Assessing arbuscular mycorrhizal fungi and sediment organic carbon density in Nova Scotia salt marshes

By
Kendra Sampson

A Thesis Submitted to
Saint Mary's University Halifax, Nova Scotia
In Partial Fulfillment of the Requirements for
The Degree of Master of Science in Applied Science

February 2023, Halifax, Nova Scotia

Approved: Dr. Danika van Proosdij
Co-supervisor
Department of Geography
and Environmental Studies

Approved: Dr. Allison Walker
Co-supervisor
Department of Biology
Acadia University

Approved: Dr. Gavin Kernaghan
External Examiner
Department of Biology
Mount Saint Vincent University

Approved: Dr. Jeremy Lundholm
Supervisory Committee

Approved: Dr. Erin Cameron
Supervisory Committee
Department of Biology

Date: February 13th, 2023

Abstract

Assessing arbuscular mycorrhizal fungi and sediment organic carbon density in Nova Scotia salt marshes

By Kendra Sampson

This research examines the carbon accumulation potential of salt marshes in the Bay of Fundy by analyzing sediment organic carbon (OC) and arbuscular mycorrhizal fungi (AMF) within the root zone of *Sporobolus* (*Spartina*) sp. at 11 salt marsh sites. Sediment was analyzed for organic matter (OM) by loss on ignition (LOI) and carbon was quantified using elemental analysis. AMF colonization rates were highly variable among sites and plant species, with high rates at restored marshes and in *Sporobolus michauxianus* roots (94%). Previously used LOI to OC conversion equation overestimated sediment OC densities. Regression analyses determined a quadratic equation that best described the relationship between OC and OM for most sites. Salt marshes are environmentally variable indicating that the relationship between OC and OM could differ geographically and should be measured independently. This study is the first to assess salt marsh AMF root colonization and sediment OC densities at multiple sites.

February 13th, 2023

Acknowledgements

As for all, theses come with many hurdles that must be jumped over and challenges that must be faced. In the midst of this program, I lost my person to a short-lived battle with cancer. My nana was my world, and I hers - I dedicate this work to her. I wrote many pages of this thesis at her kitchen table, in a kitchen filled with so many memories.

I am forever grateful for my supervisors Danika and Allison. The support, time, and guidance you have both provided is unmatched and cherished. Danika, you are a wealth of knowledge! Allison, your encouragement is incredibly uplifting and appreciated!

Thank you to my committee members, Jeremy Lundholm for your R knowledge and guidance throughout this process, and Erin Cameron for your positive feedback and suggestions; to external reader Gavin Kernaghan for your critique and suggestions; to NSERC ResNet for funding this project and supporting ecosystem services across Canada, as well as a Nova Scotia Graduate Scholarship and NSERC Discovery Grant #NSERC—2017-04325 (Walker) for providing additional funding. To CB Wetlands and Environmental Specialists for providing previous data required for the success of this project and Jennie Graham for your assistance in the field with site selection and pulling me out of the mud when I was stuck, thank you. To Ducks Unlimited Canada for allowing me to do field work at the Belcher DUC restoration site. To TransCoastal Adaptions Centre team for the use of equipment and Emma Poirier for helping me with protocols. To the Walker Lab members, Tyler d'Entremont for your microscopy expertise and assistance with root colonization rates, and Sunidhi Narayanan for help with processing samples. To Jesse MacDonald for assisting in the creation of my maps. To Trevor Avery for meeting with me to discuss stats and R. To Acadia University and Saint Mary's University both essential in the success of this project. To the K.C. Irving Centre and staff for allowing me to use your facilities at the peak of COVID-19 when restrictions were put into place and to Sarah Hines who was instrumental in the organization of equipment use.

Thank you to Sarah Adams - for your endless support in lab and out, you are always an excellent listening ear and provide great advice. To Kayla Williams, I would not have made it through 2020 field season without you. You are a hard worker and incredibly knowledgeable, I appreciate your advice and friendship. To Brittany Roughan, for your expertise especially on all things carbon related. To Jenna Jackson, for simply everything. Finally, I would like to thank my family, my mother, father, brother, sister-in-law, and papa for guiding me along the way, endlessly supporting all my hopes and dreams, and being there when needed.

List of Abbreviations

C	Carbon
CO_2	Carbon dioxide
OC	Organic carbon
OM	Organic matter
LOI	Loss on ignition
DW	Dried weight
NON	Onslow North River Restoration site
BEL	Belcher Restoration site
DUC	Belcher DUC Restoration site
CON	Converse Restoration site
SCW	St Croix West Restoration site
COG	Cogmagun Restoration site
CHV	Cheverie Restoration site
WAL	Walton Restoration site
COR	Cogmagun Reference site
CHVR	Cheverie Reference site
WALR	Walton Reference site

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Natural carbon (C) sinks have been recognized for their ability to rapidly store substantial amounts of C belowground, reducing atmospheric greenhouse gases and contributing to climate change mitigation (Howard et al., 2017). This includes blue C ecosystems (mangroves, seagrass beds, and salt marshes) a concept which highlights vegetated coastal ecosystems' contribution to organic C sequestration (McLeod et al., 2011). These systems are efficient at sequestering C internally and externally through suspended sediments which reduces atmospheric C (Macreadie et al., 2019). This reduction is essential for targeting the Intergovernmental Panel on Climate Change's (IPCC) goal of net zero carbon dioxide (CO_2) emissions by 2050 (IPCC, 2021). The protection and restoration of blue C ecosystems is imperative to overcome the consequences of anthropogenetic and natural threats to usable area, ultimately leading to the re-release of greenhouse gases (Millard et al., 2013; Macreadie et al., 2013). Salt marshes surrounding the Bay of Fundy benefit from restoration tools such as managed realignment which aims to re-introduce tidal flow and restore previously lost wetland habitat (Bowron et al., 2011; van Proosdij et al., 2010; Wollenberg et al., 2018). A local study looked at this dynamic post-restoration and found elevated allochthonous sediments and C (Wollenberg et al., 2018), however further analyses examining vegetation and microbial contributions to C sequestration require more research to understand internal C contributions and represent a current knowledge gap.

1.1 Salt marsh carbon accumulation and storage

Salt marshes are coastal wetlands found predominantly in the upper intertidal zone. These ecosystems are highly productive due to increased primary production, low rates of decomposition and suppression of microbes due to frequent inundation (Chmura et al., 2003;

Bulseco et al., 2019). Salt marshes are blue C ecosystems known for being net C sinks. Previous research suggests they can accumulate and store CO_2 faster than terrestrial ecosystems (Macreadie et al., 2013). Although occupying a small percentage of marine sediments (0.1-2%), salt marshes can bury C 55x faster than tropical rainforests which are considered the most efficient terrestrial C sink (Macreadie et al., 2013). C accumulation increases with allochthonous sources via tidal flow and suspended sediments or *in situ* from organic autochthonous sources (Drexler et al., 2020; Wollenberg et al., 2018). Allochthonous accumulation varies with marsh elevations; higher sedimentation occurs in the lower marsh zone due to frequent tidal flooding (Chmura et al., 2001; Gailis et al., 2021). Allochthonous sediments introduced with inundation influence restoration recovery as they typically have a positive ecological response with a change in surface elevation, vegetation establishment, and eventually higher species richness (Bowron et al., 2011; van Proosdij et al., 2010). The increase in tidal flow facilitates new marsh sediment deposits including C, which are assumed allochthonous (Wollenberg et al., 2018). Established (natural) marshes situated higher in the tidal frame rely on autochthonous C sources for organic carbon (OC) due to lack of suspended sediment from infrequent or introduced tidal flow (Drexler et al., 2020). These elevations may exhibit higher belowground organic production and biomass by microbes, including mycorrhizal fungi, whose contribution will influence the concentration of C in wetland soils (Elsey-Quirk et al., 2011; Parihar and Bora, 2019).

1.2 Salt marsh arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are beneficial belowground endomycorrhizal fungi that form symbiotic relationships with terrestrial and coastal plant roots, colonizing the cortical root tissue (Bonfante and Genre, 2010; Parihar and Bora, 2019). The plant host provides fixed C while AMF extended root-like structures, hyphae, uptake nutrients such as nitrogen (limited in salt marshes; Crain, 2007) and phosphorus for the plant. Plants can allocate 10-30% of their photosynthate C to AMF, which promotes hyphal proliferation and an increase in spore volume (Morton et al., 2004; Lanfranco et al., 2016).

AMF have shown tolerances to salinity and plant benefits beyond nutrient acquisition by promoting plant health and photosynthetic activity, a contributing factor to organic C conversion in salt marshes (Parihar and Bora, 2019). These are important benefits to consider for salt marsh vegetation in the Bay of Fundy region where tidal ranges and ice often affect vegetation and soil accretion (Lundholm et al., 2021). Salt marsh vegetation inoculated with AMF potentially promote early growth and prolonged survival of *Sporobolus* sp. (formerly *Spartina*) (d'Entremont et al., 2021). AMF have been found within the roots of perennial *Sporobolus* species which are key salt marsh grass species (Burcham et al., 2012; Burke et al., 2003; Cooke and Lefor, 1990; d'Entremont et al., 2018). Previous studies have found AMF colonization in *S. alterniflorus* to be inconsistent (Burcham et al., 2012; Daleo et al., 2008; d'Entremont et al., 2018). Seasonal and habitat variation could impact mycorrhizal colonization in salt marsh species (Cooke et al., 1993), explaining inconsistencies among various studies. Burcham et al. (2012) found a significant AMF colonization rate of 39.7% in *S. pumilus* roots and a 2.4% colonization rate in *S. alterniflorus* roots selected from three salt marsh sites in coastal Louisiana. These species are separated by elevation within the marsh. *S. alterniflorus* can withstand extended seawater inundation periods and is found in the lowest elevations of the marsh (Veldhuis et al., 2019). *S. pumilus* is not as inundation-tolerant as lower marsh species, constraining this species to higher elevations (d'Entremont et al., 2018, 2021). *S. michauxianus* is the least inundation-tolerant, placing it at the upper high elevations of the marsh (Maricle et al., 2007).

1.3 Project context

This research is driven by the high C storage and accumulation abilities of salt marshes to contribute to climate change mitigation. The Nova Scotia climate change plan has committed to achieving net 0 emissions by 2050 (McCoy and Hughes, 2021). However, there are limited C studies surrounding Nova Scotia's highly productive salt marshes. This includes the Bay of Fundy, a hypertidal bay which is globally known for extreme tidal ranges (exceeding 15 m) (Desplanque and Mossman, 2004). This research was completed in the upper Bay of Fundy where sedimentation is much higher compared to sites within the lower Bay which have less sediment deposition (CBWES Inc, 2020). A map of selected sites is included in Chapter 2 of this thesis. This project focused on CB Wetlands and Environmental Specialists (CBWES) monitored restoration and reference (natural) sites. Specifically, the restored sites aim at re-introducing tidal flow and sediment back into these ecosystems that have been declining in area since the arrival of the 17th Century Acadian settlers. There has since been an ~80% decrease of functional salt marshes in the Bay of Fundy due to land reclamation (Bowron et al., 2011). Presently, we have a greater knowledge on the productive role salt marshes play in mitigating climate change by sequestering and storing C (Howard et al., 2017; McLeod et al., 2011; Wollenberg et al., 2018). This thesis focused on the internal (autochthonous) accumulation of C and how dominant vegetation and mycorrhizae are contributing to C storage within the salt marsh.

This thesis is part of a larger research project funded by NSERC ResNet, a Canada-wide research coordination network composed of multiple landscapes for monitoring, modelling, and managing Canadian ecosystem services for sustainability and resilience (ResNet, 2022). Our Landscape 1 team focused specifically on dykeland and salt marsh systems in the Bay of Fundy. Our lab within the research centre, TransCoastal Adaptations: Centre for Nature-Based Solutions

(TCA), engages in research, projects, and partnerships that contribute to climate change initiatives. We worked closely with CBWES, a company devoted to collaborating and leading projects on habitat restoration (i.e. tidal wetlands), climate change adaptation, and more (CBWES Inc., 2020). Their seasonal monitoring data from all restoration sites have been influential in the creation of protocols, site selection and further data collections. CBWES, TCA, along with the Nova Scotia Department of Agriculture (NSDA) aim to protect salt marshes and dykeland habitats by implementing managed realignment projects to promote salt marsh restoration and succession, both influential to this project.

