



Decomposition Rates of Surficial and Buried Organic Matter and the Lability of Soil Carbon Stocks Across a Large Tropical Seagrass Landscape

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Abstract

The paradigm for understanding the accumulation of organic carbon in vegetated coastal “blue carbon” habitats holds that burial of organic carbon (C_{org}) slows decomposition and leads to stability of carbon stocks. Further, it is generally assumed that the presence of the plant communities contributes to the buried organic matter and the stability of the carbon stocks. In this study, these assumptions were tested and the lability of soil organic carbon was examined as a function of environmental and plant community drivers. Samples of surficial sediment and seagrass community characteristics were collected at 93 locations across the ca. 15,000 km² of seagrass beds in south Florida. Ramped pyrolysis was used to describe the relative lability of soil organic carbon across the landscape. Organic matter (OM) was lost at all temperatures from 180 to 600 °C, suggesting that even the relatively high combustion temperature of 550 °C underestimates OM content by $\approx 10\%$ on average. Additionally, deployments of model substrates (canvas strips) were used to examine decomposition rates of buried and surficial organic material at a subset of these sites. On average, finer, muddier soils contained slightly higher C_{org} stocks than coarser sediment sites, but the relationships between sediment grain size and seagrass community structure was weak. The lability of soil organic carbon varied with sediment grain size; as much as 80% of the C_{org} was refractory in coarse-grained soils compared with less than 30% in muddy soils. In muddy soils, burial decreased cellulose decomposition rate by an average of 22–39% compared with surficial breakdown, but in coarse-grained soils, burial enhanced cellulose decomposition rate by at least 55%. Taken as a whole, this study suggests that burial does not enhance C_{org} storage in all blue carbon environments, and that soil C stores are only weakly correlated with seagrass biomass at the landscape scale.

Keywords Coastal ecosystems · Blue carbon · Loss on ignition · Ramped pyrolysis · Stable isotopes · Soil carbon

Introduction

The capability of some coastal ecosystems to sequester CO₂ and store large carbon stocks is drawing increasing attention as a potential means of conservation-based climate change mitigation (IPCC 2014). The term “blue carbon” is used to

describe the organic carbon (C_{org}) stocks associated with coastal vegetated ecosystems (seagrass meadows, mangrove forests, and tidal marshes) that could be added to by net CO₂ uptake or lost and emitted as CO₂ during habitat destruction or degradation (McLeod et al. 2011). The term is also applied to those carbon finance policies and frameworks under development to maximize carbon sequestration through the protection and promotion of carbon-rich ecosystems, collectively called “blue carbon” strategies (Pendleton et al. 2012). The need to quantify blue carbon stocks and assess the relative risk of CO₂ emissions from degraded sites has spurred a flood of investigations into the causal connections between manageable ecosystem attributes and stable, long-term carbon sequestration (Howard et al. 2017; Macreadie et al. 2017).

Seagrass meadows are declining globally (Waycott et al. 2009) and their associated carbon stocks are large enough to be considered in global budgets, making them prime

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candidates for inclusion in blue carbon strategies (Fourqurean et al. 2012a). Unlike most other ecosystems incorporated into climate change mitigation strategies, seagrasses are included for their existing soil carbon stocks rather than living biomass or current production rates (Fourqurean et al. 2012a). An estimated 299 Tg carbon per year could be lost globally from remineralized soil carbon stocks resulting from the current rates of seagrass ecosystem loss (Fourqurean et al. 2012a); the focus of blue carbon strategies is to mitigate such CO₂ loss through ecosystem management and conservation. For those preliminary C_{org} loss projections to be accurate and meaningful, the presence of soil C_{org} stocks must be causally linked to the presence of intact seagrass meadows. Further, C_{org} lost from the ecosystems upon seagrass destruction must be released into the atmosphere, thus exacerbating greenhouse gas emissions, if seagrass blue carbon is to have value in mitigation strategies (Lovelock et al. 2017a, b).

Soil C_{org} stocks associated with seagrass ecosystems vary greatly among sites, influenced by local seagrass-related features, but also the local geomorphological and hydrological setting and soil characteristics (Serrano et al. 2016b). When considered broadly, seagrass ecosystems promote C_{org} storage, with studies showing that seagrass presence (Macreadie et al. 2015; Mazarrasa et al. 2015), density and productivity (Serrano et al. 2014), and recolonization (Greiner et al. 2013; Marba et al. 2015) are all positively correlated with soil carbon storage. However, the soil retention of C_{org} derived from both seagrass and non-seagrass sources relies on a depositional environment that is determined only in part by seagrasses (Miyajima et al. 2017). The canopy's complex matrix of seagrass leaves serves to decrease current velocity and alter the turbulence in a way that encourages deposition (Ward et al. 1984; Hendriks et al. 2008). However, the flexibility of seagrass shoots that bend over in the current results in a non-linear relationship so that above some modest shoot density additional shoots may not further reduce shear stress on the bottom (Fonseca et al. 2019). It is important to note that this hydrological effect of the seagrass canopy on sediment deposition/erosion can only occur when the attenuation effects of seagrasses decrease local water velocity below thresholds needed for C_{org} deposition and accumulation (see Hansen and Reidenbach 2012), which varies dependent on sediment grain size and density (Belshe et al. 2018; Bergamaschi et al. 1997). When water velocity is too great for seagrass attenuation to induce deposition, seagrass-derived C_{org} and other organic particulates may be exported, accounting for a portion of the carbon budget that is often overlooked (Duarte and Krause-Jensen 2017).

At the same time, other sites have water velocity low enough to encourage deposition and accumulation without the influence of seagrasses. For example, Serrano et al. (2016a) found that C_{org} stocks at sites where seagrasses were small or absent were controlled by the contribution of finer

particles to sediments, rather than the presence or density of seagrasses. This may not be surprising as finer particles (mud, silts, clays) have greater surface area to volume ratios which often have higher C_{org} content and can accumulate in some depositional environments without the presence of seagrasses (Bergamaschi et al. 1997; Keil and Hedges 1993). Miyajima et al. (2017) found that the areal extent of seagrass meadows was positively correlated with the surface area to volume ratio of sediment particles and C_{org} storage. However, nearby offshore sites void of vegetation exhibited sediment C_{org} storage greater than seagrass meadows due to local geophysical constraints. The relative importance of seagrass presence versus local geomorphological drivers can also vary depending on the scale at which measurements are made. For example, seagrass density positively affects C_{org} accumulation at the plot level, although these biological drivers can be outweighed by those related to location at the meadow scale (Oreska et al. 2017). The opposite is true in Florida Bay, where the importance of seagrass abundance has measurable effects on soil C_{org} stocks at the regional scale but not at the plot level (Armitage and Fourqurean 2016; Howard et al. 2016). As we seek to understand how to manage C_{org} storage in seagrass ecosystems, we have to expand on relationships between seagrass abundance and C_{org} storage to form a more holistic understanding of soil C_{org} drivers.

Mapping dense seagrass C_{org} stocks has taken primacy to date in blue carbon efforts, although for stocks to be relevant in discussions of greenhouse gas emissions, there must be evidence that seagrass presence is linked to a reduction of organic matter (OM) remineralization, and, thus, CO₂ production rates. Seagrass ecosystems are thought to create relatively stable, low redox, anoxic soil environments that reduce microbial activity and impede remineralization (Duarte et al. 2011; Fourqurean et al. 2012a). Anoxia has been shown to develop millimeters beneath the sediment surface in seagrass soils (e.g., Borum et al. 2005) and plant density reduces the oxygen input via pore-water advective transport (Burdige and Zimmerman 2002). The loss of seagrasses is suggested to be accompanied by an erosional environment that would expose once anoxic, buried C_{org} to oxic conditions, leaving it vulnerable to decomposition (Lovelock et al. 2017a, b; Pendleton et al. 2012). Furthermore, exposure of buried material to labile OM associated with dissolved organic carbon and fresh detritus of the sediment-water interface may induce a priming effect, where the decomposition of recalcitrant OM is enhanced by labile organic inputs (Trevathan-Tackett et al. 2018). These soil conditions that are promoted by seagrasses through anoxia and stability in sediments can also be reached independently by mechanisms like restricted hydrological movement, supply of low [O₂] overlying water, and decomposition of autochthonous and allochthonous organic matter (see Middelburg and Levin 2009).

Stable, anoxic soil conditions are understood to promote soil C_{org} preservation, although there is no consensus supporting this mechanism in the literature. Some experimental evidence (e.g., Dauwe et al. 2001; Moodley et al. 2005) suggests that remineralization rates of OM in marine sediments are suppressed under anoxic conditions, while other work suggests that anoxic conditions may even accelerate breakdown of some OM (Harrison 1989). More likely, the sensitivity of OM remineralization rates to oxygen availability is dependent upon the chemical composition and therefore the recalcitrance of the OM, where the decomposition rate of fresh, labile OM is independent of O_2 availability and breakdown of “aged,” recalcitrant OM is accelerated with oxygen (Burdige 2007; Hulthe et al. 1998; Kristensen et al. 1995; Kristensen and Holmer 2001). Given the discrepancies in previous literature and the importance of OM burial to blue carbon strategies, the degree of preservation remains an unresolved but necessary research question.

The organic component of soils underlying seagrass meadows is a diverse mixture of macromolecules that vary in their resistance to remineralization (Burdige 2007). The constituents of soil C_{org} vary spatially and temporally, determined by the source and type of OM inputs, as well as the degree and milieu of processing (Arndt et al. 2013; Burdige 2007). These contributing OM fractions exist on a scale of recalcitrance that is complicated by environmental factors that can augment a macromolecule’s inherent recalcitrance. Chemical stabilization (Mayer 1995), physical protection (Keil et al. 1994), anaerobic metabolism (Nierop et al. 2017), nutrient limitation (Enriquez et al. 1993; López et al. 1998), and unachieved energetic thresholds (Guenet et al. 2010) can limit microbially driven degradation and remineralization, thus changing the realized recalcitrance of soil OM. Blue carbon discussions often consider the recalcitrance of seagrass soil C_{org} as dependent on the stable, low-nutrient, anoxic environment in which it is buried (Duarte et al. 2011; Fourqurean et al. 2012a) rather than inherent properties of the OM, although this may be an oversimplification given the complicated mixtures of organic compounds that make up soil C_{org} and the complex environmental factors that influence the refractory nature of each of those compounds.

The composition of OM in seagrass tissue varies greatly in its inherent recalcitrance, varying between species, tissue component, and latitudinal origin (Trevathan-Tackett et al. 2017a). Moreover, seagrasses have a distinctive $\delta^{13}\text{C}$ signature and stable isotopic evidence suggests that the OM of seagrass soils can contain substantial OM fractions that derived from non-seagrass production (Kennedy et al. 2010). Other contributors to those soil OM stocks, like terrestrial inputs and algae, vary even more in their ability to be decomposed (Enriquez et al. 1993). This being the case, it is unlikely that litter quality and the inherent recalcitrance of OM are equal across sites inhabited by seagrasses,

potentially altering the importance of environmental conditions to the long-term preservation of C_{org} . The degree of inherent recalcitrance of soil OM could influence the risk of remineralization that blue carbon stocks face (Lovelock et al. 2017b) potentially limiting the importance of environmental setting to preservation. On the other hand, identifying sites where vulnerable, inherently labile C_{org} remains in the soil could identify priority sites for blue carbon conservation.

