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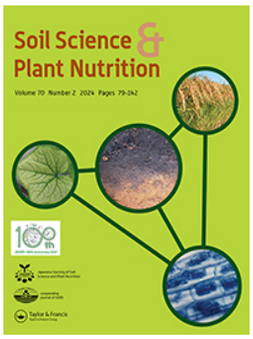

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RESEARCH ARTICLE

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Functional organic matter components in mangrove soils revealed by density fractionation

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ABSTRACT

The mechanisms underlying stabilization of soil organic matter (SOM) in vegetated coastal ecosystems, including mangrove forests, are poorly understood, limiting our ability to predict the consequences of disturbances. Here, we introduce density fractionation to mangrove soils to identify the distribution and properties of the functional components of SOM regarding degradation state, stability, and origin, namely, the free low-density fraction (f-LF), mineral-associated LF (m-LF), and high-density fraction (HF). Three soil cores (1 m) were collected in a mangrove forest on Ishigaki Island, Japan, and cut into 10 cm intervals and analyzed. Although HF was the most abundant, the massive production of mangrove fine roots resulted in a high abundance of LFs throughout the cores, which markedly differed from terrestrial soils. Relative abundance of LFs together accounted for 38–66% of total soil C. The m-LF was as abundant as f-LF and 1.6 times higher in relative abundance than the global average of terrestrial soils. The C/N ratios and $\delta^{13}C$ values clearly increased with depth in all fractions, which was attributed to the increased contribution from roots. We found a consistent pattern in $\Delta^{14}C$ values of density fractions. HF was the oldest with $\Delta^{14}C$ between −149‰ and −97‰ followed by m-LF (between −130‰ and −87‰) and then f-LF (between −89‰ and 78‰), suggesting that mineral association may be pivotal in long-term carbon storage in the mangrove mineral soil. Our analysis successfully identified meaningful functional components of mangrove SOM, yet several questions remained unanswered, including a large variability in $\Delta^{14}C$ in different cores. Future studies would benefit from a coupled analysis of the quantity and quality of density fractions and geochemical factors in mangrove soils.

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Blue carbon; vegetated coastal ecosystem; persistence; physico-chemical protection; radiocarbon

1. Introduction

Mangrove forests exhibit a high capacity for soil organic matter (SOM) storage (Bouillon et al. 2008; Kristensen et al. 2008), with SOM accounting for about 75% of total carbon stocks in mangrove forests (Alongi 2014). The global average carbon stock in the top 1 m of mangrove forest soils is estimated to be 283 ± 193 Mg C ha^{-1} (Atwood et al. 2017). Furthermore, regardless of the soil type (i.e., peaty or mineral), the average carbon stock including deeper soils (~3 m) exceeds 900 Mg C ha^{-1}, making mangrove forests among the most carbon-rich ecosystems in the tropics (Donato et al. 2011; Kida et al. 2021). Vegetated coastal ecosystems, known as 'Blue Carbon' ecosystems, including mangrove forests, are known to accumulate SOM at rates tens of times faster than terrestrial ecosystems (McLeod et al. 2011Alongi 2014)Ongoing climate change and anthropogenic disturbances such as deforestation, land reclamation, urbanization, and land use change pose significant threats to these ecosystems (Adame et al. 2021; Richards and Friess 2016). These disturbances can significantly impact carbon sequestration in coastal ecosystems and existing soil carbon pools. However, the mechanisms of SOM stabilization in these ecosystems are poorly understood, limiting our ability to predict the consequences of disturbances (Kida and Fujitake 2020).

Some stabilization mechanisms must be present for mangrove SOM to remain stable over the long-term. However, research on this topic is still scarce for mangrove soils and Blue Carbon ecosystems in general. While Blue Carbon studies have gathered significant information on the global C stocks and their regional variations in the last decade, a mechanistic understanding of SOM stabilization in these systems has been comparatively much less developed (Kida and Fujitake 2020). Anoxia has been considered a dominant factor in SOM stabilization in these vegetated coastal ecosystems. Certain organic compounds, particularly lignin, are degraded less efficiently in the absence of oxygen, and it is most likely the primary reason behind the millennial-scale accumulation of mangrove peat on oceanic islands (McKee, Cahoon, and Feller 2007). However, evidence is accumulating that SOM in mineral soils of

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vegetated coastal ecosystems consists of a myriad of different organic compounds (Dodla, Wang, and Cook 2012; Kida, Kondo, et al. 2019; Santín et al. 2008; Zhang et al. 2016). Previous studies have shown that labile organic components including many small organic compounds, tissues of micro autotrophs such as algae and phytoplankton, and fresh plant litter degrade at similar rates regardless of the presence or absence of oxygen (Lee 1992). Therefore, factors contributing to the stabilization of these otherwise labile compounds in mangrove mineral soils are of particular interest.

Several common mechanisms underlying SOM stabilization have been identified in terrestrial soils and marine sediments. Broadly, these include: (1) recalcitrance of organic matter due to its chemical structural properties, (2) physical protection (inaccessibility) of organic matter from microbial degradation within aggregates and pore spaces, and (3) chemical interactions with soil minerals and metals (Sollins, Homann, and Caldwell 1996). The physical and chemical stabilization of SOM reduces its availability to microorganisms and enzymes (Lützow et al. 2006; Marschner et al. 2008). Vegetated coastal soils, located in the transitional zone between terrestrial and marine environments, may also experience the same mechanisms of SOM stabilization. However, contrasting evidence has been found regarding the potential mechanisms that contribute to SOM stabilization in these ecosystems (Table 1). It is important to investigate whether physical and chemical stabilization of potentially labile organic matter is also present in mangrove mineral soils and coastal sediments, but few related studies have been conducted to date (Dicen et al. 2019; Shields et al. 2016; Zhao et al. 2018).

Density fractionation, which has been used by soil scientists for nearly 50 years, physically fractionates SOM into functional fractions of varying characteristics using a heavy liquid based on particle density (Crow et al. 2007). Chemical and molecular analysis of organic matter after density fractionation allows for the acquisition of higher-resolution data about the spatiotemporal distribution and properties of the functional components of SOM regarding stability and origin. The particle density of freshly incorporated, plant-derived particulate organic matter is less than 1.6 g cm^{-3}, considerably smaller than that of soil minerals (2–4 g cm^{-3}). As a result, density fractionation yields two fractions based on density: the low-density fraction (LF, also known as particulate organic matter) and high-density fraction (HF, also known as mineral-associated organic matter). The interaction between organic matter and minerals increases as the microbial degradation of plant-derived LF progresses. LF can be further divided into free LF (f-LF), a roughly mineral-free fraction consisting mainly of fresh, coarse organic materials such as plant residues, and mineral-associated LF (m-LF) which is LF attached to or embedded in soil minerals or

Table 1. Mechanisms that contribute to stabilization of soil organic matter (SOM) in coastal vegetated soils and evidence for and against each mechanism.