1.4 Thesis organization

This thesis is organized in a manuscript format and examined the C accumulation potential of salt marshes in the Bay of Fundy by analyzing autochthonous C and AMF in the root zone of *Sporobolus* vegetation. Chapter 2 focuses on C accumulation by analyzing organic carbon (OC) density using different analytical techniques within three vegetation regions in Bay of Fundy salt marshes. Two different processing techniques, loss on ignition and elemental analysis, were used to analyze sediment OC. This manuscript will be used in collaboration with other OC research at Saint Mary's University focusing on OC densities at varying depths. Chapter 3 assesses a correlation between AMF colonization and sediment OC densities in salt marshes of various ages. Additionally, AMF root colonization rates are given for the three dominant *Sporobolus* salt marsh grass species. This manuscript chapter has been submitted for publication to *FACETS*. Chapter 4 integrates the results from Chapters 2 and 3 to create broader suggestions for future salt marsh carbon and mycorrhizal studies. References for all chapters are found at the end of the thesis.

Chapter 2: Accurate estimation of sediment organic carbon densities using loss on ignition and elemental analysis

2.1 Introduction

Elevated CO_2 concentration in our atmosphere is alarming and places us in a global climate crisis. The Intergovernmental Panel on Climate Change (IPCC, 2021) estimates that by the year 2100, if global warming increases by 1.5˚C, there will be long-lasting changes and irreversible ecosystem losses. To prevent this, there must be an 85% reduction in emissions by the year 2050 (McLeod et al., 2011). Tidal ecosystems such as salt marshes, can sequester C at increased rates and reduce C in our atmosphere (Chmura, 2013). The ability of salt marshes to capture large amounts of C through vegetation and suspended sediments has switched our focus to their role in climate mitigation.

Salt marshes are found on sheltered marine coastlines above mean sea level, along tidal rivers, in bays and estuaries and are considered intertidal ecosystems (Broome et al., 1988; Chmura et al., 2003; Teal, 2001; Waltham et al., 2021). Geographically, they are found in temperate climates (Chmura, 2013). Globally, salt marsh ecosystems are facing serious anthropogenic and natural threats. Rising sea-levels, land reclamation for dykelands, landscape conversion, human impacts, and coastal development have contributed to the decline of approximately 50% of all marsh area worldwide (Broome et al., 1988; Connor et al., 2001; Chmura et al., 2003; Macreadie et al., 2013). In Canada, there is an estimated decline of 77% of pre-existing salt marsh fringing the Bay of Fundy while there is an estimated 25-50% loss of global salt marsh ecosystems over the past century, and further loss of 30-40% over the next century (Burden et al., 2019; Wollenberg et al., 2018).

Nature-based processes involving marsh restoration aim to restore a marsh back to their natural conditions and decrease marsh loss (Burden et al., 2019). Marshes differ in their structure and function; site-specific planning is more practical and profitable in comparison to standard restoration designs (Waltham et al., 2021). In many circumstances, restoration efforts may be influenced directly or indirectly. Direct action includes dredging, dyke removal and construction which may influence sedimentation, erosion, or drainage (Broome et al., 1988). Bowron et al. (2011) directly replaced a culvert in a Nova Scotian marsh fringing the Bay of Fundy which increased tidal flow by 88%. Indirect action can involve changes in salinity levels, sedimentation rates and nutrient pulses, all of which can alter the composition and C storing capacity of the marsh. Additionally, another widely used technique for salt marsh restoration is managed realignment. This involves breaching, removal and/or moving of sea defence structure, including dykes, to restore tidal flow and promote the restoration of the marsh (Burden et al., 2019; French, 2006). Managed realignment often used by CBWES to restore salt marshes along the Bay of Fundy in Nova Scotia (Bowron et al., 2011; Bowron et al., 2015a; Bowron et al., 2015b; Bowron et al., 2020; Neatt et al., 2013).

The appreciation of the importance of salt marsh restoration has been increasing due to the outcome of beneficial ecosystem and social services (Waltham et al., 2021). Composed of mainly grasses and sedges, salt marshes provide refuge for many invertebrates, birds and fishes (Chmura, 2013; d'Entremont et al., 2018; Waltham et al., 2021). They further provide ecological value in nutrient cycling due to tidal flow, shoreline stabilization by trapping sediments, storm buffering, and C sequestration (Chmura, 2013; Grimsditch et al., 2013; Wollenberg et al., 2018). Salt marshes are known for being "net C sinks" that can sequester C in the form of CO_2 and methane (CH_4) and other greenhouse gases (i.e. nitrous oxide N_2O) from the atmosphere (Broome et al., 1988). There has been a particular interest in the role salt marshes play in climate mitigation (Broome et al., 1988; Howard et al., 2017). Salt marshes store organic C as "blue carbon" (Macreadie et al., 2013; Wollenberg et al., 2018). Blue C accumulation increases with autochthonous C storage within the marsh via fixed CO_2 resulting from photosynthesis occurring by marsh vegetation and subsequent burial and slow decomposition of plant biomass (Wollenberg et al., 2018). Higher elevations and established marshes heavily rely on autochthonous C sources for organic carbon (OC) due to lack of suspended sediment from infrequent or introduced tidal flow (Drexler et al., 2020). C can be laterally import by tides into the system which adds allochthonous sediment and organic C from outside the marsh, with marsh vegetation trapping and storing the suspended sediment (Wollenberg et al., 2018). Recent research in a Bay of Fundy salt marsh found a rapid increase in allochthonous sediments 6 years post managed realignment, resulting in C burial rates exceeding those of mature Bay of Fundy marshes (Wollenberg et al., 2018). An influx of allochthonous sediments commonly occurs in managed realignment salt marsh sites where tidal flow is re-introduced, including sites found in the upper Bay of Fundy where concentrations of suspended sediments are high (Bowron et al., 2011).

Blue C can be sequestered in coastal ecosystems over a short term (decennial) and stored as biomass, or over longer scales (millennial) within the sediment (McLeod et al., 2011). In comparison to other "blue carbon ecosystems", salt marshes heavily contribute to long-term C sequestration and have a higher rate of blue C within their sediments (McLeod et al., 2011). Salt marshes can bury C 55x faster than tropical rainforests, which are considered the most efficient terrestrial C sink (Macreadie et al., 2013). Salt marsh global C burial rate reaches 87.2 ± 9.6 Tg C yr^{-1}, which exceeds that of rainforests (53 ± 9.6 Tg C yr^{-1}) (Macreadie et al., 2013).

Sediment OC density has commonly been quantified using two parameters: loss on ignition (LOI) and bulk density (Connor et al., 2001; Howard et al., 2014; Wollenberg et al., 2018). Another method commonly used to quantify % C is elemental analysis. This quantitative method provides an accurate measure of C content in percentage; however, it requires specialized costly instrumentation (Howard et al., 2014). LOI is a relatively inexpensive combustion semi-quantitative measurement for organic matter (OM) %. Following this, OM % may be used with previously established OC conversion equations, such as Craft et al. (1991) or % OC may be determined from derived relationships between % C and % OM (Howard et al., 2014). Once bulk density is determined ($g \cdot cm^3$), it is multiplied by % OC/100 to determine OC density at specific sediment depths (Howard et al., 2014). This study measured OC density using multiple methodologies: LOI, bulk density, and elemental analysis. The OC conversion equation from Craft et al. (1991) was used in this study to measure OC densities and to contrast with accurate OC conversion from regression analysis using elemental analysis.

This research examined the C storage potential of salt marshes in the Bay of Fundy by analyzing autochthonous C storage in the root zone surrounding dominant salt marsh vegetation. To determine an accurate OC conversion factor for the study sites, elemental analysis and organic matter analysis were conducted using a similar methodology to Craft et al. (1991). This chapter explores C accumulation by determining how organic carbon (OC) density at Bay of Fundy salt marshes varies with vegetation type and analytical method used. The research objectives of this chapter were to: (1) quantify OC densities within the root zone of *Sporobolus* species, (2) determine if OC densities vary between restored and reference (natural) salt marshes and (3) assess if OC densities vary using two analytical techniques (LOI & bulk density, and elemental analysis).

2.2 Study Sites

In 2020, 11 salt marsh sites were sampled in the Bay of Fundy (Figure 1; Figure 2). This included four brackish and seven saline marshes. The salt marsh sites were all located in the upper Bay of Fundy where sedimentation rates are higher. They were chosen according to whether they were previously restored, their age and presence of dominant *Sporobolus* species: *Sporobolus alterniflorus* (Loiseleur-Deslongchamps) (Smooth Cordgrass), formerly *Spartina alterniflora* (Loisel), grows in the low marsh zone, *Sporobolus pumilus* (Roth) (Salt Marsh Hay), formerly *Spartina patens* (Aiton), is found in the mid-to-high marsh zone, and *Sporobolus michauxianus* (Hitchcock) (Prairie Cordgrass), formerly *Spartina pectinata* (Bosc ex Link), is found at the highest marsh elevations (Kim et al., 2012; Porter et al., 2015; d'Entremont et al. 2018).

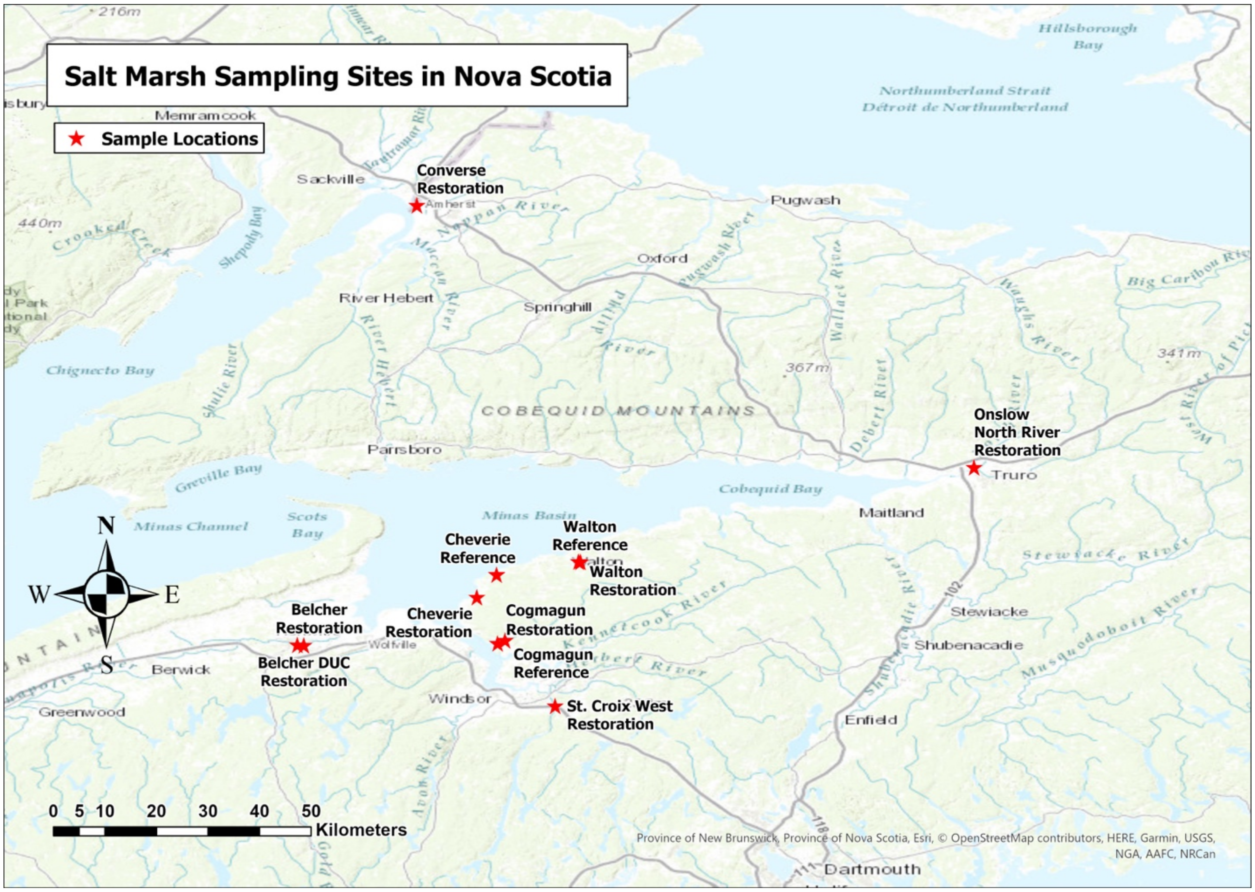

Figure 1. Salt marsh sampling locations fringing the upper Bay of Fundy in Nova Scotia.

Most of our study sites have long-term monitoring data available from CBWES and Saint Mary’s University aside from DUC Belcher which is owned by Ducks Unlimited Canada and unmonitored (Table 1, 2). In 2021, the Onslow North River Restoration site was added to our study and the sites Belcher Restoration, St. Croix West, Walton Restoration and Reference were not revisited due to having weak a weak AMF and OC density relationship, or low AMF root colonization and OC densities in 2020 (Appendix A).

