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MARIE FRANTZLIE MILIEN

CHARACTERISTICS OF MANGROVE SOIL ORGANIC MATTER IN A RESTORATION SCENARIO: A CASE STUDY IN THE PACOTI RIVER ESTUARY, CEARÁ, NE-BRAZIL

FORTALEZA

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Master's Dissertation submitted to the Graduate Program in Soil Science (Soil Chemistry, Fertility, and Pollution) at the Federal University of Ceará, as a partial requirement for the degree of Master of Science. Concentration area: Soil Chemistry, Fertility, and Biology.

Advisor: Prof. Dr. Gabriel Nuto Nóbrega

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I dedicate this work:

To God, who is always by my side. All honor and glory to Him.

To my parents, for their unconditional support, for always believing in me, and for helping me overcome every difficulty in the pursuit of my dreams. You are the source of my dedication and energy, despite the distance. May God bless you and keep you always in good health.

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"Avoiding deforestation of mangroves in northern Brazil prevents emissions equivalent to those produced by over 200.000 gasoline-powered cars each year" (Gabriel Nuto Nóbrega)

RESUMO

Os manguezais se caracterizam como um dos ecossistemas mais produtivos do planeta, produzindo grandes quantidades de matéria orgânica (MO), que são consumidas pela macrofauna, exportadas pelas correntes de maré ou aportadas no solo, onde são parcialmente mineralizadas por microrganismos aeróbios ou anaeróbios. Devido ao predomínio das vias de decomposição anaeróbias, os manguezais são capazes de acumular grandes conteúdos de carbono (C) no solo. Esse carbono armazenado nos sedimentos costeiros, principalmente na forma de resíduos vegetais parcialmente decompostos, é conhecido como carbono azul.. Nestes ecossistemas, as raízes e exsudatos radiculares constituem as principais formas de aporte de C, compondo a fração de C orgânico particulado (COp), encontrado livre ou associado às partículas do solo da fração areia, e o C orgânico associado aos minerais (COam), que é a fração da MOS fortemente ligada às partículas minerais "silte e argila". Neste sentido, estratégias de recuperação de manguezais têm sido utilizadas para mitigar o aumento da concentração de gases de efeito de estufa. Entretanto, é necessário aprofundar o entendimento da dinâmica do acúmulo e das mudanças nas características das diferentes frações da MO visando aprimorar o entendimento e vulnerabilidade desses ecossistemas para mitigar as mudanças climáticas. Nesse sentido, este estudo teve por objetivo avaliar os impactos da colonização de manguezais em áreas degradadas nas características da matéria orgânica dos solos em um contexto semiárido. Para tanto, amostras de solos foram coletadas no Estuário do Rio Pacoti, em três condições (área degradada - HTF, área de manguezal em recuperação - RM, e manguezal maduro - MM), onde foram implementadas estratégias para a recuperação de áreas degradadas, nas quais foram analisados o potencial redox (Eh), pH, densidade do solo, granulometria, carbono orgânico total, estoque de carbono equivalente e determinação do grau de piritização para entendimento das vias metabólicas de decomposição. Além disso, foi realizado um fracionamento físico da matéria orgânica para determinar a fração de carbono orgânico, bem como uma caracterização molecular (FTIR-ATR) e isotópica. Os resultados mostraram uma predominância de areia (HTF: 87.0±10.7%, RM: 87.3±5.3%, MM: 82.4±4.8%), com maiores valores de Eh observados em HTF (+236±14 mV), indicando condições subóxicas, e menor no MM (+25±38 mV), refletindo condições anóxicas favorecidas pelo acúmulo de matéria orgânica e saturação de água. Os valores de pH foram alcalinos no HTF (7.24±0.14), devido à precipitação de carbonato, enquanto o RM (6.76±0.10) e MM (6.75±0.21) apresentaram valores semelhantes ligeiramente ácidos. Em relação às formas de ferro, não foram observadas diferenças significativas nas formas reativas de ferro entre as áreas; no entanto, as formas de Fe pirítico e o grau de piritização (DOP) apresentaram um aumento significativo ao longo do gradiente de restauração (HTF: 0.1 ± 0.3 e 0.0 ± 0.1 %µmol g^{-1}; RM: 2.1 ± 3.1 e 1.1 ± 1.6 % µmol g^{-1}; MM: 26.6 ± 30 e 6.8 ± 6.9 % µmol g^{-1}), indicando uma predominância de processos metabólicos anaeróbicos em manguezais maduros. Esse processo é essencial para o acúmulo de carbono e a estabilização de nutrientes e contaminantes no solo. Os conteúdos de C e N foram mais altos na área MM (1.32% C e 0.10±0.01% N), indicando maior armazenamento de matéria orgânica devido ao maior aporte de MO e às condições favoráveis em comparação com RM (0.40±0.1% C e 0.04±0.01% N) e HTF (0.18±0.1% C e 0.02±0.01% N). Os valores médios de $\delta^{13}C$ foram mais negativos na área MM (média: -19 ± 4.29‰, -23 ± 3.16‰ e -26 ± 1.12‰), indicando uma maior contribuição de matéria orgânica de plantas do tipo C3, típicas de ecossistemas florestais e alagados. Já os valores mais elevados de $\delta^{15}N$ na área MM sugerem processos microbiológicos, como nitrificação e desnitrificação. A análise (FTIR/DRIFT) da matéria orgânica do solo (MOS) nas três áreas de estudo (HTF, RM e MM) revelou diferenças na composição química das frações de matéria orgânica particulada (POM) e matéria orgânica associada a minerais (MAOM). Os espectros de FTIR mostraram grupos funcionais, como hidroxilas (OH), grupos alifáticos (C-H) e grupos aromáticos, com variações marcantes entre áreas e profundidades estudadas (0-50 cm). A análise de componentes principais (ACP) destacou diferenças significativas entre as frações POM e MAOM, particularmente na zona de manguezal maduro (MM), que tinha uma composição orgânica mais complexa, indicando maior estabilidade e acumulação de carbono. Este estudo revela que, mesmo após 7 anos, os manguezais restaurados não recuperaram totalmente suas funções ecológicas, como a piritização e a estabilização das frações de MOP e MOAM. Prevê-se que sejam necessários 40 anos para que o DOP se recupere totalmente, o que destaca a importância do controle e das intervenções de longo prazo para acelerar a restauração.

Palavras-chave: recuperação ambiental; carbono azul; mudanças climáticas; solos hidromórficos.

ABSTRACT

Mangroves are characterized as one of the most productive ecosystems on the planet, producing large amount of organic matter (OM), which are consumed by macrofauna, exported by tidal currents or incorporated into the soil, where they are partially mineralized by aerobic or anaerobic microorganisms. Due to prevailing anaerobic decomposition pathways, mangroves are recognized by storing a great amount of carbon (C) on its soils. This carbon stored in coastal sediments, mainly in the form of partially decomposed plant residues, is known as blue carbon. In these ecosystems, roots and exudates constitute the main forms of C supply, which can be fractionated into particulate organic carbon (COp), consisting in free or associated with soil particles related to the sand fraction, and organic carbon associated with minerals (COam) which is the fraction of soil OM strongly bound to the silt and clay particles. In this sense, mangrove recovery strategies have been used to mitigate the increase in the concentration of greenhouse gases. However, it is necessary to improve the understanding of the changes in the characteristics of OM in different fractions to improve the understanding of these ecosystems as allies to climate change mitigation. In this sense, this study aimed to evaluate the impacts of mangrove restoration on the characteristics of soil organic matter in a semi-arid context, covering 3 conditions: degraded area (HTF), restored mangroves (RM) and mature mangroves (MM). Thus, soil samples were collected in the Pacoti River Estuary and characterized the redox potential (Eh), pH, soil density, granulometry, total organic carbon, stock and equivalent carbon, determination of the degree of pyritization to understand the metabolic pathways of decomposition. Additionally, physical fractionation of organic matter was performed to determine organic carbon fractionation for molecular (FTIR-ATR) and isotopic characterization. The results showed a predominance of sand (HTF: 87.0±10.7%, RM: 87.3±5.3%, MM: 82.4±4.8%), with higher redox potential (Eh) observed in HTF (+236±14 mV), indicating suboxic conditions, and lower in MM (+25±38 mV), reflecting anoxic conditions favorable for organic matter accumulation. The pH values were alkaline in HTF (7.24±0.14) due to carbonate precipitation, while RM (6.76±0.10) and MM (6.75±0.21) exhibited similar slightly acidic values. Regarding the iron forms, no significant differences were observed in reactive iron among the areas; however, pyritic iron forms and the degree of pyritization (DOP) showed a significant increase along the restoration gradient (HTF: 0.1 ± 0.3 and 0.0 ± 0.1 % μmol g^{-1}; RM: 2.1 ± 3.1 and 1.1 ± 1.6 % μmol g^{-1}; MM: 26.6 ± 30 and 6.8 ± 6.9 % μmol g^{-1}), indicating a predominance of anaerobic metabolic processes in mature mangroves. This process is essential for carbon accumulation and the stabilization of nutrients and contaminants in the soil. The C and N contents were higher in the MM area (average (1.76±0.32% C and 0.10±0.01% N), indicating greater storage of organic matter due to the greater contribution of OM and favorable environmental conditions compared to RM (average: 0.40±0.1% C and 0.04±0.01% N) and HTF (0.18±0.1% C and 0.02±0.01% N). The average $\delta^{13}C$ values were most negative in the MM area (average: -19 ± 4.29‰, -23 ± 3.16‰ and -26 ± 1.12‰), indicating a greater contribution of organic matter from C3 plants, typical of forest and flooded ecosystems. The higher $\delta^{15}N$ values in the MM area suggest a possible influence of microbiological processes, such as nitrification and denitrification. Analysis (FTIR/DRIFT) of soil organic matter (SOM) in the three study areas (HTF, RM and MM) revealed differences in the chemical composition of the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions. The FTIR spectra showed functional groups such as hydroxyls (OH), aliphatic groups (C-H) and aromatic groups, with marked variations between areas and depths. Principal component analysis (PCA) highlighted significant differences between the POM and MAOM fractions, particularly in the mature mangrove zone (MM), which had a more complex organic composition, indicating greater stability and carbon accumulation. This study reveals that even after seven years, restored mangroves have not fully recovered their ecological functions, such as pyritization and stabilization of the POM and MAOM fractions. It is estimated that 40 years will be required for the DOP to fully recover, highlighting the importance of long-term monitoring and interventions to accelerate restoration.

Keywords : environmental restoration ; blue carbon ; climate change ; hydromorphic soils.

RÉSUMÉ

Les mangroves sont caractérisées comme l'un des écosystèmes les plus productifs de la planète, produisant de grandes quantités de matière organique (MO), qui sont consommées par le macrofaune, exportées par les courants de marée ou incorporées dans le sol, où elle est partiellement minéralisée par des micro-organismes aérobies ou anaérobies. En raison de la prédominance des voies de décomposition anaérobie, les mangroves sont capables d'accumuler de grandes quantités de carbone (C) dans le sol. Ce carbone stocké dans les sédiments côtiers, principalement sous la forme de résidus végétaux partiellement décomposés, est connu sous le nom de carbone bleu. Dans ces écosystèmes, les racines et les exsudats radiculaires constituent les principales sources de C, qui peuvent être fractionnées en carbone organique particulaire (COP), libre ou associé aux particules du sol, lié à la fraction sableuse, et en carbone organique associé aux minéraux (COAM), fortement lié aux particules de limon et d'argile. Grâce à leur grande capacité à accumuler du C, en particulier dans leurs sols, des stratégies de restauration des mangroves ont été utilisées pour atténuer l'augmentation de la concentration de gaz à effet de serre. Cependant, il est nécessaire de mieux comprendre la dynamique de l'accumulation et les changements dans les caractéristiques des différentes fractions de la MO afin d'améliorer la compréhension et la vulnérabilité de ces écosystèmes à l'atténuation du changement climatique. Dans ce contexte, cette étude visait à évaluer les impacts de la restauration des mangroves sur les caractéristiques de la matière organique des sols en contexte semi-aride. A cette fin, des échantillons de sol ont été prélevés dans l'estuaire de la rivière Pacoti en trois conditions (zone dégradée - HTF, zone de mangrove en cours de récupération - RM, et mangrove mature - MM), où des stratégies de récupération des zones dégradées ont été mises en œuvre, dans lesquelles le potentiel d'oxydoréduction (Eh), le pH, la densité du sol, la granulométrie, le carbone organique total, le stock et le carbone équivalent et la détermination du degré de pyritisation ont été analysés afin de comprendre les voies métaboliques de la décomposition. De plus, un fractionnement physique de la matière organique a été effectué pour déterminer la fraction du carbone organique, ainsi que la caractérisation moléculaire (FTIR-ATR) et isotopique. Les résultats ont montré une prédominance de sable (HTF : 87.0 ± 10.7 %, RM : 87.3 ± 5.3 %, MM : 82.4 ± 4.8 %), caractérisant un sol à texture grossière. Le potentiel redox (Eh) était plus élevé dans HTF (+236 ± 14 mV), indiquant des conditions suboxiques, et plus faible dans MM (+25 ± 38 mV), reflétant des conditions anoxiques favorisées par l'accumulation de matière organique et la saturation en eau. Les valeurs de pH étaient alcalines dans HTF (7.24 ± 0.14) en raison de la précipitation des carbonates, tandis que RM (6.76 ±

0.10) et MM (6.75 ± 0.21) présentaient des valeurs similaires légèrement acides. Concernant les formes de fer, aucune différence significative n'a été observée dans les formes réactives de fer entre les zones ; cependant, les formes de fer pyritique et le degré de pyritisation (DOP) ont montré une augmentation significative le long du gradient de restauration (HTF : 0.1 ± 0.3 et 0.0 ± 0.1 % μmol g^{-1} ; RM : 2.1 ± 3.1 et 1.1 ± 1.6 % μmol g^{-1} ; MM : 26.6 ± 30 et 6.8 ± 6.9 % μmol g^{-1}), indiquant une prédominance de processus métaboliques anaérobies dans les mangroves matures. Les analyses des compositions isotopiques stables du carbone (δ^{13}C) et de l'azote (δ^{15}N), ainsi que les concentrations de carbone et d'azote dans la matière organique particulaire (POM) et la matière organique associée aux minéraux (MAOM) dans les zones d'étude ont révélé des variations significatives. Les valeurs moyennes du δ^{13}C étaient plus négatives dans la zone MM (moyenne : -19 ± 4.29‰, -23 ± 3.16‰ et -26 ± 1.12‰), ce qui indique une plus grande contribution de la matière organique provenant de plantes en C3, typiques des écosystèmes forestiers et inondés. Les valeurs δ^{15}N plus élevées dans la zone MM suggèrent une influence possible de processus microbiologiques, tels que la nitrification et la dénitrification. L'analyse (FTIR/DRIFT) de la matière organique du sol (SOM) dans les trois zones d'étude (HTF, RM et MM) a révélé des différences dans la composition chimique des fractions de matière organique particulaire (POM) et de matière organique associée aux minéraux (MAOM). Les spectres FTIR ont révélé la présence de groupes fonctionnels tels que les hydroxyles (OH), les groupes aliphatiques (C-H) et les groupes aromatiques, avec des variations marquées d'une zone à l'autre et d'une profondeur à l'autre. L'analyse en composantes principales (ACP) a mis en évidence des différences significatives entre les fractions POM et MAOM, en particulier dans la zone de mangrove mature (MM), qui présentait une composition organique plus complexe, indiquant une plus grande stabilité et une plus grande accumulation de carbone. Cette étude révèle qu'après sept ans, les mangroves restaurées n'ont pas encore entièrement retrouvé leurs fonctions écologiques, telles que la pyritisation et la stabilisation des fractions de POM et MAOM. On estime que 40 ans seront nécessaires pour une récupération complète du DOP, ce qui souligne l'importance d'un suivi à long terme et d'interventions pour accélérer la restauration.

Mots-clés : restauration environnementale ; carbone bleu ; changement climatique ; sols hydromorphes.