Given the importance of the assumptions that characteristics of seagrass meadows influence OM deposition and preservation and that buried OM decomposes more slowly when buried in seagrass soils, we examined the relationships among seagrass bed characteristics, sediment grain size, soil C_{org} content, and the lability of soil C_{org} across the landscape of seagrasses in south Florida. The aim of this study was to identify correlates of seagrass soil C_{org} stocks, lability, and breakdown rates across a spectrum of seagrass characteristics and environmental conditions. Specifically, this study addresses the importance of seagrass species, seagrass density, and soil type to C_{org} storage and breakdown rates. As buried C_{org} stocks would likely face erosion and exposure to oxygen following seagrass ecosystem loss, we also investigate how the degree of exposure (buried conditions versus surficial conditions) affects breakdown rates. The south Florida seascape hosts thousands of square kilometers of semi-continuous seagrass meadows, with high variability in seagrass abundance, productivity, and community type as well as sediment grain size, nutrient availability, and soil C_{org} storage. We document 93 sites across the diverse seascape, hypothesizing that soil C_{org} density would be tightly correlated with sediment grain size, and we expected sediment grain size would be determined by a combination of seagrass canopy-induced effects and local environmental characteristics. We expected areas with high preservation of soil C_{org} stocks to have higher fractions of labile OM preserved in the soil. Considering previous observations that sites with muddy, finer grained sediments have higher soil C_{org} content, lower decomposition, and higher preservations, we expect these sites to contain higher fractions of inherently labile OM. And since seagrass biomass is considered to be more labile than other OM sources, we expected that there would be stable isotopic evidence of a higher proportion of seagrass C_{org} in sites with more labile soil OM. As the benefits of burial to OM preservation are a foundational assumption of seagrass blue carbon strategies, we also address the hypothesis that breakdown rates of OM are suppressed in buried conditions compared with those on the sediment surface at 23 sites across the range in sedimentary environments of our study area. Given the likely variability in the lability of existing sediment OM across sites, we use the decomposition rate of a model substrate (canvas strips) across sites for these comparisons.

Methods

This study was conducted across the seagrass meadows of Florida Bay and the Florida Keys that together form one of the largest documented continuous seagrass ecosystems in the world, with seagrasses occurring across 75% of an area of nearly 20,000 km² (Fourqurean et al. 2002). Seagrass soils across the region consist primarily of biogenic carbonate sediments, with sediment grain size reflecting the wave energy of the environment. Monospecific and mixed species meadows of *Thalassia testudinum*, *Halodule wrightii*, and/or *Syringodium filiforme* are commonly encountered across the region. Especially in deeper or lower light locations, *Halophila decipiens* and occasionally *Halophila engelmanni* are found. In fringe areas close to the Florida mainland, *Ruppia maritima* is often a component of the seagrass assemblage (Fourqurean et al. 2002). The species composition of the seagrass meadows depends on local nutrient availability, sediment type, herbivory, salinity, and light availability, among other factors (Armitage and Fourqurean 2006; Fourqurean et al. 2003; Fourqurean et al. 1995). Most of the seagrasses in the study region fall within two management areas, the Florida Keys National Marine Sanctuary (FKNMS) and Everglades National Park (ENP) (Fig. 1). The species composition and seagrass abundance across this landscape is a function of phosphorus availability, with generally higher P

availability offshore in the FKNMS and increasing P limitation of biomass towards the interior of Florida Bay in ENP (Fourqurean and Rutten 2003; Fourqurean and Zieman 2002; Fourqurean et al. 1992). We used a randomization protocol to locate our sampling sites across the landscape.

Characterizing the Seagrass Community During the summer and winter seasons of 2015 and 2016, 46 long-term monitoring sites across the FKNMS and Florida Bay were surveyed for water depth, sediment type, average canopy height, and species-specific seagrass abundance, as part of ongoing seagrass monitoring programs that have been underway for over 20 years (Fourqurean et al. 2002). We did not collect similar information from 47 additional sites within Florida Bay from which sediments were collected, indicated with the symbol “+” on Fig. 1. For all surveys at each of our long-term monitoring sites, ten quadrats (0.25 m²) were placed along a permanent 50-m transect at predetermined, random distances from the primary marker. In each quadrat, all seagrass species were listed and scored for areal coverage using a modified Braun-Blanquet scale: 0 = absent; 0.1 = one individual and < 5% cover; 0.5 = few individuals and < 5% cover; 1 = many individuals and < 5% cover; 2 = 5–25% cover; 3 = 25–50% cover; 4 = 50–75% cover; and 5 = 75–100% cover (Fourqurean et al. 2001). Each of these same quadrats were also assessed for average seagrass canopy height

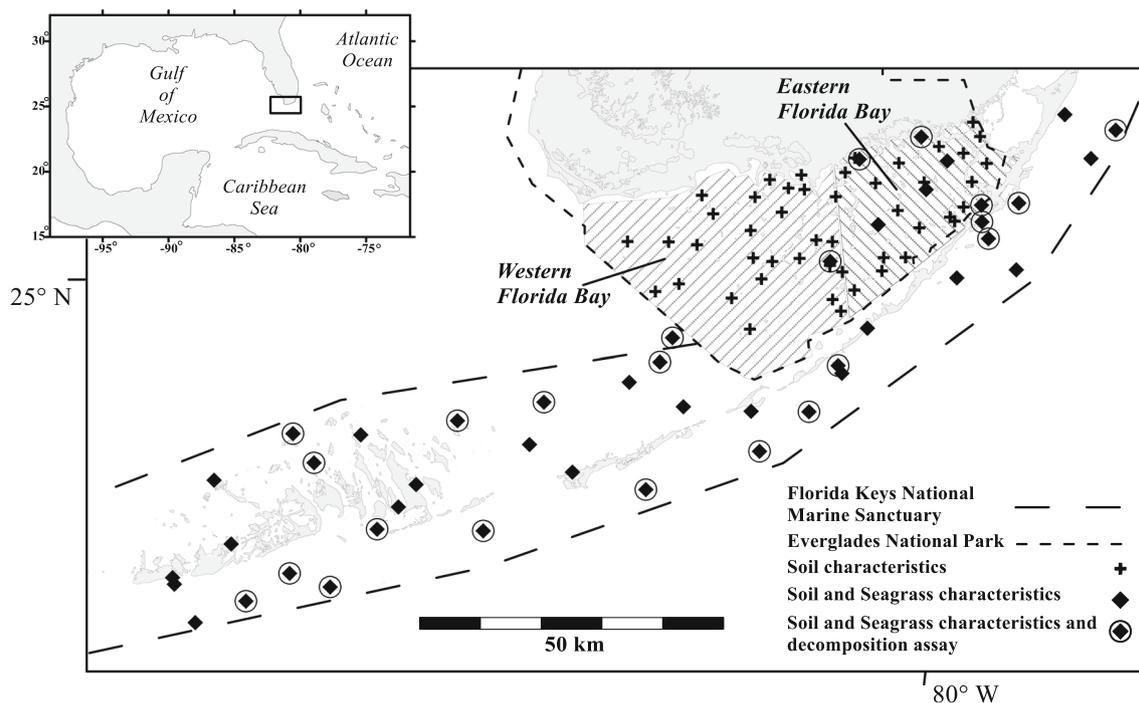


Fig. 1 Map of sampling locations across the south Florida seascape showing site types coded by observations collected and management areas of Everglades National Park and the Florida Keys National Marine Sanctuary. Within Everglades National Park, the high-

phosphorus Western Florida Bay region and the low-phosphorus Eastern Florida Bay region are indicated. The inset map puts the study area into regional geographic perspective

(measured in situ with a ruler) as well as visual/tactile classification of sediments into one of 9 categories of increasing grain size (Table 1).

Seagrass $\delta^{13}\text{C}$ values were assessed at our long-term monitoring sites during both winter and summer months of 2015 and 2016, for a total of four samples per site. At each site, short shoots of each seagrass species present were haphazardly collected, placed on ice, and transported back to the laboratory for analyses. In the lab, seagrass leaves were separated by species, cleaned of epiphytes by scraping with a razor blade and dried to a constant weight at 70 °C. Prior to analyses, seagrasses tissue was ground to a fine powder with an automated mortar and pestle. An elemental analyzer was used to combust these samples and to subsequently reduce the formed carbon-containing gases to CO_2 , which was then measured on a Finnigan MAT Delta C IRMS in a continuous flow mode. Isotopic values were reported in the standard delta notation with respect to the international standard Vienna Pee Dee Belemnite (‰). Analytical reproducibility of the reported values, based on sample replicates, was better than ± 0.08 ‰. An average $\delta^{13}\text{C}$ value for each site was calculated using values of the dominant species.

Characterizing Seagrass Soils Soil cores taken in quadruplicate from our 46 long-term sampling locations were collected in January 2016 using 60-mL plastic syringes that were modified into small piston cores (~2.6-cm diameter) that extracted surface soil from 0 to 20 cm deep. Samples collected at the 47 additional sites within Florida Bay were collected from September to October 2016, using a 2" hand sediment corer that was repeatedly used to sample the top 20 cm of soil until pooled samples of ~1000 ml soil per site were retrieved, this method precluded using those soils for some analyses that require samples be collected without changing the volume

they displaced in situ. Living seagrass biomass was rarely encountered in our cores because of their small diameter, but when we did collect living plant tissue in the soil, it was discarded before subsequent analyses.

Two cores from each long-term site were dried at 70 °C, weighed for dry bulk density (DBD), then homogenized before C_{org} , loss on ignition (LOI) and $\delta^{13}\text{C}$ analyses. The samples from the 47 additional Florida Bay cores were also analyzed for C_{org} , LOI, and $\delta^{13}\text{C}$, but were not collected in a manner conducive to assessing DBD, calculated as the dry weight of the soil divided by the volume of the original soil sample (g cm^{-3}). C_{org} content was calculated using an instrumental analyzer dry oxidation method (Fourqurean et al. 2012b). Briefly, powdered samples were analyzed for total carbon (C_{total}) using a CHN analyzer. Subsamples of dried material were weighed, ashed at 550 °C for 4 h, and reweighed, enabling OM content to be calculated as LOI. The ashed soil samples remaining after the LOI technique were reanalyzed using a CHN analyzer to quantify the C content of the inorganic fraction. C_{org} was calculated by difference between C_{total} and inorganic carbon (C_{inorg}), using LOI values to scale C_{inorg} measurements to the original, pre-ashed soil mass. Carbon density, presented as C_{org} (mg cm^{-3}), was calculated as the product of C_{org} content and DBD. Samples were processed in duplicate for each site then used to calculate site mean. For determining $\delta^{13}\text{C}_{\text{org}}$ of soils, subsamples of dry, homogenized soil were fumed with HCl for more than 14 days until a constant weight was reached to remove C_{inorg} . Samples were analyzed for $\delta^{13}\text{C}$ using the same procedures and precision as described for seagrass tissues above.

Sediment Grain Size Analyses The remaining two soil cores from each long-term monitoring site, as well as the pooled samples collected at the additional Florida Bay sites, were

Table 1 Sediment texture categories for rapid field observations of seagrass soils

Grain size category	Numeric value	Description
Mud	1	Individual grains indistinguishable, easily compress in hand, sediment remains clumped after compression
Sandy mud	2	Majority of grains indistinguishable but textured upon touch, easily compress in hand, sediment remains clumped after compression
Muddy sand	3	Sandy texture upon touch but compresses in hand, sediment dissociates upon release with most grain falling in water column
Sand	4	Clearly distinguishable grains, difficult to compress in hand, grains fall quickly in water column
Coarse shell	5	Shell and shell remains dominate sediments (approx. 5–10 mm in size)
Halimeda-hash	6	Remains of carbonate segments from <i>Halimeda</i> detritus (approx. 5–10 mm in size)
Rubble	7	Medium size rock or coral skeletal fragments (approx. 10–25 mm in size)
Live coral	8	Living hard coral
Rock	9	Bedrock or solid biogenic carbonate formations

used for grain size analysis by wet sieving (Erfteimeijer and Koch 2001). Approximately 60 mL of wet sediment was treated with 20–30 mL of 30% H₂O₂ to remove organics. After bubbling subsided, the slurry was sieved using a vibrating shaker plate and rotating shower head that supplied a continuous flow of water. Sieve sizes 4 (> 4.76 mm), 10 (> 2.00 mm), 40 (> 0.42 mm), and 200 (> 0.07 mm) were chosen for easy comparison with other South Florida research projects and used to separate gravel, coarse, medium, and fine sand fractions. Water and all sediments passing through the #200 sieve were collected in 2000 mL graduated cylinders for pipette analysis to separate silt from clay contributions (Folk 1980). The combination of procedures yielded percent contributions (by weight) of five sediment categories (clay, silt, fine sand, medium sand, coarse sand, and gravel). Clay and silt were combined for a “mud” category. Grain size analyses were done in duplicate for each site. At our long-term monitoring sites, we also collected a visual estimate of the soil texture using rapid field assays (Table 1).