Mechanisms	Evidence	References	Counter-evidence	References
Reducing condition	• Kinetic limitation of lignin degradation ("Enzyme-latch") • Millennial-scale stability of mangrove peat	McKee et al., (2007); Saraswati et al. (2016)	• Variable relationships between saturation conditions and OM decomposition rates • Leaching of water-soluble compounds • Iron-mediated organic matter decomposition • Iron dissolution and decline in physico-chemical protection	Chen et al. (2020); Huxham et al., (2010); Romero et al. (2005)
Recalcitrance of roots	• Relative preservation of roots compared to leaves	Liu et al., (2017); Middleton and McKee (2001)	• Only explains at most ~10s-years persistence	Same references as the left
Physical protection by minerals	• Iron accumulation around root wall • Coaggregation of OM and Fe at the redox interface • Correlation between clay and OM content • Correlation with specific surface area of sediment	Miyajima et al., (2017); Riedel et al., (2013); Yarwood (2018)	• No relationship between clay and OM content	Kida et al., (2021)
Chemical interaction with minerals	• Association between OM and active Fe in coastal sediments via ligand exchange	Dicen et al., (2019); Lalonde et al., (2012); Shields et al., (2016)	• Competition between SO_4^{2-} and OM during ligand exchange • Iron-mediated organic matter decomposition • Iron dissolution and decline in physico-chemical protection	Chen et al. (2020); Jia et al., (2020); Kooner et al. (1995)
Salinity-induced immobilization	• Flocculation of dissolved OM at the estuary • Mobilization of SOM by reduction in salinity	Kida et al. (2017); Sholkovitz (1976)	None reported	
Nutrient limitation	• Enhanced decomposition by nutrient enrichment	Huxham et al., (2010)	Variable effects of nutrient enrichment	Keuskamp et al. (2013); Lovelock et al., (2014)

aggregates, by disruption of soil aggregates and mineral association through mechanical shaking with glass beads or ultrasonication (Wagai, Mayer, and Kitayama 2009). Conceptually, f-LF is labile and fast-cycling due to the lack of protection by minerals, while HF is the most persistent, cycled at centuries to millennium time-scale, due to physico-chemical protection by soil mineral matrix, and m-LF often exhibits properties intermediate between the two (Wagai, Mayer, and Kitayama 2009). Density fractionation thus physically divides bulk SOC into pools directly associated with specific mechanisms and processes that affect its decomposition rate (Heckman et al. 2022). While density fractionation has been used in other coastal ecosystems such as seagrass meadows (Miyajima et al. 2017), it has not yet been applied to mangrove soils. Considering the massive production and turnover of fine roots in mangroves soils and their major contribution to SOM accumulation (Arnaud et al. 2021; Liu, Xiong, and Liao 2017; Muhammad-Nor et al. 2019) and recently proposed possible roles of soil physico-chemical factors in stabilizing SOC in vegetated coastal ecosystems (Dicen et al. 2019; Kida and Fujitake 2020; Shields et al. 2016; Zhao et al. 2018), density fractionation appears particularly useful in studying SOM stabilization mechanisms in mangrove soils.

The aim of this study was to identify the quantitatively important fractions for carbon storage in mangrove soils using density fractionation, to examine the differences in organic matter characteristics by fraction and depth, and to estimate the origin of organic matter in each density fraction. Previous research has shown that approximately 80% of fine root biomass is found within the top 30 cm of terrestrial soils (Hashimoto and Hyakumachi 1998), while in mangrove forests, fine root production is likely much greater and fine roots are widely distributed to deeper depth (Arnaud et al. 2021; Tabuchi 1983). Therefore, we hypothesized that f-LF is more abundant in mangrove soils compared to terrestrial soils. We also hypothesized that HF exhibits more non-mangrove (microbes or marine origin) signatures compared to other LFs because HF can capture other sources in water through organo-mineral interactions (Kida and Fujitake 2020).

2. Materials and methods

2.1. Study area and sampling

Samples were collected in a mangrove forest located along the Fukido River on Ishigaki Island, Okinawa Prefecture, Japan (24° 29′N, 124°13′ E, Figure 1). This forest covers an area of approximately 19 ha around the mouth of the Fukido River (Kida, Tanabe, et al. 2019), and are dominated by two mangrove species, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*. The study area has a subtropical monsoon climate, with an average annual precipitation of 2107 mm and an average annual temperature of 24.3°C from 1981 to 2010 (Ishigaki Island Local Meteorological Observatory, Japan Meteorological Agency). The watershed (approximately 2.4 km^2) receives little human activity, and broadleaf forests occupy about 95% of the area, with the rest of land use being sparse sugar cane and paddy fields. The soil in the catchment is red-yellow soil (Ultisols) with a thin A horizon and low SOM content (Kida, Tanabe, et al. 2019). A previous study has detailed the species composition, biomass, and aboveground net primary productivity of this mangrove forest (Ohtsuka et al. 2019). The Fukido mangrove exhibits a clear semidiurnal tide, with maximum tidal height reaching over 1 m at spring tide (Ohtsuka et al. 2019). At high tide, the area is inundated with seawater, whereas at low tide, the soil surface is exposed to the air. The soil is mineral and tentatively classified as gley soil. The mineral composition within the mangrove forest is spatially relatively constant, with only a minimal contribution from calcite, indicating that the minerals were mainly derived from the catchment (Kinjo et al. 2005).

Soil samples were collected in August 2015 at five points within a permanent quadrat (80 m × 80 m) established at the site in 2014 (Ohtsuka et al. 2019) using an open-face stainless core sampler with minimum compaction (1 m long, 27 cm^2 cross-sectional area; Handy GeoSlicer, Fukken Research and Design Co.) (Figure 1). The cores were cut into 10 cm intervals at the field using a metal spatula and transported to the laboratory under cool, dark conditions. Upon arrival, the cores were immediately air-dried at 60°C until a constant weight was obtained, and bulk density was determined (Donato et al. 2011;

