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**IMPACTS OF LAND USE ON THE DYNAMICS, STOCKS AND PHYSICAL
FRACTIONS OF SOIL ORGANIC MATTER IN TROPICAL ECOSYSTEMS**

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PAULO HENRIQUE FERREIRA DE BRITO

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Thesis submitted to the Post-Graduation Program in Program in Ecology and Natural Resources Federal University of Ceará, in partial fulfillment of the requirements for obtaining the title of Doctor Scientiae. Focus area: Ecology and Natural Resources.

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ABSTRACT

Soil organic matter (SOM) is a critical element for sustaining the fertility of tropical soils and addressing climate change. However, the dynamics of SOM in response to changes in land use are still poorly understood, especially in highly threatened tropical ecosystems such as the Atlantic Forest. The main objective of this study was to evaluate the impact of different land use systems on carbon (C), nitrogen (N) and sulfur (S) stocks and on the distribution of the physical fractions of SOM, including particulate organic matter (POM), mineral-associated organic matter (MAOM) and dissolved organic matter (DOM), in representative areas of the Atlantic Forest in southeastern Brazil. The chapters of this work addressed the following specific aims: i) to review the theoretical framework and mechanisms governing the formation, persistence and dynamics of the physical fractions of SOM, highlighting knowledge gaps; ii) to quantify the stocks of C, N and S in different fractions of SOM at surface (0-5 cm) and subsurface (50-60 cm) depths in different land use systems, including native forests, pastures and agricultural crops. The soils analyzed belong to the area of the Federal University of Viçosa (UFV), located in the Zona da Mata region of Minas Gerais, Brazil, with a tropical high-altitude climate (Cwa) and soils classified as typical dystrophic Latossolos Vermelho-Amarelos (Oxisols). Seven land-use systems were evaluated: native forest, *Araucaria* forest, *Eucalyptus* and rubber plantations, pasture, coffee and corn. Soil samples were collected in representative trenches and subjected to analysis of the physical fractionation of SOM, quantification of C, N and S stocks and chemical and physical soil characterization. The results showed that systems with perennial vegetation, such as native forests and *Araucaria* plantations, had higher stocks of C and N, especially in the more stable fractions (MAOM). On the other hand, agricultural systems, such as coffee and corn, resulted in lower SOM accumulation, especially in the more labile fractions (POM and DOM). In addition, C stocks in the subsoil were more homogeneous between the systems, suggesting that sustainable management practices can mitigate carbon loss. This study contributes to the understanding of the impacts of changes in land use on the dynamics of SOM in tropical ecosystems and provides fundamental information for the development of public policies and management practices that promote soil conservation, carbon sequestration and environmental sustainability.

Keywords: carbon, nitrogen and sulfur stocks; physical fractions of SOM; land use; environmental sustainability.

RESUMO

A matéria orgânica do solo (MOS) é um componente essencial para a manutenção da fertilidade dos solos tropicais e a mitigação das mudanças climáticas. No entanto, a dinâmica da MOS em resposta a mudanças no uso da terra ainda é pouco compreendida, especialmente em ecossistemas tropicais altamente ameaçados, como a Mata Atlântica. Este estudo teve como objetivo principal avaliar o impacto de diferentes sistemas de uso da terra nos estoques de carbono (C), nitrogênio (N) e enxofre (S) e na distribuição das frações físicas da MOS, incluindo a matéria orgânica particulada (MOP), matéria orgânica associada a minerais (MOAM) e matéria orgânica dissolvida (MOD), em áreas representativas da Mata Atlântica no sudeste do Brasil. Os capítulos deste trabalho abordaram os seguintes objetivos específicos: i) revisar os conceitos teóricos e mecanismos que regem a formação, persistência e dinâmica das frações físicas da MOS, destacando lacunas no conhecimento; ii) quantificar os estoques de C, N e S nas frações físicas da MOS em profundidades superficiais (0-5 cm) e subsuperficiais (50-60 cm) em diferentes sistemas de uso da terra, incluindo florestas nativas, pastagem e cultivos agrícolas. Os solos analisados pertencem à área da Universidade Federal de Viçosa (UFV), localizada na Zona da Mata mineira, com clima tropical de altitude (Cwa) e solos classificados como Latossolos Vermelho-Amarelos distróficos típicos (Oxisols). Foram avaliados sete sistemas de uso da terra: floresta nativa, floresta de araucária, plantações de eucalipto e seringueira, pastagem, café e milho. Amostras de solo foram coletadas em trincheiras representativas, sendo posteriormente caracterizadas física e quimicamente, além de serem submetidas ao fracionamento físico da MOS e posterior determinação dos estoques de C, N e S. Os sistemas com vegetação perene, como a floresta nativa e floresta de araucária, apresentaram maiores estoques de C e N, especialmente na fração mais estável (MOAM). Por outro lado, os sistemas agrícolas resultaram em menor acúmulo de MOS, principalmente nas frações mais lábeis (MOP e MOD). Além disso, os estoques de C no subsolo foram mais homogêneos entre os sistemas, sugerindo que práticas de manejo sustentável podem mitigar a perda de carbono. Este estudo contribui para o entendimento dos impactos das mudanças no uso da terra na dinâmica da MOS em ecossistemas tropicais e fornece informações fundamentais para o desenvolvimento de políticas públicas e práticas de manejo que promovam a conservação do solo, o sequestro de carbono e a sustentabilidade ambiental.

Palavras-chave: estoques de carbono, nitrogênio e enxofre; frações físicas da MOS; uso da terra; sustentabilidade ambiental.

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1 INTRODUÇÃO GERAL

A matéria orgânica do solo (MOS) é amplamente reconhecida como um componente essencial para a funcionalidade dos ecossistemas terrestres, desempenhando papéis fundamentais nos processos físicos, químicos e biológicos. Em solos tropicais, como os encontrados na Mata Atlântica brasileira, a MOS é um indicador crítico da qualidade do solo e da sustentabilidade ambiental devido à sua influência nos ciclos biogeoquímicos e na regulação climática (LEHMANN; KLEBER, 2015). Esse bioma, apesar de sua rica biodiversidade e importância ecológica, enfrenta ameaças significativas de mudanças no uso da terra e práticas de manejo insustentáveis, que impactam diretamente a quantidade e a qualidade da MOS, bem como os estoques de carbono (C), nitrogênio (N) e enxofre (S) no solo (RUMPEL et al., 2020; SIMON et al., 2021, 2020; SIMON et al., 2022).

A MOS é composta por uma ampla gama de substâncias orgânicas em diferentes estágios de decomposição, incluindo resíduos vegetais, organismos vivos, compostos microbianos e materiais altamente transformados, como aqueles associados a minerais (STEVENSON, 1994; BALDOCK; NELSON, 2000). Sua composição é organizada em frações físicas, como matéria orgânica particulada (MOP), matéria orgânica associada a minerais (MOAM) e matéria orgânica dissolvida (MOD), cada uma com funções e dinâmicas específicas. A MOP é composta de partículas facilmente decomponíveis de origem vegetal e microbiana e desempenha um papel crucial no fornecimento de nutrientes de curto prazo e no sequestro inicial de carbono (COTRUFO et al., 2013; LAVALLEE et al., 2020). A MAOM, por outro lado, é composta de compostos orgânicos quimicamente estáveis que se ligam aos minerais do solo, contribuindo para a retenção de carbono a longo prazo e para a proteção contra a rápida mineralização (LEHMANN ; KLEBER, 2015). A MOD, devido à sua alta mobilidade e rápida mineralização, desempenha um papel central no transporte de nutrientes e no enriquecimento das camadas mais profundas do solo (KALBITZ et al., 2000; MARSCHNER & KALBITZ, 2003). Desse modo, compreender o comportamento dessas frações em ecossistemas tropicais, como as florestas da Mata Atlântica, é essencial para avaliar os impactos das mudanças no uso da terra sobre os processos biogeoquímicos do solo e a estabilidade dos estoques de carbono.

A Mata Atlântica é considerada um dos *hotspots* mundiais de biodiversidade (MYERS et al., 2000), mas já perdeu mais de 80% de sua cobertura original devido à urbanização, à expansão agrícola e ao desmatamento (ROSA; DE MARCHI; DE OLIVEIRA, 2022). Essas mudanças promovem alterações significativas nos estoques e na dinâmica da

MOS, especialmente nas frações mais sensíveis, como MOP e MOD, que são diretamente influenciadas pelo aporte de resíduos orgânicos, pela atividade microbiana e pelas práticas de manejo (FROUZ, 2018; RUMPEL et al., 2020). Em áreas de floresta nativa, a MOS desempenha um papel essencial na manutenção da biodiversidade do solo, regulando o microclima e sequestrando o carbono atmosférico. No entanto, a conversão dessas áreas em sistemas intensivos de agricultura ou silvicultura geralmente resulta em perdas significativas de carbono e nutrientes, especialmente nas camadas superficiais do solo (SMITH et al., 2021).

O estudo das frações físicas da MOS tem ganhado destaque na literatura científica, pois fornece informações valiosas sobre os mecanismos de formação e proteção da MOS, bem como sobre sua resposta às mudanças no uso da terra e nas condições climáticas (LAVALLEE et al., 2020). A separação granulométrica e densimétrica dessas frações permite avaliar como diferentes práticas de manejo afetam a estabilidade do carbono, a fertilidade do solo e a dinâmica dos nutrientes (CAMBARDELLA; ELLIOTT, 1992; POEPLAU et al., 2017). Além disso, a compreensão da interação entre as frações físicas da MOS e os minerais do solo é essencial para o desenvolvimento de estratégias de manejo que promovam o sequestro de carbono em sistemas agrícolas e florestais (KAISER; KALBITZ, 2012).

Nesse contexto, esta tese buscou investigar como os diferentes sistemas de uso da terra na Mata Atlântica influenciam os estoques e a distribuição de C, N e S nas frações físicas da MOS. O primeiro capítulo aborda os conceitos teóricos e os avanços científicos relacionados à formação, persistência e dinâmica das frações da MOS, destacando lacunas no conhecimento e oportunidades para pesquisas futuras em solos tropicais. O segundo capítulo explora os impactos do uso da terra, incluindo florestas nativas, plantações comerciais e áreas agrícolas, sobre os estoques de C, N e S nas profundidades da superfície e da subsuperfície, com ênfase na relação entre o manejo e a conservação do solo. Por fim, nos concentramos nas interações entre as frações SOM e os fatores ambientais, avaliando como as mudanças no uso da terra afetam a dinâmica das frações físicas MOP, MOAM e MOD.

Com base nesses resultados, esperamos fornecer suporte científico para o desenvolvimento de políticas públicas e práticas de gestão que promovam a conservação do solo, a mitigação das mudanças climáticas e a sustentabilidade dos ecossistemas tropicais. A preservação e o manejo sustentável da MOS são estratégias indispensáveis para enfrentar os desafios impostos pela degradação ambiental e pelas mudanças climáticas.

2 GENERAL INTRODUCTION

Soil organic matter (SOM) is widely recognized as an essential component for the functionality of terrestrial ecosystems, playing fundamental roles in physical, chemical and biological processes. In tropical soils, such as those found in the Brazilian Atlantic Forest, SOM is a critical indicator of soil quality and environmental sustainability due to its influence on biogeochemical cycles and climatic regulation (LEHMANN; KLEBER, 2015). This biome, despite its rich biodiversity and ecological importance, faces significant threats from changes in land use and unsustainable management practices, which directly impact the quantity and quality of SOM, as well as carbon (C), nitrogen (N) and sulfur (S) stocks in the soil (RUMPEL et al., 2020; SIMON et al., 2021, 2020; SIMON et al., 2022).

The SOM is composed of a wide range of organic substances in different stages of decomposition, including plant residues, living organisms, microbial compounds and highly transformed materials, such as those associated with minerals (STEVENSON, 1994; BALDOCK; NELSON, 2000). Its composition is organized into physical fractions, such as particulate organic matter (POM), mineral-associated organic matter (MAOM) and dissolved organic matter (DOM), each with specific functions and dynamics. POM is made up of easily decomposable particles of plant and microbial origin and plays a crucial role in providing short-term nutrients and initial carbon sequestration (COTRUFO et al., 2013; LAVALLEE et al., 2020). MAOM, on the other hand, is composed of chemically stable organic compounds which bind to soil minerals, contributing to long-term carbon retention and protection against rapid mineralization (LEHMANN; KLEBER, 2015). DOM, due to its high mobility and rapid mineralization, plays a central role in transporting nutrients and enriching deeper soil layers (KALBITZ et al., 2000; MARSCHNER and KALBITZ, 2003). Thus, understanding the behavior of these fractions in tropical ecosystems, such as the Atlantic Forest, is essential for assessing the impacts of changes in land use on soil biogeochemical processes and the stability of carbon stocks.

The Atlantic Forest is considered one of the world's biodiversity hotspots (MYERS et al., 2000), but has already lost more than 80% of its original cover due to urbanization, agricultural expansion and deforestation (ROSA; DE MARCHI; DE OLIVEIRA, 2022). These changes promote significant changes in SOM stocks and dynamics, especially in the most sensitive fractions, such as POM and DOM, which are directly influenced by the input of organic residues, microbial activity and management practices (FROUZ, 2018; RUMPEL et al., 2020). In native forest areas, SOM plays an essential role in maintaining soil biodiversity,

regulating the microclimate and sequestering atmospheric carbon. However, the conversion of these areas into intensive agriculture or forestry systems often results in significant losses of carbon and nutrients, especially in the surface layers of the soil (SMITH et al., 2021).

The study of the physical fractions of SOM has gained prominence in the scientific literature, as it provides valuable information on the mechanisms of formation and protection of SOM, as well as on its response to changes in land use and climatic conditions (LAVALLEE et al., 2020). The granulometric and densimetric separation of these fractions makes it possible to assess how different management practices affect carbon stability, soil fertility and nutrient dynamics (CAMBARDELLA; ELLIOTT, 1992; POEPLAU et al., 2017). In addition, understanding the interaction between the physical fractions of SOM and soil minerals is essential for developing management strategies that promote carbon sequestration in agricultural and forestry systems (KAISER; KALBITZ, 2012).

In this context, this thesis sought to investigate how different land use systems in the Atlantic Forest influence the stocks and distribution of C, N and S in the physical fractions of SOM. The first chapter addresses the theoretical concepts and scientific advances related to the formation, persistence and dynamics of SOM fractions, highlighting gaps in knowledge and opportunities for future research in tropical soils. The second chapter explores the impacts of land use, including native forests, commercial plantations and agricultural areas, on C, N and S stocks at surface and subsurface depths, with an emphasis on the relationship between soil management and conservation. Finally, we focus on the interactions between SOM fractions and environmental factors, assessing how changes in land use affect the dynamics of the physical fractions POM, MAOM and DOM.

Based on these results, we hope to provide scientific support for the development of public policies and management practices that promote soil conservation, climate change mitigation and the sustainability of tropical ecosystems. The preservation and sustainable management of SOM are indispensable strategies for facing the challenges posed by environmental degradation and climate change.

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3 CHAPTER 1: GENERAL CONCEPTS FOR THE FORMATION, PERSISTENCE AND DYNAMICS OF SOIL ORGANIC MATTER IN DISSOLVED, PARTICULATE AND MINERAL-ASSOCIATED FORMS - A REVIEW

Abstract

Soil organic matter (SOM) is understood to refer to all the fractions of organic compounds present in the soil matrix, from water-soluble compounds to living organisms and decomposing plant residues. Research on SOM in Brazil encounters numerous challenges due to the country's vast territory, diverse soil types, varying climatic conditions, management practices, and differing opinions on SOM separation schemes, which complicate comparisons between studies. In tropical environments, where high temperatures and precipitation rates significantly influence SOM dynamics, the decomposition process occurs at faster rates. This increased decomposition rate has profound effects on both the turnover and stability of SOM, making its study particularly complex in such regions. Therefore, it is essential to understand the dynamics of SOM and its stability in ecosystems, as this allows us to evaluate its influence on biogeochemical cycles and on soil and environmental conservation. The physical fractioning of SOM allows us to access different fractions with varying responses to management, climatic conditions, potential accumulation, protection mechanisms, and cycling rates, besides allowing us to make inferences about different formation pathways. However, most studies conducted in Brazil do not link the concepts of the physical fractions of SOM in a simplified way. There is a scarcity of information linking soil physical fractions to SOM formation mechanisms in different systems. Here, we synthesize information from the literature to identify and discuss the main concepts, importance, dynamics, persistence, and transformations of SOM. We also discuss the importance of these fractions in C cycling and other soil properties and processes, emphasizing the importance of physical fractionation for estimating C stocks in soils. Gaps and opportunities were identified to encourage future studies involving the SOM physical fractions in Brazilian soils.

