

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL**

**SEWAGE SLUDGE-BASED FERTILIZER AND ITS EFFECTS
ON SOIL MICRONUTRIENTS AVAILABILITY AND CARBON
QUANTITY AND QUALITY IN A NO-TILLAGE SYSTEM IN
THE BRAZILIAN CERRADO**

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THE BRAZILIAN CERRADO**

Luana Corrêa Silva

Orientador: Prof. Dr. Thiago Assis Rodrigues Nogueira

Coorientadora: Aline Renée Coscione

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Agronomia (Ciência do Solo).

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IMPACTO ESPERADO NA SOCIEDADE

A pesquisa representa um avanço para a agricultura tropical ao demonstrar que o composto de lodo de esgoto (CSS) é uma alternativa viável, segura e eficiente como fertilizante orgânico no Cerrado. Quando utilizado em sistema plantio direto (SPD), o CSS supre adequadamente Cu, Mn e Zn sem riscos de toxicidade ou contaminação, ao mesmo tempo em que aumenta o sequestro e a estabilidade do carbono no solo. Essa combinação melhora atributos do solo, favorece a produtividade de culturas como capim, trigo e soja e converte um resíduo urbano em um insumo agrícola valioso. Em escala global, o estudo valida uma solução de economia circular que contribui para a sustentabilidade agrícola (ODS 2 e 12), a mitigação das mudanças climáticas via acúmulo de carbono (ODS 13) e a conservação dos ecossistemas terrestres (ODS 15).

EXPECTED IMPACT ON SOCIETY

This research represents a significant advancement for tropical agriculture by demonstrating that sewage sludge compost (CSS) is a viable, safe, and efficient organic fertilizer for use in the Brazilian Cerrado. When applied under no-tillage systems (NTS), CSS adequately supplies essential micronutrients (Cu, Mn, and Zn) without causing toxicity to plants or posing risks of contamination along the food chain. Concurrently, the CSS–NTS combination enhances soil carbon sequestration and carbon stability, reducing the soil carbon saturation deficit and indirectly contributing to climate change mitigation. These improvements translate into better soil attributes and increased productivity of major Cerrado crops such as pasture grass, wheat, and soybean. At a global scale, this study validates a circular-economy solution that transforms urban waste into an agricultural asset (SDG 12), strengthens sustainable agriculture and food security (SDG 2), promotes climate mitigation through soil carbon accumulation (SDG 13), and supports stable carbon dynamics in terrestrial ecosystems (SDG 15).

CERTIFICADO DE APROVAÇÃO

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Câmpus de Jaboticabal



Jaboticabal, 02 de março de 2026.

DADOS CURRICULARES DA AUTORA

Luana Corrêa Silva – brasileira, nascida em 22 de novembro de 1995, natural de São Luís, Maranhão, filha de Diana do Socorro Serrão Corrêa e Marconi de Jesus Chaves Silva. Em 2011, iniciou o ensino médio técnico em informática no Instituto Federal de Rondônia – campus Ariquemes IFRO. Em 2014, concluiu o ensino médio no Colégio Maria Aragão em São Luís, Maranhão. Em 2015, ingressou no Curso de Engenharia Agrônômica na Universidade Estadual do Maranhão – UEMA. Durante a graduação foi bolsista de Iniciação Científica na área de Microbiologia do solo (2014–2018). Em 2019, foi monitora na disciplina de Melhoramento Genético Vegetal. Desenvolveu pesquisa com micorrizas e suas associações a plantas de cobertura e adubação verde, defendeu seu TCC com “Fungos micorrízicos arbusculares em ambientes dunares da Ilha de Upaon Açu: diversidade e atividade”. Graduiu-se em 2020. No mesmo ano ingressou no curso de mestrado no Programa de Pós-Graduação em Agronomia – Ciência do Solo na área de Biologia do Solo na Universidade Federal Rural do Rio de Janeiro UFRRJ. Obteve a titulação de Mestre em 2023, com sua dissertação intitulada “Caracterização in vitro de linhagens de fungos endofíticos de raízes de cana-de-açúcar dos gêneros *Periconia* e *Trichoderma*”. No ano de 2022, iniciou o curso de Doutorado pelo programa de Pós-graduação na Unesp/FCAV, desenvolvendo pesquisas sobre dinâmica de micronutrientes e carbono em solos do Cerrado sob aplicação de composto de lodo de esgoto.

“Em tudo daí graças, porque esta é a vontade de Deus em Cristo Jesus para convosco.”

1 Tessalonicenses 5:18

"Por vezes, sentimos que aquilo que fazemos não é, senão, uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota".

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SUMÁRIO

RESUMO.....	v
ABSTRACT	vi
CHAPTER 1 – General considerations.....	7
1 Introduction.....	7
2 Literature Review	9
2.1 Agriculture in the Brazilian Cerrado.....	9
2.2 No-till system in the Cerrado.....	11
2.3 Sewage Sludge (SS) and Sewage Sludge Compost (CSS).....	12
2.4 Micronutrients.....	13
2.5 Carbon Quantity and Quality.....	16
3 References	20
CHAPTER 2 – Long-term impacts of a composted sewage sludge–based Class B organic fertilizer on soil micronutrients and crop production in the Tropics	32
RESUMO.....	32
ABSTRACT	33
1 Introduction.....	34
2 Material and Methods	35
2.1 Experimental area.....	35
2.2 Experimental design and treatments.....	38
2.3 Characterization of Class B organic material	39
2.4 Installation and development of the experiment.....	42
2.5 Development of the experiment	43
2.6 Cu, Mn and Zn dynamics in the soil	48
2.6.1 Sampling and preparation of soil samples	48
2.6.2 Determination of Total and Available Cu, Mn, and Zn Content	48
2.6.3 Sequential extraction of Cu, Mn, and Zn in the soil.....	48