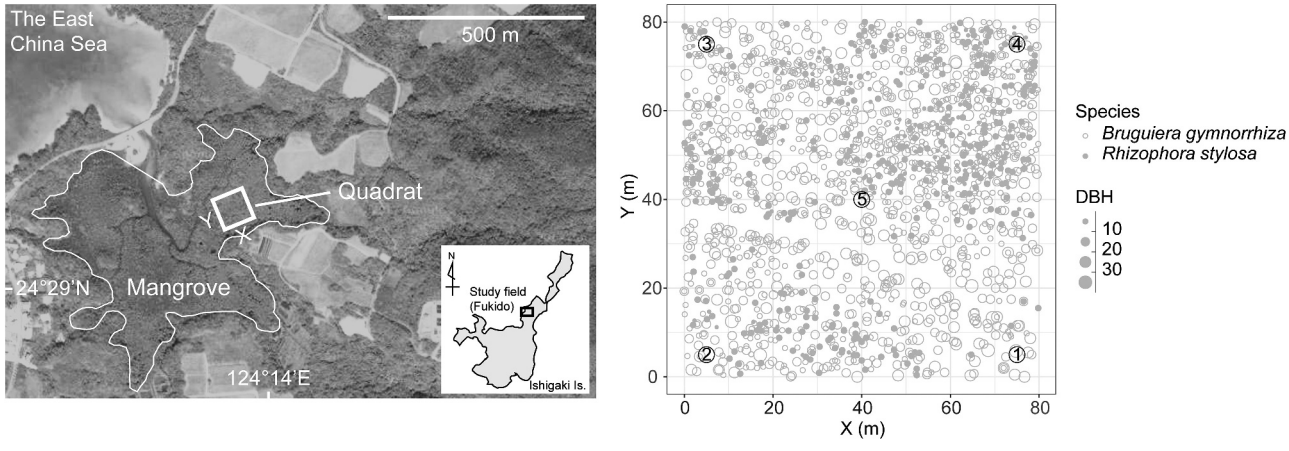

Figure 1. Map of the Fukido River mangrove forest site. The white square in the left panel indicates the 80 m × 80 m permanent quadrat, while the right panel shows the quadrat with sampling points denoted with numbers. DBH = diameter (in cm) at breast height.

Kida, Kondo, et al. 2019). The air-dried soil was then passed through a 2 mm mesh size sieve. No gravel was present in any of the samples. Soil texture is sandy clay loam with relative sand content of 60–70% (Kida, Kondo, et al. 2019). A leaf and fine root of *Bruguiera gymnorrhiza*, which was the dominant species in the quadrat (Ohtsuka et al. 2019), were also collected from each of four different standing trees. These samples were dried at 60°C and finely ground using a white porcelain mortar and pestle.

In this study, soil samples from the cores 2, 3, and 5, the deepest of the five cores, were analyzed (Figure 1). Approximately 5.5 g of the air-dried samples were carefully subsampled using the conical quadrant method to ensure representative subsamples. The samples from a depth of 70–94 cm at the point 3 were treated with 2 M HCl overnight to remove inorganic carbon because some shell fragments were visually observed. The treated sample was carefully collected using a metal spoon and analyzed for density fractionation.

2.2. Density fractionation

Density fractionation was performed using an aqueous solution of sodium polytungstate (SPT-0, TC-Tungsten Compounds; SPT) with a density of 1.6 g cm^{-3} in accordance with previous studies (Golchin et al. 1994; Wagai et al. 2008). Despite the assumption that SPT readily produces insoluble precipitates in the presence of calcium ion and thus cannot be used in Ca-rich soils without prior washing, little literature evidence was found to support this argument. In fact, the original support for this notion appeared to be pers. comm. reported in Six (1999) (Six 1999). We thus first examined whether marine-derived Ca^{2+} in mangrove soil samples interferes with the density fractionation experiment using two types of solutions: (1) a filtrate by 0.45 µm PTFE membrane filter (Omnipore, Merck) of one of the Fukido mangrove soil samples mixed with deionized water with approximately twice the solid-to-liquid ratio as the density fractionation experiment, and (2) a 0.6 M $CaCl_2$ solution. These solutions were each mixed with the same volume of a 3.2 g cm^{-3} SPT solution, making a 1.6 g cm^{-3} SPT solution with Ca^{2+} concentrations representative of the Fukido samples and unlikely high 0.3 M, respectively. In both cases, no precipitates were found after 24 h. With 0.3 M $CaCl_2$, a small amount of white precipitates (presumably Ca-PT) was observed only after 72h. The density of the supernatants remained unchanged during these experiments when there were no precipitates. We thus concluded that a prior desalinating washing step was not necessary for mangrove soils and coastal sediments in general. Omitting the washing step can alleviate the risk of material loss and saves time.

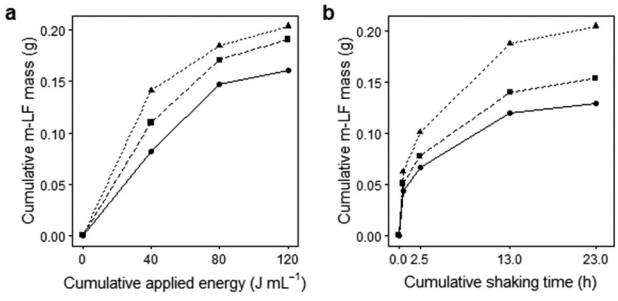

Figure 2. Comparison of mineral-associated low-density fraction (m-LF) recovery by sonication (a) and mechanical shaking (b). Surface (triangle), medium (square), bottom (circle) samples of the core 3 from the fukido mangrove soils used in the study were tested, where the corresponding line types in (a) and (b) represent the identical samples.

In this study, m-LF was collected through mechanical shaking with glass beads (φ6 mm) at 120 rpm, which facilitated the breakdown of aggregates and detachment of minerals attached to plant residues. We optimized the duration of shaking by comparing m-LF recovery with that obtained through sonication (Figure 2). Approximately 90% recovery was achieved through 24 h of shaking compared to sonication with a total energy of 120 J mL^{-1} in ice water, which itself showed maximal recovery of m-LF (Figure 2). The m-LF recovery through shaking also reached a plateau after 24 h, thus this duration was selected for the shaking process.

In density fractionation, 5 g of the soil sample was weighed in a 50 mL conical tube and 20 mL of a SPT solution was added, followed by gentle turning over 20 times. The sample was then centrifuged at 700 G for 5 min, and the suspended materials were collected as f-LF using a poly dropper and metal spoon onto a suction filtration device with a 0.45 µm PTFE membrane filter (Omnipore, Merck). This procedure was performed three times in total. In order to compensate for the SPT solution lost during the f-LF collection, the SPT solution was replenished every procedure. The collected f-LF was washed three times with 5 mL of 1M KCl to prevent possible inorganic nitrogen contamination from the SPT solution (Rota Wagai, pers. comm.), rinsed with deionized water until the electrical conductivity (EC) was less than 50 µS cm^{-1}, and dried at 80°C for 48 hours. Subsequently, glass beads and the SPT solution were added to the remaining soil, and the sample was shaken reciprocally at 120 rpm for 24 hours. The sample was then centrifuged at 8700 G for 10 min, and the material floating (m-LF) was recovered using the same procedure as the f-LF recovery. Finally, the sample residue in the conical tube (HF) was transferred to a 250 mL centrifuge tube with deionized water, centrifuged at 13,000 G for 20 min, and the supernatant was carefully discarded as much as possible using a Komagome pipette. Glass beads were removed with a care not to lose any sample soil after centrifugation. The soil was then treated with 100 mL of 1M KCl (shaken reciprocally for 10 min and centrifuged at 13,000 G for 25 min), washed several times with 100 mL of deionized water until the EC reached less than 50 µS cm^{-1}, and freeze-dried. The weight of each fraction was measured and mass recovery was calculated. Using a stereomicroscopy (×20), we observed the morphology of each density fraction. Photographs of representative samples were taken using density fractionation samples from another nearby mangrove forest on Ishigaki Island (m-LF collected by sonication) and provided in Figure 3d because we couldn't take photographs of Fukido samples. The morphological features of the density fractions from these mangroves were almost identical.

