CARBON STORAGE IN TIDAL MARSH SEDIMENTS IN THE BAY OF FUNDY: THE ROLE OF VEGETATION AND DEPTH

by

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ABSTRACT

Tidal marshes have the ability to sequester and store atmospheric CO_2 and thus contribute a valuable ecosystem service. Globally, tidal marshes have declined due to environmental damage and habitat conversion—however, restoration has become a promising mode of revitalization of these ecosystems. Little is known about carbon storage differences between restored and natural marshes or the factors that influence carbon storage in these systems. This study compares belowground carbon stocks in three tidal marshes (new restoration, old restoration, natural reference). Carbon content was sampled using a Russian peat corer at three locations in *Spartina alterniflora* vegetation at each marsh. Two sediment cores were taken at each sampling location, one from an area with live plants and one from bare mud, and each core subdivided into three depths: surface (<3cm), rhizosphere (3cm-30cm) and below-rhizosphere (<30cm). Statistical analysis showed that depth had no significant effect. Given this, it appears that the depth at which carbon is buried does not impact long-term carbon storage within tidal marshes. The older restoration and natural sites contained a similar amount of buried carbon as the new restoration site. There was no significant difference in carbon storage between vegetated versus unvegetated areas across all marshes. Further studies should explore the role of sedimentation and its influence on carbon storage within these systems. In addition, the impact of climate change should also be monitored within tidal marshes to ensure correct methods for conservation and restoration are being employed.

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INTRODUCTION

1.1 Blue carbon

We are facing a climate crisis which is the result of an increase in CO_2 in the atmosphere (McLeod et al., 2011; IPCC, 2018). Factors such as the combustion of fossil fuels, deforestation, and agriculture are drivers of this net increase in CO_2 being released into the atmosphere (McLeod et al., 2011; Pendleton et al., 2012). The Intergovernmental Panel on Climate Change (IPCC) has stated that to prevent climate-related risks to natural and human populations, the global mean temperature cannot rise more than 1.5^{o}C by the year 2100 or the damage done to specific ecosystems would be irreversible (IPCC, 2018). At the current rate of warming, models suggest that the Earth will warm by the 1.5^{o}C between 2030 and 2052 (IPCC 2018). Previously, it was believed that the only way to prevent the Earth from warming would be to drastically reduce CO_2 emissions by overhauling the mechanisms which released the greatest amount of CO_2 into the atmosphere (McLeod et al., 2011). Within the last decade, a multifaceted approach involving the reduction of CO_2 emissions, the conservation of ecosystems which ‘sequester’ or remove high volumes of atmospheric CO_2, and the restoration of ecosystems to increase their net carbon accumulation rate has been suggested and implemented as a more effective method to maintain our global climate (McLeod et al., 2011; Wollenberg et al., 2018; Burden et al., 2019).

The carbon stored in the plant material and sediment of a coastal ecosystem is collectively referred to as ‘blue carbon’ (Wollenberg et al., 2018). Ecosystems can accumulate carbon in their vegetation and sediments over time, making them efficient natural carbon sinks (Howard et al., 2017). In addition, coastal ecosystems can accumulate carbon through minimal human intervention, making them valuable as potential nature-based solutions to mitigate climate change through carbon storage (Smeaton et al., 2020). Studies on carbon storage have primarily focused on oceans and terrestrial forests (Howard et al., 2017). While research continues in these ecosystems, coastal ecosystems, such as tidal marshes and mangrove forests, are now being recognized for their potential to store sequestered carbon long-term (Howard et al., 2017). While both these methods sequester carbon from the atmosphere, carbon in the biomass of the plant is only stored temporarily as it is released when the plant dies at the end of the growing season (Howard et al., 2017; Abbott et al., 2019). However, long-term storage of carbon in coastal ecosystems occurs in the sediment.

1.2 Tidal marshes and their global decline

Tidal marshes are highly efficient at sequestering and storing blue carbon (Wollenberg et al., 2018). Periods of flooding from the rise and fall of the tides result in slow rates of decomposition due to low oxygen, which prevents microbes from breaking down the debris stored in marshes (Pendleton et al., 2012; Howard et al., 2017; Wollenberg et al., 2018). Coastal ecosystems store organic matter, which is largely composed of plant debris (van Ardenne et al., 2018). Of this stored organic matter, approximately 55%-60% is buried carbon within tidal marshes (Cagnarini et al., 2019). In addition to carbon storage, tidal marshes provide other ecosystem services essential to human life, such as pollutant collection, flood defense, erosion control, and they provide a habitat for many species of animals and invertebrates (Burden et al., 2013).

As tidal marshes begin to expand vertically and laterally through the accumulation of stored carbon and debris, deeper depths within the marsh begin to accumulate larger amounts of carbon (Wollenberg et al., 2018; Cagnarini et al., 2019). Overtime, a gradient is established within the layers of sediment found in tidal marshes. Globally, soils above 100 cm depth can contain approximately 1500 Pg-C (10^{15} grams of carbon) of buried carbon and soils between depths of 150 cm to 300 cm can store between approximately 1778 Pg-C to 3000 Pg-C (Cagnarini et al., 2019). The storage capability of a tidal marsh is dependent on factors such as allochthonous carbon, autochthonous carbon, physical soil, plant and microbial activity, and tidal inundation or flood patterns (McLeod et al., 2011; Wilson et al., 2018; Owers et al., 2020).

Allochthonous carbon refers to the carbon which was introduced to the marsh from another location and can be deposited onto the marsh through tidal inundation (Owers et al., 2020). Therefore, marsh sites which experience greater levels of inundation possibility experience higher rates of sedimentation and organic matter deposition (Owers et al., 2020). In contrast, autochthonous carbon describes the carbon deposited into the soil via the roots of the vegetation in the rhizosphere (Kritzberg et al., 2004; Jones et al., 2009). Within the rhizosphere, the soil is altered chemically, biologically, and physically due to the release of carbon through areas of the roots such as the root cap and border cells (Jones et al. 2009). In addition, symbiotic relationships exist between plants and microbes in the soil (mycorrhizas): between 10% and 30% of photosynthate carbon can be allocated to mycorrhizae (Morton et al. 2004; Lanfranco et al. 2016). The rhizosphere is an important location for carbon fixation within tidal marshes and within the sediment. Despite their importance as a valuable ecosystem, tidal marshes are increasingly declining.

Globally, tidal marshes are being lost or damaged; this is due to the conversion of marshland into agricultural land (Burden et al., 2013; Wollenberg et al., 2018) and other anthropogenic or human derived factors such as urbanization, deforestation, eutrophication, reclamation, and pollution (McLeod et al., 2011; O’Connor et al., 2020). Additional damage comes in the form of dredging, dyking, drainage, and invasive species (McLeod et al., 2011). Locally, approximately 80% of the salt marshes in the Bay of Fundy and 50% of the salt marshes in Nova Scotia have already been lost (Gallant et al., 2020). Coastal ecosystems are particularly sensitive to changes in climate as even the smallest change can lead to large-scale landscape changes and loss of function within the ecosystem (Osland et al., 2018). To combat the decline of the tidal marsh area, effort and resources are being focused on the restoration and rehabilitation of tidal marshes across the globe (Burden et al., 2013).

1.3 Restored marshes vs. natural marshes

A restored tidal marsh is a marshland in which the tidal flow has been re-introduced, such as areas which had been ‘reclaimed’ for agriculture in the past (Wollenberg et al., 2018). Contrary to this, a natural marshland is relatively untouched. Many coastal systems have man-made defenses in place to prevent erosion, flooding and the effects of rising sea levels; however, over time these defenses become costly to maintain and replace (Burden et al., 2013). These man-made defenses can include dykes, originally built for conversion of marshland into agricultural land, and seawalls, which prevent flooding and erosion (Wollenberg et al., 2018). One of the most popular and widely used techniques for tidal marsh restoration is called managed realignment. This method involves breaching or moving existing dykes to change or reintroduce tidal flow into the site (Wollenberg et al., 2018). Rather than hard defenses, nature-based solutions, such as a ‘living’ shoreline, mimic natural processes in order to protect and stabilize the newly restored marsh until vegetation develops and sedimentation begins (Burden et al., 2013; CBWES, 2020).