REZIME

Mangwòv yo karakterize kòm youn nan ekosistèm ki pi pwodiktif sou planèt la, ki pwodwi gwo kantite matyè òganik (OM), ke konsome pa bèt ki pi gwo yo, ekspòte pa kouran lanmè a oswa depoze nan tè a, kote yo pasyèlman mineralize pa mikwo-òganis ki bezwen oksijèn oswa ki pa bezwen oksijèn yo. Akòz chemen dekonpozisyon san oksijèn ki plis domine, mangwòv yo gen kapasite pou akimile gwo kantite kabòn (C) nan tè a. Kabòn sa a ki estoke nan sediman kotyè yo, prensipalman nan fòm rezidi plant ki pasyèlman dekonpoze, yo konnen l kòm kabòn ble. Nan ekosistèm sa yo, rasin ak Sibstans ki sòti nan rasin yo se prensipal fòm kontribisyon C, ki fòme fraksyon C òganik patikilè (COp), ki ka jwenn lib oswa asosye ak sab nan tè a, epi C òganik asosye ak mineral (COam), ki se fraksyon MOS ki fòtman lye ak patikil mineral tankou limon ak ajil. Nan sans sa a, estrateji rekiperasyon mangwòv yo te itilize pou diminye ogmantasyon nan konsantrasyon gaz efè tèmik yo. Sepandan, li nesesè pou konprann pi byen dinamik akimilasyon ak chanjman ki fèt nan diferan fraksyon matyè òganik yo, pou nou kapab evalye vilnerabilite kijan ekosistèm sa yo yo fonksyone e kijan yo ka ede konbat chanjman klimatik. Nan sans sa a, etid sa te gen pou objektif evalye kijan kolonizasyon mangwòv nan zòn degrade afekte karakteristik matyè òganik nan tè a, nan yon kontèks semi-arid. Pou rezon sa, echantiyon tè yo te kolekte nan antre larivyè Pacoti a, nan twa sit diferan : (zòn degrade - HTF, zòn mangwòv an rekiperasyon - RM, ak zòn mangwòv ki gen matirite - MM), kote yo te mete estrateji pou rekipere zòn ki te degrade yo, epi yo te analize plizyè paramèt tankou potansyèl redòks (Eh), pH, dansite tè, gwosè patikil tè (granilometri), kantite kabòn òganik total, estòk kabòn ekivalan, ak nivo piritizasyon pou konprann chemen metabolik dekonpozisyon yo. Anplis de sa, yon analiz fraksyonman fizik matyè òganik te fét pou detèmine fraksyon kabòn òganik yo, osi byen ke karakterizasyon molekilè (FTIR-ATR) ak izotopik li. Rezilta yo te montre tè a te gen plis sab ladan (HTF: 87.0±10.7 %, RM: 87.3±5.3 %, MM: 82.4±4.8 %), ak valè potansyèl redòks (Eh) yo te pi wo nan HTF (+236±14 mV), sa ki montre kondisyon ki gen oksijèn (siboksik), epi li te pi ba nan MM (+25±38 mV), sa ki endike kondisyon san oksijèn (anoksik), yon sitiyasyon ki reflete akimilasyon matyè òganik ak satirasyon dlo.Valè pH yo te alkalin nan HTF (7.24±0.14), akòz presipitasyon kabonat, pandan ke RM (6.76±0.10) ak MM (6.75±0.21) te gen valè yo ki sanble(similè) e ki lejèman asid. Konsènan fòm fè yo, pa te gen gwo diferans ki te obsève nan fòm fè reyaktif yo ant zòn yo ; sepandan, fòm fè piritik yo ansanm ak degre piritizasyon an (DOP) te montre yon ogmantasyon enpòtan sou tout etap restorasyon an (HTF : 0.1 ± 0.3 ak 0.0 ± 0.1 % µmol g^{-1} ; RM : 2.1 ± 3.1 ak 1.1 ± 1.6 % µmol g^{-1} ; MM : 26.6 ± 30 ak 6.8 ± 6.9 % µmol g^{-1}), sa ki montre dominasyon pwosesis metabolik san prezans oksijèn nan mangwòv ki gen matirite yo. Pwosesis sa a esansyèl pou akimilasyon kabòn ak estabilizasyon eleman nitritif ak kontaminan nan tè a. Valè C ak N yo te pi wo nan zòn MM (1.32 % C ak 0.10±0.01 % N), sa ki endike gwo depo matyè òganik akoz plis kontribisyon matyè òganik (MO) ak kondisyon ki pi favorab, konpare ak RM (0.40±0.1 % C ak 0.04±0.01 % N) e HTF (0.18±0.1 % C ak 0.02±0.01 % N). Valè mwayèn $\delta^{13}C$ yo te plis negatif nan zòn MM (mwayèn : -19 ± 4.29‰, -23 ± 3.16‰ ak -26 ± 1.12‰), sa ki montre plis kontribisyon matyè òganik ki soti nan plant tip C3, ki tipik nan ekosistèm forè ak zòn inonde yo. Sepandan, pi gwo valè $\delta^{15}N$ nan zòn MM yo bay siy ke gen pwosesis mikwobyolojik kap fèt, tankou nitrifikasyon ak nitrifikasyon. Analiz (FTIR/DRIFT) matyè òganik tè (SOM) nan twa (3) zòn etid yo (HTF, RM ak MM) revele diferans ki genyen nan konpozisyon chimik matyè òganik patikilè (POM) ak fraksyon òganik ki asosye ak mineral (MAOM) yo. Refraksyon FTIR yo te montre prezans gwoup fonksyonèl tankou idroksil (OH), gwoup alifatik (C-H), ak gwoup awomatik, ak varyasyon ki make ant zòn ak pwofondè ki te etidye (0-50 cm) yo. Yon analiz konpozan prensipal (ACP) te mete an evidans diferans enpòtan ant fraksyon POM ak MAOM, espesyalman nan zòn mangwòv ki gen matirite yo (MM), ki te gen yon konpozisyon òganik pi konplèks, ki endike plis estabilite ak plis akimilasyon kabòn. Etid sa a moutre ke menm apre 7 ane, mangwòv yo ki te restore yo pap rive rekipere tout fonksyon ekolojik yo, tankou piritizasyon ak estabilizasyon fraksyon MOP ak MOAM. Yo prevwa ke li pral pran 40 ane pou DOP an konplètman retabli, sa ki mete aksan sou enpòtans ki genyen nan kontwòl alontèm ak entèvansyon pou akselere restorasyon an.

Mo kle : rekiperasyon anviwònman ; kabòn ble ; chanjman klimatik ; tè idromòfik.

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AFS	Air-dried fine soil
CBD	Convention on Biological Diversity
CO_2	Carbon dioxyde
DRIFT	Diffuse Reflectance Fourier Transform Infrared
Fe	Iron
FTIR	Fourier Transform Infrared Spectroscopy
GHG	Greenhouse gases
HTF	Hypersaline tidal flat
IEA	International Energy Agency
IPCC	Intergovernmental Panel on Climate Change
MAOM	Mineral-associated organic matter
MOC	Mineral-associated organic carbon
MM	Mature mangrove
Pg	Pentagram
POC	Particulate organic carbon
POM	Particulate organic matter
pH	Hydrogen potential
PCA	Principal Component Analysis
RM	Restored mangrove
SOM	Soil organic matter
TN	Total nitrogen
TOC	Total organic carbon

LIST OF SYMBOLS

$ - Dollar

% - Percentage

SUMÁRIO

1 INTRODUCTION

Mangroves are coastal ecosystems located in the transition between terrestrial and marine environments, subject to the tidal regimes, characteristic of tropical and subtropical regions (Solano e al., 2024; Vikou et al., 2023; Friess et al., 2022; Nóbrega et al., 2016; Ola et al., 2019). These ecosystems are composed of plant species adapted to varying salinity conditions that colonize sedimentary environments with low oxygen levels caused by tidal inundation, having a large and diverse fauna, but a low plant diversity, though presenting soils rich in organic matter (Owuor et al., 2024; Jia et al., 2023; Souza et al., 2018). Globally, these ecosystems cover 145,068 km2 (Jia et al., 2023), with Brazil standing out for having about 8.5% of the world's mangrove areas (Rovai et al., 2022).

These ecosystems play an important role in regulating the biogeochemical cycles of carbon and nutrients (such as nitrogen and phosphorus) and in mitigating climate change Nóbrega et al., 2019; Friess et al., 2022; Macreadie et al., 2021; Suarez-Abelenda et al., 2014). In fact, mangrove forests contain some of the highest organic carbon (C) stocks among forest ecosystems (Richards et al., 2020), storing between 237 and 283 Mg C ha^{-1} or 1.9 to 2.6 Pg of C globally in the upper soil layers (Ouyang and Lee, 2020; Atwood et al., 2017). Thus, mangroves are among the most efficient ecosystems in combating climate change due to their high carbon sequestration capacity, acting as a natural sink (Nóbrega et al., 2019; Gu et al., 2022; Kauffman and Bhomia, 2017). Their organic-rich soils, which range in depth from 0.5 to over 3 meters, account for around 49% to 98% of total carbon storage in the ecosystem (Donato et al., 2011). This recognition has led to the concept of "blue carbon," which refers to carbon stored in vegetated coastal ecosystems such as mangroves, seagrasses, and salt marshes (Nóbrega et al., 2019; McLeod et al., 2011). This storage capacity is due to high primary productivity and dominant anaerobic decomposition processes, in particular iron and sulfate reduction (Nóbrega et al., 2016). Soil organic carbon (SOC) is a crucial component of the terrestrial carbon cycle and plays a vital role in enhancing soil fertility, global carbon budgets, and carbon sequestration in mangrove ecosystems (Wu et al., 2025; Nóbrega et al. 2019; Abbaszad et al. 2024).

Additionally, these ecosystems provide numerous environmental goods and services, being recognized as nutrient exporters responsible for enriching estuarine and coastal waters, directly influencing fish production, and also providing spawning and nursery grounds for various species (Souza et al., 2018; Mélo et al., 2016). Due to their complex root systems, mangroves modify the hydrodynamic and physical-chemical conditions of the environment, creating a low-energy environment by dissipating wave energy and reducing currents, preventing coastal erosion and reducing the impacts of tsunamis (Weaver, et al., 2024).

However, the capacity of these ecosystems to perform such functions depends on the degree of conservation development in the forests, which in turn can influence the composition and properties of their soils (Nóbrega et al., 2017; Lee et al., 2014). Despite this, mangrove ecosystems have been undergoing intense degradation worldwide. In recent years, around 30-50% of their total area has been lost, which created projections indicating the risk of their disappearance within the next century (Donato et al., 2011). In Brazil, according to a survey by the Climate Observatory (2017), between 2001 and 2015, about 20% of this ecosystem was lost, mainly in the Northeast and Southeast regions, where urban expansion and estuarine silting are the main causes. This can be explained by the increase in the population residing near mangrove areas in the country. According to data presented in the United Nations Environment Program’s (UNEP) Latin America and the Caribbean Regional Report (2016), between 1945 and 2014, the number of people residing in these areas increased by 122.5 million. Fortunately, due to the recognition of the importance of these ecosystems and the implementation of restoration programs around the globe have been declining the mangrove loss worldwide (Goldberg et al., 2020; Hagger et al., 2022).

According to Nóbrega (2016) and Macera et al. (2024), the main causes of degradation in these environments are related to aquaculture, agriculture, urbanization, and coastal landfills. The shrimp farming has become one of the most harmful activities for mangrove ecosystems, mainly due to abrupt changes in land cover and the discharge of effluents with high polluting potential. In mangroves of Ceará, Brazil, carbon stocks in abandoned shrimp ponds were found to be only 37 Mg C/ha — representing a loss of about 72% compared to adjacent natural mangroves. Therefore, the estimated average cumulative carbon emissions resulting from mangrove conversion to shrimp farms is approximately 1,371 Mg CO_2/ha (Neto et al., 2023; Kauffman et al., 2018a, b).

Studies have also revealed the detection of organic contaminants, such as pesticides and antibiotics, in mangrove environments in India, Indonesia, China, and Vietnam (Bayen, 2012). Accordingly, the conversion of mangrove forests to agricultural, aquacultural, and urban areas led to the loss of 158 million tons of carbon stocks (Ruiz et al., 2024; Chien et al., 2024). In an effort to promote the protection and restoration of ecosystems, the United Nations (UN), through its General Assembly during the Convention on Biological Diversity (CBD), established the beginning of the Decade on Ecosystem Restoration in 2021, complementing the previous Decade of Biodiversity (2011-2020). In Brazil, the creation of the National Program for the Conservation and Sustainable Use of Mangroves (ProManguezal; Brazil, 2024) was established to promote the conservation, restoration and sustainable use of biodiversity and ecosystems services. This new proposal aims to strengthen the restoration of degraded ecosystems, as such actions have been proven to be effective in combating climate change. In this regard, mangrove restoration has been globally encouraged, primarily to mitigate climate change (Howard et al., 2014). Thus, by restoring mangroves, in addition to promoting carbon sequestration, ecosystem services are reestablished (Casasco et al., 2014).

If the overall objective is achieved, it could restore 350 million hectares of degraded ecosystems by 2030, removing between 13 and 26 billion tons of greenhouse gases (GHGs) from the atmosphere. According to the UN, 57 countries, including subnational governments and private organizations, have committed to this goal. Thus, due to their environmental and economic importance, as well as their intense degradation, mangroves show particular potential for restoration and reforestation systems (Alongi, 2022; Ferreira et al., 2015).

However, to adequately adopt the processes of mangrove restoration aimed at mitigating climate change, it is necessary to deepen the understanding of the accumulation and changes in the characteristics of organic matter, since it may present a marked control on organic carbon retention. This involves not only understanding how different characteristics of the organic matter interact with the mineral phase promoting its stabilization, but also considering how these changes affect the ability of mangroves to provide essential ecosystem services, such as coastal protection, biodiversity, and carbon capture. Thus, this study aimed to evaluate the accumulation and the changes in the organic matter characteristics in three conditions, e.g., i) a pristine mangrove, ii) a former mangrove area that was degraded and converted into a hypersaline tidal flat; and iii) a restored mangrove that developed after the implementation of restoration practices in the hypersaline tidal flat, to assess the carbon sequestration potential and the stabilization of organic matter.

2 HYPOTHESES

1. As the vegetation is restored, there will be an accumulation of reduced forms of iron and sulfur in the mangrove environment.
2. The restoration of mangroves should increase the contents of total and particulate organic carbon, such as aromatic, phenolic, and polysaccharide groups, promoting its stabilization.

3. The recovery of particulate organic matter content will be faster than the recovery of organic matter associated with minerals.

3 OBJECTIVES

3.1 General Objective

To evaluate how the mangrove restoration promotes organic matter accumulation and stabilization in soils, through changes in physical fractionation, molecular composition and thermal recalcitrance, aiming to improve the comprehension of the carbon sequestration in restored mangroves and to provide basis for future mangrove restoration programs.

3.2 Specific Objectives

To characterize the physical-chemical properties and predominant decomposition metabolic pathways by determining the degree of pyritization in soils of non-impacted mangroves and compare them with degraded and recovering mangrove areas.

To quantify the contents of soil organic carbon, the origin of organic matter, and to evaluate the physical and molecular characteristics of the accumulated organic matter in non-impacted, impacted, and recovering mangrove areas.

4 THEORETICAL FRAMEWORKS

4.1 Climate Change and Carbon in Soil

The increase in carbon dioxide (CO_2) in the atmosphere is one of the main drivers of global climate change. The concentration of CO_2 in the atmosphere rose from approximately 278 parts per million (ppm) in 1750 (Lindsey, 2024; Gulev et al., 2021), at the start of the industrial era, to 414.7±0.1 ppm in 2021 (Friedlingstein, 2022), reaching 422 ppm in October 2024 (NOAA Trends in atmospheric Carbon dioxide). According to data from the International Energy Agency (IEA), in 2021 alone, approximately 36.3 billion tons of CO_2 were emitted worldwide, with an increase of 2 billion tons. As a result of the increased CO_2 concentration and the climatic changes, millions of people will face hunger, disease, droughts, and extreme climate conditions in the coming decades (IPCC, 2022). The global surface concentration of CO2, averaged across all 12 months of 2023, was 419.3 parts per million (ppm), an increase of 2.8 ppm during the year (Lindsey R, 2024).

In this context, the concern with the global carbon (C) cycle, greenhouse gas emissions, and their impacts on the climate has led researchers and public agencies to apply policies to mitigate the increase in these concentrations in the atmosphere (Howard et al., 2014;

Murdiyarso et al., 2015). Among these strategies, the implementation of nature-based solutions can substantially sequester CO_2 from the atmosphere through plants that store C in biomass, but especially in the soil (Taillardat et al., 2018; Bossio et al., 2020; Zeng et al., 2021). In fact, soil carbon sequestration is one of the few technologies that can be applied on a large scale to mitigate climate change (Wang et al., 2021). Among the natural carbon reserves, coastal ecosystems such as mangroves, saltmarshes, and seagrass areas play an important role in global C sequestration. The recent recognition of the relevance of these areas in carbon sequestration led to the creation of the term "blue carbon," referring to C stocks in coastal areas (Ola et al., 2019; Nellemann et al., 2009). Thus, blue carbon is accumulated in the aboveground biomass, underground biomass, or dead biomass (litter and dead wood), but mainly in the soils of these ecosystems (McLeod et al., 2011). Despite covering a small portion of the Earth's surface (1.7 million km^2 of the 510 million km^2 of the Earth's surface), it is estimated that vegetated coastal ecosystems (mangroves, marshes, and seagrasses) store about 50% of all terrestrial organic carbon (Hopkinson et al., 2012).

However, the capacity for C accumulation in mangroves varies widely due to several factors, such as the primary productivity, constantly changing geomorphological conditions, and the climatic and hydrological characteristics of the environment. These factors play diverse and interconnected roles in the sequestration of organic C in the soils of these ecosystems (Alongi et al., 2014). In this sense, mangroves have been recognized as priority areas for climate change mitigation due to their ability to store high carbon stocks, especially in their soils (IPCC, 2019). Carbon (C) sequestration in the soil can bring important benefits, such as improved soil health and the provision of ecosystem services (Wiesmeier et al., 2019).

4.2 Characteristics of Mangroves

Mangroves are ecosystems strongly influenced by seawater, which, during high tide, floods the lower areas of the landscape and conditions the occurrence of pedogenetic processes typical of thionic hydromorphic soils (Gomes et al., 2016; 2021). Due to the saline conditions, this ecosystem hosts adapted shrub/tree vegetation that can grow and complete its life cycle in a substrate containing high concentrations of soluble salt (Souza et al., 2018). These plants have special adaptations to survive in environments with high salinity, such as tolerance to flooding and salinity, including ionic compartmentalization (the ability to regulate ion distribution within cells to ensure a healthy balance), osmoregulation (the ability to control osmotic pressure to prevent dehydration and maintain a proper water balance), selective transport and ion absorption (mechanisms to selectively absorb essential ions while avoiding excessive salt absorption) (Souza et al., 2018).

