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#### ORIGINAL ARTICLE

Soil and Ecosystem Processes

## Can fine sediment addition promote carbon stabilization in coastal wetland soils? A laboratory experiment

Anthony J. Mirabito<sup>1</sup> | Jason A. Anandappa<sup>1</sup> | Nia R. Hurst<sup>2</sup> | Jacob F. Berkowitz<sup>2</sup> | Lisa G. Chambers<sup>1</sup>

#### Correspondence

Lisa G. Chambers, Department of Biology, University of Central Florida, 4000 Central Florida Blvd. Bldg 20, BIO 301, Orlando, FL 32816, USA.

Email: lisa.chambers@ucf.edu

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## Abstract

Scientists are increasingly exploring soil carbon (C) stabilization processes that protect soil organic C from microbial decomposition. Mineral-associated organic matter (MAOM) is considered one of the most protected pools of soil C, but remains understudied in wetland soils relative to upland soils. Using complementary intact soil core and bottle incubation studies, this research investigated how fine sediment addition, in the form of dredged sediment, impacts soil respiration and MAOM pools in coastal wetland soils that vary by soil organic matter (SOM) content and particle size. We hypothesized fine sediment additions would reduce soil respiration and increase MAOM pools, with the greatest response to fine sediment addition being in the high SOM soil. Contrary to our prediction, addition of fine sediments to high SOM intact soil cores did not change respiration rate, but CO<sub>2</sub> production rate decreased by 21% in low SOM cores, and the mass of MAOM-C increased by 23% after receiving fine sediment additions. In the bottle study of high SOM soils only, the response to fine sediment addition varied by initial soil particle size. Specifically, the largest soil particles (>2 mm) showed a 1727% increase in MAOM-C, which coincides with a 49% decrease in CH<sub>4</sub> production rate. Overall, soil C protection resulting from fine sediment addition differed by soil type and SOM texture, suggesting initial soil properties (e.g., existing mineral sediment content, nutrient availability, and degree of decomposition) influence C stabilization through fine sediment addition, and these properties should be considered when choosing potential coastal restoration sites.

#### **INTRODUCTION** 1

Soils provide important ecosystem functions and services, such as carbon (C) storage, that help moderate global temperatures. Subsequently, the Intergovernmental Panel on Climate Change has suggested soil organic matter (SOM) management

Abbreviations: HF, heavy fraction; LF, light fraction; LOI, loss on ignition; MAOM, mineral-associated organic matter; POM, particulate organic matter; SOM, soil organic matter; USACE-ERDC, U.S. Army Corps of Engineers Engineer Research and Development Center.

influences carbon capture and carbon dioxide (CO<sub>2</sub>) flux rates (Naburrs et al., 2022). Soil C stability, or how susceptible soil C is to mineralization, has been heavily researched by soil scientists (Beare et al., 1994; Hassink, 1995; Huang et al., 2020; Kalbitz et al., 2005; Mazzilli et al., 2014; Poeplau et al., 2023; Six et al., 2002) with an emphasis on determining which pools of soil C are less susceptible to microbial decomposition. This resulted in the categorization of soil C based on the physical, chemical, and biochemical properties (Six et al., 2002; von Lützow et al., 2006). A popular method categorizes soil

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<sup>&</sup>lt;sup>1</sup>Department of Biology, University of Central Florida, Orlando, Florida, USA

<sup>&</sup>lt;sup>2</sup>Environmental Laboratory, U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, Mississippi, USA

C stability as either particulate organic matter (POM), which contains more complex plant-derived molecules with higher C:N ratios, or mineral-associated organic matter (MAOM) composed of simple, low molecular weight compounds, often from microbial origin and lower C:N ratios (Cambardella & Elliot, 1992; Cotrufo et al., 2012; Lavallee et al., 2020, 2022; Yu et al., 2022). Despite numerous methods for isolating POM and MAOM pools (Poeplau et al., 2018), POM can broadly be defined as materials >53 µm (via physical fractionation) and/or within the light fraction (LF; via density fractionation). In contrast, MAOM is typically defined as <53 µm and/or being in the heavy fraction (HF) (Cambardella & Elliot, 1992; Cotrufo et al., 2015; Hall et al., 2015; Mikutta et al., 2019).

Research suggests C mineralization rates are higher, and turnover rates quicker, for larger size fractions and LF fractions (i.e., POM) compared to smaller, HF pools (e.g., MAOM; Feller & Beare, 1997; Hassink, 1995; von Lützow et al., 2007). Furthermore, a laboratory incubation of agricultural soil demonstrated MAOM had lower mineralization rates and was less sensitive to temperature changes than POM pools (Benbi et al., 2014). Diverse methods for comparing C mineralization of MAOM and POM (e.g., incubations, <sup>13</sup>C isotopes tracing, and <sup>14</sup>C dating) consistently show lower turnover rates for MAOM than POM, regardless of fractionation method applied (i.e., by aggregate size or soil texture size; Feng et al., 2016). The increased mean residence time of MAOM is attributed to the chemical interactions between SOM and mineral surfaces and/or SOM being entrapped within microaggregates that inhibit microbial decomposition (Lavallee et al., 2020; Liang et al., 2017; Six et al., 2002; Totsche et al., 2017).

Wetlands contain some of the most organic C-dense soils on Earth (Mitsch & Gosselink, 2015), but little research has been conducted to quantify how much wetland SOM is protected as MAOM. A recent study demonstrated high variability in the percent of soil C protected as MAOM among different wetland soil types (e.g.,  $\sim 0.6\%$  to 25%), and suggested an inverse relationship between total SOM content and the abundance of MAOM-C (Mirabito & Chambers, 2023). This finding prompted the current research question: can MAOM formation be stimulated in wetland soils with the addition of fine mineral sediments? We are particularly interested in coastal wetland soils because they contain such a disproportionately large quantity of soil C relative to their areal coverage, which has earned them the nickname "blue carbon" ecosystem (McLeod et al., 2011). Additionally, their location makes them particularly vulnerable to disturbance and environmental change, increasing the need to prompt stable forms of soil C.

Coastal wetland soils span a gradient from being highly mineral dominated, to being highly organic matter dominated, with the prevailing stabilization mechanisms likewise ranging from physicochemical mineral associations to bio-

### **Core Ideas**

- Mineral-associated organic matter (MAOM) increased when adding fine sediment.
- Fine sediment reduced soil CO<sub>2</sub> up to 21% in the intact core study.
- Fine sediment reduced soil CO<sub>2</sub> and CH<sub>4</sub> flux rates up to 6% and 49%, respectively, in the bottle incubation study.
- Larger soil size fractions generally had higher rates of CO<sub>2</sub> and CH<sub>4</sub> production.
- Coastal wetland restoration techniques adding fine sediment may contribute to MAOM.

chemical recalcitrance, respectively (Kida & Fujitake, 2020). SOM properties are important for MAOM formation based on prior research proposing more labile plant litter promotes an increase in microbial activity and byproducts, thus promoting more stable MAOM, a theory known as microbial efficiencymatrix stabilization (Cotrufo et al., 2012). However, MAOM cannot form without the fundamental presence of fine minerals <53 µm (silt and clay). Indeed, a positive correlation between silt and clay particles present, and the amount of C associated with the MAOM pool, has been observed in upland grassland soils (Hassink, 1997; Yu et al., 2022). In coastal wetland soils, a positive correlation between fine minerals and both total C and non-mineralizable C was observed (Lewis et al., 2021). However, research is lacking in quantifying the relationship between SOM textures (e.g., fibric, hemic, and sapric) and compositions, C mineralization rates, and fine mineral content in wetland soils.