Assessing the Lability of Soil Organic Matter Homogenized soil samples were re-dried at 70 °C for a minimum of 2 weeks followed by a stepwise, ramped temperature analysis of C loss to investigate the relative recalcitrance of soil C_{org} using a method modeled after thermogravimetric analysis and ramped pyrolysis (Capel et al. 2006; Kristensen 1990; Trevathan-Tackett et al. 2015; Trevathan-Tackett et al. 2017a). We used sequential mass loss on ignition at set temperatures to remove organics combined with the pre- and post-ignition C content as determined with an elemental analyzer (Thermo Flash 1112). This allowed for both weight loss and C loss to be calculated across a range of temperatures.

Prepared samples were analyzed using a CHN analyzer to measure C_{total} in the soil samples (which included both C_{org} from OM and C_{inorg} from carbonate minerals). Subsamples of dried material (1–1.5 g) were added to preashed, dry, preweighed vials and weighed to the nearest 0.1 mg. Samples were then heated at 180 °C for 4 h and reweighed for the calculation of temperature-specific LOI (LOI₁₈₀). A small (~ 20 µg) subsample of oxidized material was then analyzed for post-oxidation carbon content. The remaining oxidized material was reweighed and oxidized at the subsequent temperature (300 °C) for 4 h. The LOI₃₀₀ was calculated and a subsample was then taken for elemental analysis. This sequential process was conducted at 180 °C, 300 °C, 400 °C, 500 °C, 550 °C, and 600 °C, corresponding to primary OM categories of marine macroalgae and seagrass (Trevathan-Tackett et al. 2015; Trevathan-Tackett et al. 2017a). Each temperature step yielded temperature-specific LOI and C content values that were scaled to calculate the C lost at each temperature step relative to initial sample weight. C loss across temperatures were used to create a Recalcitrance Index (RI), defined as

C lost at 500 and 550 °C, representing the high, most recalcitrant C_{org}, divided by total C_{org} lost between 180 and 550 °C:

$$RI = \frac{C_{org} \text{ lost at } 500^{\circ}\text{C} + C_{org} \text{ lost at } 550^{\circ}\text{C}}{C_{org} \text{ lost between } 180^{\circ}\text{C and } 550^{\circ}\text{C}}$$

The 600 °C temperature step was excluded from RI calculation because common methods of C_{org} determination used in the literature are calculated at 550 °C and lower.

Canvas Strip Decomposition Assays We assessed local OM breakdown rates beginning during the January 2016 campaign using a canvas strip assay, where the loss of tensile strength of a standardized cellulose material was used as a proxy for OM decay (Harrison et al. 1993; Tiegs et al. 2013). We used Fredrix brand 12-oz unprimed, undyed, raw cotton duck, style number 548 artist canvas for our assays, because it has been found to perform similarly to previously used fabrics for such assays that are no longer in production (Slocum et al. 2009). This fabric was cut into uniform strips, 10 × 2 cm, each with the same number of warp threads in the long direction. These strips were deployed at each site where sediments allowed, with 10 deployed on the surface of the sediment and another 10 below the rhizosphere at 20-cm depth. Some sites had shallow to no sediment, preventing assay deployment. Surface-deployed strips were tethered to the bottom with a piece of nylon cord and they rested on the top of the sediment-water interface within the seagrass canopy. For deployment below the rhizosphere, sediment cores (4 cm wide × 20 cm deep) were extracted followed immediately by the insertion of buried strips and the return of extracted sediment. Buried strips were tethered to surface strips and a small foam buoy using a 5-mm nylon cord to facilitate their retrieval. Strips were deployed in January 2016 and recovered between June and July 2016 for incubation times spanning from 154 to 179 days. After the deployment period, strips were retrieved and kept on ice until processing. In the lab, strips were brushed gently to remove sediments and epiphytes, soaked in freshwater for 20 min to remove salts, and air dried. Strip tensile strength was determined using a Dillon Quantrol Snapshot tensometer set to an expansion rate of 250 mm min⁻¹ and reported in Newtons (N).

To relate loss of tensile strength to weight loss, a separate, controlled experiment was conducted. Identical strips of artist canvas ($n = 190$) were cut, weighed to the 0.1 mg, and individually labeled. To induce controlled oxidation equally across replicates, strips were incubated at ambient outdoor temperature under low light in 30% H₂O₂. Fifteen strips were removed from the H₂O₂ bath daily for 12 days. Removed strips were lightly rinsed with water to stop the oxidation reaction, air dried, and reweighed. Tensile strengths for the

180 H₂O₂-treated strips and a control group of 10 strips were measured in Newtons (N) using a tensometer set up as described for the field experiment. We fit the relationship between tensile strength and weight lost to a logarithmic model:

$$\text{Wt loss} = a - b \log_{10}(\text{tensile strength})$$

where a is the y intercept and b is the slope of the relationship. We then applied this model to convert measurements of tensile strength of field-deployed canvas strips to mass loss.

Data Processing The Braun-Blanquet scale is an effective method for the rapid assessment of benthic coverage but it is both non-linear and categorical. Species-specific Braun-Blanquet scores were converted to percent coverage by assigning the median percent cover of each score's coverage range for each quadrat along the transect. Thus, a score of "5," representing 75%–100% coverage, was converted to 87.5% coverage. The calculated species-specific percent coverages at each quadrat were added together for total seagrass coverage. Species-specific and total seagrass coverage percentages were averaged across all quadrats from each site's 50-m transect ($n = 10$), then site-specific coverage density averages (in % cover) were averaged again across sampling campaigns over 2 years ($n = 4$) to account for both minor spatial and temporal variations in a site's seagrass coverage. Similar procedures were applied to categorical sediment type data; sediment categories were assigned numbers one through nine of increasing coarseness, where "1" is mud and "9" is rock (Table 1). These scores were averaged across a site's transect, then across sampling campaigns for a representative sediment score. Numerical scores were back-calculated to original categorical nomenclature for easy interpretation. Average canopy height for each site was calculated by averaging measurements across each site's transect, then averaging across sampling campaigns.

Data Analysis When making pairwise comparisons of continuous variables (seagrass coverage, C_{org} content, sediment mud content, etc), linear regressions among sites were used, abiding by assumptions of the analysis. C_{org} and breakdown rates were analyzed across gradients of sediment type, a categorical variable, thus ANOVA was used. The average tensile strength of recovered strips from each site and placement (exposed vs buried) was calculated and translated to percent weight loss. Breakdown rates (k , in units % day⁻¹) were then calculated using a first-order exponential decay function ($X_t = X_0 e^{-kt}$, where X was mass at time = 0 or time = t) (Olson 1963). Even though we only had initial and final weights, we used the first-order exponential decay model for easy comparison of breakdown rates to existing literature (Online Resource 1).

Correlations between RI values and mud content were tested using linear regressions, as were values of $\delta^{13}\text{C}$ of soil OM

compared with soil C_{org} content. The comparisons of $\delta^{13}\text{C}$ of soil OM to seagrass leaf tissue $\delta^{13}\text{C}$ were tested similarly where data was available. The assumptions of linear regression were not met for testing the correlation between C_{org} and RI. Considering the very different upper and lower boundaries of the relationship, a quantile regression was used, testing the correlation of quantiles ranging from 5 to 95%. Quantile regression estimates multiple slopes from the minimum to maximum response, providing a description of the relationships between variables that are missed by regression methods focused on prediction of the mean values (Ellison and Gotelli 2004).

Results

Seagrass Community Characteristics Sites sampled had water depths between 0.6 and 11 m deep (Table 2). Seagrass was present at 96% of sites during sampling period. *Thalassia testudinum* was the most commonly found species, present at 94% of sites, with *Syringodium filiforme* and *Halodule wrightii* found less frequently (at 49% and 34% of sites, respectively). At least one species of seagrass occurred along the transects at 95.7% of surveyed sites. Seagrass abundance ranged from 0 to 76.8% cover; the highest cover was found at a site dominated by *S. filiforme*. The canopy height of the seagrass beds ranged from 7.9 to 41.2 cm, with an average of 18.8 cm. Seagrass leaves exhibited a broad range in $\delta^{13}\text{C}$, with site averages ranging from -12.77 to -6.91‰ , with an average value of -9.35‰ .

Seagrass Soil Characteristics The soils across the south Florida seagrass landscape ranged in texture from fine muds in shallow, protected locations to coarse shell/gravel in more exposed locations (Table 3). On average, sediments were dominated by muds (silt + clay) in the soils across the landscape, but some sites had substantially coarser sediments with high sand and gravel content and as low as 1.4% mud grains. Our rapid field assessments of sediment texture correlated well with our grain size analyses. Sites with lower sediment index

Table 2 Seagrass community characteristics at study sites

	n	Mean	SE	Min	Max	Median
Water Depth (m)	46	3.8	0.4	0.6	11.0	3.0
<i>Thalassia</i> cover (%)	46	17.8	2.3	0.0	61	16
<i>Syringodium</i> cover (%)	46	7.9	2.2	0.0	73.3	0.4
<i>Halodule</i> cover (%)	46	1.5	0.6	0.0	22.3	0.0
Total seagrass cover (%)	46	27.1	3.0	0	76.8	20.7
Canopy height (cm)	44	18.8	1.2	7.9	41.2	17.3
Seagrass leaf $\delta^{13}\text{C}$ (‰)	42	-9.35	0.21	-12.77	-6.91	-9.08

Table 3 Soil characteristics at study sites. Units are percent of the dry mass of the original soil, by weight, unless otherwise noted