The tolerance of mangroves to a highly saline environment is also closely linked to genetic expression regulation (allowing plants to adjust their responses to environmental conditions) and aerial roots that enable respiration in flooded soils, supplying oxygen to the underground parts of the plant (Souza et al., 2018). The main vegetation species found in Brazil are *Rhizophora mangle* (red mangrove), *Laguncularia racemosa* (white mangrove), *Avicennia schaueriana* (black mangrove) (Lacerda, 1984). These are accompanied by other invasive species, such as *Acrostichum aureum* (swamp fern), Spartina grass, mosses, orchids, among others (Rodrigues, 1995). Additionally, plant species like *Hibiscus tiliaceus* (seaside cotton or mangrove embira), and *Conocapus erectus* (buttonwood mangrove) are commonly found in transitional zones between mangrove and upland areas (Schaeffer-Novelli, 1990).

Mangrove soils are formed from fluvial-marine sediments mixed with organic materials of diverse origin and grain size; they are saline and thionic, classified as Gleysols or Organosols depending on the organic carbon content (to Souza-Júnior, 2001). According to Souza-Júnior et al. (2008), the texture of these soils can vary from sandy (in regions influenced by wave action or sand dunes) to clayey (in more protected regions with lower hydrodynamics). Additionally, mangrove soils are considerable nutrient retainers, deriving nutrients from tidal inflow but primarily from organic matter due to low decomposition rates and anthropogenic activities (Reef et al., 2010).

In mangroves, salinity varies daily according to flow of tides, which in turn depends on estuarine geomorphology, determining the input of seawater moving inland and the amount of freshwater (or continental water) flowing toward the sea (Atekwana et al., 2022). Thus, at high tide, the environment becomes predominantly saline, while at low tide, freshwater invades the riverbanks covered by mangroves, generating lower salinity. Another important aspect is the ability of seawater, with its salts and nutrients such as nitrogen (N), phosphorus (P), micronutrients, and trace elements, to act as a buffer solution to keep the pH relatively stable. Tidal action also promotes the export of large amounts of dissolved inorganic carbon (DIC), total alkalinity (TA), and dissolved organic carbon (DOC) to adjacent marine systems through tidal dynamics (Braga et al., 2023; Fennel et al., 2019).

Although the gradual increase of anthropogenic CO_2 leads to a decrease in ocean pH, pH variations and long-term trends in coastal regions are complex, influenced by changes in nutrient concentration, organic matter input, productivity, and respiration (Ma et al., 2023). The influence of these processes on CO_2 capture/emission and on the natural balances of production and mineralization of organic matter is not yet fully understood (Braga et al., 2023). In Brazil, areas with mangroves are considered permanent preservation zones, supported by Law No. 4,771 of September 15, 1965. However, these ecosystems have been threatened by a wide range of anthropogenic impacts, such as the installation of petrochemical and mining-metallurgical industrial complexes, shrimp farming, and the expansion of urban areas, often driven by real estate speculation (Havens et al., 2015; Diegues, 2008).

Mangroves offer a range of economic, social, and environmental benefits to populations, commonly referred to as ecosystem goods and services. These benefits are categorized into regulating, supporting, provisioning, and cultural services (Costanza et al., 2014). The flow and provision of these ecosystem goods and services do not solely depend on the presence of mangroves in a particular area but also on the species composition, population size, and other ecological factors such as soil and water quality, tidal patterns, and the influence of extreme climatic events. Factors like regulatory policies, geographic location, sociocultural context, harvesting methods, associated biodiversity (which influences resilience and the ecosystem's ability to provide essential services, such as fisheries, storm protection, and water purification), and the presence of anthropogenic chemicals or toxins also play crucial roles.

The value of mangroves varies significantly worldwide and is influenced by cultural heritage and the level of local dependency on mangroves for livelihood and well-being (UNEP, 2014). Consequently, mangroves are considered one of the planet’s most valuable ecosystems, with an estimated worth of $193,843 per hectare per year (2007 values), making them approximately 36 times more valuable than tropical forests (Costanza et al., 2014).

4.3 Carbon Sequestration in Mangroves

Mangroves and their associated soils can sequester around 22.8 million tons of carbon per year (Santos et al., 2019). In line with this, ICMBio (2018) also affirms that mangroves are important carbon stores and sequestration agents, as their carbon stock is significantly higher than that of other terrestrial forests, considering the stock in soil and subterranean biomass. Supporting this, McLeod et al. (2011) found that coastal vegetation ecosystems (salt marshes, mangroves, and seagrass beds) have higher rates of carbon sequestration and sediment accumulation than those found in soils of three forest types (temperate, tropical, and boreal). The carbon stored, sequestered, and released by coastal ecosystems is referred to as blue carbon (Santos et al., 2019; Herr et al., 2012; Giri et al., 2011), accounting for 47% of organic carbon (OC) in ocean sediments, with an estimated accumulation of 0.07–0.22 Pg C year^{-1} (Bauer et al., 2013; Xia et al., 2021). This plays a significant role in carbon sequestration and the regulation of global climate change.

Mangroves occupy only 0.5% of the global coastal area but have the highest organic carbon density of any ecosystem. Their average global stock is 692.8±23.1 Mg C ha^{-1} (Donato et al., 2011), of which approximately 76.5% of the OC is stored in the soil (Kida et al., 2021). A key reason for this high soil carbon accumulation is the slow decomposition of organic matter due to the saltwater hydromorphism typical of mangroves (Nóbrega et al., 2016; 2019).

Additionally, organo-mineral associations have long been considered an important mechanism for the stabilization and preservation of OC in soils and sediments (Kleber et al., 2021). This has led to the development of soil organic matter fractionation schemes to separate particulate organic matter (POM) from mineral-associated organic matter (MAOM) to better understand the processes associated with C accumulation (Cambardella and Elliott, 1992; Lavallee et al., 2020; Poeplau et al., 2018). However, the occurrence of organo-mineral interactions has been poorly understood in mangroves ecosystems (Ruiz et al., 2024).

One of the most important soil mineral groups for OC stabilization is reactive iron (FeR). FeR is operationally defined as iron (oxy) hydroxides reducible by sodium dithionite (Assavapanuvat et al., 2024; Chen et al., 2024; Lalonde et al., 2012; Li et al., 2023; Ye et al., 2022), which differs from the classic definition of reactive iron in marine sediments, as a fraction of iron readily reacts with sulfide to form various iron sulfide minerals (Ye et al., 2022). The formation of FeR-associated reactive OC (FeR-MAOC) is a major stabilization mechanism for OC in terrestrial, coastal, and marine environments (Chen et al., 2020; Lalonde et al., 2012; Li et al., 2023; Ye et al., 2022).

The association between organic matter and FeR prevents hydrolysis or crystallization of FeR, ensuring the stability of FeR-MAOM (Adhikari et al., 2017). In addition, FeR-MAOM formation is probably favored by acidic wetland conditions due to the occupation of mineral surfaces by protonated hydroxyl groups (Iroshaka et al., 2025). However, under anoxic conditions, FeR-MAOM may become unstable due to increased solubility of reduced iron species releasing organic matter (Chen et al., 2020; Duan et al., 2020; Kida and Fujitake, 2020). FeR exists in two main phases in mangrove environments: (a) crystalline species such as ferrihydrite and lepidocrocite and (b) hematite and goethite. The poorly crystalline FeR is abundant in variable redox environments (Iroshaka et al., 2025) and in mangroves this can be approximately >65% of the total FeR content in soil (Ferreira et al., 2021; Iroshaka et al.., 2025). As poorly crystalline phases have a high specific surface area compared to their crystalline counterparts (Pinsonneault et al., 2020), they can contribute significantly to the FeR-MAOM fraction in mangrove environments.

Due to differences in their origins and composition, the stability of POC depends on its intrinsic chemical recalcitrance and the potential for microbial enzyme inhibition under oxygen-depleted redox conditions (Lavallee et al., 2020). The persistence of mineral-associated OC (MAOC), in contrast, is regulated by chemical stabilization of soil minerals (Lavallee et al., 2020; Sokol et al., 2022). For example, OC associated with reactive iron (FeR-MAOC) is generally stabilized under oxic conditions but destabilized under anoxic conditions due to different solubility properties of reduced and oxidized iron (Chen et al., 2020; Dong et al., 2024; Kida and Fujitake, 2020).

In coastal wetland soils, previous studies have estimated a wide range of POC, MAOC, and FeR-MAOC fractions (Assavapanuvat et al., 2024). This large variation may depend on different extraction methods (e.g., particle size versus density), varying plant productivity along latitudinal gradients (Fu et al., 2024), site-specific hydrodynamic factors, and plant species-specific influences. The latter is not only regulated by broad plant groups (e.g., mangroves vs. salt marshes) but also by taxon-specific effects (e.g., *A. germinans* vs. *R. mangle*). For example, enrichment of recalcitrant organic compounds (e.g., lignin and tannins) in *R. mangle* can promote POC accumulation (Friesen et al., 2018; Saraswati et al., 2016). In contrast, increasing soil redox potential (Eh) due to oxygen release by the *A. germinans* rhizosphere can facilitate FeR-MAOC formation (Comeaux et al., 2012; Friesen et al., 2018; Krauss et al., 2006; Perry and Mendelssohn, 2009; Thibodeau and Nickerson, 1986).

Besides organo-mineral fractionation, in these ecosystems, organic matter decomposition occurs mainly by utilizing iron oxides or sulfate as the final electron acceptor instead of oxygen, thus with lower energy yield (Nóbrega et al., 2016; 2019). As a consequence of these anaerobic metabolic pathways, mangrove soils have low redox potential but also accumulate reduced forms of Fe and S, forming metal sulfides, mainly pyrite (Nóbrega et al., 2013; Ferreira et al., 2022). This decrease can alter soil biochemical processes and microbial activities in this environment.

The soil organic matter (OM) reservoir is a mixture of simple compounds with varying residence times due to physical interactions (i.e., with the mineral matrix) that can inhibit OM decomposition and its controls (Abrar et al., 2020; Arndt et al., 2013) or chemical protection against decomposition, as well as more complex compounds with inherently low reactivity that require high activation energy for decomposition (Arndt et al., 2013). Another relevant factor is the high productivity of this ecosystem, making it one of the most productive among coastal and terrestrial ecosystems, contributing large amounts of C to the soil (Iroshaka et al., 2025; Alongi, 2020; Schmidt et al., 2011; Rovai et al., 2021)._Mangrove sediments receive organic matter from both autochthonous and allochthonous sources. However, the humic-like components of mangrove SOM may have an autochthonous origin. For instance, roots are a primary constituent in mangrove peat (Iroshaka et al., 2025) and the dominant autochthonous contributor to SOC stock (Iroshaka et al., 2025; Ouyang et al., 2017).

Studies on SOM fractions in mangrove environments are multiplying, and the two-group classification of SOM into POM and MAOM has become the basis of many of these studies. The MAOM fraction can account for a significant proportion of SOM in mangroves. However, the contribution of POM cannot be ignored, particularly in mangrove environments where sediment supply conditions are poor. Through various mechanisms, both MAOM and POM are responsible for the long-term storage of SOC in mangrove substrates. However, the individual contributions of some of these mechanisms have yet to be fully explored. For example, while the reactive Fe (FeR) phases of MAOM in mangrove sediments have received greater attention, the contribution of FeS, FeS2 and clays to the MAOM fraction remains unknown. The contribution of FeS, FeS2 and clays to the MAOM fraction remains largely unexplored.

Indeed, different SOC fractions react differently to environmental conditions. For example, metal-mediated organo-mineral complexes are particularly vulnerable to humidity and redox variations. Exposure of organometallic complexes such as FeS-MOM and pyrite-MAOM to oxygen causes desorption and dissolution of these complexes, removing both metals and organic compounds from mangrove wetlands. In addition, mangrove POMs can undergo rapid decomposition (due to the lack of physical protection) in the presence of oxygen. Groundwater contributes to maintaining mangrove soils in suboxic and anoxic conditions, particularly when the wetland surface is exposed during low tides (Sippo et al., 2020; Iroshaka et al., 2025; Xiao et al., 2019).

4.4 Methods for Characterizing Soil Organic Matter

Soil organic matter (SOM) represents a biochemically active environmental compartment that has an impact on ecosystems services and biodiversity (Jimenez et al., 2021; Xu et al. 2023). As important as determining the organic matter (OM) content is understanding its influence on soil quality, especially regarding physical fractions and land use changes. Combining OM fractionation with analytical techniques, such as spectroscopy, provides valuable insights into OM dynamics, including its decomposition, stabilization, functionality, and structure (Salgado et al., 2025; Xu et al. 2023).

Despite the highly relevant aspects related to organic matter dynamics, studies on this topic have not yet elucidated all aspects of SOM dynamics in soil, which is incredibly complex and involves various interactions between soil organic and mineral components (Ingaki et al., 2020; Schmidt et al., 2011; Lehmann et al., 2015; Kleber et al., 2021). Minerals play a fundamental role in this dynamic, acting as adsorption sites for organic compounds and as catalysts for chemical reactions involving organic matter (OM) (Kleber et al., 2021).

To understand SOM dynamics, it is crucial to assess it using various procedures to study its origin, chemical nature, and recalcitrance. A comprehensive approach may require a set of methodologies that consider the relationship between organic matter accumulation and its physical location in the soil matrix, as well as the characterization of its fractions. This study aims to evaluate the impacts of mangrove colonization in degraded areas on soil organic matter characteristics (such as input, total organic carbon content in compartments, and molecular characteristics) to provide more information on the carbon sequestration process in recovering mangroves.

Studies indicate that certain SOM compartments can more quickly detect changes in carbon (C) levels associated with soil management. Reductions in these compartments are generally greater than those observed when only total C content is considered (Pinheiro et al., 2004; Conceição, 2013; 2014). Short-term changes can be verified through physical particle size and/or density fractionation of OM, with coarse and particulate fractions of C being sensitive in the first order, and occluded light fraction C (FLO) being an intermediate sensitivity measure (Freixo et al., 2002a, b).

To evaluate the chemical composition of these fractions, among the available techniques, Fourier-Transform Infrared Spectroscopy (FTIR) stands out. This analytical technique, which involves the interaction of infrared light with a sample, allows for the identification of functional groups present in POM and MAOM, such as carboxylic acids, amines, amides, hydroxyl groups, as well as aliphatic and aromatic structures, among others (Yeasmin et al., 2020; (Pärnpuu et al., 2022; Lehmann et al., 2020; Rowley et al., 2021). Understanding the functional groups present in SOM can help detect changes in SOM composition due to various factors, such as increased C and nitrogen concentrations in the soil (Calderon et al., 2013; Stehlíková et al., 2016).

FTIR spectroscopy has been successfully used to describe the degradation state of OM at different soil horizons in the forest, with deeper soil horizons showing decreasing absorption intensities for functional groups (Haberhauer et al., 1998). In an FTIR spectrophotometer, the percentage of radiation transmitted through the sample over a wavelength range of 2.5 to 25 μm (fundamental infrared, wavenumber 4000 to 500 cm-1) is recorded, resulting in a continuous absorption spectrum (Helms et al., 2008). In this technique, infrared light interacts with the sample and is then scattered by its particles in all directions (Griffiths et Haseth, 2007). The amount of diffusely reflected light depends greatly on the nature of the sample, such as the shape, size, and compactness of particles in the sample and the sample's refractive index. The quality of the DRIFTS spectrum is directly related to the amount of diffusely reflected light detected (Smith et al., 2011).

Another tool used in SOM characterization is thermogravimetric analysis, which allows estimating SOM aromaticity by observing the position of mass loss peaks or exothermic reaction peaks (López-Capel et al., 2005a;). The labile SOM fraction decomposes during this analysis between 300–350 °C, while more recalcitrant SOM decomposes around 400–500 °C (López-Capel et al., 2005a, b). It has been reported that residual organic matter (ROM) decomposes at 430–530 °C and consists of artificial or organic substances not naturally occurring in the soil, but introduced through human activities, such as the use of synthetic fertilizers, pesticides, industrial waste, among others (López-Capel et al., 2006) or natural aromatic compounds (López-Capel et al., 2006). Inorganic C, such as carbonate, can also be determined from decomposition between 700 and 900 °C.

Expanding the capacity for thermal analysis, coupling an isotope ratio mass spectrometer (IRMS) and a quadrupole mass spectrometer (QMS) with a thermal analysis system (TG-DSC) allows the proportions of the main components, evolved gas composition, and carbon isotope ratios of organic/inorganic composite materials to be determined during a single heating experiment (López-Capel et al., 2006). The distinction of isotopic signatures of labile, recalcitrant, and refractory components can provide new insights into the dynamics of organic matter in sediments (López-Capel et al., 2006).

Due to the multiple inputs of OM in estuarine regions, it is necessary to use tools capable of identifying the source and fate of OM in these areas. Isotopic analysis has been a useful tool in inferring the sources and cycling pathways of OM in aquatic ecosystems, providing clues about its origin (McCallister et al., 2006; Koszelnik et al., 2018). Different OM sources and biogeochemical reactions can result in distinct isotopic compositions that can be used to quantify the relative contribution of these different sources and provide qualitative-quantitative information on the spatial and temporal changes in OM origin. Studies utilizing the isotopic composition of sedimentary OM, POM and DOM have been conducted over time as useful tools for identifying the origin of OM in riverine, estuarine, and marine environments (Giarrizzo et al., 2011; Tue et al., 2012). Carbon isotopic ratios are useful for distinguishing OM sources and identifying different types of terrestrial plants (Meyers, 1994). Terrestrial plants preferentially incorporate the lighter carbon isotope (^{12}C) over the heavier isotope (^{13}C), thereby presenting lower (more negative) $\delta^{13}C$ values than their source. The atmospheric carbon source for terrestrial plants is atmospheric CO_2, which has $\delta^{13}C$ values ranging from -7 to -8‰.

