 100% of water depth enhanced the decomposition of the plant residue, driving the lower ALIFS and maintaining the labile and recalcitrant C contents closer to those found in soils under native vegetation. Conversely, excess moisture stimulated the decomposition of labile compounds and, consequently, the aromatization of SOM. Consequently, reusing water to irrigate forage cactus and sorghum consortium can be an alternative for sustainable agricultural management, particularly in tropical regions facing water scarcity.

Keywords: Decomposition rate. Residue half-life. Abiotic stress. Fluorescence spectroscopy.

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Acronyms: Soil Organic Matter (SOM), Crop evapotranspiration (ETc), Carbon (C), Nitrogen (N), Phosphorus (P), Microbial biomass (C-mic), Basal soil respiration (C-CO<sub>2</sub>), Microbial quotient (qmic-C), Metabolic quotient (qCO<sub>2</sub>), Laser-induced Fluorescence Spectroscopy (LIFS), Aromatization index (A<sub>LIFS</sub>), Soil organic carbon (SOC).

#### **3.1. Introduction**

The semiarid region covers 12% of the national territory and is dominated by the Caatinga biome, exclusive to Brazil. This area experiences intensive use of its vegetation for agriculture and human subsistence, as well as extreme climatic conditions such as high temperatures, irregular rainfall, and shallow soils with low soil carbon stocks (SOC) and biomass production, thereby jeopardizing the soil fertility (ALTHOFF et al., 2018; GOMES et al., 2019; NAOREM et al., 2023). To mitigate the negative impacts of soil degradation, the adoption of plant residues as ground mulch associated with irrigation to enhance microbial activity and turnover of nutrients has been used (GIONGO et al., 2020; DANTAS et al., 2023). However, there is a notable gap in research concerning the decomposition dynamics of mulch in semiarid tropical soils due to limited water resources. Remarkably, the use of treated domestic sewage water can be an effective practice to improve the decomposition of organic matter and boost soil carbon (C) sequestration in intercropped systems, moderately recovering floristic potential and agricultural productivity (LI et al., 2022; MISHA et al., 2023).

The decomposition rate of plant residues is responsive to extreme climatic factors that act in northeastern Brazil and the presence of phenolic compounds that determine the C: N ratio of the residual plant material used as mulch in the soil (DE CARVALHO et al., 2022; DANTAS et al., 2023; PEREIRA et al., 2023). Residues with a high C/N ratio (>25:1) have a slower decomposition speed and longer persistent time in the soil due to nitrogen (N) contents that are lower than those required by microorganisms, which immobilize this element in their biomass (LI et al., 2022; THAPA et al., 2022; WANG et al., 2023). Additionally, the use of sewage water as irrigation in soils under arid and semiarid climates can trigger the decomposition of soil organic matter (SOM) by providing labile compounds and ensuring the stability and protection of physical occlusions of aggregates that avoid excessive decomposition of SOM (LUO et al., 2021; DE ALMEIDA et al., 2022; LI et al., 2022; THAPA et al., 2022). However, it is necessary to adapt the quality of domestic sewage water through treatment methods to prevent the accumulation of metals, pathogenicity, and excess eutrophic nutrients (COELHO et al., 2020; DE LEMOS et al., 2021), since it is a sustainable method of soil management meeting the UN Sustainable Goals 2 (zero hunger and sustainable agriculture), 12 (responsible consumption and production) and 13 (action against global climate change) (UN, 2015; ELCOSSY et al., 2020).

Aromatization of SOM is prone to be higher in tropical climate conditions due to high temperatures and humidity and can be enhanced with the use of wastewater (SENESI et al., 2018). In intensive tillage systems, the SOM aromatization index (A<sub>LIFS</sub>), previously called humification index, exceeds that of undisturbed environments due to higher contents of particulate C or in the initial stages of decomposition of native vegetation (TIVET et al., 2013; TADINI et al., 2018, 2021); however, nothing has been reported in the Caatinga biome, where soils are more susceptible to erosion processes (ALTHOFF et al., 2018; MENEZES et al., 2021; TOMAZ et al., 2024). Yet, it is possible that the excess moisture caused by heavy rainfall or excessive irrigation can induce oxidative stress in the soil microorganisms, reducing its biomass and the production of its extracellular enzymes (ZHANG et al., 2019; GAO et al., 2021; HONG et al., 2021; FURARK; WOLINSKA, 2023).

In this sense, we postulated that supplying moisture with reused water through irrigation at 80 and 100% of water depth would increase the decomposition rate of plant residues. However, excessive humidity at 120% would intensify aromatization, potentially inhibiting microorganismal biomass and nutrient turnover. Thus, we aimed to evaluate the decomposition rate of plant residues used as soil mulch, the C and N contents of the remaining residue, the contents of exchangeable NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and available phosphorus (P), and to estimate the C of microbial biomass and C-CO<sub>2</sub> emission by soil microbial community activity, thus obtaining the microbial and metabolic quotients. Additionally, we determined the degree of aromatization of SOM in a forage cactus and sorghum intercropping system irrigated with treated domestic sewage water under tropical semiarid climate conditions.

#### 3.2. Material and Methods

#### 3.2.1. Study area

The study was conducted at the Mutuca Hydroagricultural Reuse Unit, in the municipality of Pesqueira, located in the Agreste Mesoregion and Ipojuca Valley Microregion of Pernambuco State, Brazil, at 8°16'50.94"S and 36°34'17.63" W and altitude of 654 meters. The region is inserted in the Borborema Plateau, which has high massifs and hills, with rough relief, deep and narrow dissected valleys, and the vegetation is composed of deciduous forests (CPRM, 2005). The climate is hot semiarid, according to the Köppen classification, with an average temperature of 26°C, relative humidity of 73%, and average annual precipitation of 670 mm. The highest rainfall rates were recorded between May and August. The soil of the experimental region is Haplic Planosol Hypereutrophic (DOS SANTOS et al., 2018).

Soil samples were characterized in 0-0.10 and 0.10-0.20 m respectively as pH: 7.4 and 7.9; electrical conductivity (EC): 1.0 and 1.4 ds m<sup>-1</sup>; Ca<sup>2+</sup>: 5.0 and 4.7 cmolc dm<sup>-3</sup>; Mg<sup>2+</sup>: 1.2

and 1.5 cmolc dm<sup>-3</sup>; K<sup>+</sup>: 54.4 and 58.7 mg kg<sup>-1</sup>; Na<sup>+</sup>: 41.5 and 45.5 mg kg<sup>-1</sup>; Available phosphorus (P): 33 and 26 mg kg<sup>-1</sup>; Total N (TN): 0.9 and 0.7 g kg<sup>-1</sup>; Soil organic C (SOC): 9.5 and 8.9 g kg<sup>-1</sup>; Microbial biomass (C-mic): 70.9 and 54.4 mg kg<sup>-1</sup>; metabolic quotient (qCO<sub>2</sub>): 52.7 and 45.0 mg CO<sub>2</sub> kg soil day<sup>-1</sup>; Sand, silt, and clay: 734, 119, 147 g kg<sup>-1</sup> and 724, 107 and 169 g kg<sup>-1</sup> respectively; soil and particle density: 1.3 and 2.6 g cm<sup>-3</sup> and 1.5 and 2.6 g cm<sup>-3</sup>



Figure 1. Rainfall in the years 2021, 2022 and 2023 in the municipality of Pesqueira-Pe

#### 3.2.2. Experimental design and timeline of experiment

The experimental design was in randomized blocks with four replicates and irrigation with water depth (0, 80, 100, and 120%) determined from the crop evapotranspiration (ETc) of sorghum according to Carvalho et al. (2021) and mulch at a density of 8 Mg ha<sup>-1</sup> intercropped with forage cactus and sorghum. Each block consisted of 4 plots formed by four simple rows of both crops measuring 3 m long and 5 m wide, thereby constituting 15 m<sup>2</sup>. The forage cactus was planted in simple rows with spacing of 1.0 m between rows and 0.2 m between plants, while the sorghum was sown 0.5 m away. The plant mulch was composed of different species of spontaneous grasses from the Caatinga biome identified as current grass (*Urochloa mosambicensis*), carrapicho grass (*Cenchrus echinatus*), chicken foot (*Eleusine in*dica) and Chichá (*Sterculia striata*).

This experiment lasted eighteen months and the evaluation of residue decomposition began after the third addition of mulch at the end of August 2022 (Figure 2).



Figure 2. Timeline of experiment

#### 3.2.3. Irrigation system management

The irrigation system is based on dripping and the management of the daily evaporation of the Class A Tank. To determine the ETc, the sorghum coefficients (Kc) were used, considering its phenological phases: sowing and establishment – phase I (0.4); vegetative growth – phase II (1.1); flowering and production formation – phase III (1.0); and maturation – phase IV (0.7) (COSTA et al., 2017). The reference evapotranspiration (ETo) was determined from evaporation measurements of the Class —A Tank (EV) and using the tank coefficient (Kp). The drip system was installed in the sorghum sowing line. The applied water depth was based on the sorghum evapotranspiration, with a two-day irrigation shift. The domestic effluent used in irrigation comes from 150 residences corresponding to the production of 3000 L day<sup>-1</sup>. The raw effluent was initially treated by boxes with grids and sand, followed by an Upflowed Anaerobic Sludge Blanket (UASB) reactor combined with an up-flow anaerobic filter, and then to the polishing pond.

#### 3.2.4. Assessment of mulch decomposition

The litter bag method was used to evaluate the decomposition process of plant residue. This material was made of nylon with 0.15 x 0.15 m and 1 mm mesh dimensions. Ten grams of field-dried material were placed in each litter bag. During installation, 10 g subsamples were collected and dried at 65°C to correct moisture. The litter bags were installed under the plant residue in contact with the soil, next to the sorghum line. and the evaluation periods were 0, 10, 25, 65, and 165 days. At these times, the litter bags were weighed and collected to determine moisture and C and N contents by wet combustion and the Kjeldahl method, respectively (TEIXEIRA et al., 2017). Using the data obtained, the C/N ratio of the material was calculated over time. Furthermore, the decomposition rate of the plant residue was estimated using the Olson exponential model (1963). The times required to decompose 50, 95, and 99% of the residue, half-life, were also calculated, according to Zhao et al. (2014).

#### 3.2.5. Chemical, physical and microbiological analysis of soil

Disturbed samples were collected in the 0-0.10, and 0.10-0.20 m depths at the five sampling times (0, 10, 25, 65, and 165 days) to determine C-mic and C-CO<sub>2</sub> emission, according to Mendonça and Matos (2017). The *q*-CO<sub>2</sub> was calculated by the C-CO<sub>2</sub>/C-mic ratio (ANDERSON; DOMSCH, 1993), in addition to the microbial quotient (*q*-Mic) by the C-mic/SOC ratio (BINI et al., 2014). The soil exchangeable  $NH_4^+$  and  $NO_3^-$  contents and available P contents were also determined (MENDONÇA; MATOS, 2017). The SOC content was obtained by the Walkley-Black method (TEIXEIRA et al., 2017; WALKLEY; BLACK, 1934).

#### 3.2.6. Laser-induced fluorescence spectroscopy (LIFS)

Pellets were prepared with 0.5 g of soil samples sieved at 100 mesh (0.125 mm of open) (also carried out in the five sampling periods). The samples were subjected to a pressure of 5 tons for two minutes. The samples were excited by a 405 nm laser beam to obtain the laser-induced fluorescence spectra, and the emission was recorded in the range of 465 to 800 nm. With the data obtained by LIFS, it was possible to calculate the  $A_{LIFS}$  of the soil organic matter through the ratio between the integrated area of the spectrum emitted in the region of 465-800 nm and the SOC of the soil sample (MILORI et al., 2006). The  $A_{LIFS}$  index was previously stated as a humification index (MILORI et al., 2006); however, to assess the SOM persistence as proposed by Lehmann and Kleber (2015), here we refer to this index as the aromatization index ( $A_{LIFS}$ ), since the humification index was mainly calculated based on the degree of aromatic C found in the sample.

#### 3.2.7. Statistical analysis

Discrepant data for all variables were removed using boxplots, and normality was assessed using the Shapiro-Wilk test. Data on microbial activity,  $NO_3^-$ ,  $NH_4^+$ , available P, SOC, and the A<sub>LIFS</sub> were subjected to analysis of variance (F test) and, when significant, were

compared using the Tukey test at a significance level of 5% (FERREIRA, 2010). Data on the decomposition rate were subjected to regression analysis at a significance level of 5%. Statistical analysis was conducted using SISVAR 5.0 software. Additionally, we performed a principal components analysis (PCA) using the package FactoMineR in R software (LÊ et al., 2008) to evaluate the relationship among chemical attributes of soil and microbial variables affected by the different water depth treatments and the time of experimentation.

#### 3.3. Results

## 3.3.1. Chemical properties and decomposition rate of plant residue under different irrigation depth of reused water

The decomposition assay was conducted over 165 days, during the progressively decomposed phytomass added. After 165 days, significant decomposition of 45%, 48%, 47%, and 49% was exhibited for the 0, 80, 100, and 120% water depth, respectively (p < 0.001). The highest decomposition rate was observed after 10 days for 80% (16%), and after 25 days for 80, 100 and 120% (24, 22 and 21% respectively). In the first 30 days, the 80% water depth showed a higher decomposition rate. Posteriorly, the 100% depth had a similar result. After 65 days, the irrigated treatments with 80 and 100% depth was significant with a higher decomposition rate, with 39 and 35% in decomposition rate of the mulch (Table 1 and Figure 1).

mulch											
Irrigation depth	Added phytomass	Decomposed phytomass			Decomposition rate at 165 days		К	t/50%	t/95%	t/99%	
%			kg ha <sup>-1</sup>				0/ 11			1	
_	0 day	10 days	25 days	65 days	165 days	kg day <sup>-1</sup>	% day <sup>-1</sup>		days		
0	7840 a	531 b	1166 b	2084 b	3538 a	21.4 <sup>ns</sup>	0.27 <sup>ns</sup>	0.00353	196	850	1416
80	7840 a	1237 a	1862 a	3033 a	3751 a	22.7	0.29	0.00358	194	838	1397
100	7840 a	700 b	1763 a	2955 a	3702 a	22.4	0.29	0.00371	187	809	1348
120	7840 a	704 b	1672 a	2332 b	3818 a	23.1	0.30	0.00380	182	789	1316

**Table 1**. Decomposed phytomass, decomposition rate and half-life of plant residue used as soil mulch

Lower case letters indicate differences between depths by Tukey's test at 5% significance.

**Figure 3**. Soil mulch decomposition in a consortium with palm and forage sorghum irrigated with 0, 80, 100, and 120% of water depth of sorghum evapotranspiration in the Brazilian semiarid



The highest daily decomposition rate was found for the 80 and 120% water depth after 165 days (22.7 and 23.1 g day<sup>-1</sup>). The time required for 50% decomposition was 196, 194, 187 e 182 days, respectively, to 0, 80, 100 and 120%. To reach 95 and 99% decomposition of the plant residue are necessary 850, 838, 809 and 709 for 0, 80, 100 e 120% respectively, and 1416, 1397,1348 and 1316 days, respectively to 0, 80, 100 e 120% (Table 1). The initial N concentration in the plant residue was 16.2 g kg<sup>-1</sup> and the C content was 404.7 g kg<sup>-1</sup>. Residual N concentration exhibited losses after 10 days in the irrigated treatments (1.2 g kg<sup>-1</sup>, 1.3 g kg<sup>-1</sup>, and 1.8 g kg<sup>-1</sup> for the 80, 100, and 120% depth, respectively). The effect of irrigation was significant in all treatments after 10 days, thus resulting in a significant decline in N content compared to the initial time, which led to an increase in the C: N ratio (Table 2).