	<i>n</i>	Mean	SE	Min	Max	Median
Loss on ignition (%)	93	8.4	0.4	3.3	20.9	7.0
C_{org} content (%)	93	2.8	0.2	0.7	8.6	2.2
Dry bulk density (g cm^{-3})	46	0.7	0.01	0.2	1.5	0.7
Soils organic C density (mgC cm^{-3})	46	13.8	0.8	6.2	27.7	12.9
Soil organic $\delta^{13}\text{C}$ (‰)	50	-15.0	0.2	-17.7	-11.2	-14.8
Gravel (%)	92	2.2	0.3	0	12.4	0.8
Coarse sand (%)	92	7.7	0.5	0.9	22.7	6.8
Medium sand (%)	92	19.8	1.2	1.4	56.9	17.7
Fine sand (%)	92	29.6	1.7	4	81.9	26.7
Silt (%)	92	25.1	1.7	0	60.1	21.8
Clay (%)	92	15.5	1.0	1.4	39.3	14.5
Mud (%)	92	40.6	2.3	1.4	90.1	39.2
Field classification of grain size	44	2.7 = muddy sand	0.2	1 = Mud	5.3 = coarse shell	2.6 = muddy sand

scores (i.e., mud and sandy mud) had higher fractions of mud (Fig. 2a; ANOVA, $p < 0.05$) and generally lower dry bulk densities (Fig. 2b; ANOVA, $p < 0.05$). Organic matter content, as assessed by LOI, ranged from 3.3–20.9%, with a mean of 8.4%. C_{org} content averaged 2.8% with a range of 0.7–8.6% (Table 3). Organic carbon content was lower ($< 2\%$ dry wt) in waters deeper than 4 m and in more exposed areas found off the oceanside of the Florida Keys, whereas shallow waters nearshore and within Florida Bay generally had higher soil C_{org} . Soil DBD ranged from 0.2–1.5 g cm^{-3} , and soil C_{org} density averaged 13.8 mgC cm^{-3} , with a range of 6.6–27.7 mgC cm^{-3} . Sites with lower sediment index scores had higher C_{org} content and C_{org} density (Fig. 2c, d; ANOVA, $p < 0.05$). DBD was positively correlated with water depth (Fig. 3b; linear regression, $p < 0.01$, $r^2 = 0.55$) and lower mud content (Fig. 3d; linear regression, $p < 0.001$, $r^2 = 0.29$) with deeper sites consistently having coarser, denser sediments. Sites with a deeper water column had lower C_{org} stock than shallow sites nearshore and within Florida Bay (Fig. 3c; linear regression, $p = 0.006$, $r^2 = 0.16$). Soils from Western FL Bay had higher C_{org} content compared with Eastern Florida Bay and the Florida Keys (4.0 ± 0.3 compared with 2.3 ± 0.2 and $2.4 \pm 0.3\%$ dry wt, respectively; ANOVA, $p < 0.05$, Fig. 4a). Both Eastern and Western Florida Bay had higher mud content than sites along the FL Keys (ANOVA, $p < 0.05$; Fig. 4b). Mud content correlated positively with soil C_{org} content, but only for Florida Keys and Western Florida Bay regions (linear regression, $p < 0.05$, Fig. 4c). Soil organic matter had a wide range of $\delta^{13}\text{C}$ values, with a range from -17.7 to -11.2‰ with a mean of -15.0‰ (Table 3), which was $> 6\%$ lower than the mean seagrass leaf $\delta^{13}\text{C}$ (Table 2).

Relationships Between Seagrass Community and Soil Characteristics

There was no correlation between the

abundance (assessed as percent cover) and mud content, C_{org} content, or density of the soils for any of the three individual seagrass species (linear regression, $p > 0.10$). When species identity was disregarded and seagrasses were considered collectively, there was a weak, positive correlation between total seagrass abundance and mud content (Fig. 5b; linear regression, $p < 0.05$, $r^2 = 0.19$) and between canopy height and mud content (Fig. 5d; linear regression, $p < 0.05$, $r^2 = 0.39$). Sites with muddier sediments had higher C_{org} density (linear regression, $p < 0.05$) and mud content alone explained 34% of the variability in C_{org} stock among sites. There was a positive correlation between plant abundance and C_{org} density (Fig. 5a; linear regression, $p < 0.05$) with seagrass density explaining 13% of the variation in C_{org} density. Average canopy height was also positively correlated with soil C_{org} density (Fig. 5c; linear regression, $p = 0.001$, $r^2 = 0.24$). Even though the $\delta^{13}\text{C}$ of both the seagrass leaves and the sediment organic carbon varied by over 6‰, there was no relationship between the $\delta^{13}\text{C}$ of soil organic matter and the $\delta^{13}\text{C}$ of the seagrasses found at the sites, but there was a positive relationship between soil C_{org} content and soil $\delta^{13}\text{C}_{\text{org}}$ values (Fig. 6; linear regression, $p < 0.001$).

Assessing the Lability of Soil Organic Matter There was weight loss at all temperature steps from 180 to 600 °C in the ramped pyrolysis measurements of seagrass soils (Fig. 7a). The largest decreases in weight occurred between 180 and 300 °C and 400–500 °C where an average of $22.4 \pm 0.5\%$ and $25.1 \pm 0.7\%$ of the total weight loss occurred, respectively. The sequential LOI was low at 180 °C, and at higher temperatures there was a loss of mass of between 1.5 and 2.75% at each step. Mirroring the loss in dry mass, there was loss of C at every step in the ramped pyrolysis above 180 °C (Fig. 8a). Very little C was lost from samples at 180 °C, and the largest

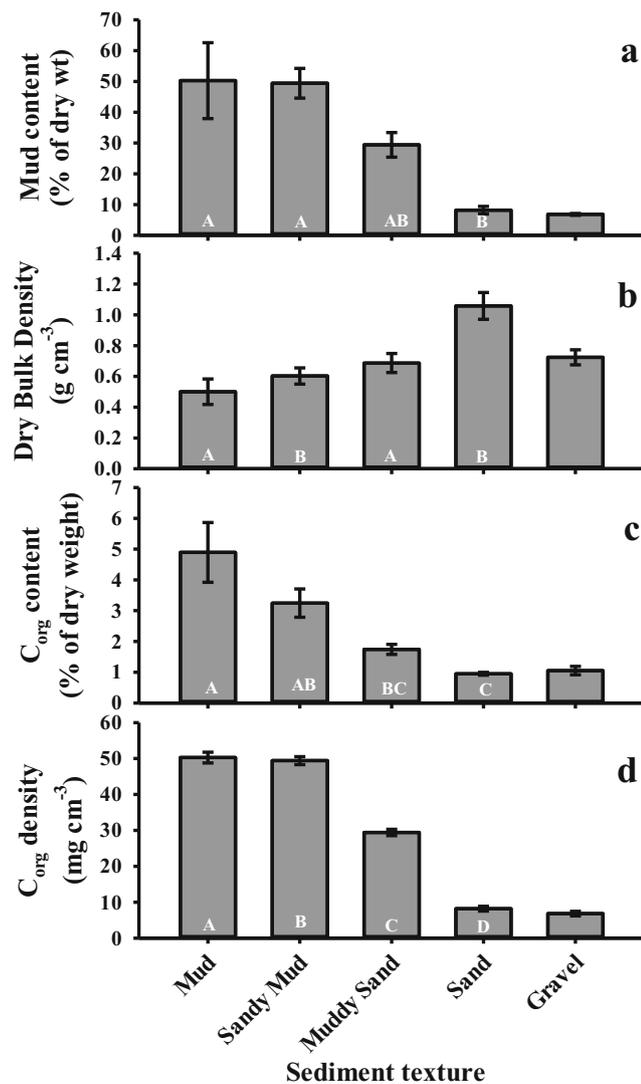


Fig. 2 Relationship between rapid field assessments of soil texture and sediment characteristics, as assessed with ANOVA. Soil texture represents averages across ten quadrats per site and four sampling periods from January 2015 and July 2016. Letters inside the bars represent groupings from Tukey post hoc tests. Only two sites had mean soil texture rated as “gravel” so this category was excluded from statistical analyses. Error bars indicate ± 1 SE

fraction of total C was lost at the 300 °C step ($30.0 \pm 0.9\%$, Fig. 8b). The average amount of C lost generally decreased at temperatures above 300 °C. The small dry mass lost when dry samples were treated at 180 °C was only ca. 10% C, compared with carbon accounting for $38.9 \pm 1.1\%$ of the mass lost at 300 °C (Fig. 8c). Above 300 °C, the ratio of C lost to mass lost generally decreased.

Recalcitrant organic carbon, as assessed by RI, made up between 28 and 80% of the total C_{org} at the sampled sites, with an average of $44.7\% \pm 1.2\%$ ($n = 92$). Muddy soils contained relatively labile C_{org} compared with soils with coarser sediments (Fig. 9a; linear regression, $p < 0.05$). RI values tended to decrease with increasing soil C_{org} content (Fig. 9b),

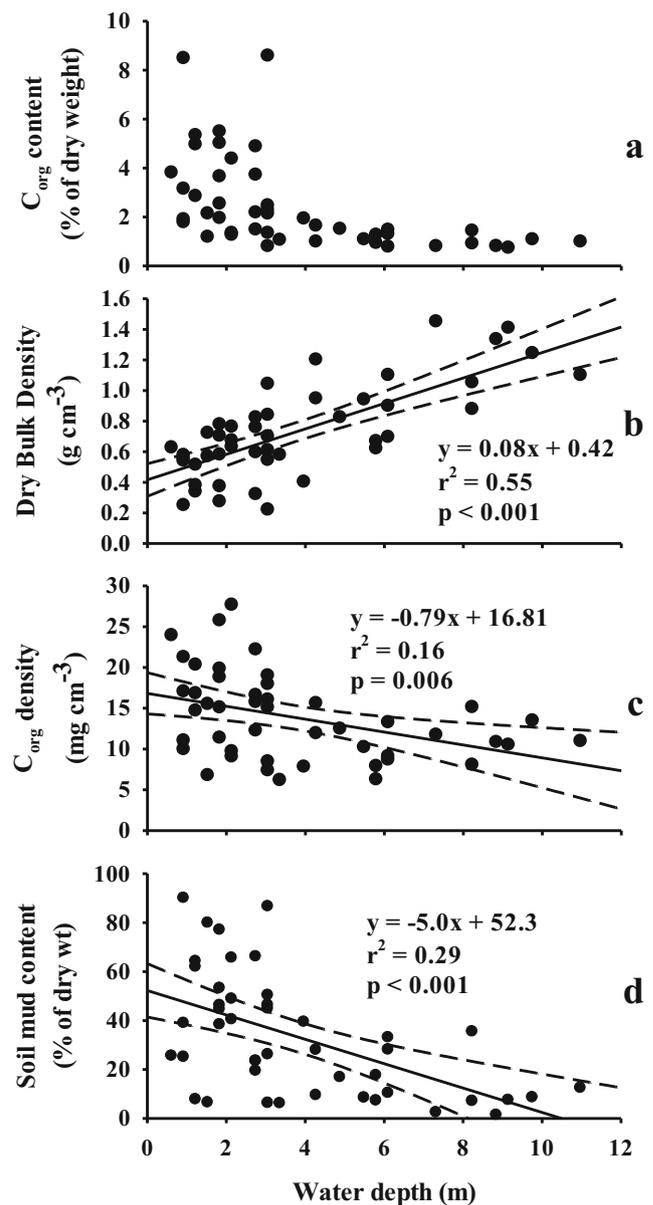


Fig. 3 Organic carbon content (a), dry bulk density (b), organic carbon density (c), and mud content (d) of soils as a function of water column depth. Linear relationships ($p < 0.05$) are indicated by fitted model, with 95% confidence interval displayed. No regression is shown for panel (a) because data did not meet the statistical assumptions

although this was not linear and the assumption of heteroskedasticity was not met. As C_{org} content increased, the range of RI values decreased, with most RI values being low when C_{org} content was high. In other words, there was a negative relationship between soil C_{org} and RI, but this relationship was not consistent across all study sites. Seagrass soils with a large fraction of seagrass-derived OM in them were more labile than those soils whose $\delta^{13}\text{C}$ values indicated their organic matter contained a smaller fraction of seagrass-derived OM. RI decreased (Fig. 10a) as the $\delta^{13}\text{C}$ of the soil C_{org} increased to approach the mean value of seagrass leaf