2.7 Crop productivity	49
2.8 Statistical analysis	49
3 Results	49
3.1 Total soil contents of Cu, Mn, and Zn.....	49
3.2 Available Cu, Mn, and Zn levels in the soil.....	52
3.3 Chemical fractionation of Cu, Mn, and Zn in the soil	54
3.4 Crop Productivity.....	55
3.5 Correlation of total and available contents and productivity	57
4 Discussion	58
4.1 Micronutrient accumulation and soil dynamics	58
4.2 Geochemical fractionation and element mobility	61
4.3 Agronomic performance and sustainable production	63
5 Conclusions	67
6 References	67
CHAPTER 3 – Successive composted sewage sludge applications reduce carbon saturation deficit and sustain crop yields in a tropical conservation system	76
RESUMO.....	76
ABSTRACT	77
1 Introduction.....	78
2 Materials and Methods	79
2.1 Description of the Experimental Area.....	79
2.2 Experimental Design and Treatments	81
2.3 Characterization of Sewage Sludge Compost.....	84
2.4 Experiment Installation and Development.....	87
2.5 Experiment Development in the 2017 to 2023 Growing Seasons	93
2.6 Soil Carbon Dynamics.....	93
2.6.1 Sampling and Sample Preparation.....	93

2.6.2 Determination of Soil Carbon Contents and Stock.....	93
2.6.3 Physical Fractionation of Organic Matter	94
2.6.4 Assessment of Soil C Saturation Level	94
2.7 Enzymatic and Microbial Activity	95
2.8 Porosity	95
2.9 Wheat and Soybean Analysis	95
2.10 Statistical Analysis	96
3 Results	96
3.1 Soil C and N content and stocks	96
3.2 Effect of organic fertilizer on the physical quality of SOM	98
3.3 SOC stability: enzymatic activity and microbial carbon	101
3.4 Carbon Saturation Potential and Deficit in Soils under Organic Fertilization	104
3.4 Wheat x grass x soybean: yield and production components	104
3.4 Principal component analysis.....	105
4 Discussion	107
4.1 Organic fertilizer combined with crop succession contributes to SOC and N stocks.....	107
4.2 Soil organic matter stability under the influence of sewage sludge compost	108
4.3 Carbon saturation deficit reveals high untapped sequestration potential	108
4.4 Soil carbon (C) saturation potential and deficit.....	109
4.5 How do organic fertilizer and crop rotation modulate soil microbial efficiency and activity?	110
4.4.1 Enzymatic activity.....	110
4.4.2 Microbial biomass and respiration.....	110
4.4.3 Microbial metabolic efficiency.....	111
4.5 Sustained crop productivity driven by organic fertilization	111

4.6 Linking soil carbon sequestration and biological health to crop productivity: a multivariate perspective	112
4.7 Broader implications for soil C management and circular fertilization	113
5 Conclusions	114
6 References	114

APLICAÇÕES SUCESSIVAS DE COMPOSTO DE LODO DE ESGOTO NA DISPONIBILIDADE DE MICRONUTRIENTES E NA QUANTIDADE E QUALIDADE DO CARBONO EM ÁREA SOB PLANTIO DIRETO NO CERRADO

RESUMO

A reciclagem do lodo de esgoto compostado (CSS) na agricultura tem sido uma alternativa para reduzir a dependência de fertilizantes minerais e pressões ambientais. O CSS possui quantidades consideráveis de matéria orgânica (MO) e nutrientes, com possibilidade de ser utilizado como fertilizante orgânico. Quando o CSS é associado a sistemas conservacionistas, plantio direto (SPD), pode contribuir para melhorar a saúde do solo, em especial, em áreas de intenso cultivo e naturalmente inférteis no Cerrado brasileiro. As aplicações de CSS podem proporcionar adequada ciclagem de nutrientes no solo, aumentando a produtividade das culturas e o estoque de carbono (C). Já o incremento de metais como cobre (Cu), manganês (Mn) e zinco (Zn), mesmo essenciais as plantas, devem ser monitorados para otimizar as doses e reduzir os impactos do uso do CSS no sistema solo-planta. Todavia, ainda são escassas informações sobre aplicações sucessivas de CSS e seus efeitos na quantidade e qualidade do C e na disponibilidade de micronutrientes em solos da região do Cerrado cultivados sob SPD. Diante da escassez de dados, esta pesquisa objetivou avaliar os efeitos de sucessivas aplicações de CSS em um sistema de rotação de culturas sobre a dinâmica do C, o fracionamento de micronutrientes e o desempenho agronômico. O experimento foi a campo, conduzido em Selvíria (MS) sob delineamento em blocos casualizados, foi estruturado em dois estudos. O estudo (I) testou, ao longo de seis anos de cultivo, cinco doses de CSS (0 a 12,5 Mg ha⁻¹) aplicadas nos anos 1, 2 e 5. Foram monitorados os teores disponíveis e totais de Cu, Mn e Zn no solo (0–0,2 m), bem como a distribuição desses nutrientes entre frações móveis, trocáveis e ligadas a óxidos de Mn e Fe, matéria orgânica e residuais. O estudo (II) analisou, no sexto ano, os estoques e o fracionamento de C, a atividade microbiana e enzimática em diferentes profundidades sob doses acumuladas de CSS (22,5 e 37,5 Mg ha⁻¹), comparando-as à adubação mineral e à vegetação nativa. Aproximadamente 85% dos teores de Cu, Mn e Zn estão ligados a fração residual no solo. O CSS aumentou a disponibilidade de Cu e Zn no solo em até 67% e 600%, respectivamente na dose de 12,5 Mg ha⁻¹. Os teores totais de Cu e Mn apresentaram resposta quadrática, com ligeiras reduções na dose máxima. A produtividade do milho e do trigo correlacionou-se positivamente com a disponibilidade de Cu e Zn. No aspecto da dinâmica do C, a dose acumulada de 37,5 Mg ha⁻¹ aumentou os teores de C_{org} em 31% e o (MAOM) em 19% na camada de 0,1–0,2 m, além de incrementar a biomassa microbiana (61,23 µg g⁻¹) e reduzir em 5% o déficit de saturação de C na fração MAOM. O sistema trigo-soja foram próximos ao tratamento com fertilizante mineral. Conclui-se que a aplicação de CSS, associada à SPD, constitui uma estratégia agronomicamente eficaz e ambientalmente segura para o sequestro de carbono e o fornecimento de Cu, Mn e Zn. A prática promove a qualidade do solo e mantém a segurança alimentar, alinhando-se aos Objetivos de Desenvolvimento Sustentável (Agenda 2030 da ONU); contudo, a sustentabilidade do sistema a longo prazo depende do monitoramento contínuo dos metais no sistema solo-planta.