The residual C concentration was affected by N availability, thus increasing the C: N ratio from 25:1 due to the significant variations in the proportions of C and N. After 165 days,

there was a significant effect of irrigation on all sample depths, except for the 0%, which presented lower C contents compared to the initial time (Table 2 and Figure 3).

over 105 days							
Irrigation depths (%)	0 day	10 days	25 days	65 days	165 days		
	C of the residue (g kg <sup>-1</sup> )						
0	$404.7^{(\pm 12.0)Aa}$	$398.5^{(\pm 4.0)Aab}$	$399.7^{(\pm 1.0)Aab}$	$390.0^{(\pm 4.0)\mathrm{Ab}}$	390.0 <sup>(±10.0)</sup> Aab		
80	404.7 <sup>(±12.0)</sup> Aa	$399.5^{(\pm 5.0)}$ Aa	$374.1^{(\pm 8.0)Bb}$	$352.5^{(\pm 4.0)}$ Cc	352.8 <sup>(±5.0)</sup> Bc		
100	404.7 (±12.0) Aa	$404.0^{(\pm 3.0)\mathrm{Aa}}$	$403.5^{(\pm 1.0)\mathrm{Aa}}$	$403.0^{(\pm 6.0)}$ Aa	$354.0^{(\pm 9.0)}{}^{\mathrm{Bb}}$		
120	$404.7 \ ^{(\pm 12.0)Aa}$	$404.2^{(\pm 7.0)Aa}$	$390.5^{(\pm 16.0)Aab}$	$381.0\ ^{(\pm 8.0)Bb}$	$360.7 \ ^{(\pm 2.0) Bc}$		
		N of the residu	e (g kg <sup>-1</sup> )				
0	16.2 <sup>(±0.1)</sup> Aa	15.2 <sup>(±0.4)</sup> Ab	15.9 <sup>(±0.3)</sup> Aa	$14.8 \ ^{(\pm 0.2)  Abc}$	$14.5^{(\pm 0.5) \text{Ac}}$		
80	$16.2 \ ^{(\pm 0.1)  Aa}$	$15.0^{(\pm 0.5)\mathrm{Ab}}$	$14.9~^{(\pm0.1)~Bb}$	$14.8^{(\pm 0.2)}$ Ab	$13.8 \ ^{(\pm 0.2) Bc}$		
100	16.2 <sup>(±0.1)</sup> Aa	$14.9^{(\pm 0.4) \text{ ABb}}$	$14.5^{(\pm 0.1)}$ Bb	$14.6^{(\pm 0.2)Ab}$	$14.5 \ ^{(\pm 0.2)}{}^{\text{Ab}}$		
120	16.2 <sup>(±0.1)</sup> Aa	$14.4^{(\pm 0.5)}$ Bb	14.5 <sup>(±0.5) Bb</sup>	$14.4^{(\pm 0.5)}$ Ab	$14.6^{(\pm 0.3)\mathrm{Ab}}$		
		C:N rat	io				
0	$25:1^{(\pm 0.3)Ab}$	$26:1^{(\pm 2.1)\mathrm{Ba}}$	$25:1^{(\pm 0.3)}$ Cb	$26:1^{(\pm 0.2)Ba}$	$27{:}1^{(\pm 0.9)\mathrm{Aa}}$		
80	$25:1^{(\pm 0.3)Ab}$	$27:1^{(\pm 1.3)\mathrm{Ba}}$	$25:1^{(\pm 0.6)}$ Cb	$24:1^{(\pm 0.8) Cc}$	$26{:}1^{(\pm 0.6)Bb}$		
100	$25:1^{(\pm 0.3)Ab}$	$28:1^{(\pm 0.4)\mathrm{Aa}}$	28:1 <sup>(±0.6) Aa</sup>	$27:1^{(\pm 1.5)Aa}$	$24:1^{(\pm 0.6)Cb}$		
120	$25:1^{(\pm 0.3)\mathrm{Ac}}$	28:1 <sup>(±1.0)</sup> Aa	$27:1^{(\pm 0.5) Bb}$	$26:1^{(\pm 1.4)Bb}$	$25:1^{(\pm 0.1) BCc}$		

**Table 2.** Carbon (C), nitrogen (N) content and C/N ratio of plant residue used as soil mulch over 165 days

Depth 0, 80, 100 and 120% calculated based on sorghum Etc. Capital letters indicate statistical differences between slides. Lower case letters indicate differences over time by Tukey's test at 5% significance.

#### 3.3.2. Microbial C and soil C-CO<sub>2</sub> emission

A decreasing trend in C-mic at 0-0.10 and 0.10-0.20 m was observed, particularly at 65 days (82, 91, and 85% for the 0, 80, 100 and 120% depth, respectively, at 0-0.10 m, and 90, 93%, 90 and 85% at 0.10-0.20 m). This is due to the high rainfall of 100 mm (Figure 1). At 165 days, 80 and 100% depth presented higher C-mic (Figure 4 a and b). The highest *q*-Mic was also observed in the first 30 days, with a significant reduction after 65 days in all treatments and depths (Figure 4 c,d).

In the 0-0.10 m depth, there was an increase in C-CO<sub>2</sub> emission after 10 and 25 days, with a significant decrease after 65 days. In the 0.10-0.20 m depth, a decrease in this emission was also observed after 65 days (Figure 4 e and f), while the qCO<sub>2</sub> had an increase at 71, 91, 88, and 86% for the 0, 80, 100, and 120% depth, respectively, compared to the 0 day at 0-0.10 m depth. At 0.10-0.20 m, there was a significant increase of 53, 91, 88, and 77% relative to baseline for the 0, 80, 100, and 120% depth, respectively (Figure 4 g, h).



**Figure 4**. Microbial biomass carbon (a,b), microbial quotient (c,d), basal respiration (e,f), and metabolic quotient (g,h) of soil under different irrigation depth with reused water in the Brazilian semiarid

Capital letters indicate statistical differences between depths. Lowercase letters indicate differences over time by Tukey's test at 5% significance.

#### 3.3.3. Soil available ammonium, nitrate, and phosphorus

The  $NH_4^+$  content decreased at 0-0.10 and 0.10-0.20 m depths over time. Conversely, the  $NO_3^-$  contents were higher than  $NH_4^+$ , however, after 10 and 65 days there was a significant decrease in both depths. During this period, a reduction in  $NO_3^-$  of 48, 35, 35, and 62% was

observed in the 0-0.10 m, and 24, 21, 15, and 15% in the 0.10-0.20 m for the 0, 80, 100, and 120% depth, respectively (Table 3). Notably, in the 0-0.10 m depth, up to 65 days, the 100% irrigation level exhibited significant potential in the supply of  $NO_3^-$ . Available P was significantly higher from 10 to 65 days for the 80% irrigation level at 0-0.10 m depth. In 0.10-0.20 m, higher P levels were observed at 65 days for the 100% depth (Table 3). The treatments with the highest P availability were the 80 and 100% depth.

**Table 3**. Ammonium, nitrate and phosphorus contents available in the soil over 165 days of evaluation of the decomposition rate of plant residue under different irrigation depth with reused water

Irrigation depths	Depth (m)	0 day	10 days	25 days	65 days	165 days		
(%)								
0		24.5 <sup>(±2.0)</sup> Ba	19.8 <sup>(±2.1) Ab</sup>	NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> ) 18.0 <sup>(±0.7) Ac</sup>	$6.4^{(\pm 0.9)}$ Bd	3.6 <sup>(±0.1) Ce</sup>		
80		24.3 29.3 <sup>(±0.90)</sup> Aa	15.1 <sup>(±1.0)</sup> <sup>Bb</sup>	$7.4^{(\pm 0.5)}$ Bc	$7.0^{(\pm 0.4)}$ Bc	15.2 <sup>(±0.9) Ab</sup>		
100	0-0.10	$17.5^{(\pm 0.6) \text{ Ca}}$	7.4 <sup>(±0.5)</sup> Cc	$7.9^{(\pm 1.0)}$ Bc	$7.4^{(\pm 0.5)}$ Bc	10.6 <sup>(±0.9) Bb</sup>		
120		$17.3^{(\pm 0.7) \text{ Ca}}$	$7.4^{(\pm 0.5) \text{ Cc}}$	3.8 <sup>(±0.4) Cd</sup>	$11.0^{(\pm 0.5)}$ Ab	11.6 <sup>(±0.1) Bb</sup>		
120		10.1	7.4	5.0	11.0	11.0		
0		17.3 <sup>(±0.8)</sup> Ba	10.5 <sup>(±0.8) Ab</sup>	$17.8^{(\pm0.4)\mathrm{Aa}}$	$7.0^{(\pm 0.5)\mathrm{Ac}}$	$7.2^{(\pm 0.1)\mathrm{Bc}}$		
80	0.10-0.20	$28.5^{(\pm 0.8)\mathrm{Aa}}$	$3.8^{(\pm0.5)\mathrm{Cc}}$	$6.3^{(\pm0.7)\mathrm{Cb}}$	$7.0^{(\pm0.4)\mathrm{Ab}}$	$6.0^{(\pm0.4)\mathrm{Cb}}$		
100		$15.8^{(\pm 1.3) \text{ Ca}}$	$7.0^{(\pm 0.4)\mathrm{Bc}}$	$7.4^{(\pm 0.5) \text{ BCc}}$	$3.8^{(\pm0.4)\mathrm{Bd}}$	$9.9^{(\pm0.8)\mathrm{Ab}}$		
120		$14.7^{(\pm 0.4)Ca}$	$7.0^{(\pm 0.4)\mathrm{Bc}}$	$8.3^{(\pm0.9)\text{Bb}}$	$3.6^{(\pm0.1)Bd}$	$9.6^{(\pm0.8)Ab}$		
				NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )				
0	0-0.10	65.9 <sup>(±3.0)</sup> Ba	38.0 <sup>(±1.5) Cc</sup>	68.6 <sup>(±6.1) Bb</sup>	$34.5^{(\pm 3.2)\mathrm{Bc}}$	91.2 <sup>(±1.7) Aa</sup>		
80		$77.1^{(\pm 2.2)Aa}$	$25.6^{(\pm2.6)\mathrm{Dc}}$	$48.1^{(\pm 3.8)}$ Cb	49.8 <sup>(±2.2) Ab</sup>	71.1 <sup>(±4.7) Ba</sup>		
100		$73.4^{(\pm 1.6)\mathrm{Aa}}$	$60.5^{(\pm 3.7)Ab}$	81.6 <sup>(±7.9) Aa</sup>	$49.0^{(\pm 3.5)\mathrm{Ac}}$	$45.4^{(\pm 2.5) \text{ Dc}}$		
120		$73.4^{(\pm 3.7)Aa}$	$50.4^{(\pm1.5)Bb}$	$49.8^{(\pm2.2)\text{Cb}}$	$25.6^{(\pm1.6)\mathrm{Cc}}$	$52.3^{(\pm3.2)\text{Cb}}$		
0		48.1 <sup>(±3.1) Aa</sup>	24.0 <sup>(±1.6) Cc</sup>	49.6 <sup>(±0.1) Aa</sup>	36.4 <sup>(±1.6) Ab</sup>	38.6 <sup>(±2.7) Bb</sup>		
80		46.4 <sup>(±2.7)</sup> Aa	33.3 <sup>(±3.1) Bb</sup>	37.2 <sup>(±0.1) Bb</sup>	36.4 <sup>(±1.6) Ab</sup>	$24.8^{(\pm 0.1)}\mathrm{Dc}$		
100	0.10-0.20	31.0 <sup>(±7.2) Bb</sup>	52.8 <sup>(±2.4)</sup> Aa	49.8 <sup>(±2.2) Aa</sup>	$25.6^{(\pm1.6)Bb}$	30.8 <sup>(±1.1) Cb</sup>		
120		$28.8^{(\pm3.1)\text{Bb}}$	$24.8^{(\pm0.1)\text{Cb}}$	45.7 <sup>(±3.1) Aa</sup>	$24.8^{(\pm 0.1)Bb}$	48.9 <sup>(±1.6)</sup> Aa		
			Р	available (mg kg	<sup>-1</sup> )			
0		183.0 <sup>(±16) Bab</sup>	179.0 <sup>(±10) Bab</sup>	229.8 <sup>(±39)</sup> Ba	155.2 <sup>(±23)</sup> Db	$162.3^{(\pm 30) BCb}$		
80	0-0.10	$221.3^{(\pm23)}{}^{\mathrm{Bb}}$	$332.3^{(\pm 30)\mathrm{Aa}}$	$344.7^{(\pm 29)Aa}$	$329.4^{(\pm 43) Ba}$	$246.7^{(\pm 26)Ab}$		
100		$274.0^{(\pm 19)Abc}$	$313.8^{(\pm32)Ab}$	$231.8^{(\pm 31)Bcd}$	$490.3^{(\pm44)\mathrm{Aa}}$	$207.9^{(\pm 30)Abd}$		
120		$171.8^{(\pm18)Bbc}$	$215.9^{(\pm 32)Bab}$	$224.0^{(\pm35)Bab}$	$229.4^{(\pm 40)Ca}$	$152.1^{(\pm 13) \text{ Cc}}$		
0		228.0 <sup>(±29) Ca</sup>	137.3 <sup>(±27) Bb</sup>	161.1 <sup>(±41) Bb</sup>	136.5 <sup>(±21) Cb</sup>	$170.8^{(\pm 30)}$ Cb		
80		$306.4^{(\pm 17)}$ Bb	261.5 <sup>(±11) Ab</sup>	110.1 <sup>(±12)</sup> Cc	377.8 <sup>(±47)</sup> Ba	$259.9^{(\pm41)Bb}$		
100	0.10-0.20	$378.8^{(\pm 14)}$ Ab	$305.5^{(\pm 30)\mathrm{Ac}}$	308.0 <sup>(±11)</sup> Ac	449.0 <sup>(±21) Aa</sup>	$349.2^{(\pm 26)Abc}$		
120		$215.7^{(\pm 16)\text{Cb}}$	$265.4^{(\pm 31)Aa}$	$188.6^{(\pm18)Bb}$	$132.8^{(\pm 28)}$ Cc	$132.9^{(\pm 30) \text{ Cc}}$		

Depth 0, 80, 100, and 120% were calculated based on sorghum Etc. Capital letters indicate statistical differences between slides. Lowercase letters indicate differences over time by Tukey's test at 5% significance.

#### 3.3.4. Fluorescence spectra, ALIFS, and SOC content

The emission spectra exhibited a broad band with maximum intensity around 525-550 nm for all irrigation depth over time, with higher intensity at the 120% depth after 10, 65, and 165 days. Additionally, pronounced shoulders were observed near 550 nm and 620 nm at both depths (Figure 7). Regarding  $A_{LIFS}$ , it was also significantly higher after 10 days for the 120% depth, mainly at 0-0.10 m. The  $A_{LIFS}$  was lower for the 80 and 100% depth, thereby being those that most closely resembled natural vegetation in both depths. These results were consistent with those of SOC. The 80 and 100% depth showed the highest SOC contents, with a significant increase at 25 days and 10 days in the 0-0.10 and 0.10-0.20 m depths respectively for the 80% depth (Figures 5 and 6).





Capital letters indicate statistical differences between depths. Lowercase letters indicate differences over time by Tukey's test at 5% significance.



**Figure 6**. Soil organic carbon (SOC) contents over 165 days under different irrigation depth with reused water at 0, 80, 100, and 120%, in the Brazilian semiarid

Capital letters indicate statistical differences between depths. Lowercase letters indicate differences over time by Tukey's test at 5% significance.

#### 3.3.5. Principal Components Analysis

Principal Components Analysis assessed the interactions among all variables influenced by the different water depth applications and the duration of the experiment. Particularly, the PCA analysis revealed that the water depth treatments mainly influenced SOC, A<sub>LIFS</sub>, and available P, while microbial activity, nutrient availability, and biomass properties were more influenced by the time of experimentation (Figure 8).

**Figure 7**. Average LIFS emission spectra with excitation wavelength at 405 nm obtained from soil pellets collected in a sorghum and palm intercrop irrigated with different depth of reused water at 0, 80, 100 and 120% of the sorghum Etc, over 165 days of evaluation of the decomposition rate of the residue used as soil mulch.



**Figure 8.** Principal components analysis (PCA) of all the variables analyzed in experiment (a). The discriminant analysis comprises the time groups (b) and water depth (c).



#### 3.4. Discussion

## 3.4.1. Dynamics of decomposition of plant residue and remaining N in different irrigation depth with reused water

The use of vegetative waste for mulch only and its consequent decomposition is essential for the turnover of nutrients and replenishment of energy in the trophic chains of the ecosphere. Notably, the adoption of grass as a mulch in several crops can benefit soil health through the prevention of soil erosion and the stabilization of aggregates, thus allowing the protection of organic matter and the storage of C (XAVIER; OLIVEIRA; SILVA, 2017; ADHIKARI et al., 2024).