2.3. Elemental analysis

The elemental composition of bulk soil, each density fraction, leaves, and fine roots was determined using an elemental

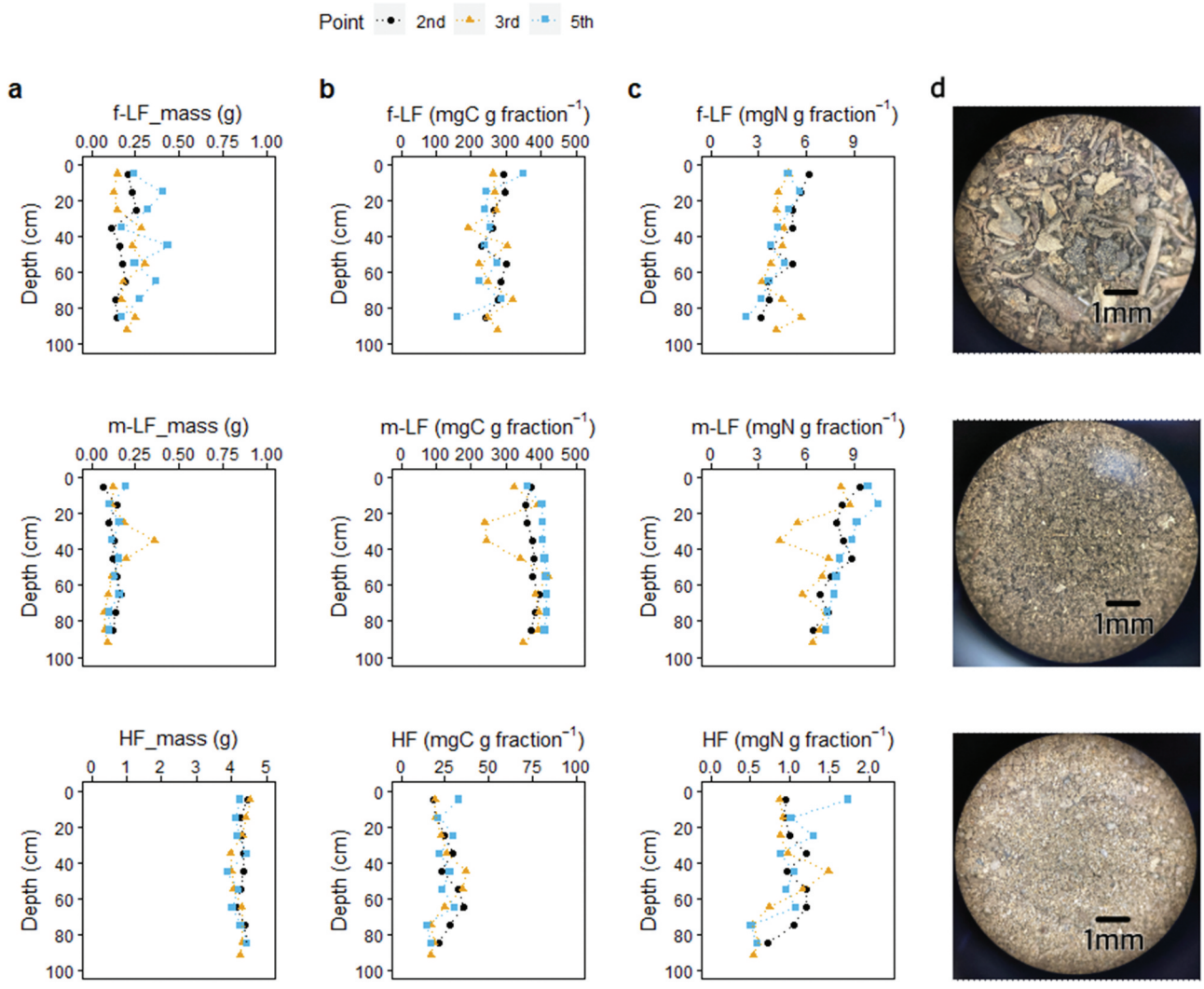

Figure 3. Contribution of each density fraction to total soil mass (partitioned from 5 g) (a) and C and N concentrations of each fraction on fraction basis (b, c) with depth. The stereomicroscopic photographs (×40) of representative samples of each fraction are also provided in (d). Note the different x-scale between fractions. In (a), the x-axis of f-LF and m-LF was magnified by a factor of five compared to that of HF, while in (b) and (c), the x-axis of HF was magnified by a factor of five. f-LF: free low-density fraction, m-LF: mineral-associated low-density fraction, and HF: high-density fraction.

analyzer (PE2400 series II; PerkinElmer). Following grinding and drying at 80°C for 12 h, the soil samples were sealed in tin capsules (Tin Capsule Foil, 8 × 5 mm, Exeter Analytical Inc.). Bulk soil and HF fractions were encapsulated in approximately 15 mg, whereas the organic materials including LF fractions were encapsulated in approximately 2–4 mg. Measurements were performed in duplicate and the average value was used for the results. If the coefficient of variation (CV) between two measurements exceeded 10%, a third measurement was performed and the most outlier was removed. The overall analytical precision for C and N was 1.2% and 2.5% by CV, respectively. From the obtained C and N content, the mass recovery of C and N by density fractionation, the contribution of each density fraction to the bulk C and N content, and the C/N ratio were calculated.

2.4. Stable carbon isotope analysis

In mangrove soils, stable carbon isotopes have been used to identify major carbon sources of SOM, to investigate patterns of organic carbon utilization by microbial and animal communities, and to track organic matter exchange between adjacent ecosystems (Bouillon, Connolly, and ShingY 2008), but never for density fractions. In this study, carbon stable isotope ratios were measured for each density fraction using a continuous flow elemental analyzer/isotope ratio mass spectrometer (EA/IRMS; FLASH EA 1112 series + Thermo Finngan DELTA plus, Thermo Scientific, U.S.A.). The carbon stable isotope ratios were expressed in the common delta (δ) notation as the per mil (‰) difference of the $^{13}C/^{12}C$ ratio in a sample relative to the Vienna Pee Dee Belemnite standard. Peach Leaves ($\delta^{13}C$: −26.06‰ ± 0.05‰, NIST1547, Sigma-Aldrich) and Glycine ($\delta^{13}C$: −32.3‰ ± 0.2‰, Aminostandard, Shoko Science) were used as calibration standards. To check the scale-dependent variation of the $\delta^{13}C$ values, the amounts of the standards were varied from 0.03 to 0.07 mg. The calibration curve using the Peach Leaves and Glycine standards was prepared at approximately 0.07 mg and 0.03 mg, respectively, to correct for the deviation of the measured

values from the true values of the standard samples. A 5-point calibration with the standards was used to calibrate and normalize the measured isotopic ratios to the international scale. Two standards were run for every 20 samples, and 2 blanks and conditioning and calibration standards were included at the beginning and end of each run. Measurement precision and trueness were both within ± 0.14‰ for $\delta^{13}C$ of the laboratory standards.