Currently, efforts are underway in many locations to manage or manipulate coastal wetlands to increase coastal resiliency, restore degraded ecosystems, and maximize C storage to help reduce risks associated with climate perturbations (Abbott et al., 2019). One wetland creation and restoration technique involves the placement of dredged sediments from nearby navigation channels to construct new wetlands or increase elevation of existing wetland surfaces. The goal is to prevent wetland submergence from rising sea levels, attenuate wave energy to reduce flood risk, and promote continued ecosystem functions, including C sequestration and storage (Yozzo et al., 2004). The use of dredged sediment has been shown to increase the bulk density and mineral content, resulting in improved vegetation growth (Berkowitz et al., 2016; VanZomeren et al., 2018) and increasing clay content at restored wetlands (Edwards & Proffitt, 2003). If fine minerals are a limiting factor for MAOM formation in coastal wetland soils, dredged sediment addition could stimulate its formation, enhancing soil C stabilization as MAOM by increasing the availability of active clay and silt binding sites (Islam et al., 2022). Fu et al. (2023) observed a positive correlation between vertical accretion rates (contributed by mineral deposition) and MAOM pools in both mangrove and saltmarsh soils. Restored wetlands typically have lower total soil C pools compared to unmanaged areas (Fenstermacher et al., 2016), likely because they remain substantially younger than natural wetlands (Moreno-Mateos et al., 2012). We propose that the increase in fine mineral availability from the addition of dredged sediment could result in created/restored coastal wetlands have less total C, but a larger proportion of the total C may be more stable within the MAOM-C pool, compared to natural wetlands.

This study investigated the impact of fine mineral addition (using dredged sediment) on soil C mineralization rates and MAOM formation in coastal wetland soils within intact soil core and bottle incubation laboratory experiments. The studies sought to test how soil properties interact with fine mineral sediment additions based on (1) the abundance of organic versus inorganic material in the soil, and (2) the degree of decomposition of the existing SOM, ranging from large fresh litter with minimal decomposition ("fibric" texture, >2 mm size fractions) to small highly decomposed muck ("sapric" texture, <53 µm size fractions). Specifically, the intact core experiment quantified how the addition of fine mineral sediments to coastal wetland soils of different SOM contents affected CO2 fluxes and MAOM pools, while also investigating related biogeochemical properties. We predicted soils receiving fine sediment additions would increase the MAOM pool during the incubation, which would correlate with a lower CO<sub>2</sub> flux, and that this would be amplified in wetland soils characterized by a higher percentage of SOM. Within the bottle incubation experiment, bulk coastal wetland soils were physically fractionated into four size classes to provide new data on how particle size (a proxy for SOM texture and degree of decomposition) relates to mineralization rates, and determine if size fraction influences the fine sediment addition response. Based on prior research in upland soils, we expected the largest soil size fractions (i.e., POM) would have higher rates of mineralization and produce the greatest change in the size of the MAOM pool with sediment addition, while the smallest (<53 µm) size fraction would have the lowest rate of C mineralization due to more C being physically and chemically protected as MAOM to begin with.

## 2 | MATERIALS AND METHODS

## 2.1 | Site selection

Soil samples were collected from two brackish coastal wetlands in Apalachicola, FL, chosen based on differing soil characteristics such as SOM, silt, clay, and sand content (Table 1) while being in close proximity to each other. Cat Point (84°53.4938509′ W, 29°43.3836508′ N) was dominated by *Juncus roemerianus* (black needle rush) and *Bolboschoenus robustus* (sturdy bulrush) and has been a designated reference site for a nearby wetland previously created using dredged sediment (Berkowitz et al., 2021), while East Bay (84°52.7597208′ W, 29°46.0836862′ N) was dominated by *J. roemerianus* (Steinmuller et al., 2022). Soil collection points were chosen using a stratified random point selection in ArcGIS to target only the common plant community, *J. roemerianus*, to keep plant community consistent across East Bay and Cat Point samples.

Soil samples for intact cores were collected using the push core method to a target depth of 30 cm using a 10-cm-diameter polycarbonate tube. A total of 25 intact soil cores were collected (n = 15 at East Bay, n = 10 at Cat Point) with the soil profile and water column kept intact. Additional site water was collected in two 208.2-L containers and used to keep the intact soil cores inundated during the experiment (Supporting Information). Aboveground plant biomass of the dominant vegetation at each site (J. roemerianus) was collected within  $1 \text{ m} \times 1 \text{ m}$  quadrats from five of the 25 cores.

Soil samples for bottle incubation were collected at East Bay using the same sampling methods. Soil cores were collected at three different points to the depth of 30 cm and composited together in the field, sealed in Ziplock bags, and stored on ice. East Bay was chosen for the bottle incubation experiment because it contained greater variability in soil particle size fractions, as compared to Cat Point. All materials were transported to the University of Central Florida in Orlando, FL, for analysis and experimentation.

## 2.2 | Intact core: Experiment design and monitoring

To optimize space and resource availability, an unbalanced experimental design was chosen where intact cores from Cat Point (10) and East Bay (15) were randomly assigned to two treatment conditions (control [5] and layer fine sediment [5]), with a third treatment for East Bay being mixed fine sediment (5). All cores received 0.15 g of plant litter from their respective sites, which had been oven-dried at 70°C until constant weight and homogenized using a SPEX 8000 M Mixer/Mill (SPEX Sample Prep). Litter was added across all cores (including the control cores) to ensure an adequate supply of fresh C was available to support the microbial community while the variable of interest (dredged sediment addition) was manipulated. The chemical composition of the plant litter added was  $44.51 \pm 0.15\%$  C and  $0.7851 \pm 5.0 \times 10^{-3}\%$  N. Fine dredged sediment was supplied by the U.S. Army Corps of Engineers Engineer Research and Development Center (USACE-ERDC), and was collected

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Physicochemical properties of soils from intact core study (n = 5 independent replicates for each treatment and depth, except for redox potential where n = 3). TABLE 1