Notably, the highest decomposition rate occurred in the first 25 days for treatments at 80 and 100% depth due to the greater mineralization of N (Figure 3 and Table 3) and the consequent decomposition of easily decomposable labile compounds, such as unbranchedchain carbohydrates, single-chain amino acids, phospholipids, and diester compounds, among other non-phenolic substances (HONG et al., 2021; THAPA et al., 2022; ADHIKARI et al., 2024). This contributed to the non-oligotrophic (*i.e.*, r-strategist organisms) microbial community, where proteobacteria could predominate (HU et al., 2023). Thus, there is a boost in microbial biomass, whose decomposition activity depends upon the extreme abiotic conditions, which predominate in the tropical semiarid environment.

The highest N contents are immobilized mainly after the addition of the residue. They could be related to the priming effect, which accelerates decomposition and C-CO<sub>2</sub> emission by microbial activity during the decomposition of organic matter less recalcitrant present in sewage water (RAKESH et al., 2021; ADHIKARI et al., 2024). The decrease in the decomposition rate over time may be associated with the persistence of more recalcitrant compounds, such as lignin and hemicellulose, and the lower availability of N, which decreases over time, thereby being a limiting factor for decomposition (HONG et al., 2021; THAPA et al., 2022). Therefore, in this period of the experiment, we postulate that the microbial community of proteobacteria will be replaced by oligotrophs such as actinobacteria and mainly fungi (i.e. k-strategists' organisms), which have a metabolism capable of degrading the phenolic compounds (HU et al., 2024). Hence, the soil C-mic, where the fungi are predominant, showed a fast increase.

Conversely, the significant decrease in the content of N promoted the increase of the C: N ratio of the mulch residue, which contributed to the lower decomposition rate over time (Figure 1 and Table 3). The C: N ratio is an inherent factor in the quality of the residue as it is responsible for more than 45% of the decomposition rate of plant residues, and its increase over time is associated with the presence of more resistant compounds such as holocellulose, which are complex compounds present in the cell wall of the plant, and the microorganisms demand more energy to decompose it than easily decomposable labile carbohydrates present at the beginning of the decomposition of the plant residue (HONG et al., 2021; THAPA et al., 2022).

Treatments irrigated with reused water exhibited a higher decomposition rate over time (Figure 1). In environments with ideal humidity, decomposition is favored, as there is a gradual increase in biomass and microbial activity and the production of extracellular degradative enzymes (ANNALA et al., 2022). We observed that both excess and lack of humidity directly affected the microbial indicators, consequently decreasing the decomposition of organic compounds (HONG et al., 2021). In semiarid regions, there is a positive correlation between the decomposition rate and the supply of moisture, as it is known that a reduction in plant mass occurs by more than 7% when water is available (WANG et al., 2020; LI et al., 2022;). On the other hand, in the absence of moisture, suppression of microbial physiological activity may occur through a reduction in the activation of extracellular enzymes such as lignin and manganese peroxidase produced by fungal cells, leading to a reduction in microbial biomass, which is established mainly by fungi that inhabit the soil microbiome (GAO et al., 2021; CHEN et al., 2023).

Then excess moisture through natural precipitation or irrigation tends to enhance microbial activity, thereby intensifying the decomposition process (DE ALMEIDA et al., 2022). Thus, in the case of more frequent and less intense rainfall, the decomposition process occurs more effectively, because the heavy rain can leach nutrients and make the soil more anoxic, where anaerobic microsites are formed, thus reducing the enzymatic activity of fungal cells, which mostly oxidize organic compounds (JOLY; KURUPAS; THROOP, 2017; LUO et al., 2021; THAPA et al., 2021; THAPA et al., 2022). In addition, the diversity of species used as plant mulch actively interferes with the decomposition process by directly interfering with the structure of the microbial community. In this sense, plants with a high C: N ratio and high biomass production, such as mulch grasses, can decompose more slowly and gradually recycle nutrients in the soil, affecting the dynamics of the aromatization index of organic matter (THAPA et al., 2021).

### 3.4.2. Effect of irrigation with reused sewage water on microbial activity and $NH_4^+$ , $NO_3^-$ and available P levels in the soil

In arid and semiarid tropical environments, extreme weather conditions and intense rainfall can jeopardize the development and activity of soil microbial biomass (GOLLA, 2021). Our experiment showed that heavy rains at 65 days of caused excessive moisture, leading to a significant decrease in C-mic due to leached wastewater. This could cause the soil to break down quickly, exposing organic content that was previously protected within aggregates (DE OLIVEIRA FERREIRA et al., 2018). This disturbance in the soil ecosystem at the microbial habitat level likely accelerated the degradation of labile organic compounds. Hence, the increase in metabolic quotient indicated high energy expenditure per unit of microbial biomass, leading to intensified  $CO_2$  emissions and soil C depletion (RIDGEWAY et al., 2022).

The intense rainfall at 65 days also affected the mineralization of the residue and C, N, and P contents. The increase in soil moisture may have reduced the diffusion of oxygen and nitrogen, with the greatest reduction in the first 24 hours. After this period, microorganisms living under these conditions exhibit greater energy expenditure and become morphologically and genetically adapted to the excessive humidity of the environment (FURARK; WOLINSKA, 2023). Subsequently, the SOM aromatization index indicated an increasing trend especially at 0.10-0.20 m for 120% water depth. Aromatization transforms SOM into stable forms with a high degree of aromaticity, reducing the accessibility of microorganisms to these compounds (TIVET et al., 2013; TADINI et al., 2018; 2021). In addition, aromatic compounds are recognized to have a higher affinity to Fe forms, which can potentially contribute to the formation of organo-mineral associations in soil, especially under acidic conditions (SCHEEL et al., 2007).

The highest NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents were found in the 0-0.10 m depth, with a predominance of nitrate, due to the intense mineralization and nitrification process, which are stimulated by moisture. In addition, the influence of residue and microbial activity is greater in the surface layer of the soil (ADROVER et al., 2017). Although the predominant form of N is NO<sub>3</sub><sup>-</sup>, at 0-0.10 m there is a significant decrease in its content, indicating its immobilization by microbial biomass and the possibility of leaching. In addition, as the environment was reducing, with high water retention, the nitrate can be used by microorganisms as an electron acceptor in the respiratory chain, allowing denitrification reactions, which reduce nitrate into atmospheric N with nitrous oxide emissions (HERZOG et al., 2016; GRZYB; WOLNA-MARUWKA; NIEWIADOMSKA, 2020; FURARK; WOLINSKA, 2023). Therefore, in addition to the

damage to the crop, the local ecosystem may be contributing to the emission of greenhouse gases, which disqualifies the management system as sustainable, even temporarily.

The higher contents of available phosphorus in the first 65 days for irrigation depth of 80 and 100% at 0-0.10 m depth are related to the higher decomposition rate during this period. This is mainly due to the availability of labile compounds for microorganisms, resulting in the release of this nutrient (HONG et al., 2021). In addition, there may be an additional supply of P by irrigation since this water comes from domestic sewage (SINGH; DESHBHRATAR; RAMTEKE, 2012; OFORI et al., 2021). In the 0.10-0.20 m depth, there is greater availability of P for 100% irrigation level, indicating the availability of this nutrient at depth, which may benefit crop growth and productivity (OFORI et al., 2021).

### 3.4.3. Impact of different irrigation depth with sewage reuse water on Fluorescence Spectra, A<sub>LIFS</sub> and SOC

The spectral region of wavelength 525-550 nm suggested the presence of molecular components characterized by a resistant polycondensation of the aromatic structure with a high degree of conjugation and their ability to carry substituents, such as carbonyl and carboxylic groups. Similar results have already been demonstrated for soils under tropical conditions (GONZALÉZ-PÉREZ et al., 2006; MILORI et al., 2006; FAVORETTO et al., 2008; MILORI et al., 2011; TADINI et al. 2018, 2021). The 80 and 100% treatments showed lower intensities and lower  $A_{LIFS}$  ratios, indicating a lower degree of aromatization and the presence of higher concentrations of labile organic matter.

Such labile forms are recognized to be contained in the soil aggregates, improving the persistence of SOM (GONZALÉZ-PERÉZ et al., 2006; TIVET et al., 2013; TADINI et al., 2018, 2021), a fact also observed for native vegetation soils (MARTINS et al., 2011). The higher aromatization rates in the 0 and 120% treatments suggest that the absence or the excess of sewage water does not favor the presence of labile fractions in the soil, as noted by the higher fluorescence intensity in the 120% depth, which may be related to a more notable number of aromatic substances (TADINI et al., 2021).

The presence of shoulders in the region near 620 nm suggests fluorescence emissions from groups even more condensed. This shift to longer wavelengths may have led to a higher concentration of highly condensed aromatic chains (TIVET et al., 2013; TADINE et al., 2021). A<sub>LIFS</sub> may increase with increasing content of these structures, which are considered indicators of the aromatization process. Particularly under the 120% depth, more intense aromatization may be related to a greater supply of moisture, which favors the rapid decomposition of labile

compounds and the loss of C from the soil. Regarding SOC contents, the 80 and 100% depth presented higher contents, which could be related to higher labile C contents in these treatments. Despite being higher, both are still lower than the SOC contents of native vegetation.

#### **3.5.** Conclusion

The decomposition of plant residues is quite high at the beginning of the experiment and is strongly influenced by N contents. Furthermore, the irrigated treatments exhibited the highest decomposition rates with greater efficiency under the 80 and 100% Etc irrigation depth, after 10 and 25 days. In this case, the supply of constant moisture at ideal rates through irrigation favored microbial activity and residue decomposition.

The intense rainfall negatively affected microbial activity, resulting in a decrease in their biomass and an increase in the metabolic quotient. Thus, this period was marked by the disturbance in microbial oxidative metabolism, which affected the residue decomposition rate. However, the 80 and 100% depth were more efficient in the availability of C, N, and P.

The 80 and 100% depth presented lower aromatization indexes associated with higher SOC contents, being the treatments that most closely resembled native vegetation. Hence, the combination of irrigation depth with plant residues as a mulch can contribute to the increase in C content in cultivated soil under the extreme climatic conditions of the semiarid region.

All treatments exhibited higher intensity in the spectral range of 525-550 nm, focusing on the 120% depth, indicating the presence of aromatic structures with a high degree of conjugation. In this sense, understanding the structural composition of SOM in semiarid regions is an essential tool for improving the understanding of the dynamics of C in the soil and for evaluating the use of sustainable soil management practices to meet the sustainable objectives proposed by the United Nations.

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#### CAPÍTULO 3: RESERVATÓRIOS DE CARBONO EM CONSÓRCIO DE PALMA E SORGO IRRIGADO COM ÁGUA DE REUSO NO SEMIÁRIDO COM DIFERENTES TAXAS DE COBERTURA DO SOLO

#### Resumo

Em regiões semiáridas tropicais, os estoques de carbono (C) orgânico do solo (COS) e os reservatórios de matéria orgânica do solo (MOS) tendem a ser baixos devido à entrada limitada de biomassa e práticas inadequadas de manejo do solo. Postulamos que a combinação de água de reuso com a cobertura morta do solo (8 Mg ha<sup>-1</sup>) em sistemas de consórico de palma forrageira e sorgo armazena mais C, particularmente no compartimento estável de MOS. Assim, este estudo teve como objetivo avaliar os estoques de COS e os diferentes reservatórios de MOS em um sistema de consórcio de palma forrageira (Opuntia stricta (Haw.)) e sorgo (Sorghum sudanense (Piper) Stapf) irrigado com água de esgoto tratada sob diferentes taxas de cobertura morta na região Semiárida no nordeste brasileiro. O experimento consistiu em um delineamento de blocos casualizados com quatro repetições, com parcelas subdivididas contendo quatro níveis de irrigação (0, 80, 100 e 120% da evapotranspiração da cultura (ETc) do sorgo) e a presença (8 Mg ha<sup>-1</sup>) e ausência (0 Mg ha<sup>-1</sup>) de cobertura morta. Amostras de solo foram coletadas nas profundidades de 0-0,10, 0,10-0,20 e 0,20-0,40 m, e foram determinados os estoques de COS, C extraível com água quente (C-AQ), C oxidável com permanganato de potássio (C-OXP), C orgânico particulado (COP), C orgânico associado a minerais (MAOC) e substâncias húmicas (SH) sendo humina (HU), ácido fúlvico (AF) e ácido húmico (AH). Além disso, foi determinada a produtividade do sistema de cultivo consorciado. Os maiores estoques de COS, COP, C-OXP e C-AQ foram observados nos tratamentos com 8 Mg ha<sup>-1</sup> de cobertura morta, combinados com 80 e 100% de ETc de sorgo, particularmente nas frações MAOC e SH, com a HU sendo a mais proeminente. Esses resultados sugerem maior proteção e estabilidade de C nesse sistema. Os conteúdos de COS nos reservatórios MAOC e HU foram significativamente maiores em comparação à vegetação nativa (NV). Para o MAOC, um aumento de 19, 16 e 66% foi observado em 0-0,10, 0,10-0,20 e 0,20-0,40 m de profundidade, respectivamente, no tratamento com 80% ETc e 8 Mg ha<sup>-1</sup> de cobertura morta, e 46 e 58% a mais em 0,10-0,20 e 0,20-0,40 m no tratamento com 100% ETc e 8 Mg ha<sup>-1</sup> de cobertura morta. Na fração HU, um aumento de três vezes foi observado em 0-0,10 m em ambos os tratamentos de 80 e 100% ETc com 8 Mg ha<sup>-1</sup> de cobertura morta, ou cerca de três e quatro vezes maior em 0,10-0,20 m, respectivamente, e sete e seis vezes mais em 0,20-0,40 m nos mesmos tratamentos. Os aumentos observados em frações estáveis de C sugerem que a irrigação com água de reúso, juntamente com o uso de cobertura morta, aumentou significativamente o armazenamento de COS, particularmente nas frações MAOC e HU, facilitando assim o sequestro de C em solos de regiões tropicais semiáridas.

**Palavras-chave**: Matéria orgânica do solo. Agricultura sustentável. Sequestro de carbono. Agricultura conservacionista.

#### 4. CHAPTER III: CARBON POOLS IN INTERCROPPING OF FORAGE CACTUS AND SORGHUM IRRIGATED WITH REUSED WATER UNDER DIFFERENT SOIL MULCH IN THE SEMIARID REGION

#### Abstract

In tropical semiarid regions, soil organic carbon (SOC) stocks and soil organic matter (SOM) pools tend to be low due to limited biomass input and inadequate soil management practices. We postulated that the combination of reused water and mulching (8 Mg ha<sup>-1</sup>) in intercropping systems of forage cactus and sorghum stores more carbon (C), particularly in the stable SOM compartment. Thus, this study aimed to evaluate SOC stocks and different SOM pools in an intercropping system of forage cactus (Opuntia stricta (Haw.)) and sorghum (Sorghum sudanense (Piper) Stapf) irrigated with treated sewage water under varying mulching rates in a Brazilian northeastern region. The experiment was based on a randomized block design with four replications, with split plots containing four irrigation depth (0%, 80%, 100%, and 120% of the crop evapotranspiration (ETc) of sorghum) and the presence or absence of mulch (8 Mg ha<sup>-1</sup>). Soil samples were collected at 0-0.10, 0.10-0.20, and 0.20-0.40 m depth, and SOC stocks were determined along hot water-extractable C (HWEO-C), potassium permanganate-oxidizable C (POX-C), particulate organic C (POC), mineral-associated organic C (MAOC), and humic substances (HS) being humin (HU), fulvic acid (FA), and humic acid (HA). Additionally, the productivity of the intercropping system was assessed. The highest SOC, POC, POX-C, and HWEO-C stocks were observed in treatments with 8 Mg ha<sup>-1</sup> of mulch, combined with 80 and 100% irrigation depth, particularly in the MAOC and HS fractions, with HU being the most prominent. These results suggest increased C protection and stability over time. SOC contents in the MAOC and HU pools were significantly higher compared to native vegetation (NV). For MAOC, an increase of 19, 16, and 66% was observed at 0-0.10, 0.10-0.20, and 0.20-0.40 m depth, respectively, in the treatment with 80% ETc and 8 Mg ha<sup>-1</sup> of mulch, and 46 and 58% more at 0.10-0.20 and 0.20-0.40 m in the treatment with 100% ETc and 8 Mg ha<sup>-1</sup> of mulch. In the HU fraction, a threefold increase was observed at 0-0.10 m in both 80 and 100% ETc treatments with 8 Mg ha<sup>-1</sup> of mulch, or *ca*. three and four times higher at 0.10-0.20 m, respectively, and seven and six times more at 0.20-0.40 m in the same treatments. The observed increases in stable carbon fractions suggest that irrigation with reused water, coupled with mulching, significantly enhanced SOC storage, particularly in the MAOC and HU fractions, thereby facilitating C sequestration in soils of semiarid tropical regions.