Palavras-chave: *Glycine max* L.; Micronutrientes; Sequestro de carbono; Fertilizantes orgânicos; Economia circular.

SEWAGE SLUDGE-BASED FERTILIZER AND ITS EFFECTS ON SOIL MICRONUTRIENTS AVAILABILITY AND CARBON QUANTITY AND QUALITY IN A NO-TILLAGE SYSTEM IN THE BRAZILIAN CERRADO

ABSTRACT

The recycling of composted sewage sludge (CSS) in agriculture has been an alternative to reduce the dependence on mineral fertilizers and environmental pressures. CSS has considerable amounts of organic matter (OM) and nutrients, with the possibility of being used as an organic fertilizer. When CSS is associated with conservation systems, such as the no-tillage system (NT), it can contribute to improving soil health, especially in areas of intense cultivation and naturally infertile soils in the Brazilian Cerrado. CSS applications can provide adequate nutrient cycling in the soil, increasing crop yield and carbon (C) stocks. However, the increase in metals such as copper (Cu), manganese (Mn), and zinc (Zn) even though they are essential to plants must be monitored to optimize doses and reduce the impacts of CSS use on the soil-plant system. Nevertheless, information on successive CSS applications and their effects on C quantity and quality, as well as the availability of micronutrients in soils of the Cerrado region cultivated under NT, is still scarce. Given the scarcity of data, this research aimed to evaluate the effects of successive CSS applications in a crop rotation system on C dynamics, micronutrient fractionation, and agronomic performance. The field experiment, conducted in Selvíria (MS) under a randomized block design, was structured into two studies. Study (I) tested, over six years of cultivation, five CSS doses (0 to 12.5 Mg ha⁻¹) applied in years 1, 2, and 5. The available and total contents of Cu, Mn, and Zn in the soil (0–0.2 m) were monitored, as well as the distribution of these nutrients among mobile, exchangeable, Mn and Fe oxide-bound, organic matter-bound, and residual fractions. Study (II) analyzed, in the sixth year, C stocks and fractionation, microbial and enzymatic activity at different depths under accumulated CSS doses (22.5 and 37.5 Mg ha⁻¹), comparing them to mineral fertilization and native vegetation. Approximately 85% of the Cu, Mn, and Zn contents are bound to the residual fraction in the soil. CSS increased the availability of Cu and Zn in the soil by up to 67% and 600%, respectively, at the dose of 12.5 Mg ha⁻¹. Total Cu and Mn contents showed a quadratic response, with slight reductions at the maximum dose. Maize and wheat yields correlated positively with Cu and Zn availability. Regarding C dynamics, the accumulated dose of 37.5 Mg ha⁻¹ increased SOC (Soil Organic Carbon) contents by 31% and MAOM (Mineral-Associated Organic Matter) by 19% in the 0.1–0.2 m layer, in addition to increasing microbial biomass (61.23 µg g⁻¹) and reducing the C saturation deficit in the MAOM fraction by 5%. The wheat-soybean system results were close to those of the mineral fertilizer treatment. It is concluded that CSS application, associated with NT, constitutes an agronomically effective and environmentally safe strategy for carbon sequestration and the supply of Cu, Mn, and Zn. The practice promotes soil quality and maintains food security, aligning with the Sustainable Development Goals (UN Agenda 2030); however, the long-term sustainability of the system depends on the continuous monitoring of metals in the soil-plant system.

Keywords: *Glycine max* L.; Micronutrients; Carbon sequestration; Organic fertilizers; Circular economy.

CHAPTER 1 – General considerations

1 Introduction

Sewage sludge (SS) treatment systems raise the challenge of better final disposal of SS. The recycling of this waste in agriculture stands out, provided it is used as a base for an organic fertilizer (Rodrigues et al., 2023; Mbasabire et al., 2024). Sewage sludge contains high levels of organic matter (OM) and nutrients, which can contribute to reducing the amount of mineral fertilizers applied (Zomer et al., 2018). When SS is properly treated through composting, it is called composted sewage sludge (CSS). Research has evaluated the agronomic viability of using CSS, especially in supplying phosphorus (P) (Lanno et al., 2021), nitrogen (N) (Elsalam et al., 2021), and micronutrients (Prates et al., 2020, 2022; Oliveira et al., 2023).