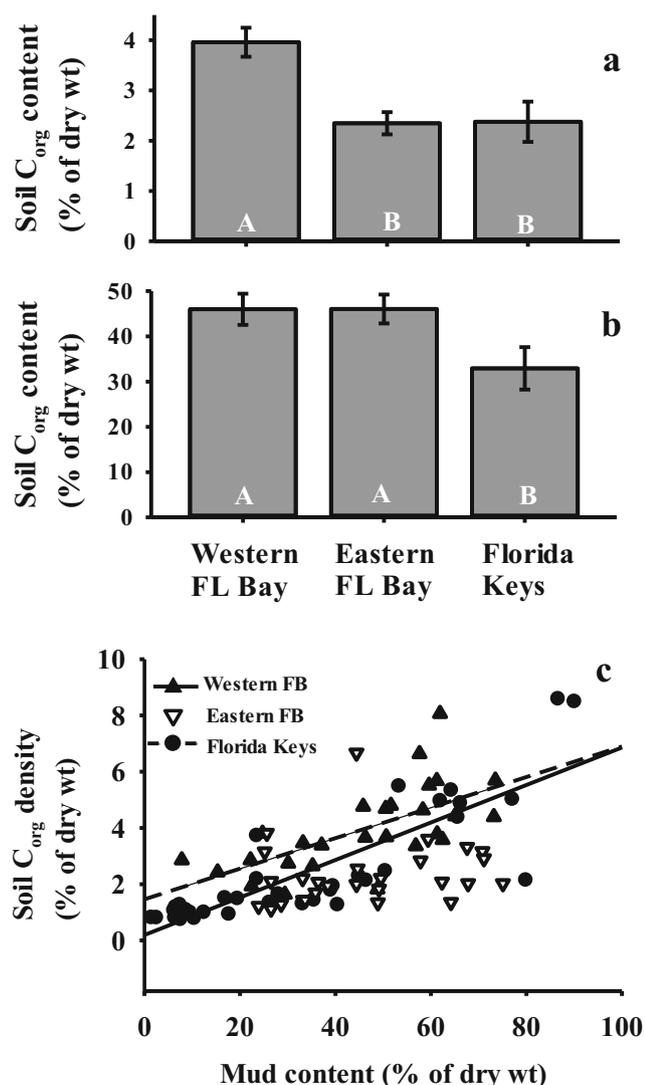


Fig. 4 Comparison of surface soil characteristics by geographic region in the Florida Keys. **a** Organic carbon content (C_{org}) and **b** mud content between three sampling regions. Figures represent mean \pm SE with letters representing groups determined by Tukey post hoc test. **c** Relationship between surface soil mud content and C_{org} content for three study regions. Lines represent significant correlations. Solid line shows relationship for the Western Florida Bay region, $y = 0.05x + 1.43$, $r^2 = 0.40$, $p = 0.002$; dashed line shows the relationship for the Florida Keys region, $y = 0.07x + 0.19$, $r^2 = 0.71$, $p < 0.001$. No regression line is shown for the Eastern Florida Bay region because the slope of that regression was not significantly different from 0

$\delta^{13}C$ found across our sites ($-9.35\text{‰} \pm 0.21\text{‰}$, Table 2). The greater the fraction of soil C_{org} that came from more depleted, non-seagrass sources (as indicated by the difference between the $\delta^{13}C$ of the seagrass leaves and the soil C_{org} at a site), the higher the RI of the bulk soil OM (Fig. 10b).

Organic Matter Decomposition Assays During the longest exposure to hydrogen peroxide in the lab calibration experiment (12 days), canvas strips decreased in tensile strength from an average of 238.7 ± 7.2 to 10.7 ± 1.4 N, and decreased in mass

from 741.9 ± 4.6 to 613.8 ± 8.4 mg. A logarithmic model was used to explain the relationship between tensile strength and weight loss, yielding a best fit equation of $w_{loss} = 29.5 - 12.4 \log_{10}(tensstrgth)$ with $R^2 = 0.77$, where w_{loss} represents percentage of weight loss of canvas strip and $tensstrgth$ represents the final tensile strength of deployed strip (Fig. 11).

Canvas strips were successfully retrieved from 23 of the sites, accounting for sites unsuitable for deployment and sites where deployed strips were lost. Based on the tensile strength of the recovered strips and the calibration curve above, breakdown rates, k , averaged $0.090 \pm 0.005\% \text{ day}^{-1}$ across all sites and conditions, ranging from 0.0019 to $0.162\% \text{ day}^{-1}$. Considering all 23 sites, there was not an effect of burial on breakdown rates (paired t test, $p > 0.05$; Fig. 12a), nor was there an effect of seagrass density (on surface or buried breakdown rates; linear regression, $p > 0.05$). Breakdown rates of surface-deployed strips were generally lower than buried strips on the southeastern, ocean-exposed side of the Florida Keys archipelago. Breakdown rates were generally lower for buried strips than surface strips in the areas of Florida Bay, the Gulf of Mexico side of the lower Keys, and other shallow sites. When sites were divided into sediment type categories, breakdown rates of buried strips increased with increasing sediment coarseness while rates for surface-deployed strips decreased with increasing coarseness (Fig. 12b; ANOVA, $p < 0.05$ for both comparisons). Breakdown rates in sediments categorized as “mud” and “sandy mud” were 39 and 22% lower for buried canvas strips relative to those deployed on the sediment surface (Fig. 12b; ANOVA, $p < 0.05$). For sites with coarser sediments (muddy sand, sand, and gravel), the opposite was true: breakdown rates of buried strips were at least 55% faster than surface-deployed strips (Fig. 12b; ANOVA, $p < 0.05$).

The breakdown rates of the model substrate buried in seagrass meadows were influenced by the characteristics of the soil. C_{org} content of the seagrass soils decreased as breakdown rates increased (Fig. 13a, linear regression; $p = 0.04$). Conversely, the recalcitrance of the C_{org} in seagrass soils increased as breakdown rates increased (Fig. 13b, linear regression, $p < 0.001$). At sites with high breakdown rates of buried OM, the soil C_{org} had less isotopic similarity to the seagrasses at the site than found at sites with low breakdown rates (Fig. 13c, linear regression, $p = 0.10$).

Discussion

Here, we showed that seagrass density and canopy height are related to surface soil C_{org} density, thus likely C_{org} stocks, across the South Florida seascape. However, sediment type and grain size (not necessarily driven by seagrasses) better explained variation in C_{org} soil density. We found that C_{org} source and sediment grain size both influenced soil C_{org} in

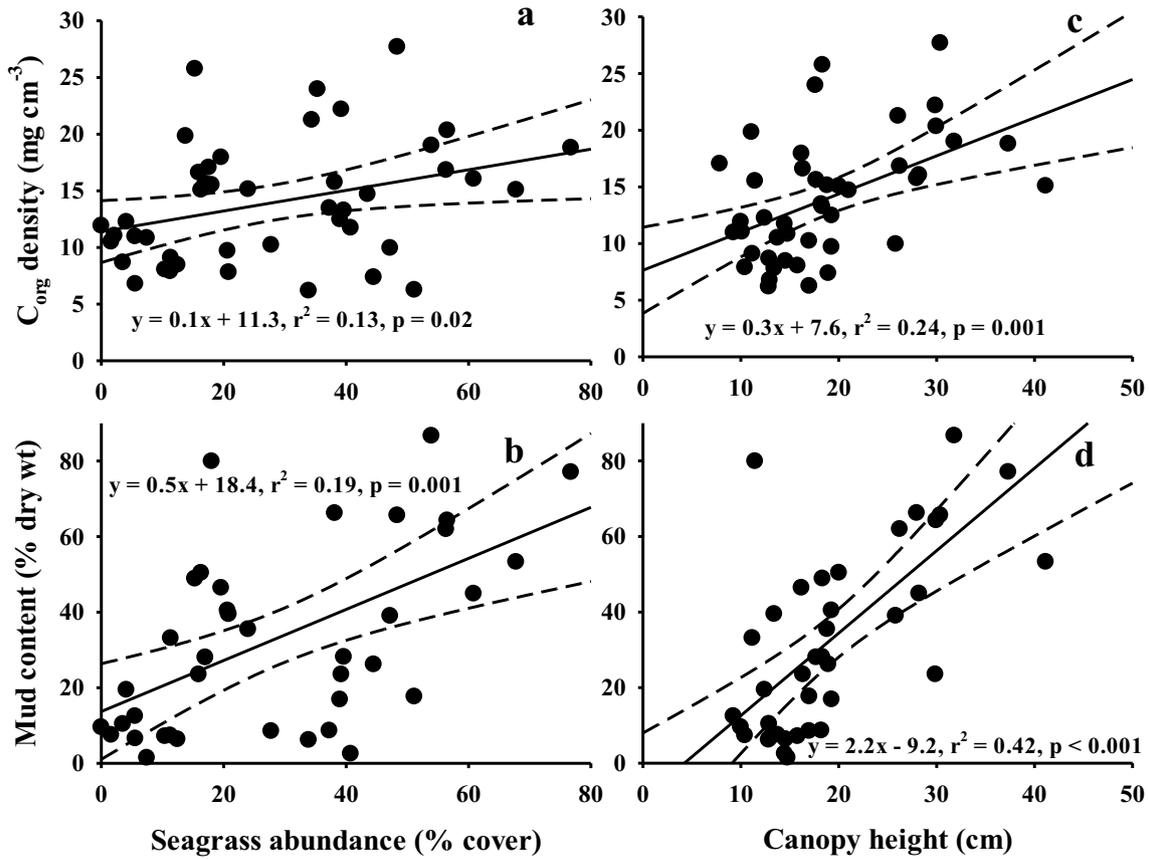


Fig. 5 Relationships between the soil characteristics of organic matter density (a) and mud content (b) and seagrass abundance expressed as percent cover, and relationships between the soil characteristics of

organic matter density (c) and mud content (b) and seagrass canopy height. Linear relationships are indicated by fitted model showing 95% confidence intervals

south Florida seagrasses communities, although the importance of each driver varied geographically. We also showed that not all soil C_{org} is equally at risk of remineralization, and that the fraction of soil C_{org} that is labile decreased as sediment

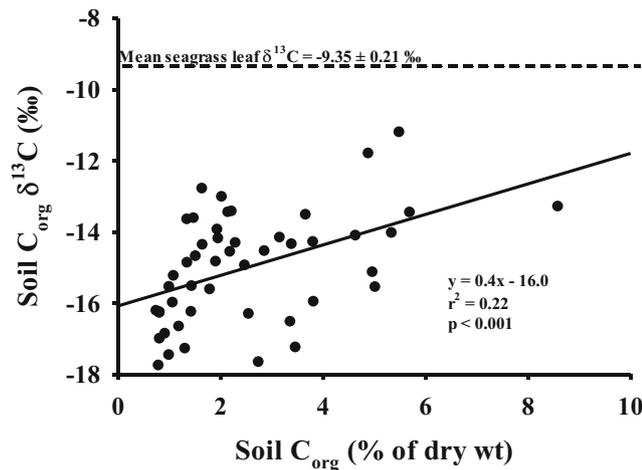


Fig. 6 Comparison of $\delta^{13}C$ values of soil organic matter compared with the C_{org} content of the soil, as % of dry weight. Mean $\delta^{13}C$ for seagrass leaves from all sites sampled included for reference. Trend line is linear regression

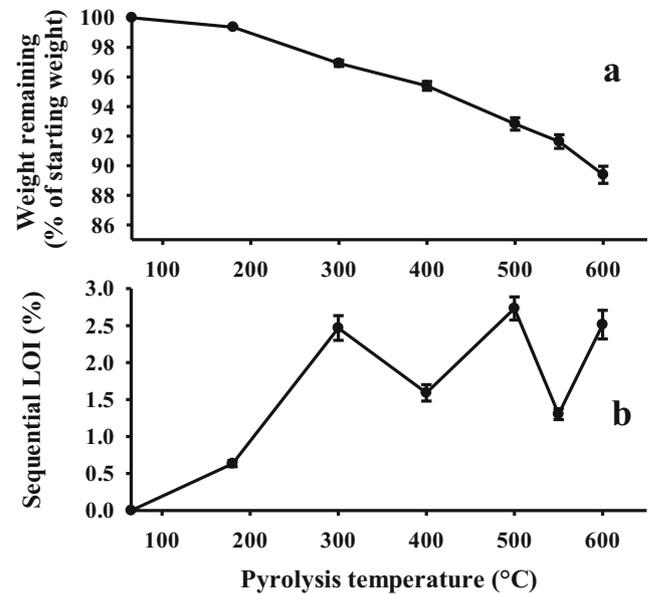


Fig. 7 Ramped pyrolysis of seagrass soils. **a** Average weight remaining (± 1 SE) following pyrolysis at increasing temperatures, $n = 92$. **b** Average weight loss at each step in the ramped pyrolysis (± 1 SE) as a percentage of the weight remaining at the end of the previous step