Keywords: POM, MAOM, physical fractionation, biogeochemical cycles

3.1 Introduction

Soil organic matter (SOM) is an indicator of soil quality, responsible for maintaining the ecosystem equilibrium because it is related to the chemical, physical, and biological processes that occur in the soil (SIMON et al., 2022). It is an important reservoir of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) for crops, natural environments, and areas undergoing environmental recovery (SARKER et al., 2018). In addition, the SOM influences soil structure, soil organisms and biogeochemical cycling, acting on aggregation, aeration, water retention, promoting plant health, nutrient mineralization, sequestering C and retaining toxic compounds, protecting the soil from erosion, increasing soil biodiversity, increasing primary production, acting on climate regulation and improving water quality (FROUZ, 2018; HOFFLAND et al., 2020).

Thus, SOM has a key role to play in climate change by acting as a C sink, storing organic carbon in the soil, and contributing to the reduction of carbon dioxide (CO₂) concentrations in the atmosphere. Additionally, it influences greenhouse gases (GHGs) emissions from the soil and helps to improve soil quality and regulate soil temperature, playing a significant role in the resilience of terrestrial ecosystems to climate change. Therefore, proper management of SOM is essential to mitigate climate change and promote environmental sustainability (RUMPEL et al., 2020).

In general, there are various types of fractionations of SOM that seek to separate it into fractions that are homogeneous in terms of their nature, dynamics and function in the soil. Historically, chemical fractionation constituted the first efforts to separate SOM into significant components, seeking to better characterize and chemically identify its components by determining the fractions of humic acids, fulvic acids and humin by alkaline extraction (MINASNY et al., 2020). However, the physical fractionation of SOM has emerged as an innovative approach in soil science to overcome limitations of conventional analysis (CAMBARDELLA; ELLIOTT, 1992), allowing the separation of organic matter into distinct fractions based on their physical properties, such as density and particle size. In this context, there are two types of physical fractionation: granulometric and densimetric. The physical fractions of SOM in the first fractionation are: mineral-associated organic matter (MAOM < 0.053 mm), particulate organic matter (0.053 mm < POM < 2 mm) and dissolved organic matter (DOM < 0.45 µm), the latter being the most mobile fraction in the soil and can coexist in POM or MAOM (LAVALLEE; SOONG; COTRUFO, 2020a). In the second fractionation, the fractions are classified as: free light fractions, occluded light fractions and heavy fractions

(HAO et al., 2023). In this way, the physical fractionation of SOM has enabled a detailed understanding of stabilization processes, C cycling and organic-mineral interactions (CARVALHO et al., 2023).

According to Lavalley et al (2020a), in a review on the conceptualization of the physical fractions of SOM, there is a wide and little-explored gap in research, both critically and conceptually. This is because most studies focused on specific scientific issues have resulted in confusing approaches, i.e. there is currently no consensus on SOM separation schemes (POEPLAU; VOS; DON, 2017), which makes comparisons between studies difficult (ALMEIDA et al., 2021; MANTOVANI et al., 2024; MEDEIROS; CESÁRIO; MAIA, 2023).

Thus, it becomes necessary to contextualize SOM and standardize studies of the physical fractionation of SOM and its formation in different use systems to provide simple and effective answers to predict large-scale changes in SOM dynamics in the context of climate change challenges.

Therefore, in this literature review, we investigate discussions in previous studies on the physical fractions of SOM and highlight the current interest in this research topic in Brazil. To this end, we searched the literature to describe the importance, origin, formation, transformation processes, and future of SOM in the soil-root systems, with an emphasis on the quality and quantity of organic matter input to the soil in different systems under tropical conditions. Finally, knowledge gaps and opportunities were outlined to guide future research for a better understanding of the composition, structure, and implications of changes in physical fractions in the tropical soils of Brazil.

3.2 Definition of SOM and SOM physical fractions

The SOM consists of a continuum of organic compounds in different stages of decomposition, from intact plant/animal/microbial material to highly oxidized carbon (C) (LEHMANN; KLEBER, 2015a). Stevenson (1994) complements this view by detailing that the main components of organic matter, such as humic and fulvic acids, are formed by chemical and biological transformation processes, which influence cation exchange capacity and nutrient availability. Furthermore, SOM not only improves the physical and chemical properties of the soil, but also contributes to climate regulation, promoting the resilience and sustainability of agricultural and natural systems (TELO DA GAMA, 2023). As such, the SOM is an important reservoir of C, nutrients, and energy within the C global cycle, so understanding its composition is used to predict responses to future climate change (HUR; LEE; SHIN, 2011; KUZUYAKOV;

BLAGODATSKAYA, 2015).

Classic textbooks in Soil Science describe SOM as a complex system, composed not only of humic substances (HS), but also of C present in soil organisms, their metabolites, and plant exudates. Additionally, SOM includes C from non-humified substances, such as biologically relevant macromolecules (proteins, amino acids, lipids, sugars, among others), which possess well-characterized chemical and physicochemical properties. It also encompasses C derived from plant and animal residues at various stages of decomposition (STEVENSON, 1994). These compounds play essential roles, serving as sources of nutrients and energy for soil organisms, while also contributing to critical processes such as nutrient complexation and the immobilization of potentially toxic elements and compounds for plants, including aluminum, heavy metals, and persistent organic pollutants in the environment (BALDOCK; NELSON, 2000).

The primary process of SOM formation is photosynthesis (NOBRE; REID; VEIGA, 2012). Vegetation is the main source of organic material deposition in the soil. SOM is composed of C, O, and H, associated with other elements such as N, P, and S (SILVA et al., 2023).

The content and characteristics of SOM are influenced by various factors, including input sources, the frequency of production and incorporation of residues, decomposition and transformation processes, mineralization rate, and topography, which interact with specific environmental conditions (LAGANIÈRE et al., 2022; VITTORI ANTISARI et al., 2021). The dynamics of SOM in ecosystems are primarily regulated by climatic conditions and the biochemical composition of organic inputs. These key factors directly affect microclimatic conditions and the soil microbiome, influencing microbial diversity and activity, which impact nutrient cycling rates, soil moisture availability, and ultimately, crop productivity (BROWN; BAROIS; LAVELLE, 2000; FIERER, 2017).

The SOM content of a given soil class and/or ecosystem tends towards an equilibrium value, depending on the environmental conditions (FERREIRA et al., 2012; KOTHAWALA et al., 2021; PATERSON; SIM, 2013). Despite the equilibrium trend, even in natural ecosystems, there is variability in the monthly averages of the soil CO₂ flux due to the influences of edaphoclimatic factors. Therefore, there is a very large variation in SOM contents: from < 1 g/kg in desert soils to 1000 g/kg in organic soils. Although its concentration does not exceed the value of 50 g/kg in relation to the topsoil layer, it is its existence that makes the soil alive, and its absence turns the soil into a merely physical support (DELARMELINA et al., 2022).

3.2.1 *Particulate organic matter (POM) and mineral-associated organic matter (MAOM)*

The SOM can be separated into POM and MAOM, which can be differentiated by their formation and persistence in the soil (LAVALLEE; SOONG; COTRUFO, 2020a).

POM is the fraction consisting of organic fragments such as plant and animal remains partially decomposed by soil organisms, resulting in small organic particles (ranging between 53 and 2000 μm) and, for this reason, end up being easily available for microorganisms (COTRUFO et al., 2013; LAVALLEE; SOONG; COTRUFO, 2020a). This fraction is a key component of soil C stocks and can comprise 6 to 37% of total SOC in agricultural soils (GREGORICH et al., 2006). The POM enters the soil from the litter (top organic layer) and from the rhizosphere, through root fragmentation or even dead roots. Its accumulation is generally considered an early stage of C sequestration in soils. Thus, POM concentrations are useful to assess fertility status and C sequestration potential in soils (KRAVCHENKO et al., 2014). In addition, POM can be a sensitive indicator of changes in soil quality resulting from land management practices (LEUTHOLD et al., 2024).

The MAOM fraction is derived mainly from microbial necromass and its by-products (chemically transformed by the soil biota) and from certain plant compounds (leached directly from the plant material) and is in an advanced stage of decomposition by the microbial community in the form of microscopic organic molecules that have a high affinity for the surface of minerals (LAVALLEE; SOONG; COTRUFO, 2020b). MAOM is formed via the adsorption of organic compounds to soil minerals during the decomposition process. The amount of MAOM formed in soil depends on a series of factors, including soil type, texture, climate, vegetation, and soil management practices. The addition of organic matter to the soil can increase the formation of MAOM. Therefore, knowing the MAOM contents in soils allows us to understand several functions and processes related to soil health and productivity, such as soil fertility, water storage, soil structure, and soil erosion (LAVALLEE; SOONG; COTRUFO, 2020).

The main difference between POM and MAOM is the persistence of each of them in the soil. The persistence of POM in soil is controlled mainly by physical protection in aggregates, while that of MAOM is controlled mainly by intense association with minerals through chemical bonds, and in both fractions by the biochemical protection associated with the inherent quality of the material that contributed to these fractions (YU et al., 2022).

3.2.2 *Dissolved organic matter (DOM)*

Dissolved organic matter (DOM) is the fraction of SOM most sensitive to the different managements applied to the soil (SANTOS et al., 2022), being composed mainly of materials leached into the profile by the action of rainfall, leaf litter, root deposits, microorganisms, organic molecules dissolved in rainwater and even the SOM itself (GMACH et al., 2019; KALBITZ et al., 2000). It plays an important role in several physical and chemical processes, e.g., translocation and leaching of nutrients (due to the higher mobility of DOM), mineral weathering, and nutrient availability (by mineralization of nutrients by microorganisms). Importantly, recent findings emphasize that the dynamics of dissolved organic carbon, nitrogen, and phosphorus (DOC, DON, and DOP, respectively) in soils is a major pathway of element cycling by the action of microorganisms and mineralization to plants (KALBITZ et al., 2000).

The DOM is part of the sorption and desorption processes of the MAOM fraction. Due to this mobility and interaction with the mineral matrix, DOM is considered one of the pathways to increase the C stock in deeper soil layers (LEINEMANN et al., 2016, 2018). It is an important resource for soil microorganisms, being considered a readily available source (MARSCHNER; KALBITZ, 2003) and continuously formed, transformed, and consumed in the soil (DING et al., 2020; ROTH et al., 2019).

The organic matter inputs to the soil can be through animal and plant residues and microorganisms as can be seen in the formation of POM and MAOM; as well as in the increase in soluble organic compounds leached by rainwater. These DOM inputs modify the SOM values of the soil.

Operationally, the DOM is the fraction of organic carbon that passes through a 0.45 μm filter after extraction with water or dilute saline solution (KALBITZ et al., 2000). It is a fraction that can vary widely in terms of the method used for extraction or determination; as stated by Zsolnay (2003), who reflects on the DOC levels found by different analysis methods. DOM is the most mobile fraction of the SOM and is therefore of fundamental importance in the dynamics of SOC and other nutrients. Therefore, DOM in soils plays an important role in the biogeochemistry of C, N, P and S, as well as in the transport of pollutants in the soil (KAISER; KALBITZ, 2012; KALBITZ; KAISER, 2008; THURMAN; THURMAN, 1985; ZSOLNAY, 2003)

The DOM originates from the interrelationship between primary productivity (photosynthesis) and the metabolism of soil organisms and compounds from external sources,

such as atmospheric deposition, water from streams and rivers, wetlands and marshes, irrigation channels, groundwater resurgence and mineral sources, including anthropogenic contributions (MARSCHNER; KALBITZ, 2003). The formation of DOM is characterized by the microbial transformation of plant residues in the environment, where environmental and physical-chemical factors, such as climatic conditions, vegetation, SOM quality, pH, soil-mineral interaction, etc., play a fundamental role (D'ANDRILLI et al., 2022).

The conceptual framework for the construction of DOM dynamics proposed by Kaiser and Kalbitz (2012) facilitates a more comprehensive understanding of the processes influencing this fraction. One of the ways in which SOM is enriched in the subsoil is through DOM, where the degradation of biomass generates dissolved organic compounds, which in turn bind to the mineral component. The addition of new residues leads to competition for binding sites with older organics compounds, which, when not fully oxidized (by decomposition, for example), migrate to lower layers (DUBEUX, et al., 2024). However, the greatest production of DOM occurs in the surface layers, due to the constant input of plant residues (DON et al., 2012). According to Saidy et al., (2013), DOM can be derived from different sources (inputs), such as atmospheric C dissolved in rain, litter and crop residues, manure, root exudates and decomposition of SOM (KALBITZ et al., 2004; KALBITZ; KAISER, 2008; SAIDY et al., 2013).

DOM is characterized by the heterogeneity of soluble organic compounds from the decomposition of carbon-rich materials, such as microbial metabolites from the organic layers. These processes are evident in forest ecosystems, where there is a continuous input of organic matter (MENG et al., 2024). Thus, DOM constituents can be grouped into labile and recalcitrant (MARSCHNER; KALBITZ, 2003). Labile DOM consists mainly of simple carbohydrate compounds (i.e., glucose and fructose), low molecular weight organic acids, amino sugars, and proteins (JIANG et al., 2017; LI et al., 2020; WANG et al., 2018). Recalcitrant DOM consists of polysaccharides (i.e., cellulose and hemicellulose degradation products) and compounds from other plants and/or degradation products derived from microbes (IDE et al., 2020; LI et al., 2019a; SHARMA; CHAUHAN; KUMAR, 2021).

In summary, the formation of DOM is a complex and multifaceted process, influenced by a combination of environmental, chemical, and biological factors. Understanding these factors is essential to understanding how DOM affects soil health, water quality and biogeochemical cycles in terrestrial ecosystems.

3.3 Scientific interaction with the SOM fractions

Since studies of the SOM fractions (POM, MAOM, and DOM) were introduced between 1980 and 1990, the interest in this topic in terrestrial systems has increased exponentially (SOKOL et al., 2022). However, interlinked studies of the fractions in soil systems, especially in soils of tropical regions, are uncommon in the world. To illustrate this contrast between the number of publications on POM, MAOM, and DOM in Brazil and abroad, we performed a simplified bibliometric systematic study in the Web of Science database.

Initially, searching for the term "organic matter fractions" as a "topic" from 1980 to 2024 yielded 35,003 publications. When the word "soil" was added to the searches, the total number of publications dropped to 18,672 for the same period. For comparison purposes, only the topics "particulate organic matter", "mineral-associated organic matter " and "dissolved organic matter" were used in conjunction with the word "soil" to avoid overlapping results, the results found were 1,589, 275, and 5,172; respectively, however, a search was carried out for "particulate organic matter" and "mineral-associated organic matter" and "dissolved organic matter", and "soil" in order to find studies on the three fractions of SOM together, and only 708 were found. When the searches were restricted to studies conducted in Brazil, the terms "particulate organic matter", "mineral-associated organic matter", "dissolved organic matter", and "Brazil" yielded only 83, 23, and 108 publications respectively, the five most recent publications on each topic are listed in Table 1. In another search for "particulate organic matter", "mineral-associated organic matter", "dissolved organic matter" and "USA" 608, 139 and 1,342 publications were found for the same period, respectively. Furthermore, the same search in the sequence of the physical fractions of the SOM added the word "China" which showed 245, 51, and 1,739 publications, and by "Europe" 652, 125, and 2,173 publications were found, respectively. The terms searched in the three scientific databases are detailed in Table 2 of the Appendix A. Supplementary data.

Table 1 – More recent studies evaluating the physical fractions of SOM in Brazil.