**Keywords:** Soil organic matter. Sustainable agriculture. Carbon sequestration. Conservation tillage.

**Acronyms**: Soil organic carbon (SOC), Soil Organic Matter (SOM), Carbon (C), Crop evapotranspiration (ETc), hot water-extractable carbon (HWEO-C), potassium permanganate-oxidizable carbon (POX-C), particulate organic carbon (POC), mineral-associated organic

carbon (MAOC), humic substances (HS), humin (HU), fulvic acid (FA), humic acid (HA) and native vegetation (NV).

#### 4.1. Introduction

The Caatinga is an exclusive Brazilian biome, covering an area of 734,000 km<sup>2</sup>, classified as a dry tropical forest and one of the largest semiarid regions in the world (FERNANDES et al., 2020). This biome is predominantly located in the northeastern region of Brazil, characterized by high population density and rich biodiversity. The vegetation consists of deciduous plants, annual herbs, succulents, predominantly shrubs, and small trees (LONDE; GOMES; MARTINS, 2023). The biome experiences high temperatures, with yearly averages ranging from 25°C to 30°C, and precipitation between 400 and 800 mm, reaching up to 1,000 mm in border areas. Precipitation is irregular and often intense, while potential evapotranspiration ranges from 1,500 to 2,000 mm annually, resulting in a water deficit semiarid with a precipitation/evapotranspiration ratio below 0.65 (GARIGLIO, 2010).

The Caatinga exhibits one of the lowest soil organic carbon stocks (SOC), estimated at 4.8 PgC, compared to other biomes such as the Atlantic Forest (11.49 PgC), Cerrado (17.07 PgC), and the Amazon Rainforest (36.10 PgC) (GOMES et al., 2019). This, combined with traditional farming practices, has contributed to a decline in SOC stocks, with potential reductions exceeding 50% (LACERDA et al., 2023; MENEZES et al., 2021; SÁNCHEZ-GONZÁLEZ et al., 2017; TOMAZ et al., 2024). Therefore, the adoption of practices that enhance carbon (C) sequestration and storage in the semiarid region is critical for advancing sustainable agricultural systems. Additionally, it is essential to examine C dynamics across various soil organic matter pools and evaluate how different management strategies impact C distribution within these pools. In this context, the use of reused water for soil irrigation combined with mulching has been explored, particularly in intercropping systems involving economically and socially significant crops for local populations, such as forage cactus (*Opuntia stricta*) and sorghum (*Sorghum sudanense*).

Reused water in agriculture offers several benefits for regions facing extreme climate conditions, including increased nutrient availability for crops, reduced production costs, protection of aquatic ecosystems, and enhanced soil organic matter content due to higher biomass production (ADROVER et al., 2017). Moreover, mulching contributes to the control of water erosion in semiarid regions (LIMA et al., 2020), maintenance of soil moisture, provision of energy for microorganisms, promotion of soil aggregation, increased nutrient availability, regulation of soil temperature, control of runoff, surface sealing, and C sequestration in the soil (SILVA et al., 2019).

The application of mulching enhances POC stocks, particularly in sandy loam soils, as this fraction plays a critical role in SOC storage (NISAR; BENBI, 2024). This effect is attributed to the increased stability of macroaggregates and the promotion of root system development (DE OLIVEIRA FERREIRA et al., 2018; SIX et al., 2004). Furthermore, C derived from decomposed residue fractions, processed by microorganisms, actively contributes to POC formation, while the sorption or fixation of residue fractions aids in the formation of MAOC (VIRKI et al., 2021). Drip irrigation, in turn, can accelerate the conversion of POC into MAOC, enhancing the decomposition process and influencing other fractions, such as POX-C and HWEO-C (CHATTERJEE et al., 2018; LIU et al., 2024; NÚÑEZ et al., 2022). The integration of drip irrigation with mulching and minimum tillage represents a system that improves soil structure and facilitates C sequestration in semiarid regions, particularly in soils susceptible to salinization and degradation (GARCIA-FRANCO et al., 2021).

Short-term studies, such as those by Chatterjee et al. (2018), indicate increases in SOC, particularly in the 0-0.05 m layer, with the combination of irrigation and mulching. On the other hand, cultivation and irrigation can increase the exposure of labile fractions, such as POX-C and POC, to microorganisms, accelerating the oxidation of SOM (MANDAL; TOOR; DHALIWAL, 2020). More soluble fractions, such as HWEO-C, may be mobilized by irrigation water, promoting their leaching and conversion into MAOC (NÚÑEZ et al., 2022). Therefore, evaluating the impact of reused water irrigation and mulching on SOM pools and C sequestration is crucial for achieving the United Nations Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger and Sustainable Agriculture), SDG 6 (Clean Water and Sanitation), and SDG 13 (Climate Action) (UN, 2015).

In this context, we hypothesize that the combination of reused water and mulching (8  $Mg ha^{-1}$ ) in intercropping systems of forage cactus and sorghum contributes to C sequestration, particularly in the stable pool of SOM. Thus, this study aims to evaluate the effect of different irrigation depth with reused water and mulching on SOC stocks and the distribution of labile and stable SOM pools in intercropping systems of forage cactus and sorghum in the Brazilian semiarid region.

#### 4.2. Materials and Methods

The study was conducted at the Mutuca Hydroagricultural Reuse Unit, located in Pesqueira, within the Agreste Mesoregion and the Vale do Ipojuca Microregion of Pernambuco, Brazil. The experimental area is at coordinates 8°16'50.94"S and 36°34'17.63" W, at an elevation of 654 meters. The region is part of the Borborema Plateau geoenvironmental unit,

characterized by high massifs and hills, rugged terrain, deep and narrow dissected valleys, and vegetation composed of sub-deciduous and deciduous forests (CPRM, 2005). The climate is classified as hot semiarid according to the Köppen classification, with an average temperature of 26°C, relative humidity of 73%, and an average annual precipitation of 670 mm, with the highest rainfall recorded between May and August. The soil in the sampled area is classified as Haplic Salic Sodic Hyperutrophic Planosol (DOS SANTOS et al., 2018).

The Mutuca experimental unit covers an area of 3,614 m<sup>2</sup> and has been used for experimental research since 2009. In the experiment initial soil preparation involved disking, followed by organic fertilization with bovine manure, and planting of rainfed and irrigated cotton (*Gossypium hirsutum*), cultivar BRS Safira, using reused water. Cultural practices included weed control and insecticide applications to manage aphids. In 2015, moringa (*Moringa oleifera*) was planted, irrigated with reused water using a drip irrigation system, and fertilized with bovine manure. In 2018 and 2019, forage sorghum (*Sorghum bicolor*) was planted, also irrigated with reused water, with various materials, such as coconut husk and ground moringa, used as soil mulch (Figure 9).

Figure 9. History of use of the experimental area.



#### 4.2.1. Experimental design and management system of the study area

The experimental design consisted of a randomized block with four replications. The treatments included four irrigations depth (0, 80, 100, and 120%) based on the crop evapotranspiration (ETc) of sorghum, combined with mulching at two densities (0 and 8 Mg ha<sup>-1</sup>) in an intercropping system of forage cactus (*Opuntia stricta* (Haw.)) and sorghum

(*Sorghum sudanense* (Piper) Stapf). Each block consisted of four plots, each with four single rows of forage cactus and four rows of sorghum, with a plot size of 3 m in length and 5 m in width, corresponding to 15 m<sup>2</sup>. For the forage cactus, planting was done in single rows with a spacing of 1.0 m between rows and 0.2 m between plants. Sorghum was seeded 0.5 m from the cactus planting rows, in furrows 0.5 m deep. The subplots with 0% irrigation were left uncultivated, as sorghum can not tolerate severe water stress (Figure10). The experiment commenced with planting forage cactus, and after its establishment, eleven months later, sorghum was sown and irrigation of the intercropping system began.

**Figure 10.** Experimental design in split plots with four irrigation depths with reused water in the plots (0, 80, 100 and 120% of the sorghum Etc) and two mulch rates in the subplots (0 and 8 Mg ha<sup>-1</sup>) in a consortium of forage palm and sorghum in the Semiarid region



Mulching was applied in the experiment at three distinct times: initially, seven months after the planting of the forage cactus in September 2021; following sorghum sowing in April 2022; and one month after the first sorghum harvest in August 2022. The mulch material consisted of grasses identified as *Urochloa mosambicensis*, *Cenchrus echinatus*, *Eleusine indica*, and *Sterculia striata*, and was applied three times, totaling 24 Mg ha<sup>-1</sup> of mulch. Sorghum thinning was carried out in all plots to ensure treatment uniformity. Sowing occurred in April 2022, with a cultivation period of 90 days, culminating in the first harvest in July 2022. The second cycle began in July 2022, 60 days after the first harvest, and the third cycle commenced in October 2022, 60 days after the second harvest, also lasting 60 days. The forage cactus was harvested at the end of the experiment, 18 months after planting (Figure 11).



Figure 11. Timeline of experiment

The irrigation system used was drip irrigation, and the management was based on the daily evaporation data from a Class A Pan. The crop evapotranspiration (ETc) was determined using sorghum crop coefficients (Kc) for different phenological stages: sowing and establishment – stage I (0.4); vegetative growth – stage II (1.1); flowering and production formation – stage III (1.0); and maturation – stage IV (0.7) (COSTA et al., 2017). The reference evapotranspiration (ETo) was calculated based on evaporation measurements from the Class A Pan (EV) and the pan coefficient (Kp). The applied water depth was calculated based on sorghum evapotranspiration, with a two-day irrigation cycle. The reused water was sourced from the domestic effluent of 150 local households, with an average production of 3000 L/day. The effluent underwent treatment, initially through grids and sand filtration, followed by an Upflow Anaerobic Sludge Blanket (UASB) reactor, combined with an up-flow anaerobic filter, and finally, a polishing lagoon. The irrigation water depths were: i-for the first sorghum harvest: 80%: 26.65 mm; 100%: 33.06 mm; 120%: 39.67 mm; ii-for the second harvest: 80%: 77.66 mm; 100%: 97.07 mm; 120%: 116.48 mm; iii-for the third harvest: 80%: 63.98 mm; 100%: 79.97 mm; 120%: 95.96 mm.

#### 4.2.2. Sampling

The soil samples were collected from the 0-0.10, 0.10-0.20, and 0.20-0.40 m layers, followed by chemical and physical characterization (Table 4).

Sorghum productivity was determined after each cutting. Three plants were collected from each subplot, forming a composite sample, followed by drying in an oven at 65°C to determine dry matter content. The cactus cutting was carried out 18 months after planting. Concurrent with the sorghum cuttings, soil samples were collected from the 0-0.10, 0.10-0.20, and 0.20-0.40 m layers between the cactus and sorghum lines to determine SOC levels in the different pools of carbon. In addition, soil sampling was carried out in native vegetation paired with the experiment at the same depths.

#### 4.2.3. Total soil C stock

The SOC contents were determined using the method proposed by Yeomans and Bremner (1988). This variable was assessed at the end of the experiment. The SOC stocks were calculated using the soil equivalent mass method using soil bulk density from native vegetation (reference) (ELLERT; BETTANY, 1995). SOC stocks of soil carbon pools were also obtained by the same method.

SOC stock (Mg ha<sup>-1</sup>) = SOC (kg ha<sup>-1</sup>) \* soil bulk density (Mg m<sup>-3</sup>) \* layer volume (m<sup>3</sup>)

Where: SOC = soil organic carbon.

Additionally, the total organic C contents of the reused water and mulch were also determined, yielding values of 24.8 mg  $L^{-1}$  and 404.7 g kg<sup>-1</sup> of C, respectively. Furthermore, the C input into the soil via reused water and mulch during the experimental period was also calculated (Table 5).
Variables	Units	Experiment	NV
	0-0.10 m		
pH	water (1:2,5)	7.4	6.5
EC*	ds m <sup>-1</sup>	1.0	-
Р	mg kg <sup>-1</sup>	33.0	55.0
K	cmol <sub>c</sub> dm <sup>-3</sup>	0.40	0.06
Na	cmol <sub>c</sub> dm <sup>-3</sup>	0.18	0.00
Ca	cmol <sub>c</sub> dm <sup>-3</sup>	5.0	4.4
Mg	cmol <sub>c</sub> dm <sup>-3</sup>	1.2	2.1
SOC stock	Mg ha <sup>-1</sup>	12.6	22.0
HWEO-C stock	Mg ha <sup>-1</sup>	0.22	0.42
POC stock	Mg ha <sup>-1</sup>	2.7	5.9
POX-C stock	Mg ha <sup>-1</sup>	2.4	3.2
MAOC stock	Mg ha <sup>-1</sup>	9.9	16.0
HU	Mg ha <sup>-1</sup>	9.1	3.1
НА	Mg ha <sup>-1</sup>	1.4	5.8
FA	Mg ha <sup>-1</sup>	1.0	5.9
Sand	g kg <sup>-1</sup>	734	822
Silt	$g kg^{-1}$	119	115
Clay	$g kg^{-1}$	147	63
Soil bulk density	g cm <sup>-3</sup>	1.3	1.3
bon bunk density	0.10-0.20 m	1.5	1.5
pH	water (1:2.5)	7.9	6.5
EC	$ds m^{-1}$	1.4	-
P	$mg kg^{-1}$	26.0	- 56.0
r K		0.15	
к Na	cmol <sub>c</sub> dm <sup>-3</sup> cmol <sub>c</sub> dm <sup>-3</sup>		0.07
		0.20	0.00
Ca	cmol <sub>c</sub> dm <sup>-3</sup>	4.7	3.8
Mg	cmol <sub>c</sub> dm <sup>-3</sup>	1.5	1.6
SOC stock	Mg ha <sup>-1</sup>	9.3	16.4
HWEO-C stock	Mg ha <sup>-1</sup>	0.22	0.28
POC stock	Mg ha <sup>-1</sup>	1.6	3.6
POX-C stock	Mg ha <sup>-1</sup>	1.7	3.0
MAOC stock	Mg ha <sup>-1</sup>	7.7	12.8
HU	Mg ha <sup>-1</sup>	5.5	2.9
НА	Mg ha <sup>-1</sup>	0.6	4.5
FA	Mg ha <sup>-1</sup>	1.4	4.1
Sand	g kg <sup>-1</sup>	724	795
Silt	g kg <sup>-1</sup>	107	156
Clay	g kg <sup>-1</sup>	169	49
Soil bulk density	g cm <sup>-3</sup>	1.5	1.4
	0.20-0.40 m		
pH	water (1:2.5)	8.1	6.6
EC	ds m <sup>-1</sup>	2.4	-
Р	mg kg <sup>-1</sup>	18	58.0
K	cmol <sub>c</sub> dm <sup>-3</sup>	0.13	0.06
Na	cmol <sub>c</sub> dm <sup>-3</sup>	0.20	0.00
Ca	cmol <sub>c</sub> dm <sup>-3</sup>	3.6	2.2
Mg	cmol <sub>c</sub> dm <sup>-3</sup>	2.0	0.8
SOC stock	Mg ha <sup>-1</sup>	14.5	24.2
HWEO-C stock	Mg ha <sup>-1</sup>	0.25	0.25
POC stock	Mg ha <sup>-1</sup>	1.7	6.6
POX-C stock	Mg ha <sup>-1</sup>	1.7	5.3
MAOC stock	Mg ha <sup>-1</sup>	12.8	17.7
HU	Mg ha <sup>-1</sup>	9.8	2.5
НА	Mg ha <sup>-1</sup>	1.9	4.5
FA	Mg ha <sup>-1</sup>	1.9	4.1
Sand	g kg <sup>-1</sup>	704	763
Silt	g kg g kg <sup>-1</sup>	119	183
Clay	g kg g kg <sup>-1</sup>	119	185 54
	g kg g cm <sup>-3</sup>	1.7	1.4
Soil bulk density	g cili	1./	1.4

**Table 4**. Physical and chemical characterization of soil and native vegetation (NV) and initial carbon contents of the different organic matter reservoirs evaluated

\*Electrical conductivity

	C (Mg ha <sup>-1</sup> )					
Irrigation depths (%)	Cut 1	Cut 2	Cut 3	Overal		
80	0.0066	0.0196	0.016	0.0422		
100	0.008	0.0248	0.02	0.0528		
120	0.001	0.0395	0.023	0.0635		
Mulch	1st addition	2nd addition	3rd addition			
8 Mg ha <sup>-1</sup>	3.3 Mg ha <sup>-1</sup>	3.3 Mg ha <sup>-1</sup>	3.3 Mg ha <sup>-1</sup>	9.9		

**Table 5.** Carbon input to soil way reused water and mulch in semiarid region of Brazilian Caatinga biome

The data presented correspond to the duration of the experiment.