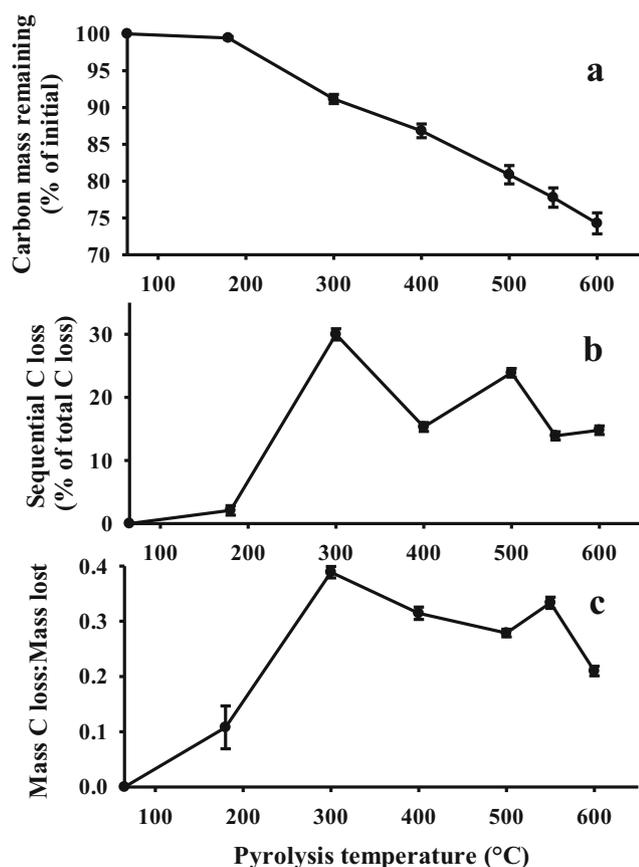
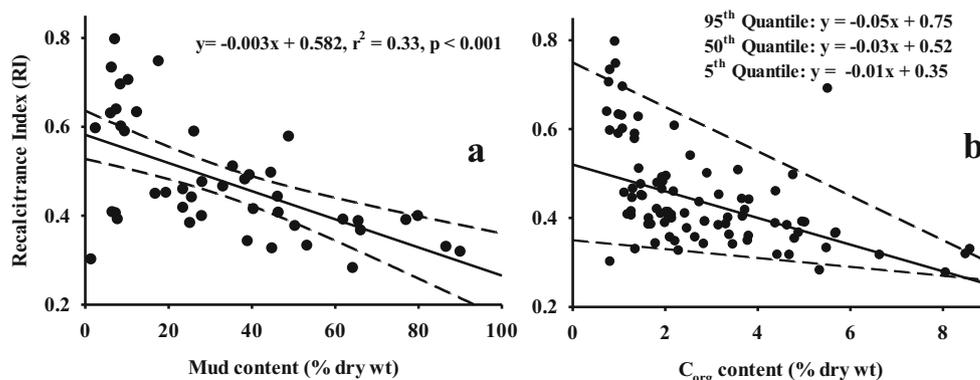


Fig. 8 Ramped pyrolysis of seagrass soils. **a** Average (± 1 SE, $n = 92$) fraction of the original C in the dry soils remaining after each temperature step. **b** Average (± 1 SE, $n = 92$) fraction of the total carbon loss occurring at each of the temperature steps ($n = 92$). **c** The average (± 1 SE, $n = 92$) ratio of mass of C lost to dry mass lost at each step in the ramped pyrolysis

grain size increased and as total C_{org} stocks decreased so that the C_{org} stocks in coarser soils, while lower than those in muddy soils, are more resistant to decomposition. Stable isotopic evidence suggested that seagrass-derived C_{org} is more labile than C_{org} derived from other sources in the south Florida seagrass landscape. Further, we found that soil characteristics, rather than seagrass characteristics, controlled OM breakdown rates. The general hypothesis that burial enhances OM preservation was not supported at all of our sites, rather only at a

Fig. 9 Ratio of C_{org} oxidized at 500 °C + 550 °C to the total organic C lost from 180–550 °C in the ramped pyrolysis (Recalcitrance index, RI) as a function of **a** mud content of the soils (% of mass by dry weight) and the **b** C_{org} content of the soils. Lines represent the **a** fitted linear regression and **b** fitted for regression for the 5%, 50%, and 95% quantiles



subset of sites with fine-grained, high C_{org} soils. Conversely, the breakdown of buried OM was enhanced compared with OM on the soil surface in coarse-grained, low C_{org} seagrass environments. We also found that soil C_{org} was more refractory when the decomposition rates of OM were rapid and that the contribution of seagrass-derived C_{org} to soil C_{org} stocks decreased as cellulose decomposition rates increased. These findings have direct bearing on the development of Blue Carbon strategies, as they bring into question the categorical importance of seagrass abundance and burial to C_{org} preservation, suggesting that only sites with fine sediments, regardless of seagrass presence, enhance preservation of C_{org} through burial.

C_{org} Storage in Seagrass Meadows of South Florida The soil C_{org} content across South Florida seagrass beds spanned the entire range of measurements taken globally with a similar median (2.2% vs 1.8% dry wt, respectively), and the DBD of south Florida seagrass soils was relatively low (0.70 ± 0.0 compared with 1.03 ± 0.02 g cm⁻³; Fourqurean et al. 2012a); these values agree closely with other surveys of local C_{org} and DBD in the region (Fourqurean et al. 2012b). Sediment grain size was a primary driver of soil C_{org} stock in seagrasses in our study, as found elsewhere (Dahl et al. 2016; Miyajima et al. 2017; Rohr et al. 2016), but in south Florida, the relationship was limited to Western Florida Bay and the FL Keys regions. Not only was there a lack of relationship between sediment grain size and C_{org} content in Eastern FL Bay, the high C_{org} content predicted by the high average mud content was absent. Eastern FL Bay is phosphorus limited, restricting autochthonous production and overall C_{org} input for the region (Armitage and Fourqurean 2016; Fourqurean et al. 1992). The fine sediments of the region have high surface area to volume ratios suitable for C_{org} sorption (Mayer 1994), although general lack of OM in the region causes C_{org} concentration to be low regardless of particle surface area and local hydrology. In contrast, Western Florida Bay has relatively high P availability and autochthonous seagrass production (Zieman et al. 1989) and is accumulating the fine carbonate sediments produced in the bay (Wanless and Taggett 1989)

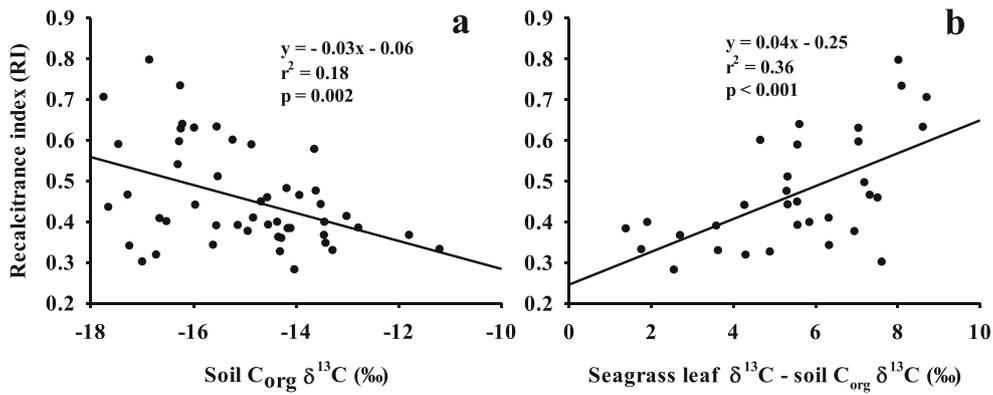


Fig. 10 Soil organic matter recalcitrance (as Recalcitrance Index, RI, the fraction of organic C that oxidizes at the highest temperatures) as a function of **a** the $\delta^{13}\text{C}$ signature of the soil organic matter, and **b** the difference between the $\delta^{13}\text{C}$ signatures of the seagrass leaves at the site

and the soil organic matter. Note that seagrass tissue has less negative $\delta^{13}\text{C}$ signature than other sources of organic carbon, hence the decrease in RI as $\delta^{13}\text{C}$ increases suggests that seagrass OM is more labile than that produced by other primary producers

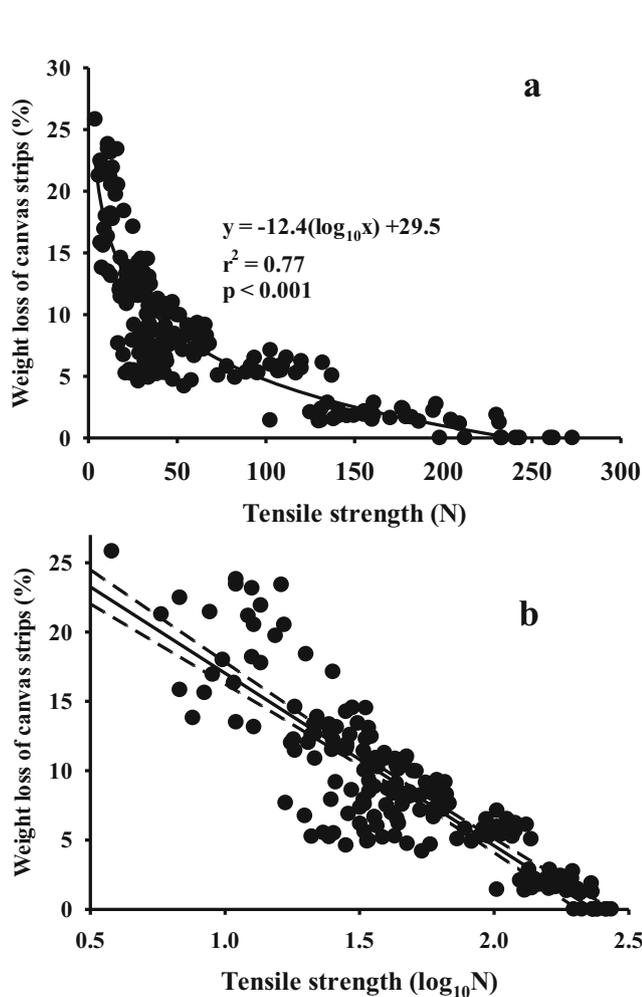


Fig. 11 Relationship between tensile strength and weight loss of canvas strips incubated in H_2O_2 for bench top calibration experiment. **a** Weight loss plotted as a function of final tensile strength, indicating the regression trend line of the data as a function of the \log_{10} (tensile strength), and **b** \log_{10} -transformed tensile strength as a predictor of weight loss, showing the regression line and the upper and lower 95% confidence intervals around the regression