Year	Authors	Papper	Site	System	OM fractions evaluated
(2023)	Vicente et al.	Chemical Composition of Organic Carbon in Aggregate Density Fractions Under Cacao Agroforestry Systems in South Bahia, Brazil.	Bahia, Brazil	Cocoa agroforestry systems (AFSs)	POM and MAOM
(2023)	Valente et al.	Native multispecies and fast-growing forest root biomass increase C and N stocks in a reclaimed bauxite mining area.	Minas Gerais, Brazil	Mixed forest planting	POM and MAOM
(2023)	dos Santos at al.	Selective sorption and desorption of DOM in Podzol horizons-DOC and aluminium contents of leachates from a column experiment.	São Paulo, Brazil	Tropical forest	DOM
(2023)	da Silva et al.	Influence of land use and different plant residues on isotopic carbon distribution of total and water extractable organic matter in an incubation experiment with weathered tropical soil	Rio de Janeiro, Brazil	Natural forest and sugarcane plantation	DOM
(2022)	Ribeiro et al.	Physical fractionation and carbon and nitrogen stocks in soil after poultry waste applications.	Goiás, Brazil	Agricultural land	POM
(2022)	Medeiros et al.	Soil carbon stocks and compartments of organic matter under conventional systems in Brazilian semi-arid region.	Alagoas, Brazil	Secondary native vegetation in conventional systems (agricultural land and pasture)	POM and MAOM
(2022)	Farias et al.	Physical fractions of organic matter and mineralizable soil carbon as quality indicators in areas under different forms of use in the Cerrado-Pantanal Ecotone.	Mato Grosso do Sul, Brazil	Different uses in the Cerrado-Pantanal Ecotone	POM and MAOM

(2022)	Locatelli et al.	Soil strength and structural stability are mediated by soil organic matter composition in agricultural expansion areas of the Brazilian Cerrado Biome. Carbon saturation deficit and litter quality drive the stabilization of litter-derived C in mineral-associated organic matter in long-term no-till soil.	Maranhão, Brazil	Different land uses (native Cerrado, pasture and agricultural land)	POM and MAOM
(2022)	Rodrigues et al.	Soil organic matter fractions in an Oxisol under tillage systems and winter cover crops for 26 years in the Brazilian subtropics.	Rio Grande do Sul, Brazil	No-till	MAOM
(2022)	Amadori et al.	Selective sorption and desorption of DOM in podzol horizons-FTIR and Py-GC/MS of leachates from a column experiment	Paraná, Brazil	Agricultural land	POM and MAOM
(2022)	dos Santos et al.	Soil organic carbon mobility in equatorial podzols: soil column experiments	São Paulo, Brazil	Tropical forest	DOM
(2021)	Merdy et al.		Amazonas, Brazil	Tropical forest	DOM

Most recent articles found in the Web of Science database using the terms “particulate organic matter” **OR** POM; “mineral-associated organic matter” **OR** MAOM; “dissolved organic matter” **OR** DOM; **AND** “Brazil” **AND** “Soil” (Accessed May 01, 2024).

The number of publications decreased even more when the word "litter decomposition" was added (i.e., "Organic matter fractions" and "litter" and "soil"), resulting in 127 publications by 2023, but only 18 publications showed results from soil and litter experiments carried out in Brazil. Most studies focused on understanding the dynamics of the physical fractions of SOM in temperate regions and humid areas; however, results with studies of the formation processes of the physical fractions along the soil profile are still scarce in the literature (VAN GAELEN et al., 2014), especially under tropical conditions. We also tried to find publications with similar terms; however, the sum of the terms "physical fractionation", "soil", "Brazil" and "litter bags" showed few publications for the terms together.

Complementary to this search in the Web of Science database, the same search was performed in the ScienceDirect and Google Scholar databases. The results found in both were very different to those found in Web of Science. This result is because Web of Science uses a more focused and specific search system, with advanced filters, which can reduce the number

of irrelevant results, while Google Scholar and ScienceDirect can return a larger volume of publications, but with a smaller proportion of articles directly related to the subject of this review. Web of Science also has filters that allow searches to be refined by country, which makes it easier to organize the literature based on scientific production in different regions. Searching by country makes it possible to identify regional patterns and areas of research concentration, which can reveal which nations are leading advances in a given area of study.

These searches in the main scientific databases showed the lack of studies in Brazil on the processes of formation of SOM fractions along the soil profile. While the international scientific community is concerned with understanding the implications of SOM fractions in the functioning of natural and anthropic ecosystems, in Brazil there is much to advance in understanding the dynamics of these fractions, especially in systems of different land uses.

3.4 Theoretical-conceptual construction of SOM: dynamics and persistence in soil

According to the synthesis model proposed by Gross and Harrison (2019) on the dynamics of SOM, there are several mechanisms and processes involved in its persistence in the soil. Soil is a complex system in which numerous interactions occur between organic and mineral-organic components (especially chemical bonds with clay minerals, iron oxides, and aluminum oxides). Changes in environmental constraints affect the ecological cycling of SOC, altering soil properties and carbon inputs, which in turn alter the communities of soil organisms and the processes of access to SOM and other nutrients.

Thus, it has been shown that the fraction of SOC adsorbed to minerals increases proportionally with depth and comprises most of the recalcitrant organic C stored in soils. Although the age of SOC at surface is primarily influenced by climatic and land use factors, the age of deep SOC is more strongly associated with soil type. This highlights the significant role of clay texture and soil mineralogy, particularly the clay fraction, in promoting long-term SOC storage through its interactions with SOM, especially the DOM fraction (GROSS; HARRISON, 2019).

However, there are ways to protect SOM in face of the functional complexity of SOC (LEHMANN et al., 2020). This functional complexity involves a spatial separation between the microorganisms and their substrate. A spatial-temporal variation of the substrate and the microorganisms in the soil provides a cost-benefit ratio to be met by the construction of the enzymatic machinery for the degradation of the organic compounds of the SOM (WOLNA-MARUWKA et al., 2023). Molecular biodiversity is the richness of molecules found

in living organisms, so a high molecular diversity can lead to a high cost-benefit ratio. A low molecular diversity, in turn, can lead to a good cost-benefit ratio for microorganisms to receive more energy than they can expend. The mechanisms of organic C enrichment from soil to subsurface generally include bioturbation, the contribution of roots and root-associated microorganisms, and the release of DOM (GROSS; HARRISON, 2019; LEHMANN et al., 2020). Plant diversity on the surface contributes to increased DOM concentration and biodegradation on the surface and the formation of POM and the transformation of MAOM fraction (LANG et al., 2023).

Overall, the climate and the quality of organic matter directly influence the dynamics and permanence of SOM fractions in the most diverse ecosystems (KALBITZ et al., 2000). The desorption of SOM from the surface layers occurs due to the stronger binding forces of newer inputs. This leads to the migration of older compounds to deeper layers. As soil depth increases, microbial decomposition alters the DOM, synthesizing medium-sized molecules while decreasing the proportions of small and large molecules. Consequently, the proportion of microbial-derived compounds increases (ROTH et al., 2019).

3.5 Importance of SOC and the physical fractions for the terrestrial carbon cycle

Soils represent the largest reservoir of terrestrial organic carbon, storing more C than the phytomass and the atmosphere (LORENZ; LAL, 2022; SCHARLEMANN et al., 2014). According to Stevenson and Cole (1999) SOM influences soil structure and thus soil water availability, porosity, penetrability, nutrient retention, as well as nutrient resources for microbial and faunal communities. Twenty seven recent approaches have estimated that the average global SOC stock is around 1500 Pg (CROWTHER et al., 2019). Some uncertainties about the most accurate method and the exact estimate are questioned, including possible overestimations by misuse of bulk density and rock content parameters (POEPLAU; VOS; DON, 2017; TARNOCAI et al., 2009).

The SOC stocks have the potential to offset 5-15% of fossil fuel emissions and can contribute to improved agricultural yields on a global scale (LAL, 2004). The highest concentrations of SOC are typically found in the topsoil, while subsoils often contain less available carbon. However, when SOC stocks are present in subsoils up to 2 meters deep, they account for more than half of the total stored SOC due to the large volume and bulk density of these deeper layers. Subsoils are an important factor in long-term carbon sequestration (JOBÁGY & JACKSON, 2000; RUMPEL & KÖGEL-KNABNER, 2011). The relative

importance of subsoil carbon increases because these layers store significant amounts of carbon, which can remain stable over long periods, playing a key role in mitigating climate change.

In the literature, SOM has been described as a diverse combination of organic substances from various sources, such as growing plants, microbial activity, animal and plant residue. This mixture ranges from simple molecules, such as organic acids and monomers, to complex mixtures of biopolymers, extracellular enzymes, surfactant proteins and chelating compounds (PAUL, 2016; SENESI; LOFFREDO, 2018; ZHAI et al., 2022).

Traditionally, one of the most important processes in the carbon cycle is decomposition and subsequent humification, whereby various organic molecules found in decomposing plant and animal remains are transformed into a more humified material, resulting in significant changes in the compositional structure of SOM (STEVENSON, 1994). This view has recently been challenged by investigations showing the high chemical complexity coming from a mixture of simple molecules (OHNO et al., 2014) and the high spatial complexity at fine scales (LEHMANN et al., 2008). New evidence supports the theory that SOM is an assembly of molecular species. Therefore, this assembly behaves either as a high-weight macromolecular species or as a collection of low-weight molecular species (GALICIA-ANDRÉS et al., 2021).

Finally, the importance of SOC for the global carbon cycle, especially with a focus on climate change and food security, is widely agreed upon (LAL, 2013). In this context, the study of the total organic carbon stocks of the physical fractions establishes the development of climate mitigation and sustainable management strategies for terrestrial ecosystems. Therefore, analyzing the physical fractions of SOM offers a more detailed view of soil carbon dynamics, especially in response to changes in land use and management practices. This information is critical for the development of sustainable management strategies aimed at increasing carbon sequestration and mitigating climate change, while simultaneously promoting ecosystem resilience and global food security (SMITH et al., 2015).

3.6 Quantity and quality of organic matter and its fractions

The quantity and quality of SOM can be evaluated in terms of its fractions, which are different components of organic material with different characteristics. Thus, their characteristics are influenced by several factors, such as soil type, climate, vegetation, soil management, and the quantity and quality of organic material added to the soil. Sandy soils tend to store less SOM, while clayey soils tend to store a greater amount of it, due to a greater capacity to adsorb organic compounds and their aggregation capacity (LEHMANN et al., 2015).

resulting in greater physical and chemical protection of the SOM.

The quality of SOM can be evaluated through several parameters, such as the C/N ratio (ratio between organic carbon and nitrogen content), lignin content, hydrophobicity and aromaticity. Soils that have SOM with a recalcitrant biochemical composition, high resistance and a high C/N ratio are more resistant to the decomposition process and thus accumulate higher levels of C throughout the soil profile (KAISER; KALBITZ, 2012).

SOM is essential for assessing soil health and quality, and various chemical and structural aspects are used as indicators for this purpose. The elemental chemical composition, which includes carbon, hydrogen, sulfur, oxygen, nitrogen and phosphorus, provides a detailed insight into the quality of the SOM and its ability to supply the plants' nutritional needs. In addition, the chemical composition of aggregates, which encompasses the mineral and particulate fractions, is crucial to understanding soil structure and stability, influencing water and nutrient retention. The spectroscopic properties of SOM, such as those obtained by infrared absorption spectroscopy and nuclear magnetic resonance, offer insights into the complexity and composition of SOM, helping to infer about its stability and function in the soil. These combined characteristics are fundamental for an accurate assessment of SOM and efficient soil management (CHENU et al., 2024).

The diversity of compounds along the decomposition and size continuum of SOM, together with its complex chemical and spatial characteristics, represent significant challenges for analysis and contributes to a still limited understanding of its complexity, dynamics and stability (CHENU et al., 2024). Consequently, investigating the composition of SOM and its impacts on the global biogeochemical carbon cycle presents significant difficulties (ARTEMYEVA et al., 2021). These challenges have led to the development of recent analytical techniques that have advanced to better understand the processes underlying their transformations, forcing many authors to re-examine some of the long-standing theories of SOM formation and persistence in soils (HATCHER et al., 1983). Lehman and Solomon (2010) emphasized that the characterization of SOM could benefit even more from the progress made in non-destructive X-ray spectroscopy techniques at the micro and nanoscale to gain new insights into the reactivity, composition, microheterogeneity, physical location of organic materials and their interaction with soil minerals. In this sense, synchrotron radiation has opened new opportunities for the study of C in soils, as the high energy provided significantly improves the spectral and spatial resolution in the physical fractions of SOM (LEHMANN; SOLOMON, 2010). However, despite these advances, a clear standardization in the analytical protocols for SOM fractionation and characterization is still lacking, which limits comparisons

across studies and biomes. It is crucial that future research not only adopts advanced techniques, but also works towards harmonizing methodologies to enable the integration of data. Thus, it is recommended to standardize analytical protocols to facilitate comparisons across studies and biomes. The creation of open and collaborative data repositories can enhance data sharing. Research networks and technical training are essential to disseminate advanced methodologies. The use of artificial intelligence can support data harmonization and interpretation.

Traditional paradigms viewed SOM stability as primarily dependent on the intrinsic chemical recalcitrance of organic compounds, suggesting that certain molecules—like lignin—were inherently resistant to decomposition. However, new evidence suggests that persistence is more strongly influenced by physical protection mechanisms, such as organo-mineral associations and spatial inaccessibility to decomposers (SCHMIDT et al., 2011; LEHMANN & KLEBER, 2015). This shift in perspective emphasizes that SOM dynamics are controlled more by the context of interactions within the soil matrix than by molecular structure alone, requiring analytical approaches that can resolve these interactions at fine spatial scales.

3.7 Concluding remarks

The C contents and composition in the physical fractions of SOM are essential to better understand the processes of formation, persistence, and the ecological functions they exert in the soil. However, these properties are highly variable and influenced by local soil characteristics, climate, vegetation, and land management (SPARLING et al., 2016). While a considerable body of research on SOM fractions has been developed in temperate soils—often treating the fractions in isolation tropical soils remain underexplored, despite their distinct features such as deep weathering, high acidity, and elevated contents of aluminum and iron oxides and hydroxides. These characteristics substantially affect organic matter dynamics by enhancing organo-mineral interactions and influencing the distribution, stabilization, and persistence of SOM components.

Our research highlights a clear knowledge gap regarding the formation, transformation, and interaction of particulate organic matter (POM), mineral-associated organic matter (MAOM), and dissolved organic matter (DOM) in Brazilian soils. This lack of integrated information hinders the development of more robust models for predicting SOM behavior under different land-use scenarios in tropical regions. Given the increasing pressure on tropical ecosystems, addressing this gap is essential.

Moreover, traditional concepts that attributed SOM stability mainly to the chemical

recalcitrance of organic compounds have been challenged by recent findings. The emerging paradigm emphasizes the primacy of physical and physicochemical protection—such as microaggregation, mineral adsorption, and spatial inaccessibility—as the dominant mechanisms controlling SOM persistence (SCHMIDT et al., 2011; LEHMANN & KLEBER, 2015). This conceptual shift requires analytical approaches capable of resolving microscale processes and molecular interactions within the soil matrix.

In this context, we recommend that future studies on SOM characterization, particularly in tropical soils, adopt integrative and high-resolution techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Near-Edge X-ray Absorption Fine Structure (NEXAFS), X-ray fluorescence microscopy (μ -XRF), nanoscale Secondary Ion Mass Spectrometry (nanoSIMS), and synchrotron-based X-ray spectroscopies. These methods allow for non-destructive, spatially resolved analysis of SOM composition, reactivity, and localization, offering new insights into the eco-functionality and stabilization pathways of different SOM fractions.

Standardizing SOM fractionation protocols and analytical workflows across studies and regions is also a key step to enable comparability and meta-analytical approaches. Ultimately, deepening our understanding of SOM dynamics in tropical soils is vital for developing evidence-based strategies to mitigate climate change, enhance soil health, and promote sustainable land use in some of the world's most vulnerable ecosystems.