Terrestrial plants fall into three main categories (López-Capel et al., 2005a): C3 plants (trees and shrubs), C4 plants (mainly grasses), and CAM plants (cacti and bromeliads). This distinction is based on the different photosynthetic pathways in carbon uptake by the three plant groups. C3 plants have a lighter (more negative) isotopic composition, being depleted in ^{13}C, with $\delta^{13}C$ values between -30 and -25‰. C4 plants have a heavier (less negative) isotopic composition, being enriched in ^{13}C relative to the number of ^{12}C atoms, with a $\delta^{13}C$ value ranging between -15 and -10‰, while CAM plants exhibit metabolism similar to both C3 and C4 plants, thus presenting a wide range of carbon isotopic composition. Marine OM (represented by phytoplanktonic organisms) has $\delta^{13}C$ values between -22 and -20‰ (Meyers, 1994).

These differences in $\delta^{13}C$ values are crucial for tracing the carbon cycle, showing how carbon is absorbed, released, and transferred between different environmental compartments, which is essential for assessing the role of ecosystems in climate regulation and mitigating climate change. Furthermore, tracking these isotopic compositions can provide insights into carbon transport patterns and the carbon sources sustaining primary production in terrestrial and aquatic ecosystems. This deeper understanding is essential for developing effective strategies for conserving and managing natural resources, as well as for predicting and mitigating the impacts of global environmental changes.

5 MATERIALS AND METHODS

5.1 Study Area

This study was carried out in an Environmental Protection Area in the Pacoti River Estuary (Ceará, Northeastern Brazil). The Pacoti River stretches 150 km, originating in the Baturité mountain range in the municipality of Guaramiranga. The river's flow is regulated by two major reservoirs for human consumption, which has led to an increase in soil salinity within the estuary (Barbosa et al., 2016; Ferreira et al., 2022a; Lacerda et al., 2007). The estuary is influenced by a semi-arid tropical humid climate with a rainy season between January and May and a dry season between June and December. Rainfall rates in the coastal region reach around

1,400mm/year, associated with an average temperature of 27°C with a low amplitude of variation (Carvalho et al., 2022; IPECE, 2018). Most of the time, the dynamics of the northeast coast are controlled by sea waves, but it is also strongly influenced by dispersive waves or swell (Carvalho,2022). The estuary is dominated by a semi-diurnal and mesotidal regime, with tidal variations ranging from 2 to 3 meters, and astronomical tides reaching maximum and minimum amplitudes of 3 m and 0.2 m, respectively (Carvalho, 2022; Eduardo et al., 2021). Mangrove vegetation is predominant along the estuary margins, extending approximately 362 km² inland from the river mouth and covering an area of 160 hectares (Barbosa et al., 2016).

Before 1985, the areas along the Pacoti River estuary were occupied by salt flats established on original mangroves in the 1960s (Lacerda et al., 2007). Major changes in mangrove areas occurred between 1968 and 1999, primarily due to extinction of salt production, abandonment of salt ponds, and construction of river dams. During this period, the mangrove area increased by 1.42 km², mainly due to the colonization of 0.56 km² of abandoned salt ponds. Most of this new mangrove area (approximately 69%) developed in areas previously occupied by salt ponds. Additionally, an extra 0.15 km² (about 21% of the initial mangrove area in 1958) grew in areas not previously occupied by salt ponds. Likewise, an additional 0.2 km² growth observed between 1999 and 2004 also occurred outside the old salt ponds. Thus, most of the total increase in mangrove area resulted from the conversion of higher-elevation areas previously occupied by salt ponds. Mangroves also developed on newly formed islands along the riverbanks that had not been converted to salt flats.

The abandoned ponds allowed the formation of hypersaline tidal flats (locally known as *apicuns*), which highly saline areas with sparsely (or devoid) vegetation (Albuquerque et al., 2014). In the hypersaline tidal flats (HTF), species such as *Batis marítima* (C. Linnaeus), *Blutaparon portulacoides* (St. Hil), and *Sporobolus virginicus* (C. Linnaeus) K. Kunth colonized the area in an irregular pattern (Cabral et al., 2020). These areas correspond to the supratidal region, where the less frequent flooding prevent mangroves colonization, and thus are found in intertropical regions worldwide (Lacerda et al., 2007; Ferreira et al., 2022; Albuquerque et al., 2014). Since the HTF are subjected to much less frequent tidal flooding periods compared to mangrove, also coupled with high evaporation and low precipitation rates, these ecosystems are characterized by the occurrence of hypersaline soils (Albuquerque et al., 2014).

Aiming to restore the degraded HTF areas, in 2017, five channels were constructed perpendicular to the riverbed to promote water flow and circulation, supporting the colonization of the area by mangrove propagules (Ferreira et al., 2022). In addition to the construction of the channels, *Rhizophora mangle* (L.) propagules were planted with 30 cm spacing between plants (Ferreira et al., 2023). After 32 months, successful restoration practices were observed, with specimens of R. mangle, *Avicennia germinans,* and *Laguncularia racemosa* reaching heights between 58.3±15.8 and 80.8±35.5 cm (n=381); 85.6±23.2 and 110.6±30.7 cm (n=463), and 110.9±40.0 and >200 cm (n=290), respectively (Ferreira et al., 2022a).

To assess the influence of restoration practices for C dynamics and organic matter characteristics, soil samples from degraded areas (e.g., *apicum*), restored mangroves and pristine (e.g., old mangroves forests) were sampled using a 3 x 5 x 5 factorial scheme, totaling 75 samples, covering three different conditions (hypersaline tidal flat; five depths, and five replicates. Samples were collected over two days during the dry season, totaling two sampling activities.

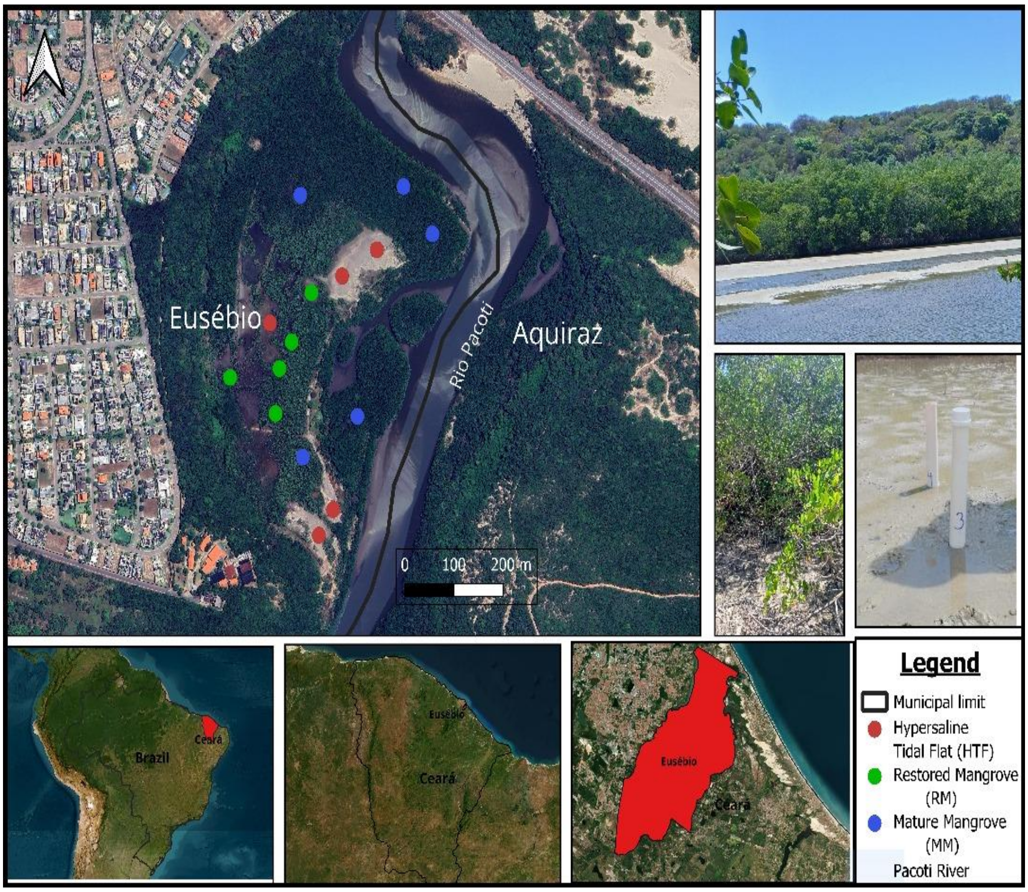

Figure 1: Map of the location of the studied estuary (Source: author).

Table 1. Coordinates of the sampled sites for each condition (HTF, RM and MM)

Points	Hypersaline tidal flat (HTF)	Restored Mangrove (RM)	Mature Mangrove (MM)

1	3º 49’57.27” S 38°25’15.93” W	3º 49’50.38” S 38°25’18.92” W	3º 49’ 53.22”S 38°25’17.07” W
2	3º 49’55.90” S 38°25’14.93” W	3º 49’48.07” S 38°25’18.66” W	3º 49’ 38.85”S 38°25’03.61” W
3	3º 49’45.90”S 38°25’11.38” W	3º 49’47.09”S 38°25’17.01” W	3º 49’ 41.42” S 38°25’07.80” W
4	3º 49’41.82”S 38°25’12.10” W	3º 49’44.82” S 38°25’14.54” W	3º49’50.84” S 38°25’13.30” W
5	3º 49’45.86”S 38°25’19.56” W	3º49’49.30”S 38°25’22.53” W	3º 49’39.08”S 38°25’17.26” W

5.2 Sample Collection

The sampling was carried out on January 15, 2024, at three different sites (HTF, RM, MM). Fifteen sampling points were selected and sampled during the dry season, at five different depths. At each sampling site, two soil samples were collected using PVC tubes (70 cm in length, 50 mm in diameter) attached to a flooded soil sampler. Once collected, the samples were hermetically sealed and transported vertically. They were then sectioned into intervals of 0-5, 5-10, 10-20, 20-30, and 30-50 cm, stored in labeled plastic bags, and sent under refrigeration to the laboratory for analysis. One sample from each site was oven-dried with forced circulation at 50°C until constant weight to determine soil density (ρ = soil volume divided by collected layer thickness) and to prepare for the analysis of organic matter, particle size distribution, and carbon content (C) quantification. The other sample was used to obtain Eh (redox potential) and pH values and was then frozen for subsequent pyritization analysis. Eh and pH values were measured using portable electrodes, with pH values obtained via a calibrated glass electrode using pH 4.0 and 7.0 standard solutions, and Eh values measured with a platinum electrode, adjusted by adding +244 mV for the standard calomel electrode value (Nóbrega, 2016).

5.3 Soil Characterization

Soil samples from each site were characterized for sand, silt-clay content, total organic carbon (TOC), total nitrogen (TN), pyritization degree, soil carbon stock (SCS), and equivalent soil carbon stock (SCSEQV) (Nóbrega, 2016). The Particle size analysis was performed using a combination of physical (2500 rpm shaking for 16 hours) and chemical methods (sodium hexametaphosphate solution - Na (PO$_3$)$_6$, 5 g/L - for dispersion, at a ratio of

20 g soil to 60 mL dispersant solution). The sand fraction was determined by sieving through a 53 µm mesh and washed with distilled water, while fine fractions (silt and clay) were determined using a densimeter. The soil suspension density (1000 mL) was determined at two time points: 30 seconds (D1) and 2 hours (D2). Particle fractions were calculated using the following equations:

% Clay + % Silt = ((D1–blank)/TFSE) ×100×factor"f"((D1 – blank) / TFSE) \times 100 \times factor "f"((D1–blank)/TFSE) ×100×factor"f"

% Clay = ((D2–blank)/TFSE) ×100×factor"f"((D2 – blank) / TFSE) \times 100 \times factor "f"((D2–blank)/TFSE) ×100×factor"f"

% Silt = ((D1–blank)–(D2–blank)/TFSE) ×100×factor"f"((D1 – blank) – (D2 – blank)/ TFSE) \times 100 \times factor "f"((D1–blank)–(D2–blank)/TFSE) ×100×factor"f"

% Sand = 100 – ((D1–blank)/TFSE) ×100×factor"f"((D1 – blank) / TFSE) \times 100 \times factor "f"((D1–blank)/TFSE) ×100×factor"f"

After separation, the particle size fractions were dried at 60°C until constant weight to preserve organic matter integrity. Carbon and nitrogen contents corresponding to particulate organic carbon (C_{POM} and N_{POM}) and mineral-associated organic carbon (C_{MAOM} and N_{MAOM}) were then determined (Cambardella & Elliott, 1992). TOC and TN were determined using 12 mg of soil, weighed in tin capsules (EURO EA) on a precision scale (Mettler TOLEDO) and analyzed in an elemental analyzer (Euro Victor) at the Chemistry Laboratory of the Federal University of Ceará (UFC, PICI campus). The following procedure was used to remove the inorganic fraction (Carbonate) from the soil: 5 g of dry soil was weighed and transferred to centrifuge tubes, to which 10 ml of a 1 mol L^{-1} HCl solution was added. The samples were left to stand for 24 hours to permit reaction between the acid and the samples. The samples were then centrifuged for 15 minutes. After removal of the acid, the soil was washed three times with distilled water to remove all acid residues. The samples were then oven-dried to constant weight. After drying, the soils were stored for 15 days before analysis to ensure complete removal of the inorganic fraction.

Thus, Soil carbon stock (SCS) was quantified down to a depth of 50 cm using ρ and TOC content ($SCS = TOC \times \rho \times 50$ cm; Nóbrega et al., 2016) for the three conditions. Equivalent soil depths were calculated to compare carbon stock in soils from impacted and regenerating areas, using mangrove soil bulk density as a reference (Ellert et al., 1995). The use of equivalent depths for soil carbon stock calculations is beneficial when comparing ecosystems with differing soil bulk densities, as higher stock values may be related to denser soils (e.g., due to particle size composition, compaction, subsidence) rather than higher organic carbon content (Nóbrega et al., 2016; Ellert & Bettany, 1995).

The quantification of reactive iron (FeREACTIVE) and pyrite iron (FePYRITE) was performed using frozen samples, following the sequential extraction method of Nóbrega et al. (2016) as proposed by Huerta-Diaz and Morse (1990, 1992). FeREACTIVE was initially extracted with 1 M HCl solution (16-hour shaking), followed by pre-treatment to remove iron associated with silicates using 10 M HF and organic matter with H_2SO_4. FePYRITE was then extracted with concentrated nitric acid (2-hour shaking). The degree of pyritization (DOP) was calculated according to Berner (1970) as: DOP (%) = (FePYRITE × 100) / (FeREACTIVE + FePYRITE).

5.4. Analysis of the isotopic composition of particulate organic matter (C_{POM} and N_{POM}) and organic matter associated with minerals (C_{MAOM} and N_{MAOM}).

To determine stable isotopes of carbon and nitrogen (isotopic ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$), around 5 mg of organic matter fractions (POM and MAOM) were weighed into tin capsules. These isotopic ratios were determined using an elemental analyzer Isolink (Themo), connected to a Conflo IV interface and then to a Delta V Advantage (Thermo Scientific Isotope Ratio Mass Spectrometer, IRMS). The results for carbon ($\delta^{13}C$) are expressed using a conventional notation relative to the Vienna Pee-Dee Belemnite standard (VPDB ±2SD‰ (95% confidence level). Currently, the following certified reference materials are used: urea from IVA, fructose from IPR/PUCRS and proteins from Elemental Microanalysis for $\delta^{13}C$ and $\delta^{15}N$ analysis.

The reference materials now include urea from IVA, N-2 from IAEA and proteins from Elemental Microanalysis for $\delta^{13}C$ and $\delta^{15}N$ analysis. For samples where errors are not indicated, a standard deviation (SD) of SD(1s) < 0.1% is assumed. Precision is reported as $\delta^{13}C$ = 0.05% and $\delta^{15}N$ =0.01%. This system includes an inline dual furnace setup, with an oxidation column at 1020°C and a reduction column at 650°C. The gases obtained (CO_2 and NO_X) were chemically dried, separated chromatographically, and injected into the mass spectrometer for isotopic ratio determination. The isotopic ratio is calculated as follows: $\delta NE‰ = (Rsample/Rstandard - 1) \times 1000$. Where N is the heavier isotope of element E (carbon or nitrogen), and R represents the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of these elements (Hoefs, 2018).

5.5 Fourier Transform Infrared Spectroscopy / Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFT/FTIR)

For Fourier Transform Infrared Spectroscopy (FTIR) by diffuse reflectance (DRIFT), samples of different organic carbon fractions (POM and MAOM) were ground in a grinder for analysis. FTIR is widely used in soil science for its speed and low overall expense in characterizing the composition (Baumann et al., 2016) and reactivity of organic matter fractions. This technique enables the identification of reactive groups, such as COOH (carboxyl), OH (phenolic), NH_2 (amines), C=O (carbonyl), and aromatic rings (Stevenson, 1994). These functional groups are indicative of the presence of aromatic and aliphatic structures, as well as polysaccharides, esters, and nitrogenous compounds (He x et al., 2017).

Samples were analyzed with a Cary 630 FTIR spectrometer (Agilent®) to identify functional groups (e.g., carboxylic acids, amines, amides, and hydroxyl groups) across a wavenumber range of 4000 to 500 cm^{-1}, using a resolution of 4 cm^{-1} (Costa et al., 2016). Reflectance data were used to process the spectra, and Principal component analysis (PCA) was used to check the degree of dissimilarity between the three areas studied.