The micronutrients copper (Cu), manganese (Mn), and zinc (Zn) are present in high levels in CSS (Prates et al., 2020). Although required in smaller amounts by plants, when in excess, they cause functional disruption of cells and reduced productivity (Chrysargyris et al., 2022). Furthermore, they can alter the environment's fauna, flora, and biota, affecting ecosystem functions and creating risks to water quality and public health (Su et al., 2014).

A precise method for evaluating the available forms of micronutrients and/or heavy metals found in agricultural soils is the use of sequential extraction (Nogueira et al., 2010; Fontes et al., 2021). This technique is used in locations that have received applications of waste with high levels of these elements and with the possibility of environmental pollution risk or food contamination (Gomes et al., 2021). Therefore, before promoting the use of CSS as an organic fertilizer, it is necessary to monitor these micronutrients to ensure soil quality and food safety (Quan et al., 2020).

The Cerrado holds strategic importance for the Brazilian economy due to the production of important agricultural commodities (Alencar et al., 2020). Among these, soybean stands out for its high production and extensive cultivated areas in Brazil, occupying 45 million hectares and producing 160.2 million tons in the current 2023/24 crop season (CONAB, 2023). Among winter crops, wheat has received significant national visibility. National wheat production reached a new record of 10.8 million tons, representing a 2.5% increase compared to the last crop (CONAB, 2023). Future prospects predict an increase in the production area in the Cerrado region, which currently estimates four million hectares suitable for wheat cultivation, including

irrigated and rainfed systems (Chagas et al., 2021). However, the Cerrado has highly weathered soils, with high acidity and low nutrient availability, limiting agricultural production (Gonçalves 2021). Generally, these soils are highly dependent on acidity corrective materials and fertilizers to increase production (Vieira et al., 2022).

No-till (NT) systems with crop rotation associated with the use of organic fertilizer (e.g., CSS) as a soil conditioner and/or fertilizer can be interesting alternatives for greater nutrient cycling and improvement of soil OM quality (Luz et al., 2019). OM is considered one of the main reservoirs of soil carbon (C), and its loss alters global C dynamics (Lal 2020; Maia et al., 2022). OM is known to be an important indicator of soil quality, as it encompasses the main reserves of nutrients for plants and microorganisms (Urra et al., 2019). OM can reduce the bioavailability of heavy metals in the soil. This occurs because the soluble (fulvic acid) or insoluble (humic acid) forms convert the elements into less soluble fractions, forming stable metal-organic complexes (Nogueira et al., 2010).

Among the mechanisms that best explain C stabilization in soil, the physical fractionation of SOM stands out, which will allow the interpretation of the C stability level of a given soil by measuring its fractions of particulate organic matter (POM) and mineral-associated organic matter (MAOM) (Lavalée et al., 2020). POM is generally a very dynamic C reservoir because of its faster turnover, and MAOM has slow turnover and is basically associated with microbes and minerals like clay and silt. Therefore, C sequestration in agricultural soils is directly influenced by the capacity of a given soil to stabilize C, which is often directly associated with the physical protection of this SOM in aggregates and chemically, as it is bound to silt and clay particles (Veloso et al., 2020).

Only through the physical fractionation of SOM into particulate organic carbon and mineral-associated organic carbon is it possible to evaluate the level of C saturation, i.e., its maximum point in C in soils of agroecosystems with different management conditions and SOM accumulation (Rodrigues et al., 2022). Every soil will present its C saturation point, and this explains the lack of response in C quantities to varying levels of this element's input over many years of cultivation with the incorporation of different types of crop residues. The apparent dependence between C stabilization efficiency and soil C content suggests the possibility of an upper limit or "saturation level" for soil C (Di et al., 2018; Guillaume et al., 2022). Consequently, there

is a need for further studies on the soil C saturation level, aiming to understand how and when to change management in order to increase the level of this saturation point.

Soil quality bioindicators, as well as the quantity and quality of C, allow for the monitoring of soil health. Enzymatic activity and microbial biomass, in particular, show greater sensitivity to changes in the soil due to the management system used. Thus, their measurement aids in evaluating the magnitude of the effects of soil management on OM cycling, through the interpretation of specific critical levels of the indicator (Mendes et al., 2019; Mendes et al., 2020).

In this context, due to the need for more sustainable approaches to agricultural practices, this study expected to obtain new information on successive fertilization using an organic fertilizer based on sewage sludge. Our results can serve as a basis for agronomic recommendations, especially the rates and frequency of CSS application in fertilization in areas cultivated under NT in the Cerrado, seeking to reduce the input of mineral sources. Such information can also assist in better understanding the phytoavailability of Cu, Mn, and Zn in the soil, as well as the quantity and quality of C in NT systems in the Cerrado, in addition to increasing the productivity of wheat and soybean crops, ensuring economic profitability for rural producers.

This research is aligned with the National Fertilizer Plan (PNF), which aims to reduce national dependence on fertilizer imports, with the '2030 Agenda' of the 'United Nations' (UN) to promote the '17 Sustainable Development Goals' (SDGs), highlighting 'SDG2' – 'Zero hunger and sustainable agriculture', 'SDG13' – 'Action against global climate change', and 'SDG15' – 'Life on land' (UN 2019), with the COP26 and IPCC initiatives in combating climate change, and adheres to the priority technological areas for sustainable development and quality of life – MCTIC no 1.122/2020 and no 1.329/2020, “Technologies for Sustainable Development: Treatment and Recycling of Solid Waste” and “Technologies for Quality of Life: Basic Sanitation”.

2 Literature Review

2.1 Agriculture in the Brazilian Cerrado

The Cerrado is the second largest Brazilian biome, covering an extensive area of about 2 million km², which corresponds to 25% of the national territory. The biome possesses one of the highest levels of biodiversity on the planet, characterized by stressful environments for plant development due to naturally more acidic soils, with low nutrient availability and a limited water regime (Sauer and Cabral 2022).