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4 CHAPTER 2: CARBON, NITROGEN, AND SULFUR STOCK IN THE SOIL ORGANIC MATTER PHYSICAL FRACTIONS UNDER DIFFERENT LAND USES IN THE ATLANTIC FOREST BIOME

Abstract

Land-use changes significantly impact the stocks of carbon (C), nitrogen (N), and sulfur (S) in tropical soils, particularly in Brazil's Atlantic Forest due to its high sensitivity, intense fragmentation and history of deforestation. This study evaluated the effects of different land-use systems, as well as the physical fractions of soil organic matter (SOM). Soil samples were collected at two depths, topsoil (0-5 cm) and subsoil (50-60 cm), in a region in southeastern Brazil. The results showed that the *Araucaria* forest exhibited the highest C stocks in the topsoil (22.95 Mg C ha⁻¹), followed by *Eucalyptus* plantations (17.14 Mg C ha⁻¹). In the subsoil, C stocks were more homogeneous across land uses, ranging from 13.64 to 9.00 Mg C ha⁻¹, with *Eucalyptus* plantations showing the highest values. N stocks also varied, with the highest values observed in *Eucalyptus* plantations (3.09 Mg N ha⁻¹ in the topsoil), while coffee showed the lowest N stocks (1.24 Mg N ha⁻¹). Regarding S, *Eucalyptus* plantations had the highest topsoil stocks (1.00 Mg S ha⁻¹), whereas pasture S stocks were below detection limits in the subsoil. The SOM fractions showed distinct responses to different land uses. Particulate organic matter carbon (POM-C) stocks were highest in native forests (6.65 Mg C ha⁻¹), while maize had the lowest values (0.78 Mg C ha⁻¹). Mineral-associated organic matter carbon (MAOM-C) stocks were greatest in *Araucaria* forests and pastures, while agricultural systems had lower stocks. No significant differences were found in Dissolved organic matter (DOM) fractions between land-use systems. Thereby, we conclude that the results support our hypothesis: land-use systems with perennial vegetation and reduced physical disturbance, such as native forests and silvicultural systems, promote greater accumulation and stabilization of SOM, particularly in the MAOM fraction, throughout the soil profile. This is likely driven by the higher quality of organic residues, increased soil cover, and the presence of deeper root systems in these environments. In contrast, intensive agricultural systems showed lower SOM stocks and a predominance of labile fractions.

Keywords: stock; land uses; soil organic carbon; tropical forest.

4.1 Introduction

Although tropical forests provide higher quality and quantity of ecosystem services than non-tropical forests, the services they provide are decreasing at a faster rate with the increasing temperature of the planet (ALAMGIR et al., 2016). Soil organic matter (SOM) plays numerous roles in the physical, chemical, and biological attributes of soils. Tropical soils with higher SOM contents show greater soil microbial activity, better physical conditions in terms of structure (aggregates), higher ion adsorption capacity, greater supply of plant-available nutrients, soil protection against physical impacts and radiation, higher soil water retention, higher groundwater recharge, less soil degradation, higher productivity, and a pathway of carbon stock (BRADY; WEIL, 2013). Therefore, maintaining adequate levels of SOM is critical for ecosystem sustainability. However, management and changes in land use influence the amount of SOM (CARTER, 2002).

Changes in land use and land cover have generated major transformations in the landscape, directly affecting natural resources and biodiversity at local and global scales, significantly influencing ecosystem functions and services (MORI; ISBELL; SEIDL, 2018). The negative effects of changes land use and cover can be enhanced in tropical regions, which have limiting climatic conditions for SOM accumulation due to its fast cycling (TERRADO et al., 2014). However, few studies have analyzed in detail changes in land use and land cover in these regions and correlated them with SOM dynamics and dissolved organic matter (DOM) as indicators of existing biotic and abiotic ecological processes (HUANG; SPOHN, 2015; KOUBA et al., 2018). Thus, understanding continuous land use changes is critical for understanding degradation processes, and serves as an important tool for environmental monitoring (GABRIELE et al., 2022).

The SOM fractions are considered good indicators of how the land use and management alter the biogeochemical cycles of soil nutrients. They make it possible to assess the availability of resource for microorganisms and the appropriate incorporation of phytomass for soil protection (CERIOTTI; TANG; MAGGI, 2020). Additionally, SOM fractions contribute to increasing carbon stocks, as soil organic matter is the largest terrestrial carbon reservoir and also contains organic forms of many essential nutrients (BONGIORNO et al., 2019).

Studies into the physical fractions of SOM is important to provide simple and effective answers (GMACH et al., 2019; KALBITZ et al., 2000). Among these fractions, particulate organic matter (POM) and mineral-associated organic matter (MAOM) are usually

studied. Lavallee et al., (2020a) propose standardizing studies of MAOM and POM fractions (differences in density and size) and when evaluated together, they are important to provide information regarding the change in SOM stocks and input due to various effects, including land-use changes.

In addition to these fractions separated by physical parameters, the authors report that DOM interacts directly with MAOM and POM. This fraction represents an important part of the carbon cycling and other nutrients in the soil, such as nitrogen, phosphorus, and sulfur. DOM is a source of enrichment of the subsoil through percolation (KALBITZ et al., 2000) and is associated with the loss of carbon from soil into water bodies. It is ecologically and economically important because of this dynamic and because it is considered a complex mixture of substances with varied and little-known chemical compositions and properties (LI et al., 2019b; MOPPER, 1987).

Thus, it is essential to understand the ecological, chemical, and physical controls on SOM stability and its correlation with POM, MAOM and DOM fractions to maintain the balance of the C, N and S cycles, increasing food production, as well as protecting biodiversity and ecosystem services (LEHMANN; KLEBER, 2015b). Despite the recognition of their importance, SOM stocks have been largely lost or degraded due to land use change and unsustainable forest and agricultural management practices (JACKSON et al., 2017). Therefore, understanding how land use in different ecosystems affects the formation of SOM in the subsoil becomes crucial to developing practices that accumulate more stable forms of carbon in the deeper layers of soil. In this context, sustainable land use management practices and the study of SOM quantity and quality are important tools to slow the rise of atmospheric CO₂ and to understand the chemical balance of carbon and other nutrients to mitigate climate change, ecological impacts, and changes in soil carbon stocks (BERG, 2014).

In agricultural systems in southeastern Brazil, for example, physical, chemical, and biological constraints of the horizons restrict root growth and water and nutrient extraction (EVANGELISTA SILVA et al., 2021). These are mainly relevant in the subsoil, defined as all material below the arable layer (in most countries found from 20 cm depth) (ADCOCK et al., 2007; PEIGNÉ et al., 2013). According to these authors, the higher carbon stocks are probably due to the POM, a greater diversity of cultivated species, and the deposition of plant residues on the soil. However, further research is needed regarding the contribution of residue incorporation, plant diversity and soil tillage in the investigation of increased S, N and C stocks, both in the topsoil and in the subsoil (COSER et al., 2018).

The stocks of carbon (C), nitrogen (N), and sulfur (S) vary widely across different land uses in Brazil in the Atlantic Forest. In areas of native vegetation, such as forests, there is a greater accumulation of C and N, especially in the topsoil layer (0-5 cm), due to the high organic matter content and intense biological processes (COSER et al., 2018; FONTANA et al., 2011; PEREIRA et al., 2020; SALES et al., 2020). However, with the conversion of forests to other land uses, such as agriculture and pasture, these stocks tend to decrease, particularly in deeper soil layers (50-60 cm), where organic matter is less dynamic (RAMOS et al., 2013; VALLADARES et al., 2016). Studies on S stocks are less abundant, but it is known that its cycling is also affected by land-use change (SCHLESINGER et al., 2011; DAVIDSON & JANSSENS, 2006; LEHMANN & KLEBER, 2015). Although there is already data on the effects of deforestation and agricultural management on C and N stocks, knowledge about how different land uses affect the SOM physical fractions in tropical soils is still limited, especially at greater depths.

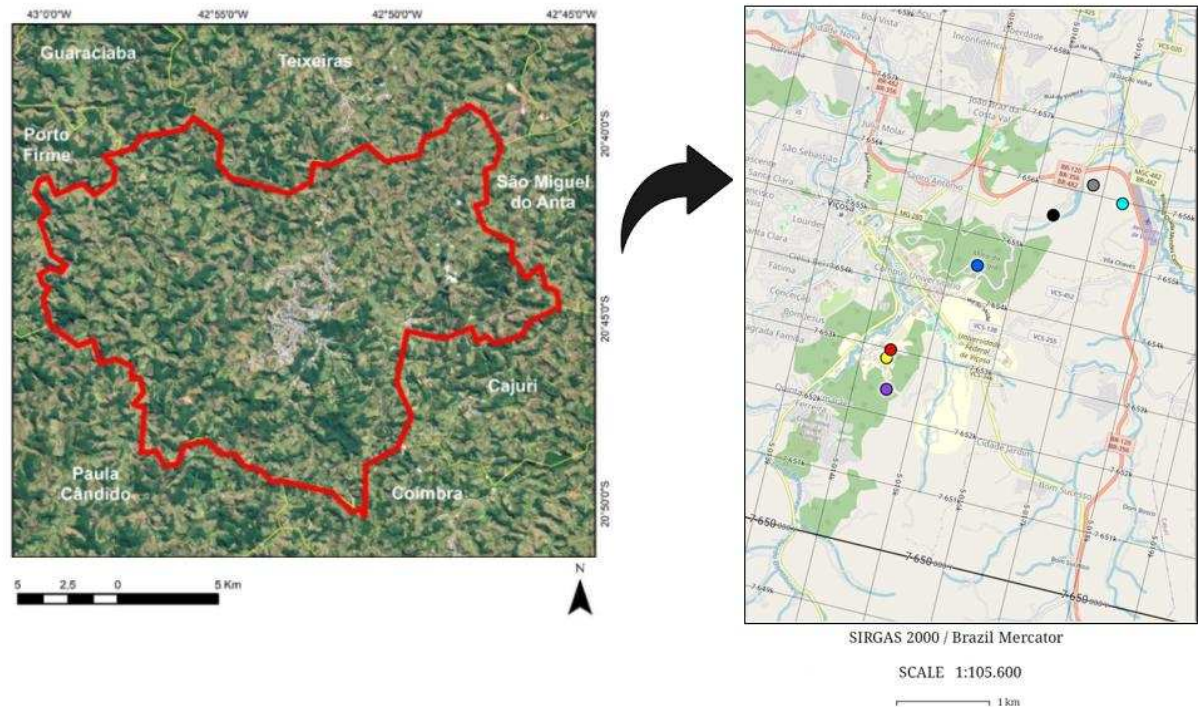
Our aim was to assess the influence of different land uses on C, N and S stocks and SOM physical fractions (POM, MAOM and DOM), both at surface (0–5cm) and subsoil (50–60cm), in southeastern Brazil. We hypothesized that perennial land-use systems with reduced physical disturbance, such as forestry and silviculture, would promote greater accumulation and stabilization of soil organic matter, especially in the mineral-associated organic matter (MAOM) fraction, throughout the soil profile, compared to intensive agricultural systems. This is primarily due to higher quality organic inputs, continuous soil cover, and deeper rooting systems. This study will help inform land management strategies that enhance SOM conservation, promote sustainable nutrient cycling, and mitigate environmental degradation.

4.2 Materials and Methods

4.2.1 Study site

The study areas were defined in different land use systems of areas belonging to the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, southeastern Brazil (42° 52' W and 42° 50' W longitude, and 20° 44' S and 20° 47' S latitude), totaling an area of 1,359 hectares (Figure 1).

Figure 1 – The following colors correspond to the collection areas for each type of land use: purple - Eucalyptus; yellow - Native forest; red - Rubber tree; blue - Araucaria; light blue - maize; gray - coffee and black - pasture.



Source: Author

The UFV is located in the municipality of Viçosa, in the Zona da Mata region, at an altitude of 649 m. The climate of the region according to the Köppen classification is tropical altitude with warm and rainy summers and cold and dry winters (Cwa). Following data from the Climatological Normal of the National Institute of Meteorology (2020), Viçosa-MG registered an average annual rainfall of 1,289 mm, an average monthly temperature ranging in the rainy (November-March) period between 20.3 °C and 22.3 °C, and in the dry (April-September) period between 15.4 °C and 18.3 °C. As for the minimum temperature, the month of July registered the lowest values, with an average of 10 °C, while the maximum temperature ranged from 25 °C to 35 °C during summer, and in winter, from 23 °C to 25 °C (INMET, 2020).

The soil throughout the area is a *Latossolo Vermelho-Amarelo distrófico típico* according to the Brazilian Soil Classification System (Embrapa, 2018) which corresponds to an Oxisol (Hapludox, in the Soil Taxonomy). This highly weathered soil is characterized by its well-developed structure and good water retention capacity but has a low nutrient retention ability due to its low cation exchange capacity. It can be vulnerable to degradation when exposed to inappropriate land use practices.

In addition to the physical and climatic characteristics, it is important to note that the study areas include different land use systems, which reflect the diversity of agricultural

and silvicultural practices in the region. This diversity is crucial to understanding how the different covers affect the dynamics of soil organic matter (STAFF, 2014).

4.2.2 Land-use systems

For this research, seven types of land use were selected that represent forest areas, agricultural and silvicultural practices in the Atlantic Forest (Table 1). These systems were chosen not only because of their representativeness in the region, but also because of their complex interactions with SOM dynamics and their implications for environmental sustainability and carbon.

Table 1 – Management characteristics, land use history and organic matter quality across different systems in the Atlantic Forest sequestration.

Land Use	Crop Type	Hectares	Years	Management practices	Organic matter lability
Native Forest (dense ombrophilous forest)	Natural vegetation	~18 ha	~100	No anthropic intervention; high litter diversity and biological activity.	Predominantly stable and recalcitrant.
Eucalyptus: <i>Eucalyptus grandis</i> W.Hill	Perennial monoculture	~8.3 ha	~30	Liming and NPK fertilization before planting; moderate litter input (lignified).	Intermediate to low; slower turnover.
Rubber tree: Rubber tree (<i>Hevea brasiliensis</i> L.)	Perennial crop	~9.3 ha	~30	Occasional fertilization and liming; low soil disturbance.	Medium; residues moderately recalcitrant.
Araucaria: Araucaria plantation (<i>Araucaria angustifolia</i> (Bertol.)	Planted forest	~10.4 ha	~70	Low-intensity management; needle litter (high lignin).	Stable; highly recalcitrant litter.
Maize: Annual field of maize crop (<i>Zea mays</i> L.)	Annual crop	~0.5 ha	~30	Intensive tillage; frequent fertilization and liming; high turnover of crop residues.	High; very labile residues, rapid SOM decomposition.

Coffee: (<i>Coffea arabica</i> L.)	Perennial crop	~1.5 ha	~30	Fertilization with macro/micronutrients; liming; pruning and leaf litter input.	Intermediate; contributes to both POM and MAOM fractions.
Pasture: Brachiaria grass (<i>Urochloa ruziziensis</i> (R.Germ.& Evrard) Crins)	Perennial grass	~7.1 ha	~30	Occasional fertilization and liming; moderate grazing; high root biomass input.	Medium; root inputs promote partial stabilization.

Source: Author

The study areas are located on lands belonging to the Universidade Federal de Viçosa (UFV), in the Atlantic Forest biome. Seven representative land use systems were selected based on their relevance to regional agricultural and forestry practices and their contrasting impacts on soil organic matter (SOM) dynamics. Below is the detailed description of the land-use systems, their history, and management practices:

Native Forest (*Dense Ombrophilous Forest*): This area represents a remnant of primary Atlantic Forest, preserved under legal protection. The site is characterized by native vegetation with a dense canopy and rich biodiversity. There is no history of soil amendment or nutrient addition, and the SOM input originates from natural litterfall and root turnover. The organic matter tends to be more stable and less labile due to the slow decomposition of recalcitrant compounds under a closed canopy. No mechanical disturbance or fertilization occurs in this area (VELOSO; RANGEL-FILHO; LIMA, 1991).

Eucalyptus (*Eucalyptus grandis*): This eucalyptus plantation was established around 30 years ago for timber and pulpwood production. The area had previously been used for pasture. Management includes periodic liming, application of mineral fertilizers (N-P-K), and mechanical harvesting. High biomass production and frequent harvesting lead to rapid nutrient cycling (RODRIGUES et al., 2019). However, due to the low quality of eucalyptus litter and high C:N ratio, the SOM is typically more recalcitrant, with slower decomposition rates (DAI et al., 2023). The plantation's high water uses and allelopathic litter components may reduce microbial diversity and alter nutrient cycling (PEREIRA et al., 2020; ZHOU et al., 2021).

Rubber Tree (*Hevea brasiliensis*): Rubber cultivation has been maintained for approximately 30 years. The area was converted from pasture and has received periodic fertilization and

liming, based on soil test recommendations. In some sections, intercropping with legumes or cover crops has been introduced. Rubber tree plantations can enhance carbon sequestration in the long term, especially when understory management avoids intensive soil disturbance. However, conventional practices may include herbicide applications and pruning residues left on the soil surface, which influence SOM quality and decomposition dynamics (YANG et al., 2019).

Araucaria (*Araucaria angustifolia*): The Araucaria plantation is approximately 70 years old and was established in a previously forested area. The site has experienced minimal human intervention since plantation. There has been no recent application of fertilizers or lime, and ground vegetation is rarely managed. The SOM tends to be moderately labile, derived from the decomposition of needle litter and understory inputs (ZINN; FIALHO; SILVA, 2024). The slower decomposition under coniferous canopy contributes to a gradual accumulation of MAOM in subsurface layers (SILVA et al., 2022).