Figure 2. Topography of new (A = Converse Restoration, B = Belcher DUC Restoration), intermediate (C = Cogmagun Restoration), old (D = Cheverie Restoration) restoration sites and reference sites (E = Cheverie Reference, F = Cogmagun Reference).

Table 1. Data collected by CBWES from restored salt marsh sites in the Bay of Fundy, Nova Scotia. Years since restoration refer to the respective CBWES report. Marsh zones are defined differently for newly restored Converse and Belcher sites as distinct zones have not yet been achieved. *Sporobolus alterniflorus* and *Sporobolus pumilus* were not present at St. Croix West. DUC Belcher is not included as it is maintained by Duck Unlimited Canada and not CBWES (Bowron et al., 2013, 2015a, 2015b, 2021; Graham et al., 2021; Neatt et al., 2013).

Restored site	Converse			Belcher			St. Croix West			Cogmagun			Cheverie			Walton		
Years since restoration	2 (2018)			3 (2017)			5 (2009)			5 (2009)			7 (2005)			7 (2005)		
Age of marsh during sample collection	2 (2020) & 3 (2021)			3 (2020) & 4 (2021)			11 (2020) & 12 (2021)			11 (2020) & 12 (2021)			15 (2020) & 16 (2021)			15 (2020)		
Type of managed realignment	Dyke realignment and breach			Dyke realignment			Dyke breach			Dyke breach			Culvert replacement			Water control structure removal and dyke breach		
Mean water salinity (ppt)	N/A			N/A			4.7 (Year 5)			29.05 (Year 5)			30.15 (Year 7)			27.9 (Year 7)		
Mean pore water salinity (ppt)	N/A			N/A			1.75 (Year 5)			N/A			10.39 (Year 7)			7.58 (Year 7)		
Marsh zone	***S. a***	***S. p***	***S. m***	***S. a***	***S. p***	***S. m***	**LM**	**MM**	**HM (*S. m*)**	**LM**	**MM**	**HM**	**LM**	**MM**	**HM**	**LM**	**MM**	**HM**
Net change in surface elevation (cm/yr ± SE)	3.1 (± 1.4) (Year 2)	N/A	6.4 (± 2.7) (Year 2)	23.4 (± 2.4)	N/A	35.3 (± 1.8)	46.15 (±0.26)	RSET-3: 16.63 (±0.28) RSET-4: 38.40 (±0.29)	35.24 (±0.25)	1.92 (±0.23)	3.22 (±0.25)	1.43 (±0.21)	RSET-1: 6.05 (±0.10) RSET-2: 15.44 (±0.10)	N/A	RSET-3: 6.64 (±0.22) RSET-4: 3.67 (±0.10)	RSET-1: 8.78 RSET-3: 1.09	N/A	4.04
Net sediment accretion (cm/yr)	-1.64 (MH2)	N/A	-1.40 (MH13)	2.3 (MH1A)	N/A	N/A	45.98 (±2.10)	RSET-3: 16.20 (±0.55) RSET-4: 41.83 (±0.21)	30.18 (±1.12)	5.28	5.81	5.48	RSET-1: 5.93 RSET-2: 6.26	N/A	RSET-3: 6.33 RSET-4: 2.86	RSET-1: 6.00 RSET-3: 4.59	N/A	3.29
Inundation frequency at sampling area	~18.9% (RSET1)	N/A	~37.8% (T2S3)	~20.2%	N/A	~14.3%	75.1-100%	13.1-33%	0-33%	34.5-38.9%	25.4-34.4%	14.3-16.2%	39.61-70.3%	31.91-39.6%	17.6-24.2%	63.71-81.3%	63.71-81.3%	30.71-47.2%

Table 2. Net change in surface elevation and sediment accretion collected by CBWES from reference salt marsh sites in the Bay of Fundy, Nova Scotia (Bowron et al., 2013, 2015b; Neatt et al., 2013).

Reference site	Cogmagun			Cheverie			Walton		
	LM	MM	HM	LM	MM	HM	LM	MM	HM
Net change in surface elevation (cm/yr ± SE)	0.67 (± 0.07)	0.828 (±0.15)	0.696 (±0.14)	2.19 (±0.06)	0.294 (±0.06)	0.18 (±0.12)	2.42	N/A	0.377
Net sediment accretion (cm/yr)	0.788	1.12	0.67	0.403	0.266	0.382	3.21	N/A	0.91

2.3 Materials and Methods

Sampling locations within each site were selected based on vegetation zones: *S. alterniflorus, S. pumilus, S. michauxianus*. Three sediment cores were taken within each vegetation zone across salt marsh sites to increase coverage and when applicable, in correspondence with CBWES transects. In August 2020 and 2021, cores were collected with a 50 cm Russian peat auger within the root zone at a depth of 15 cm. This depth was targeted to fulfill Chapter 3 requirements which targeted the sediment surrounding *Sporobolus* roots and mycorrhizal hyphae. AMF require soil aeration, which is why they remain in the upper portions of the soil matrix as anoxia can be a limiting factor for their growth and survival (Maček, 2017). Root samples were transported in Ziploc bags and stored at the K.C. Irving and Environmental Centre at Acadia University at 4˚C; sediment cores were stored at Saint Mary's University at -20˚C.

2.3.1 Loss on ignition and bulk carbon density

To accurately determine soil C density, bulk density and OM content were quantified. A drying oven, desiccator and muffle furnace were required to process sediment samples for OM, a procedure completed over three days. The process of loss on ignition (LOI) described below, was initiated by determining water and OM content in samples heated at high temperatures which burned off any volatile substances and determined the amount of sample lost by comparing preand post-ignition weight (Wollenberg et al., 2018). LOI and bulk density were completed at Saint Mary's University in the In_CoaST lab in 2020 and at Acadia University for the remainder of 2020 (due to COVID-19) and in 2022. Prior to processing on day one, cores collected both years (2020 and 2021) were thawed at 4 ˚C for 24 hours. Two subsamples from the same depth (~15 cm) were processed and the average was used for analyses. On day one, all samples were processed and weighed in labelled porcelain crucibles. Approximately 2.5 g of sediment (for each subsample) were added to the pre-weighed crucible and placed in a 95˚C oven for 24 hours to initiate LOI. The following day, the samples were removed from the drying oven and placed in a desiccator for a minimum of one hour to cool and remove any additionally moisture from the samples. The samples were re-weighed to receive their dry weight (DW_{95°). Using a mortar and pestle, the samples were ground into a fine powder and returned to their respective crucibles and placed into a muffle furnace (Fisher Scientific Isotemp Muffle Furnace 550 Series). The furnace parameters were set at 550˚C and samples were left in for approximately 2 hours and 40 minutes (furnace took ~40 minutes to reach 550˚C). After this time, the furnace was turned off and samples were allowed to cool in the furnace overnight. On the final day, the samples were removed from the muffle furnace and placed in a desiccator for a minimum of one hour. The samples were weighed (DW_{550°) and then discarded.

Bulk density was determined by using a known syringe volume of sediment in aluminum crucibles and dehydrating in a drying oven for 24 hours at 95˚C. After 1 hour in the desiccator, dry weights were recorded in $g \cdot cm^{-3}$.

2.3.2 Sediment analysis

In February 2022, 10 g of dried powder sediment subsamples from each root zone sample (10-15cm) collected in 2021 were quantified in a CHN analyzer (Perkin Elmer 2400 Series II) using cystine as a standard calibration (Elemental MicroAnalysis) for total %C at the Centre for Environmental Analysis and Remediation at Saint Mary's University.

2.3.3 Calculation of organic carbon

Organic carbon density

Once LOI was completed in 2020 and 2022, OM content within the soil was determined using the Heiri et al. (2001) equation:

$$LOI\ fraction\ =\ (DW_{95^{\circ}} - DW_{550^{\circ}}\ /\ DW_{95^{\circ}}) * 100 \qquad (2.1)$$

To convert from OM to OC, a conversion equation from Craft et al. (1991) was used:

$$OC\ conversion\ =\ 0.40(LOI)\ +\ (0.025 * LOI)^2 \qquad (2.2)$$

This equation has been previously used in research quantifying C in the Bay of Fundy (Connor et al., 2001; Wollenberg et al., 2018). To determine OC density, the value from the OC conversion equation was multiplied by the bulk density ($g \cdot cm^3$) for each sample.

Organic carbon conversion factor

To predict accurate soil OC %, regression analysis using OM and elemental analysis results were quantified (Craft et al., 1991). The relationship between OM and OC was used for each salt marsh site to determine if one or more equations are required for the accurate measurements of OC % at each of our Bay of Fundy salt marsh sites.

2.4 Results

2.4.1 Organic carbon density

Samples were collected 2020 and 2021 and pooled (Figure 3). There were no large significant differences between years. Sediment OC densities ($g \cdot C \cdot cm^{-3}$) varied in each vegetation zone in 2020 using Craft et al. (1991) OC conversion equation: between 0.0147 to 0.0580 $g \cdot C \cdot cm^{-3}$ in the *S. alterniflorus* region 0.0147 to 0.0400 $g \cdot C \cdot cm^{-3}$ in the *S. pumilus* region and 0.0150 to 0.0515 g·C·cm^{-3} in the *S. michauxianus* region (Appendix A; Figure 3). OC densities in *S. alterniflorus* region were highest at newly restored sites, Belcher DUC and Converse (0.0187 to 0.0580 g·C·cm^{-3}). In the *S. pumilus* region, OC densities were highest at an intermediate aged restoration site (Cogmagun Restoration) with 0.0400 g·C·cm^{-3}. One old restoration site (Walton Restoration) had the highest OC density in the *S. michauxianus* marsh zone (0.0515 g·C·cm^{-3}) (Figure 3).

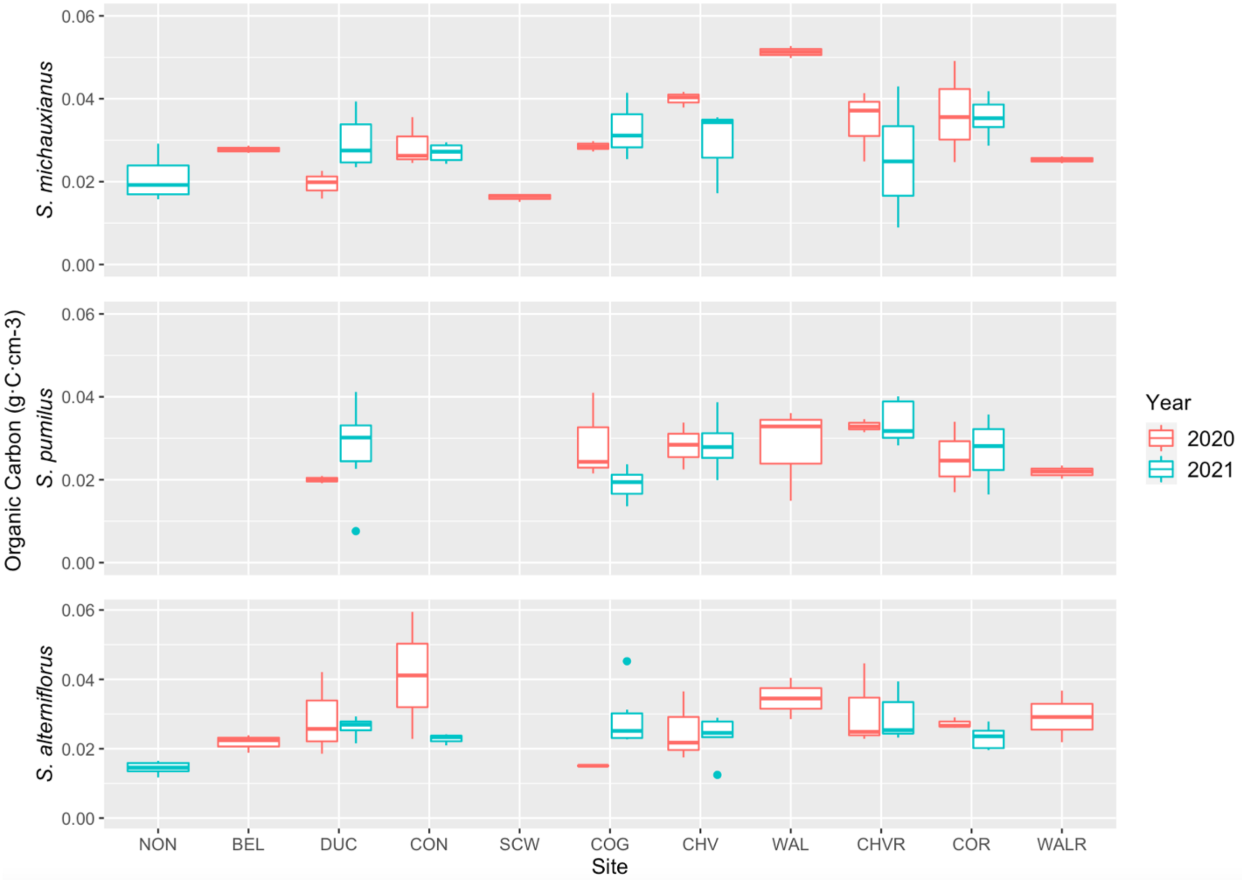

Figure 3. Organic carbon (g·C·cm^{-3}) 2020 and 2021 results in vegetation zones at Bay of Fundy salt marsh sites using conversion equation by Craft et al. (1991) arranged from new to old restoration, and reference sites: NON = Onslow North River Restoration, BEL = Belcher Restoration, DUC = Belcher DUC Restoration, CON = Converse Restoration, SCW = St. Croix West Restoration, COG = Cogmagun Restoration, CHV = Cheverie Restoration, WAL = Walton Restoration, CHVR = Cheverie Reference, WALR = Walton Reference, and COR = Cogmagun Reference. Error bars represent 95% confidence intervals.