## 4.2.4. Particulate Organic Carbon and Mineral-Associated Organic Carbon

The POC was obtained using the method proposed by Cambardella and Elliott (1992), employing 20 g of soil and dispersion with sodium hexametaphosphate at 5 g L<sup>-1</sup>, with shaking for 16 hours on a horizontal shaker. This was followed by washing through a 53  $\mu$ m sieve, drying at 65°C, and the C content was determined by combustion according to Yeomans and Bremner (1988). The MAOC in the < 53  $\mu$ m fraction was obtained by the difference between SOC and POC.

#### 4.2.5. Hot Water Extracted Carbon and Potassium Permanganate Oxidized Carbon

The HWEO-C (Hot Water Extracted Organic Carbon) was determined according to the methodology adapted from Ghani, Dexter and Perrott (2003), where C determination was performed according to Yeomans and Bremner (1988). The POX-C (Potassium Permanganate Oxidized Carbon) was determined following Blair et al. (1995), adapted by Shang and Tiessen (1997) using a spectrophotometer.

## 4.2.6. Carbon of the HU, HA, and FA Fractions

The extraction was carried out with 1 g of TSFA and 10 mL of 0.1 mol L<sup>-1</sup> NaOH, followed by shaking for 1 hour on a vertical shaker, resting for 24 hours, and then centrifuged for 20 minutes. The alkaline extract corresponded to the HA and FA fractions and had its pH adjusted to 2.0 using 20% H<sub>2</sub>SO<sub>4</sub>. The content was transferred to centrifuge tubes and left to rest for 18 hours to allow the HA fraction to precipitate. After centrifugation for 5 minutes, the supernatant, corresponding to the FA fraction, was transferred to 50 mL flasks. To the precipitate, 30 mL of 0.1 mol L<sup>-1</sup> NaOH was added. Then, the SOC contents of the HA and FA fractions were determined by wet digestion. The remaining material, corresponding to the humin fraction, was transferred to an oven at 45°C, and the C content was subsequently

determined by acid digestion with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (TEIXEIRA et al., 2017; YEOMANS; BREMNER, 1988).

#### 4.2.7. Data analysis

The SOC stocks and the pools of SOC were tested for distribution using the Shapiro-Wilk test, and they presented a normal distribution. They were then subjected to an analysis of variance (F-test) using the SISVAR 5.0 software (FERREIRA, 2010), at a 5% significance level. When significant, the means were compared using Tukey's test at the 5% significance level. Additionally, the data were compared with native vegetation also using Tukey's test at the 5% significance level.

## 4.3. Results

## 4.3.1. SOC stocks

The treatments with 8 Mg ha<sup>-1</sup> of mulch under irrigation presented higher SOC stocks in most of the evaluated layers, particularly for the 80 and 100% irrigation depth. In the 0-0.10 m layer, the treatments with 80 and 100% irrigation depths and 8 Mg ha<sup>-1</sup> of mulch presented 10 and 24% more SOC, respectively, compared to the 0 Mg ha<sup>-1</sup> treatment. In the 0.10-0.20 and 0.20-0.40 m layers, the SOC increments were 5, 40, 37, and 17%, respectively, to the 8 Mg ha<sup>-1</sup> mulch treatments under 80 and 100% irrigation depths. The treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> mulch was significantly higher than the treatment with 120% irrigation and 8 Mg ha<sup>-1</sup> at subsoil depths (0.10-0.20 and 0.20-0.40 m) (Figure 12A).

Considering the SOC stock in the 0-0.40 m layer, the increments in C for treatments with 0, 80, and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch were 8, 21, and 25%, respectively, compared to the same treatments with 0 Mg ha<sup>-1</sup>. In contrast, the 120% irrigation treatment with 0 Mg ha<sup>-1</sup> showed 9% more SOC compared to 8 Mg ha<sup>-1</sup> (Figure 2A). When comparing these results to native vegetation (NV), in the 0.10-0.20 m layer, the treatment with 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch exhibited 4.9 Mg ha<sup>-1</sup> more in SOC stock, or approximately 23%. In the 0.20-0.40 m layer, the 80% and 100% irrigation treatments with 8 Mg ha<sup>-1</sup> of mulch significantly exceeded the SOC stock of NV, with 9.0 and 9.2 Mg ha<sup>-1</sup> more SOC (37 e 38%), respectively (Figure 12Bb). To treatments with 0 Mg ha<sup>-1</sup> of mulch, irrigation alone was sufficient to surpass the SOC stocks of NV, except at the 0.20-0.40 m depth in the 100% irrigation treatment (Figure 12Ba).

**Figure 12.** SOC stock under different irrigation depth with reused water (0, 80, 100 and 120% of sorghum Etc), with different soil mulch rates (0 and 8 Mg  $ha^{-1}$ ) in a consortium of forage palm and sorghum), and SOC stocks compared with native vegetation (B)



Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

## 4.3.2. SOC Stock in the labile reservoirs

The POC stocks (Figure 13) were significantly higher in treatments with 8 Mg ha<sup>-1</sup> of mulch irrigated with reused water. The highest POC values during the first sorghum cut were observed in the 80 and 100% irrigation treatments with 8 Mg ha<sup>-1</sup> of mulch at all depths. In the 0-0.10 m layer, the treatments with 8 Mg ha<sup>-1</sup> presented significantly higher values, with 32, 43, 42, and 17% more POC for 0, 80, 100, and 120%, respectively, compared to the same treatments without mulch. In the 0.10-0.20 m layer, only the 0 and 100% treatments with 8 Mg ha<sup>-1</sup> were significant, with 5 and 12% more POC compared to the same irrigation depths with 0 Mg ha<sup>-1</sup> of mulch. In the 0.20-0.40 m layer, the 80, 100, and 120% treatments with 8 Mg ha<sup>-1</sup> were significantly higher compared to the irrigation depths with 0 Mg ha<sup>-1</sup> of mulch. In the 0.20-0.40 m layer, the 80, 100, and 120% treatments with 8 Mg ha<sup>-1</sup> were significantly higher compared to the irrigation depths with 0 Mg ha<sup>-1</sup> of mulch. In the 0.20-0.40 m layer, the 80, 100, and 120% treatments with 8 Mg ha<sup>-1</sup> were significantly higher compared to the irrigation depths with 0 Mg ha<sup>-1</sup> of mulch. In the 0.20-0.40 m layer, the 80, 100, and 120% treatments with 8 Mg ha<sup>-1</sup> were significantly higher compared to the irrigation depths with 0 Mg ha<sup>-1</sup> of mulch, with 36, 51, and 20% more POC.

In the second cut at 0-0.10 m, the treatments with 8 Mg ha<sup>-1</sup> of mulch except the 120% irrigation depth were significantly higher, with 9, 21, and 15% more POC for the 0, 80, and 100% irrigation depth, respectively. The 100% irrigation depth was the significantly higher treatment. At 0.10-0.20 m, the 0 and 100% irrigation treatments with 8 Mg ha<sup>-1</sup> of mulch presented 34 and 30% more POC, respectively, compared to the 0 and 100% treatments with 0 Mg ha<sup>-1</sup> of mulch. At 0.20-0.40 m, only the 100% treatment with 8 Mg ha<sup>-1</sup> of mulch was significantly higher, with 42% more POC compared to the 100% treatment with 0 Mg ha<sup>-1</sup> of mulch.

The POC stock in the 100% treatment with 8 Mg ha<sup>-1</sup> of mulch, in the third cut, was higher than the other treatments in the order of 21 to 91% in 0-0.10 m; 22 to 100% in 0.10-0.20 m; 29 to 73% in 0.20-0.40 m. A variation of the POC was observed throughout the sorghum cuts, with a decreasing trend in all treatments, and no treatment in the third cut exceeded the NV values (Figure 14).

The POC fraction in the first sorghum cut, in the 0-0.10 m layer, represented 21 to 37% of the SOC; in the second cut, it represented 13 to 27%; and in the third cut, it represented 2 to 19%. In the 0.10-0.20 m layer, in the first cut, it represented 19 to 29%; in the second cut, it represented 15% to 28%; and in the third cut, it represented 0 to 12%. In the 0.20-0.40 m layer, in the first cut, it ranged from 14 to 28%; in the second cut, from 18 to 25%; and in the third cut, from 7 to 16%. A variation in POC is observed throughout the sorghum cuts, with a tendency to decrease in all treatments, and no treatment in the third cut exceeds the NV values (Figure 14).



**Figure 13**. POC stock under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum Etc), with different mulch rates (0 and 8 Mg ha<sup>-1</sup>) of the soil over three harvests of the sorghum crop

Cut 1: after 90 days from sowing; cut 2: after 150 days from sowing; cut 3: after 210 days from sowing. Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

**Figure 14**. POC and MAOC stocks under different irrigation depth with reused water (0, 80, 100 and 120% of sorghum Etc), with different mulch rates (0 Mg ha<sup>-1</sup> (a,b) and 8 Mg ha<sup>-1</sup> (c,d)) compared with native vegetation



Letters indicate differences between treatments by Tukey's test at 5% significance.

For POX-C, there was a significant interaction effect in the first cut, but only in the 0-0.10 m layer. The 80% irrigation depth with 8 Mg ha<sup>-1</sup> of mulch presented the highest values,

with 26% more POX-C. In subsoil depths, there was a significant effect of mulch (p < 0.05) and of the 80% irrigation depth (p < 0.05) (Figure 15).

**Figure 15**. POX-C stock under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum Etc), with different mulch rates (0 and 8 Mg ha<sup>-1</sup>) of the soil over three harvests of the sorghum crop



Cut 1: after 90 days from sowing; cut 2: after 150 days from sowing; cut 3: after 210 days from sowing. ns= no significance for interaction. Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

In the second cut, in the 0-0.10 m layer, the 0, 80, and 100% depths irrigation with 8 Mg ha<sup>-1</sup> of mulch presented significantly higher POX-C values compared to the treatments with 0 Mg ha<sup>-1</sup> of mulch, with 14, 18, and 22% more C in this fraction. In the 0.10-0.20 m layer, only the 0 and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch were significantly higher compared to the

same depths with 0 Mg ha<sup>-1</sup> of mulch, with 11 and 46% more POX-C. In the 0.20-0.40 m layer, only the 100% treatment with 8 Mg ha<sup>-1</sup> of mulch was significantly higher compared to the treatment with 0 Mg ha<sup>-1</sup> of mulch, with 28% more POX-C (Figure 15).

In the third cut, the POX-C values were higher in the 0-0.10 m layer, with increments of 13 and 7% for the 80 and 100% irrigation depths with 8 Mg ha<sup>-1</sup> of mulch, respectively, compared to the same irrigation depths with 0 Mg ha<sup>-1</sup> of mulch; in the 0.10-0.20 m layer, 19 and 48% for the 80 and 100% irrigation depths with 8 Mg ha<sup>-1</sup> of mulch, respectively, compared to the same irrigation depths with 0 Mg ha<sup>-1</sup> of mulch; and in the 0.20-0.40 m layer, 27, 54, and 22% for 0, 80, and 100% with 8 Mg ha<sup>-1</sup> of mulch, respectively (Figure 15). None of the treatments exceeded the POX-C values of NV (Figure 16).

**Figure 16**. POX-C stocks under different irrigation depths with reused water (0%, 80%, 100% and 120% of sorghum Etc), with different mulch rates (0 Mg  $ha^{-1}$  (a,) and 8 Mg  $ha^{-1}$  (b)) compared with native vegetation.



Letters indicate differences between treatments by Tukey's test at 5% significance.

For HWEO-C in the three sorghum cuts, in the 0-0.10 m layer, the treatment irrigated with 80% and 0 Mg ha<sup>-1</sup> of mulch was significantly higher. In 0.10-0.20 m in the first cut, the 100% irrigation depth with 0 Mg ha<sup>-1</sup> of mulch was significantly higher, and in the second and third cuts, the treatment irrigated with 80% with 8 Mg ha<sup>-1</sup>. In 0.20-0.40 m in the second and third harvests, the highest treatment was 80% with 0 Mg ha<sup>-1</sup> (Figure 17). In 0-0.40 m, in the

second cut, the treatment with 8 Mg ha<sup>-1</sup> and 80% of irrigation depth stands out. When compared with NV, a significant effect in subsoil was observed, as well as the effect of using mulch in the first and third cuts in all irrigation depths (Table 6). In this study, the HWEO-C fraction in the 0-0.10 m layer represented between 0.4% and 1.4% of the SOC stock; at 0.10-0.20 m, between 0.5% and 1.9% of the SOC stock; and at 0.20-0.40 m, between 0.5% and 2.3% of the SOC stock.

**Figure 17**. Organic carbon stock extracted in hot water (Mg ha<sup>-1</sup>) under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum Etc) with different soil mulch rates



Cut 1: after 90 days from sowing; cut 2: after 150 days from sowing; cut 3: after 210 days from sowing. Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