Maize (*Zea mays L.*): This is an intensively managed annual system, with maize cultivation practiced in rotation or succession over the past 30 years. The soil undergoes frequent disturbance due to plowing and harrowing. Fertilization follows technical recommendations, including urea, phosphorus, potassium, and micronutrients. Herbicides are applied before planting, and crop residues are often removed or burned. These practices accelerate SOM decomposition and promote loss of labile fractions such as POM and DOM, especially in surface layers. Organic matter in this system is of low stability and highly vulnerable to mineralization (COSER et al., 2018).

Coffee (*Coffea arabica L.*): Coffee has been grown in this area since 2003. Initially, the area was pasture, with the native vegetation removed in the early 1990s. Coffee cultivation, especially in agroforestry systems, can promote biodiversity conservation and soil sustainability. However, intensive coffee growing practices can lead to erosion and a decrease in SOM stocks if they are not accompanied by conservation strategies. Fertilization is based on annual soil analyses, and lime and gypsum are applied as needed. This system promotes carbon inputs and improves SOM quality when managed with low chemical inputs and good ground cover (MELO et al., 2021; HADDAD et al., 2022).

Pasture (*Urochloa ruziziensis*): This Brachiaria pasture has been established for approximately 30 years on land previously used for annual crops. The area receives periodic fertilization with

N-P-K and liming based on soil tests. Grazing intensity is moderate. SOM in this system is characterized by relatively labile fractions due to root turnover and organic inputs from the grass canopy. However, intensive grazing without rotation can lead to compaction and SOM loss over time (FERNANDES et al., 2019).

4.2.3 Soil sampling

In each of the seven chosen study areas, located on slopes, six trenches were opened, with an approximate length of 1 x 1 m, totaling 42 trenches. The deformed soil samples were collected at depths of 0-5 (topsoil) and 50-60 cm (subsoil).

For the DOM analysis, ~ 500 g, were placed in a dark plastic bag to avoid photodegradation, and placed in a thermal box with chemical ice, kept at 4°C for no more than two months, and taken to the laboratory for analysis. Another subsample, ~ 200 g was air dried and sieved (< 2 mm for physical fractionation by granulometry and subsequent analyses).

4.2.4 SOM fractionation and analysis

Deformed dry soil samples (< 2 mm) were used for the SOM physical fractionation. POM, which is associated with the sand fraction (> 53 µm); and MAOM, associated with the silt + clay fractions (< 53 µm), were extracted according to Cambardella and Elliott (1992). In short, sub-samples of 10 g soil were dispersed in 30 ml of sodium hexametaphosphate (5g L⁻¹) stirred for 15 h in a horizontal shaker (Tecnal, TE-145/176). Samples were then passed through a 53 µm sieve after several times washing with milli-Q water. The two fractions were oven-dried with forced circulation at 60°C until constant weight and ground to determine their carbon (C), nitrogen (N) sulfur (S) (BREMNER; TABATABAI, 2015; NELSON; SOMMERS, 1980; SNYDER; TROFYMOW, 1984) contents on an elemental analyzer (EA 2400 SERIES II, Perkin Elmer).

For the (DOM, fraction < 0.45 µm) extraction in water, we used the method adapted from Jones and Willett (2006) with optimization by Xie et al. (2017) and Xie et al.(2020). Subsamples were standardized by sieving on 4 mm mesh, removing coarse roots present. The extraction of DOM in water was performed from triplicates of each subsample of 50 g of fresh soil in 100 mL of Milli-Q water, correcting for the gravimetric humidity, in a solid-liquid ratio of 1:2. Then the samples were stirred for 24 h at 130 rpm and room temperature (25° C) in 150 mL Erlenmeyer flask. This procedure was performed in the absence of light (DING et al., 2020).

The soil suspensions were centrifuged at 3500 rpm for 10 min. The supernatant was filtered through a 0.45 μm polyethersulfone (PES) filter which does not release or adsorb DOM (ZSOLNAY et al., 1999), and stored in amber plastic headspace bottles in a freezer at -20°C (DING et al., 2020) for subsequent determination of total organic carbon (TOC) and total nitrogen (TN).

The stocks of C, N, and S in the POM and MAOM were calculated based on the specific mass of the soil (ELLERT; BETTANY, 1995), as well as for the stocks of C and N of DOM. This method considers the equivalent soil mass of the areas of use, corrected the thickness of the systems with the changes in soil density (Ds), using as a reference the average density of the natural vegetation area in its respective depth. The soil mass at each depth of each area was determined by the equation:

$$M_{soil} = Lt \times ha \times Ds \quad (1)$$

Where: M_{soil} is the soil mass per hectare in each layer evaluated (Mg ha^{-1}); Lt is the soil thickness for each layer (m); ha is the area employed ($1 \text{ ha} = 10,000 \text{ m}^2$); and, Ds is the soil density in each layer sampled (g cm^{-3}). Then, the thickness of the layer to be deducted or added in the systems in relation to natural vegetation was determined by the equation:

$$Lt_{(add\backslash ded)} = (M_{ref} - M_{soil}) \times fha / Ds \quad (2)$$

Where: $Lt_{(add\backslash ded)}$ is the thickness of the soil layer to be added to (+) or deducted from (-) the system layer (m); M_{ref} is the average equivalent mass of the reference area (Mg ha^{-1}); M_{soil} is the equivalent soil mass of each area (Mg ha^{-1}); fha is the conversion factor from ha to m^2 (0.0001 ha m^{-2}); Ds is the soil density for the sampled layer area (Mg m^{-3}). After determining the thickness, the C, N and S stocks were calculated by the following equation:

$$S = (C \times Ds \times Lt \pm E_{(add\backslash ded)}) \times 10 \quad (3)$$

Where: S the stock of C, N or S in the SOM fractions (Mg ha^{-1}); Lt is the soil layer thickness (m); Ds is the soil density of the cropping system in the chosen layer ($\text{g cm}^{-3} = \text{Mg m}^{-3}$); C is the C, N or S contents in the SOM fraction (g kg^{-1}); and 10 is the conversion factor from kg m^{-2} to Mg ha^{-1} .

The stocks of the reference area were calculated only by the product of the content, density, and thickness of the soil layer, by the equation:

$$S = C \times Ds \times Lt \times 10 \quad (4)$$

Where: S is the stock of C, N or S in the SOM fractions of the reference area (Mg ha^{-1}); Lt is the thickness of the soil layer (m); Ds is the soil density of the land use in the chosen layer ($\text{g cm}^{-3} = \text{Mg m}^{-3}$); and 10 is the conversion factor from kg m^{-2} to Mg ha^{-1} .

4.2.5 Physical and chemical analyses

Soil moisture was determined in deformed samples collected in centrifuge tubes. Moisture was determined by the freeze-drying process (freezing the soil and then sublimating the water content of the samples) using FreeZone LABCONCO® 6 L equipment, with the mass of the set (soil, tube and lid) being weighed before and after the process. Soil density was determined using the volumetric ring method (TEIXEIRA, et al., 2017).

Soil texture was determined using the pipette method (CAMARGO. et al., 2009) and classified according to the United States Agriculture (USDA) classification (Table 2). Sand, silt and clay determined by mechanical dispersion by orbital shaking for 16 hours at 140 rpm and chemically with NaOH 1 mol L^{-1} (TEIXEIRA, et al., 2017).

Table 2 – Physical properties of an Oxisol soil from different land uses in the Atlantic Forest.

Granulometric analysis							
Area	Coarse sand	Fine sand	Silt	Clay	Textural features	WDC	ρS
	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}		kg kg^{-1}	g cm^{-3}
0-5cm							
<i>Eucalyptus</i>	0.190	0.147	0.208	0.454	Clay	0.158	2.50
Native forest	0.325	0.148	0.092	0.436	Sandy clay	0.133	2.60
Rubber tree	0.255	0.168	0.112	0.465	Clay	0.222	2.63
<i>Araucaria</i>	0.263	0.138	0.159	0.440	Clay	0.108	2.47
Maize	0.266	0.218	0.104	0.411	Sandy clay	0.138	2.53
Coffee	0.197	0.172	0.074	0.557	Clay	0.217	2.56
Pasture	0.285	0.135	0.090	0.490	Clay	0.175	2.50
50-60cm							
<i>Eucalyptus</i>	0.167	0.121	0.191	0.521	Clay	0.136	2.56
Native forest	0.246	0.156	0.103	0.495	Clay	0.168	2.60

Rubber tree	0.177	0.122	0.100	0.601	Highly clay	0.021	2.56
<i>Araucaria</i>	0.300	0.108	0.155	0.437	Clay	0.168	2.53
Maize	0.191	0.106	0.082	0.621	Highly clay	0.208	2.63
Coffee	0.155	0.116	0.067	0.662	Highly clay	0.015	2.53
Pasture	0.204	0.123	0.084	0.589	Clay	0.221	2.63

WDC = Water dispersed clay; ρS = Particle density

The Table 3 show the chemical characterization of the soil samples collected was carried out by determining Hydrogen potential (pH) in water: determined in a 1:2.5 solution (soil-solution) by potentiometry; P-Rem: remaining P extracted with CaCl_2 and determined by molecular absorption; available K and P: extracted with the Mehlich-1 solution, with P determined by colorimetry and K by flame emission photometry; Ca^{2+} , Al^{3+} e Mg^{2+} : extracted with KCl 1 mol L^{-1} , in the ratio 1:10, with Ca^{2+} and Mg^{2+} and determined by atomic absorption spectrometry and Al^{3+} by titration with NaOH 0.025 mol L^{-1} ; H+Al: extracted with calcium acetate 0.5 mol L^{-1} , in the ratio 1: 15 and determined by titration with NaOH $0.0606 \text{ mol L}^{-1}$; SB = sum of exchangeable bases ($\text{Mg}^{2+} + \text{K} + \text{Ca}^{2+}$); T = cation exchange capacity at pH 7.0 which is the sum of Ca^{2+} , Mg^{2+} , K^{2+} and Al^{3+} e H^{+} ; t = cation exchange capacity at pH 7.0 which is the sum of de Ca^{2+} , Mg^{2+} , K^{2+} e Al^{3+} ; V = base saturation $V(\%) = (100 \cdot \text{SB}) t^{-1}$; m = aluminum saturation where $m = (100 \cdot \text{Al}^{3+}) (\text{SB} + \text{Al}^{3+})^{-1}$, P-rem and the other analyses were determined according to Alvarez, et al., (2000)..

Table 3 – Chemical properties of an Oxisol soil from different types of land use in the Atlantic Forest.

Land use	pH H ₂ O	P	K	Ca ²⁺	Mg ²⁺	Al ³⁺	H+Al	SB	t	TpH ₇	V	m	P-Rem
	(1:2,5)	mg dm ⁻³ ₃	mg dm ⁻³ ₃	cmol ^{lc} dm ⁻³	cmol ^c dm ⁻³	cmol ^{lc} dm ⁻³	cmol ^c dm ⁻³	cmol ^c dm ⁻³	cmol ^c dm ⁻³	cmol ^c dm ⁻³	%	%	mg L ⁻¹
0-5cm													
<i>Eucalyptus</i>	4.8	4.1	87	2.77	1.55	0.31	7	4.54	4.85	11.54	39.3	6.4	34.5
Native forest	4.9	5.5	57	2.31	0.6	0.37	7	3.06	3.43	10.06	30.4	10.8	30.4
Rubber tree	5.09	4.6	49	1.95	0.74	0.2	5.2	2.82	3.02	8.02	35.2	6.6	31.2
<i>Araucaria</i>	5.15	2.1	37	5.72	1.79	0.06	5.7	7.6	7.66	13.3	57.1	0.8	32.7
Maize	5.32	10.3	57	2.37	1.18	0.06	3.9	3.7	3.76	7.6	48.7	1.6	34.7
Coffee	5.04	24.3	123	2.87	0.94	0.12	5.3	4.13	4.25	9.43	43.8	2.8	30.1
Pasture	5.02	1.5	77	1.38	0.99	0.35	6.7	2.57	2.92	9.27	27.7	12	21.2
50-60cm													
<i>Eucalyptus</i>	4.89	0.6	3	0.49	0.35	0.51	3.4	0.85	1.36	4.25	20	37.5	21
Native forest	4.6	0.8	9	0.14	0.08	0.9	4.1	0.24	1.14	4.34	5.5	78.9	23
Rubber tree	4.83	0.8	7	0.08	0.03	0.66	3.6	0.13	0.79	3.73	3.5	83.5	17.9
<i>Araucaria</i>	5.38	0.3	5	0.9	0.26	0.47	2.6	1.17	1.64	3.77	31	28.7	30.5
Maize	5.06	0.9	17	1.31	0.68	0.2	3.7	2.03	2.23	5.73	35.4	9	18.8
Coffee	4.49	1.2	73	0.35	0.12	0.98	4.3	0.66	1.64	4.96	13.3	59.8	14.2
Pasture	5.21	0.6	11	0.96	0.22	0.45	4.9	1.21	1.66	6.11	19.8	27.1	10.6

H+Al = potential acidity; SB = Base Saturation; t = capacity of ionic exchange; V = Base Saturation Index; m = Aluminum Saturation Index; P-Rem = remaining P; TpH₇ = cation exchange capacity at pH₇

4.2.6 Statistical Analyses

The variables studied were analyzed using an entirely randomized design, in a factorial arrangement (7 x 2), with the factors being seven different land use systems and two depth (0-5 and 50-60 cm). The normality was tested using the Shapiro-Wilk test (p-value < 0.05) and the homogeneity of variances was tested using the Levene's test (p-value < 0.05). A two-way ANOVA was applied to investigate the effects of different land-use systems on C, N, and S stocks in the topsoil and subsoil. When the assumptions of parametric statistics were not met, the variables were transformed using the logarithm transformations plus a constant ($\log(x) + 1$). were used for the stocks: N-MAOM and N-POM in the 0-5 cm depth and, POM-C and S in the 50-60 cm depth. Tukey's post hoc Test was used to test for significant differences between areas (p < 0.05). A principal component analysis (PCA) was carried out to explore general trends in soil attributes (chemical and physical parameters) and to assess which main variables associated with SOM and its physical fractions could be grouped or separated according to land use.

These statistical analyses were performed with R version 4.3.0 software (R Core Team, 2023). In addition to the base R packages, *ggplot2* (WICKHAM 2017), *easyanova* (ARNHOLD, 2022) and *tidyverse* (WICKHAM et al., 2019) were used.

4.3 Results

4.3.1 Carbon, Nitrogen and Sulfur stocks

The SOC content as well as the stocks of C, N, and S in the MAOM, POM, and DOM fractions in the different land use systems can be seen in Figures 2, 3, and 4, respectively.