Furthermore, the biome includes the main hydrographic basins of South America, namely: the Amazon, Paran , and S o Francisco. The Cerrado is responsible for the water cycle and aquifer recharge, a fundamental role for rain formation and the maintenance of food production (Lopes et al., 2021; Garcia et al., 2024).

The soils found in the Cerrado are highly weathered, with low organic matter content, which limits their nutrient and water retention capacity, making the soil highly dependent on correctives and fertilizers. In addition, the lower amount of OM in these soils facilitates the leaching of macronutrients, which hinders the retention of nutrients applied through fertilization (Silva, 2019). Organic matter (OM) aids in the improvement of the soil's chemical, physical, and biological attributes, especially in soils from tropical and humid subtropical climate regions (Martin-Neto et al., 2023). Thus, alternatives that promote an increase in the OM content in these regions become fundamental, such as the use of organic residues and the adoption of the NTS.

Despite its environmental importance and unfavorable soil characteristics, the Cerrado, or Brazilian savanna, has now become a driving economic force, being responsible for the production of most of the country's agricultural commodities, receiving international attention as one of Brazil's main agricultural frontiers, elevating the country to the largest producer and exporter of soybeans on a global scale (Voora et al., 2020). There has also been significant production, mainly of maize, beans, and recently wheat production has been gaining ground in the Cerrado (Hershaw and Sauer 2023).

Brazil is among the top five producers and exporters of grains in the world ranking, especially maize and soybeans (USDA 2024). However, unlike other countries, it still has significant room for agricultural expansion. In this context, agribusiness advanced rapidly in the Cerrado, and this agricultural expansion was accompanied by environmental degradation, driven by the expansion of monocultures and extensive cattle ranching, both causing extreme deforestation (Hershaw and Sauer 2023). Given this scenario, the demand for conservation management techniques is growing; among sustainable agricultural practices, the no-till system (NTS) stands out, covering about 33 million hectares of the cultivated area in Brazil (FEBRAPDP 2022).

2.2 No-till system in the Cerrado

The NTS is a conservation practice that consists of direct seeding and fertilization into soil not disturbed by a plow or harrow (Lopes & Guimarães Guilherme, 2016; Silva et al., 2022). Adopting NTS helps improve the soil's chemical (nutrient cycling, aluminium complexation, CTC, and redox potential) (Table 1), physical (aggregation, porosity, particle density, infiltration, and penetration resistance) (Passos et al., 2018), and biological (biodiversity, decomposition and mineralization rate) attributes (Luz et al., 2019).

NTS reduces greenhouse gas emissions, soil water and wind erosion, the use of fossil fuels and pesticides, increases water infiltration and fertilization efficiency, in addition to recovering biodiversity and increasing soil organic matter, making it a practice for sustainable agriculture (FEBRAPDP 2022). NTS aids the productive capacity of degraded or conventionally managed soils through the use of cover crops, which are fundamental for maintaining soil moisture, mitigating erosive processes, improving soil structural quality (Cherubin et al., 2016; Bordonal et al., 2018), increasing OM, C stock, increasing energy for microbial activity (Pereira et al., 2017), increasing humic acid and particulate C fractions, which are components of OM and soil quality indicators (Duval et al., 2020). For the implementation of NTS, it is necessary to choose species with high biomass production, rapid growth, and high persistence of plant residues on the soil. Species with soil persistence have been successful in NTS in Cerrado regions, due to high C/N and lignin/N ratios, slowing their decomposition (Teixeira et al., 2011).

Carbon indices indicate that the absence of soil disturbance under NTS and the abundance of crop residues, based on legumes and nitrogen fertilization, are key strategies for the effective conservation of soil functions and crop yields (Zanatta et al., 2019).

Crop rotation and plant diversity in NTS can also provide more favorable conditions for the accumulation of carbon (C) and nitrogen (N) in the soil and for nutrient cycling, increasing the productivity of agricultural systems by improving soil quality (Luz et al., 2019). The inclusion of legumes in crop rotations provides N to microorganisms that synthesize stable fractions of soil organic matter (SOM), in addition to promoting C protection and stability, consequently reducing greenhouse gas (GHG) emissions. The practice of NTS with crop residue mulch has been identified

as an effective way of storing soil C, thus reducing net CO₂ emissions (Maia et al., 2022).

In the Cerrado, NTS is characterized by the low use of cover crops and crop rotation and, consequently, little straw on the soil surface, with soybean monoculture and/or the continuous summer soybean/second-crop maize succession system predominating (Silva et al., 2022b). Adequate crop rotation in this region, even with low soil cover maintenance, favors nutrient cycling, which can ultimately increase crop productivity and biomass production in agroecosystems (Silva et al., 2022b).

The main adaptation strategies for reducing greenhouse gas (GHG) emissions lie in the use of renewable energies, the development of public policies that prevent deforestation, and the adoption of conservation practices in agricultural production systems (Hong et al., 2021). With this, the search for soil management strategies that store maximum C, reducing GHG emissions, and thus mitigating climate change in Brazil, intensifies; among the strategies, we have NTS and integrated production systems (Maia et al., 2022).

2.3 Sewage Sludge (SS) and Sewage Sludge Compost (CSS)

Sewage sludge (SS) is an organic residue produced at Wastewater Treatment Plants (WWTPs), organic in nature, semi-solid, with variable levels of inorganic components, and rich in nutrients; it is mostly destined for sanitary landfills (Lacerda et al., 2022). Due to population growth, it is understood that the volume of SS produced will increase progressively. An annual dry mass production of SS is estimated at around 376.5 thousand and 3 million tons in Brazil. Therefore, the challenges for the production and sustainable use of biosolids are increasing (Araújo et al., 2022; Paes et al., 2023).