Depth	Mulch rate	Irrigation depths (%)					
(m)	$(Mg ha^{-1})$	0	80	100	120	NV	
			Cut 1				
0-0.10		$0.10^{\pm 0.08} \mathrm{B}$	$0.25^{\pm 0.04}$ B	$0.11^{\pm 0.05} \mathrm{B}$	$0.18^{\pm0.1}$ B	$0.42^{\pm 0.03}$ A	
0.10-0.20		$0.09^{\pm 0.02}$ D	$0.15^{\pm 0.03}$ C	$0.23^{\pm 0.02}$ B	$0.21^{\pm 0.02}$ B	$0.28^{\pm 0.01}$ A	
0.20-0.40	0	$0.16^{\pm 0.04}$ ns	$0.17^{\pm 0.01}$ ns	$0.16^{\pm 0.01}$ ns	$0.15^{\pm 0.02}$ ns	$0.25^{\pm 0.02}$ ns	
0-0.40		$0.36^{\pm 0.03}$ B	$0.57^{\pm0.06}$ B	$0.53^{\pm0.05}$ B	$0.57^{\pm 0.05}$ B	$0.95^{\pm0.04} A$	
0-0.10		$0.14^{\pm 0.063} BC$	$0.19^{\pm 0.04}$ B	$0.09^{\pm 0.02}$ C	$0.16^{\pm 0.04} BC$	$0.42^{\pm 0.03}$ A	
0.10-0.20	8	$0.19^{\pm 0.01}$ B	$0.16^{\pm 0.05}$ B	$0.16^{\pm 0.03}$ B	$0.14^{\pm 0.05}$ B	$0.28^{\pm0.01} \mathrm{A}$	
0.20-0.40	8	$0.35^{\pm 0.05}$ ns	$0.37^{\pm 0.02}$ ns	$0.39^{\pm 0.02}$ ns	$0.39^{\pm 0.02}$ ns	$0.25^{\pm 0.02}$ ns	
0-0.40		$0.68^{\pm 0.12}$ B	$0.72^{\pm 0.10}$ B	$0.65^{\pm0.04}\mathrm{B}$	$0.72^{\pm0.03} AB$	$0.95^{\pm0.04} A$	
			Cut 2				
0-0.10		$0.07^{\pm 0.02}$ D	$0.25^{\pm 0.04}$ B	$0.20^{\pm 0.0}$ C	$0.20^{\pm 0.0}$ C	$0.42^{\pm 0.03}$ A	
0.10-0.20	0	$0.07^{\pm 0.02}$ C	$0.15^{\pm 0.03}$ B	$0.18^{\pm0.02}\mathrm{B}$	$0.16^{\pm 0.01}$ B	$0.28^{\pm 0.01} \mathrm{A}$	
0.20-0.40		$0.15^{\pm 0.04}$ C	$0.49^{\pm 0.05}$ A	$0.27^{\pm 0.05} \mathrm{B}$	$0.25^{\pm 0.05} BC$	$0.25^{\pm 0.02} BC$	
0-0.40		$0.32^{\pm 0.07}$ C	$0.89^{\pm 0.04}$ A	$0.65^{\pm 0.06} \mathrm{B}$	$0.61^{\pm 0.05}$ B	$0.95^{\pm0.04} \mathrm{A}$	
0-0.10		$0.10^{\pm 0.04}$ C	$0.20^{\pm 0.04}$ B	$0.12^{\pm0.02}$ BC	$0.10^{\pm 0.04}$ C	$0.42^{\pm 0.03}$ A	
0.10-0.20	Q	$0.23^{\pm0.03}$ AB	$0.20^{\pm 0.03} BC$	$0.20^{\pm0.03}$ BC	$0.17^{\pm 0.05}$ C	$0.28^{\pm 0.01}$ A	
0.20-0.40	8	$0.29^{\pm0.05}$ ABC	$0.38^{\pm0.04}AB$	$0.37^{\pm 0.05}$ A	$0.27^{\pm 0.05} BC$	$0.25^{\pm 0.02}$ C	
0-0.40		$0.62^{\pm0.07}\mathrm{BC}$	$0.77^{\pm 0.06}$ B	$0.69^{\pm 0.09}$ B	$0.53^{\pm 0.08}$ C	$0.95^{\pm0.04} A$	
Cut3							
0-0.10		$0.11^{\pm 0.07}$ C	$0.28^{\pm 0.03}$ B	$0.19^{\pm0.05}$ BC	$0.19^{\pm 0.02} BC$	$0.42^{\pm 0.03}$ A	
0.10-0.20	0	$0.08^{\pm 0.03}$ B	$0.08^{\pm 0.02}$ B	$0.13^{\pm 0.02}$ B	$0.13^{\pm 0.02}$ B	$0.28^{\pm0.01} \mathrm{A}$	
0.20-0.40	0	$0.09^{\pm 0.06}$ C	$0.47^{\pm 0.09}$ A	$0.43^{\pm 0.06}$ A	$0.48^{\pm 0.13}$ A	$0.25^{\pm 0.02}$ B	
0-0.40		$0.28^{\pm 0.09}$ C	$0.83^{\pm 0.10}$ B	$0.75^{\pm0.08}$ B	$0.79^{\pm 0.13}$ B	$0.95^{\pm0.04} A$	
0-0.10		$0.08^{\pm 0.02}$ C	$0.18^{\pm 0.03}$ B	$0.19^{\pm 0.02}$ B	$0.18^{\pm 0.05}$ B	$0.42^{\pm 0.03}$ A	
0.10-0.20	8	$0.10^{\pm 0.01}$ C	$0.26^{\pm 0.04}$ A	$0.16^{\pm0.01}\mathrm{B}$	$0.16^{\pm 0.01}$ B	$0.28^{\pm0.01} \mathrm{A}$	
0.20-0.40		$0.41^{\pm0.06}$ AB	$0.35^{\pm0.05}AB$	$0.40^{\pm 0.09} \mathrm{A}$	$0.27^{\pm 0.06}$ B	$0.25^{\pm0.02}$ B	
0-0.40		$0.60^{\pm0.05}\mathrm{BC}$	$0.78^{\pm0.11} \mathrm{AB}$	$0.78^{\pm0.11}$ ABC	$0.61^{\pm 0.11}$ C	$0.95^{\pm0.04}$ A	

**Table 6**. Organic carbon stock extracted in hot water (Mg ha<sup>-1</sup>) under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum Etc) with different soil mulch rates compared to native vegetation.

Cut 1: after 90 days from sowing; cut 2: after 150 days from sowing; cut 3: after 210 days from sowing. Letters indicate differences between treatments by Tukey's test at 5% significance. ±Standard deviation of the mean.

#### 4.3.3. SOC stock in the stable reservoir

The stock of MAOC increased with the reduction of POC, showing higher values after the third cut. The highest concentrations were observed in the treatments with 8 Mg ha<sup>-1</sup> of mulch at the 80 and 100% irrigation depth (Figure 18).



**Figure 18**. MAOC stock under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum  $Et_c$ ), with different mulch rates (0 and 8 Mg ha<sup>-1</sup>) of the soil over three cuts of the sorghum crop

Cut 1: after 90 days from sowing; cut 2: after 150 days from sowing; cut 3: after 210 days from sowing. Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

In the first cutting, at 0-0.10 m, the treatments with 0 and 80% irrigation and 0 Mg ha<sup>-1</sup> of mulch were significantly higher compared to the same treatments with 8 Mg ha<sup>-1</sup> of mulch, showing 26 and 3% more MAOC, respectively. On the other hand, the treatments with 100 and 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch were significantly higher than the same irrigation depth with 0 Mg ha<sup>-1</sup> of mulch, with increases of 17 and 9% in MAOC stock, respectively. At 0.10-0.20 m, the treatments with 0 and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch, showing

32 and 47% more MAOC, respectively. Conversely, the treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> of mulch was significantly higher than 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch, with 16% more MAOC.

At 0.20-0.40 m, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch showed 17, 37, and 7% more MAOC, respectively, compared to the same irrigation depth with 0 Mg ha<sup>-1</sup> of mulch. However, the 120% treatment exhibited the opposite trend, with 30% more MAOC in the treatment with 0 Mg ha<sup>-1</sup> of mulch. A similar pattern was observed for the accumulated MAOC (0-0.40 m), with increases of 10, 18, and 21% in the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch, respectively, compared to the treatments with 0 Mg ha<sup>-1</sup> of mulch. In contrast, the 120% treatment with 0 Mg ha<sup>-1</sup> of mulch.

In the second sampling, at 0-0.10 m, the treatments with 80, 100, and 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch showed significantly higher MAOC stocks compared to the treatments with 0 Mg ha<sup>-1</sup>, with increases ofb7, 26, and 14%, respectively. On the other hand, the treatment with 0% irrigation and 0 Mg ha<sup>-1</sup> of mulch was significantly higher than the 0% treatment with 8 Mg ha<sup>-1</sup>, with 15% more MAOC. In the 0.10-0.20 m layer, the treatments with 0, 80, and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch also presented significantly higher MAOC stocks compared to the treatments with 0 Mg ha<sup>-1</sup>, with increases of 31, 8, and 42%, respectively. Conversely, the treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> of mulch was significantly higher than the 120% treatment with 8 Mg ha<sup>-1</sup>, with 18% more MAOC.

For the treatments with 0, 80, and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch, the increases in MAOC stock were 19, 42, and 14%, respectively, for the 0.20-0.40 m layer, and 14, 24, and 25%, respectively, for the accumulated 0-0.40 m stock. The 120% treatment with 0 Mg ha<sup>-1</sup> of mulch was significantly higher, with 25 and 11% more MAOC in the 0.20-0.40 m layer and the 0-0.40 m accumulated stock, respectively, compared to the 120% treatment with 8 Mg ha<sup>-1</sup>.

In the third sampling, at 0-0.10 m, the treatments with 80, 100, and 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch showed significantly higher MAOC stocks compared to the treatments with 80, 100, and 120% irrigation and 0 Mg ha<sup>-1</sup>, with increases of 7, 25, and 8%, respectively. On the other hand, the treatment with 0% irrigation and 0 Mg ha<sup>-1</sup> of mulch showed a 32% higher MAOC stock than the 0% treatment with 8 Mg ha<sup>-1</sup>. In the 0.10-0.20 m layer, the treatments with 0 and 100% irrigation and 8 Mg ha<sup>-1</sup>, with increases of 29 and 39%, respectively.

Conversely, the treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> of mulch was significantly higher than the 120% treatment with 8 Mg ha<sup>-1</sup>, with a 13% increase in MAOC stock.

In the 0.20-0.40 m layer, the treatments with 0, 80, and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch showed significantly higher MAOC stocks compared to the treatments with 0, 80, and 100% irrigation and 0 Mg ha<sup>-1</sup>, with increases of 13, 38, and 15%, respectively. However, the treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> of mulch showed 17% more MAOC compared to the treatment with 120% irrigation and 8 Mg ha<sup>-1</sup>. In the accumulated 0-0.40 m stock, the results followed the same trend observed in the 0.20-0.40 m layer. The treatments with 0, 80, and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch showed increases of 10, 19, and 24%, respectively, in MAOC stock compared to the same irrigation depth with 0 Mg ha<sup>-1</sup>. Conversely, the treatment with 120% irrigation and 0 Mg ha<sup>-1</sup> of mulch showed 8% more MAOC compared to the treatment with 120% irrigation and 8 Mg ha<sup>-1</sup>.

Compared to NV, the 80 and 100% irrigation depth demonstrated a significant potential for increasing C in this fraction, even exceeding it. In the 0-0.10 m layer, the 80% treatment with 0 Mg ha<sup>-1</sup> and the 80% treatment with 8 Mg ha<sup>-1</sup> of mulch showed 10 and 19% more MAOC, respectively, compared to NV. In the 0.10-0.20 m layer, the 80% treatment with 0 Mg ha<sup>-1</sup> of mulch and the 80 and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch exhibited increases of 18, 16, and 46%, respectively, relative to NV. In the 0.20-0.40 m layer, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch of NAOC, respectively, relative to NV. In the 0.20-0.40 m layer, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of MAOC, respectively, compared to NV. In the 0.20-0.40 m layer, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of MAOC, respectively, compared to NV. In the 0.20-0.40 m layer, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of MAOC, respectively, compared to NV. In the 0.20-0.40 m layer, the 0, 80, and 100% treatments with 8 Mg ha<sup>-1</sup> of MAOC, respectively, compared to NV.

For HU, at 0-0.10 m, the treatments with 80 and 100% irrigation and 8 Mg ha<sup>-1</sup> of mulch showed higher SOC stock values (p < 0.05), being significantly higher than the treatments with 0 and 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch, with 41 and 18% more C for the 80% irrigation treatment compared to 0 and 120%, and with 36 and 12% more C for the 100% irrigation treatment compared to 0 and 120%. In this layer, the treatments with 80 and 120% irrigation and 8 Mg ha<sup>-1</sup> of mulch were significantly higher than the treatments with 80 and 120% irrigation and 0 Mg ha<sup>-1</sup>, with 11 and 13% more carbon, respectively. The 0% treatment with 0 Mg ha<sup>-1</sup> of mulch was significantly higher than the 0% treatment with 8 Mg ha<sup>-1</sup>, with 18% more C (Table 7).

At depths of 0.10-0.20 m, the treatment with 100% mulch at 8 Mg ha<sup>-1</sup> was significantly higher compared to the 0, 80, and 120% treatments at 8 Mg ha<sup>-1</sup>, with increases of 55, 15, and 36% in C content, respectively. Only the 80 and 100% treatments with 8 Mg ha<sup>-1</sup> were significantly higher than the 80 and 100% treatments with 0 Mg ha<sup>-1</sup>, showing increases of 13 and 27%, respectively. In contrast, the 0 and 120% treatments with 0 Mg ha<sup>-1</sup> were significantly

higher than their counterparts with 8 Mg ha<sup>-1</sup>. At depths of 0.20-0.40 m, the 80% treatment with 8 Mg ha<sup>-1</sup> mulch exhibited significantly higher C content compared to the 0, 100, and 120% treatments with 8 Mg ha<sup>-1</sup>, with increases of 2.1, 1.2, and 2.4 times, respectively. The 80% treatment with 8 Mg ha<sup>-1</sup> was significantly higher than the 80% treatment with 0 Mg ha<sup>-1</sup>, with a 47% increase in C content. Conversely, the 0 and 120% treatments with 0 Mg ha<sup>-1</sup> were significantly higher than the 0 and 120% treatments with 8 Mg ha<sup>-1</sup>.

When considering the 0-0.40 m depth, the 80 and 100% treatments exhibited significantly higher C levels, with 27 and 18% more carbon, respectively, compared to the treatment without irrigation. Regarding HA, at the 0-0.10 m layer, the 80 and 100% treatments with 8 Mg ha<sup>-1</sup> of mulch were significantly higher than the 80 and 100% treatments with 0 Mg ha<sup>-1</sup>, showing increases of 39 and 11%, respectively. In contrast, the 120% treatment with 0 Mg ha<sup>-1</sup> was significantly higher than the 120% treatment with 8 Mg ha<sup>-1</sup>, with a 25% increase in C. At the 0.10-0.20 m layer, the 0 and 100% treatments with 8 Mg ha<sup>-1</sup> were significantly higher than the 0% and 100% treatments with 0 Mg ha<sup>-1</sup>, with increases of 79 and 46%, respectively. Conversely, the 80% and 120% treatment with 0 Mg ha<sup>-1</sup> was significantly higher than the 80 and 120% treatment with 8 Mg ha<sup>-1</sup>, with a 2 and 100%, respectively, increase in C. The HA content was particularly high at the 0-0.40 m layer in the treatment with 100% and 8 Mg ha<sup>-1</sup>, which showed 9.3 Mg ha<sup>-1</sup> of C, significantly higher than all other treatments (Table 7).

Depth	Mulch rate	Irrigation depths (%)					
(m)	Mg ha- $1$	0	80	100	120		
		HU					
0-0.10		$9.1^{\pm0.6}$ Ab	11.3 <sup>±1.3</sup> Ba	$10.4^{\pm0.6}$ Aab	$9.0^{\pm0.5}$ Bb		
0.10-0.20	0	$5.5^{\pm0.4}$ Ac	$9.9^{\pm 1.3}$ Ba	$9.9^{\pm0.4}$ Bab	$8.9^{\pm0.8}$ Ab		
0.20-0.40	0	$15.0^{\pm 1.0}$ Aa	$12.0^{\pm 0.4}$ Bb	$16.3^{\pm 0.8}$ Aa	$14.1^{\pm0.6}$ Aat		
0-0.40		27.5 <sup>±3.7</sup> Ab	33.2 <sup>±2.2</sup> Ba	34.6 <sup>±1.7</sup> Ba	32.2 <sup>±1.4</sup> Aa		
0-0.10		$7.4^{\pm 0.5}$ Bc	12.7 <sup>±0.9</sup> Aa	$11.6^{\pm0.9}$ Aab	$10.3^{\pm 0.7}$ Ab		
0.10-0.20	8	$6.0^{\pm0.5}$ Ad	$11.4^{\pm 1.2}$ Ab	13.5 <sup>±0.7</sup> Aa	$8.6^{\pm0.4}$ Ac		
0.20-0.40	0	$9.8^{\pm 1.2}$ Bc	$20.6^{\pm0.8}$ Aa	$17.0^{\pm 0.7}$ Ab	$8.6^{\pm 2.7}$ Bc		
0-0.40		$23.3^{\pm 1.2}$ Bb	45.3 <sup>±1.4</sup> Aa	42.0 <sup>±1.9</sup> Aa	27.2 <sup>±3.8</sup> Bb		
			Н	ÍA			
0-0.10		$1.4^{\pm0.3}$ Aab	1.1 <sup>±0.2</sup> Bb	$1.4^{\pm0.1}$ Ab	$1.8^{\pm 0.2}$ Aa		
0.10-0.20	0	$0.6^{\pm0.1}\mathrm{Bc}$	$1.7^{\pm 0.2}$ Aa	$1.3^{\pm0.5}$ Bab	$1.0^{\pm0.2}$ Abc		
0.20-0.40	0	$1.4^{\pm1.1}$ Ab	$1.1^{\pm0.4}$ Ab	$2.8^{\pm1.1}$ Ba	$0.2^{\pm 0.2}$ Bc		
0-0.40		$3.4^{\pm 1.1}$ Bb	3.9 <sup>±0.7</sup> Aab	$4.8^{\pm 0.3}$ Ba	$3.1^{\pm0.4}$ Ab		
0-0.10		$1.2^{\pm0.1}$ Ab	$1.8^{\pm 0.3}$ Aa	$1.5^{\pm0.3}$ Aab	$1.4^{\pm0.3}$ Bab		
0.10-0.20	8	$2.9^{\pm 0.3}$ Aa	$1.3^{\pm0.2}$ Bb	$2.4^{\pm0.3}$ Aa	$0.0^{\pm0.0}$ Bc		
0.20-0.40	0	$1.9^{\pm0.4}$ Ab	$1.5^{\pm0.5}$ Ab	$5.3^{\pm 1.0}$ Aa	$2.1^{\pm0.1}$ Ab		
0-0.40		$6.0^{\pm0.4}$ Ab	$4.5^{\pm0.9}$ Abc	9.3 <sup>±0.9</sup> Aa	$3.5^{\pm0.3}$ Ac		
			F	A			
0-0.10		1.0 <sup>±0.1ns</sup>	4.0 <sup>±0.4***</sup>	<u>A</u> $2.5^{\pm 0.2^{**}}$	2.3 <sup>±0.5**</sup>		
0.10-0.20	0	$2.4^{\pm 0.1}$ Aa	$2.3^{\pm0.4}$ Aa	$1.4^{\pm0.2}$ Bb	$2.3^{\pm0.1}$ Aa		
0.20-0.40	U	$1.7^{\pm 1.1}$ Ac	$4.7^{\pm0.2}$ Aab	$3.5^{\pm0.7}$ Ab	4.9 <sup>±0.4</sup> Aa		
0-0.40		$5.1^{\pm 1.3}$ B	$11.0^{\pm 0.7}$ A	$7.6^{\pm 0.4}$ B	$9.7^{\pm0.5}$ A		
0-0.10		$2.6^{\pm 0.1}*$	4.9 <sup>±0.9</sup>	4.1 <sup>±0.6</sup>	$4.6^{\pm 0.5}$		
0.10-0.20	8	$2.5^{\pm0.4}$ Ab	$2.3^{\pm 0.1}$ Ab	3.3 <sup>±0.2</sup> Aa	$2.2^{\pm0.0}$ Ab		
0.20-0.40	0	$1.8^{\pm0.4}$ Ac	$3.5^{\pm0.6}$ Bab	$4.3^{\pm0.4}$ Aa	$2.6^{\pm0.4}$ Bbc		
0-0.40		$6.9^{\pm0.7}$ Ac	$10.7^{\pm 1.5}$ Aab	$11.7^{\pm 0.9}$ Aa	$8.8^{\pm1.5}$ Abc		