A study by Burden et al. (2019) stated that it takes approximately 100 years for a restored marsh to accumulate the same amount of carbon as a reference or natural marsh. This study examined the carbon storage of a restored tidal marsh through a series of models which predicted carbon accumulation overtime (Burden et al., 2019). However, this study focuses on tidal marshes in Eastern England, which would have a different tidal range than the large tides of the Bay of Fundy. There are different dominant plant species in English tidal marshes in comparison to the marshes along the East coast of Canada and additionally, the soil composition is different (Adam, 1981). Due to these differences, it is not possible to apply this research and their conclusions to local marshes in the Bay of Fundy, meaning researchers are not certain how long it takes for a restored tidal marsh to transition to a natural, reference tidal marsh.

1.4 Tidal marshes as net carbon sinks

When discussing carbon sequestration in tidal marshes and other coastal ecosystems, there is often a question concerning whether they are a true net carbon sink; other gases, such as nitrous oxide (N_2O) and methane (CH_4), can also be sequestered and re-released. Nitrous oxide can be released into the atmosphere from the use of manure, the production of fertilizer, and land-use changes (van Amstel and Swart, 1994). Sources of methane include cattle, animal waste, landfills, and the burning of biomass (van Amstel and Swart, 1994). During processes such as denitrification and the breakdown of organic matter, these gases can be re-released into the atmosphere (Adams et al., 2012). Initially, there was not much known about whether the release of these gases would offset the carbon accumulation of tidal marshes. However, studies have been published which highlight tidal marshes as net carbon sinks (Magenheimer et al., 1996).

A local study in the Bay of Fundy by Magenheimer et al. (1996) compared methane and carbon dioxide fluxes in tidal marshes. It was reported that while CH_4 fluxes were observed on the sites, they were much smaller than fluxes observed in other temperate regions and marshes along the eastern coast of the United States (Magenheimer et al., 1996). CO_2 fluxes in these sites were attributed largely to plant respiration and the decomposition of organic matter (Magenheimer et al., 1996). Both fluxes were small in these sites and did not outweigh the net productivity of the sites (Magenheimer et al., 1996). A study by Adams et al., (2012) examined gaseous releases of tidal marshes compared to their carbon burial and presented their results as gross carbon accumulation against the fluxes of CH_4 and N_2O. They concluded that tidal marshes are net sinks for CH_4 and N_2O despite their fluxes and while carbon burial would be much higher without the reduction due to the gas fluxes, tidal marshes are still able to accumulate large amounts of carbon (Adams et al., 2012). Overall, the fluxes of methane and other gases within tidal marshes are negligible in comparison to the benefits of carbon sequestration.

1.5 Importance of vegetation in blue carbon storage

Native plant species are important to the health and future of an ecosystem and provide many ecosystem services (Osland et al., 2018). Vegetation aids in carbon storage by accumulating carbon short term in the biomass of the plant through plant productivity (Abbott et al., 2019). Existing vegetation on a tidal marsh is the source of near-surface carbon accumulation (Owers et al., 2020). A study by Abbott et al., (2019) concluded that long-term carbon accumulation rates in restored tidal marshes may be influenced by the same conditions needed to facilitate the growth of late-succession vegetation, such as *Spartina patens* and *Distichlis spicata*, which is important information for future planning of restoration projects. This study highlights the importance of vegetation and its development on restoration sites, indicating that research is important to understanding the processes occurring on restored tidal marshes and the overall health of the ecosystem.

1.5.1 *Spartina alterniflora* zone

Tidal marshes can be divided into zones based on elevation and flood pattern (Janousek et al., 2019; Owers et al., 2020). *Spartina alterniflora* is a dominant low marsh plant, usually found in the lowest level of the intertidal zone (Perry et al., 2020).

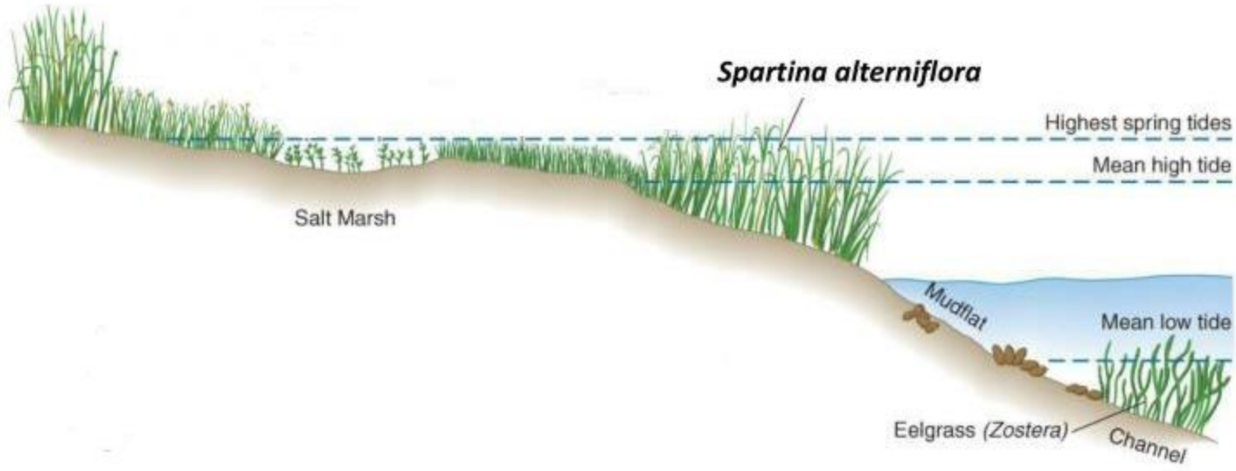


Figure 1. Tidal marsh zonation, highlighting the *Spartina alterniflora* zone (http://diagramland.arte-viaggi.it/diagram/salt-marsh-diagram)

Within tidal marshes, *S. alterniflora* is the first colonizer on a restored marsh and often covers the entire marsh prior to the formation of zones and elevation changes (Perry et al., 2020). This zone is often characterized by patchy vegetation and exposed mudflats due to tidal sediment deposition and prolonged periods of inundation.

1.6 Knowledge gap and local research

Most published studies focusing on coastal wetlands described their sustainability and resilience to threats of exploitation and landscape changes (Burden et al., 2019). Recent studies have shown that climate change may increase carbon sequestered by coastal ecosystems however, there is a lack of evidence and data supporting this claim (Mao et al., 2020). Changes in climate have the potential to severely damage the function and productivity of a tidal marsh and the uncertainty around the long-term effects of climate warming on buried carbon makes it difficult to manage restoration sites (Osland et al., 2018; Mao et al., 2020). Overall, few studies provided information regarding carbon stocks in restoration sites compared to natural sites and studies that addressed this idea were not long-term nor large scale (O’Connor et al., 2019).

There have been previous studies in the region which have assessed carbon storage in the Bay of Fundy. In 2018, Wollenberg et al. published a study which assessed the carbon storage of a restored marshland six years after the reintroduction of tidal flow onto the site. Their study site was located in Aulac, New Brunswick along a section of the Aulac dyke system and focused on one site which was divided into two ‘cell’ by a channel (Wollenberg et al., 2018). Wollenberg et al. concluded that sedimentation begins at once after realignment and there were no significant differences between the carbon densities of vegetated versus unvegetated cores (Wollenberg et al., 2018). Carbon storage is driven by the influx of sediment and associated carbon rather than vegetation however, vegetation traps sediment and thereby indirectly aids carbon storage (Wollenberg et al., 2018). The issue with this study, however, is that it focused on only one site and there was a lack of comparison between restoration sites to natural sites. While their findings do contribute to the knowledge gap within tidal marshes, further research is needed to validate their findings across other marsh bodies.

1.7 Study objectives

This research was intended to expand knowledge around carbon sequestration and how carbon is stored across restored and natural tidal marshes over time. With that in mind, this research had the following objectives:

1) In the *S. alterniflora* zone, to determine if below ground carbon stocks differ among vegetated and unvegetated areas.

    Finding unvegetated areas on an older restoration site and a natural reference marsh is much more difficult at higher elevations and in zones other than the *S. alterniflora* zone. I focused on the *S. alterniflora* zone, as the likelihood of finding unvegetated areas is much higher than in other zones. I predict that vegetation will impact carbon storage across the marshes.

2) To determine if organic carbon content varies by depth.

    Previous studies, such as Cagnarini et al. (2019), have observed carbon sequestration within larger sections of soil however, few focus on comparing how much carbon is in the rhizosphere versus below the rhizosphere. I predict that there will be higher levels of carbon in the rhizosphere.

3) To determine if organic carbon content varies between restored and natural sites.