According to CONAMA resolution 498/2020, SS is a class B biosolid; the term "biosolid" is defined as the product of sanitary SS treatment that meets microbiological and chemical criteria determined by it, thus being suitable for application on soils (BRASIL 2020). After the sanction of the Legal Framework for Basic Sanitation in the country, there was greater incentive for sustainable alternatives for the adequate final disposal of SS (Paes et al., 2023).

However, there are restrictions on the use of SS in agricultural soils, due to the possible increase in the bioavailability of heavy metals and pathogenic microorganisms in the soil when applied at high doses and without agronomic criteria (Amorim Junior

et al., 2021). In order to reduce the negative impacts of SS on soil and food quality, some countries adopt the composting process, originating sewage sludge compost (CSS), which aims to reduce pathogenic organisms and bioavailable metals (Nafez et al., 2015; Picariello et al., 2021; Paes et al., 2023).

The organic fertilizer CSS, that has high levels of organic matter in its composition, also presenting macro and micronutrients that perform important functions in plants and soil (BRASIL, 2020b). In Brazil, CSS can be registered as a Class B organic fertilizer if it meets the criteria established in Normative Instruction No. 61 of July 8, 2020, from the Ministry of Agriculture, Livestock and Supply (MAPA), (BRASIL, 2020a). Some studies have already proven the viability of using CSS as an organic fertilizer (Jakubus and Bakinowska, 2018; Prates et al., 2020, 2022; Silva et al., 2022; Oliveira et al., 2023).

The use of CSS as an organic fertilizer, in addition to improving the soil's chemical, physical, and biological attributes, can prevent the contamination and degradation of water resources (Jakubus and Graczyk 2020). When applied at agronomic rates in agricultural areas, besides increasing the input of organic matter to the soil, CSS can provide large amounts of nitrogen (N) and phosphorus (P), as well as micronutrients like iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), reducing production costs due to lower use of mineral fertilizers (Jakubus and Bakinowska 2018; Prates et al., 2020; Prates et al., 2022), in addition to providing an increase in productivity (Prates et al., 2020; Prates et al., 2022; Oliveira et al., 2023).

2.4 Micronutrients

The use of CSS as an organic fertilizer, in addition to improving the soil's chemical, physical, and biological attributes, can prevent the contamination and degradation of water resources (Jakubus and Graczyk 2020). When applied at agronomic rates in agricultural areas, besides increasing the input of organic matter to the soil, CSS can provide large amounts of nitrogen (N) and phosphorus (P), as well as micronutrients like iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), reducing production costs due to lower use of mineral fertilizers (Jakubus and Bakinowska 2018; Prates et al., 2020; Prates et al., 2022). In addition to providing an increase in productivity and microbiological activity (Prates et al., 2022, 2020; Silva et al., 2022).

These micronutrients can be found in high concentrations in organic compounds used in agriculture such as organic fertilizer (Jakubus and Graczyk 2020). A recent

study showed that CSS has the following levels of Cu (191.2 mg kg^{-1}), Mn (310 mg kg^{-1}), and Zn (684 mg kg^{-1} , Prates et al., 2020).

The elements Cu, Mn, and Zn are considered micronutrients classified as heavy metals; they are required in smaller amounts by plants but are fundamental for their development. These micronutrients act as constituents of cell walls and membranes, acting as enzyme activators, and are involved in the reproductive phase, plant growth, productivity, crop quality, and resistance to biotic and abiotic stresses (Kirkby and Römheld 2004; Tavares et al., 2015).

However, in excess, these elements are phytotoxic and can cause disturbances; for example, excess Cu can inhibit the absorption of other nutrients and water, reducing plant growth and affecting the root system's morphology (Ambrosini et al., 2015; Andreola et al., 2021). Zn toxicity can cause a reduction in dry matter in both the shoot and root biomass, root necrosis, seedling death, and growth inhibition (Souza et al., 2020). Excess Mn, on the other hand, interferes with the absorption of other nutrients like Fe and Mg, causes lower synthesis of chlorophyll and auxins, which reduces production (Silva and Berti 2022).

The micronutrients Cu, Mn, and Zn are classified as heavy metals. Unlike organic contaminants, metals do not decompose or degrade in the soil, accumulating in various areas and causing an impact on the environment (Weil and Brady 2016; Sousa et al., 2021). The term heavy metal still lacks a clear definition, but different parameters are used to define them, such as density (metal when greater than 5.0 mg m^{-3}), mass or atomic number, chemical properties, or toxicity (Duffus 2002; Moschem and Gonçalves 2020; Kolesnikov et al., 2022). Among the chemical peculiarities of heavy metals are their high level of reactivity and capacity for bioaccumulation; they can cause numerous adverse chemical reactions in living organisms (Cruz et al., 2021).