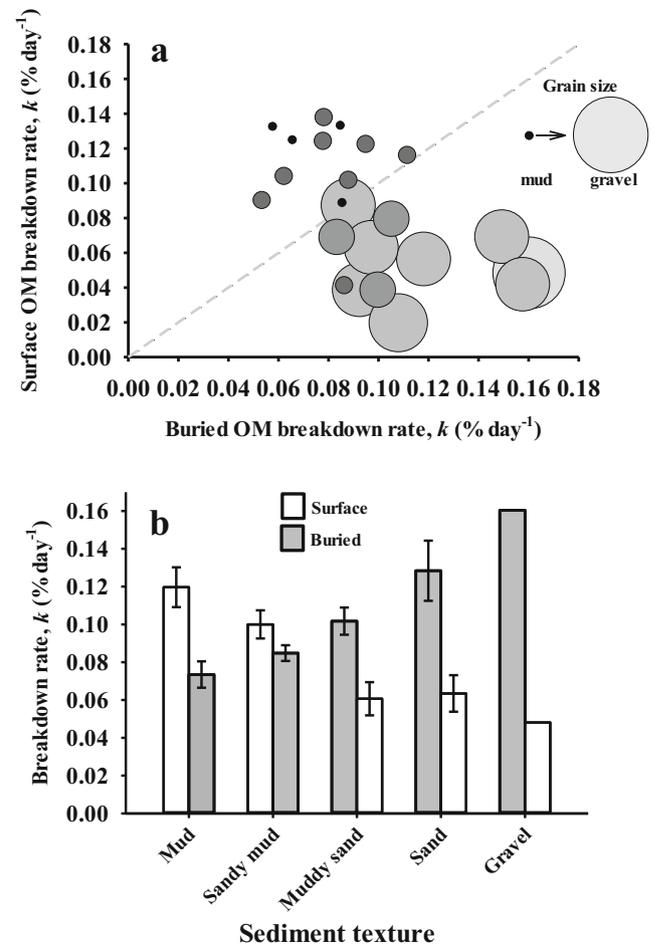


Fig. 12 Comparison of breakdown rates for canvas strip assays deployed at 20-cm depth (buried) and at the sediment-water interface (surface). Dashed gray line indicates 1:1. **a** Comparison between decay rates for buried and surficial samples by site, with the size of the symbol scaled by relative sediment grain size, and **b** comparison between decay rates for buried and surficial samples binned into categories of sites with different sediment texture. Bar heights are means, error bars represent ± 1 SE. No error bars are given for the “gravel” category because only one site fell into this category

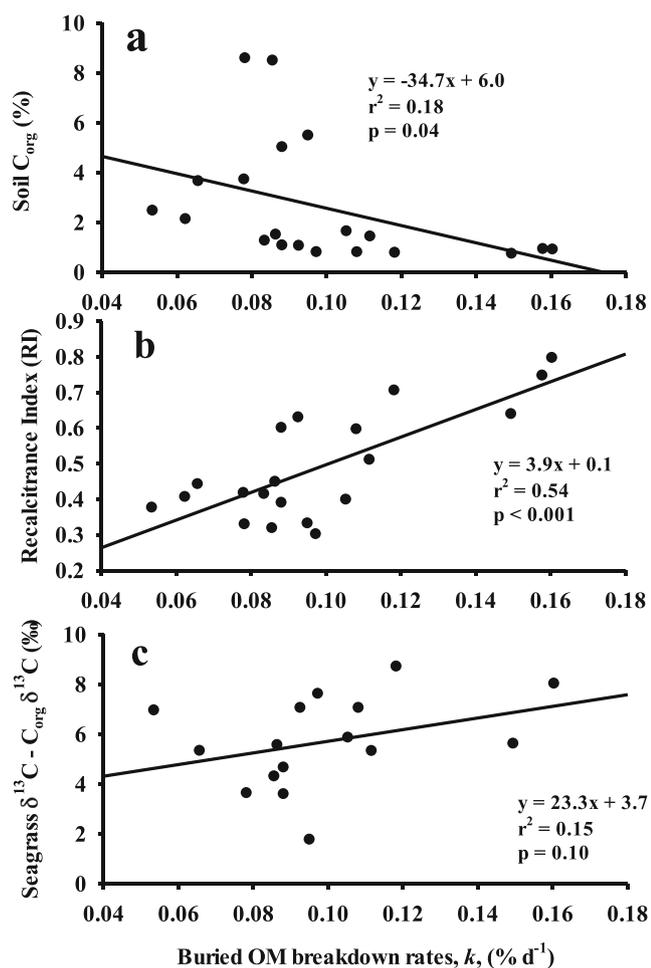


Fig. 13 Relationships between breakdown rates of a buried model organic substrate (canvas strips) and soil properties across the seagrass landscape. **a** Site soil C_{org} content as a function of the breakdown rate ($\% \text{ day}^{-1}$) of canvas strips; **b** recalcitrance of remaining soil C_{org} as a function of the breakdown rate ($\% \text{ day}^{-1}$) of canvas strips at sites; **c** the difference between the $\delta^{13}\text{C}$ signatures of seagrass leaves and soil C_{org} at a site as a function of the breakdown rate ($\% \text{ day}^{-1}$) of canvas strips. Greater differences between $\delta^{13}\text{C}$ of seagrass leaves and soil suggest higher contribution and/or preservation of OM derived from other primary producers. Trend lines are the linear regressions, regression statistics given in the panels

which results in high soil C_{org} content. The Florida Keys region varies greatly in benthic ecology and sedimentology, although the lower average mud content and larger grain size in the region may explain the lower C_{org} content of Eastern compared with Western Florida Bay. All of our study sites had at least 0.7% C_{org} by weight, even in nutrient limited regions. C_{org} delivery may be low in some of our study area, but the carbonate sediment particles found here should accumulate some background C_{org} concentration because C_{org} can be incorporated into their mineral matrix (Mayer 1994).

The positive correlation between seagrass presence and C_{org} storage we documented across South Florida has been noted elsewhere (Armitage and Fourqurean 2016; Macreadie et al. 2015). In our work, this trend was only significant when

total seagrass density, rather than species-specific densities, was considered. Seagrass species vary in C production, plant C allocation, and tissue recalcitrance (Howard et al. 2016; Trevathan-Tackett et al. 2017a); thus, we expected C_{org} stocks to relate to seagrass community composition as this has been observed elsewhere (Lavery et al. 2013). However, if soil C content was driven primarily by the decrease in shear stress at the sediment surface provided by seagrass canopy complexity, individual species presence or density would not matter as much as the overall canopy complexity produced. The presence of seagrass has been shown to decrease grain size in sediments, in turn altering the soil C_{org} content (Bos et al. 2007; Miyajima et al. 2017); we noted positive, albeit weak, relationships between both seagrass density and canopy height with mud content in our study region. Alternatively, seagrass characteristics and C_{org} density could be strictly correlative, where proximity to land and water depth affect seagrass morphology, density, and sediment characteristics simultaneously via altered hydrodynamics, nutrient availability, and light availability (Hemminga and Duarte 2000; Schanz and Asmus 2003). If this is the case, both seagrass characteristics and C_{org} stocks could be dependent on local landscape configuration and geomorphology. Sediment characteristics better explained variations in C_{org} stocks than did seagrass density. We found that finer, less dense sediments contained higher C_{org} density, supporting previously published work (Dahl et al. 2016; Gullstrom et al. 2018; Rohr et al. 2016; Serrano et al. 2016a). Like Serrano et al. (2014), we found that deeper sites contained lower C_{org} density, although we explain the low soil C_{org} content soils at these sites by their coarse sediment resulting from a higher energy environment, rather than the lower carbon inputs stemming from lower light availability. Conversely, many of the shallow muddy sites found sheltered between small islands and embayments had the highest C_{org} stocks. These high C_{org} sites are positioned within a matrix of land, protecting sediments from high wave energy and water velocity which promotes an environment more conducive to the settling of fine, low DBD sediment particles (Rohr et al. 2016). Two sites within the extremely P-limited portion of Florida Bay where water hydrodynamics allow for the accumulation of fine particulates but nutrient limitation suppresses C_{org} production (Fourqurean and Zieman 2002) were exceptions to this general pattern. Apparently, P availability in inner Florida Bay is so severe that soil C_{org} density can be driven by seagrass characteristics and primary productivity rather than hydrologically driven sediment characteristics that dominate the South Florida seascape more broadly (Armitage and Fourqurean 2016).

Assessing the Lability of Soil Organic Matter Popular methods of OM determination of biomass and soils rely on LOI, but different oxidation temperatures are used in different

laboratories, ranging from 360 to 550 °C (Dean 1974; Fourqurean et al. 2012a; Howard et al. 2014; Kristensen and Andersen 1987; Wang et al. 2011). While the loss-on-ignition determination method is used to partition organic from inorganic material, loss of volatile salts, structural water of clay minerals (not clay-sized particles as present in our samples), and/or metal oxides can also confound LOI data as they are also lost during thermo-oxidative processes (Heiri et al. 2001). This could result in weight loss at temperatures lower and higher than the assumed oxidation temperature for organics (Kristensen 1990; Wang et al. 2011). Our samples subjected to stepwise higher temperatures continued to lose mass at every temperature step, and concomitant with mass loss was C loss, indicating that this mass lost at high temperatures was at least partly organic matter. At all temperatures above 180 °C, the C lost:Mass lost was 30–40%, consistent with the loss of high C content organic matter. Interestingly, this ratio decreased with increasing temperature, perhaps giving clues to the changing nature of the organic matter oxidized at each step. Organic matter was lost at 600 °C from samples that had been previously combusted at 550 °C, suggesting that even the relatively high combustion temperature of 550 °C underestimates OM content by $\approx 10\%$ on average. Hence, the temperature of combustion must be considered when comparing LOI data between studies.

Thermal stability of biogenic material has been used as a proxy for its propensity for decomposition, where higher oxidation temperatures correspond to higher recalcitrance (Capel et al. 2006; Kristensen 1990; Persson et al. 1986; Trevathan-Tackett et al. 2017a). Known discrepancies between LOI and C_{org} content (Wang et al. 2012), along with a primary interest in the C_{org} portion of OM (Fourqurean et al. 2012a), have led the LOI methodology to be coupled with elemental analysis to measure C loss rather than weight loss alone (e.g., Campbell et al. 2015; Fourqurean et al. 2012b). There was a tight correlation between temperature-specific weight loss and computed C loss at all temperatures besides 160 °C, and mass lost was only about 10% C at 160 °C, suggesting that weight loss at this low T can be partially attributed largely to water loss. Temperature steps at 300 °C and 400 °C had the highest C loss:Mass loss ratios indicating that weight loss at these temperatures also included the highest fractions of C loss. These are the temperatures at which C-rich organic matter (i.e., cellulose) oxidizes (Kristensen 1990). Higher temperatures (500 and 550 °C) have lower C loss:Mass loss, suggesting that C is still lost but less of the total weight can be attributed to C-rich organics.

There were two peaks of carbon loss during the ramped pyrolysis (300 and 500 °C), consistent with previous reports of bimodal combustion patterns for seagrass tissue (Trevathan-Tackett et al. 2017a) and biogenic material in marine sediments (Kristensen 1990). We interpreted this pattern as two primary OM oxidation points with the lower peak

corresponding to low molecular weight volatile compounds and relatively more labile macromolecules. The second peak was interpreted as the oxidation point of char and polyphenolic compounds like lignin, humic substances, and kerogens that are thermally more stable. These compounds are also more resistant to microbial driven decomposition (Arndt et al. 2013; Burdige 2005). We quantified the relative fraction of the C_{org} that was composed of the more recalcitrant compounds with our RI. There was a negative correlation between mud content and RI values, suggesting that soils with a higher mud content and lower average grain size have a relatively higher fraction of labile C_{org} . Many authors have attributed low OM breakdown in muddy sites to decreased microbial decomposition compared with sites with coarser sediments (e.g., Burdige 2007; Cook et al. 2007; Huettel et al. 2014; Silburn et al. 2017), which could explain why such high proportions of labile, microbially accessible C_{org} remained at muddy sites. There was also a negative relationship between RI and total soil C_{org} content. At our sites, high C_{org} soils had proportionally more labile C_{org} , but low C_{org} sites exhibited a large range in RI. Sites with high C_{org} content are likely to contain C_{org} that is vulnerable to remineralization during environmental change or over time. Low C_{org} may be similar, although higher RI values at some sites suggest that soil C_{org} may be resistant to microbial decomposition regardless of environmental setting. Our minimum measured RI value of approximately 0.3 suggested that approximately 30% of C_{org} is relatively refractory. This may be related to a background level of recalcitrant C_{org} associated with mineral surface area and the carbonate matrix (Mayer 1994). Seagrass soil C_{org} content varied 18-fold among sample sites, although the differences in C_{org} recalcitrance among sites suggest an even greater variance in potential CO_2 produced during remineralization.