It is unknown how long it takes for locally restored marshes along the Bay of Fundy to accumulate as much carbon as a reference site. I aim to determine this within my project by studying carbon accumulation in a newly restored, older restored, and natural tidal marsh along the Bay of Fundy. I predict that there will be higher levels of carbon in the natural site compared to the restoration sites.

2. MATERIALS AND METHODS

2.1 Study sites

The three study sites are located around the New Minas Basin in the Annapolis Valley, Nova Scotia, Canada; their respective site names are Belcher, Cogmagun Restoration, and Cogmagun Reference (Figure 2).

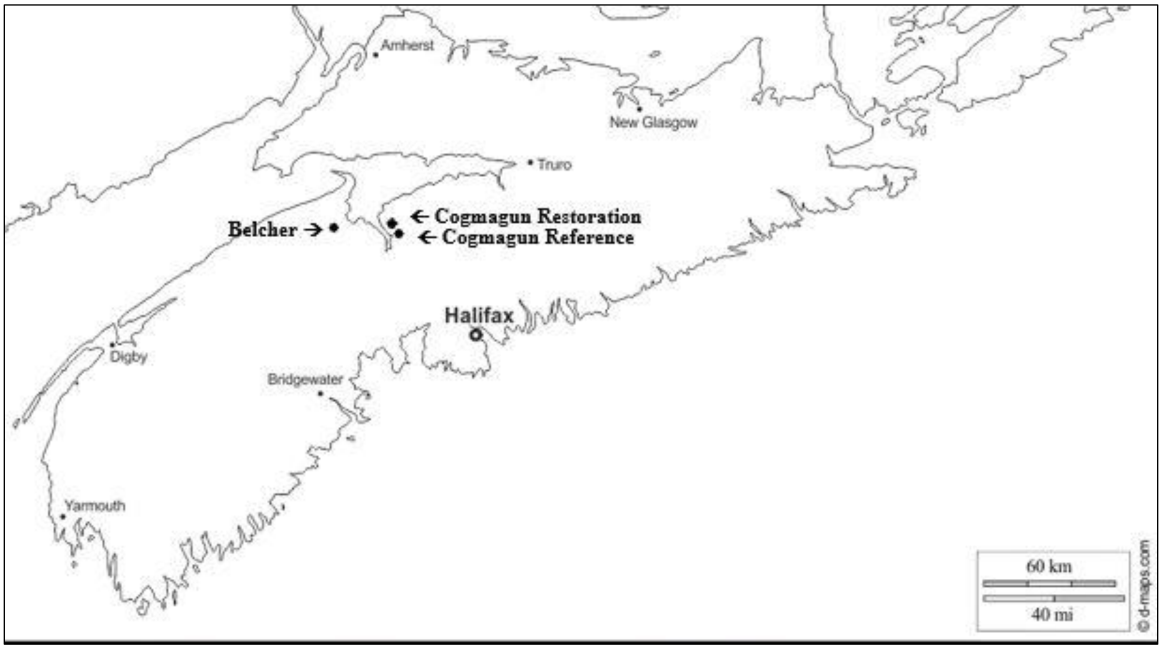


Figure 2. Locations of the sampling sites in the Annapolis Valley, NS. In total, there are three sites: one site is located in Belcher while the other two sites are found along the Cogmagun river.

In the spring of 2018, Belcher (45°04'25.9"N 64°28'26.6"W), located in New Minas, N.S, was restored (CB Wetlands and Environmental Specialists, 2020). The Cogmagun Restoration site (45°04'45.5"N 64°07'51.4"W) was restored in 2009 and is located along the Cogmagun River, N.S (CBWES, 2020). There is little known about the Cogmagun Reference site (45°05'02.6"N 64°07'01.1"W). However, it has likely been naturalized since before the 1950's (CBWES, 2020). Belcher and the Cogmagun Restoration site both had dyke systems which were breached to allow the return of the tide; these structures are not visible on the Cogmagun Reference site.

2.2 Field sampling

Prior to coring, sample locations were selected based on the vegetation and were marked with a bamboo stake and flagging tape. Sampling locations were randomly sampled based on the presence or absence of *Spartina alterniflora*, the dominant low marsh species. Site boundaries were previously determined by the managers of the sites—CB Wetlands and Environmental Specialists. At each study site, there were three sampling locations, with two cores—vegetated and unvegetated—being taken at each sampling location. Exact coordinates were taken using a GNSS Rover (Leica Geosystems) provided by Saint Mary's University. The sites were flagged in June and July 2020 and sampling occurred during the last two weeks of August 2020. A Russian peat corer (WaterMark®) was used to retrieve the cores with minimal compaction of the sediment; it takes the core beside where it was driven into the ground (Wollenberg et al., 2018) (Figure 3). At each sample location, a core was taken in an unvegetated area and a vegetated area. The corer was pushed into the ground manually and a rubber mallet was used, if needed, to push the corer fully into the ground. The cores measured approximately 50 cm long and were 3.8 cm wide. To collect the sample, the corer was twisted clockwise to pull the sample into the collection tube. After collection, the corer could be pulled out of the ground.

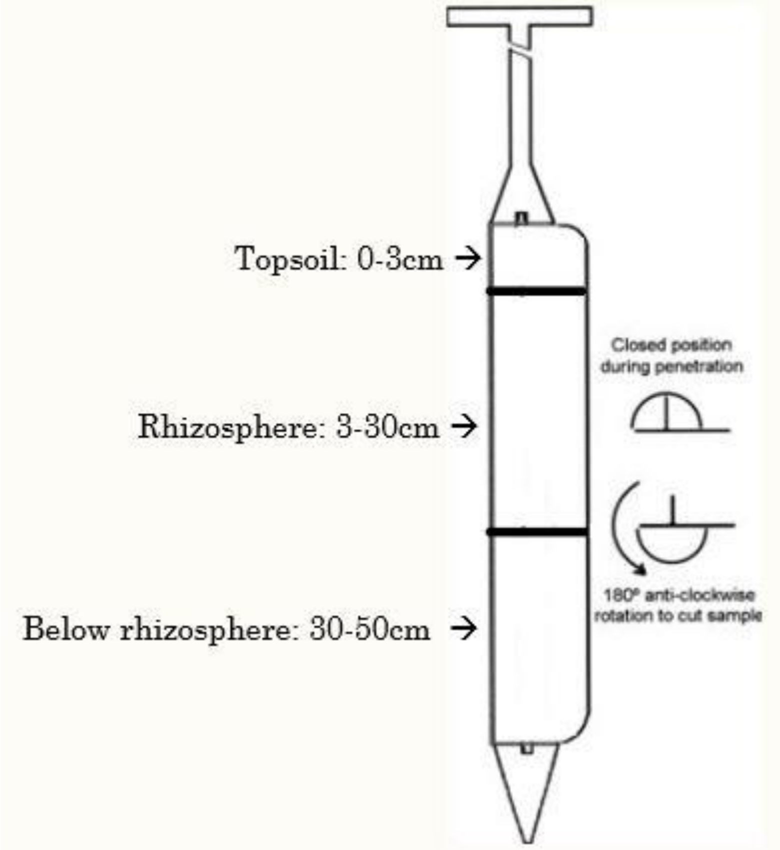


Figure 3. A diagram of the Russian peat corer used to collect the samples. This corer is designed to retrieve a sample with a minimal amount of compaction as it samples to the back of the blade once turned counterclockwise. The dark black lines indicate where the core was later sub sectioned in the laboratory.

The core was removed from the collection tube by pushing counterclockwise on the blade. A ruler and whiteboard with the core details was placed above the core and a photograph of the core was taken. Each core was pushed off the corer blade, using a trowel to free the edges, into a PVC tray which measured the length of the core and was wrapped in plastic cling wrap (No Name). The core was placed in a transparent garbage bag (AL-PACK, 20in x 22in) and the excess plastic was wrapped around the core. The bag was secured in place using Duct Tape® and the sample details were written on the tape. This procedure was followed for each of the 18 total cores. Until the cores were taken to Saint Mary's University, they were stored on ice packs while in the field and were stored at Acadia University, located in Wolfville, N.S, during the sampling week.