5.6 Statistical Analysis

A statistical analysis was conducted to compare soil samples from three types of vegetation (hypersaline tidal flat, restored mangrove, and pristine mangrove). The average values for each variable were evaluated using ANOVA and subjected to Tukey’s test. The areas and the five sampling depths were also compared with the same test at a 0.05 probability level. All statistical operations were performed using AgroEstat software version 1.1.0.712, and graphs were generated with SigmaPlot version 14.5. FTIR graphs were created using R Studio version 4.4.0 (Antoine Stevens, 2024), applying Savitzky-Golay smoothing. Principal component analysis (PCA) was used to assess relationships among soil functional groups in the three study areas.

6 RESULTS AND DISCUSSION

6.1 Characterization of study areas

Considering the grain size composition, no significant differences ($p = 0.05$) were recorded for the grain size fractions among studied sites and depths (Figure 2). Sand was the most important fraction for all the samples, with averages ranging from 87.0±10.7, 87.3±5.3 and 82.4±4.8% for HTF, RM and MM, respectively. This predominance of sand suggests a coarse-textured sediment profile, characteristic of high-energy depositional environments, where finer particles (silt - clay) are often removed by hydrodynamic processes such as wave action, tidal currents or wind transport (Yuan et al., 2020). The mineralogical composition of the clay fraction is largely dominated by kaolinite (>80%), with minor contributions from illite, 2:1 clay mineral, and small amounts of iron and aluminum oxides (Costa et al., 2020). Aeolian sand dunes surrounding the sampling sites (Figure 1) also influences grain size composition of the studied soils. This coarse-textured profile aligns with observations from studies in similar environments, where sediment dynamics are governed by energy regimes that favor the accumulation of larger particles due to their higher settling velocity (Blott & Pye 2001, 2012). The silt-clay content, although insignificant, could indicate less energetic conditions or the influence of flocculation processes, particularly in areas where fine particles are transported in suspension and settle under more stable conditions (Yuan et al., 2020).

When compared the soil bulk density, higher bulk density was recorded for the HTF (average: 1.42±0.29 g cm^{-3}), followed by the RM site (average: 1.24±0.22g cm^{-3}) and MM (average: 0.85±0.20 g cm$^{-3;}$ figure 3). Soil density varied distinctly among these areas due to the specific physical-chemical characteristics and composition of each site. As a consequence of greater organic matter input and lower organic matter decomposition there is an accumulation of low-density components reducing soil bulk density (Angst et al., 2021; Lavallee et al., 2018, 2020).

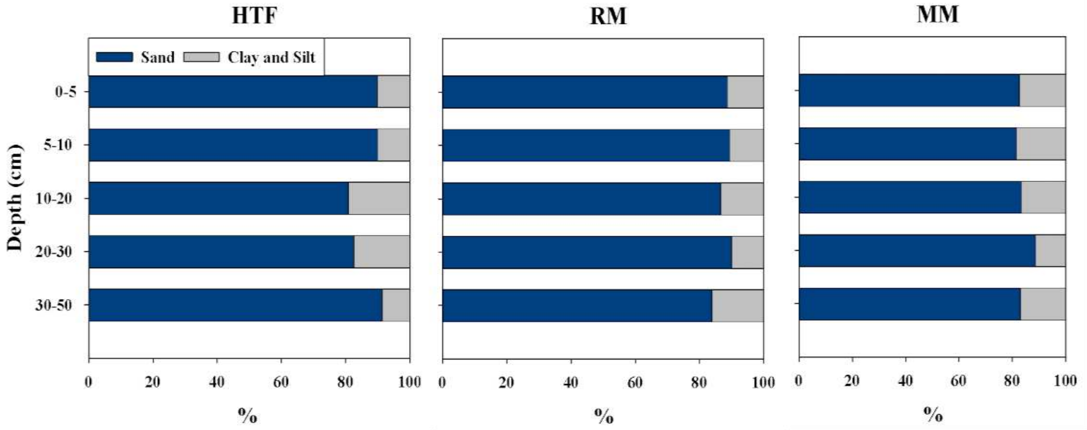

Figure 2. Sand, Silt and Clay contents for the three areas (hypersaline tidal flat - HTF, Restored mangrove - RM, and mature mangrove - MM) and depths studied, showing no statistically significant difference.

Regarding the physico-chemical conditions, the results indicate the influence of regenerating vegetation on soil physico-chemical properties, in particular the redox potential (Eh) and pH, with significant difference ($p < 0.0001$) between the three studied areas. The Eh is typically controlled by the tidal flooding, oxygen availability, organic matter (OM) content, salinity, iron oxides content, coastal morphology, and microtopography (Ferreira et al., 2007; Nóbrega et al., 2021; Andreote et al., 2012). When compared the studied sites, the hypersaline tidal flat (HTF) exhibited the highest redox potential, indicating a sub-oxic condition, with an average of +236±14 mV (Figure 3), followed by the Restored mangrove (RM) (average: +150±31 mV) and Mature Mangrove (MM; average: +25±38 mV), indication an anoxic condition (Otero et al., 2017). These conditions reflect the oxygen availability, influenced by physiographic position, and the OM present in these areas (Albuquerque et al., 2014; Ferreira et al., 2024). For example, whereas in the HTF the higher physiographic position favors soil drainage increasing the redox potential (Sartor et al., 2018), in the MM the prolonged flooding and the higher organic matter content rapidly consumes the available oxygen leading to anaerobic decomposition pathways (e.g., iron and sulfate reduction) with lower energy yield with lower redox potential (Nóbrega et al., 2016). In all three areas, the Eh values did not differ significantly between soil depths.

As for the pH values, the HTF exhibited the highest pH value, with an average of 7.24±0.14, In contrast, the RM and MM had similar pH values (6.76±0.10 and 6.75±0.21, respectively). No significant differences in pH were observed when comparing the soil layers in any of the three areas, suggesting that pH remained relatively consistent throughout the depths. The higher alkalinity observed in the HTF, compared to the other two areas, is attributed to the precipitation of carbonates occurring in this environment, which is due to the high content of exchangeable sodium (Na) (Albuquerque et al., 2014; Marques, 2010). The cation exchange capacity (CEC) values measured at pH 7.0 and ~5.6, attributable to soil organic matter (SOM), are 408.6 and 148.7 cmolc kg^{-1} of carbon, respectively. SOM contributes between 32% and 84% of the total CEC (with an average of 52%). In contrast, the CEC values of the inorganic fraction are significantly lower, with values of 2.32 and 0.78 cmolc kg^{-1} of minerals, respectively. The CEC of the clay fraction increases with total organic carbon (TOC), but decreases exponentially with increasing clay content. Furthermore, total carbon content shows a linear increase in relation to the C/N ratio (Costa et al., 2020).

When compared Fe forms, in order to improve the comprehension of predominant metabolic pathways, no significant differences were assessed between reactive Fe forms (average: 303.3±156.8, 299.1±135.9 and 386.1±194.7 μmol g^{-1} for HTF, RM and MM respectively (Figure 4). On the other hand, significant differences were observed between pyritic Fe forms, with lowest values recorded for the HTF (average:0.1±0.3 μmol g^{-1}), followed by RM (2.1±3.1 μmol g^{-1}) then MM (26.6±30 μmol g^{-1}; Figure 4). Thus, as the mangrove is restored, there is an increase in the degree of pyritization (DOP), with averages ranging from 0.0±0.1% at HTF, 1.1±1.6% at RM and 6.8±6.9% at MM (Figure 4). As vegetation develops and, presumably, there is a higher input of organic matter into the soil, anaerobic pathways (mostly Fe and sulfate reduction) predominate increasing DOP (Ferreira et al., 2024; Jimenez et al., 2022). The recovery of pyritization plays a key role in immobilizing contaminants in mangrove soils. Pyrite is an important trace metal-binding phase within mangrove soils; therefore, its presence is directly associated with the regulating pollutant removal ecosystem service (Ferreira et al., 2024).

For the study site, even 7 years after restoration practices, the restored mangroves are not able to completely perform the ecological services (Ferreira et al., 2024) since DOP and pyritic Fe are statistically different between sites and thus, indicating that mangroves restoration takes more than 7 years. In fact, after 7 years of restoration, only 17% of the DOP was recovered and, considering a linear increase in DOP, it would take 39,9 years for a complete restoration of the pyritization process and the ecosystems services related to pyritization. This reflects the complexity of biogeochemical processes in mangrove soils, which depend on a delicate balance between vegetation growth, organic matter inputs and microbial activity. The slow recovery can also be attributed to external factors such as hydrological changes, climatic variability or anthropogenic disturbance, which can impede a faster re-establishment of optimal conditions for mangrove functioning (Jimenez et al., 2022).

Furthermore, the slow reintegration of key biogeochemical processes, such as sulfate reduction and pyrite formation, highlights the need for long-term monitoring of restoration efforts, which will enable us to assess not only short-term changes, but also the long-term trajectory of ecosystems being restored. Complementary approaches, such as the controlled introduction of organic matter, fertilization, or the enhancement of hydrological connectivity, could accelerate the restoration process by stimulating microbial activity and creating favorable reducing conditions (Macera et al., 2024). Therefore, understanding these SOM fractions in mangrove settings can be important to determine SOC storage longevity, SOC dynamics under different environmental conditions, and responses of SOC to climate change (Iroshaka et al., 2025). This has led to the need to assess soil organic matter fractionation schemes to separate particulate organic matter (POM) and mineral-associated organic matter (MAOM) (Lavallee et al.,2018, 2020; Poeplau et al., 2018). Mangrove conservation and restoration still requires considerable effort and investment, to maintain the low rates of net loss in mangrove carbon stocks, and perhaps increase those stocks through restoration. The high densities of carbon present in mangroves provide good carbon returns per unit area conserved (Richards et al., 2020).

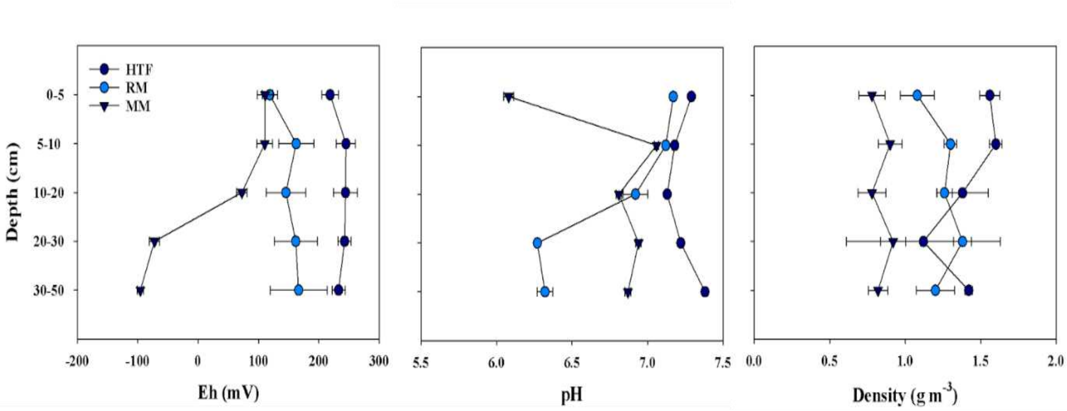

Figure 3. Physicochemical characterization of redox potential Eh and pH of the sampling areas during the wet season and variation in Soil Density Across the Three Study Areas (HTF, RM and MM), Showing distinct patterns among them.

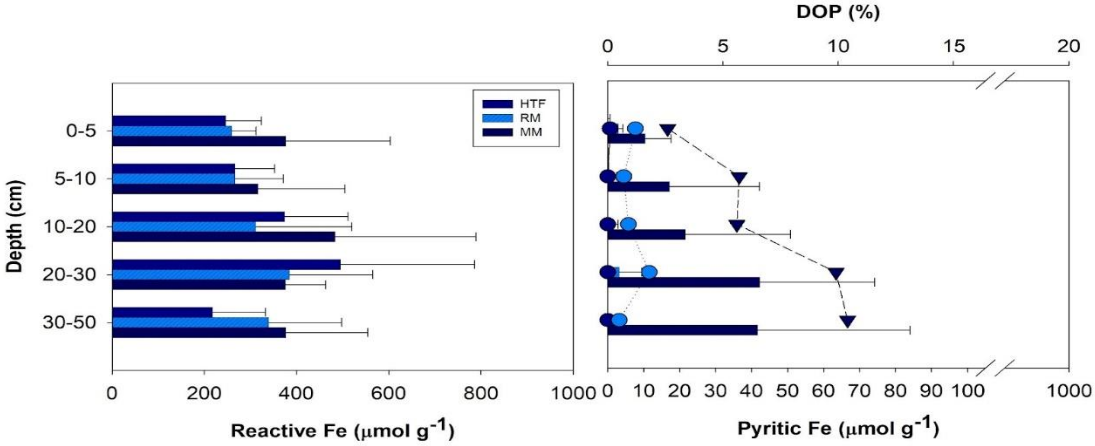

Figure 4. Solid-phase Fe fractionation, defining 2 fractions (reactive and pyritic Fe) and the degree of pyritization (DOP), according to Berner et al. (1970)**.**

6.2. Total Organic Carbon (TOC), Total Nitrogen (TN)

When comparing the Total Organic Carbon (TOC) and total nitrogen (TN) contents, there were significant differences ($p < 0.0001$) between the studied areas. The MM presented the highest average (1.76±0.32% C and 0.10±0.01% N), compared to RM (average: 0.40±0.10% C and 0.04±0.01% N) and HTF (0.18±0.1% C and 0.02±0.01% N) confirming the greater ability of preserved mangroves to store large amount of carbon and nitrogen (Figure 5) due to anoxic conditions and the occurrence of favorable conditions for organic matter stabilization and preservation (Nóbrega et al., 2019; Ruiz et al., 2024). As for the depths, there were no significant differences ($p = 0.05$), with averages varying between 1.16%, at a depth of 0-5 cm and 0.58% at 30-50 cm, showing that the impact of the area on carbon accumulation does also vary with depth. On the other hand, TN contents presented a significant difference between depths (Figure 5) showed that the 0–5 cm layer had the highest average (0.08%), while the 30–50 cm depth had the lowest (0.04%). Depth effects were significant ($p < 0.0001$), suggesting that soil depth may influence nitrogen availability, possibly due to variations in N absorption and retention into organic forms. This results also indicate differences for the C:N ratio among sites, where MM presented the higher ratio (average: 16.2±3.3), which was significantly higher than those recorded for the RM (average: 7.8±3.0) and HTF (average: 6.0±2.5). Determining the concentration of C in relation to N to obtain the C/N ratio is the main method for assessing the origin of organic matter in an ecosystem (Tang et al., 2023). A high C/N ratio, greater than 15, characterizes terrestrial organic matter, given that terrestrial vegetation is mostly composed of lignin and cellulose, which are poor in nitrogen (Leng and Lewis, 2017). Thus, the difference in C:N ratio among sites can also be observed when plotting the correlation among TOC vs. TN (Fig. 6) for each site. The lower TOC:TN ratio for the HTF and RM indicates a terrigenous/riverine source, whereas the TOC:TN ratio ranging between 15 and 20 indicates a mangrove source of the OM (Carneiro et al., 2021). Organic matter of allochthonous origin, on the other hand, is characterized by low C/N ratios (generally less than 10) and is mainly produced by local algae, which have more nitrogen than terrestrial plants because they contain a higher concentration of proteins and nucleic acids (Leng and Lewis, 2017).

There is often a positive correlation between TOC and TN, as both are closely associated with organic matter (Saavedra-Hortua et al., 2023). In this study, areas with higher TOC levels, such MM also presented higher TN levels. This relationship suggests that factors which enchance carbon accumulation under anoxic conditions also promote nitrogen retention. Carbon sequestration is greater in mangrove soils than in salt marshes due to reduced water flux through plant roots and shoots, increasing particle trapping capacity, and nitrogen retention is an important coastal ecosystem service that reduces the negative effects of high nitrogen concentrations in coastal waters and the ocean (Saavedra-Hortua et al., 2023; Santos et al., 2019). Mangroves are capable of retaining more nitrogen and for longer time periods than salt marshes, and appear to have higher nitrogen contents in soils (12.2-15.4 Mg N ha^{-1}) due to a bigger and more robust plant structure (Saavedra-Hortua et al., 2023; Reis et al., 2017).

Comparing 0-50 cm SOC stocks, significant differences were observed ($P < 0.0001$) among the three study areas, with the highest values found in the MM area (6.59±0.35 kg m^{-2}), followed by RM (average: 1.95±0.73 kg m^{-2}) and HTF (average: 0.95±0.14 kg m^{-2}; Figure 7). This same pattern occurred when accounted the equivalent soil C stocks, using the MM bulk density as a reference, with lowest equivalent stocks recorded for the HTF (average: 0.67±0.07 kg m^{-2}), which were significantly different from those recorded for RM (average: 1.47±0.76 kg m^{-2}) and MM (used as reference). Considering the initial 7 years of restoration, we can calculate that the restoration practice resulted in a sequestration rate of 0.52±0.38 kg CO_2 m^{-2} yr^{-1} when considering only the soil C stocks at the 0-50 cm depth. Also, assuming a linear increase in the C stocks following mangrove restoration, it would also be necessary approximately 40 years for a complete C stock restoration. However, the time length for C stock restoration may be shorter, considering that as forest biomass increases, there is presumably higher organic matter input into the soil accelerating C stocks recovery. Thus, it is crucial long term ecological study to improve the quantification of the sequestration rate.