Site	Treatment	Depth (cm)	Bulk density (g cm <sup>-3</sup> )	Soil moisture (%)	Redox potential (mV)	Hq	Organic matter (%)	Clay (%)	Silt (%)	Sand (%)
Cat Point	Control	0-10	$0.12\pm0.00a$	$87.7 \pm 0.6a$	$-66.5 \pm 19.8a$	$4.34 \pm 0.26a$	$41.6 \pm 6.8a$	$3.4 \pm 0.5a$	$50.4 \pm 5.5a$	$4.6 \pm 1.3a$
	Layer		$0.36 \pm 0.03b$	$66.6 \pm 1.1b$	$-81.0 \pm 20.4a$	$5.20 \pm 0.36b$	$23.5 \pm 5.3b$	$6.0 \pm 0.4b$	$68.9 \pm 4.7b$	$1.6\pm0.7a$
	Control	10-20	$0.36\pm0.04a$	$69.2 \pm 3.0a$	NA	$5.18\pm0.28a$	$14.9\pm1.5a$	$5.8 \pm 0.3a$	$66.0 \pm 2.3a$	$13.4 \pm 2.9a$
	Layer		$0.52 \pm 0.08b$	$62.3 \pm 5.2b$	NA	$4.24 \pm 0.44b$	$14.3 \pm 2.3a$	$4.5 \pm 0.1b$	$59.0\pm1.5a$	$22.1 \pm 3.4a$
East Bay	Control	0-10	$0.12\pm0.00a$	$87.0 \pm 0.8a$	$67.3 \pm 87.2a$	$5.70\pm0.13a$	$59.6\pm1.2a$	$2.0 \pm 0.1a$	$37.8\pm1.0a$	$0.5 \pm 0.2a$
	Layer	0-10	$0.24 \pm 0.01b$	$79.6 \pm 1.3b$	$101.5 \pm 142.5a$	$5.70\pm0.18a$	$34.0 \pm 0.6b$	$4.3 \pm 0.5b$	$60.8 \pm 0.6b$	$0.8 \pm 0.2a$
	Mix	0-10	$0.26 \pm 0.01b$	$75.5 \pm 1.2b$	$0.7 \pm 81.3a$	$5.52 \pm 0.14a$	$29.2 \pm 1.3b$	$5.2 \pm 0.2b$	$65.2 \pm 1.2b$	$0.5\pm0.1\mathrm{a}$
	Control	10-20	$0.14 \pm 0.01a$	$86.8 \pm 0.7a$	NA	$5.43 \pm 0.09a$	$46.5 \pm 2.9a$	$3.2 \pm 0.3a$	$50.0 \pm 2.7a$	$0.3 \pm 0.1a$
	Layer	10-20	$0.14 \pm 0.01a$	$86.9 \pm 0.8a$	NA	$5.45\pm0.21a$	$54.0 \pm 2.4a$	$2.6\pm0.1a$	$42.5 \pm 2.1a$	$0.8 \pm 0.2a$
	Mix	10-20	$0.13 \pm 0.01a$	$86.6\pm0.7a$	NA	$5.34 \pm 0.14a$	$51.4 \pm 2.3a$	$2.8\pm0.1a$	$45.1 \pm 2.2a$	$0.8 \pm 0.2a$
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from the Mobile River (Mobile, AL) using hand tools and stored in 22-L containers at 4°C for 6 months at the dredged sediment management facility prior to arriving at the University of Central Florida, which is the same way the dredged sediment is handled and stored prior to restoration projects (Sapkota & Berkowitz, 2024; Hurst, personal communication, 2024). For all control cores, plant litter was applied directly to the soil surface. For layer treatments, a slurry of 250 g (wet weight) of fine dredged sediment, 150 mL of site water, and 0.15 g of plant litter was applied to the soil surface. The application of a slurry approximates real-world dredged sediment applications. Finally, for the East Bay mix treatment, fine dredged sediment slurries were made in the same quantities as described above, but the slurry was mechanically mixed into the top 10 cm of the soil core. The mix treatment simulates coastal wetland restoration projects because extensive bioturbation mixing naturally occurs in the field following dredged sediment introduction. Site water, which was stored in the dark and gently mixed using aquarium pumps, was continuously pumped from a water reservoir and recycled through the intact cores while maintaining a 10 cm water column. This water was regularly sampled throughout the experiment to ensure no significant changes in chemistry occurred. Intact core setup is described in more detail in Supporting Information.

Carbon dioxide fluxes were monitored for 154 days, and each flux measurement was collected for 90 s using a LI-COR 8100 portable infrared gas analyzer with a 10-cm-diameter chamber (LI-COR Biosciences). Flux measurements were collected daily for the first 14 days, then gradually decreased to once weekly. Water samples (25 mL) were collected weekly to monitor ammonium (NH<sub>4</sub><sup>+</sup>), nitrite + nitrate (NO<sub>3</sub><sup>-</sup>), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC) in the water reservoirs described in more detail in Section 2.3.

#### 2.3 **Intact core: General soil properties and** nutrients

After the last sampling, platinum electrodes were placed 10 cm below the soil surface within the intact cores (n = 3per treatment). The platinum electrodes were used to quantify redox potential against a reference electrode filled with AgCl Orion filling solution (Thermo Fisher Scientific) and a voltmeter (Megonigal & Rabenhorst, 2013). The probes were placed at 10 cm due to a barrier between initial soil and fine dredged sediment occurring here to ensure redox conditions were not impacted by the treatment. Measurements were taken on days 3 and 5 after electrode installation and were corrected to the standard hydrogen electrode. Intact soil cores were then destructively sampled, dividing the soil core into 10-cm depth segments.

Subsamples from each depth segment (0–10 cm and 10–20 cm) were oven-dried at 70°C until constant weight to determine soil moisture content and bulk density. Due to resource constraints and the lack of direct manipulation to the 20- to 30-cm depth segment, this bottom segment was not analyzed. Measurements of pH were made by creating a 1:5 soil to solution slurry with deionized (DI) water and then recording the pH value after 30 min using an Accument XL 200 benchtop pH probe (Thermo Fisher Scientific).

SOM content and particle size analysis were measured using a sequential process. A homogenized subsample (2 g) of oven-dried soil was used to determine the percent SOM using loss on ignition (LOI) by combusting all SOM in a muffle furnace at 550°C for 3 h. For particle size analysis, 40 mL of 4% sodium hexametaphosphate was added to 1 g of ashed sediment and shaken on an orbital shaker for 24 h at 150 revolutions per minute (RPM) and 25°C. Next, 5-10 mL of sample was pipetted into a Cilas 1190 Particle Size Analyzer (Cilas) to measure clay, silt, and sand content, which was categorized using USDA specifications (<0.02 µm, 0.02– 0.50 μm, >50 μm, respectively; USDA NRCS, 2011). Soil composition was calculated for SOM, clay, silt, and sand content to equal 100% of the soil (Table 1). The composition of the organic matter of the soil was characterized using a modified acid-detergent fiber analysis to quantify cellulose, hemicellulose, lignin, and ash content (Chambers et al., 2024).

Soil nutrients were extracted within 24 h of destructively sampling the intact cores. Briefly, 4 g of field moist soil and 25 mL of 2 M KCl shaken for 1 h at 150 RPM and 25°C and then centrifuged at 5000 RPM at 10°C for 10 min. The supernatant was filtered through a 0.45-µm polyvinylidende diflouride (PVDF) membrane filter using a vacuum filtration system and acidified using double distilled H<sub>2</sub>SO<sub>4</sub> for sample preservation. Routine water samples collected from the intact core reservoirs were also filtered and preserved with double distilled H<sub>2</sub>SO<sub>4</sub>. All water and soil extractions were analyzed for NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and SRP by colorimetric analysis using USEPA Methods 231-A Rev.0, 210-A Rev.1, and 204-A Rev.0, respectively (USEPA, 1993), using an AQ2 Automated Discrete Analyzer (Seal Analytical). Water and soil extractions were analyzed for DOC using a Shimadzu TOC-L Analyzer (Shimadzu Scientific Instruments).