2.3 Lab analyses

2.3.1 Core processing – water and organic matter content

A drying oven, a muffle furnace, and a desiccator were used to process the cores. The cores had to be processed in batches due to the limited space in certain equipment; each batch took three days to process. The limiting factor was the muffle furnace as it can only hold 12 crucibles therefore, two cores could be processed at a time**.** It took nine batches to process all 18 cores. Batches were run simultaneously to allow for faster analyses (i.e one batch in oven, one in the furnace, etc). Cores were stored in the freezer before processing and had to be moved to the fridge 24 hours before processing could begin. Water and organic matter content were determined by loss on ignition, which involves heating a sample to a high temperature to burn off any volatile substances (Wollenberg et al., 2018). The amount 'lost' from the pre-ignition weight to the post-ignition weight is what the researcher is trying to determine (Wollenberg et al., 2018). All crucibles were weighed using a Denver Instrument SI-234 weigh scale and all values were recorded to the fourth decimal place.

On day one, clean porcelain crucibles were labelled, weighed, and checked to ensure that they had a unique identification number on the bottom. The unique core ID, the subsection, and whether it is vegetated or unvegetated was recorded, then the section was weighed and recorded. Meanwhile, the oven (Fisher Scientific 3511FS Gravity Convection oven) was being preheated to 105^{o}C. The core was unwrapped, making a note of which end was the top and the bottom, and was photographed as a whole. Following this, the core was sub sectioned into top (<3cm), rhizosphere (3cm-30cm) and below-rhizosphere (<30cm) based on the presence of roots within the middle, or rhizosphere, section. Sub sectioning was done using a standard ruler and a serrated knife: at the desired measurement needed, the core was cut. Next, two 2.5g samples of each subsection were taken by randomly sampling along the section and then were placed in a pre-weighed crucible. The crucible was reweighed with the sample, the value was recorded, and the sample was placed in the drying oven for 24 hours. These steps were repeated with a second core on the same day.

On day two, the samples were removed from the drying oven and placed in the desiccator (Fisher Scientific) for an hour to cool. After one hour, the samples were weighed in the crucible and the weight was recorded; this is the dry weight. The samples were ground into a fine powder with a mortar and pestle and returned to their crucible. Using gloves and tongs, the sample was moved into the muffle furnace (Fisher Scientific Isotemp Muffle Furnace 550 Series). Once all the samples were in the furnace, it was turned on to 550^{o}C and took approximately 40 minutes to heat to 550^{o}C. Once the furnace reached 550^{o}C, the samples were left in for two hours. After two hours, the furnace was turned off and the samples, in the crucibles, were left to cool in the furnace overnight.

On day three, the samples were removed from the muffle furnace using tongs and placed in the desiccator for one hour. After one hour, the samples were weighed in the crucible and the weight was recorded; this is the 550^{o}C weight. These samples could not be used for any further analysis once they went through the furnace therefore, they were thrown out and the crucibles were washed before being used again.

2.3.2 Core processing – bulk density

During the procedure for the water and organic matter content samples on day one, a sample was also taken to allow for the calculation of bulk density. A clean porcelain crucible was weighed, and its weight was recorded. As with the other samples, each crucible was labelled with a unique label. Using a syringe, a 5mL sample was taken from each subsection of the core, ensuring not to compact the sediment. Carefully, the sample was pushed from the syringe into a pre-weighed crucible. The sample was weighed, and the value was recorded. The bulk density samples were placed in the drying oven at 105^{o}C for 24 hours, alongside the samples for water and organic matter content.

The following day, the samples were allowed to cool in the desiccator for one hour. After one hour, the bulk density samples were weighed, and their weight was recorded. This was the final step for the bulk density samples; they did not go into the muffle furnace. After they were weighed, they were individually placed in plastic Ziplock bags labelled with my name, the date, their core identification, their subsection, and whether they were vegetated or unvegetated. They were returned to the freezer in case they were needed again.

2.4 Statistical analysis

All statistical analyses were performed in RStudio (RStudio Team, 2020)**.** The replicates of each sub section were used to take averages for organic matter content (OM), the loss on ignition fraction (LOI), and subsequently, organic carbon content (OC). To convert from organic matter content to organic carbon content in tidal marshes, the calculation used by Wollenberg et al., (2018) was used: Organic C fraction = 0.40(LOI fraction) + $(0.025*\text{LOI fraction})^2$ (Wollenberg et al., 2018). A three-way mixed model ANOVA was used to look at the interaction between the site, vegetation, and depth. A pairwise post-hoc comparison was completed following the ANOVA tables. In addition, depth was analyzed across all three sites by subsection (top, middle or bottom) to assess whether there was any statistical significance associated with a specific depth. OC was also examined by subsection alone across all sites to test for significance.

3. RESULTS

Since OM was used to obtain OC, only the statistical results from the analysis of OC will be stated and subsequently discussed. Water content ranged from 27.15 to 54.24% across the three sites, with the lowest value being recorded at Belcher and the highest value being found at the Cogmagun Restoration site. OC content ranged from 0.73 to 1.38 OC g cm^{-3}, respectfully, which subsequently yielded organic carbon densities ranging from 0.016 to 0.034 g OC cm^{-3} (Table 1). Bulk density ranged from 0.734 g cm^{-3} to 1.390 g cm^{-3}.

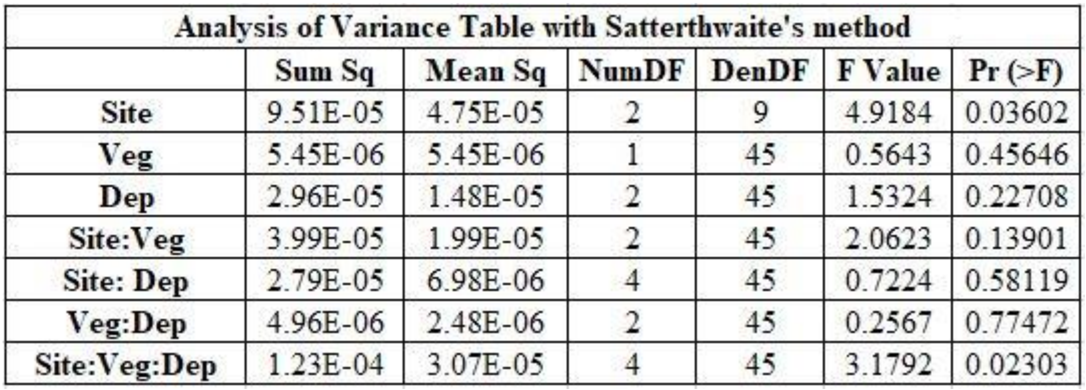
Table 1. Bulk density and organic carbon content from cores across all sites. Cog. Ref refers to the Cogmagun Reference site, Cog. Res refers to the Cogmagun Restoration site, and Bel refers to Belcher. V indicates the core was vegetated and UV indicates the core was taken in an area where no vegetation was present.

ID	Site	V or UV	Subsection	Bulk Density (g cm^{-3})	Organic Carbon (g cm^{-3})
CORK7	Cog. Ref	UV	Top	1.085	0.022
CORK7	Cog. Ref	UV	Middle	0.833	0.015
CORK7	Cog. Ref	UV	Bottom	1.296	0.025
CORK7	Cog. Ref	V	Top	1.132	0.022
CORK7	Cog. Ref	V	Middle	1.377	0.024
CORK7	Cog. Ref	V	Bottom	1.298	0.026
CORK3	Cog. Ref	UV	Top	1.250	0.027
CORK3	Cog. Ref	UV	Middle	0.979	0.020
CORK3	Cog. Ref	UV	Bottom	1.120	0.022
CORK3	Cog. Ref	V	Top	0.966	0.020
CORK3	Cog. Ref	V	Middle	1.053	0.023
CORK3	Cog. Ref	V	Bottom	1.060	0.020
CORK6	Cog. Ref	UV	Top	0.984	0.018
CORK6	Cog. Ref	UV	Middle	0.997	0.019
CORK6	Cog. Ref	UV	Bottom	1.059	0.023
CORK6	Cog. Ref	V	Top	1.390	0.026
CORK6	Cog. Ref	V	Middle	1.093	0.034
CORK6	Cog. Ref	V	Bottom	1.192	0.020
COGK5	Cog. Res	UV	Top	1.035	0.033
COGK5	Cog. Res	UV	Middle	0.973	0.034
COGK5	Cog. Res	UV	Bottom	1.179	0.033
COGK5	Cog. Res	V	Top	0.860	0.030
COGK5	Cog. Res	V	Middle	0.734	0.022
COGK5	Cog. Res	V	Bottom	1.140	0.028
COGK2	Cog. Res	UV	Top	1.384	0.025
COGK2	Cog. Res	UV	Middle	1.211	0.032
COGK2	Cog. Res	UV	Bottom	0.982	0.016
COGK2	Cog. Res	V	Top	1.378	0.027
COGK2	Cog. Res	V	Middle	1.104	0.022
COGK2	Cog. Res	V	Bottom	1.059	0.017
COGK7	Cog. Res	UV	Top	1.237	0.023
COGK7	Cog. Res	UV	Middle	0.952	0.018
COGK7	Cog. Res	UV	Bottom	1.306	0.022