Table 1. Main chemical reactions of copper, manganese, and zinc in the soil

Reaction/Process	Influence in soil	Bibliography
Adsorption	Cu, Mn, and Zn are strongly adsorbed by clays, oxides (Fe, Al, Mn), and hydroxides. Adsorption is influenced by pH, CTC, and mineral type, with the priority order of adsorption generally being Cu > Zn > Mn.	(Fernández et al., 2015; Campillo-Cora et al., 2020)
Complexation	SOM, in the form of humic and fulvic acids, forms complexes with Cu and Zn, affecting their mobility and availability to plants.	(Fulda et al., 2013; Wang et al., 2024)
Precipitation	At high pH or under reducing conditions, micronutrients can precipitate as carbonates, hydroxides, oxides, or sulfides, becoming less available.	(Wang et al., 2024)
Oxidation/Reduction	Mn is sensitive to redox processes, alternating between soluble and insoluble forms. Cu and Zn also undergo redox transformations, influencing their solubility and mobility, in flooded environments or those with oxygen variation.	(Ma et al., 2020; Wang et al., 2024)

Several chemical reactions occur with the elements Cu, Mn, and Zn in the soil, including: adsorption on clay surfaces, complexation with humic and fulvic acids, organic and inorganic ligands, precipitation with carbonates, hydroxides, oxides, sulfides, oxidation, and reduction (Fulda et al., 2013; Campillo-Cora et al., 2020; Wang et al., 2024). The chemical equilibrium of these reactions defines the availability, toxicity, solubility, and leaching of the metal in the soil (Gomes et al., 2021). Furthermore, they can be found in various forms in the soil, such as: soluble, exchangeable, complexed, specifically adsorbed, bound to organic materials, and bound to precipitates (Pires et al., 2006). As a result of this variability in the available forms of micronutrients found in the soil, there is a growing demand for more precise estimates of these fractions, since part of these nutrients becomes unavailable for plant absorption. This partially invalidates the use of only the total concentration of metals in

the soil as an index to estimate their absorption by plants growing in contaminated soils.

Sequential extraction is a technique that makes it possible to evaluate the proportions of elements associated with each component of the soil's solid phase and, thus, predict their availability to the crop (Mattiazzo et al., 2001), being: 1) in soluble form, i.e., in the fraction readily available to plants; 2) in exchangeable form, retained or adsorbed on negative charge sites present in organic matter or clay minerals. Their binding energy is low, so the exchange with the soil solution fraction is rapid; 3) in specifically adsorbed form, in which they will be adsorbed by covalent bonding to phyllosilicates, oxides, and/or organic matter. Because their reaction involves high energy, the release of cations occurs more slowly than in the exchangeable form; 4) bound to insoluble organic materials, i.e., resistant to microbial degradation or present in recently dead cells, where they are complexed; and 5) bound to precipitates, in cations precipitated in the form of carbonates, sulfates, phosphates, hydroxides, among others. They can also form precipitates directly with anions and co-precipitates with elements of low solubility such as Ca, Mg, Fe, and Al (Alloway 1995; Pires et al., 2006).

The soils of the Brazilian Cerrado are prone to micronutrient deficiencies because they lack an important source of these nutrients, which is organic matter. Leaching in these soils is facilitated by the lack of electrical charges that would allow the retention of micronutrients added via fertilization (Silva, 2015).

2.5 Carbon Quantity and Quality

Organic matter is of great importance for improving the chemical attributes of the soil, especially in soils from humid tropical and subtropical climate regions of Brazil. High temperatures and humidity increase the decomposition rates of residues, making the accumulation of OM in the soil difficult (ANTOLÍN et al., 2005). Thus, alternatives that provide an increase in OM content in these regions become fundamental, such as the use of organic residues and the adoption of the no-till (NT) system.

In terms of carbon sequestration, the remaining native vegetation of the Cerrado contains about 3.5 billion tons of carbon above ground and almost three times more in the subsoil (Freitas et al., 2018). This total of 12.8 billion tons of CO₂eq (after converting C to CO₂eq) is equivalent to almost seven years of Brazil's total greenhouse gas emissions (Russo et al., 2018; SEEG, 2020).

Between 2003 and 2017, a total of 13 Mg ha⁻¹ of Cerrado deforestation was caused by soybean expansion (Zu Ermgassen et al., 2020), resulting in the emission of over 1.6 billion tons of CO₂ from land-use change (Noojipady et al., 2017). Although soils are recognized as one of the main C reservoirs, soils can also be sources of atmospheric C, depending on soil management practices (Lal et al., 2021). This storage helps reduce atmospheric CO₂ concentrations, which is the main driver of the greenhouse effect (Rodrigues and Rodrigues 2024).

C-rich soils promote resilience to extreme climatic disturbances, as they have the capacity to partially mitigate environmental stress, due to the supply of nutrients, expansion of the rhizosphere, improvement of soil structure, water retention, and interactions. The combination of these characteristics makes deep C sequestration an important tool in combating climate change, reinforcing the importance of integrated strategies for mitigation and adaptation (Bieluczyk et al., 2020; Bieluczyk et al., 2023).

Deep soil C sequestration is emerging as a promising strategy (Georgiou et al., 2022; Pimentel et al., 2024; Tenelli et al., 2025). This process, which consists of capturing and storing organic C in deeper soil layers, contributes to mitigating global warming and promoting improvements in ecosystem health (Bieluczyk et al., 2023). However, the soil will contribute to climate change mitigation provided it is managed efficiently (Lal, 2021). Furthermore, C stabilization in the soil not only contributes to climate control but also improves soil health and fertility, directly benefiting crop productivity.

The soil C stock is a fundamental strategy for facing environmental and socioeconomic challenges. This process not only helps mitigate climate change by reducing atmospheric CO₂ concentrations but also improves soil health and productivity (Lavallee et al., 2020; Khan et al., 2021). The formation of soil organic C depends on the balance of residue inputs and outputs and, mainly, on the stabilization mechanisms of soil OM (SOM). Some mechanisms explain SOM accumulation, namely: i) selective preservation or biochemical recalcitrance of organic molecules, ii) spatial inaccessibility of decomposers to organic compounds within aggregates, and iii) the interaction of organic substances with clay, minerals, and metals found in soils (Kögel-Knabner e Amelung, 2021; Martin-Neto et al., 2023).