## 2.4 | Intact core: Soil physical fractionation

The MAOM pool was analyzed using a physical and density fractionation method modified for wetland soils (Mirabito & Chambers, 2023). Briefly, field moist soil equivalent to 15 g of dry soil weight was dispersed using 0.5% sodium hexametaphosphate (1:8 soil to solution ratio) with 10 glass beads on an orbital shaker at 150 RPM at 25°C for 18 h. Following dispersion, samples were wet sieved using a 53-µm sieve at a

rate of 50 reps within 2 min. Soil remaining on top of the sieve (>53  $\mu$ m) and soil that passed through the sieve (<53  $\mu$ m) were oven-dried at 70°C.

Following physical fractionation, 0.5–1.0 g of <53 µm soil was weighed and 20 mL of 1.85 g cm<sup>-3</sup> sodium polytungstate was added. Samples were shaken on an orbital shaker at 150 RPM at 25°C for 18 h. Samples were centrifuged at 3400 RPM for 30 min at 20°C, separating the LF from the HF and decanting the LF onto a 0.45-µm nylon membrane filter and rinsing with DI water. Once all the LF was removed, 30 mL of DI water was added to the remaining HF soil and centrifuged at 5000 RPM for 10 min at 20°C. The solution was decanted and this process was repeated two times. All LF soil and HF soils were oven-dried at 70°C. The <53 µm LF was combined with the >53 µm soil (henceforth referred to as POM) with the <53 µm HF being MAOM. All dried MAOM and POM samples were homogenized in a SPEX 8000 M Mixer/Mill (SPEX Sample Prep) and analyzed for total C using an Elementar Vario Micro Cube (Elementar Americas Inc.). Prior to analysis, samples were visually assessed for the presence of carbonates by using 1 M HCl (Lacroix et al., 2025).

## 2.5 | Bottle incubation: Experimental design

A total of 500 g of the composited 0-30 cm soil samples from East Bay were physically fractionated into four size fractions  $(>2 \text{ mm}, 2 \text{ mm}-250 \mu\text{m}, 250-53 \mu\text{m}, \text{ and } <53 \mu\text{m})$ . These size fractions were chosen to represent different possible soil types found in coastal wetland soils based on texture and degree of decomposition, ranging from soils composed of large, free POM, to soils dominated by fine minerals and DOC. To minimize impact to the natural soil microbial community, a dispersing agent was avoided, and soil was wet sieved at a rate of 50 reps within 2 min using DI water. It is acknowledged that the physical fractionation prior to incubation is a destructive process and may have impacted the biological and chemical properties, but since all size fractions received the same disturbance, comparisons could still be made among the fractions. Soil in each size fraction was air-dried until they reached soil moisture content similar to field conditions. A subsample of this soil was oven-dried at 70°C, homogenized, and measured for total C and nitrogen (N) using an Elementar Vario Micro Cube (Elementar Americas Inc.).

Next, air-dried soil (7 g) from each size fraction was transferred into 120-mL serum bottles and randomly assigned as a control (soil + water only; n = 5) or a treatment (soil + water + 0.5 g sediment; n = 5). The sediment within the treatment was the same fine dredged sediment the USACE-ERDC provided referenced in Section 2.3, but with SOM removed by combustion at 550°C for 3 h (Xu et al., 2022) with the goal to increase the available binding sites of the minerals for new SOM to form MAOM. A subsample of the fine dredged

sediment was analyzed for total C and N after LOI, and the remaining C content was  $0.24 \pm 0.02\%$  and the N content was  $0.03 \pm 0.001\%$ .

Bottles were sealed with rubber septa and purged with 99%  $O_2$ -free  $N_2$  gas for 3 min. Next, 14 mL of purged (99%  $O_2$ -free  $N_2$  gas), filtered (0.45-µm PVDF membrane filter) site water was added to each bottle. Bottles were placed on an orbital shaker at 150 RPM at 25°C, and headspace was sampled on days 1, 4, 8, 10, 17, 37, 43, 58, 72, 87, and 121 and analyzed for CO<sub>2</sub> and CH<sub>4</sub> production using a GC-2014 gas chromatograph (Shimadzu Scientific Instruments). Headspace purges with 99% O<sub>2</sub>-free N<sub>2</sub> gas were done for each bottle after sampling to prevent gas concentrations from inhibiting microbial activity (Bridgham & Ye, 2013). Soil respiration rates were calculated over time using Henry's law and the ideal gas law and were calculated using linear regression (Bridgham & Ye, 2013). MAOM pools were quantified at the end of the incubation period using the same method outlined in Section 2.4.

## 2.6 | Statistical analysis

All statistical analysis was done using R version 4.03 (R Foundation for Statistical Computing) within RStudio (RStudio Team, 2020). All data visualization was done using R package "ggplot2" (Wickham, 2016) and all data wrangling was done using R package "tidyverse" (Wickham et al., 2019). Generalized linear models (glms) with an interaction between site, treatment, and soil depth as predictor variables were used to compare physicochemical properties, extractable nutrients, and fractionation pools data from the intact core study. Redox potential was the one physicochemical property exception that used only site and treatment as predictor variables within the glm. All water nutrients used site and treatment as predictor variables within their glm's. Soil CO<sub>2</sub> flux rates from the intact core experiment were averaged over the length of the study and compared using generalized additive models within the "mgcv" package (Wood, 2017). Parameters of treatment and site were used as fixed predictors, and soil core replicates as a random effect to account for repeated measurements. Pearson correlations were calculated and used to compare MAOM and CO<sub>2</sub> produced to various soil parameters outlined above.

Soil respiration rates for the bottle incubation were normalized per gram of total C within each bottle, and analyzed using generalized linear mixed models (glmm) using the "lme4" package (Bates et al., 2015), where fixed effects included treatment (control or + sediment) and soil size fraction (>2 mm, 2 mm–250  $\mu$ m, 250–53  $\mu$ m, and <53  $\mu$ m); bottle replicates were considered random effects to account for repeated sampling. Also, through the "performance" package (Lüdecke et al., 2021), conditional and marginal Nakagawa's  $R^2$  values were calculated for the mixed models,

where the conditional  $R^2$  values consider both the fixed and random effects and the marginal  $R^2$  values consider only the fixed effects based on Nakagawa et al. (2017). Adjusted  $R^2$ values for each treatment were >0.83 for all CO<sub>2</sub> flux rates and >0.72 for CH<sub>4</sub> flux rates except for treatment 250–53 µm + sediment ( $R^2 = 0.15$ ). Soil C within MAOM pool of the bottle study was calculated in the units of mg C g<sup>-1</sup> C of the entire C in the sample (C from soil and sediment, if applicable) and analyzed using a glm with treatment and size fraction as predictor variables. Models were selected based on model performance using the R packages "AICcmodayg" package (Mazerolle, 2020) to compare Akaike information criterion scores and "performance" package, which included testing for normality, homogeneity, and collinearity. Pairwise comparisons were made using the Tukey method within the "emmeans" package (Lenth, 2020). Results are reported using mean values and standard errors.