The average SOC stocks of the 0-5 cm layer (topsoil) varied from 10.46 to 22.96 Mg C ha⁻¹ in the different land-use systems under study. The average SOC values of the 50-60 cm layer (subsoil) ranged between 13.64 and 9.00 Mg C ha⁻¹ (Figure 2a). The SOC stocks in the 0-5 cm soil depth under the different land use systems follow the following order, with corresponding quantities (Mg C ha⁻¹): *Araucaria* forest plantation (22.95), *Eucalyptus* forest plantation (17.14), pasture (14.26), native forest (14.0), coffee (13.70), rubber tree (12.44), maize (10.45). At the soil depth of 50-60 cm, SOC stocks were in the order (Mg C ha⁻¹): *Eucalyptus* (86.17), pasture (81.81), maize (80.23), rubber tree (76), *Araucaria* (71.91), native forest (64.67), coffee (58.89) (Fig. 2a). The *Araucaria* forest had 120% greater soil SOC stocks

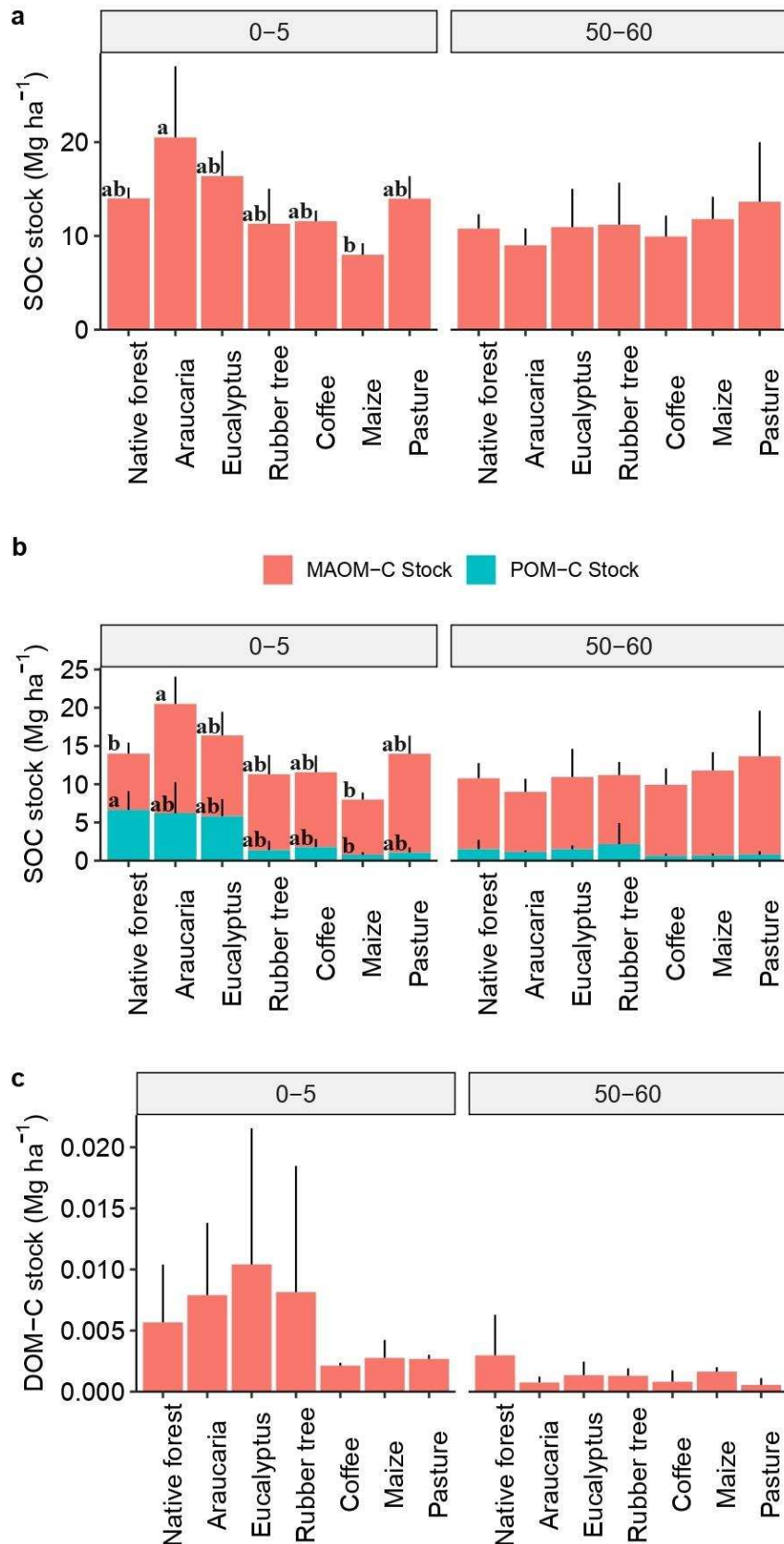
for 0-5cm depth than the maize area ($p < 0.05$), while the other areas did not show significant differences ($p > 0.05$) in carbon stock values (Fig. 2a). However, in the subsoil the average SOC stocks did not differ between land uses (Fig. 2a).

The average POM-C stocks of the 0-5cm layer varied from 0.78 to 6.65 Mg C ha⁻¹. The average POM-C values of the 50-60cm layer were between 0.63 and 2.15 Mg C ha⁻¹ (Fig. 2b). Overall, no statistical difference in POM-C stocks was observed between land-uses in the 50-60 cm depth classes, however for the 0-5cm depth, the average POM-C stocks were significantly different only between native forest (6.65 Mg C ha⁻¹) and maize (0.78 Mg C ha⁻¹).

The average MAOM-C stocks of the 0-5 cm layer varied from 7.20 to 14.28 Mg C ha⁻¹. The highest stocks were observed for the *Araucaria* forest (14.28), which were statistically different from areas of native forest and maize. The average MAOM-C values of the 50-60 cm layer varied between 7.87 and 12.87 Mg C ha⁻¹ (Fig. 2b). However, there were no statistical differences between the types of land use in the SOC stock in this fraction.

The DOM-C stocks ranged from 0.002 (coffee) to 0.0104 (*Eucalyptus*) Mg C ha⁻¹ for the 0-5 cm depth over all land uses. At depths of 50-60cm, the average DOM-C stock was between 0.001 Mg C ha⁻¹ and 0.003 Mg C ha⁻¹. There were no differences ($p > 0.05$) between land uses (Fig. 2c).

Figure 2 – The effect of various land-use systems on (a)- SOC stock (b) POM-C and MAOM-C stock (c) DOM-C stock at depths 0 to 5 cm and 50 to 60cm. Values are means \pm SDs of three replicates ($p < 0.01$). Different letters indicate significant differences at $p < 0.05$.



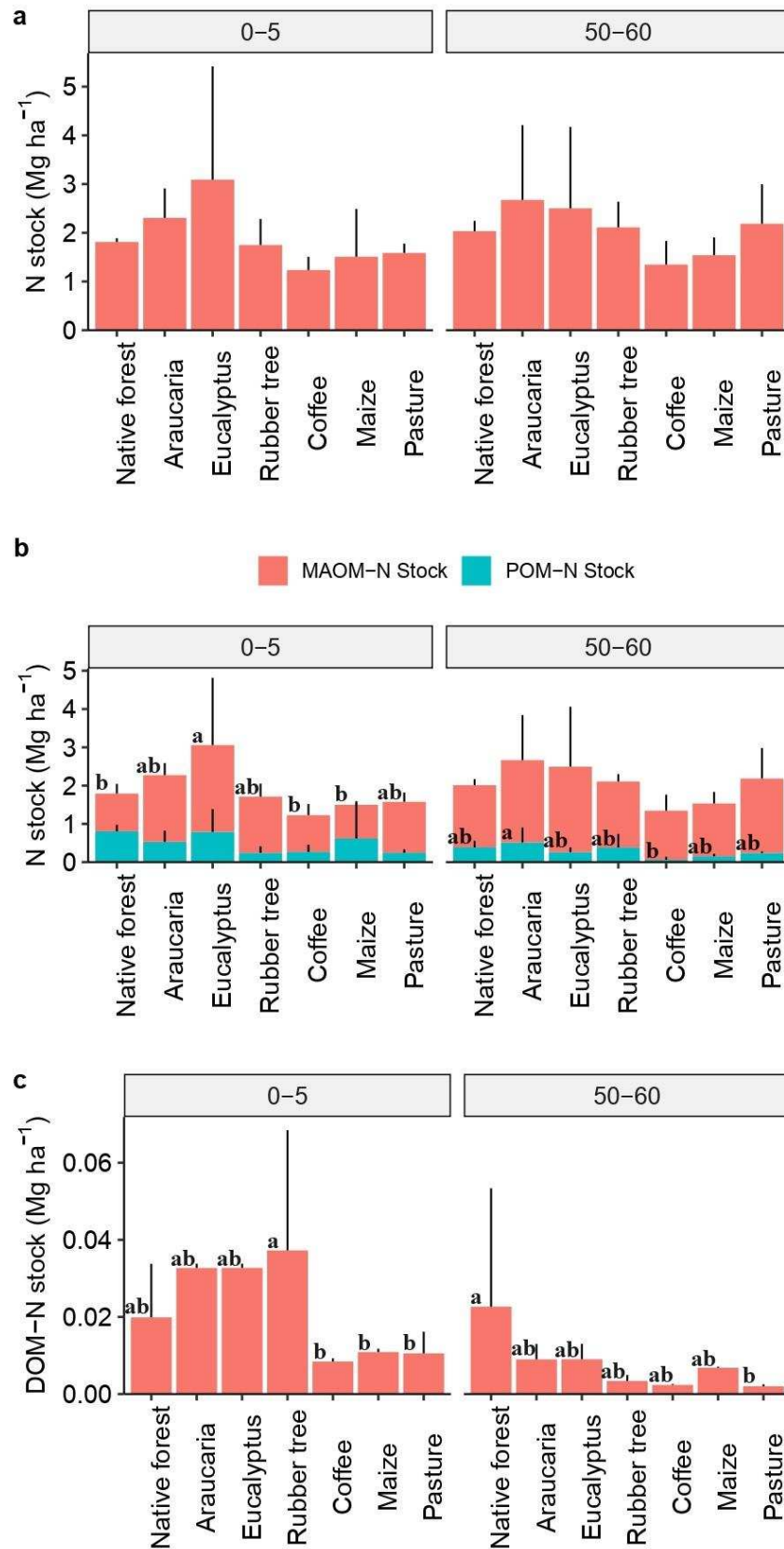
The average N stocks of the 0-5cm layer ranged from 1.24 (coffee plantation) to 3.09 (*Eucalyptus* forest) Mg N ha^{-1} , and from 1.35 (maize crop) to 2.68 Mg N ha^{-1} (*Araucaria* forest) for the 50-60cm layer (Figure 3a). The nitrogen obtained at a depth of 0-5 in the different systems studied corresponded to the following amounts (Mg N ha^{-1}): *Eucalyptus* forest (3.09), *Araucaria* forest (2.30), maize (1.81), pasture (1.75), rubber tree (1.58), native forest (1.50), coffee (1.24). At 50-60cm, nitrogen was in the order (Mg N ha^{-1}): *Araucaria* (2.68), *Eucalyptus* (2.50), rubber tree plantation (2.19), pasture (2.11), native forest (2.03), maize (2.54), coffee (1.35). There were no differences between land uses regarding average N stocks for neither of the two depths ($p > 0.05$).

The average POM-N stocks of the 0-5cm layer varied from 0.24 - 0.81 Mg N ha^{-1} . The average POM-N values of the 50-60cm layer were between 0.06 and 0.51 Mg N ha^{-1} (Fig. 3b). Overall, no statistical differences in POM-N stocks were observed between land-uses in the 0-5cm depth, however for the 50-60cm depth class, the mean POM-N stocks were significantly different following the order, in Mg N ha^{-1} : *Araucaria* (0.51) ~ native forest (0.39) ~ rubber tree (0.38) ~ *Eucalyptus* (0.26) ~ pasture (0.24) ~ maize (0.16) > coffee (0.06).

The highest MAOM-N stocks were in the *Eucalyptus* plantation at 0-5 cm (Fig 3b). At the same time, the native forest, coffee and maize showed lower MAOM-N stock values of approximately 60% lower in relation to the higher stocks evaluated. There were significant differences as shown in Figure 3b. In the lower soil layer, the areas did not differ for the MAOM-N fraction.

The DOM-N stocks ranged from 0.010 to 0.040 Mg N ha^{-1} for depth 0-5 cm. At the 50-60 depth the mean N-DOM stock values ranged from 0.002 (pasture) to 0.023 (native forest) Mg N ha^{-1} . In the DOM fraction, the average N stocks were greatest for the areas with rubber trees at 0-5cm soil depth, and the areas of pasture, coffee and maize had the lowest DOM-N stock value at this depth (Fig. 3c).

Figure 3 – The effect of various land-use systems on (a)- N stock (b) POM-N and MAOM-N stock (c) DOM-N stock at depths 0 to 5 cm and 50 to 60 cm. Values are means \pm SDs of three replicates. Different letters indicate significant differences at $p < 0.05$.



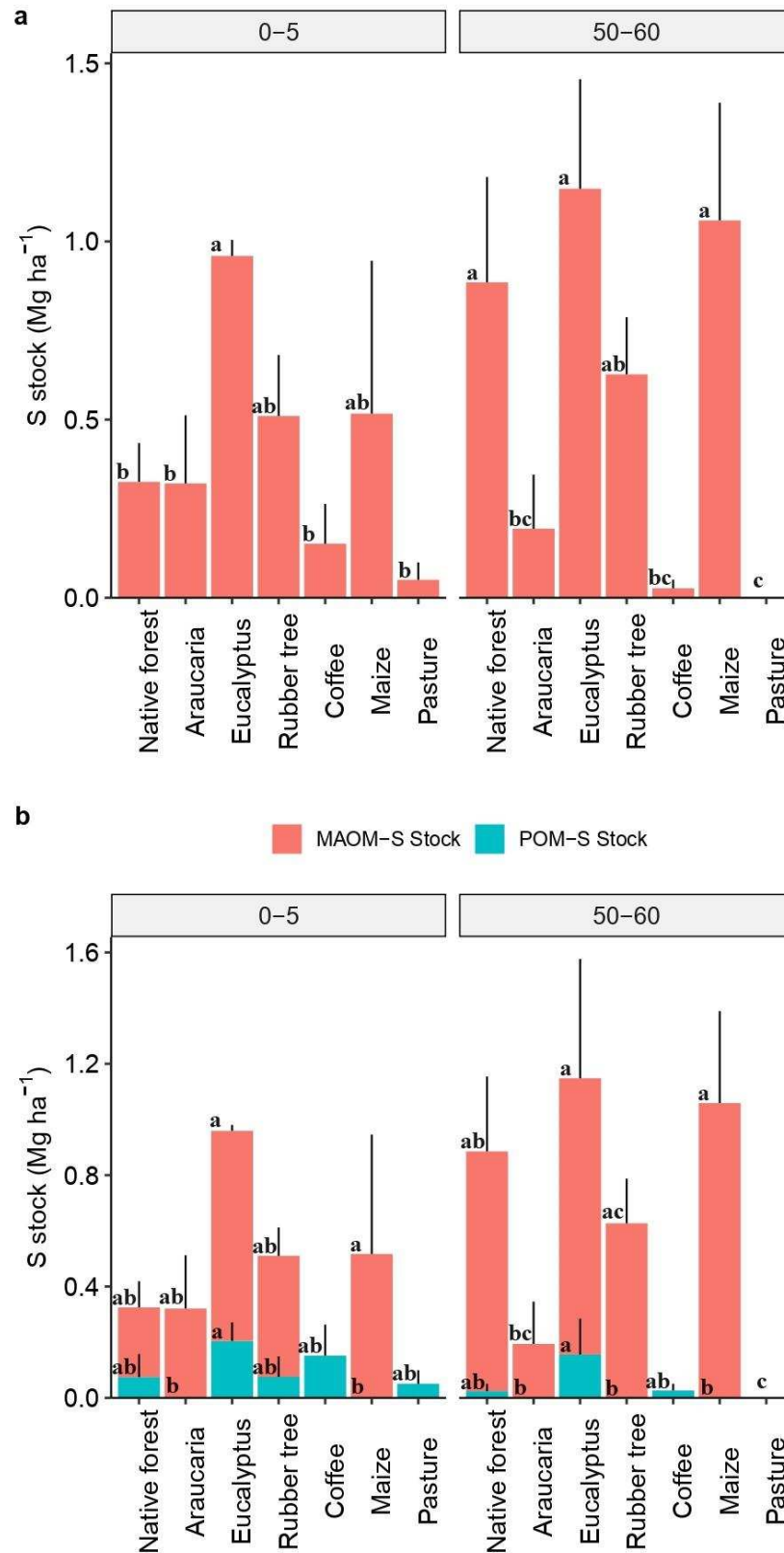
The average S stocks in the 0-5 cm layer ranged from 0.06 to 1.0 Mg S ha⁻¹. *Eucalyptus* (1.00 Mg ha⁻¹) stood out with the highest ($p < 0.05$) S stocks when compared to the other land uses at this depth. In the 50-60 cm layer the highest S stocks were of *Eucalyptus*, maize, Native forest and rubber tree but the pasture's S stock value was below the detection limit, so it was considered zero (Fig. 4a). The stocks of *Araucaria*, coffee and pasture did not differ from each other, however, when compared to the four aforementioned stocks, they had the lowest S stocks.

The average POM-S stocks of the 0-5 cm layer varied from 0.05 to 0.21 Mg S ha⁻¹. The average POM-S values of the 50-60 cm layer were between 0.02 and 0.15 Mg S ha⁻¹. Some values were below the detection limit (Figure 4b).

The S stocks in the MAOM fraction were as follows: in the *Eucalyptus* (0.76 Mg S ha⁻¹), maize (0.52 Mg S ha⁻¹), rubber tree (0.44 Mg S ha⁻¹) and *Araucaria* plantations (0.32 Mg S ha⁻¹) and native forest (0.25 Mg S ha⁻¹) at the depth of 0-5 cm. The pasture and coffee system showed values below the detection limit (Fig. 4b).