The stable carbon isotopic composition of seagrass biomass and soil C_{org} across our sampling landscape suggest that seagrass-derived organic matter is relatively labile and that OM produced either by other primary producers within the landscape or imported from allochthonous sources is more recalcitrant. Seagrass tissue $\delta^{13}\text{C}$ values across our landscape averaged $-9.35 \pm 0.21\text{‰}$, comparable with previously reported values for the region (Campbell and Fourqurean 2009; Fourqurean et al. 2005). Seagrass $\delta^{13}\text{C}$ values are typically distinct from other sources of OM, thus their relative contribution to higher trophic levels and OM pools can be determined (Fry et al. 1977). Using a stable isotope mixing model approach, on average the C_{org} in seagrass soils, is about 50% derived from seagrass primary production globally (Kennedy et al. 2010). Correlations between seagrass $\delta^{13}\text{C}$ and soil $\delta^{13}\text{C}_{\text{org}}$ have been shown globally (Kennedy et al. 2010) and regionally (Howard et al. 2018) but we found no such correlation across our seagrass landscape. The lack of correlation could be due to a high homogenization and mixing of

sediments in the region; this was the suggested mechanism for similar soil $\delta^{13}\text{C}$ values within plots of differing macrophytes at the scale of meters (Howard et al. 2016), although is questionable given the wide geographic range of our study sites. The lack of correlation between seagrass and soil $\delta^{13}\text{C}_{\text{org}}$ could also be related to spatial variation in non-seagrass inputs (Oreska et al. 2017), but it is more likely that seagrass organic matter is more labile than the isotopically distinct non-seagrass components of the soil C_{org} . We did find a correlation between soil C_{org} content and soil $\delta^{13}\text{C}_{\text{org}}$. At high C_{org} concentration, soil $\delta^{13}\text{C}_{\text{org}}$ approached the typical seagrass $\delta^{13}\text{C}$ value suggesting that at sites with high C_{org} that seagrasses were the major contributor to soil C_{org} . RI decreased with increasing soil C_{org} content, and sites with low RI values were ones with smaller differences between the $\delta^{13}\text{C}$ of seagrasses and the $\delta^{13}\text{C}$ of soil C_{org} . Seagrasses do produce a lot of organic matter, but we conclude that seagrass-derived biomass is relatively labile compared with other sources that end up preserved in the sediments in places where decomposition leads to low remnant C_{org} content of the soils. It is also possible that decomposition itself could drive seagrass-derived C_{org} to be less enriched in ^{13}C than fresh seagrass biomass. Differences in the $\delta^{13}\text{C}$ values of polysaccharides and lignin across various plants could result in shifts in the isotopic composition of bulk OM and may occur as particular OM fractions are preferentially decomposed (Benner et al. 1987). Further, early diagenesis of newly buried seagrass biomass leads to a preferential loss of ^{13}C and a decrease in the remaining $\delta^{13}\text{C}_{\text{org}}$ (Fourqrean and Schrlau 2003).

Controls of Organic Matter Decomposition Burial in stable, anoxic environments understood to be created in seagrass soils is thought to suppress decomposition and remineralization of OM, particularly that of more refractory compounds (Burdige 2007; Lovelock et al. 2017b). However, we found no general trend for the preservation of buried OM compared with exposed material, with approximately half of sites displaying faster breakdown rates at 20-cm depth, below the rhizosphere, compared with the sediment-water column interface. The categorical importance of burial to OM decomposition has been repeatedly questioned, giving way to a more complex hypothesis where enhanced decomposition by O_2 exposure and the priming effect is only important to certain types of OM (Bianchi 2011; Burdige 2007; Hulthe et al. 1998; Kristensen et al. 1995).

The vast majority of previous studies of OM decomposition use litter bags to measure breakdown rates, despite well-known weaknesses of the approach (Wieder and Lang 1982). Variations of the litter bag methodology make use of raw, natural materials that are naturally inconstant between species and sources. We avoided these obstacles by minimizing any variance in recalcitrance of the starting OM through the use of standardized organic material, artist's canvas (Tiegs et al.

2007). The litter bag method may further introduce measurement variation by the change in material mass not related to microbially mediated decomposition, like material gain and loss through pores and detritivorous feeding, that could lead to a misestimation of decay rates (Moran and Hodson 1989; Valiela et al. 1985). The rates of decomposition we measured with our cotton strip assay were relatively low compared with those previously reported for seagrass ecosystems, although still within the reported range of 0.02–3.94% day^{-1} , mean = 1.2% day^{-1} . (Online resource 1). Unintended weight loss from litter bags, the common methodology in the literature, could explain our comparably low decay rates, although there could be another explanation. The standard organic substrate used for the cotton strip assay is > 95% cellulose (Tiegs et al. 2013), dominated by a single type of OM found among a plethora of other compounds found in soils, all varying greatly in their recalcitrance. Canvas strips do not capture the full range of recalcitrance of naturally occurring seagrass OM, particularly the more labile OM fractions. These fractions of fresh OM can be leached out or quickly mineralized regardless of redox conditions (Harrison 1989). The lack of these OM fractions in our canvas strips would likely result in decay rates lower than typically found in previous studies that use fresh and whole tissue OM. Trevathan-Tackett et al. (2017b), for example, used dead leaf detritus rather than fresh material, resulting in a lower leaf decay rate. Given their rapid loss rates, these labile OM fractions excluded from our experimental design are unlikely to be important for long-term C_{org} storage. However, the canvas strip assay is not without its methodological shortcomings. While cellulose is one of the more recalcitrant organic compounds produced by seagrasses, the canvas strips also lack the complex, even more recalcitrant OM fractions that could be important to long-term storage (Trevathan-Tackett et al. 2017a). The canvas strip assay has been used previously in comparative studies in multiple ecosystems (Harrison et al. 1988; Kirwan et al. 2014; Tiegs et al. 2007), although never before has it been converted to the commonly used units of weight loss. Our lab bench calibration experiment using H_2O_2 as an oxidant yielded a clear correlation between tensile strength and weight loss ($r^2 = 0.77$), though the mechanisms of chemical oxidation versus enzymatically mediated breakdown of cellulose polymers are likely very different, which could lead to different relationships between tensile strength and mass loss in photo-oxidized or enzymatically decomposed samples.

Burial did not decrease decomposition rates at all of our sites. The seagrass literature contains decay rates spanning two orders of magnitude (see Online resource 1 and reviews by Harrison 1989; Trevathan-Tackett et al. 2020), although the variation relies on study methodology, type of substrate, and environmental conditions that do not typically address burial. In Florida Bay, decomposition rates differ among plant types but not between buried and surface-deployed litter bags

(Fourqurean and Schrlau 2003), although Godshalk and Wetzel (1978) did note faster decomposition rates in aerated conditions, but only at the highest experimental temperature. The stable, anoxic conditions of seagrass soil may encourage the long-term preservation of soil C_{org} , although there is little empirical support of universality or effect size.

In our work, burial depressed OM breakdown rates by an average of 39% at sites with muddy sediments and 22% at sites with sandy mud. Sites with coarser sediments (muddy sand, sand, and gravel) showed the opposite trend, where buried canvas strips had accelerated rates of breakdown compared with strips deployed on the sediment surface. This may be explained by advective processes occurring in highly permeable, sandy sediments that allow solutes (O_2 , nutrients, labile DOM, etc) to be transported through the sediment depth profile and to decrease OM preservation (Burdige 2007; Janssen et al. 2005). In the sandy, coarse-grained carbonate sands of the Bahamas (geologically similar to the oceanside of the Florida Keys in our study area), O_2 is supplied to the soils via pore-water advection to depths over 20 cm (Burdige and Zimmerman 2002). Pore-water exchange of solutes through coarse sediments leads to faster turnover rates of organics introduced to sediments and lower C_{org} retention (Huettel et al. 2014). Fine-grained, muddy sediments have lower porosity and pore-water exchange, leading to decreased solute penetration and a reliance on sulfate reduction rather than aerobic decomposition (Cook et al. 2007; Silburn et al. 2017). Breakdown of buried OM (including deployed canvas strips) in fine muds is thus only a fraction of that in coarse sediments. The maintenance of uneroded, buried OM is important to its preservation at some sites as previously suggested (Fourqurean et al. 2012a; Lovelock et al. 2017b), although possibly only at those with stable, fine-grained sediments.

Breakdown rates of surface-deployed canvas strips were notably faster in fine compared with coarse sediments, suggesting that decomposition rates of OM are not universally slower in fine-grained depositional environments. This pattern is the opposite of what would be expected if settling of fine-grained material in more quiescent environments would lead to accumulation of fines and subsequent anoxia of surface-deployed strips compared with higher energy, coarser grain sites. This pattern also indicates that physical abrasion of the surface-deployed strips did not lead them to break down faster in higher energy environments. Oxygen uptake rate by microbial decomposition on the sediment surface is negatively correlated with grain size, thus sediments containing higher C_{org} content and greater surface area for microbial substrate have faster breakdown rates (Deflaun and Mayer 1983; Hargrave 1972; Huettel et al. 2014). Faster decomposition and consumption of solutes at the sediment surface in fine sediments can lead to fewer solutes being transported through the sediments for bacteria to use when remineralizing buried C_{org} ; this

compounded with limited permeability of muds creates a large gradient in oxygen consumption and decomposition down the sediment profile (Silburn et al. 2017). As a result, soils with higher decomposition rates had lower C_{org} content, and the C_{org} that remained at such sites was more refractory than that from sites with slower decomposition rates.

Implications for Management and Blue Carbon Strategies

Degradation of seagrass ecosystems may lead to significant carbon losses and emissions of CO_2 as carbon is oxidized, thus a primary justification for seagrass conservation in blue carbon strategies is to maintain the stable, anoxic sediments they create (Lovelock et al. 2017a, b). Our results indicate that neither seagrass presence nor abundance is consistent drivers of C_{org} density in coastal environments, and that burial of OM does not necessarily decrease its breakdown rate and enhance OM storage. For C_{org} stocks to be important to climate mitigation and blue carbon management, we need to understand whether C_{org} stocks can be remineralized, the environmental conditions that control remineralization, and best management practices that could keep C_{org} sequestered. Here, we found that environmental context can be more important than plant characteristics to C_{org} storage and preservation, and only at a subset of sites does seagrass density directly influence soil C_{org} stocks, where seagrass presence leads to a decrease in sediment grain size *and* where grain size is fine enough to slow decomposition of buried compared with surficial soil OM. Burial of OM in coarse-grained sediments does not enhance C_{org} preservation, and the fine-grained sediments that promote OM preservation can be established in certain locations without the presence of plants. We also show that differences in C_{org} vulnerability are linked to molecular characteristics inherent to OM. The environmental context provided by seagrasses may help preserve C_{org} stocks in some areas, although large fractions of C_{org} stocks in may be too recalcitrant to be oxidized, regardless of changes in the surrounding environment. The relative contribution of labile C_{org} varies with mud and total C_{org} content, suggesting that soils with fine grain sizes have more C_{org} as well as relatively more labile C_{org} than their sandy counterparts, making them priority targets for conservation under blue carbon strategies.

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