## 3 | RESULTS

## 3.1 | Intact core: CO<sub>2</sub> flux rates and soil properties

During the intact core experiment, the Cat Point (low SOM) layer treatment had a lower mean  $CO_2$  flux rate (p < 0.001) of  $518.85 \pm 21.67 \text{ mg CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ , compared to the control treatment (652.98  $\pm$  22.24 mg CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>; Figure 1a), representing a 21% decline (Figure 1b). In contrast, East Bay soils (high SOM) showed no differences in average daily CO<sub>2</sub> flux by treatment (Figure 1a). Specifically, the East Bay control treatment averaged 354.99  $\pm$  26.73 mg CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>, compared to the layer treatment (319.54  $\pm$  13.16 mg CO<sub>2</sub> m<sup>-2</sup>  $day^{-1}$ , p = 0.758) and the mix treatment (373.58 ± 17.87 mg  $CO_2$  m<sup>-2</sup> day<sup>-1</sup>, p = 0.542). Site had a significant effect on CO2 flux rates, with all East Bay intact cores having lower average rates than all Cat Point cores, regardless of treatment condition (p < 0.001; Figure 1a). Layer and mix treatments increased bulk density within the 0- to 10-cm depth segment of the soil relative to their control, with the Cat Point layer treatment increasing bulk density by 209% (p < 0.001; Table 1). East Bay layer and mix treatments increased bulk density 92% and 115%, respectively, relative to the East Bay control (both p = < 0.001). Cat Point layer soils also had greater bulk density within the 10-20 cm soil (p = 0.001) than the control. Redox potential did not differ between treatments and controls, and there were no clear trends for pH (Table 1). The dredged sediment was predominantly silt (79.37  $\pm$  0.04%) with small fractions of clay  $(10.68 \pm 0.10\%)$  and SOM  $(9.99 \pm 0.08\%)$ , and the two dominant extractable nutrients were DOC (38.22  $\pm$  6.06 mg kg<sup>-1</sup>) and  $NO_3^-$  (9.315  $\pm$  1.250 mg kg<sup>-1</sup>; Table S1). Treatments generally did not impact composition of the SOM (Figure S3), but East Bay control had a slightly higher proportion of SOM

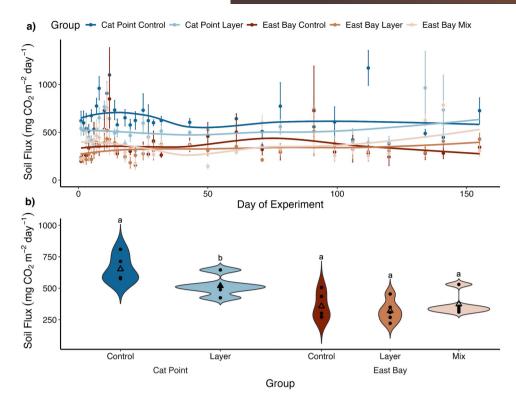


FIGURE 1 (a) Scatter plot displays mean soil flux rates from the intact soil cores. Dots represent mean value for flux rate of treatment replicates for each sampling point and standard error (n = 162 observations from five independent replicates per treatment). (b) Violin plots display soil flux CO<sub>2</sub> produced with triangle symbols representing mean and dots representing the five independent replicates per treatment. Different letters denote p < 0.05 across treatments within the same site.

being lignin (33.95  $\pm$  2.79%; p = 0.003) and hemicellulose (22.75  $\pm$  2.10%; p = 0.051) in the 10–20 cm section compared to Cat Point control (15.57  $\pm$  5.56%; 12.45  $\pm$  2.89%).

Both Cat Point and East Bay SOM content decreased in the top 0–10 cm layer and mix treatments compared to their controls (p < 0.001; Table 1). Looking at the clay, silt, and sand components of the soil composition, layer and mix treatments had the greatest impact on clay and silt content in the top 0–10 cm of the soil (Table 1). Cat Point layer had a 77% increase in clay and a 37% increase in silt, compared to the control (both p < 0.001). For East Bay soils, layer and mix treatments had 110% and 155% increase in clay, respectively, compared to the control (both p < 0.001). Silt content increased by 61% in the layer treatment and 72% in the mix treatment relative to the control (both p < 0.001).

Soil nutrients varied across site and depth with treatments (Figure 2). Cat Point control generally had greater  $\mathrm{NH_4}^+$  (p < 0.001),  $\mathrm{NO_3}^-$  (p = 0.045), SRP (p = 0.005), and DOC (p < 0.001) than the layer treatment. East Bay control had higher concentrations of DOC compared to East Bay layer (p = 0.001) and East Bay mix (p < 0.001) for 0–10 cm (Figure 2g). Water nutrient levels generally did not differ across treatments during the length of the experiment, with Cat Point soils having higher  $\mathrm{NO_3}^-$ , SRP, and DOC than East Bay soils (Figure S1).

## 3.2 | Intact core: Soil organic matter forms

Carbon quantity found within the MAOM pool showed few differences between the two sites or between treatments within a single site. The layer treatment for Cat Point generally had greater average C content in the MAOM pool (499.77  $\pm$  137.31 mg C g $^{-1}$  C) than the control (404.75  $\pm$  29.58 mg C g $^{-1}$  C) in the 0- to 10-cm depth, but high variability in the layer treatment made these results non-significant (p=0.497; Figure 3). The average C content in the East Bay MAOM fraction was greatest for the mix treatment (459.06  $\pm$  16.31 mg C g $^{-1}$  C), followed by the layer treatment and control (p=1.00), for 0- to 10-cm depth segment.

By mass, the MAOM pool contained 40% of the total C in the 0- to 10-cm depth and 37% of the total C in the 10- to 20-cm depth of the Cat Point control. Even in the high SOM East Bay soil, the control had 46% of the total C as MAOM in the 0- to 10-cm depth and 51% of the total C as MAOM at the 10- to 20-cm depth. Compared to the initial MAOM-C content of the dredged sediment used in this study, Cat Point layer, East Bay layer, and East Bay mix observed an increase in MAOM-C in the 0- to 10-cm soil depth (131%, 145%, 162%, respectively). Of note, for all treatments and depths, the variability among replicates was much lower for the controls than the layer and mix treatments (Figure 3). Overall, there was no