COGK7	Cog. Res	V	Top	1.087	0.028
COGK7	Cog. Res	V	Middle	1.233	0.024
COGK7	Cog. Res	V	Bottom	1.125	0.024
BELK2	Bel	UV	Top	0.945	0.020
BELK2	Bel	UV	Middle	0.952	0.021
BELK2	Bel	UV	Bottom	0.937	0.021
BELK2	Bel	V	Top	0.898	0.021
BELK2	Bel	V	Middle	0.953	0.022
BELK2	Bel	V	Bottom	0.932	0.021
T1S4	Bel	UV	Top	1.017	0.020
T1S4	Bel	UV	Middle	0.973	0.016
T1S4	Bel	UV	Bottom	0.942	0.016
T1S4	Bel	V	Top	0.850	0.020
T1S4	Bel	V	Middle	0.887	0.018
T1S4	Bel	V	Bottom	0.916	0.017
T4S4	Bel	UV	Top	1.165	0.021
T4S4	Bel	UV	Middle	1.102	0.024
T4S4	Bel	UV	Bottom	1.011	0.018
T4S4	Bel	V	Top	1.336	0.022
T4S4	Bel	V	Middle	1.199	0.020
T4S4	Bel	V	Bottom	1.279	0.020

From the ANOVA table with Satterthwaite’s method on OC content across all three sites and depths, site appeared to be weakly significant ($p < 0.03$) (Table 2). Statistical analysis showed that there was a significant three-way interaction between site, vegetation, and depth in carbon storage across all three sites ($p < 0.02$; Table 2). There were no other significant findings from the ANOVA test.

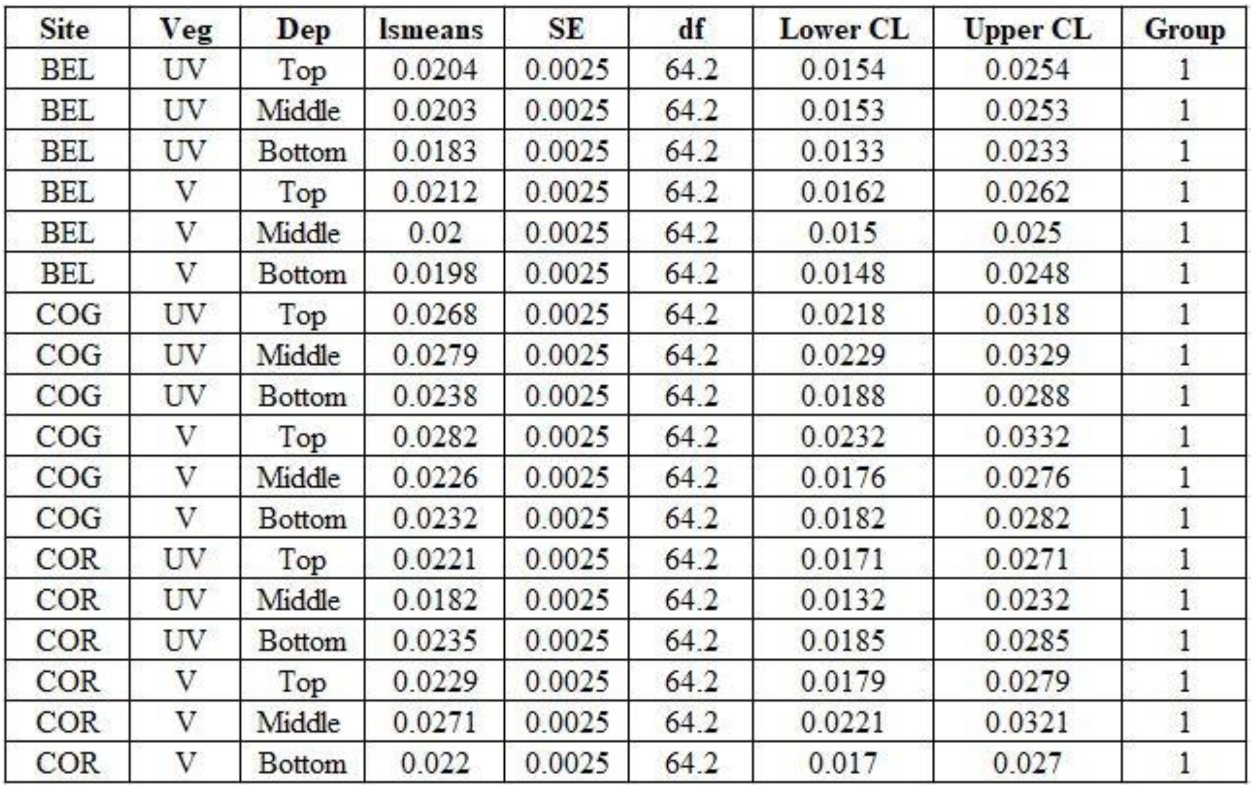
Table 2. Analysis of Variance Table with Satterthwaite's method on OC depth content.



Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F Value**	**Pr (>F)**
Site	9.51E-05	4.75E-05	2	9	4.9184	0.03602
Veg	5.45E-06	5.45E-06	1	45	0.5643	0.45646
Dep	2.96E-05	1.48E-05	2	45	1.5324	0.22708
Site:Veg	3.99E-05	1.99E-05	2	45	2.0623	0.13901
Site: Dep	2.79E-05	6.98E-06	4	45	0.7224	0.58119
Veg:Dep	4.96E-06	2.48E-06	2	45	0.2567	0.77472
Site:Veg:Dep	1.23E-04	3.07E-05	4	45	3.1792	0.02303

A pairwise post-hoc comparison test on OC content from the ANOVA table showed that there were no significant group interactions (Table 3).

Table 3. OC content pairwise post-hoc comparison test.



Site	Veg	Dep	lsmeans	SE	df	Lower CL	Upper CL	Group
BEL	UV	Top	0.0204	0.0025	64.2	0.0154	0.0254	1
BEL	UV	Middle	0.0203	0.0025	64.2	0.0153	0.0253	1
BEL	UV	Bottom	0.0183	0.0025	64.2	0.0133	0.0233	1
BEL	V	Top	0.0212	0.0025	64.2	0.0162	0.0262	1
BEL	V	Middle	0.02	0.0025	64.2	0.015	0.025	1
BEL	V	Bottom	0.0198	0.0025	64.2	0.0148	0.0248	1
COG	UV	Top	0.0268	0.0025	64.2	0.0218	0.0318	1
COG	UV	Middle	0.0279	0.0025	64.2	0.0229	0.0329	1
COG	UV	Bottom	0.0238	0.0025	64.2	0.0188	0.0288	1
COG	V	Top	0.0282	0.0025	64.2	0.0232	0.0332	1
COG	V	Middle	0.0226	0.0025	64.2	0.0176	0.0276	1
COG	V	Bottom	0.0232	0.0025	64.2	0.0182	0.0282	1
COR	UV	Top	0.0221	0.0025	64.2	0.0171	0.0271	1
COR	UV	Middle	0.0182	0.0025	64.2	0.0132	0.0232	1
COR	UV	Bottom	0.0235	0.0025	64.2	0.0185	0.0285	1
COR	V	Top	0.0229	0.0025	64.2	0.0179	0.0279	1
COR	V	Middle	0.0271	0.0025	64.2	0.0221	0.0321	1
COR	V	Bottom	0.022	0.0025	64.2	0.017	0.027	1

A similar amount of carbon was found to be buried across all three sites; however, there was a lot of variation within the treatments (Figure 5). The difference between the least and most amount of carbon present in a treatment combination varied by a factor of >2 (Figure 5). Due to the weak site significance as a main effect in the initial ANOVA results, a pairwise test was performed to determine whether there was a significance between the three sites (Table 4).