For the 50-60 cm depth, the MAOM-S stocks in the maize (1.06 Mg S ha⁻¹), *Eucalyptus* (0.99 Mg S ha⁻¹), native forest (0.86 Mg S ha⁻¹) and rubber tree (0.63 Mg S ha⁻¹) systems showed no significant differences (Fig. 4b), while the S stock values in the coffee and pasture areas were also below the detection limit.

Figure 4 – The effect of various land-use systems on (a)- S stock (b) POM-S and MAOM-S stock at depths 0 to 5 cm and 50 to 60 cm. Values are means \pm SDs of three replicates ($p < 0.01$). Different letters indicate significant differences at $p < 0.05$.



4.3.2 *C/N and C/S ratios in the POM, MAOM and DOM fractions*

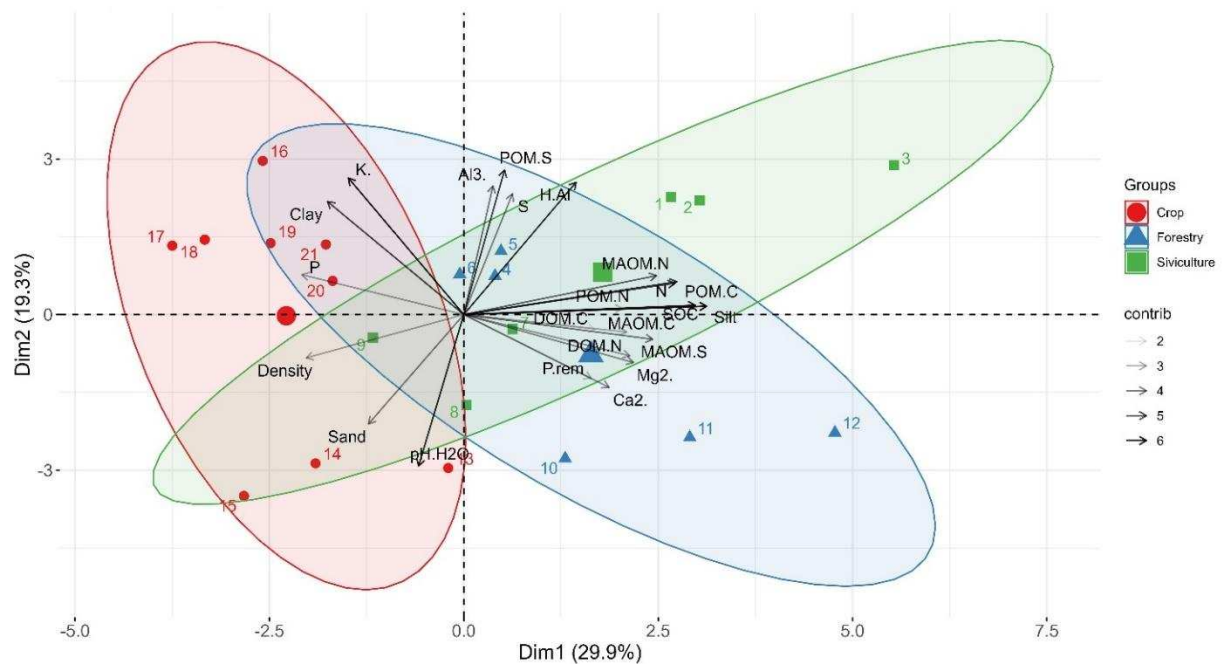
The POM fraction had the highest C/N ratio in the forest areas, with values of around 10.8 in the 0-5 cm layer. In pasture and agricultural uses, the C/N ratio in the POM fraction was reduced, reflecting accelerated decomposition and the lower contribution of carbon-rich plant residues. In the MAOM fraction, the C/N ratio was more uniform between use types and depths, ranging from 8.4 to 9.2, showing a fraction of SOM relatively stable, little affected by the type of land use, and indicating the importance of the physical protection of carbon for its long-term persistence.

The C/S ratio in the MAOM fraction was markedly higher in forests in the 0-5 cm layer (around 64), reflecting an accumulation of carbon in more protected SOM fractions in relation to sulfur, which highlights the ability of forests to conserve carbon in highly stabilized forms. In deeper layers (50-60 cm), the C/S ratio in the MAOM fraction was more homogeneous, with values close between the different land uses. The DOM, with low C/N and C/S ratio values, regardless of land use or depth, reflects the nature of soluble organic matter as a highly labile fraction susceptible to decomposition, being one of the most vulnerable SOM fractions in environments with intensive land use.

4.3.3 *Principal component analysis (PCA)*

Principal component analysis (PCA) was applied to reduce the dimensionality of data from 7 different land use areas, evaluating chemical variables and physical fractions of SOM at depths of 0-5 cm (Fig. 5) and 50-60 cm (Fig. 6). The data set included C, N and S content in the POM, MAOM and DOM fractions, as well as chemical and physical parameters such as pH, soil texture and others.

Figure 5 – Principal component analysis - PCA indicates the separation of land use groups according to soil organic matter content and soil physical and chemical variables for topsoil. Dim1 and Dim2: dimension axis.



Source: Author

The principal component analysis (PCA) carried out on the topsoil data (0-5 cm) showed well-defined patterns between the different land use systems. The first two principal axes (Dim1 and Dim2) together explained 49.2% of the total variability in the data, with Dim1 accounting for 29.9% and Dim2 for 19.3%.

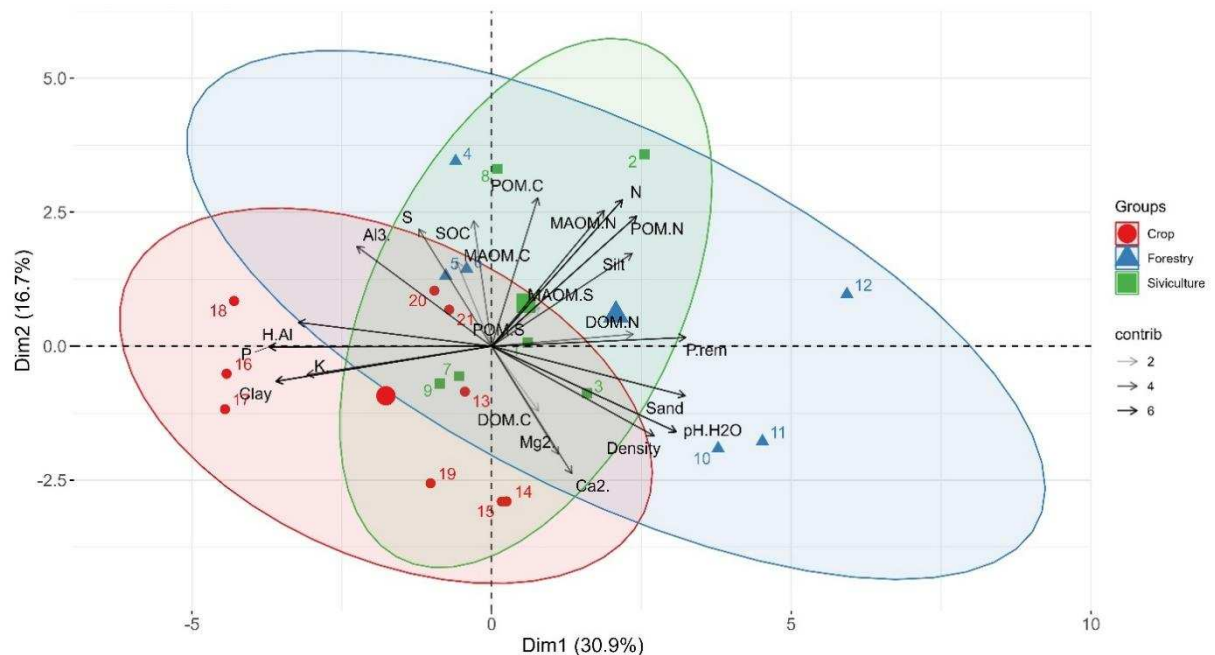
Agricultural use systems (Crop), represented by red circles, clustered predominantly in the negative region of Dim1, especially with samples associated with corn and coffee cultivation. These points showed a strong correlation with attributes such as soil density, sand content, pH in H₂O and remaining phosphorus (P rem), indicating that intensive management, with soil plowing and less accumulation of organic residues, directly influenced these variables. The grouping of the points suggests homogeneity of the soils under intensive cultivation in terms of these characteristics.

On the other hand, forestry systems, represented by the blue triangles, were strongly associated with the positive portion of Dim1, clustering around the variables related to organic matter associated with minerals (MAOM-C, MAOM-N and MAOM-S), silt content, total nitrogen and total carbon (SOC). These results indicate that forest areas, such as native vegetation and Araucaria, favour the stabilization of organic matter in more recalcitrant forms, with greater association with soil minerals, which reinforces the conservationist role of these areas in relation to nutrient cycling and accumulation.

Siviculture areas, in green (squares), were mostly positioned in the upper part of Dim2 and between the other two categories on the main axes. This intermediate position suggests that exotic tree planting systems, such as eucalyptus and rubber trees, have physical and chemical soil characteristics that share traits with both natural and agricultural systems. What stands out in the data is the moderate association with labile fractions of soil organic matter, such as POM.C and POM.N, as well as some elements such as sulfur (S) and exchangeable aluminum (Al^{3+}).

The principal component analysis (PCA) for the subsoil layer (50–60 cm) revealed a distinct variability structure compared to the surface layer (Figure 6).

Figure 6 – Principal component analysis - PCA indicates the separation of land use groups according to soil organic matter content and soil physical and chemical variables for subsoil. Dim1 and Dim2: dimension axis.



Source: Author

The first component (Dim1), which explains 30.9% of the total variance, is strongly associated with chemical and physical soil attributes. Notably, high contributions were observed from variables related to acidity and texture, such as exchangeable aluminum (Al^{3+}), clay content, and potential acidity ($\text{H}+\text{Al}$), all of which had vectors oriented negatively along the Dim1 axis. These attributes are closely correlated with agricultural systems (Crop), whose points are clustered in the lower-left quadrant of the biplot.

On the other hand, the variables that contributed most positively to Dim1 include pH in water ($\text{pH}-\text{H}_2\text{O}$), remaining phosphorus (P rem), bulk density, and sand content. These variables, with vectors pointing in the positive direction of Dim1, better characterize forest environments (Forestry), which tend to present less acidic soils, with lower aluminum saturation and sandier textures.

The second component (Dim2), accounting for 16.7% of the variability, distinguishes the systems based on properties related to soil organic matter quality. Variables such as particulate organic carbon (POM-C), particulate nitrogen (POM-N), and MAOM-N contributed positively to Dim2 and were more closely associated with silvicultural systems (Silviculture), where a greater accumulation of organic matter in stable fractions was observed, even in the subsoil.

These patterns indicate that, even at greater depths, land use systems continue to strongly influence the composition and quality of soil organic matter. Silvicultural systems stand out by promoting higher C and N contents in stabilized fractions, whereas agricultural systems show greater acidity, bulk density, and clay content-potentially linked to more intensive management practices and structural soil degradation. Details of the graphical dispersion of the physical and chemical variables for the two depths are shown in Figure 7 and Figure 8 of the Appendix B. Supplementary data.

4.4 Discussion

We found significant variation in soil organic carbon (SOC) stocks in the surface layer (0-5 cm), with *Araucaria* plantation (22.95 Mg C ha⁻¹) and *Eucalyptus* plantation (17.14 Mg C ha⁻¹) standing out, both exceeding pasture systems, native forest, and agricultural crops such as maize and coffee. This finding is consistent with the literature, which indicates that planted forests, especially in areas with high biomass like *Araucaria* and *Eucalyptus*, have a greater capacity to accumulate carbon in the topsoil due to higher production of plant residues and nutrient cycling (MAYER et al., 2020). Moreover, these ecosystems maintain favorable microclimatic conditions for organic matter accumulation, such as lower soil temperature and higher moisture. It is known that *Eucalyptus* leaf litter is not so easily decomposed and that *Araucaria* leaf litter, despite being native, is a gymnosperm, with very recalcitrant leaves (KARKI; GOODMAN, 2015).

SOC stocks in the subsoil (50-60 cm) did not show significant differences between land use systems. This suggests that, despite variations in surface carbon accumulation, subsoil carbon stocks are more stable and less influenced by land use type. Literature supports this finding, indicating that SOC accumulation processes in the subsoil are mainly related to the dynamics of more stable organic matter fractions, such as mineral-associated organic matter (MAOM), which is less sensitive to land-use changes (KALBITZ et al., 2000).

The results for particulate organic matter (POM) fractions in the surface layer indicate that native forest had the highest POM-C stocks (6.65 Mg C ha⁻¹), significantly higher than in the maize cropping system (0.78 Mg C ha⁻¹). POM is considered a labile fraction of organic matter, more susceptible to changes caused by management practices, and is strongly associated with the input of plant residues and short-term carbon cycling (BONGIORNO et al., 2019). The higher POM accumulation in native forest can be explained by the diversity of plant

species and the greater amount of organic residues being incorporated into the soil, which favors soil protection and increases carbon stocks (LEHMANN; KLEBER, 2015b).

In contrast, the MAOM fraction, which is more stable and associated with mineral adsorption processes, was predominantly higher in pasture and planted forest systems, such as *Araucaria* and *Eucalyptus*. MAOM plays a key role in carbon stabilization in soils, and its accumulation is essential for long-term carbon sequestration (LAVALLEE; SOONG; COTRUFO, 2020a). The higher MAOM-C stocks in the *Araucaria* plantations (15.99 Mg C ha⁻¹) and pasture (12.92 Mg C ha⁻¹) systems indicate that these systems are effective in storing carbon in more stable fractions, which could have positive implications for carbon sequestration and climate change mitigation. In particular, the accumulation of MAOM in pastures is due to the continuous input of grass residues, rich in simple compounds, and the dense root system which favors interaction with soil minerals. These compounds are adsorbed by clays and iron and aluminium oxides, while the formation of aggregates physically protects the MAOM from microbial decomposition, ensuring its stabilization (SIX et al., 2002).

Nitrogen stocks followed a similar pattern to SOC, with *Eucalyptus* and *Araucaria* plantations standing out in the surface layer (0-5 cm). However, at depth (50-60 cm), there were no significant differences between systems, supporting studies that show that subsoil nitrogen tends to be less influenced by land-use changes due to its association with more stable organic matter forms (HUANG; SPOHN, 2015).

Regarding sulfur, *Eucalyptus* plantations showed the highest stocks in both the surface and subsoil layers (0.99 Mg S ha⁻¹ and 1.06 Mg S ha⁻¹, respectively), followed by maize and native forest. This result reinforces the role of *Eucalyptus* plantations in sulfur cycling, possibly due to its high productivity and accumulation of organic matter rich in sulfur, as suggested by Berg (2014). On the other hand, the pasture and coffee systems showed low sulfur stocks, with values below the detection limit, suggesting that these systems may be associated with lower sulfur cycling, which could result in lower soil fertility over the long term.

We highlight the significant potential of Brazilian soils, across different land-use types, to contribute to climate change mitigation. The MapBiomass report highlights that Brazilian soils can store the equivalent of 70 years of the country's annual CO₂ emissions if properly managed. In this study, planted forest systems like *Araucaria* and *Eucalyptus* showed a greater potential for carbon storage, reinforcing the need for public policies focused on expanding forest areas and restoring degraded lands with native species. These practices could further enhance soil carbon sequestration, especially in regions that currently practice less sustainable agriculture, such as maize and coffee cultivation (MapBiomass Project, 2023).

By promoting sustainable management practices, such as adopting agroforestry systems and integrating native and exotic species, it is possible to significantly increase carbon stocks in more stable soil fractions (TSCHORA; CHERUBINI, 2020). These practices are critical for aligning Brazilian agriculture with global goals of reducing greenhouse gas emissions and promoting food security, without compromising soil fertility.

Total C/N and C/S ratios and the SOM physical fractions offer important insights into nutrient dynamics and carbon stability in Atlantic Forest soils subjected to different land uses. These parameters reflect the quality of the organic matter and indicate the potential for carbon storage and its resilience to changes in land use, which are critical factors for eco-efficiency and climate change mitigation. By comparing our data with the results of current literature, it is possible to deepen the discussion on ecological processes and the influence of management practices on the eco-functionality of SOM.