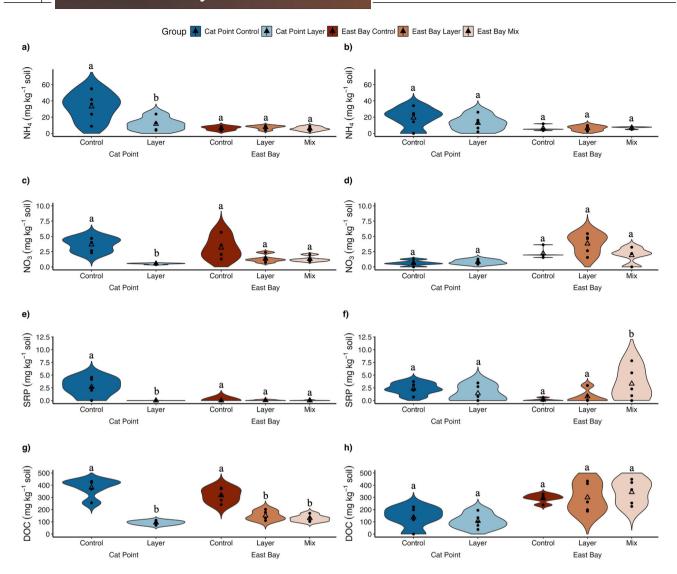


FIGURE 2 Violin plots display nutrient concentrations from soil extractions of the intact soil cores. Visuals (a), (c), (e), and (g) represent 0-10 cm soil depth, and visuals (b), (d), (f), and (h) represent 10- to 20-cm soil depth. Dots represent sampling points, and triangles represent mean values. Different letters denote p < 0.05 across treatments within the same site and depth (n = 5 independent replicates for each soil depth and treatment).

observed relationship between  $CO_2$  production and MAOM in Cat Point (r = -0.04) or East Bay (r = 0.05) shown in Figure S2a,b, respectively.

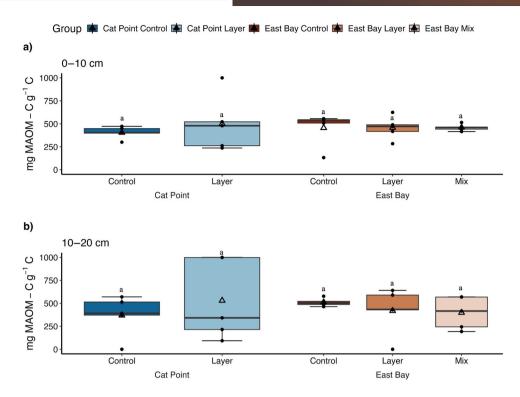
## 3.3 | Bottle incubation: Carbon flux rates

The treatment had the greatest impact on  $CO_2$  flux in the >2 mm size fraction, with the >2 mm + sediment having a flux rate of 53.81  $\pm$  13.08  $\mu$ g C-CO<sub>2</sub> g C<sup>-1</sup> day<sup>-1</sup>, representing a 6% decline compared to the control (Figure 4b; p=0.053). The rate of  $CO_2$  production generally decreased with smaller soil particle size fractions within the controls for the three largest groups; however, the  $CO_2$  flux within the <53  $\mu$ m fraction control group was greater than the 250–53  $\mu$ m fraction (p<0.001).

Methane production was below detection in all bottles until about day 40. At that point, CH<sub>4</sub> production increased rapidly in the >2 mm size fraction. The >2 mm + sediment CH<sub>4</sub> flux rate (0.54  $\pm$  0.09  $\mu g$  C-CH<sub>4</sub> g C<sup>-1</sup> day<sup>-1</sup>) was 49% lower than the >2 mm control (Figure 4d; p<0.001). In the 2 mm–250  $\mu m$  + sediment, average CH<sub>4</sub> production was also slightly lower (0.31  $\pm$  0.08  $\mu g$  C-CH<sub>4</sub> g C<sup>-1</sup> day<sup>-1</sup>) compared to the 2 mm–250  $\mu m$  control (0.37  $\pm$  0.09  $\mu g$  C-CH<sub>4</sub> g C<sup>-1</sup> day<sup>-1</sup>; Figure 4d; p=0.343).

## 3.4 | Bottle incubation: MAOM pools after sediment addition

Compared to their respective controls, the C content in the MAOM fraction was greater in the >2 mm + sediment



**FIGURE 3** Box plots display concentration of carbon per total gram of carbon in the mineral-associated organic matter (MAOM) pool through the 0–10 cm (a) and 10–20 cm (b) profile across each treatment from the intact soil cores. Dots represent each soil core replicate per treatment, and triangles represent mean value for each treatment. Different letters denote p < 0.05 across treatments within the same site (n = 5 independent replicates for each treatment).

(p=0.042) and the 2 mm-250  $\mu$ m + sediment (p=0.347; Figure 5a,b). Specifically, the >2 mm size + sediment contained 422.21  $\pm$  31.95 mg C g<sup>-1</sup> C (a 1727% increase from its control) and the 2 mm-250  $\mu$ m + sediment contained 288.90  $\pm$  96.25 mg C g<sup>-1</sup> C (a 225% increase from its control). The <53  $\mu$ m + sediment saw a slight decrease in MAOM-C compared to the control (p=0.729; Figure 5d).

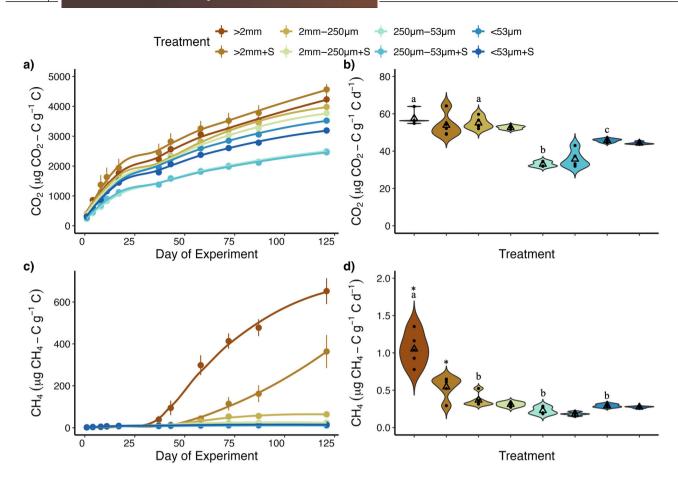
## 4 | DISCUSSION

## 4.1 | Intact core: CO<sub>2</sub> flux and MAOM pools in soils of varying SOM

Soil respiration results from the intact core study were contrary to our hypothesis, which predicted a decrease in  $\mathrm{CO}_2$  flux rate from fine sediment addition would be greater in East Bay soils due to higher SOM, and perceived limitation of minerals for MAOM formation in this organic-rich soil. However, respiration from Cat Point soils was reduced with the addition of fine sediment. Specifically, the layer treatment had 21% lower  $\mathrm{CO}_2$  flux during the experiment compared to the control (Figure 1a). The decline in  $\mathrm{CO}_2$  from the layer treatment was similar to a previous wetland intact soil core study, which demonstrated sediment addition decreased  $\mathrm{CO}_2$  flux by 36%

in an organic-rich soil (Boudreau et al., 2024). However, this trend was not observed in East Bay soils, where the control, layer, and mix treatments had statistically similar CO<sub>2</sub> flux rates. Therefore, any changes in CO<sub>2</sub> flux could be related to C protection through MAOM formation, inhibiting mineralization of C through physical (spatial inaccessibility) or chemical (adsorption to minerals) protection (Six et al., 2002). This differed from Breithaupt et al. (2019), which observed CO<sub>2</sub> flux along a gradient of wetland SOM content and found sediment addition only impacted respiration in the most organic-rich soil. All soils within the intact core study were maintained under anaerobic conditions, and there was no evidence in the redox data to suggest the sediment addition was "capping" the soil to prevent CO<sub>2</sub> from effluxing (Breithaupt et al., 2019). Although additional research is needed to disentangle the drivers of the difference in response to sediment addition between Cat Point and East Bay, it is noteworthy that Cat Point soils had overall greater microbial activity (CO<sub>2</sub> flux; Figure 1) and generally greater nutrient content (NH<sub>4</sub><sup>+</sup>, SRP, DOC; Figure 2) than East Bay soils, which may have accelerated MAOM formation through the in vivo microbial pathway with microbial by-products interacting with mineral surfaces (Cotrufo & Lavallee, 2022; Cotrufo et al., 2012; Sokol et al., 2019).