This sequestration rate is within the range of global average (ranging from 0.50 to 0.70 kg CO_2 m^{-2} yr^{-1}; Wang et al., 2021 and Breithaupt and Steinmuller, 2022), but half of the national average (1.0 kg CO_2 m^{-2} yr^{-1}; Rovai et al., 2022). However, due to limited data availability, the Brazilian average was calculated without any data from the semiarid coastal region, where mangrove biomass is considerably lower than those recorded for the humid coast (Kauffman et al., 2018a, b). Another aspect that could contribute with these differences is related to the time-frame assessed, since the national average was obtained using 210Pb and 137Cs geochronometers obtaining hundred years records (Passos et al., 2023; Rovai et al., 2022), whereas in our study only accounted 7 years. Thus, this study contributes to improving the precision of national estimates, covering poorly studied areas with specific climatic and soils conditions (Ferreira et al., 2022).

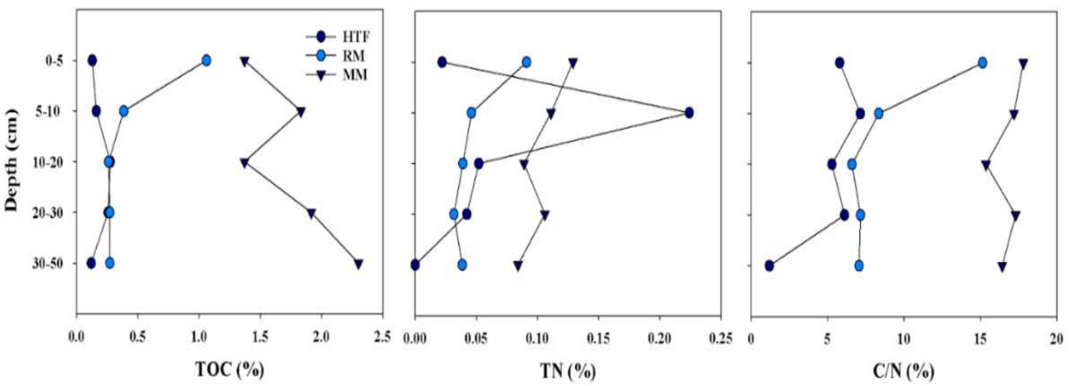

Figure 5. TOC, TN and C/N levels for the three studied mangrove areas (HTP, RM, and Mangrove), showing statistically significant differences.

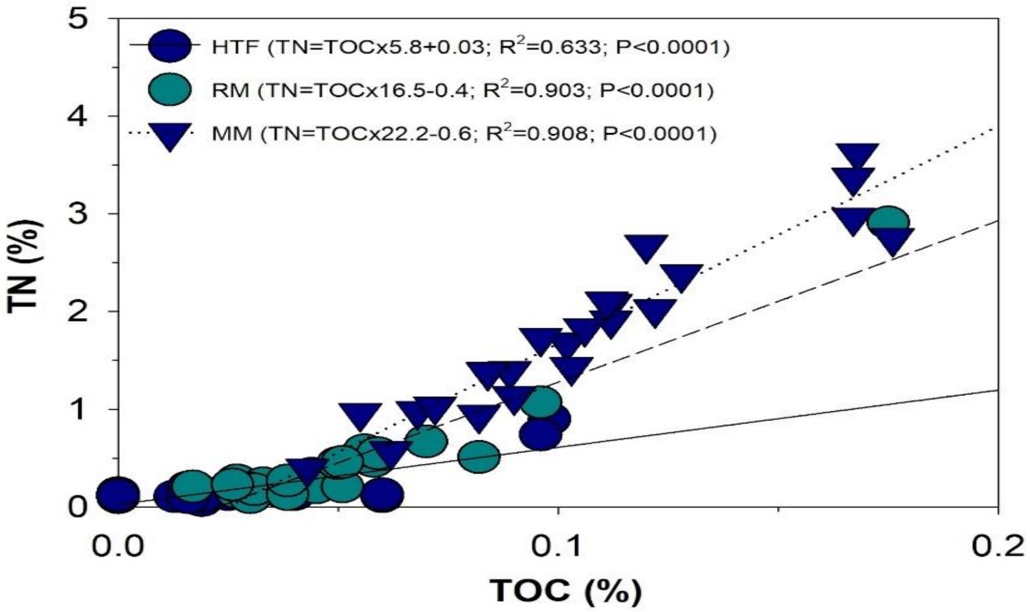

Figure 6. Correlation between TOC and TN for the three studied areas, with different angular coefficient for each site indicating different organic matter sources (Carneiro et al., 2021).

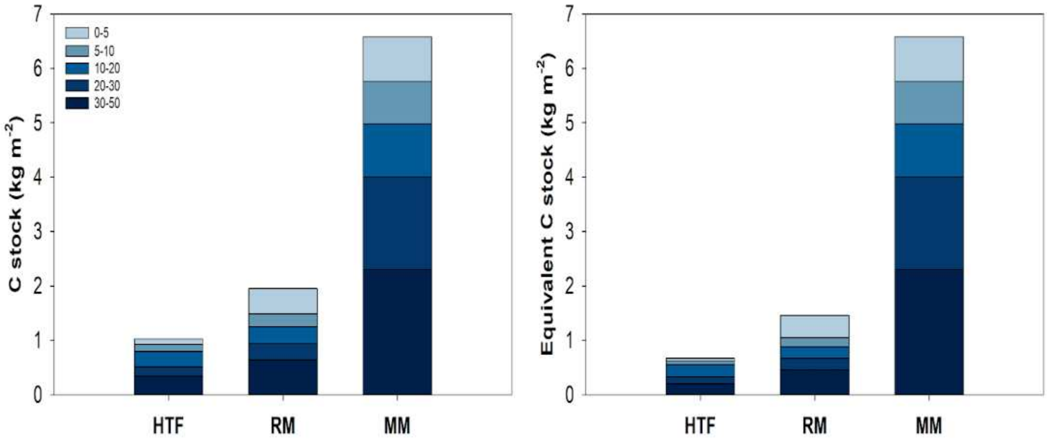

Figure 7. Soil Carbon Stock (SCS) and Equivalent Soil C stock (ESCS) for the three studied areas (HTF, RM and MM) exhibited no significant differences between the HTF and RM areas.

6.3. Physical fractionation of organic matter: Particulate Organic Matter (POM), and Mineral-Associated Organic Matter (MAOM)

When considered OM fraction for the three conditions, higher C and N contents were recorded for the MAOM (average: 2.12±1.90 and 0.13±0.09% for C_{MAOM} and N_{MAOM}, respectively) when compared to POM (average: 0.39±0.60 and 0.02±0.03% for C_{POM} and N_{POM}, respectively). When comparing the different conditions for each fraction, MM samples presented the highest C_{MAOM} (average: 4.01±2.04%; $p < 0.0001$), then RM (average: 1.55±1.03%) and HTF (average: 0.81±0.34%). This same pattern was observed for the N_{MAOM} (average: 0.19±0.11, 0.15±0.07 and 0.07±0.03 % for MM, RM and HTF, respectively).

The results also showed that the MM presented the highest particulate carbon (C_{POM} average: 1.04±0.68%) and nitrogen contents (N_{POM} average: 0.05±0.02%; Figure 8), while RM and HTF showed significantly lower C_{POM} values ($p < 0.0001$), with averages ranging from 0.08±0.03% and 0.07±0.02%, whereas N_{POM} contents were zero for both sites. Additionally, C and N contents for both fractions at the different depths showed no significant differences. Besides lower C content, C_{POM} represents 53.5±8.4% of the TOC at MM and 42.0±15.1% at HTF, whereas at the RM it represents 30.9±14.1%. For N, the particulate fraction represents 57.9±15.3% at the MM, whereas all N in the RM and HTF were associated with minerals (e.g., N_{MOAM}). In this sense, the coarse grain size composition, with sand content higher than 85%, may hinder the accumulation of mineral-associated organic matter (MAOC), which consists of smaller, more decomposed organic molecules that are tightly bound to soil minerals. This fraction is more stable, with a lifespan ranging from centuries to millennia, as the mineral association offers protection against microbial degradation (Ruiz et al., 2024; Lehmann et al., 2020).

When considered the changes during mangrove restoration, considering a linear trend, a lower increase rate was observed for C_{POM} (0.014 % yr^{-1}) compared to C_{MOAM} (0.11 % yr^{-1}), which implies that would take almost 700 years for the full restoration of C_{POM}, whereas C_{MOAM} would be restored within 31 years. On the other hand, N_{MOAM} would be restored within 11 years. The distribution of total nitrogen in marsh soils is reflected in the C:N ratio, which is lowest in the MAOM and highest in the POM (Mirabito et al., 2023). This is consistent with recent frameworks stating that microbial by-products (in vivo) and soluble organic matter (ex vivo) are the main precursors of MAOM (Mirabito et al., 2023; Cotrufo and Lavallee, 2022; Liang et al., 2017).

The functioning of SOM is critical to its role in providing ecosystem services, as POM and MAOM tend to function very differently. Although SOM performs many functions in soil, organo-mineral associations have long been considered an important mechanism for stabilizing and preserving CO in soils and sediments (Kleber et al., 2021; Lavallee et al., 2020). This has led to the development of soil organic matter fractionation schemes to separate particulate organic matter (POM) and mineral-associated organic matter (MAOM) (Cambardella and Elliott, 1992; Lavallee et al., 2020; Poeplau et al., 2018). The large, low-density, unprotected organic debris is operationally defined as POM, while the small, heavy organic matter associated with reactive mineral surfaces is defined as MAOM. Within the soil, a large proportion of particulate organic carbon is of plant origin, and is largely composed of recalcitrant complex organic molecules (e.g., lignocellulose) (Lavallee et al., 2020, 2018).

In contrast, mineral-associated organic carbon is thought to come from microbe-derived CO (also known as the in vivo pathway) and/or labile CO derived from plants, such as rhizodeposits, (also known as the ex vivo pathway) (Cotrufo et ai., 2015; Lavallee et al., 2020; Sokol et al., 2019; Villarino et al., 2021). One of the most important groups of soil minerals that can stabilize CO is reactive iron (FeR). FeR has been operationally defined as sodium dithionite-reducible (oxy) iron hydroxides (Assavapanuvat et al., 2024; Lalonde et al., 2012), which differs from the classical definition of reactive iron. Reactive iron differs from the classical definition of reactive iron in marine sediments because a fraction of the iron reacts easily with sulfide to form various iron sulfide minerals. The formation of iron-associated reactive CO (FeR-CMAOM) is an important mechanism for CO stabilization in terrestrial, coastal and marine environments (Chen et al., 2024; Li et al., 2023; Ye et al., 2022).

Due to differences in their origins and composition, the stability of POM depends on their intrinsic chemical recalcitrance and potential for microbial enzyme inhibition under oxygen-depleted redox conditions (Lavallee et al., 2020), while the persistence of MAOM is regulated by the chemical stabilization of soil minerals (Lavallee et al., 2020; Sokol et al., 2022). For example, FeR-CMAOM is generally stabilized under oxic conditions, but destabilized under anoxic conditions, due to different solubility properties of reduced and oxidized iron (Chen et al., 2020; Dong et al., 2024; Kida and Fujitake, 2020). In coastal wetland soils, previous studies have estimated a wide range of POM, MAOM and FeR-CMAOM fractions. This large variation may depend on different extraction methods (e.g. particle size versus density), different plant productivity across latitudinal gradients (Fu et al., 2024), site-specific hydrodynamic factors and plant species-specific influences.The enrichment of recalcitrant organic compounds (e.g. lignin and tannins) in R. mangle mangrove detritus can promote the accumulation of CPOM (Assavapanuvat et al., 2024; Friesen et al., 2018).

Comparing POM, MAOM compounds tend to be more nutrient dense, have lower decomposition activation energies and require less depolymerisation before microbial or plant uptake (Yu et al., 2022; 2025; Lavallee et al, 2020). Yet, despite the availability of oxygen to fuel aerobic respiration, organic carbon persists over million-year timescales. Identifying the controls on organic carbon preservation requires an improved understanding of the composition and distribution of organic carbon within deep marine sediment (Estes et al., 2019). Thus, these ecosystems effectively reduce atmospheric carbon dioxide concentrations and contribute to climate change mitigation (Ahmed et al., 2017; Kauffman et al., 2014; Rosentreter et al., 2018). The soil organic matter (OM) pool is a mixture of simple compounds that have different residence times due to physical interactions (i.e., with the mineral matrix) that can inhibit OM decomposition and its controls, or chemical protection against decomposition, and more complex compounds with intrinsically low reactivity that require high activation energies for decomposition (Santos et al., 2021; Abrar et al., 2020; Shah et al., 2021). Accordingly, soil OM exhibits a spectrum of susceptibility to microbial attack. Soils with high root litter content tend to contain compounds with high resistance to decomposition (Santos et al., 2021), while leaf, flower and fruit litter produce rich carbon composts but are susceptible to microbial decomposition (Santos et al., 2021; Friesen et al., 2018).

The anaerobic decomposition of organic matter (OM) in soils, particularly in mangroves, is mainly driven by the activity of microorganisms, contributing to its preservation and accumulation in sediments (Santos et al., 2021). Bacteria and fungi, which make up around 90% of the microbial population, play a central role in this process. They facilitate the decomposition of OM through various mechanisms such as nitrogen fixation, sulfate reduction, methanogenesis and enzyme production (Santos et al., 2021). These decomposition pathways are strongly influenced by the nature of the organic compounds present in the substrate, water availability, and the presence of various electron acceptors such as (NO_3^-, Fe^{3+}, $SO4^{2-}$). These electron acceptors often limit the complete mineralization of OM, favoring its retention in soils (Luo et al., 2019; Schmidt et al., 2011). The degree of OM stability in mangrove soils depends on both autochthonous and allochthonous sources. Indigenous sources include different mangrove species and the specific composition of their tissues, which influence OM quality and stability (Huang et al., 2019; Wang et al., 2021). In addition, allochthonous OM, consisting of free organic particles or particles associated with minerals, is transported and deposited in mangrove soils by tides and rivers. The particulate fraction of organic matter (POM) is a dynamic component of OM, composed of non-stabilized organic fragments, often derived from plant litter and residues. This fraction is particularly important in the early stages of soil restoration, as it provides immediately available nutrients for microorganisms and plants (Mirabito et al., 2023). However, POM is less stable and more susceptible to rapid decomposition. In contrast, mineral-associated organic matter (MAOM) represents the stabilized fraction of OM, where organic compounds are closely associated with mineral soil particles. This fraction is essential for long-term carbon storage, as it is more resistant to decomposition. It also contributes to soil structural stability, water retention and long-term nutrient availability (Mirabito et al., 2023).

The faster accumulation of C_{MAOM} and N_{MAOM} compared to C_{POM} indicates the importance of these fractions for carbon sequestration. In fact, organic matter associated with minerals is often associated with higher recalcitrance, in addition to being chemically more favorable to decomposition (Assavapanuvat et al., 2024). In this sense, further studies are required to improve understanding of SOM formation in mangrove soils in order to develop management strategies and practices aimed at enhancing carbon sequestration.

To maximize organic matter (OM) retention in mangrove soils, it is essential to promote rapid recovery of the particulate OM fraction (POM) promoting natural organic inputs, such as mangrove reforestation. In addition, it is crucial to stimulate the stabilization of mineral-associated organic matter (MAOM) through processes that enhance the interactions between organic compounds and soil minerals, notably through erosion reduction and controlled sedimentation management.

Our results clearly indicate that mangrove degradation is determined to cause a collapse in the capacity of these ecosystems to produce OM. In the hypersaline tidal flat (HTF) area, characterized by high salinity and limited vegetation, the retention of organic matter fractions (POM) is influenced by the absence of organic inputs and stabilizing mechanisms (Ferreira et al. 2022b). In this area, the predominant SOM is particulate organic matter (POM), which consists mainly of plant fragments transported by tidal movements (Lavallee et al., 2020). Due to reduced biological activity and the absence of stabilizing interactions with minerals, mineral-associated organic matter (MAOM) is less abundant (Cotrufo and Lavallee, 2022). During mangrove restoration, POM regeneration occurs more slowly, as it originates mainly from plant residues rich in lignin, a complex substance that is resistant to decomposition. This residue offers a carbon source that is more difficult for microorganisms to mineralize, resulting in a reduced decomposition rate and less nutrient availability for the ecosystem. In contrast to MAOM, which is stabilized through interaction with reactive minerals and plays a crucial role in long-term carbon sequestration due to its stronger physical protection and greater durability (Song et al., 2025; Cotrufo et al., 2022).

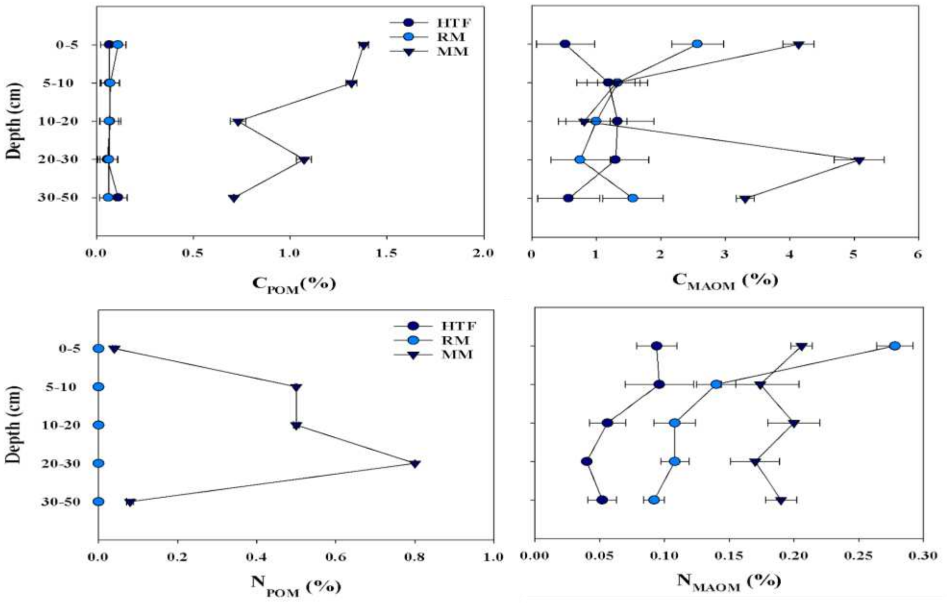

Figure 8. Particulate carbon (CPOM and CMAOM) and nitrogen (NPOM and NMOAM) in the three zones studied.