2.5. Radiocarbon analysis

Radiocarbon analysis of density fractions at the deepest part of each core were performed at Yokoyama Lab, AORI, Japan (Yokoyama et al. 2019). We provided approximately 3 mg C for radiocarbon analysis. All samples were sealed in Ag capsules, combusted into CO_2 gas in an elemental analyzer (Vario Micro Cube, Elementar), and converted to graphite by a custom-built graphitization vacuum line (Yokoyama et al. 2022). After graphitization, the radiocarbon content was measured with a single-stage accelerator mass spectrometer (NEC, U.S.A.). Radiocarbon data are expressed as ^{14}C ages, percent modern carbon (pMC), and $\Delta^{14}C$ which is the fractional deviation, in parts per thousand (‰), of the sample $^{14}C/^{12}C$ ratio relative to that of the oxalic acid international standard (National Institute of Standards and Technology) (Stuiver and Polach 1977). Analytical precision for the $\Delta^{14}C$ analysis was better than 4‰.

3. Results and discussion

3.1. General characteristics and microscopic observations of soil density fractions

The evaluation of material recovery is a crucial initial step in reporting the results of density fractionation. Our density fractionation yielded an average soil mass recovery as high as 92.2% (Table 2). The recoveries based on C and N content were also high, albeit slightly lower than those of the mass recovery (Table 2), which are commonly observed in density fractionation analysis of soils. The slight loss could be due to the loss of dissolved matter and fine colloids during the washing step (Wagai et al. 2008). Additionally, a higher loss of N might have resulted from the loss of inorganic N. Overall, these high material recovery rates assured the validity of our approach.

HF was by far the most dominant fraction in the mangrove soils in terms of mass (Figure 3a). The average mass proportions of f-LF, m-LF, and HF in the bulk soils were 4.9%, 2.9%, and 92.2%, respectively. Yet, the average percentage of the f-LF and m-LF combined (~8%) was higher than that of forest and agricultural soils (Cerli et al. 2012; Crow et al. 2007; Kölbl and Kögel-Knabner 2004; Parker et al. 2002; Swanston et al. 2005; Wagai et al. 2008). The amounts of f-LF did not show a clear change with depth and varied largely (Figure 3a). The m-LF content showed much less variation with depth but had a notably high value at 30–40 cm in core 3, accompanied by reductions in the C and N concentrations (Figure 3b,c). The HF content was almost constant with depth and between cores. According to stereomicroscopic observation, materials recovered as f-LF were mainly coarse plant residues, with relatively intact, undecomposed mangrove fine roots found in shallower (<50 cm) sections while more fragmented fine roots and bark of coarser roots in deeper sections (still, live fine roots were present in the deepest sections) (Figure 3d). Much of the plant residues in f-LF was covered with patches of fine mineral particles. In contrast, m-LF was almost free of mineral particles and consisted of much smaller plant fragments. The use of glass beads and mechanical shaking may have largely broken plant tissues and obscured the morphology. However, m-LF recovered by disruption of aggregates by sonication consisted of similarly small fragments (Figure 3d), thus this fragmented morphology may be a property of m-LF in mangrove soils. As expected, HF contained no recognizable plant tissues and was instead dominated by mineral particles (Figure 3d).

The C and N concentrations of the density fractions (Figure 3b,c) were consistent with the microscopic observations. C concentrations were one-order of magnitude higher in LF than in HF, while N concentrations were several times higher. Among LF, m-LF had almost always higher C and N concentrations compared to f-LF (Figure 3b,c). Nitrogen was relatively more enriched (by ~80%) than C (by ~50%) in m-LF than in f-LF. Higher C and N concentrations in m-LF compared to f-LF have been found in forest and agricultural soils (Wagai et al. 2008 and references there in). Interestingly, only N concentrations on fraction basis showed a clear decline with depth in all fractions (Figure 3c). This decoupling between C and N dynamics suggests selective consumption or leaching of N-rich compounds and/or selective preservation of C-rich compounds.

When evaluating on a bulk soil basis, the C concentrations (mgC g $soil^{-1}$) in all fractions varied widely and did not show a clear depth trend, while the N concentrations decreased with depth, particularly in the m-LF and HF fractions (Figure 4a,b). The depth distributions of LFs were markedly different from those typically observed in terrestrial soils which tend to decrease rapidly with depth (Luo et al. 2020; Parker et al. 2002; Swanston et al. 2005). This is presumably due to the massive production of mangrove fine roots, even in deep soils. Although data on in-situ fine root production in deep mangrove soils (>30 cm) is scarce, Arnaud et al. (2021) recently showed that a major fraction of fine root production occurs deeper than 30 cm. Other recent papers have also reported fine root production down to a depth of 50–60 cm below ground in mangrove forests (Fujimoto et al. 2021; Ono et al. 2022). Our visual inspection also revealed abundant live and dead fine roots in deep (> 50cm) soils. These results collectively support our first hypothesis.

When normalized to the total, f-LF accounted for 14–44% of total soil C and 8–32% of total N, m-LF for 15–36% and 11–23%, and HF for 34–62% and 56–75%, respectively (Figure 4c,d). HF was therefore the most abundant form of

Table 2. Mass recovery rate and weight recovery rate of C and N relative to the mass.