Sediment OC densities from 2021 were slightly less variable in comparison to 2020's results (Appendix A, B). In 2021, fewer sites were revisited (2020: 10 sites, 2021: 7 sites), although sample size was doubled for each site (2020: n = 73, 2021: n = 110). Each vegetation zone had varying OC densities: *S. alterniflorus* ranged from 0.0124 to 0.0452 g·C·cm^{-3}, *S. pumilus* ranged from 0.00760 to 0.0411 g·C·cm^{-3} and *S. michauxianus* ranged from 0.0141 to 0.430 g·C·cm^{-3} (Appendix B; Figure 3). Sediment OC densities in the *S. alterniflorus* region were highest at intermediate site, Cogmagun Restoration. In the *S. pumilus* region, OC density was highest at a newly restored site (Belcher DUC Restoration) with 0.0411 g·C·cm^{-3}, however this site also accounted for the lowest OC density as well (0.00760 g·C·cm^{-3}) (Figure 3). OC densities were mostly elevated at reference sites for the *S. michauxianus* region, as for restoration sites, Cogmagun Restoration had the highest OC density in this region (0.0414 g·C·cm^{-3}) (Figure 3). These 2021 results differed from previous 2020 results which found elevated densities at newly restored sites, Belcher DUC, and Converse in *S. alterniflorus* region, intermediate aged site, Cogmagun Restoration for *S. pumilus* and the site with the highest OC density for *S. michauxianus* (Walton Restoration) was not revisited in 2021 (Figure 3).

2.4.2 Organic carbon conversion factor

In 2021 elemental analysis was completed on all samples collected within that year. OC density from elemental analysis was significantly different among sites and vegetation type also with a site x vegetation interaction (Table 3).

Table 3. Analysis of variance showing relationship between sediment organic carbon density from elemental analysis by site and vegetation type in 2021. Significant values are represented with asterisks.

Factor	*df*	*SS*	*MS*	*F*	*P*
Site	7	0.057674	0.0082392	59.7797	2.2e-16***
Vegetation Type	2	0.002685	0.0013427	9.7419	0.0001467***
Site x Vegetation Type	10	0.002593	0.0002593	1.8811	0.00578779*
Residuals	91	0.01254	0.0001378		

A linear model illustrated a positive relationship between OC% and OM% (Figure 4). A quadratic equation from the linear model fit best for most Upper Bay of Fundy salt marshes, aside from Cogmagun Reference which required a separate equation (Figure 4, 5). This equation is also known as the OC conversion equation:

$$Organic\ C = (0.373)LOI + (-0.00510)LOI^2 \tag{2.3}$$

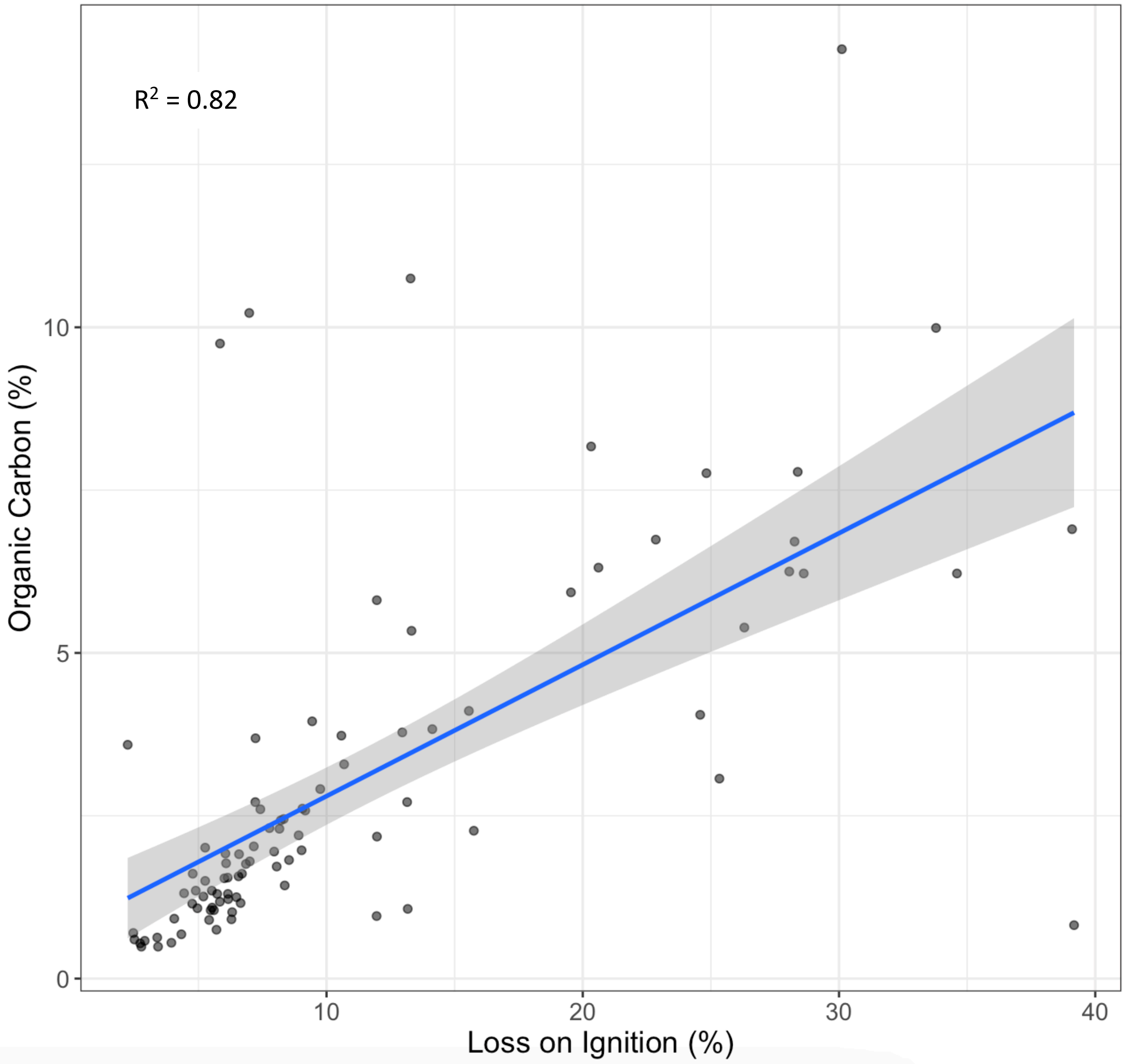

Figure 4. The relationship between organic carbon from elemental analysis and loss on ignition for all salt marsh sites excluding Cogmagun Reference (n = 92). Regression equation: Organic C = (0.373)LOI + (-0.00510)LOI^2.

Cogmagun Reference was significantly different from all other sites using regression analysis. Results from this site did not fit within the same regression, as a result, this site was given its own regression equation (OC conversion) (Figure 5):

$$Organic\ C = (0.474)LOI + (-0.00988)LOI^2 \tag{2.4}$$

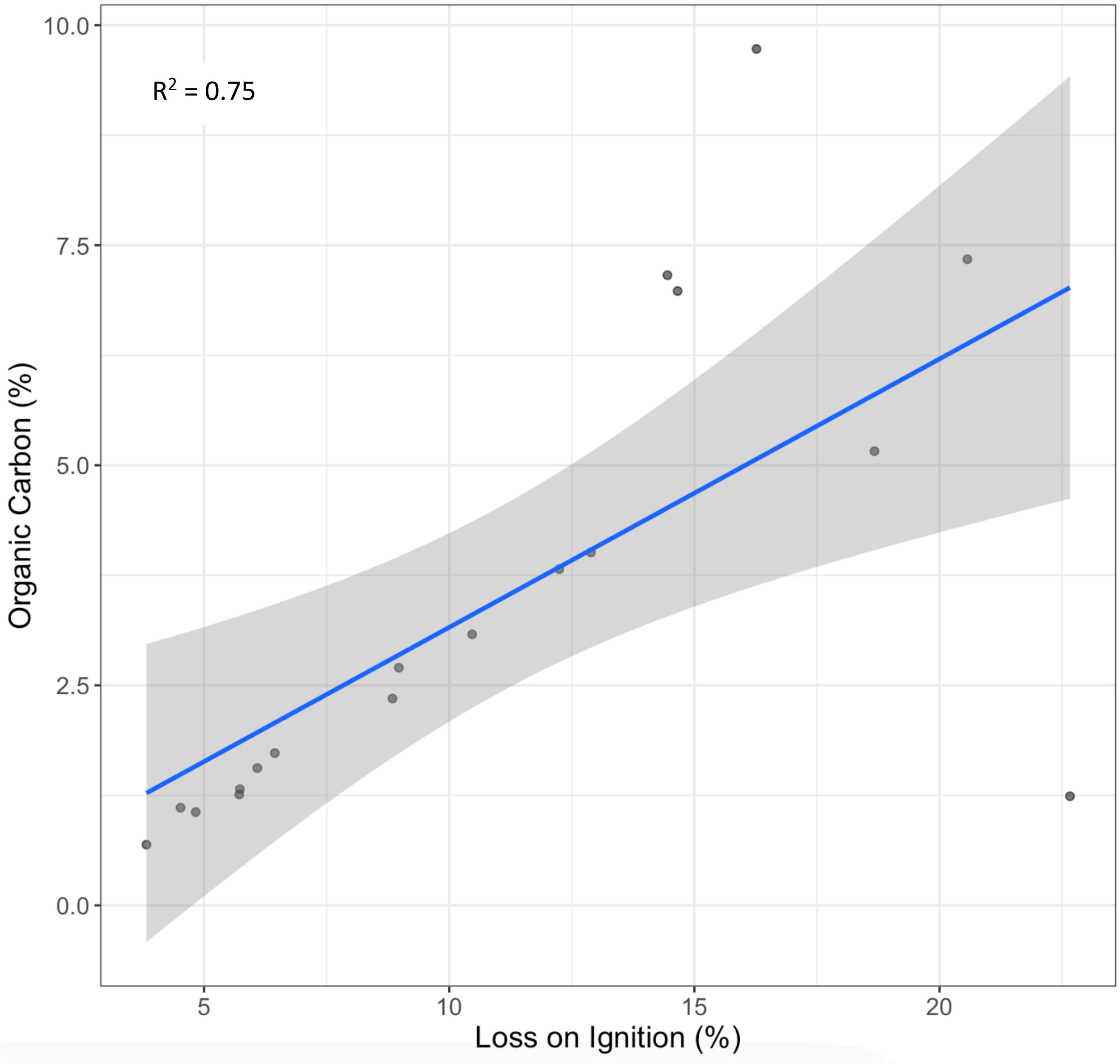

Figure 5. The relationship between organic carbon from elemental analysis and loss on ignition for Cogmagun Reference (n = 18). Regression equation: Organic C = (0.474)LOI + (-0.00988)LOI^2.