Understanding the mechanisms of soil organic matter (SOM) accumulation in the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions is essential to understand the impacts of adding organic material to the soil

and its influence on storage dynamics (Hemingway et al., 2019). This analysis provides crucial information about the functionality and stability of SOM, especially in tropical soils with unique properties (Pimentel et al., 2024; Tenelli et al., 2025).

Most soil organic C is physically and chemically associated with soil minerals (Cotrufo et al., 2019). This association restricts decomposer access to the organic material, and as a result, mineral-associated organic C (MAOM) can have its turnover period prolonged compared to particulate organic C (POM) in the same soil layer (Hemingway et al., 2019).

Although POM, which includes all decomposing organic materials $>53 \mu\text{m}$, is less protected, it manages to promote faster cycling, with a greater contribution to soil fertility and serving as an energy source for microorganisms (Cotrufo et al., 2019; Tenelli et al., 2025). From another perspective, MAOM is chemically stabilized by interactions with minerals, therefore, more resistant to decomposition. This fraction is essential for deep carbon storage, ensuring longer residence time and reducing GHG emissions (Hernandes et al., 2022). Thus, increasing MAOM may be the solution for durable C sequestration in soils. However, it is not yet clear how much MAOM currently exists and how much could be accumulated in each soil class (Lavallee et al., 2020).

Thus, understanding and managing these fractions are crucial to maximize the soil's C sequestration potential. Soil organic C (SOC) generally responds slowly to short-term changes in soil management practices, while labile organic C, including particulate organic C (POM) and microbial biomass C, is much more sensitive to changes in the soil environment and can be considered a good indicator of soil quality (Shahid et al., 2017). Labile organic C reservoirs are affected by different soil management practices, such as soil tillage, soil water conditions, and organic fertilizer applications (Cotrufo et al., 2019; Yu et al., 2020; Hernandez et al., 2022).

In agricultural systems, contributed crop residues are the main source of soil C, and their chemical composition influences C and N dynamics and the rate of decomposition and formation of microbial biomass and its byproducts (Bruijn et al., 2011; Schmatz et al., 2017). Thus, not only the total SOM contents are essential, but its fractions can express changes in soil quality more quickly (Duval et al., 2020). Different vegetation covers, and conversion from native vegetation to agroecosystems, alter this C storage in the soil, including its most available fractions (Oliveira et al., 2017; Tenelli et al., 2021). In this sense, crop residues with higher proportions of labile

constituents are converted more quickly into microbial products; they are also the main contributors to the soil organic C stock (Cotrufo et al., 2013).

C stabilization occurs through mechanisms of biochemical recalcitrance of soil organic molecules, physical protection by soil aggregates, and, finally, chemical association between C and fine soil particles such as silt, clay, and Fe and Al oxides. These mechanisms protect soil C against microbial oxidation, resulting in different levels of stabilization. Thus, analyses that can cover the physical, chemical, and biochemical fractions of SOM can improve scientific knowledge of different C stabilization mechanisms in soils and, mainly, provide a better understanding of the impact of soil management practices on C stocks, in addition to helping identify management strategies to increase soil C sequestration and mitigate anthropogenic CO₂ emissions (Rodrigues et al., 2022). Also, integrating physical, chemical, and density fractionation methods provides a better understanding of the mechanisms that interfere with the level of C sequestration and saturation by management systems. This identification of soils far from the C saturation level makes it possible to increase C contents by managing agroecosystems with soils of high C storage capacity.

The various forms of organic matter input, both through the NT system with diverse plant covers, crop rotation, and different doses of CSS, mainly interfere with the quality of the organic matter and its distribution and mineralization in the soil. Thus, there is a growing demand for information about the effects of the adopted management on the soil. For this, it becomes necessary to use indicators capable of clearly estimating the impacts of these systems on the soil. Microbiological attributes, such as enzymatic activity, microbial biomass, and accumulated soil respiration, are considered sensitive to environmental changes resulting from agricultural use in the short term (Epelde et al., 2014; Mendes et al., 2020).

Soil microbial biomass is usually evaluated based on its C, N, and P contents, in which the measurement of soil microbial biomass C (SMBC) is important in regulating soil organic C dynamics, being considered a very sensitive indicator of decreases in the quantity of SOM (Nunes et al., 2009; Tenelli et al., 2021).

Another indicator of this OM dynamic is enzymatic activity, in which most soil enzymes are of microbial origin, although plants and animals also contribute as possible sources. Microorganisms produce extracellular enzymes to degrade high-molecular-weight biomolecules (Mendes et al., 2020). Enzymes from the glycosidase group are widely found in nature, playing an important role in the C cycle, because the

products obtained by the action of these enzymes are energy sources for soil microorganisms (Rodrigues et al., 2022). The β -glucosidase enzyme acts in the final stage of cellulose decomposition, being responsible for the hydrolysis of cellobiose residues, forming the simple sugar β -D-glucose, which plays an essential role in C cycling in the soil, especially in the decomposition of cellulosic plant components (Tabatabai 1994).

The use of CSS as an organic fertilizer in crops under NT with crop rotation can become an advantageous practice, providing benefits such as nutrient supply, input and improvement in the soil C stock, and enabling the adsorption of part of the Cu, Mn, and Zn, which are phytotoxic in high soluble concentrations. Although there is progress in research on the use of organic fertilizers in plant nutrition and soil fertility, studies related to the effect of CSS application on the soil in long-term experiments and with variation in application rates are still scarce, with emphasis on the interactions between CSS and the quantity and quality of C, on the dynamics of Cu, Mn, and Zn in the soil, and on the development of plant species cultivated in the Cerrado region.

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