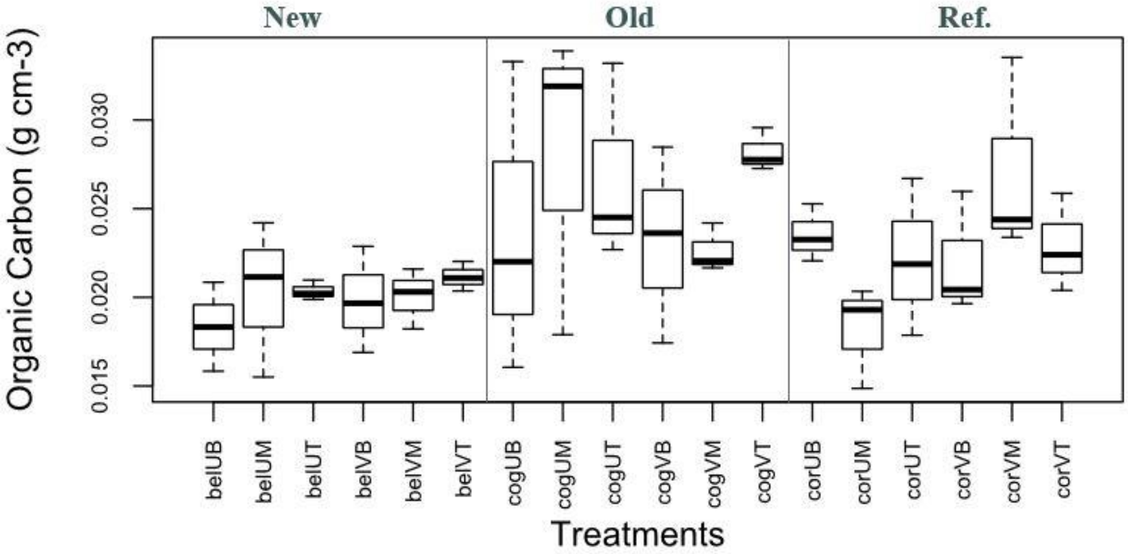
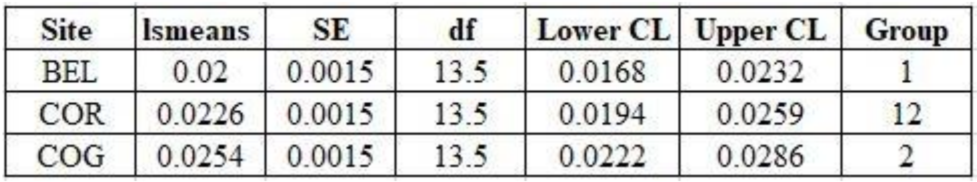


Figure 5. Organic carbon content from the treatments across the three sites: Belcher (bel), Cogmagun Restoration (cog) and Cogmagun Reference (cor). There were three sampling locations at each site, with two cores taken from each location (n= 18). Treatment codes list the site name first, followed by whether its vegetated (V) or unvegetated (U), and which depth it was taken from (Top, Middle or Bottom). Pairwise groups are represented with ‘a’—bars which share letters are not significantly different.

Table 4. Pairwise post-hoc comparison test on site significance across all sites.



Site	lsmeans	SE	df	Lower CL	Upper CL	Group
BEL	0.02	0.0015	13.5	0.0168	0.0232	1
COR	0.0226	0.0015	13.5	0.0194	0.0259	12
COG	0.0254	0.0015	13.5	0.0222	0.0286	2

There were significant site differences observed in our results. The older restoration site, Cogmagun restoration, had higher values of buried OC than the new restoration site, Belcher, but both restoration sites overlapped with the reference site, Cogmagun reference (Figure 6).

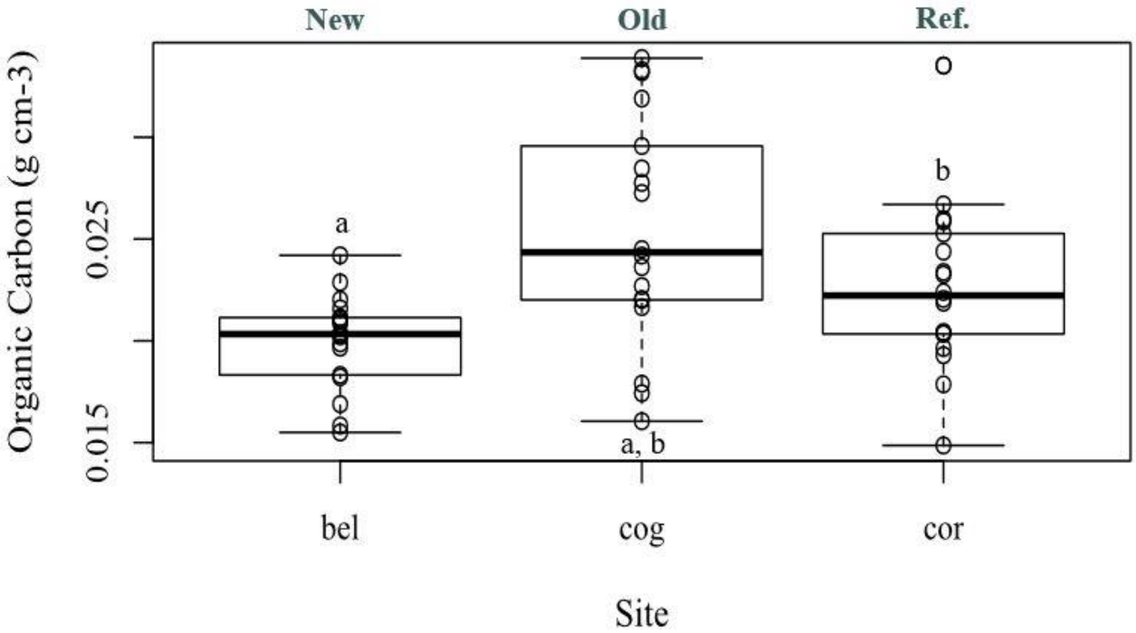
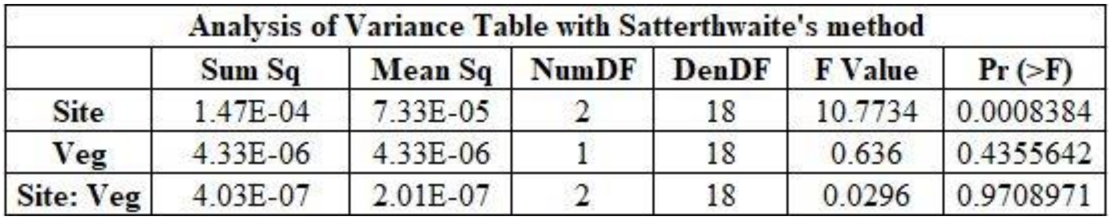


Figure 6. Organic carbon content from the top, middle, and bottom section of all the cores from the three field sites: Belcher (bel), Cogmagun Restoration (cog), and Cogmagun Reference (cor). Site differences were observed, with the older restoration site containing more buried carbon than the new restoration and reference site.

Depth was examined at each section across all sites to determine if there was any significance. From the ANOVA table with Satterthwaite's method on OC content for the top section of the core, site was determined to be significant ($p < 0.0008$; Table 5). There were no other significant interactions.

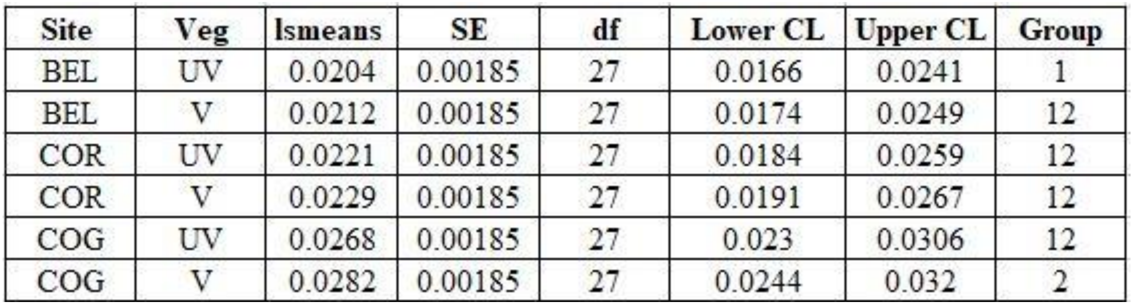
Table 5. Analysis of Variance Table with Satterthwaite's method on OC top across all sites.



Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F Value**	**Pr (>F)**
Site	1.47E-04	7.33E-05	2	18	10.7734	0.0008384
Veg	4.33E-06	4.33E-06	1	18	0.636	0.4355642
Site: Veg	4.03E-07	2.01E-07	2	18	0.0296	0.9708971

A pairwise post-hoc comparison test on OC top showed that OC at cog V (0.0282 ± 0.0038) was significantly greater than bel UV (0.0204 ± 0.00375) (Table 6).