Core	Mass recovery (%)	C recovery (%)	N recovery (%)
2	92.6±1.0	85.2±12.5	81.8±8.1
3	92.4±1.5	89.6±11.9	83.8±6.7
5	91.7±2.0	97.0±8.5	86.6±4.4

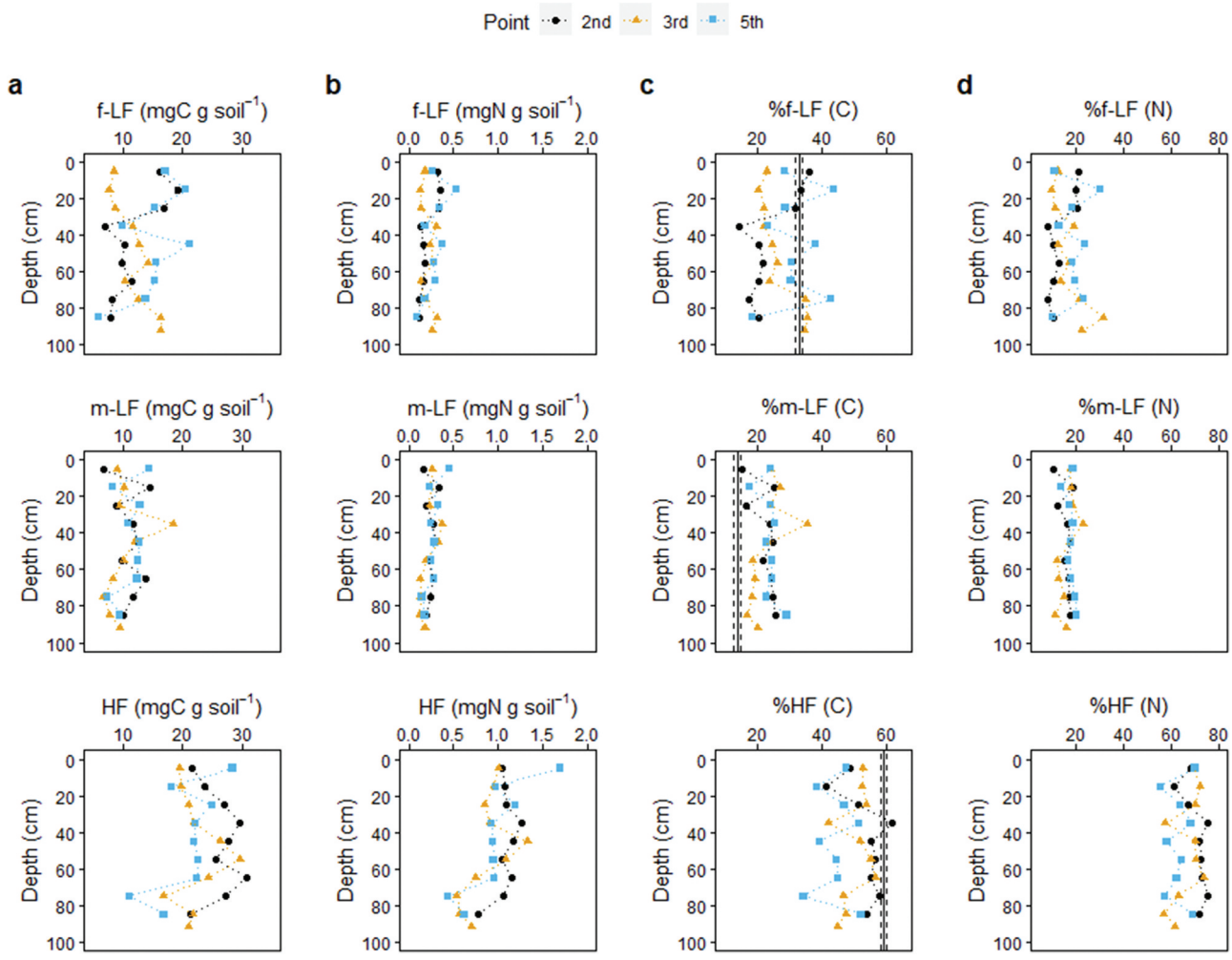

Figure 4. Contribution of each density fraction to total soil C (a, c) and N (b, d) content with depth. Results are presented in C and N concentrations of each fraction on bulk soil basis (a, b) and relative abundance of each fraction in bulk C and N abundance (c, d). The vertical lines in (C) represent means (solid line) and one standard errors (dashed line) of the relative abundance of density fractions in 1222 terrestrial soils reported by Heckman et al. (2022). f-LF: free low-density fraction, m-LF: mineral-associated low-density fraction, and HF: high-density fraction.

SOM in the mangrove soils, and its contribution was more prominent as N. All fractions did not exhibit a clear depth trend, while the variations of %m-LF (CV of 18% and 17% for C and N, respectively) were relatively smaller than that of %f-LF (CV of 28% and 39% for C and N, respectively). A recent comprehensive meta-analysis of data obtained by density fractionation of terrestrial soils (n=1222) (Heckman et al. 2022) reported that the mean contribution of f--LF, m-LF, and HF to the total soil C was 33%, 14%, and 59%, respectively, indicating m-LF is the minimal functional component of SOM in terrestrial soils. Contrarily, our analysis revealed that m-LF was as abundant as f-LF (particularly as N) in the mangrove soils throughout the 1-m cores (Figure 4c,d). The average relative contribution of m-LF carbon in the mangrove soils was 1.6 times higher than that in terrestrial soils (Heckman et al. 2022). In mangrove soils, the longer residence time of plant residues (f-LF, mainly as fine roots) under suboxic conditions may have resulted in greater associations with soil minerals, providing physico-chemical protection for such plant residues from microbial degradation.

3.2. Organic matter early diagenesis inferred from C/N ratios and $\delta^{13}C$ values

The C/N ratios of the density fractions showed clear differences between fractions (Figure 5). The C/N ratios of the f--LF, m-LF, and HF ranged from 43.2 to 78.5, 32.0 to 66.8, and 16.8 to 33.8, respectively (Figure 5a). Those of leaves and fine roots of *Bruguiera gymnorrhiza* ranged from 31.6 to 42.6 (mean ± standard deviation of 36.0 ± 4.9, n = 4) and 47.9 to 57.6 (52.3 ± 4.3, n = 4), respectively. The lower C/N ratio of HF compared to the other low-density fractions is similar to that observed in terrestrial soils, suggesting that microbial degradation of HF was more progressed and that plant-derived components contributed more to the LFs (Liao, Boutton, and Jastrow 2006; Tan et al. 2007). There was no obvious difference in $\delta^{13}C$ values between fractions (overall −29.7 to −27.3‰) (Figure 5b), indicating that the major origin of organic matter differed only little among the fractions. Previous studies have shown that mangrove tissues, microalgae, macroalgae, and seagrasses are important sources of SOM in mangroves (Bouillon, Connolly, and ShingY 2008).