6.4. Stable isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in particulate organic matter (POM) and mineral-associated organic matter (MAOM)

The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic compositions of particulate organic matter (POM) in the three study areas (HTF, RM, MM) varied between -27 and -8‰ for $\delta^{13}C$, and -0.8 to 0.2‰ for $\delta^{15}N$, with averages of -23.16‰ and -0.20‰, respectively (Fig.

9). The average $\delta^{13}C$ and $\delta^{15}N$ values for the three areas (HTF, RM, MM) were as follows: $\delta^{13}C$ values of -19±4.29‰, -23±3.16‰, and -26±1.12‰, and $\delta^{15}N$ values of -0.5±0.41‰, 0±0.56‰, and -0.1±0.25‰, respectively, with a significant difference ($p < 0.05$) observed in the MM area for carbon ($\delta^{13}C$) compared to the other two areas. Regarding depth, no significant differences were observed, with averages of -24±1.50 ‰, -25±1.27‰, -22±3.12‰, and -19±0.05‰ for $\delta^{13}C$, and -0.1±0.29‰, -0.0±0.26‰, -0.4±0.26‰, and -0.3±0.24‰ for $\delta^{15}N$, at the depths of 0-5 cm, 5-10 cm, 10-20 cm, and 30-50 cm, respectively.

Regarding MAOM, the isotopic compositions varied between -26‰ and -18‰ for $\delta^{13}C$ and -1‰ and 2‰ for $\delta^{15}N$, with averages of -23.41‰ and 0.80‰, respectively (Fig. 9) considering the studied sites (HTF, RM, and MM). There were significant differences ($p < 0.0001$ for $\delta^{13}C$ and $p < 0.05$ for $\delta^{15}N$) between the studied areas. The MM presented the lowest average (-26.10±0.37‰ $\delta^{13}C$), compared to RM (average: -23.88±0.65‰ $\delta^{13}C$) and HTF (-20.27±0.70‰ $\delta^{13}C$) and highest average (1.89±0.55‰ $\delta^{15}N$), being significantly different from RM (-0.35±0.71$\delta^{15}N$), but statistically similar to HTF (0.87± 1.43 $\delta^{15}N$). No significant differences were found in $\delta^{13}C$ and $\delta^{15}N$ values across different depths (Fig. 9).

The terrestrial plants use atmospheric CO_2, preferably ^{12}C, resulting in ^{13}C depleted organic matter. Around 90% of plants are of the C3 type, and have $\delta^{13}C$ between -32‰ and -21‰; C4 plants have $\delta^{13}C$ between -17‰ and -9‰ (Leng and Lewis, 2017). Aquatic plants, in turn, can use both bicarbonate (HCO_3^-) and dissolved CO_2, which has a lower $\delta^{13}C$ value. As the ratio between CO_2 and HCO_3^- is a function of pH, where lower pH favors the formation of CO_2, there is a lower concentration of CO_2 in marine environments than in freshwater. Thus, freshwater algae of the C3 type tend to have lower $\delta^{13}C$ (-26 ‰ to -30 ‰) than marine algae (-16 to -23‰; Leng and Lewis, 2017).

Comparing the organic matter fractions analyzed (POM and MAOM), significant variations in isotopic $\delta^{13}C$ and $\delta^{15}N$ values are observed in the three areas studied. In the MM area, the more negative average $\delta^{13}C$ values (-26.78‰ for POM and -26.10‰ for MAOM) indicate a greater contribution of organic matter from C3 plants, which are characteristic of forested and flooded ecosystems (Carneiro et al., 2021). In general, terrestrial organic matter, when compared to marine organic matter, has lower values of $\delta^{13}C$ and $\delta^{15}N$ (Carneiro et al., 2021; Li et al., 2016). The $\delta^{15}N$ values depend on the characteristics of the local nitrogen cycle. Variations in the rate of nitrogen fixation of the atmosphere, mineralization, nitrification and denitrification determine the nitrogen isotopic fractionation (Carneiro et al., 2021; Spanó et al., 2014).

The higher average value of δ¹⁵N observed in the MM area may indicate a higher rate of microbial degradation of organic matter and/or the influence of nitrogen sources enriched in ^{15}N. This enrichment occurs because, during the mineralization and denitrification processes, microorganisms preferentially use the lighter isotope (^{14}N), leaving the residual fraction more enriched in ^{15}N Li et al., 2022; Liu et al., 2021a, b; Collins et al., 2019). The negative $\delta^{15}N$ values observed in HTF and RM suggest a strong influence of biological nitrogen fixation (BNF) as a dominant nitrogen source in these areas. BNF introduces new nitrogen into the ecosystem, and since atmospheric N_2 has an isotopic signature of ~0‰, the nitrogen incorporated into organic matter generally exhibits $\delta^{15}N$ values close to or slightly below 0‰ (ranging from -2‰ to 0‰) (Liu et al., 2022). This predominance is probably due to limited external nitrogen inputs, anoxic sedimentary conditions and a microbial community well adapted to nitrogen depletion. In contrast, the MM may receive greater nitrogen inputs from external sources, resulting in distinct $\delta^{15}N$ signatures.

The results reveal a strong correlation positive between $\delta^{13}C$, $\delta^{15}N$, and the $\delta^{13}C/\delta^{15}N$ ratio, with both elements closely linked to organic matter and displaying similar R-regression values (*R*= 0.842 and 0.98), with the exception of MM showing a higher value in POM fraction R=0.88 (Figure 10).

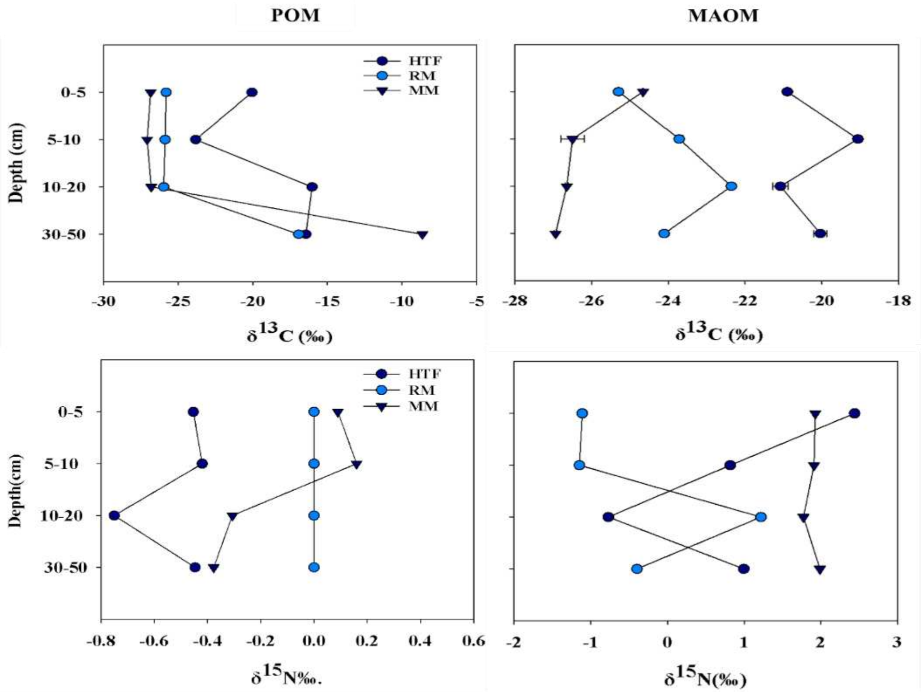

Figure 9. The average values of δ^{13} Cand $\delta^{15}N$ in POM and MAOM, showing statistically significant differences between the three areas studied (HTF, RM and MM).

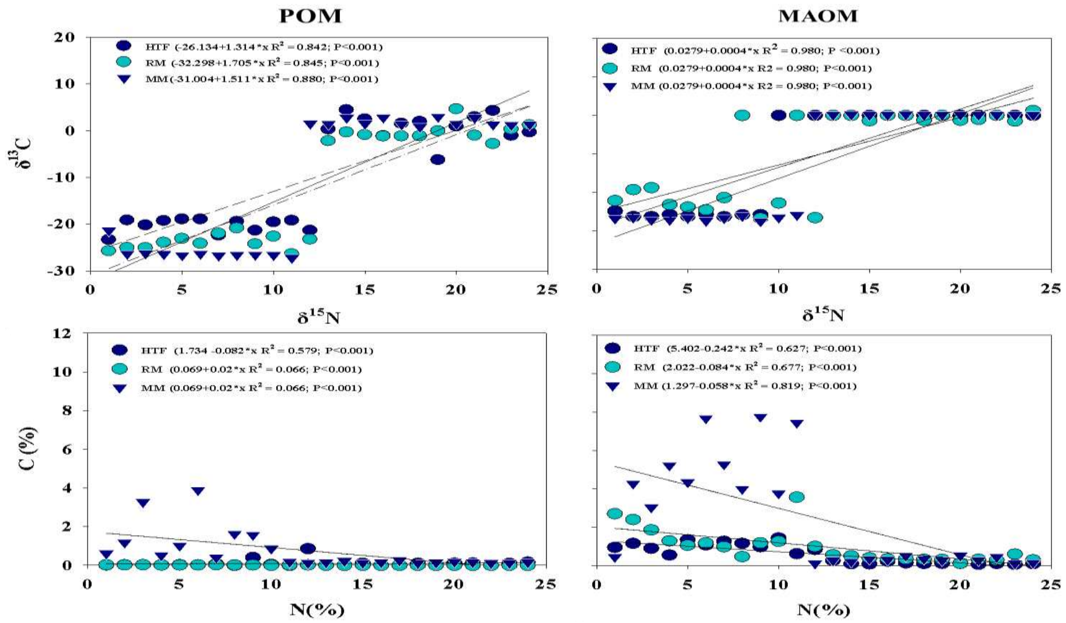

Figure 10. Correlation between ($\delta^{13}C_{POM}$, $\delta^{15}N_{POM}$), ($\delta^{13}C_{MAOM}$, $\delta^{15}N_{MAOM}$) and $\delta^{13}C/\delta^{15}N$ ratio for the three studied areas, with different angular coefficient $P < 0.001$; for each site indicating different organic matter sources (Carneiro et al., 2023; Li et al 2022).

Table 2: Isotopic comparison between the Particulate Organic Matter (POM) and Mineral-Associated Organic Matter (MAOM) fractions under MM, RM and HTF conditions.

PARAMETER	POM	MAOM
$\delta^{13}C$	Presence of C3 (flooded forests), C4 plants or freshwater algae.	Predominantly C3 plant origin/more stable.
Origin	Plant residues (fragments)	Microbial by-products and root-derived soluble compounds
Stability	Low susceptibility to decomposition	Organic matter protected by mineral association
Chemical composition	Rich in carbohydrates and lipids; low degree of humification	Rich in aromatic compounds; highly humified
Ecological importance	Immediate nutrient source during ecosystem restoration	Long-term carbon and nitrogen storage

6.5. Characterization of soil organic matter using Fourier Transform Infrared Spectroscopy (FTIR) by diffuse reflectance (DRIFT).

The analysis of soil organic matter (SOM) composition across the three studied areas (HTF, RM, and MM) revealed distinct patterns in the FTIR-DRIFT spectra (Figure 11), indicating the presence of various functional groups associated with the POM and MAOM fractions. The characteristic bands reflect the diversity of organic compounds in the samples, with similarities observed between areas and depths. This observation is in accord with previous studies, which have shown that POM has a similar molecular composition to MAOM under a range of vegetative, soil and environmental conditions (Dhillon et al., 2017). The FTIR spectra of POM showed the same pattern within the three study areas (Figure 11). In the region between 3700-3500 cm^{-1}, stretching vibrations of hydroxyl groups (O-H), present in alcohols, phenols, and water molecules, were identified. This result showed a similarity between the three areas studied (HTF, RM and MM), likely due to the common presence of hydroxylated organic compounds, derived from shared organic inputs or similar environmental conditions (Dhillon et al., 2017; Baumann et al., 2016).

The bands located at 3500 to 2500 cm^{-1} are often attributed to the stretching of C-H bonds in aliphatic groups, indicating the presence of saturated hydrocarbons, which are probably due to the decomposition of plant organic matter, root exudation or microbial activity in the soil, which facilitate their production and accumulation (Li et al., 2023; Silva et al., 2022). In the HTF and RM areas, a similar pattern was observed, with higher reflectance in this spectral region. This suggests a relatively high concentration of aliphatic compounds, probably due to a constant supply of organic matter and partially advanced transformation processes (Silva et al., 2022). Soil-organic interactions in these areas are influenced by specific environmental conditions, such as moisture and nutrient availability, which favor the accumulation and persistence of saturated hydrocarbons (Dhillon et al., 2017; Li et al., 2023). In contrast, a significant difference was noted in the MM area, which showed greater absorption in this spectral region indicating a higher concentration of saturated hydrocarbons, probably due to more advanced degradation or stabilization processes within the particulate organic matter fraction, reflecting the long-term retention of aliphatic compounds in mature mangrove soils (Li et al., 2023; Baumann et al., 2016). Regarding the depths studied, the 0-5 cm, 5-10 cm and 10-20 cm layers show similar characteristics between the HTF and RM areas. In comparison, the MM area shows significant differences over the entire depth studied (0-50 cm), reflecting the dynamics of stabilization and long-term storage in the deeper layers (Ruiz et al., 2024).

In the 2000-1500 cm^{-1} range, C=O bond stretching vibrations are mainly attributed to carbonyl, carboxyl (-COOH) and amide (-CONH$_2$) groups, commonly found in various organic compounds. These vibrations result from the degradation and transformation of organic matter in soils and sediments, and provide valuable information on the chemical composition of soil organic matter (SOM) in different ecosystems. In the areas studied (HTF, RM and MM), these vibrations showed similar patterns across the five depths (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, and 30-50 cm), suggesting a consistent presence of these functional groups in organic matter across different soil layers and ecosystems (Margenot et al., 2017; Bartos et al, 2020; Sandra P. et al., 2022). The presence of these functional groups reflects ongoing processes of microbial degradation and transformation of particulate organic matter (POM), mainly derived from plant and root inputs (Margenot et al., 2017; Bartos et al., 2020). The carbonyl (C=O), carboxyl (-COOH), and amide (-CONH$_2$) groups play a key role in the degradation of POM (Carvalho et al., 2020). For instance, carboxylic acids are frequently formed during the breakdown of complex compounds such as lignin and cellulose in plant debris (Estes et al., 2019), while amides and carbonyls are associated with microbial and plant residues (Margenot et al., 2015). This chemical composition indicates that POM, although a transient fraction of organic matter, actively participates in biological transformation processes, thus contributing to the overall carbon dynamics in soils (Ruiz et al., 2024; Bartos et al., 2020; Sandra P. et al., 2022).

The region between 1100-500 cm^{-1}, often referred to as the soil organic matter (SOM) 'fingerprint' region, is a key spectral range that reflects a variety of functional groups commonly found in organic matter (Weng et al., 2022). This region showed no significant differences between the three areas (HTF, RM, MM) and five depths (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm and 30-50 cm) studied. It includes absorption bands associated with various molecular vibrations characteristic of organic matter composition, such as those of polysaccharides and aliphatic compounds (Yu W et al., 2022; Margenot et al., 2015, 2016). These functional groups are essential to the structural composition of organic matter, and their presence in sediment samples from different areas and depths suggests similar chemical characteristics in all the regions studied, indicating that organic carbon sources and microbial degradation processes affecting organic matter composition are likely to be comparable in these environments (Yu et al., 2022; Ruiz et al., 2024; Carvalho et al., 2020).

Furthermore, the absence of significant differences between regions and depths indicates that SOM composition remains relatively stable and uniform with regard to the main organic functional groups. This stability could reflect long-term processes such as microbial decomposition, which leads to the transformation and retention of organic matter that has been subjected to similar environmental conditions, such as nutrient availability, moisture content and microbial activity (Li et al., 2023; Weng et al., 2022). The consistency of these chemical patterns across depths and areas highlights the persistence of these functional groups in soil organic carbon reservoirs, which plays an important role in understanding the stability and cycling of organic carbon in different ecosystems (Ruiz et al., 2024; Bartos et al., 2020).

Regarding MAOM, there are two general pathways for the formation of MAOM: adsorption reactions and coprecipitation by iron and aluminum (Nguyen et al., 2019; Kleber et al., 2015). In adsorption reactions, the SOM interacts with an existing mineral substrate forming bonds of varying strength. Adsorption is sensitive to a variety of different environmental factors such as pH, temperature, and SOM chemistry (Li Q et al., 2023; Kleber et al., 2015). In the coprecipitation pathway, iron or aluminum cations hydrolyze and interact with organic molecules to form metal-organic complexes. The nucleation and crystal growth of these complexes in addition to the adsorption of SOM leads to the formation of MAOM (Nguyen et al., 2019; Kleber et al., 2015). Iron (Fe) has a significant association with a large part of the associated mineral organic matter (MAOM) through adsorption processes, forming highly stable organic-mineral complexes. These complexes help to increase the stability of organic carbon (OC) and are considered one of the main forms of carbon storage in the soil, helping to prevent quick decomposition by microorganisms. The bond between organic compounds and iron oxides occurs mainly through ligand exchange, where functional groups such as hydroxyl (OH) and carboxyl (COOH) present in organic matter (Qi Li et al., 2023; Chen et al., 2020). Analysis of FTIR spectra has revealed that certain functional groups present in organic matter, such as carboxylic acids and aromatic compounds, have a stronger interaction with iron oxides. For example, polysaccharides show a greater affinity for iron oxides, while compounds such as lignin show a less intense interaction (Han et al., 2019).