Sediment OC densities using the OC conversion equation from this study differed in comparison to results using Craft et al. (1991) equation (Appendix B). Overestimations in Figure 6 are noted at Onslow North River Restoration, Belcher DUC Restoration, Converse Restoration (*S. michauxianus* region), Cogmagun Restoration (*S. alterniflorus* and *S. pumilus* regions), Cheverie Restoration (*S. alterniflorus* and *S. michauxianus* regions), Cheverie Reference, and

Cogmagun Reference (*S. pumilus* and *S. michauxianus* regions). An overestimation of 0.022 $g \cdot C \cdot cm^{-3}$ at Cogmagun Restoration is also noted in Appendix B (0.066 $g \cdot C \cdot cm^{-3}$ compared to 0.044 $g \cdot C \cdot cm^{-3}$), a max Y-axis limit of 0.06 was set for Figure 6 to ensure proper spread of boxplot. However, the Craft et al. (1991) equation did show an underestimated of OC densities for the remaining sites and vegetation regions: Converse Restoration (*S. alterniflorus* region), Cogmagun Restoration (*S. michauxianus* region), Cheverie Restoration (*S. pumilus* and an outlier in the *S. alterniflorus* region), and Cogmagun Reference (*S. alterniflorus* region). Overall, there were more OC density overestimations than underestimations for the 2021 sample sites using the Craft et al. (1991) OC conversion equation.

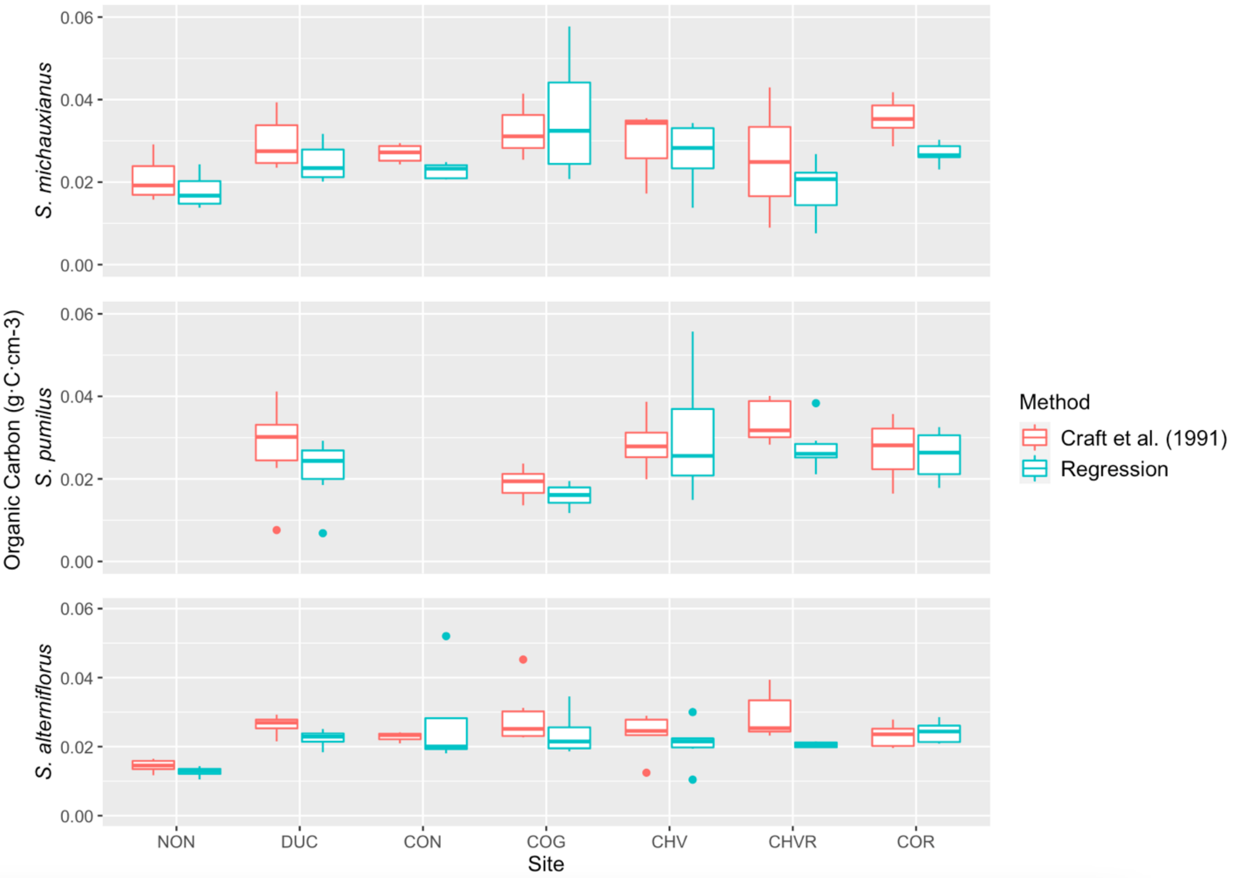

Figure 6. Organic carbon (g·C·cm^{-3}) 2021 results in three vegetations zones at Upper Bay of Fundy salt marsh sites using regression conversion equation (Organic C = (0.373)LOI + (-0.00510)LOI^2) for all sites excluding Cogmagun Reference, requiring a separate equation (Organic C = (0.474)LOI + (-0.00988)LOI^2) and Craft et al. (1991) organic carbon conversion equation, arranged from new to old restoration, and reference sites: NON = Onslow North River Restoration, DUC = Belcher DUC Restoration, CON = Converse Restoration, COG = Cogmagun Restoration, CHV = Cheverie Restoration, CHVR = Cheverie Reference, and COR = Cogmagun Reference. Error bars represent 95% confidence intervals.

2.5 Discussion

2.5.1 Organic carbon density

Restoration sites yielded higher sediment OC densities than reference sites. Three years post dyke realignment and breach, Converse Restoration had the most elevated OC densities across all sites in *S. alterniflorus* sediments (0.0580 g·C·cm^{-3}) (Bowron et al., 2020). These high carbon densities are likely due to increased bulk densities from allochthonous sediments, as incoming sediments tend to be mineral base composed of sand, silt, and clay, which also provide lower C densities between 0.01 and 0.02 $g \cdot C \cdot cm^{-3}$ (Appendix A, B; Wollenberg et al., 2018). Allochthonous sediments are often heavier with more material per unit volume which explains high OC densities in newer restored sites. Autochthonous sediments are highly composed of organic material including marsh vegetation roots which is lighter per unit volume (Howard et al., 2014). Sediment accumulation is much more rapid in the lower marsh zone due to consistent tidal flow and vegetation contributions causing sediment and OC to accumulate significantly compared to high and upper high elevated zones (Chmura et al., 2001; van Proosdij et al., 2006). The suspended sediment through regular tidal flow contributes to allochthonous C storage by transporting sediment and OC from outside of the marsh system and trapping it via vegetation (Gonneea et al., 2019; Wollenberg et al., 2018). With managed realignment, particularly post-dyke realignment and breaching, there is an increase in tidal flow which provides longer inundation times and can lead to rapid sediment accumulation (Byers and Chmura 2007; van Proosdij et al., 2010; Wollenberg et al., 2018). Introduction of tidal flow in marshes post-restoration contributes to sediment accretion leading to an accumulation of allochthonous OC (Wollenberg et al., 2018). This explains rapid sediment and C accretion in newer restored marshes such as Belcher Restoration, Converse Restoration and Belcher DUC Restoration in comparison to sites such as Cheverie Restoration which has been restored for a much longer timeframe (Table 1). Older restored sites have longer to equilibrate post-restoration which provides more consistent values year to year (Table 1). The newer restored sites require similar timeframes until equilibrium is reached and consistent C values can be measured temporally (Bowron et al., 2013).

OC densities in the *S. alterniflorus* vegetation zone were comparable across most sites except for Onslow North River Restoration which had lower OC sediment densities in *S.*

alterniflorus and *S. michauxianus* zones. This is a new site to CBWES and this project, so extensive monitoring data were not available, however this site is known to have high freshwater input (Bowron et al., 2019). Current data suggest that OC decreases with increasing salinity (Van de Broek et al., 2016), although that trend was not applicable to this study. Lower OC densities were observed at most study sites with freshwater input, which included lower mean water salinities and mean pore water salinities (Table 1; Appendix A, B). St. Croix West (2020) had the lowest OC density in the *S. michaxianus* region. There is little research known on freshwater tidal wetlands, no global inventory of these systems and no global OC stock measurement have been created (Van de Broek et al., 2016). These decreased OC densities in marshes with freshwater input could be impacted by depth limitations. When quantifying C specifically in freshwater marshes, deeper cores are essential as a large portion of the C may be unaccounted for in deeper soils (>30 cm) due to large peat reserves (Loder and Finkelstein, 2020). Although freshwater marshes are also considered net C sinks, C analysis commonly occurs short-term (year-to-year) in comparison to salt marshes which have been more readily analyzed for long-term carbon storage. Freshwater systems depend on autochthonous production, have high OM content (>50%) resulting in lower bulk density and are not as mineral-reliant. Salt marshes rely on both allochthonous input from suspended sediment and autochthonous production, and this varies depending on topography and age of the marsh (Wollenberg et al., 2018). C accumulation in freshwater marshes over short timescales are comparable to salt marshes, and temperate peatlands over longer timescales (Loder and Finkelstein, 2020).

2.5.2 Organic carbon conversion factor

OC density using elemental analysis was highly variable among sites ($p < 2.2e-16$, $\alpha = 0.05$) and vegetation types ($p < 0.000147$, $\alpha = 0.05$) (Table 3). However, despite variation among sites, analyses showed that variation was not explained by site nor vegetation type aside from Cogmagun Reference which was statistically different among all sites (Figure 3, 4). Reference sites are considered natural sites which are not monitored as closely as restoration sites, indicating less data are available. Cogmagun Reference site had a high OC density and varying OM% which could have contributed to the observed statistical difference (0.0614 $g \cdot C \cdot cm^{-3}$; 0.1038 $g \cdot C \cdot cm^{-3}$) (Craft et al., 1991). R-squared values for both Figures 3 and 4 illustrated high levels of correlation indicating a strong relationship between OC% (from elemental analysis) and OM%, allowing one equation to derive OC from LOI to be used for all sites excluding Cogmagun Reference (Craft et al., 1991).

The OC conversion equation proposed by Craft et al. (1991) has been widely used for converting OM% using LOI to OC% (Abbott et al., 2019; Connor et al., 2001; Elsey-Quirk et al., 2011; Macreadie et al., 2013; Wollenberg et al., 2018). This is a cost-effective method as elemental analysis requires specialized instrumentation and can range between $10-20 per sample (Howard et al., 2014). However, this equation was created using regression analysis from sediment samples collected from ten brackish and salt marshes in North Carolina (Craft et al., 1991). All marshes are topographically different with varying hydrological conditions, specifically marshes in the Bay of Fundy with some of the highest tides globally. Bay of Fundy marshes could potentially experience varying conditions in comparison to North Carolina marshes which are further south. As seen in Figure 6, result vary when using the Craft et al. (1991) equation in comparison to the regression equation used in this study. The Craft et al. (1991) equation overestimated OC density results at Cheverie Reference, Belcher DUC and Cogmagun Restoration sites. The equation created by Craft et al. (1991) was specific for their samples from various marshes, which had a very strong R-squared value and higher estimates;

understandably it may not suffice as an accurate conversion factor for all marshes globally. These marshes could differ in minerology for example, whereas Bay of Fundy marshes are high in iron and may alter redox potentials and intensity of anaerobic conditions within the sediments, thus affecting autochthonous C accumulation (Bowron et al., 2015b). Using elemental analysis is the most accurate and suitable method for analyzing C for individual studies and should be included when budgeting for projects.

OC analysis is increasing globally due to known benefits and supporting our understanding of climate change. In Nova Scotia, few studies have focused on quantifying C in various regions of the Bay of Fundy (Connor et al., 2001; Gallant et al., 2020; Wollenberg et al., 2018). When comparing these findings to previous results within the Bay of Fundy, there are slight variations. Studies such as Connor et al. (2001) and Wollenberg et al. (2018) sampled in a different geographical region of the Bay of Fundy and relied on Craft et al. (1991) equation for converting OC which may have overestimated their results. Sedimentation is highest in the Upper Bay of Fundy which differs geographically from their selected study sites and may have had less sedimentation compared to sites selected for this study (Wu et al., 2011). Gallant et al. (2020) measured economic valuation of C sequestered in Nova Scotian wetlands. Our research is the first to use elemental analysis to accurately quantify C in Nova Scotian salt marshes, providing novel information to better understand the mitigation capacities of marshes in our region.