**Table 7**. Carbon stock (Mg ha<sup>-1</sup>) of humin (HU). humic acid (HA) and fulvic acid (FA) fractions under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum) Etc) with different soil mulch rates

Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates. according to the Tukey test at 5% significance. \*\*\* significant effect for 80% depth; \*\* significant effect for 100 and 120% depth; \* significant effect for the soil mulch rate of 8 Mg ha<sup>-1</sup>;  $\pm$ Standard deviation of the mean.

At 0-0.10 m, a significant effect of mulch on FA was observed, with the use of 8 Mg  $ha^{-1}$  contributing substantially to increased C in this fraction. The 0, 80, 100, and 120% treatments with 8 Mg  $ha^{-1}$  showed increases of 60, 18, 39, and 50% in FA compared to the treatments with 0 Mg  $ha^{-1}$ . Additionally, the 80, 100, and 120% treatments with 8 Mg  $ha^{-1}$ 

showed significantly higher values than the 0% treatment with 8 Mg ha<sup>-1</sup>, with increments of 48, 38, and 45%, respectively. At 0.10-0.20 m, the 100% treatment with 8 Mg ha<sup>-1</sup> exhibited the highest FA values, surpassing the 0, 100, and 120% treatments with 8 Mg ha<sup>-1</sup>, with increases of 23, 28, and 32%, respectively. It was also significantly higher than the 100% treatment with 0 Mg ha<sup>-1</sup>, with a 58% increase in C. At 0.20-0.40 m, the 80 and 120% treatments with 0 Mg ha<sup>-1</sup> exhibited significantly higher FA values compared to the 80 and 120% treatments with 8 Mg ha<sup>-1</sup>, with increases of 26 and 46%, respectively. Within this layer, the 80 and 120% treatments with 0 Mg ha<sup>-1</sup>, with increases of 64 and 24% for the 80% irrigation treatment, and 66 and 27% for the 120% irrigation treatment, respectively. Finally, considering the 0-0.40 m depth, the 100% treatment with 8 Mg ha<sup>-1</sup>, on the contrary, the 100% treatment with 0 Mg ha<sup>-1</sup> (Table 7).

The values for HU were significantly higher compared to NV at all depths. At 0-0.10 m, the treatments with 0 Mg ha<sup>-1</sup> of mulch in the 0, 80, 100, and 120% irrigation depth showed increases of 3.0, 3.6, 3.3, and 3.0 times, respectively, compared to NV. The treatments with 8 Mg ha<sup>-1</sup> in the same irrigation depth showed increases of 2.4, 4.1, 3.7, and 3.3 times compared to NV. At 0.10-0.20 m, the treatments with 0 Mg ha<sup>-1</sup> in the 0, 80, 100, and 120% depth presented increases of 1.9, 3.4, 2.7, and 3.0 times, respectively, compared to NV, while the treatments with 8 Mg ha<sup>-1</sup> in the same depth showed increases of 2.0, 3.9, 4.6, and 3.0 times. At 0.20-0.40 m, the treatments with 0 Mg ha<sup>-1</sup> in the 0, 80, 100, and 120% depth presented increases of 6.8, 4.8, 6.5, and 5.6 times, respectively, compared to NV, while the treatments with 8 Mg ha<sup>-1</sup> in the same depth showed increases of 3.9, 8.2, 6.8, and 3.4 times. For HA and FA values, none of the treatments surpassed the values observed in NV (Table 8).

Depth	Mulch rate	Irrigation depths (%)						
(m)	Mg ha- $1$	0	80	100	120	NV		
	HII							
0-0.10		9.1 <sup>±0.6</sup> B	$11.3^{\pm 1.3}$ A	$10.4^{\pm 0.6} AB$	$9.0^{\pm 0.5}$ B	$3.1^{\pm 0.4}$ C		
0.10-0.20	0	$5.5^{\pm0.4}$ C	$9.9^{\pm 1.3}$ A	$7.9^{\pm0.4}$ B	$8.9^{\pm0.8}$ AB	$2.9^{\pm0.8}$ D		
0.20-0.40	0	$15.0^{\pm 1.0}$ AB	$12.0^{\pm0.4}$ C	$16.3^{\pm 0.8}$ A	$14.1^{\pm 0.6}$ B	$2.5^{\pm0.4}$ D		
0-0.40		$27.5^{\pm 3.7}$ B	$33.2^{\pm 2.2}$ A	$34.6^{\pm 1.7}$ A	32.2 <sup>±1.4</sup> AB	8.6 <sup>±1.3</sup> C		
0-0.10		$7.4^{\pm 0.5}$ C	$12.7^{\pm 0.9}$ A	$11.6^{\pm 0.9}$ AB	$10.3^{\pm 0.7}$ B	$3.1^{\pm 0.4}$ D		
0.10-0.20	8	$6.0^{\pm0.5}$ D	$11.4^{\pm 1.2}$ B	$13.5^{\pm 0.7}$ A	$8.6^{\pm 0.4}$ C	$2.9^{\pm 0.8}$ E		
0.20-0.40	0	$9.8^{\pm 1.2}$ C	$20.6^{\pm0.8} \mathrm{A}$	$17.0^{\pm0.7}$ B	$8.6^{\pm 2.7}$ C	$2.5^{\pm0.4}$ D		
0-0.40		$23.3^{\pm 1.2}$ B	$45.3^{\pm 1.4}$ A	42.0 <sup>±1.9</sup> A	$27.2^{\pm 3.8}$ B	8.6 <sup>±1.3</sup> C		
	НА							
0-0.10		$1.4^{\pm 0.3}$ B	$1.1^{\pm0.2}$ B	$1.4^{\pm 0.1}$ B	$1.8^{\pm0.2}$ B	$5.8^{\pm0.4}$ A		
0.10-0.20	0	$0.6^{\pm 0.1}$ C	$1.7^{\pm 0.2}$ B	$1.3^{\pm0.5}$ BC	$1.0^{\pm0.2}$ BC	$4.5^{\pm0.6}$ A		
0.20-0.40	0	$1.4^{\pm 1.1}$ C	$1.1^{\pm 0.4}$ C	$2.8^{\pm 1.1}$ B	$0.2^{\pm 0.2}$ C	$4.5^{\pm 0.1}$ A		
0-0.40		$3.4^{\pm 1.1}$ BC	3.9 <sup>±0.7</sup> BC	$4.8^{\pm 0.3}$ B	$3.1^{\pm 0.4}$ C	$14.8^{\pm0.7}$ A		
0-0.10		$1.2^{\pm 0.1}$ B	$1.8^{\pm 0.3}$ B	$1.5^{\pm 0.3}$ B	$1.4^{\pm 0.3}$ B	$5.8^{\pm0.4}$ A		
0.10-0.20	8	$2.9^{\pm 0.3}$ B	$1.3^{\pm 0.2}$ C	$2.4^{\pm0.3}$ B	$0.0^{\pm0.0}$ D	$4.5^{\pm 0.6}$ A		
0.20-0.40		$1.9^{\pm 0.4}$ B	$1.5^{\pm0.5}$ B	$5.3^{\pm 1.0}$ A	$2.1^{\pm0.1}B$	$4.5^{\pm 0.1}$ A		
0-0.40		$6.0^{\pm0.4}$ C	$4.5^{\pm0.9}$ D	9.3 <sup>±0.9</sup> B	$3.5^{\pm 0.3}$ D	$14.8^{\pm 0.7}$ A		
	FA							
0-0.10		$1.0^{\pm 0.1}$ D	$4.0^{\pm0.4}$ B	$2.5^{\pm 0.2}$ C	2.3 <sup>±0.5</sup> CD	5.9 <sup>±1.3</sup> A		
0.10-0.20	0	$2.4^{\pm 0.1}$ B	$2.3^{\pm0.4}$ B	$1.4^{\pm 0.2}$ C	$2.3^{\pm0.1}B$	$5.4^{\pm0.8}$ A		
0.20-0.40	0	$1.7^{\pm 1.1}$ B	$4.7^{\pm 0.2}$ A	$3.5^{\pm 0.7}$ A	$4.9^{\pm 0.4}$ A	$4.1^{\pm 0.3}$ A		
0-0.40		$5.1^{\pm 1.3}$ D	$11.0^{\pm 0.7}$ B	$7.6^{\pm0.4}$ C	$9.7^{\pm 0.5}$ B	$15.3^{\pm 1.3}$ A		
0-0.10		$2.6^{\pm 0.1}$ C	4.9 <sup>±0.9</sup> AB	$4.1^{\pm 0.6}$ BC	$4.6^{\pm0.5}$ AB	$5.9^{\pm 1.3}$ A		
0.10-0.20	8	$2.5^{\pm0.4}$ BC	$2.3^{\pm0.1}$ BC	$3.3^{\pm0.2}$ B	$2.2^{\pm 0.0}$ C	$5.4^{\pm0.8}$ A		
0.20-0.40	ð	$1.8^{\pm 0.4}$ C	$3.5^{\pm0.6}AB$	$4.3^{\pm 0.4}$ A	$2.6^{\pm0.4}$ BC	$4.1^{\pm 0.3}$ A		
0-0.40		$6.9^{\pm 0.7}$ C	$10.7^{\pm 1.5}$ B	$11.7^{\pm 0.9}$ B	$8.8^{\pm1.5}$ BC	$15.3^{\pm 1.3}$ A		

**Table 8**. Carbon stock (Mg ha<sup>-1</sup>) of humin (HU). humic acid (HA) and fulvic acid (FA) fractions under different irrigation depths with reused water (0, 80, 100 and 120% of sorghum Etc) with different soil mulch rates compared to native vegetation (NV)

Letters indicate differences between treatments by Tukey's test at 5% significance. ±Standard deviation of the mean.

## 4.3.4. Crop productivity

For the forage cactus, the treatments irrigated with 100 and 120% of crop evapotranspiration (ETc) and 8 Mg ha<sup>-1</sup> of mulch showed higher productivity values (p<0.05), with 9 and 35% more dry matter, respectively, compared to the treatments irrigated with 100

and 120% of ETc and 0 Mg ha<sup>-1</sup> of mulch. In the treatments with 0 Mg ha<sup>-1</sup> of ground soil mulch, the 80 and 100% irrigation depth exhibited significantly higher productivity than the 120% irrigation depth (Figure 19)

Regarding sorghum, in the first cutting, all treatments with 8 Mg ha<sup>-1</sup> of mulch were significantly higher compared to the treatments with 0 Mg ha<sup>-1</sup> of mulch, with 3.2, 1.3, and 4.3 Mg ha<sup>-1</sup> more dry matter, corresponding to 47, 18, and 49% more productivity for the 80, 100, and 120% irrigation depth, respectively. The treatment irrigated with 120% and 8 Mg ha<sup>-1</sup> was significantly higher than the treatments irrigated with 80 and 100% and 8 Mg ha<sup>-1</sup>, with 23 and 17% more dry matter, respectively.

In the second cutting, the treatments irrigated with 80 and 120% and 8 Mg ha<sup>-1</sup> showed a significant increase of 35 and 42% in dry matter (1.9 Mg ha<sup>-1</sup>) compared to the treatments irrigated with 80 and 120% and 0 Mg ha<sup>-1</sup> of mulch. Among the treatments with 8 Mg ha<sup>-1</sup> of mulch, the treatment irrigated with 80% was significantly higher than the treatments irrigated with 100 and 120%, with 22 and 15% more dry matter, respectively.

In the third cutting, all treatments with 8 Mg ha<sup>-1</sup> of mulch showed a significant increase of 2.7, 5.3, and 2.4 Mg ha<sup>-1</sup> of dry matter (DM), corresponding to 36, 37, and 24% more DM for the 80, 100, and 120% irrigation depth, respectively, compared to the treatments with 0 Mg ha<sup>-1</sup> of mulch. Among the treatments with 8 Mg ha<sup>-1</sup>, the treatment irrigated with 100% was significantly higher than the treatments irrigated with 80 and 120%, with 48 (6.8 Mg ha<sup>-1</sup> of DM) and 30% (4.3 Mg ha<sup>-1</sup> of DM) more, respectively (Figure 19).

**Figure 19**. Dry matter of forage cactus (a) and dry matter of sorghum under different irrigation depth in three cutting seasons: (b)-after 90 days of sowing, (c)-after 150 days of sowing, (d)-after 210 days of sowing



0 Mg ha<sup>-1</sup>= without mulch; 8 Mg ha<sup>-1</sup>= with mulch. Lowercase letters indicate differences between the depth and uppercase letters indicate differences between the soil mulch rates, according to the Tukey test at 5% significance.

#### 4.4. Discussion

#### 4.4.1. SOC stocks

This study reported the considerable effect of combining domestic wastewater reuse with mulch on SOC levels and soil C pools in the tropical semiarid region. The effects of these practices were observed both in the surface layers (0-0.10 and 0.10-0.20 m) and the subsurface (0.20-0.40 m). Furthermore, it was observed that total SOC stocks and the MAOC and HU fractions exceeded the stocks of NV. Land use changes strongly impact SOC stocks when compared to NV (LACERDA et al., 2023; SÁNCHEZ-GONZÁLEZ et al., 2017). Besides, higher SOC stocks were observed in the 0.20-0.40 m layer, which exceeded the NV values, due to the translocation of soil carbon fractions, such as MAOC and HWEO-C, and higher SOC rhizodeposition by deep rooting systems of forage grass (sorghum) along the profile. The sandy texture of the soil favors this transport, and the deeper layers tend to present a greater carbon deficit, which may contribute to its accumulation at depth (DE OLIVEIRA FERREIRA et al., 2021; SÁ et al., 2022).

The highest SOC stocks observed in the treatments irrigated with 80 and 100% of ETc with 8 Mg ha<sup>-1</sup> of mulch are associated with the addition of soil mulch and the optimal irrigation rates, which favored the decomposition of residues and maintained SOC stocks in the soil. Furthermore, the absence of soil preparation after implementing the management practices may have favored the formation of aggregates that physically protect soil carbon. The crops used in the consortium (cactus and sorghum) also contribute to SOC stocks, either through root exudation or the higher root density in the surface and subsurface layers (LACERDA et al., 2023; TOMAZ et al., 2024).