When comparing the HTF, RM and MM areas, spectral analysis revealed that the 3700 cm-^{1} region is common to all, indicating the presence of hydroxyl groups in both particulate organic carbon (POM) and mineral-associated carbon (MAOM). The bands around 1500 cm-^{1} and 500 cm-^{1} of the MAOM are associated with aromatic groups, in interaction with clay minerals and other iron/aluminum minerals, showing similarities between the three areas (HTF, RM, MM) and the five depths (0-5; 5-10; 10-20; 20-30; 30-50 cm), with variations in the intensity of these bands. The presence of these minerals in the soil matrix facilitates their incorporation, which results in greater stability of the organic matter (Ruiz et al., 2023).

In this study, the predominant functional groups in the soil organic matter (SOM) fractions analyzed (POM) and (MAOM), mainly include hydroxyl (O-H), aliphatic (C-H), and aromatic (C=C) groups. Each of these groups reflects important aspects of the processes of decomposition, transformation and stabilization of organic matter in the soil. In this context, an increase in reactive minerals and metals may increase the accumulation of carbon C in MAOM compared to POM. During the decomposition of organic matter, residual carbon typically suffers greater oxidation, resulting in fewer aliphatic bonds and making it more likely to bind to minerals via aromatic or carbonyl groups (Yu et al., 2022; Ruiz et al., 2023). Consequently, we expect higher levels of reactive minerals to facilitate stronger bonds between aliphatic and carbonyl groups during the transition from POM to MAOM. Moreover, we hypothesize that an increase in reactive minerals and metals could promote the adsorption of aromatic compounds derived from soluble plant litter, likely leading to a higher aromatic/carbonyl ratio in MAOM, which reflects the balance between different functional groups in the organic matter. The carbonyl group is generally associated with more decomposed or oxidized organic matter and stronger interactions between aromatic and carbonyl structures in both POM and MAOM fractions (Yu et al., 2022).

To better understand the composition of the organic matter in the different samples, a Principal Component Analysis (PCA) was carried out, considering the fractions of Particulate Organic Matter (POM) and Organic Matter Associated with Mineral (MAOM) in the three areas studied. The PCA is a statistical technique used to reduce the dimensionality of data while preserving as much variability (information) as possible. It transforms a set of correlated variables into a smaller number of uncorrelated variables called principal components. The analysis revealed significant differences in the composition of soil organic matter (SOM) between the samples, showing variations not only between the POM and MAOM fractions, but also between the areas analyzed and the different depths (Fig. 12). The PCA results are presented by means of loadings and scores.

The loadings correspond to the values of the wavelengths, indicating the importance of different wavelengths in the main components, while the scores represent the values of the samples in the space of the main components, visualizing the relationship of each sample with these components. The principal component analysis showed that the first two components together explain approximately 98.7% of the total variation in the POM data, with 95.6% attributed to component 1 and 3.1% to component 2. For the MAOM data, component 1 accounts for 93% of the variation, while component 2 explains 5.7%. Therefore, retaining only two components is considered sufficient, as they capture nearly all of the data variability. This decision is further supported by the graphical representation shown in Figure 12. For both fractions, POM and MAOM, significant differences were observed (Figure 12).

For the POM fraction, the first principal component (PC1) showed the highest contributions in the 3000-2500 cm-¹ range. The second principal component (PC2) showed the highest contributions in the 4000-3500 cm-¹ and 2000-1500 cm-¹ ranges. In contrast, the MAOM fraction showed higher contributions around 4000 cm-¹ for PC1 and PC2, as well as in the 3500-2000 cm-¹ range for PC1 and in the 2000-1500 cm-¹ range for PC2. These observations correspond to the most distant peaks in the PC1 and PC2 analyses, as shown in Figure 12.

When comparing the two fractions (POM and MAOM) (Figure 12), the mature mangrove area (MM) showed the greatest contribution to the POM fraction in the three areas analyzed. The MM also showed a strong negative correlation in dimension 2, positioning itself in the second quadrant, where it stands out distinctly in relation to the depths studied, except for the surface layer (0-5 cm). This fraction showed greater similarity with the HTF and RM areas, at depths of 10-20 cm, 20-30 cm and 30-50 cm, grouped in quadrant 1. In quadrant 3, the MM and RM areas showed similarity with the 0-5 cm depth, with MM showing a negative correlation in dimension 2, with RM showing a positive correlation. In quadrant 4, the RM area, associated with depths of 5-10 cm and 20-30 cm, showed a high contribution. The fraction of organic matter associated with the mineral (MAOM), in quadrant 1, showed a stronger relationship between the MM and RM zones with the depths of 0-5 cm, 10-20 cm, 20-30 cm and 30-50 cm, in addition to the HTF area, which showed a positive relationship in dimension 1. In quadrant 2, the MM area showed a greater contribution at the depths of 5-10 cm and 10-20 cm, while the HTF and RM areas, grouped in quadrants 3 and 4, showed greater contributions. The DRIFT samples showed a similar spectral profile (Figure 12).

However, the MM area exhibited a distinct spectral behavior, with higher absorbance compared to the other two areas, which showed higher reflectance. Higher reflectance in HTF and RM areas suggests relatively high concentrations of aliphatic compounds, while MM showed greater absorption, reflecting higher concentrations of saturated hydrocarbons. Across all depths, similar characteristics were noted between HTF and RM, while MM exhibited significant differences. This indicates a richer composition in specific functional groups and a greater diversity of compounds in the organic matter of the mangrove soil (Ruiz et al., 2023).

The combination of the recalcitrance of POM and the stabilization mechanisms of MAOM plays a crucial role in carbon storage and the overall dynamics of organic matter in mangrove soils, highlighting its importance in the global carbon cycle (Carvalho et al., 2020).

From the analysis of the PCA data, we can see that the composition of soil organic matter varies significantly between the areas and depths studied, with emphasis on the mature mangrove area (MM), which showed a richer and more diverse composition of compounds. The distinct spectral behavior of the MM area, with higher absorbance, indicates a higher concentration of specific functional groups, which may be a reflection of the greater microbial activity and the greater supply of organic matter derived from external sources, such as mangroves and river contributions.

This factor, combined with the greater availability of organic matter, can create anaerobic conditions favorable to the preservation of organic matter at greater depths (McGivern et al., 2024). On the other hand, areas such as HTF and RM, associated with sandy soils, have a lower concentration of organic matter, which is correlated with the lower carbon retention capacity in these environments. In fact, the predominance of sandy soils tends to reduce the capacity to accumulate organic matter due to their low porosity and greater drainage, and factors which favour the mineralization of organic matter (Carvalho et al., 2020). Comparing the POM and MAOM fractions also provides important information on the mechanisms of organic matter stabilization. The MAOM fraction, with its high capacity for interaction with soil minerals, tends to be more stable over time and is associated with more complex processes for incorporating carbon into the soil. This is particularly evident in mangrove areas, where high levels of organic matter and interaction with minerals favor the formation of more stable compounds (Yu et al., 2025).

The study of POM and MAOM fractions in the different areas studied reinforces the importance of mangroves as regulators of carbon dynamics, emphasizing the necessity of preserving these ecosystems to ensure the maintenance of the ecosystem services they provide, especially with regard to carbon storage and climate change mitigation (Yu et al., 2022, 2025). The PCA carried out on the areas relative to the peaks of the organic compounds obtained from the POM clearly differentiated the three samples, indicating that the MM area was associated with a higher concentration of aliphatic compounds, being mainly related to hydrocarbons and polysaccharides. On the other hand, the variation in MAOM was more pronounced for aromatic compounds, such as lignin, and iron-derived compounds.

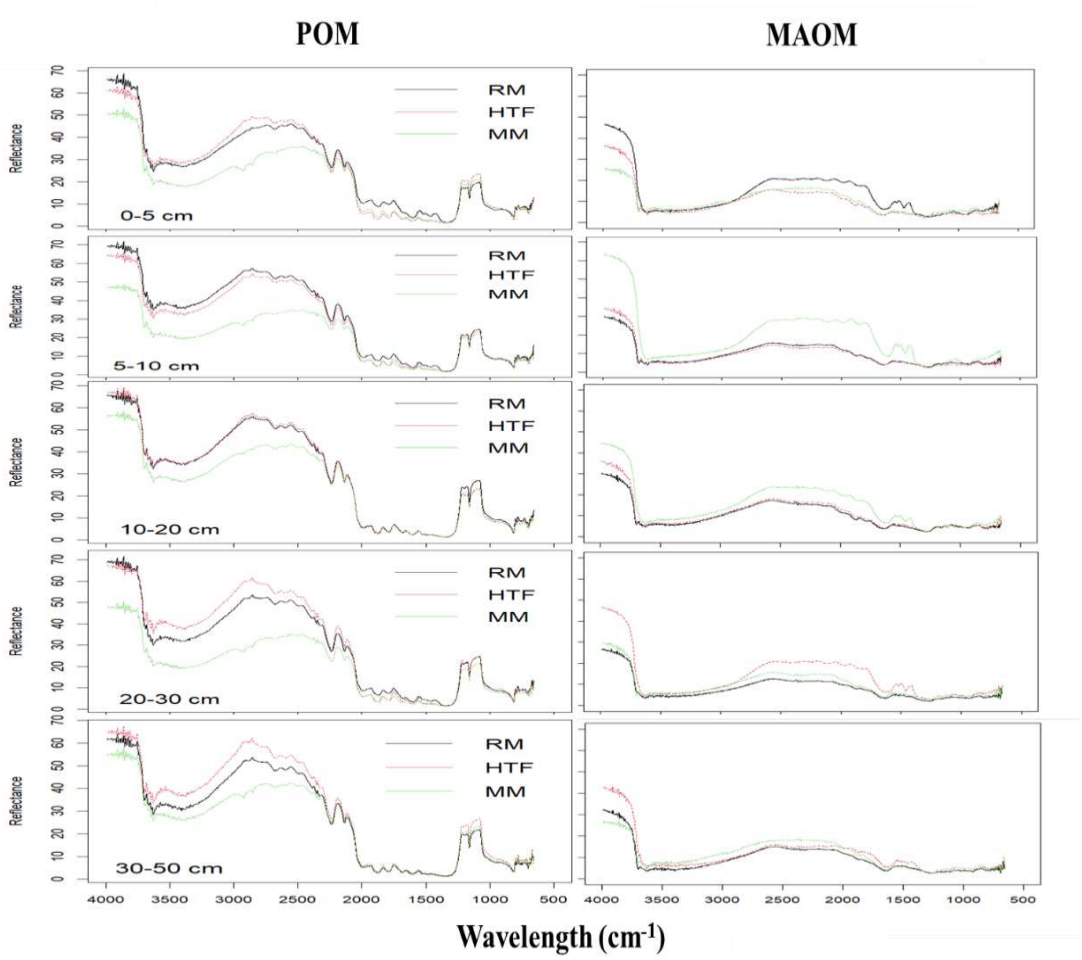

Figure 11. Identification of functional groups present in Particulate Organic Matter (POM) and Mineral-Associated Organic Matter (MAOM) in the three studied areas (HTF, RM, MM) using (FTIR) with (DRIFT), showing significant differences and similarities in the composition of the organic matter, with a predominance of aliphatic groups in the MOP and aromatic groups in the MAOM of the mature mangroves.

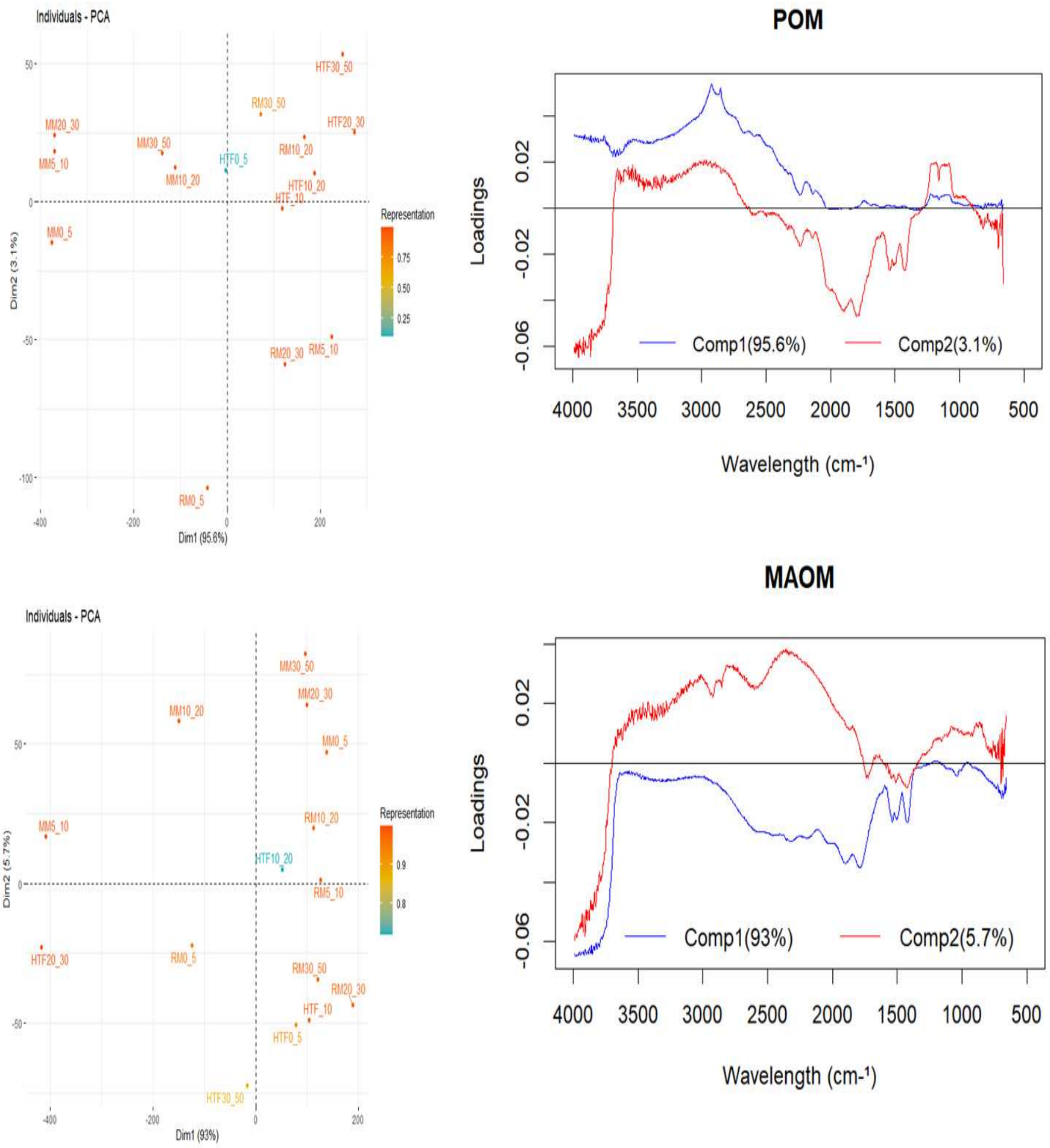

Figure 12. Principal component analysis (PCA) of POM and MAOM and FTIR peaks of the three studied areas (HTF, RM, MM), showing significant contribution and clustering among them.

7 CONCLUSIONS

The Mangrove restoration (RM) is essential for recovering key ecological functions such as carbon sequestration, nutrient cycling, and coastal protection. In high-energy and semi-arid environments, restoring degraded areas improves soil quality by increasing soil organic matter (SOM), enhancing water retention, and reducing erosion. The Mature mangroves (MM) have the highest soil organic carbon (SOC) stocks, followed by restored mangroves (RM) and Hypersaline tidal flats (HTF) (MM>RM>HM). The RM shows significant potential to recover ecosystem services, with an estimated carbon sequestration rate of 0.52 kg CO_2 m^{-2} yr^{-1}. The full SOC recovery may take around 40 years.

As vegetation is restored, there is an accumulation of reduced forms of iron and sulfur, promoting pyrite formation and organic matter stabilization. Restoration also increases total and particulate organic carbon contents, especially aromatic, phenolic, and polysaccharide compounds, which enhance carbon stabilization and soil structure. Isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$) reveal that the origin of organic matter influences carbon and nitrogen cycles. Although it is generally expected that particulate organic matter (POM) recovers faster than mineral-associated organic matter (MAOM), this study shows the opposite: MAOM recovers more rapidly than POM, likely due to differences in organic matter inputs and stabilization mechanisms in restored mangroves.

The distinct characteristics of mature mangroves (MM), restored mangroves (RM), and Hypersaline tidal flats (HTF) highlight their specific roles within the mangrove ecosystem. MM serves as key reservoirs of stable and diverse organic compounds, playing a crucial role in long-term carbon storage. The RM exhibits strong potential to restore lost ecosystem services, demonstrating the success of ecological restoration initiatives. Although HTF contains lower levels of organic matter, they still contribute meaningfully to local biogeochemical processes, emphasizing the ecological interconnection among these systems.

Finally, mangrove restoration is a key strategy to restore ecosystem functionality, mitigate climate change, and protect essential ecosystem services.

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