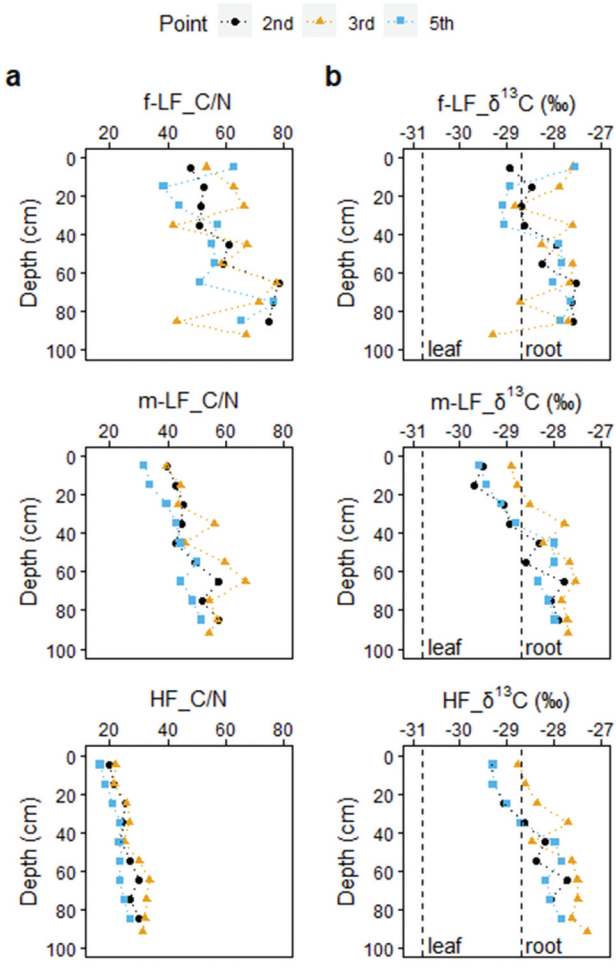

Figure 5. C/N ratio and $\delta^{13}C$ stable isotope ratio of each fraction with depth. f-LF: free low-density fraction, m-LF: mineral-associated low-density fraction, and HF: high-density fraction. The vertical dashed lines in the $\delta^{13}C$ results represent the average $\delta^{13}C$ values for leaf and root of mangroves in the fukido mangrove forest reported by Iimura et al., (2019).

Their average $\delta^{13}C$ values are −28.1‰, −20.2‰, −18.9‰, and −12.1‰, respectively (Bouillon, Connolly, and ShingY 2008), suggesting all fractions were primarily derived from mangroves in the Fukido mangrove forest. The little difference in $\delta^{13}C$ values between density fractions was against commonly observed patterns in terrestrial soils, where organic matter strongly associated with minerals (i.e., HF) is typically enriched in ^{13}C (Sollins et al. 2009), and rejected our second hypothesis.

The C/N ratio clearly increased with depth in all density fractions (Figure 5a). At the same time, the $\delta^{13}C$ values also showed clear increases with depth (Figure 5b). The simultaneous increases in the C/N ratios and $\delta^{13}C$ values with depth were against commonly observed patterns in terrestrial soils and not straightforward to interpret. In terrestrial soils under C_3 plants, C/N ratios generally decrease while $\delta^{13}C$ values increase with depth, resulting in a negative correlation between them (Lorenz et al. 2020; Paul, Balesdent, and Hatté 2020; Sollins et al. 2009; Werth and Kuzyakov 2010). Although inorganic carbon can theoretically raise C/N ratios and $\delta^{13}C$ values, we could rule out a contribution from inorganic carbon because the three samples that were subjected to a HCl treatment (70–94 cm at station 3) showed similar C and N concentrations, C/N ratios, and $\delta^{13}C$ values compared to the rest of untreated samples (Figures 2–4). A HCl test on randomly selected samples with no visible inorganic carbon fragments (such as shells or coral fragments) was indeed negative.

The reasons for the enrichment in ^{13}C with depth are not yet fully understood, but the ^{13}C enrichment can be the result of temporal changes in the initial composition of C or isotopic fractionation associated with post-photosynthesis processes in either plants or soils. We summarized possible processes that can affect C/N ratios and $\delta^{13}C$ values of SOM (Table 3). A range of processes is known to influence both parameters in either direction. Among these, only the increased relative dominance of roots compared to leaves could explain the simultaneous increases in the C/N ratios and $\delta^{13}C$ values with depth, although all processes are not mutually exclusive (Table 3). The proportion of root-derived C inputs is expected to be higher at depth, and roots generally have higher C/N ratios (by ~5–50) and $\delta^{13}C$ values (by ~1–5‰) compared to leaves because of differences in chemical composition and post-photosynthetic allocation mechanisms (Cernusak et al. 2009; Lorenz et al. 2020; Werth and Kuzyakov 2010; Zeh et al. 2020). Our data for *Bruguiera gymnorrhiza* were consistent with this general trend; the C/N ratio of fine roots was higher by approximately 16 than leaves. Furthermore, decomposed mangrove roots can exhibit a high C/N ratio. For instance, after one-year of decomposition, mangrove leaves decreased in C/N ratio (from 32 to 18) while roots considerably increased it (from 36 to 66) in

Table 3. Processes that can change C/N ratios and $\delta^{13}C$ values of soil organic matter under C_3 plants. The direction of change is expressed as downward change in a soil column.

	Downward change in a soil column		
Processes	C/N ratio	$\delta^{13}C$ value	References
Dominance of roots compared to shoots/leaves	increase	increase	Werth and Kuzyakov (2010); Zeh et al., (2020)
Selective preservation of lignin	increase	decrease	Bowling et al., (2008); Kida, Kondo, et al. (2019)
^{13}C kinetic discrimination during microbial utilization	-	variable[†]	Ågren et al. (1996); Torn et al., (2002); Werth and Kuzyakov (2010)
Increased contribution from soil microbial-derived organic matter	decrease	increase	Boström et al., (2007); Werth and Kuzyakov (2010)
Increased contribution from marine organic matter	decrease	increase	Bouillon, Connolly, et al. (2008)
Historical changes in atmospheric ^{13}C abundance (^{13}C-Suess effect)	-	increase	Francey et al. (1999)
Greater isotopic discrimination during photosynthesis under higher CO_2 levels and associated historical changes in vegetation ^{13}C abundance	-	increase	Keeling et al. (2017); Paul et al., (2020)

[†] Microbial utilization generally results in ^{13}C enrichment in residual substrates but see a review by Werth and Kuzyakov (2010).

Table 4. Radiocarbon (± 1σ) of the density fractions of fukido mangrove soil cores. The numbers after 'FUK' represent the soil core numbers and depths of the sections analyzed.