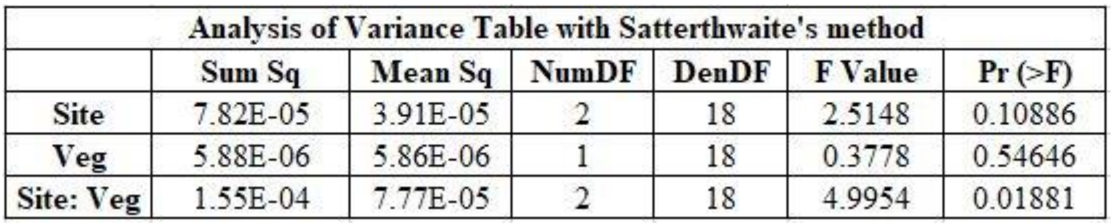
Table 6. Pairwise post-hoc comparison test on OC content from the top section of the cores.



Site	Veg	lsmeans	SE	df	Lower CL	Upper CL	Group
BEL	UV	0.0204	0.00185	27	0.0166	0.0241	1
BEL	V	0.0212	0.00185	27	0.0174	0.0249	12
COR	UV	0.0221	0.00185	27	0.0184	0.0259	12
COR	V	0.0229	0.00185	27	0.0191	0.0267	12
COG	UV	0.0268	0.00185	27	0.023	0.0306	12
COG	V	0.0282	0.00185	27	0.0244	0.032	2

From the ANOVA table with Satterthwaite’s method on OC content for the middle section of the core, there was a significant interaction between site and vegetation ($p < 0.018$; Table 7).

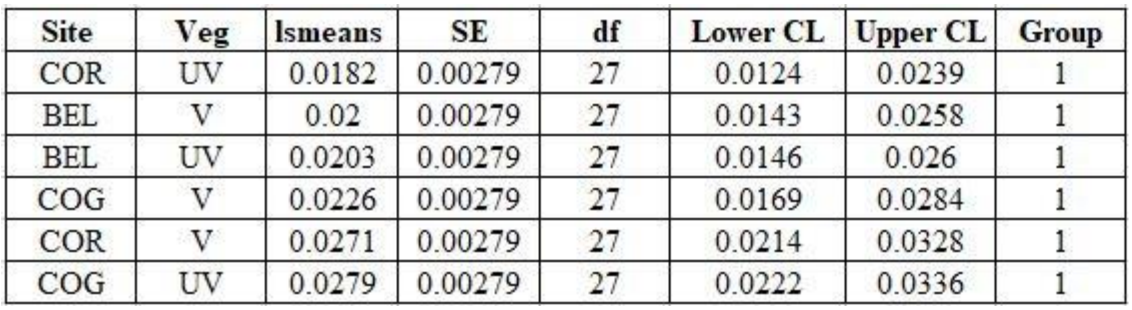
Table 7. Analysis of Variance Table with Satterthwaite's method on OC middle across all sites.



Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F Value**	**Pr (>F)**
Site	7.82E-05	3.91E-05	2	18	2.5148	0.10886
Veg	5.88E-06	5.86E-06	1	18	0.3778	0.54646
Site: Veg	1.55E-04	7.77E-05	2	18	4.9954	0.01881

A pairwise post-hoc comparison test on OC middle showed there was no significance across all groups (Table 8).

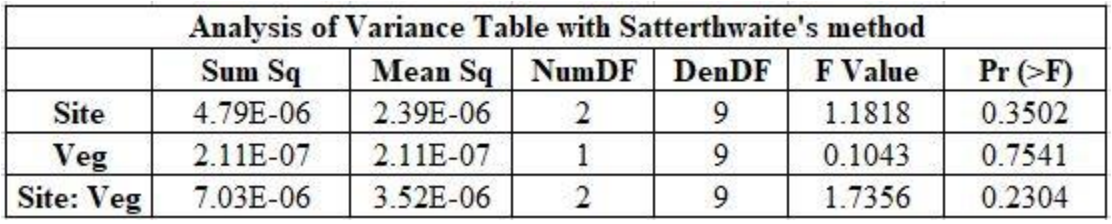
Table 8. Pairwise post-hoc comparison test on OC content from the middle section of the cores.



Site	Veg	lsmeans	SE	df	Lower CL	Upper CL	Group
COR	UV	0.0182	0.00279	27	0.0124	0.0239	1
BEL	V	0.02	0.00279	27	0.0143	0.0258	1
BEL	UV	0.0203	0.00279	27	0.0146	0.026	1
COG	V	0.0226	0.00279	27	0.0169	0.0284	1
COR	V	0.0271	0.00279	27	0.0214	0.0328	1
COG	UV	0.0279	0.00279	27	0.0222	0.0336	1

From the ANOVA table with Satterthwaite's method on OC content for the bottom section of the core, no variables were determined to be significant (Table 9). Further analysis were not conducted as there was no significant effect according to the ANOVA test.

Table 9. Analysis of Variance Table with Satterthwaite's method on OC bottom across all sites.



Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	Mean Sq	NumDF	DenDF	F Value	Pr (>F)
Site	4.79E-06	2.39E-06	2	9	1.1818	0.3502
Veg	2.11E-07	2.11E-07	1	9	0.1043	0.7541
Site: Veg	7.03E-06	3.52E-06	2	9	1.7356	0.2304

4. DISCUSSION

4.1 Vegetation and below ground carbon storage

There were no interactions detected in the pairwise analyses, indicating no groups were different—vegetation was determined to not be significant in altering the amount of stored carbon in the soil. However, our sample size was small which potentially impacts our analyses to detect whether there was a true effect of vegetation within these systems. Sediment is important for the long-term storage of buried organic carbon; however, it appears that plants may aid in carbon storage long-term as well, in addition to short-term in their biomass (McLeod et al., 2011). While the aboveground biomass from vegetation—along with the carbon stored in the biomass—is lost at the end of the growing season, carbon buried in the sediment within the rhizosphere from the roots of the plants will remain at the end of the growing season.

This thought, however, contrasts with the literature—a study published by Wollenberg et al. (2018) concluded that sedimentation and an influx of sediment from the tides had a higher impact on carbon storage than vegetation, with vegetation being an indirect aid by trapping sediment from the tide (Wollenberg et al., 2018). The study by Wollenberg et al. (2018) took place in Aulac, New Brunswick along the Bay of Fundy. Therefore, their marsh system would have experienced similar tide fluctuations and inundation as the marshes within our study. The calculated OC values from our study are comparable to those reported by Wollenberg et al. (2018) from their study in Aulac, indicating additional similarity between the different sites. As reported by Swift et al., (1973), the Bay of Fundy has a suspended sediment load which contains not only organic carbon but sand, clay, silt, and plankton (Swift et al., 1973). The Bay of Fundy has the highest tides in the world therefore, the importance of sedimentation cannot be ruled out. However, vegetation is likely impacting the system in ways which are currently not completely transparent, perhaps through carbon buried in the rhizosphere throughout the growing season.

In addition, Wollenberg et al., (2018) concluded that organic carbon associated with sedimentation is likely to be found in marshes rather than mudflats. This is due to the benefits associated with vegetation in these systems, such as roots which aid in erosion control (Wollenberg et al., 2018). This may explain the weak significance found statistically within our study regarding carbon storage and vegetation in our initial analysis—our sediment cores were only taken in the low marsh (*S. alterniflora* zone) where vegetation was sparse. The low marsh is not as established as the upper marsh therefore there are more exposed mudflats, which may impact the carbon storage abilities of the marsh. Future studies should examine carbon storage potential of tidal marshes across all three tidal zones to compare differences in vegetation and its impact on carbon storage. This would give a full profile of a site and likely direct further inquiries based on the findings.

It is important to consider the sample size and number of sites within this study. Without having multiple sites from each category (restoration or natural) or additional replicates, our statistical analyses likely lacked power thereby impacting our results. Due to COVID-19, field work was delayed and there was no time to collect more samples from our sites nor to consider expanding the number of field sites. In addition, several cores taken from areas with no visible vegetation aboveground still possessed roots below ground. This may have skewed our results when comparing vegetated versus unvegetated as there would be OC present in the unvegetated cores from the roots in the sample. A further study should be conducted to examine the same parameters at more sites with additional replicates to compare to our results for clarification.

4.2 Depth and below ground carbon storage

The effect of depth on carbon storage was not significant. Within the upper sections of the sediment is the rhizosphere, where plants push carbon into the sediment via their roots. Therefore, it was expected that this section would contain more organic carbon.