Chapter 3: Assessing *Sporobolus* (*Spartina*) mycorrhizal colonization and sediment carbon density in Bay of Fundy salt marshes

Submitted to: FACETS on 9 September 2022, Manuscript no. facets-2022-0198
Modified since submission

3.1 Introduction

Interest in tidal ecosystem restoration such as managed realignment of salt marshes (i.e. removing tidal barriers) is increasing due to the potential for climate change mitigation (Byers and Chmura, 2007; van Proosdij et al., 2010). Salt marshes can sequester carbon (C) in the form of CO_2, methane (CH_4) and other greenhouse gases (i.e. nitrous oxide (N_2O)) at increased rates and reduce C in our atmosphere, highlighting their climate mitigation value (Chmura, 2013; Howard et al., 2017). C sequestered and stored in biomass and sediment of coastal and marine ecosystems is termed ‘blue carbon’ (‘Blue C’) (Wollenberg et al., 2018; Zinke, 2020). Blue C accumulation can increase with autochthonous C storage within tidal wetlands via fixed CO_2 from marsh vegetation photosynthesis (McLeod et al., 2011; Wollenberg et al., 2018). Tidal flow is an indirect allochthonous C source, introducing sediment and organic C (OC) from outside the marsh, with marsh vegetation trapping the suspended sediment and storing C (McLeod et al., 2011; Owers et al., 2020; Wollenberg et al., 2018). This C is also essential for the survival of beneficial fungal associates which support marsh vegetation growth.

Arbuscular mycorrhizal fungi (AMF) are known beneficial plant symbionts, essential to the surrounding soil as they link the plant-soil continuum (Smith and Read, 2010; Wilson et al., 2009). The plant acts as a host and C source for the fungi, while the AMF supply nitrogen and phosphorus to their host. In this unique partnership, plants allocate 10-30% of their photosynthate C to AMF which aid in the storage of C as triacylglycerides. These compounds are transported to the extraradical hyphae within the root zone (Lanfranco et al., 2016; Morton et al., 2004; Olsson et al., 2010). In terrestrial ecosystems, AMF enhance autochthonous C storage through up-regulation of photosynthesis which increases primary production, and by translocating C away from high respiratory activity around the roots and into the soil matrix (Wilson et al., 2009; Zhu and Miller, 2003). Considering that all AMF possess similar physiological structures, it is possible that salt marsh AMF could be making equivalent contributions to C storage. We lack an understanding of the long-term effects of salt marsh AMF on C storage. AMF are often overlooked in climate mitigation studies, particularly in salt marsh ecosystems despite their involvement in the accumulation of additional photosynthetic fixed C in sediment (Burcham et al., 2012).

AMF have been found within the roots of common halophytic salt marsh *Sporobolus* (formerly *Spartina*) species, specifically, *Sporobolus alterniflorus* (Loiseleur-Deslongchamps) (Smooth Cordgrass), *Sporobolus pumilus* (Roth) (Salt marsh Hay) (Poaceae), and *Sporobolus michauxianus* (Hitchcock) (Prairie Cordgrass) (Anderson et al., 1986; Burcham et al., 2012; Burke et al., 2003; Cooke and Lefor, 1990; d'Entremont et al., 2018, 2021). The mutualistic relationship between *Sporobolus* species and salt marsh AMF is understudied in terms of its potential role in C sequestration.

Using three reference (natural) and seven restored salt marshes, the objectives of this study were to: (1) compare AMF colonization rates among three *Sporobolus* (*Spartina*) species and evaluate whether salt marsh age impacts (2) AMF colonization rate, and (3) sediment OC densities. Previous research suggests that AMF colonization and sediment C densities may differ between marsh vegetation types and sites, although these two variables have not been analyzed concurrently (Abbott et al., 2019; Burcham et al., 2012; Burke et al., 2003; d'Entremont et al., 2018, 2021; Wollenberg et al., 2018).

3.2 Study Sites

We chose 10 salt marsh sites fringing the upper Bay of Fundy, Nova Scotia based on the existence of monitoring data (Tables 1, 2; Figure 1) and presence of plant species *S. alterniflorus, S. pumilus* and *S. michauxianus*. Converse, Belcher and Belcher DUC restoration sites are newly restored salt marshes (< 5 years post-restoration), St. Croix West and Cogmagun are intermediate restored salt marshes (5+ years post-restoration), Cheverie (Bowron et al., 2011) and Walton (van Proosdij et al., 2010) are older restored salt marsh sites (15+ years post-restoration), and Cheverie reference (Bowron et al., 2011), Cogmagun reference and Walton reference (van Proosdij et al., 2010) are all considered unaltered, natural marshes which neighbour the corresponding restored marsh (Bowron et al., 2012; Porter et al., 2015). In Tables 1 and 2, mean water salinity (ppt), mean pore water salinity (ppt), inundation frequency, net change in surface elevation (cm ± SE) and net sediment accretion (cm) values were measured by CBWES (Bowron et al., 2011, 2013, 2015; Neatt et al., 2013; van Proosdij et al., 2010). Water salinity measures the salinity in the tidal floodwaters which can affect the abundance, distribution and diversity of plants present within the salt marsh (Bowron et al., 2015). Pore water salinity provides the soil salinity which influences plant and microbial abundance (Bowron et al., 2013; Maček, 2017). Both salinity measurements contribute to understanding of saline tolerances of the vegetation and AMF within our respective salt marsh sites. The inundation frequency (%) is a valuable measurement that supplies the recorded tides that flooded a sampling location (Bowron et al., 2015). This percentage highly influences both accretion and elevation of the marsh surface. Surface elevation is measured using Rod Surface Elevation Tables (RSET) and incorporates both subsurface processes including root production and sediment deposition (Cahoon et al., 2002). Sediment accretion values were derived from annual measurements from marker horizons and represent the vertical accumulation of inorganic and organic material deposited by floodwaters into the salt marsh (Bowron et al., 2013; van Proosdij et al., 2010). For detailed methods for these measurements please see: Bowron et al., 2011, 2013, 2015; Neatt et al., 2013; van Proosdij et al., 2010.

3.3 Materials and Methods

3.3.1 Sediment core and root collection

Sampling locations within each site were selected based on the presence of any targeted vegetation species: *S. alterniflorus* (*n* = 26), *S. pumilus* (*n* = 20), and *S. michauxianus* (*n* = 27). Three sediment cores were taken within each zone in each site where possible (not all vegetation zones were found at each site). In August 2020, cores were collected with a 50 cm Russian peat auger within the root zone at 15 cm depth. Cores were transported on ice and stored at -20˚C until analysis.

Root samples were collected within 1 m of each sediment core. Three root samples were collected per vegetation zone (*S. alterniflorus*, *S. pumilus*, and *S. michauxianus*) at each salt marsh site. In August 2020, samples were collected at 15 cm depth using a trowel and sterile Ziploc bags. Samples were transported on ice and stored at 4˚C until analysis.

3.3.2 Staining of arbuscular mycorrhizal fungi and gridline intersect method

In September 2020, an ink-vinegar technique from Vierheilig et al. (1998) was used to de-pigment plant root cortical cells and stain AMF in *S. alterniflorus, S. pumilus* and *S. michauxianus* roots. The gridline intersect method is an effective way to determine % AMF colonization within the roots (d'Entremont et al., 2018). Four lines were drawn 5 mm apart on the reverse of a glass microscope slide using a fine tip Sharpie. Stained roots were placed in a zig-zag arrangement on the glass slide to achieve 50 intersect points. At each intersect, transects were analyzed for stained AMF (hyphae, vesicles or any other mycorrhizal structures) (Figure 7) and given a colonization percentage (d'Entremont et al., 2018).

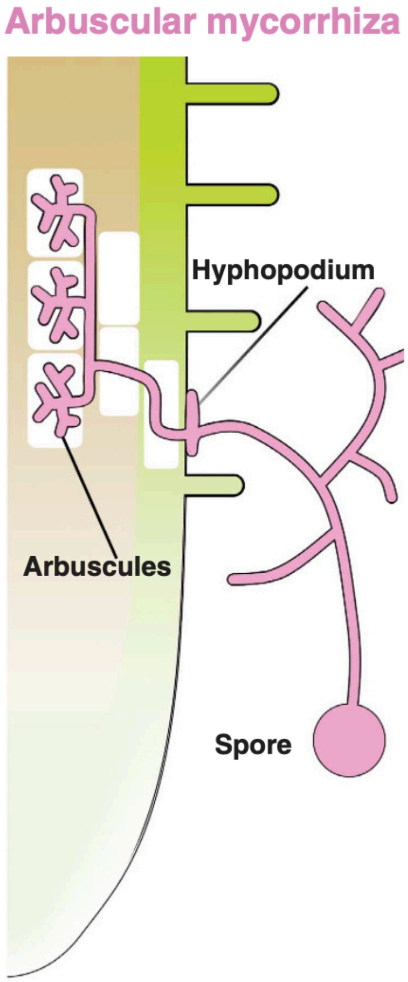

Figure 7. Illustration of mycorrhizal fungal structures found in plant roots during staining and gridline intersect method (Modified from Bonfante et al., 2010).

3.3.3 Loss on ignition and bulk carbon density

In November 2020, prior to processing, cores were thawed at 4˚C for 24 hours. Two subsamples from the same depth (~15 cm) were processed and the average were used for analyses. The processing technique to calculate loss on ignition (LOI) followed Heiri et al. (2001).

Bulk density was determined by using a known syringe volume of sediment in aluminum crucibles and dehydrating in a drying oven for 24 hours at 95˚C. After 1 hour in the desiccator, dry weights were recorded in $g \cdot cm^3$.

3.3.4 Calculation of organic carbon

Organic carbon density

The loss on ignition (LOI) fraction is a weight change measurement that is commonly used to estimate the organic content within sediments (Heiri et al., 2001). The LOI was calculated using Heiri et al. (2001) equation:

$$LOI\ fraction = (DW_{95^{\circ}} - DW_{550^{\circ}} \,/\, DW_{95^{\circ}}) * 100 \qquad (2.1)$$

To convert from organic matter to OC, two conversion equations are used. The OC equation can be determined using a calculation from Craft et al. (1991):

$$OC\ conversion = 0.40(LOI) + (0.025 * LOI)^2 \qquad (2.2)$$

Regression analysis shown in Chapter 2 provided an equation suitable for most marshes within the Upper Bay of Fundy:

$$Organic\ C = (0.373)LOI + (-0.00510)LOI^2 \qquad (2.3)$$

Samples collected from Cogmagun Reference require a different equation as this site wa statistically different than other selected study sites.

$$Organic\ C = (0.474)LOI + (-0.00988)LOI^2 \qquad (2.4)$$

These equations provide a percentage of OC within the organic matter for each sample. This fraction was then multiplied by bulk density ($g \cdot cm^{-3}$) to determine the OC density ($g \cdot C \cdot cm^{-3}$) (Wollenberg et al., 2018; Appendix B).

AMF colonization and carbon analyses

ANOVA testing using mixed models in RStudio (version 4.0.3) determined the relationship between response variable, AMF % colonization of *Sporobolus* roots and independent variables OC density, salt marsh vegetation zones per site as an interaction effect and age of the salt marsh.

3.4 Results

3.4.1 Arbuscular mycorrhizal fungal colonization of Sporobolus roots

AMF colonization was found in all three *Sporobolus* species examined and % colonization was significantly different among plant species ($p < 1.339e\text{-}06$, $\alpha = 0.05$) (Figure 8; Table 4). Colonization percentages differed significantly for each *Sporobolus* species at each site when vegetation types were considered ($p < 0.004915$, $\alpha = 0.05$) (Table 4).

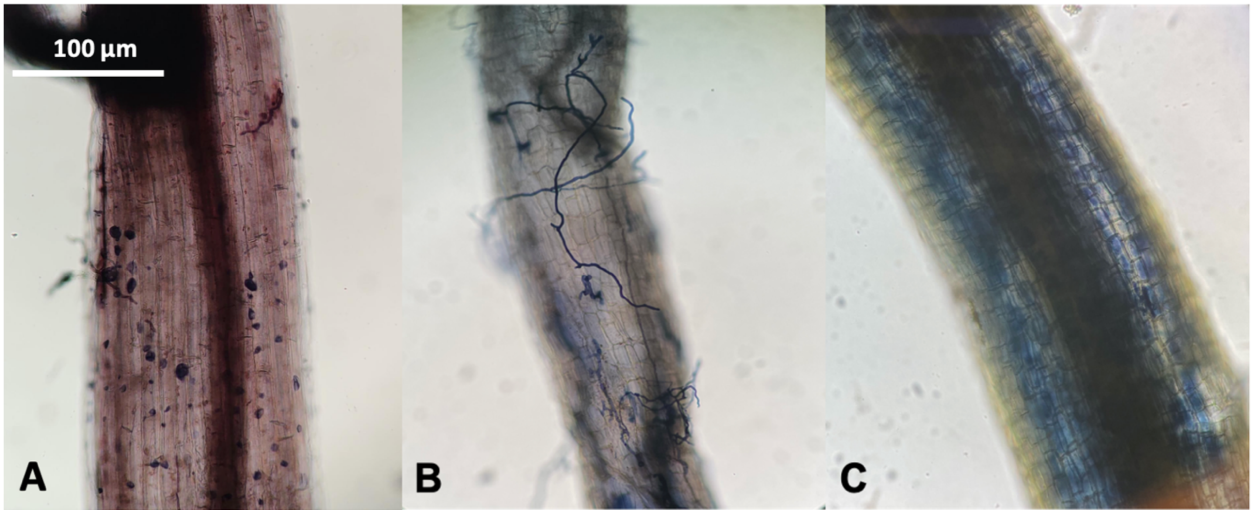

Figure 8. Roots of salt marsh plants (A) *Sporobolus alterniflorus,* (B) *Sporobolus pumilus,* and (C) *Sporobolus michauxianus* from Cogmagun Reference site viewed at 200x magnification, showing arbuscular mycorrhizal fungi stained blue.