For all treatment cores across both soils, fine sediment addition in the intact cores lowered total C (MAOM + POM)



**FIGURE 4** Scatter plots display gas production over time for  $CO_2$  (a) and  $CH_4$  (c) where dots represent the average of the five bottle replicates and error bars are the standard error from the bottle incubation. Violin plots represent mean soil flux rates for  $CO_2$  (b) and  $CH_4$  (d). Dots represent average flux per gram of carbon for each replicate. For each plot, averages are from (n = 5) independent replicates for each treatment. Different letters denote p < 0.05 across control groups of each size fraction, and asterisks represent p < 0.05 between control and treatment within the same size fraction.

compared to the control, possibly a dilution effect due to the relatively low C content in fine sediment. To compensate for the C dilution effect by mass, MAOM was viewed as a proportion of total C, rather than per gram soil. The proportion of total C found in the MAOM pool in 0- to 10-cm depth for Cat Point layer averaged 50.0%, compared to 40.5% in the control. Similarly, in the 10- to 20-cm depth, the Cat Point layer treatment contained 53.0% of total C as MAOM, while the control contained 36.9%. Therefore, the addition of fine sediment decreased overall total C within the soil due to dilution, but increased the percentage of MAOM-C relative to the total C for Cat Point soils. The positive relationship between C associated with fine minerals and the percentage of fine minerals present in the soil is consistent with Hassink (1997) and Six et al. (2002). This increase in MAOM-C following sediment addition did not occur with East Bay and was not as evident as Boudreau et al. (2024), which measured a 60% increase in MAOM-C.

# **4.2** | Bottle incubation: Carbon flux rates and MAOM by varying size fraction

In the bottle experiment, the addition of fine sediment slightly decreased the average rate of  $\rm CO_2$  in three of the four size fractions (>2 mm, 2 mm–250 µm, and <53 µm) and the average  $\rm CH_4$  production in all four fractions. However, the greatest decrease occurred in the >2 mm size fraction for both  $\rm CO_2$  (16.5%; p=0.053) and  $\rm CH_4$  (48.6%; p<0.001) compared to their controls. Few studies have investigated  $\rm CH_4$  production across soil size fractions or within the MAOM pool, as  $\rm CH_4$  production may be minimal in the aerobic upland soils typically studied (Huang et al., 2019). However, the fine sediment additions had the greatest effect on reducing  $\rm CH_4$  production within the wetland soil in this study, which is unique considering wetlands soils are estimated to emit 20%–25% of the global  $\rm CH_4$  emissions (Mitsch & Gosselink, 2015). The observed general decrease in  $\rm CO_2$  production

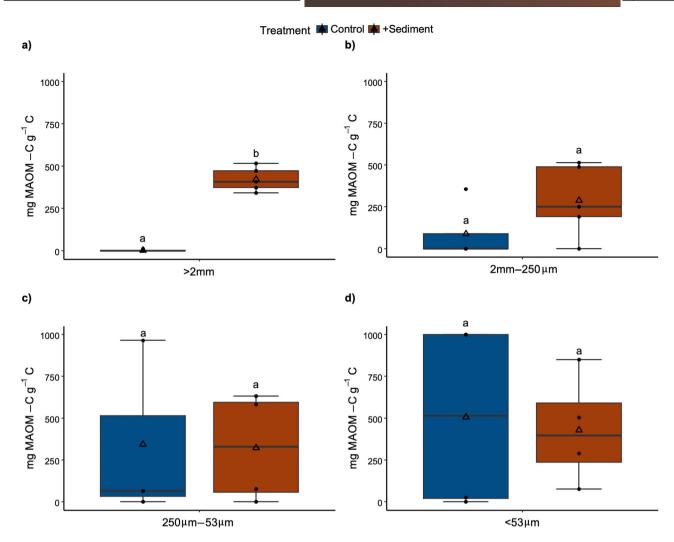


FIGURE 5 Box plots display carbon associated with mineral-associated organic matter (MAOM) pools from bottle incubation samples across different sizes fractions: >2 mm (a), 2 mm-250  $\mu$ m (b), 250-53  $\mu$ m (c), and <53  $\mu$ m (d). Triangles for each plot represent mean values. Different letters denote p < 0.05 across treatments (n = 5 independent replicates for each treatment).

with the addition of fine minerals in this study is consistent with Adhikari et al. (2019), which observed a ferrihydrite amendment decreasing CO<sub>2</sub> production by 8.1%. It has been suggested that the specific mineralogy used for an amendment can impact the effect of soil respiration (Nguye & Marschner, 2014; Six et al., 2002). Along with specific mineralogy impacting MAOM formation, it has also been observed that soil texture size (fine clay vs. coarse clay) has varied organic matter interactions based on size (Kögnel-Knabner et al., 2008), again emphasizing the importance of mineralogy in MAOM formation for future research and specifically the presence of redox active elements in the sediment that may poise the redox potential of the soil above that needed for methanogenesis.

Unexpectedly, the  $<53 \mu m$  did not have the lowest rate of  $CO_2$  or  $CH_4$ , as the existing literature has found (von Lützow et al., 2006). Rather, respiration rate declined with size for the three largest size fractions (Figure 4), as found

by others (Ashman et al., 2003; Benbi et al., 2014), but the <53 µm size fraction (control and treatment) both averaged greater CO<sub>2</sub> flux rates than the 250-53 µm fraction. This difference may be because previous research has presented respiration rates per unit of soil, while this study presented per gram of C to account for differences in C percentages between size fractions (Conant et al., 2000; Riaz & Marschner, 2020). Moreover, this study's use of <53 µm pool without density-separating the HF (MAOM) from the LF (small POM) could mean any DOC from the larger size fractions could be included in the <53 µm fraction and account for the faster mineralization rate. However, Mueller et al. (2012) observed DOC in the <53 µm pool only impacted mineralization for the first 2-3 days of the incubation. This finding that the 250-53 µm size fraction had lower average CO<sub>2</sub> or CH<sub>4</sub> flux per gram C than the <53 µm size fraction is potentially significant given all the literature promoting MAOM as the most protected form of C (Kleber et al., 2015; Kögel-Knabner

et al., 2008), but additional studies with greater statistical power are needed to validate this finding.