The continuous use of irrigation with reused water over the long term may cause a cumulative effect on SOC stocks, due to the high concentrations of organic compounds in this water, mainly contributing to the formation of more stable components of soil organic matter, such as MAOC and HU (GARCIA-ORENES et al., 2015; LIU et al., 2021; LIANG et al., 2014; WELDEWAHID et al., 2023). Specifically, irrigation with reused water made a significant contribution to SOC stocks, providing 0.0422, 0.0528, and 0.0635 Mg ha<sup>-1</sup> of C for the 80, 100, and 120% irrigation depths, respectively, over the sorghum cycles. This input via reused water is a sustainable solution for C storage in soils of the Brazilian semiarid region, which naturally experiences water deficits and extreme climatic conditions.

The deficit irrigation depth, in addition to contributing to the maintenance of SOC stocks, also enables water savings through drip irrigation (SONI et al., 2021). Furthermore, the complementary effect between irrigation and mulch is a practice that contributes to SOC sequestration in soils that are naturally characterized by low organic matter input (CHATTERJEE et al., 2018; KUMAR et al., 2024). In a study using drip irrigation combined with soil mulch in maize cultivation for just two years, there was a 6.4% increase in SOC (CHATTERJEE et al., 2018). In this work, the treatments 80 and 100% combined with mulch exceeded NV by 15 and 19%, respectively. In relation to the treatment 0% without mulch, this increase was 61 and 66%, respectively. In systems where these practices are adopted over the long term, they further contribute to increasing SOC stocks and improving soil quality (ZONG et al., 2023).

On the other hand, soils in the Caatinga biome naturally tend to have low SOC accumulation due to climatic conditions (high temperatures, poor temporal distribution of rainfall, and high evapotranspiration) and the characteristics of the Caatinga vegetation, predominantly composed of deciduous species, which results in low biomass production and high rates of soil organic matter decomposition, negatively impacting SOC accumulation (MONROE et al., 2021). Therefore, the provision of biomass through mulch, combined with

irrigation using reused water in the cactus-sorghum consortium, contributes to increasing SOC stocks and the various C pools. The plant roots and their exudates can aggregate soil particles, leading to the formation of macroaggregates (TOMAZ et al., 2024). Furthermore, improvements in the microbial community may occur, which influence aggregation through the production of extracellular polysaccharides, enzymes, and fungal mycelium (CHATTERJEE et al., 2018; KUMAR et al., 2024; LIU et al., 2021).

In the treatments without irrigation, with or without mulch application, lower SOC were observed, which can be attributed to both the reduced organic matter input and the absence of crops that typically contribute root biomass. Additionally, the lack of soil moisture, coupled with high temperatures, hinders SOC sequestration in semiarid regions, conditions that limit the formation and stabilization of organic compounds in the soil (CHEN et al., 2023; ZHOU et al., 2023).

# 4.4.2. Changes in labile and stable SOC reservoirs following the use of reused water combined with soil mulch

The labile SOC reservoirs, such as POC, POX-C, and HWEO-C, decompose relatively easily and exhibit higher turnover rates compared to more recalcitrant reservoirs. These fractions are involved in the biogeochemical transformation of nutrients such as N, P, and S and contribute to soil structure and stabilization. Furthermore, they respond more rapidly to management practices, making them valuable as early and sensitive indicators of changes in soil quality (LIANG et al., 2014). In particular, the present study demonstrated that reused water combined with mulch application significantly increased the levels of POC and POX-C compared to the non-irrigated plots.

The higher POC values observed in the treatments with 8 Mg ha<sup>-1</sup> may be associated with enhanced soil structuring and the formation of macroaggregates (NISAR; BENBI, 2024; ZONG et al., 2023). The positive effect of surface mulch on POC can be attributed to the improved stability of macroaggregates, greater C input through root systems, and, as POC is derived from the decomposition of these inputs, its short-term protection is largely controlled by stabilization within larger aggregates (DE OLIVEIRA FERREIRA et al., 2018; SIX et al., 2004). Furthermore, the use of surface mulch increases microbial activity, with microorganisms involved in the decomposition and processing of plant residues, with C from the decomposed material playing an active role in the formation of POC.

The sorption or fixation of fractions of the residue or partially oxidized material to the surfaces of minerals accelerates the formation of MAOC. Thus, the availability of C via mulch intensifies microbial activity, contributing to the increase of MAOC (BRIEDIS et al., 2012; LATIF VIRK et al., 2021). Moreover, drip irrigation can contribute to the transformation of POC into MAOC, with POC being a significant pathway for SOC sequestration, particularly in sandy soils (NISAR; BENBI, 2024). In contrast, the absence of mulch in treatments with 0 Mg ha<sup>-1</sup> may lead to soil structure disruption, reducing the physical protection of aggregates and favoring water and wind erosion processes, which expose C to oxidation and increase CO<sub>2</sub> emissions (LATIF VIRK et al., 2021).

These changes in POC stocks and the increase in MAOC across the sorghum cycles may directly influence the composition of the soil microbial community, which can shift from copiotrophic prokaryotes to oligotrophic ones, which act on the decomposition of labile and recalcitrant SOC, respectively. It is known that there is a high correlation between MAOC stocks and the relative abundance of Bacteroidetes and Basidiomycota, indicating that the increase in the oligotrophic community contributes to SOC stabilization (LIU et al., 2024). On the other hand, the use of reused water may alter the microbial community towards prokaryotes dominated by copiotrophs, which also contribute to SOC sequestration and are associated with the nutrient availability provided by reused water (SOMENAHALLY et al., 2023).

Nisar and Benbi found a small contribution of MAOC to the stabilization of SOC stocks, which contrasts with the results obtained in this study. To some extent, the sandy loam texture of the soil has a limited potential to form organomineral complexes, considering that MAOC is formed through the progressive fragmentation of POC into microbial byproducts, with intimate associations with mineral particles. Therefore, the formation of MAOC would be determined by the quantity of silt and clay particles. However, this study exhibited higher MAOC stocks, which may not be related to the quantity of silt and clay, but rather to the quality of their constituents. For example, 2:1 clay with high surface area, such as smectites, common in poorly weathered soils of the Semiarid region, can adsorb large molecules via cationic bridges, such as Ca<sup>2+</sup>, between negative charges on clay surfaces and negatively charged functional groups of organic matter, particularly carboxyl groups. Furthermore, aromatic C can form micro-sites that are hydrophobic, hindering decomposition and stimulating greater accumulation of organic matter. Positively charged edges of 2:1 clay minerals can also complex organic matter directly through rapid ligand exchange (STONER et al., 2023; WATTEL-KOEKKOEK et al., 2001).

Compared to POC, the POX-C fraction, also labile, exhibited a shorter turnover time. This fraction is primarily composed of easily oxidizable C compounds that can be strongly influenced by different land uses and management practices. When compared to NV values, the POX-C levels were significantly lower, indicating that irrigation has contributed to its mineralization and transformation into more stable C forms. Although mulch has some effect on this fraction, irrigation exposes POX-C to microorganisms, accelerating its oxidation due to its low recalcitrance (MANDAL; TOOR; DHALIWAL, 2020). In contrast, the POC fraction, due to its greater association with mineral fractions of various sizes, is less susceptible to rapid decomposition. The input of biomass via mulch plays an important role in the stability of microaggregates within macroaggregates that protect POC (DE OLIVEIRA FERREIRA et al., 2018; MANDAL; TOOR; DHALIWAL, 2020). Furthermore, the observed reduction in POX-C may be associated with the increase in non-labile fractions and the high C/N ratio (25:1) of the mulch material used, which may contain high levels of lignin and cellulose (CHATTERJEE et al., 2018).

The continuous reduction in POC levels with depth may be linked to the gradual decrease in the input of mulch, root biomass, and other forms of organic matter. On the other hand, the reduction in POX-C with depth can mainly be explained by the presence of more recalcitrant C compounds, such as lignin, suberin, and tannin, in the deeper layers (MANDAL; TOOR; DHALIWAL, 2020). These compounds are more closely related to the root system of the crops than to their aerial parts (MANDAL; TOOR; DHALIWAL, 2020).

Regarding the HWEO-C fraction, the highest concentrations observed at depth are related to the movement of this fraction, driven by the water flow provided via irrigation, which promotes the leaching of soluble C. Additionally, the high temperatures (annual averages of 25°C) of the Semiarid region also favor this process. Leaching enhances the transformation of soluble C into C associated with minerals due to its sorption onto the surfaces of clay minerals in the soil (NÚÑEZ et al., 2022). Reused water can contribute to this fraction by providing dissolved C and promoting microbial activity, stimulating the synthesis of carbohydrates, amides, and enzymes that constitute this fraction. Moreover, the exudates and lysates produced by the crops' roots can further contribute to the increase in HWEO-C (GHANI; DEXTER; PERROTT, 2003; HAYNES; BEARE, 1997).

In relation to humic substances, the SOC stocks in the HU fraction stood out. The treatment that stood out the most was the 80% irrigation level with 8 Mg ha<sup>-1</sup> across all depths studied (0-0.10, 0.10-0.20, and 0.20-0.40 m). Other treatments showed some variability across the studied layers, with some outpacing those without mulch. The predominance of the humin fraction in all treatments indicates an advanced degree of humification of soil organic matter (SOM), as humin is the most stable fraction of humic substances, with high resistance to

decomposition due to complexation with metal ions and/or clay-humic complexes (ROSSET et al., 2024). Due to these characteristics, HU is resistant to changes resulting from soil management (ALMEIDA et al., 2021; DE OLIVEIRA et al., 2022). It is noteworthy that the humin stock in the NV was significantly lower compared to the other treatments, with 80 and 100% irrigation depth with 8 Mg ha<sup>-1</sup> showing 76 and 73% more C in this fraction than NV, indicating that the use of mulch at 8 Mg ha<sup>-1</sup> associated with reused water continues to contribute to C storage in this fraction (TIWARI et al., 2023). Furthermore, in the experimental area, reused water has been used for irrigation for ten years, contributing to the increase of SOC stock in the HU fraction over time (Tables 7 and 8).

In general, higher stocks of the fulvic acid (FA) fraction were found in relation to humic acids (HA), except for NV, where the values were similar for both fractions and significantly higher than those observed in the experimental area. The supply of water through irrigation may accelerate the decomposition of mulch, which could have contributed to the higher FA concentrations compared to HA. The FA fraction has greater mobility than the others and contributes significantly to SOC over short periods (LIRA JÚNIOR et al., 2020). Additionally, the root systems of sorghum and forage cactus contribute to the increase in C concentrations in various SOC fractions (JAOUADI et al., 2019).

Another aspect to consider is that the semiarid conditions and the exposure of SOM to oxidation from cultivation practices accelerate decomposition and mineralization, contributing to the reduction of HA stocks (BEM MBAREK et al., 2024). In contrast, higher HA concentrations were observed in soils under conservation practices compared to conventional tillage (DATTA et al., 2022). Although HA concentrations are lower than those of FA, the adoption of irrigation and mulch contributed to the formation of this fraction, particularly at the 100% irrigation depth. HA plays a crucial role in maintaining soil fertility and nutrient availability, as well as contributing to the formation of stable aggregates, especially in soils with high Ca<sup>2+</sup> content, enhancing water retention, aeration, porosity, and permeability (DATTA et al., 2022; TIWARI et al., 2023). Furthermore, HA and FA fractions are less stable and subject to processes of polymerization and mineralization, moving within the soil profile, which reduces their quantities (ROSSET et al., 2024).

## 4.4.3. Crop productivity after the use of reused water associated with soil mulching

In general, the use of mulching associated with irrigation using reused water resulted in higher productivity for the cactus and forage sorghum crops, indicating that this practice contributes to increased biomass production in the aerial parts and is recommended for semiarid regions, particularly in areas where the soil is prone to intense erosive processes. In addition to providing greater water-use efficiency, reduced evaporation, and more stable soil temperature fluctuations, these factors may contribute to increased productivity (ARAÚJO FILHO et al., 2023; CARVALHO et al., 2021).

Regarding the sorghum crop, there was variability across the cuts, with the treatments at 120, 80, and 100% with 8 Mg ha<sup>-1</sup> of mulching being significantly higher for the first, second, and third cuts, respectively. These results suggest that the association of irrigation with reused water may have enhanced the productivity of the crops. Carvalho et al. (2021) observed an increase in sorghum productivity when using 140% of evapotranspiration (ETc) for irrigation with reused water and a 24% increase in the productivity of *Sorghum sudanense* due to the use of mulching in the Brazilian semiarid region. On the other hand, Araujo et al. (2023) reported that soil mulching did not significantly influence productivity in the intercropping of cactus with millet in the semiarid region, due to the rapid decomposition influenced by high temperatures (average of 26.9°C) and the constant moisture supply through irrigation and rainfall.

Regarding the forage cactus, the use of mulching favored productivity, and it was observed that the treatments with 100 and 120% of 8 Mg ha<sup>-1</sup> of mulching were more efficient than those with 0 Mg ha<sup>-1</sup> of mulching. Lemos et al. (2021) reported that irrigating the forage cactus (*Opuntia tuna L. Mill*) with reused water is viable in terms of productivity, yielding three times more labile biomass than growing cactus under rainfed conditions. In terms of dry matter, productivity was observed at around 70 Mg ha<sup>-1</sup> per year under irrigation with reused water and 44 Mg ha<sup>-1</sup> per year under rainfed conditions (LEMOS et al., 2021).

The adoption of intercropping between crops may have favored the productivity values obtained due to the water compensation characteristics provided by the distinct photosynthetic metabolisms of cactus (CAM) and sorghum (C4), which operate in gas exchange processes at night and during the day, respectively, resulting in lower water loss (ARAÚJO JÚNIOR et al., 2023). Jardim et al. (2021) reported that the forage cactus-sorghum intercropping system in the semiarid region was 47% and 3.5 times more productive in fresh and dry biomass, respectively, compared to monoculture cactus. This practice may also enhance C input into the soil by improving nutrient, water, and solar radiation use efficiency (CHIMONYO; MODI; MABHAUDHI, 2018; DINIZ et al., 2017; LIMA et al., 2018; SALVADOR et al., 2024).

## 4.5. Conclusion

The association of reused water with mulching contributed to the increase in SOC stocks and the more stable pools of SOC within the SOM. The highest SOC stock were observed in treatments with 8 Mg ha<sup>-1</sup> of mulching, associated with 80 and 100% ETc irrigation for sorghum, particularly in the mineral-associated fraction and humic substances, with a notable increase in the humin fraction. This ensures greater protection and stability of SOC stock over time.

The use of mulching combined with reused water boosted the productivity of the cactus and sorghum intercropping system. For forage cactus, treatments with 100 and 120% of 8 Mg ha<sup>-1</sup> showed the highest productivity, as well as 80% with and without the use of mulch on the soil. For sorghum, there was variability in water requirements throughout the crop cycle, with higher productivity observed in the following order: 120% irrigation in the first cut, 80% in the second cut, and 100% in the third cut, all associated with 8 Mg ha<sup>-1</sup>. This suggests that the combined adoption of irrigation with reused water and mulching promotes aboveground biomass production, ensuring sustainability for agricultural systems.

Thus, the adoption of 8 Mg ha<sup>-1</sup> of mulching combined with the use of reused water, especially at irrigation depths of 80 and 100% of the ETc of sorghum, contributes to carbon sequestration in the tropical semiarid region and increases the productivity of forage crops. Therefore, this practice can be a low-cost and easily implementable technology, viable for adoption by farmers.

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## **5. FINAL CONSIDERATIONS**

The findings of this study make a significant contribution to the advancement of lowcarbon agriculture in the Brazilian semiarid region, providing valuable insights that support the achievement of the Sustainable Development Goals (SDGs). The production of high-quality forage offers producers a reliable, year-round source of animal feed. In this context, irrigation with reused water plays a crucial role in ensuring consistent production, particularly during periods of drought.

In addition to irrigation with wastewater, the adoption of soil mulching plays a pivotal role in maintaining and enhancing soil quality. It contributes to the increase of total carbon stocks and their storage across various soil organic matter reservoirs. Furthermore, mulching helps conserve moisture, reduce erosion, improve soil structure, and promote nutrient cycling, all of which are essential for sustaining soil health and boosting agricultural productivity over time.

This study offers a comprehensive database for future research on carbon storage across different compartments of soil organic matter in the Brazilian semi-arid region, thereby supporting the development of conservation practices that enhance carbon sequestration in these areas. Additionally, it provides valuable insights into the decomposition process of plant residues and their role in fostering the growth and diversity of the soil microbial community.