Table 4. Analysis of variance showing relationship between arbuscular mycorrhizal fungal root colonization and sediment organic carbon (Craft et al., 1991) by vegetation type and site. Significant values are represented with asterisks.

Factor	*df*	*SS*	*MS*	*F*	*P*
Organic Carbon	1	112.7	112.7	0.2747	0.602724
Site	9	4353.8	483.8	1.1787	0.330953
Vegetation Type	2	15110.0	7555.0	18.4080	1.339e-06***
Site x Vegetation Type	14	15820.3	1130.0	2.7533	0.004915**
Residuals	46	18879.3	410.4		

Results from analysis of variance in Table 5 did not statistically differ from results in Table 4 where OC was converted using equation from Craft et al. (1991). However, AMF colonization was significantly different among plant species ($p < 1.369e\text{-}06$, $\alpha = 0.05$) (Table 5). Colonization percentages differed significantly for each *Sporobolus* species at each site when vegetation types were considered ($p < 0.004812$, $\alpha = 0.05$) (Table 5).

Table 5. Analysis of variance showing relationship between arbuscular mycorrhizal fungal root colonization and sediment organic carbon by vegetation type and site. Significant values are represented with asterisks.

Factor	*df*	*SS*	*MS*	*F*	*P*
Organic Carbon	1	7.0	7.0	0.0172	0.896328
Site	9	4664.5	518.3	1.2683	0.279600
Vegetation Type	2	15011.9	7505.9	18.3683	1.369e-06 ***
Site x Vegetation Type	14	15795.6	1128.3	2.7610	0.004812 ***
Residuals	46	18797.2	408.6		

AMF colonization was lower in *S. alterniflorus* but increased with *S. pumilus* and *S. michauxianus* (Figure 9). Colonization rates for all *Sporobolus* species examined were consistently higher at reference sites (Figure 9). The age effect of marshes was not consistent throughout the vegetation zones. At newly restored sites, *S. alterniflorus* % colonization was greater in comparison to intermediate and old marshes, with highest colonization (60%) at DUC Belcher Restoration (Figure 9). *Sporobolus pumilus* was absent from most new and intermediate restoration sites (Belcher Restoration, Converse Restoration, St. Croix West Restoration).

Although, when present at new and intermediate sites, colonization % was higher (52% at DUC Belcher Restoration and 57% at Cogmagun Restoration) than at older restored sites (32% at Cheverie Restoration) (Figure 9). As opposed to *S. alterniflorus* and *S. pumilus*, AMF was less abundant in *S. michauxianus* at new (0-74% at Belcher Restoration and 24-68% at Converse Restoration 38-48%) and intermediate (21-82% at St. Croix West Restoration and 26-82% Cogmagun Restoration) restoration sites and more abundant at older sites such as Cheverie Restoration (41-94%) (Figure 9).

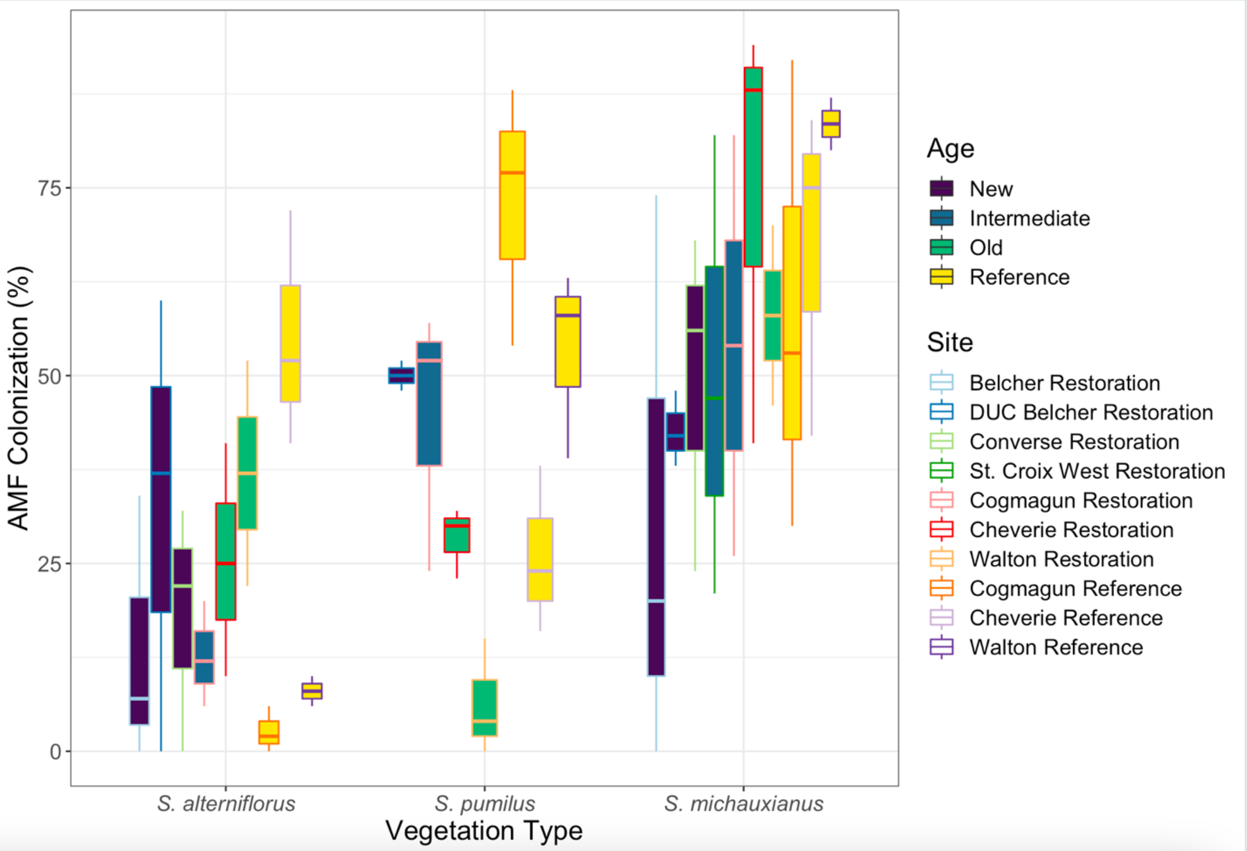

Figure 9. Arbuscular mycorrhizal fungal % root colonization in *Sporobolus alterniflorus*, *Sporobolus pumilus*, and *Sporobolus michauxianus* at salt marsh sites of various ages. Error bars represent 95% confidence intervals.

3.4.2 Organic carbon analyses

Sediment OC densities (g·C·cm^{-3}) varied in each vegetation zone using Craft et al. (1991) equation: between 0.0147 to 0.0580 g·C·cm^{-3} in the *S. alterniflorus* region 0.0147 to 0.0400

$g \cdot C \cdot cm^{-3}$ in the *S. pumilus* region and 0.0150 to 0.0515 $g \cdot C \cdot cm^{-3}$ in the *S. michauxianus* region (Figure 10). OC densities in *S. alterniflorus* region were highest at newly restored sites (0.0187 to 0.0580 $g \cdot C \cdot cm^{-3}$). In the *S. pumilus* region, OC densities were highest at an intermediate restoration site (Cogmagun Restoration) with 0.0400 $g \cdot C \cdot cm^{-3}$. One old restoration site (Walton Restoration) had the highest OC density in the *S. michauxianus* marsh zone (0.0515 $g \cdot C \cdot cm^{-3}$) (Figure 10).

Sediment OC densities using the OC conversion equation from Chapter 2 differed in comparison to results using Craft et al. (1991) equation (Appendix B; Figure 10). Overestimations in Figure 10 are noted at all sites and vegetation regions aside from Cogmagun Restoration and Reference both in the *S. alterniflorus* region. An overestimation of 0.178 $g \cdot C \cdot cm^{-3}$ at Converse Restoration is also noted in Appendix A (0.249 $g \cdot C \cdot cm^{-3}$ compared to 0.071 $g \cdot C \cdot cm^{-3}$), a maximum Y-axis limit of 0.06 was set for Figure 10 to ensure proper spread of boxplot. Overall, there were many OC density overestimations for the 2020 sample sites using Craft et al. (1991) OC conversion equation.

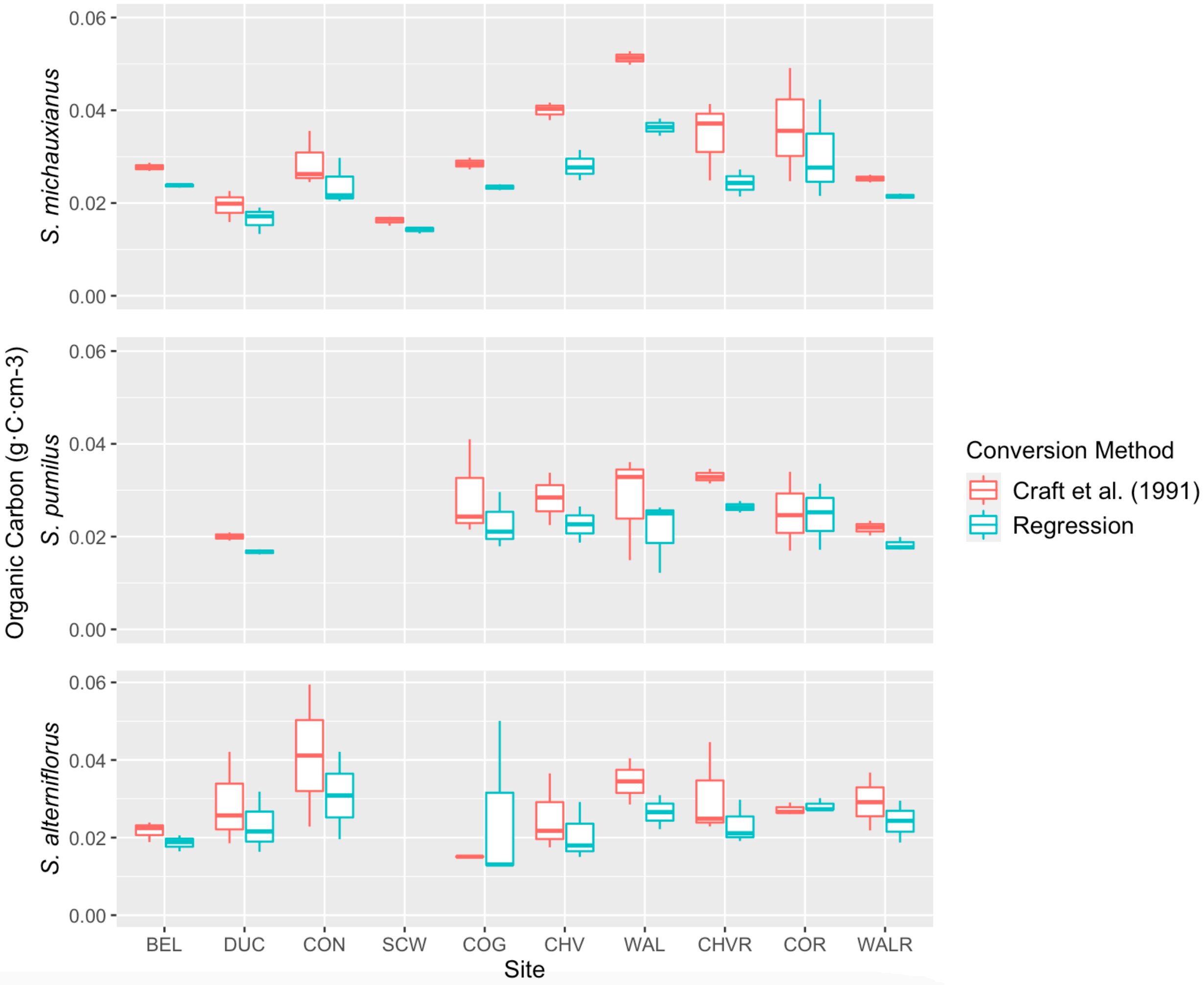

Figure 10. Organic carbon (g·C·cm^{-3}) 2020 results in three vegetations zones at Bay of Fundy salt marsh sites using regression conversion equation (Organic C = (0.373)LOI + (-0.00510)LOI^2) for all sites excluding Cogmagun Reference, requiring a separate equation (Organic C = (0.474)LOI + (-0.00988)LOI^2) and Craft et al. (1991) organic carbon conversion equation, arranged from new to old restoration, and reference sites: BEL = Belcher Restoration, DUC = Belcher DUC Restoration, CON = Converse Restoration, SCW = St. Croix West Restoration, COG = Cogmagun Restoration, CHV = Cheverie Restoration, WAL = Walton Restoration, CHVR = Cheverie Reference, WALR = Walton Reference, and COR = Cogmagun Reference. Error bars represent 95% confidence intervals.