Following the bottle incubation, soils in the two largest size fractions that received the fine sediment addition showed a general increase in mean MAOM-C, but the difference was greatest in the >2 mm + sediment, which had a 1727% increase in MAOM-C compared to the control. To note, the high organic matter fraction (>2 mm) had the greatest increase in MAOM-C and the greatest decrease in C flux due to the addition of fine sediment compared to the other size fractions. The addition of fine minerals may not have increased MAOM-C as measurable in the smaller size fractionations due to fine minerals potentially increasing soil aggregation (Boudreau et al., 2024; Even & Cotrufo, 2024). The formation of stable soil aggregates may have caused an underestimation of MAOM, such as the lower MAOM-C in the <53 µm treatment, despite using both physical and chemical dispersion techniques (Christensen, 1992). It is hypothesized that clay content and type of clay influence soil aggregations (Six et al., 2002), along with soil aggregates promoting a stable pool of POM (Fraznluebbers & Arshald, 1997), which could explain the lower MAOM-C in the <53 µm treatment. Stable soil aggregates could also explain the lower respiration of the 250-53 µm fraction compared to <53 µm fraction, as the size fractions did not undergo density fractionation and there is evidence that "coarse MAOM" or "heavy POM" could behave differently than light POM, with this pool being measurable within both upland and wetland soils (Mirabito & Chambers, 2023; Samson et al., 2020). Moreover, some have suggested that aggregation may temporally precede MAOM formation, so this experiment may have needed to run longer to document more significant MAOM formation (Lavallee et al., 2020).

# **4.3** | Comparison between intact core and bottle study

The difference in  $\mathrm{CO}_2$  flux response between Cat Point and East Bay could be attributed to an incorrect assumption about mineral availability being a limiting factor for MAOM in East Bay soils or not accounting for differing biogeochemical properties in these soils (e.g., inorganic N and P available for microbial respiration, labile C availability, or microbial community composition) limiting microbial activity. Although East Bay soils did have abundant SOM ( $\sim$ 60% SOM in the top 0–10 cm), the rest of the soil solids ( $\sim$ 40%) were fine minerals (silt and clay). Following the basic concept of the law of minimum, if fine sediment was not the limiting factor for MAOM formation in East Bay soils, the treatment addition would not promote its formation or produce the expected reduction in  $\mathrm{CO}_2$  flux. Rather, other edaphic soil properties may have lim-

ited new MAOM formation, while Cat Point demonstrated the expected result.

The increase in MAOM-C observed in the bottle study may not have been as clear in the intact core study due to methodological differences. Carbon saturation, or the maximum limit of C that can be associated within the MAOM pool (Castellano et al., 2015; Cotrufo & Lavallee, 2022; Hassink, 1997; Six et al., 2002), may have been lower for the sediment added in the bottle study. First, the fine sediment used in the bottle study had the SOM removed prior to the experiment (via volatilization), and the removal of SOM may have increased the ability to form MAOM. Fine sediment has limited surface area that can interact with SOM to form MAOM, so once the silt or clay particle reaches C saturation, Chung et al. (2008) showed that an increase in C inputs does not correspond with an increase in MAOM. Also, the ratio of added minerals to the original soil differed, with the intact core study having 2.5 g of sediment per gram soil compared to 0.07 in the bottle study. Additionally, the soils from the bottle incubation were constantly shaking, increasing the potential for fine sediment to interact with C compounds.

## **4.4** | Implications for wetland creation and restoration

Understanding how fine sediment addition may impact biogeochemical cycling in wetland creation and restoration projects utilizing dredged sediment can be informative to research scientists and project managers (Croft et al., 2006; Raposa et al., 2022; VanZomeren et al., 2018). A large number of studies have investigated how the addition of dredged sediment through restoration efforts impacts elevation change, plant and avian community dynamics, and basic soil properties such as bulk density, SOM content, total soil C and N (Berkowitz & White, 2013; Croft et al., 2006; Ford et al., 1999), but investigating the potential impacts dredged sediment has on MAOM pools of the soil remains to be studied. Results from these laboratory experiments suggest that soils with high nutrients and microbial activity (e.g., Cat Point soils) may show a greater reduction in mineralization rate, and demonstrate more short-term MAOM formation, than soils with lower nutrients and respiration. The Cat Point site may represent a coastal wetland that would benefit from dredged sediment placement because visual observations and several biogeochemical properties (Figure 2) suggest the site is experiencing excessive inundation and water stagnation because of declining elevation relative to sea level and possibly hydraulic isolation. In contrast, East Bay appeared to be a more healthy and stable wetland.

In organic-rich coastal wetland soils, the degree of decomposition of organic soils (herein represented by different size fractions) may influence how soil C dynamics are impacted by sediment addition. In this study, the coarsest POM (>2 mm) exhibited both the highest  $\mathrm{CO}_2$  and  $\mathrm{CH}_4$  flux rates, and the greatest reduction due to sediment addition. The potential for sediment addition to reduce  $\mathrm{CH}_4$  fluxes in fibric organic soils may be particularly beneficial for projects seeking greenhouse gas mitigation. Finally, the application style of the fine sediment (whether layered or mixed in the soil surface; Figure 3) showed little impact on the overall MAOM pool formation under laboratory conditions but may still be important at the field scale.

## **5** | CONCLUSION

The objective of this study was to improve the understanding of the potential for fine sediment application to reduce C mineralization rate and promote MAOM-bound C within coastal wetland soils. While similar studies in upland soils have observed positive correlations between fine minerals and mineral-bound C, testing to see if these trends occurred in wetland soils containing significantly more SOM was necessary. In the intact core study, Cat Point soils (with higher nutrients, respiration, and mineral content) demonstrated a 21% decline in CO<sub>2</sub> flux with the addition of fine sediment, while the East Bay soils did not show an effect. Cat Point also saw a slight increase of 23% in the amount of total C in the MAOM pool due to the fine sediment addition. The bottle study indicated a general trend of decreasing CO2 flux with decreasing particle size, with the exception of the <53 µm pool. The >2 mm size fraction demonstrated the greatest decline in CO<sub>2</sub> flux (6%) and CH<sub>4</sub> flux (49%) from the fine sediment addition, which corresponded to a 1727% increase in MAOM-C. This study highlights potential mechanisms for MAOM formation in wetland soils and suggests that applying fine sediment to different wetland soils induces different results based on initial soil characteristics. Additional research is needed to explore these patterns at larger scales, including field experiments and experimentation with wetland soils that have varying soil characteristics.

## AUTHOR CONTRIBUTIONS

Anthony J. Mirabito: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; visualization; writing—original draft; writing—review and editing. Jason A. Anandappa: Investigation; methodology; writing—review and editing. Nia R. Hurst: Funding acquisition; project administration; writing—review and editing. Jacob F. Berkowitz: Funding acquisition; project administration; writing—review and editing. Lisa G. Chambers: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—original draft; writing—review and editing.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## ORCID

Anthony J. Mirabito https://orcid.org/0000-0002-6209-8168

Jacob F. Berkowitz https://orcid.org/0000-0002-7226-0632

*Lisa G. Chambers* https://orcid.org/0000-0001-6432-8038

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