Given this, it appears that the depth at which carbon is buried does not impact long-term carbon storage within these tidal marshes.

Our findings are consistent with several studies in the literature. Firstly, Wollenberg et al., (2018) concluded that marshes appeared to have relatively uniform carbon densities at different depths and therefore, carbon storage did not vary with depth (Wollenberg et al., 2018). In addition, they did state that lower carbon densities would be present in the deeper sediment as it would reflect carbon being deposited prior to the establishment of vegetation (Wollenberg et al., 2018). This was found within our study therefore, vegetation may impact storage directly in some way despite our study determining that there was no direct effect of vegetation on carbon storage — our sample size or limited number of sites may be hindering the full statistical strength of our analyses.

A Russian peat corer was used to collect the sediment core in our study however, there have been conflicting opinions within the literature on the effectiveness of different coring methods. This corer was selected based on the literature, particularly a study by Smeaton et al., (2020), which found that gouge corers, such as the Russian peat corer, were more reliable than hammer corers for estimating carbon stocks due to reduced sediment compaction. However, how does a Russian peat corer compare to other estimation methods, including less invasive methods? A study by van Ardenne et al., (2018), found a similar result to Wollenberg et al., (2018) in another tidal system in New Brunswick—organic carbon content did not vary by depth across the three tidal marshes in their study. Within the study by Ardenne et al., (2018), a Russian peat corer was used to collect sediment cores however, other methods were also used to approximate carbon stocks within tidal marshes (Ardenne et al., 2018). They used GIS interpolation and estimated carbon stocks across all marshes in the study and stocks along single transects (Ardenne et al., 2018). They found different results across each method and concluded that carbon stocks may be under- or over-estimated if sampling at a depth of half a meter or one meter depending on the method being employed (Ardenne et al., 2018).

Using additional methods of estimating carbon stocks in tidal ecosystems in future studies would allow for a comparison of values within a site. This should be done with caution however, as consulting several methods may be misleading depending on the precision associated with each method. A review of all available methods for sediment coring and carbon stock estimation should be done to identify the most reliable methods used in the field to direct ongoing and future research.

4.3 Age and below ground carbon storage

Due to the pairwise tests yielding no group interactions, this means our significant finding of the three-way interaction was not strong enough to be detected. The older restoration site was significantly higher in OC than the new restoration site, but both restoration sites overlapped with the reference site; this contrasted our prediction. Organic carbon content was consistent with little variation in the new restoration site, averaging 0.021 OC g cm^{-3} and significantly lower than at the older restoration site. There were more inconsistent values and variation in results within the older restoration site and reference site, which was not expected but an area to be explored in future research.

With regards to how long it takes for a restored marsh to reach the carbon storage capabilities of a natural reference site, our study was unable to come to any conclusions regarding this question, because the reference site, which was expected to have the highest carbon storage, was intermediate between the new and old restoration sites. A study by Burden et al., (2013) concluded that the older restoration site in their study, fifteen years post-restoration, did not function biologically or chemically equivalent to the natural reference site, particularly with regards to carbon storage (Burden et al., 2013). Within the literature, there are conflicting results surrounding the importance of site age on long-term carbon storage. A study by Abbott et al., (2019) found that long-term carbon storage did not differ significantly among marshes of varying ages. They concluded that long-term carbon storage was influenced by site-specific environmental factors, such as sediment composition or stem density, rather than age alone (Abbott et al., 2019). Species such as *Spartina patens* have a high stem density, which Abbott stated may facilitate higher long-term carbon stocks. Our study did not focus on the mid and upper marsh however, as explained in the previous section, this would be a topic worth exploring in future work to encompass all zones within tidal marshes for a complete site profile.

4.4 Future research

4.4.1 Sedimentation

As expressed by Wollenberg et al. (2018), sedimentation is a more crucial factor within carbon storage than originally thought. However, there is little research, particularly within the Bay of Fundy, to quantify the overall impact of sedimentation on carbon storage and how it contributes to short-term versus long-term storage. Owers et al.,

(2020) reported that sediment and its characteristics influence buried carbon content within the near-surface soil (Owers et al., 2020). This study identified sedimentation as a factor within short-term storage—as near-surface soil is often disturbed, and organic material can be re-released back into the environment—but what about the potential influence on long-term storage? Further studies should explore the role of sedimentation and its influence on long-term carbon storage within these systems.

4.4.2 Carbon storage across all zones

Within our study, sediment cores were only taken in the low marsh or *Spartina alterniflora* zone, not within all three zones. This was largely due to time constraints and the amount of work associated with taking, at minimum, triple the number of cores and therefore our study focused on the most dynamic zone. As mentioned in a previous section, future studies should examine the role of vegetation, depth, and site age across the low, mid, and upper marsh zones within tidal marshes. This would allow for a more complete overview of carbon storage not only across tidal marshes of varying ages but also across all three zones within each site. More variables influencing carbon storage may be identified through this research which is important as environmental conditions continue to evolve.

4.4.3 Microbes

Within the sediment of tidal marshes are organisms, such as microbes and fungi, which may contribute to carbon storage through their symbiotic relationship with plants. A particular fungus, arbuscular mycorrhizal fungi (AMF), have been found to be present in most terrestrial soils including tidal marsh sediment (d’Entremont et al., 2018). AMF may be beneficial to carbon storage by increasing nutrient uptake and thereby increasing plant surface area, allowing plants to bury more carbon overtime (Lanfranco et al., 2016; Thirkell et al., 2019). The impact of microbe communities and mycorrhizal fungi on carbon storage within the tidal marshes of the Bay of Fundy have been largely overlooked and are a potential avenue for future research. Due to the dynamic environmental conditions within the Bay of Fundy, conclusions from outside studies on microbes within marshland sediments cannot be blatantly accepted—questions surrounding the influence of these communities must be explored in the context of the particular ecosystem. Plant roots and sediment cores could be used to conduct these experiments and allow for conclusions to be drawn within the Bay of Fundy. These results could then be compared to outside systems for further analysis and understanding.

4.4.4 Seasonal variation in carbon storage

In temperate regions, such as Atlantic Canada, there may exist a seasonal fluctuation in carbon storage rates within tidal ecosystems. While this may not be evident in deeper, below ground stocks, carbon values in the top layer may increase or decrease given the presence of plant biomass, organic debris from dead plants, fluctuations in sedimentation given the presence of ice in the winter, and other environmental conditions. This could be extended across sites of varying ages to also compare whether age is a relevant factor in seasonal fluctuation as well. While it may be difficult to get a full core from tidal marshes in the winter, the top layer of soil could be analyzed to explore the possibility of seasonal variation.

4.4.5 Climate change

Carbon sequestration is a valuable ecosystem service provided by tidal marshes however, there is little literature available on how climate change will impact carbon storage within these systems. A study by Mao et al., (2020) suggested that while carbon storage within tidal marshes is predicted to increase as the climate warms overtime, changes within the ecosystem due to climate change may lead to restructuring and landscape alterations. This could alter not only the methods of burying organic carbon within tidal marshes but how much carbon they are able to bury long-term (Mao et al., 2020). In addition, changing water sources (salt water versus freshwater components) and precipitation changes may alter the plant species found on marshes or change zonation, leading to changes in ecosystem services, stability of the system, and overall marsh health (Osland et al., (2018). Therefore, ongoing research should document environmental variables such as temperature, rainfall, inundation, carbon storage, marsh plant species composition, sedimentation, and other factors for reflection in the future as the global climate changes as it may help determine methods of mitigation.

5. CONCLUSION

Tidal marshes contribute a valuable ecosystem service by sequestering and storing atmospheric CO_2. The decline of tidal marsh systems globally has led to the expansion of ecosystem restoration avenues however, there is little literature exploring the factors that influence carbon storage in these systems, particularly in restored versus natural sites. Our study compared belowground carbon stocks in three tidal marshes (new restoration, old restoration, natural reference) and found that the older restoration and natural sites contained more buried carbon than the new restoration site. Vegetation and depth did not appear to influence long-term carbon storage in tidal marshes. In contrast to the literature, there was no consistent relationship between site age after restoration and the amount of carbon storage relative to a natural reference site in the three sites examined. Further studies should explore the role of sedimentation and how climate change may influence or change carbon storage rates in these systems. Expansion of our methods to the other tidal marsh zones, in addition to increased samples, within future studies may be useful for a complete assessment of carbon stocks within a tidal marsh for comparison across other sites.

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