Lab code	Sample name	Depth (cm)	^{14}C age (yr BP)	pMC (%)	Δ^{14}C (‰)
YAUT-081125	FUK_2_90_f-LF	80–90	637 ± 20	92.37 ± 0.23	−84.40 ± 2.23
YAUT-079333	FUK_2_90_m-LF	80–90	662 ± 32	92.09 ± 0.37	−87.04 ± 3.65
YAUT-080338	FUK_2_90_HF	80–90	747 ± 24	91.12 ± 0.27	−96.86 ± 2.68
YAUT-079336	FUK_3_94_f-LF	90–94	modern	108.75 ± 0.41	78.02 ± 4.08
YAUT-079337	FUK_3_94_m-LF	90–94	1051 ± 32	87.74 ± 0.35	−130.23 ± 3.48
YAUT-081124	FUK_3_94_HF	90–94	1222 ± 19	85.89 ± 0.20	−148.66 ± 2.01
YAUT-080339	FUK_5_90_f-LF	80–90	678 ± 20	91.90 ± 0.23	−89.07 ± 2.32
YAUT-079338	FUK_5_90_m-LF	80–90	827 ± 31	90.22 ± 0.35	−105.65 ± 3.47
YAUT-079339	FUK_5_90_HF	80–90	984 ± 29	88.47 ± 0.32	−122.97 ± 3.15

a mangrove forest on Pohnpei Island (Ono et al. 2015). In this regard, the decomposition of roots alone might explain the simultaneous increases in the C/N ratios and δ^{13}C values with depth (Figure 5b) if ^{13}C kinetic discrimination during microbial utilization favors the enrichment of ^{13}C in residual roots (Ågren, Bosatta, and Balesdent 1996; Torn et al. 2002). Previous research has shown that the δ^{13}C values of mangrove leaves and fine roots in the Fukido mangrove were on average −30.9‰ and −28.7‰, respectively (Figure 5b), indicating that roots had a heavier carbon isotope signature than leaves (limura et al. 2019). However, a slight (~1.5‰) but consistent deviation from the simple leaf-to-root mixing model (Figure 5b) suggests an input of ^{13}C-enriched materials, such as soil microbial-derived organic matter and marine organic matter, regardless of depth or fractions (Table 3) (Boström, Comstedt, and Ekblad 2007; Marchand et al. 2005; Werth and Kuzyakov 2010). Nonetheless, the marked difference in the C/N ratios between fractions (Figure 5a) indicates that different density fractions have undergone distinct degradation state and potentially have different ages.

Selective preservation of phenolic compounds reported in this mangrove forest (Kida, Kondo, et al. 2019) could explain the increase in C/N ratios (Figure 5a), but that would in turn lead to depleted δ^{13}C values because lignin typically is depleted in ^{13}C compared to other plant constituents such as proteins and cellulose (Bowling, Pataki, and Randerson 2008). Isotope analysis of plant organic compounds, as well as end-members such as terrestrial, mangrove, and marine sources, could provide further insight into the simultaneous increases in the C/N ratios and δ^{13}C values with depth (Figure 5). Finally, fractionation of dissolved organic carbon (DOC) can also partly account for the depth gradients in C/N ratios and δ^{13}C (Bowling, Pataki, and Randerson 2008; Kaiser et al., 2001). In terrestrial soils, material flow is typically 'top-down' due to major organic matter inputs in surface soils and subsequent translocation down to subsurface soils through water flow (Heckman et al. 2022). During passage through the mineral soil, DOC interacts with the mineral matrix through preferential sorption/desorption of compounds with specific molecular characteristics as in chromatography. These chromatographic behaviors and the decay of labile compounds can alter the molecular signatures of DOM, and in extension, associated bulk SOM because of differences in C/N ratios and δ^{13}C values among compounds (Bowling, Pataki, and Randerson 2008). However, the influence of DOC on the molecular signature of bulk SOM in mangrove soils is unknown, as material flow in these soils is not only vertical but also horizontal due to organic matter inputs throughout the soil column (~1 m) through massive fine root production (Arnaud et al. 2021; Tabuchi 1983) and advective translocation of DOC in soils by tidal water movement (Maher et al. 2013; Ohtsuka et al. 2020).

3.3. Mineral association as a key factor in long-term carbon storage in mangrove mineral soils

The major contribution of m-LF in mangrove soils (Figure 4) compared to terrestrial soils was a novel finding, but its long-term stability needs verification for m-LF, as well as HF, to be an important fraction for carbon storage in mangrove soils. We therefore conducted a radiocarbon analysis of density fractions in the deepest samples. We found a consistent pattern in Δ^{14}C values of density fractions in all the measured cores (Table 4). HF was the oldest with Δ^{14}C between −149‰ and −97‰ followed by m-LF (between −130‰ and −87‰) and then f-LF (between −89‰ and 78‰). These differences in Δ^{14}C among density fractions were consistent with the findings from terrestrial soils (Heckman et al. 2022) and suggest that mineral association may be pivotal in long-term carbon storage in mangrove mineral soils. In the LFs, m-LF was always older than the corresponding f-LF, although f-LF showed a considerable variability in their Δ^{14}C (Table 4). The lower Δ^{14}C values of m-LF indicate that on average, m-LF is more persistent than f-LF. Together with the relative enrichment of m-LF, our findings suggest that m-LF may play a more important role in carbon storage in mangrove soils than in terrestrial soils. It is likely that slower decomposition under reducing conditions due to flooding resulted in longer residence time of f-LF and greater associations with soil minerals. The positive Δ^{14}C (modern age) observed in f-LF of the core 3 was due to recent inputs of live fine roots, while negative Δ^{14}C values of f-LF in the other cores suggest limited inputs from live roots (Table 4). Questions remained, however, why there were a large variability in Δ^{14}C between the same fractions of different cores (Table 4). Molecular-level analyses of each fraction and more detailed source partitioning by measuring end-members (river, mangrove, and marine) and linking the result to geochemical factors such as specific surface area and reactive metal phases (Fe, Al), may shed light on organic carbon stabilization mechanisms in mangrove soils. A variability in environmental factors such as oxidation-reduction potential, pH, or salinity should simultaneously be considered.

4. Conclusion

We introduced density fractionation to mangrove soils in this study. The method could successfully separate meaningful functional components of SOM in mangrove soils which differed in abundance, degradation state, and age. The massive production of mangrove fine roots resulted in a high abundance of plant debris (low-density fractions) throughout the 1-m cores, which markedly differed from terrestrial soils. By analyzing elemental and isotopic signatures of density fractions, we revealed shifts in sources and degradation states within and between fractions. Although we were able to show that mineral associated fractions were the most important for carbon storage in mangrove soils both in terms of quantity and residence time, the processes generating organic matter in each density fraction and their influencing factors remain to be studied. Future studies would benefit from a coupled analysis of quantity (C/N concentrations and relative abundance) and quality (stable and radio isotopes and molecular composition) of density fractions and geochemical factors in mangrove soils. It is also needed to elucidate how natural environmental variations such as redox conditions and pH influence the association between mineral particles and reactive metal phases and SOM of different nature, on different time scales of hourly, daily, and seasonally over semi-diurnal and spring – neap tidal cycles.

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Disclosure statement

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Author contributions

MK designed the experiment and collected the samples with the help of NF. KH conducted the density fractionation experiments with an initial guidance from MK. MK and KH conducted stable isotope analysis with the help of TO. MK, YM, and YY conducted radiocarbon analysis. KH wrote an initial draft with a significant contribution from MK, and all authors have reviewed and approved the final article.

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