In forest areas, the total C/N ratio in the first 5 cm of depth was consistently higher compared to pasture and agricultural soils, a result similar to that observed by Feng et al. (2020) and Wang et al. (2022), who show that forest environments, because they receive a large amount of leaf litter and have less disturbance, accumulate SOM with a higher carbon content in relation to nitrogen. This pattern may indicate a condition of slower decomposition, typical of less anthropized environments and with a greater presence of aromatic and stable compounds, which favor long-term carbon accumulation. In agricultural systems, the reduction in the C/N ratio may be associated with accelerated decomposition, influenced by frequent exposure of the soil and greater mineralization of SOM, which is in line with the findings of Xu et al. (2021).

In the deeper layers (50-60 cm), there was greater homogeneity in the C/N and C/S ratios, regardless of land use, which may reflect a stage of greater stabilization of the SOM at these depths. According to Trumbore (2020), at subsurface depths, the accumulation of SOM depends more on processes of physical stabilization and association with minerals than on inputs from recent plant residues, which reduces the influence of variations in land use. This pattern suggests that management practices mainly impact the surface layer, which is fundamental for preserving carbon stocks.

C/N and C/S ratios in the POM, MAOM and DOM fractions

The particulate fraction (POM), which represents a more labile and biologically active form of SOM, showed higher C/N ratio values in forest areas, which corroborates studies by Lavallee et al. (2021), which point to lower nitrogen mineralization in forest environments

due to the accumulation of less decomposed organic matter. In pasture and agricultural areas, the C/N values in the POM fraction were significantly lower, indicating greater mineralization and decomposition of easily degradable organic compounds, a result consistent with intensive management practices that promote oxidation and loss of SOM (SIX et al., 2002).

In the MAOM fraction, the C/N ratio was more homogeneous between land uses and depths. This result is consistent with studies indicating that MAOM is a more stable fraction, whose formation is associated with the physical and chemical protection of carbon, regardless of recent residue inputs (LEHMANN and KLEBER, 2015). The stability of the C/N ratio in MAOM suggests that the carbon associated with minerals is less susceptible to variations in land use and offers a long-term storage mechanism, making it a critical component in mitigating climate change in tropical soils.

The higher C/S ratio in forest areas compared to pastures and agriculture reflects the ability of native vegetation to preserve carbon in a form that is relatively less influenced by sulfur. This selective retention of carbon in relation to sulfur is important for the accumulation of stable SOM, as evidenced by Don et al. (2020). The stability of the C/S ratio in the MAOM fraction between the different land uses reinforces the importance of carbon associated with minerals as a long-term stock that is less dependent on changes in use, which corroborates studies by Rumpel and Kögel-Knabner (2011).

In the dissolved fraction (DOM), the C/N ratio was low in all types of land use and depths, reflecting the more labile and easily degradable nature of this fraction, an aspect also reported by Marschner and Kalbitz (2003). The low C/N ratio in DOM at all depths reinforces the idea that this fraction is directly associated with microbial activity and mineralization, which can contribute to rapid nutrient cycling and indicates a greater vulnerability of DOM in intensively managed environments.

Implications for Conservation and Sustainable Management

The data presented indicates that the preservation of areas and the implementation of sustainable management practices in agricultural and pasture soils are fundamental for the conservation of carbon and nitrogen levels in the soil. In particular, the higher C/N ratios observed in forest areas indicate the greater efficiency of these systems in keeping carbon in more stable fractions that are less susceptible to rapid decomposition, as reported by Don et al. (2020). The homogeneity of the C/N and C/S ratios in the deeper layers (50-60 cm) between the different land uses suggests that management practices mainly affect the surface layer,

indicating the need for conservation practices aimed at protecting the most susceptible layer of the soil.

These findings corroborate the importance of practices that favor the accumulation of SOM in the more stable fractions, such as the use of cover crops, crop rotation and reduced tillage intensity, strategies recommended by Lal (2020) for soil carbon conservation. In a context of climate change, adopting these practices is essential to reduce carbon emissions from the soil and contribute to the ecoefficiency of SOM, promoting sustainability and mitigating environmental impacts in tropical ecosystems.

This study also highlights the effects of land use on soil attributes, especially in the surface layer. Agricultural systems such as maize and coffee exhibited negative correlations with variables associated with soil organic matter in the PCA and positive correlations with indicators of soil degradation, such as high bulk density and low silt and clay contents. This may be linked to the continuous use of intensive management practices, including herbicide application, frequent soil disturbance, and reduced input of organic residues, which lead to structural degradation and carbon loss (SOUZA et al., 2020).

Conversely, forested areas, particularly those with native vegetation, showed strong correlations with the most stable fractions of soil organic matter, such as MAOM. This supports the hypothesis that conserving vegetative cover fosters the accumulation of stable organic matter, thereby enhancing soil sustainability (COSER et al., 2018; SCHNEIDER et al., 2021). The accumulation of SOC and essential nutrients like nitrogen and sulfur is directly linked to the continuous input of complex organic residues, which favor humification processes and complexation with clay and iron/aluminum oxides.

Silvicultural areas exhibited intermediate characteristics, reflecting the type of management employed. For instance, eucalyptus and rubber tree systems, despite being perennial plantations with some organic residue input, may have limited diversity of organic inputs and alterations in the hydrological cycle and soil microbiota (RODRIGUES et al., 2019), potentially constraining the accumulation of stabilized SOM fractions. Their moderate association with POM (particulate fractions) suggests that much of the organic matter in these systems exists in more labile forms, which are more prone to decomposition.

The PCA for the subsurface layer revealed the persistence of land-use effects even at greater depths, supporting the hypothesis that vegetation cover and fertility management influence the vertical dynamics of SOM fractions. Soils under silviculture maintained relevant levels of carbon and nitrogen in stable fractions (MAOM), indicating greater resilience of these environments to organic matter losses. In contrast, the dominance of variables associated with

acidity and bulk density in agricultural systems underscores the need for conservation-oriented management strategies, such as crop rotation, green manuring, and reduced compaction.

The integrated analysis of the PCA results at 0–5 cm and 50–60 cm depths revealed consistent patterns related to the influence of different land-use systems on soil physicochemical properties and SOM dynamics, including particulate (POM), mineral-associated (MAOM), and dissolved organic matter (DOM) fractions. The separation among land-use groups (agriculture, silviculture, and forestry) was evident in both layers, though more pronounced at the surface, reflecting the direct influence of recent organic residue inputs and vegetation cover on soil attributes. However, even at 50–60 cm, distinct patterns among land-use types persisted, indicating that land-use effects extend beyond the surface and influence subsoil quality as well.

At the 0–5 cm depth, silvicultural systems showed higher accumulation of carbon (C) and nitrogen (N) in both POM and MAOM fractions, standing out due to the high quality of organic matter inputs and the presence of perennial vegetation with dense litter layers and functionally diverse root systems. These systems were positively correlated with indicators of SOM quality and stability, such as SOC, POM-C, POM-N, MAOM-C, and MAOM-N, highlighting a more conserved environment with greater potential for soil carbon sequestration and protection. In contrast, agricultural systems were associated with areas of the PCA biplot linked to degradation indicators, such as higher exchangeable aluminum (Al^{3+}), increased potential acidity ($\text{H}+\text{Al}$), higher bulk density, and lower silt and organic matter contents, reflecting the negative effects of land-use intensification, mechanization, frequent tillage, and reduced input of high-quality residues.

At the 50–60 cm depth, although the influence of surface inputs was reduced, patterns observed in the surface layer were partially maintained. Forest and silvicultural systems remained associated with higher C and N levels in MAOM fractions, indicating enhanced protection of SOM at depth, resulting from the incorporation of more recalcitrant organic material, greater root system complexity, and the presence of stable aggregates capable of preserving mineral-associated organic matter. This persistence of patterns across soil depths suggests that soil management and vegetation cover exert profound and lasting effects throughout the soil profile, directly influencing soil structure and functionality even in deeper layers less explored by superficial roots.

Overall, forestry and silvicultural systems demonstrated greater capacity to promote SOM accumulation and stabilization at both depths, contributing to improved soil quality and increased carbon stocks, with positive implications for climate change mitigation and

ecosystem resilience. In contrast, agricultural systems were less efficient at retaining stable organic matter, particularly in mineral-associated fractions, which could jeopardize long-term productive sustainability. These findings highlight the importance of management practices that enhance not only the input but also the quality and stabilization potential of organic matter in the soil such as crop diversification, permanent cover crops, agroforestry systems, and reduced soil disturbance.

Therefore, the integrated analysis across surface and subsurface layers underscores the importance of considering the entire soil profile when assessing land-use effects on SOM dynamics. Systems with perennial vegetation, particularly those with forest or silvicultural cover, proved more effective in promoting the accumulation of stable SOM fractions at depth, representing a critical advantage for soil conservation and combating environmental degradation in tropical regions.

4.5 Conclusion

This study demonstrated that land-use change in the Atlantic Forest biome significantly influences the distribution and stocks of carbon (C), nitrogen (N), and sulfur (S) in both surface (0–5 cm) and subsoil (50–60 cm) layers, with pronounced effects on the physical fractions of soil organic matter (SOM). Our findings confirmed that perennial land-use systems with reduced soil disturbance, such as native forest, eucalyptus, and rubber tree plantations, promoted higher SOM stabilization, particularly through the accumulation of C, N, and S in the mineral-associated organic matter (MAOM) fraction. These systems exhibited more stable SOM dynamics due to continuous litter input, deeper root systems, and minimized mechanical disturbance, contributing to the enhancement of SOM quality and long-term C sequestration.

Conversely, intensive annual systems such as maize cropping and degraded pastures were associated with lower SOM stocks and reduced stabilization, particularly in deeper soil layers, reflecting the effects of soil disturbance, lower biomass input, and higher SOM lability. The differences between surface and subsoil patterns highlight the importance of considering deeper soil horizons in SOM assessments and in designing soil conservation strategies.

These results support our hypothesis that perennial land uses under lower physical disturbance provide more favorable conditions for SOM accumulation and stabilization across the soil profile. Therefore, promoting sustainable land management practices that mimic natural

forest systems can play a crucial role in conserving SOM, maintaining soil fertility, and mitigating the impacts of climate change in tropical regions.

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5 CONCLUSION

This thesis demonstrated that the characterization of the physical fractions of soil organic matter (SOM) is essential to understanding its formation, stability, and ecological functions, especially in deeply weathered tropical soils. The unique characteristics of these soils require advanced analytical methods to unravel the mechanisms controlling SOM dynamics.

The results showed that perennial and low-disturbance land-use systems, such as forests and eucalyptus and rubber tree plantations, promote the accumulation and stabilization of carbon, nitrogen, and sulfur in SOM, particularly in the mineral-associated organic matter (MAOM) fraction. In contrast, intensive annual systems, such as maize cultivation and degraded pastures, reduced SOM stocks and stability, especially in subsoil layers. These findings reinforce the need to adopt sustainable land management practices that promote SOM conservation, contributing to soil fertility, carbon sequestration, and climate change mitigation in tropical ecosystems.

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APPENDIX A – SUPPLEMENTARY DATA (CHAPTER 1)

Table 2 – Number of publications searched in different databases.

Search terms	Science direct	Academic Google	Web of science
<i>"organic matter fractions"</i>	4226	19500	1248
<i>"soil organic matter fractions"</i>	874	11600	327
<i>"particulate organic matter" and "soil"</i>	7944	33400	1746
<i>"mineral-associated organic matter" and "soil"</i>	739	4830	382
<i>"dissolved organic matter" and "soil"</i>	19244	184000	5784
<i>"particulate organic matter" and "mineral-associated organic matter" and "dissolved organic matter" and "Soil"</i>	111	707	10
<i>"particulate organic matter" and "soil" and "Brazil"</i>	1057	8740	96
<i>"mineral-associated organic matter" and "soil" and "Brazil"</i>	95	858	32
<i>"dissolved organic matter" and "soil" and "Brazil"</i>	1773	15000	126
<i>"particulate organic matter" and "soil" and "United states of America"</i>	79	2560	658
<i>"mineral-associated organic matter" and "soil" and "United states of America"</i>	248	3170	176
<i>"dissolved organic matter" and "soil" and "United states of America"</i>	203	4430	1466
<i>"particulate organic matter" and "soil" and "Canada"</i>	2017	15000	104
<i>"mineral-associated organic matter" and "soil" and "Canada"</i>	123	1110	31
<i>"dissolved organic matter" and "soil" and "Canada"</i>	-	-	311
<i>"particulate organic matter" and "soil" and "Europe"</i>	-	-	603
<i>"mineral-associated organic matter" and "soil" and "Europe"</i>	-	-	137
<i>"dissolved organic matter" and "soil" and "Europe"</i>	-	-	1809
<i>"particulate organic matter" and "soil" and "China"</i>	-	-	294
<i>"mineral-associated organic matter" and "soil" and "China"</i>	-	-	82
<i>"dissolved organic matter" and "soil" and "China"</i>	-	-	2141
<i>"organic matter fractions" and "litter" and "soil"</i>	-	-	127

*"organic matter fractions and "litter" and
"soil" and "Brazil"*

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APPENDIX B – SUPPLEMENTARY DATA (CHAPTER 2)

PCA variables:

Figure 7 – Graphical dispersion of physical and chemical variables at a depth of 0-5cm.

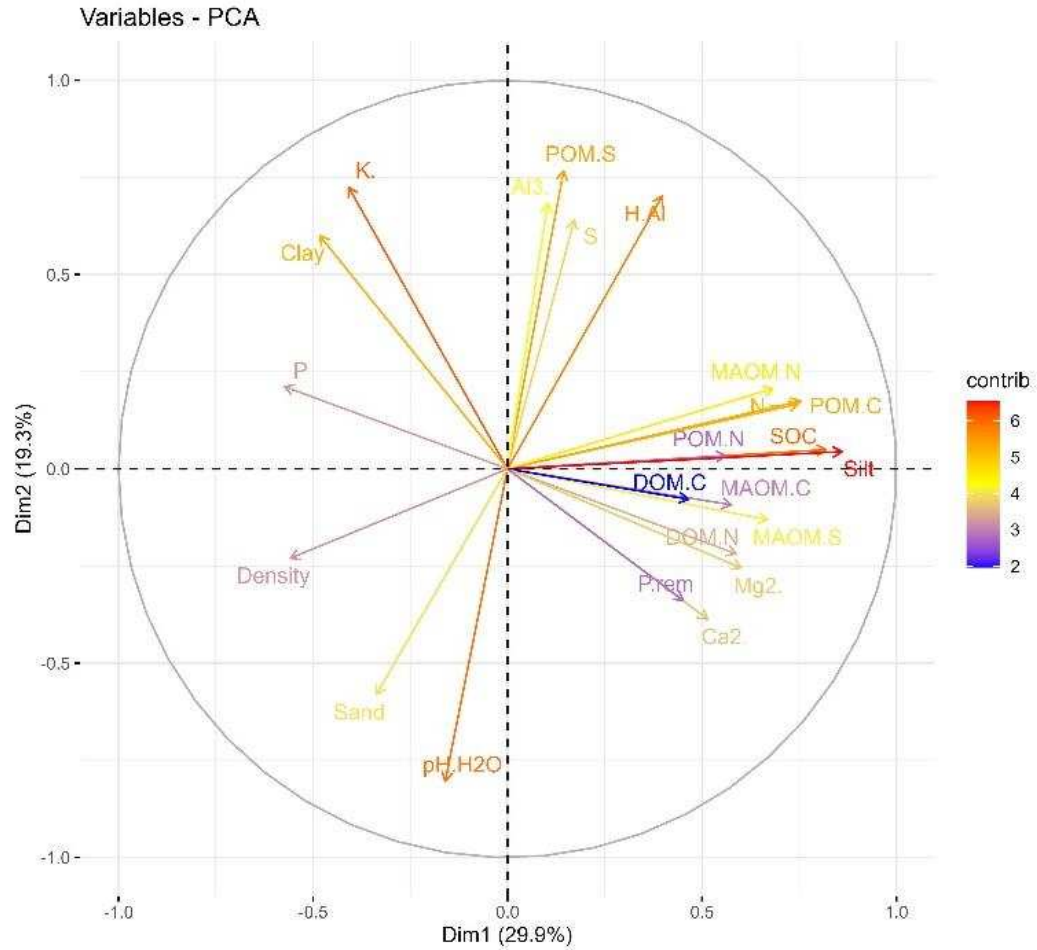


Figure 8 – Graphical dispersion of physical variables and chemical variable at a depth of 0-5cm.