3.4.3 Arbuscular mycorrhizal fungi and organic carbon

There was a positive relationship but not significant between sediment OC density (g·C·cm^{-3}) and AMF colonization within *S. alterniflorus* and *S. michauxianus* roots using both Craft et al. (1991) OC conversion equation and regression equation (ANOVA testing using mixed models). An opposite trend was noted for *S. pumilus,* with increased OC in the surrounding sediment associated with a decrease in AMF colonization. There were no significant differences between vegetation types when using regression equation and the OC conversion equation from Craft et al. (1991).

3.5 Discussion

AMF were highly abundant in roots of higher marsh plants *S. michauxianus* and *S. pumilus*. *S. michauxianus* are typically found in upper high elevations within the marsh that are only flooded at the peak of high tide; lower marsh elevations experience longer tidal inundation periods (Gonneea et al., 2019). AMF are often limited to upper soil layers as anoxia increases with depth, explaining the high abundance of AMF colonization noted in vegetation from higher marsh zones in our study (Maček, 2017; McIntosh and Wimp, 2022). The high colonization results were unexpected for *S. alterniflorus* due to their infrequent mutualisms with AMF as the low marsh is often faced with stressful conditions (Burcham et al., 2012; d'Entremont et al., 2018; Welsh et al., 2010). All sites experienced higher inundation frequencies in sediment surrounding *S. alterniflorus* (Table 1; Table 2). Salinity was highest in the lower marsh (*S. alterniflorus* zone), with often anoxic soils and long inundation timeframes (Veldhuis et al., 2019). AMF require soil aeration, and this can be a limiting factor for their growth and survival (Maček, 2017). Despite this, many of our colonization rates are notably higher than colonization rates documented in *S. alterniflorus* (9%) at a natural marsh within 50 km of our sites in the Minas Basin, Nova Scotia during a previous study (d'Entremont et al., 2018). Our high colonization rates may have potentially included other fungal structures that stain when using the ink/vinegar staining solution (John et al., 2014; Wężowicz et al., 2017). This includes non-melanized saprobes and root endophytes, which could have potentially overestimated our AMF colonization results. When using the gridline intersect method, a more specialized method (the quantitative magnified intersect (QMI)) would provide more detail and an absolute value of all AMF structures present at each intersect (John et al., 2014). By implementing this method, specific fungal structures such as arbuscules, vesicles and hyphae at each point of intersect can be recorded. This would describe low or high abundances of arbuscules indicating if symbiosis is functionally active. Vesicle frequency is often higher than arbuscules in many studies. Vesicles are the lipid stores of the fungi, which may be indicative of AMF carbon acquisition (Thirkell et al., 2019).

S. michauxianus is understudied in salt marsh systems, unlike *S. alterniflorus* and *S. pumilus* which have been investigated for AMF colonization and biodiversity (Burcham et al., 2012; d'Entremont et al., 2018; Welsh et al., 2010). *S. michauxianus* showed the highest AMF colonization rates at our older restored marsh site (CHV). The salinity of these sites could be contributing to the higher fungal colonization due to the fact sporulation is stimulated under saline conditions (Evelin et al., 2009); both Cogmagun and Cheverie sites had high water salinities (COG = 29.05 ppt and CHV = 30.15 ppt). AMF have shown tolerance to salinity while promoting plant health beyond nutrient acquisition; they also improve rhizospheric conditions, improve plant photosynthetic activity and water absorption (Parihar and Bora, 2019).

Most of our AMF colonization results were in agreement with OC densities with the exception of *S. pumilus*. When higher OC densities were documented, vegetation often had higher AMF colonization rates, particularly at reference salt marsh sites (COR, CHVR, WALR). Reference marshes with consistent surface elevation established through increased vegetation abundance and larger root masses suggest a higher dependency on autochthonous OC accumulation (Drexler et al., 2020; Saintilan et al., 2013). On average, reference sites had lower net sediment accumulation than restoration sites (Table 1; Table 2). The allocation of C from the plant to AMF at the site of C and nutrient exchange could be driving *in-situ* accumulation of organic matter, contributing to belowground C storage, as opposed to sediment accumulation through tidal flow.

High AMF colonization rates and correlating sediment OC densities were noted for each vegetation zone at restoration sites. DUC Belcher, a newly restored salt marsh site, had high AMF colonization in *S. alterniflorus* (60%) and OC densities >0.02 $g \cdot C \cdot cm^{-3}$; AMF could be contributing to the early establishment of belowground biomass by providing limiting nutrients and contributing to biomass gain (d’Entremont et al., 2018). This trend was also documented for *S. pumilus*, although in an intermediate aged marsh (COG). These colonization results are lower than previously reported at another natural salt marsh site in the Minas Basin (Wolfville, NS) (d’Entremont et al., 2018). According to Chmura et al. (2003), sediment OC densities in the Bay of Fundy are often significantly greater in the *S. pumilus* zone due to location in the tidal frame. However, an opposite trend was seen in *S. pumilus* in our study when using both Craft et al. (1991) equation and regression equation. The maximum OC density in these areas was lower than the maximum in both *S. alterniflorus* and *S. michauxianus*. Compared to *S. alterniflorus, S. pumilus* is commonly found higher in the marsh tidal frame, often with infrequent tidal flooding and lower suspended sediment (Chmura et al., 2003). It is unclear whether the negative relationship between OC and AMF in *S. pumilus* is truly representative of this species' performance in our region due to the small sample size or any errors in quantifying colonization. This species was not present at most sites which resulted in 26% fewer samples than *S. michaxianus* and 23% fewer samples than *S. alterniflorus*.

Restoration sites yielded higher sediment OC densities than reference sites. Three years post dyke realignment and breach, Converse Restoration had the most elevated OC densities across all sites in *S. alterniflorus* sediments (0.0580 $g \cdot C \cdot cm^{-3}$) (Bowron et al., 2020). OC densities were overestimated for nearly all sites when using Craft et al. (1991) conversion equation. The equation created by Craft et al. (1991) using the same methodology as Chapter 2, was specific for their samples from various marshes, which had a very strong R-squared value and higher estimates; understandably it may not suffice as an accurate conversion factor for all marshes globally. However, high C densities were noted for regression analysis as well and may be attributed to increased bulk densities from allochthonous sediments, as incoming sediments tend to be mineral base composed of sand, silt, and clay, which also provide lower C densities between 0.01 and 0.02 $g \cdot C \cdot cm^{-3}$ (Appendix A, B; Wollenberg et al., 2018). Allochthonous sediments are often heavier with more material per unit volume which explains high OC densities in newer restored sites. OC densities are likely to change temporally in newly restored marshes such as Converse Restoration, in comparison to older restored marshes as they require more time to reach an equilibrium. Older restored marshes provide yearly consistent values which can be attributed to autochthonous C production as surface elevations are higher and well established in these marshes, which makes reliance on tidally imported sediments less pertinent. Sediment accumulation is much more rapid in the lower marsh zone due to consistent tidal flow and vegetation contributions causing sediment and OC to accumulate significantly compared to high and upper high elevated zones (Chmura et al., 2001; van Proosdij et al., 2006). The suspended sediment through regular tidal flow contributes to allochthonous C storage by transporting sediment and OC from outside of the marsh system and trapping it via vegetation (Gonneea et al., 2019; Wollenberg et al., 2018). Allochthonous sediments are largely contributing to sediment and C accretion however, autochthonous belowground activity may as well be a dominant C driver in salt marshes. With managed realignment, particularly post-dyke realignment and breaching, there is an increase in tidal flow which provides longer inundation times and can lead to rapid sediment accumulation (Byers and Chmura 2007; van Proosdij et al., 2010; Wollenberg et al., 2018). Introduction of tidal flow in marshes post-restoration contributes to sediment accretion leading to an accumulation of allochthonous OC (Wollenberg et al., 2018).

To our knowledge, this chapter documented some of the highest AMF colonization rates (*S. alterniflorus* max. 74%, *S. pumilus* max. 82%, *S. michauxianus* max. 94%) compared to previous studies (Burcham et al., 2012; d'Entremont et al., 2018; Welsh et al., 2010). After analyzing AMF colonization in multiple vegetation regions within restored and reference salt marshes, our results indicate AMF may be a contributing factor to belowground autochthonous C accumulation. Using OC conversion equation from chapter 2, high OC densities were found. Additionally, using Craft et al. (1991) conversion equation, an overestimation was noted for the majority of 2020 sample sites. It is recommended to proceed with elemental analysis on sediment samples provides an accurate OC conversion equation for samples collected in the root zone within the Bay of Fundy. Our findings are the first to analyze salt marsh C and AMF root colonization in Atlantic Canada and provide a foundation for future studies on salt marsh C accumulation potential with influence from AMF.

Chapter 4: Conclusion

This thesis documented some of the highest AMF colonization rates in *Sporobolus* species (*S. alterniflorus* max. 74%, *S. pumilus* max. 82%, *S. michauxianus* max. 94%), to our knowledge. We analyzed AMF colonization in multiple dominant salt marsh vegetation species in reference and restored salt marshes of different ages. Our results indicate AMF may be contributing to belowground autochthonous C storage; a positive correlation (although not significant) was documented between *S. alterniflorus* and *S. michauxianus*, AMF colonization rates, and OC densities. *S. michauxianus* is highly understudied in marsh systems but provided the highest OC density and AMF colonization rate in this study; AMF may be playing the strongest role in C accumulation in the high/brackish marsh zone. High OC densities were recorded in *S. alterniflorus* at newly restored sites indicating that while allochthonous sediments are making large contributions to accretion, autochthonous belowground productivity may also be an important driver of high OC in this zone. Furthermore, elemental analysis on sediment samples provides an accurate OC conversion equation for samples collected in the root zone within the Bay of Fundy. Our findings provide a foundation for future studies on salt marsh C accumulation potential with influence from AMF in Atlantic Canada. Studies of salt marsh AMF are essential for understanding the health of restored marsh vegetation, which contributes to OC conversion and storage.

4.1 Future Direction

The AMF colonization rates from Chapter 3 were frequently high, indicating that AMF could be contributing to sediment OC. Previous research has found the extraradical hyphae in terrestrial AMF provide a rapid pathway for atmospheric C to enter the soil due to their chitinous cell walls; chitin is a recalcitrant carbohydrate (Solaiman, 2014). The formation of the glomalin protein occurs in the extraradical hyphae of AMF which is associated with aggregate stability and protection of soil organic C (Wright and Upadhyaya, 1996). Although the hyphae have a rapid turnover rate (5-6 days), this still allows for an accumulation of hyphal residue containing C to remain in the soil matrix. Previous research suggests a residence time of 49 ± 19 years for chitinous-derived pyrolysis product to be retained in the soil organic matter (Gleixner et al., 2002). The extraradical hyphae and glomalin protein have been highly studied in terrestrial ecosystems (Hammer and Rillig, 2011; Leake et al., 2004; Miller et al., 1995; Miller and Kling, 2000; Olsson, 1999; Solaiman, 2014; Wright and Upadhyaya, 1996; Zhu and Miller, 2003) but their role in coastal systems and the mechanisms used to contribute to C storage remains understudied. In tropical forest soil, glomalin represented ~4-5% of total C soil (Zhu and Miller, 2003), with ecosystems that contain higher amounts of C, such as salt marshes, it is possible that glomalin could be further contributing to autochthonous C storage in salt marshes. A study in North Queensland Australia by Adame et al. (2012), compared glomalin related soil protein across coastal sediments and found that the high intertidal was glomalin-rich with the influence of groundwater flow (Adame et al., 2012). According to Hammer and Rillig (2011), glomalin content significantly alters as a stress responder. This was tested under saline concentrations and found glomalin production substantially increased as a response.

Results from Chapter 3 indicate that AMF may be contributing to belowground C storage. The methodology of quantifying glomalin present in the extraradical hyphae could provide AMF’s contribution to autochthonous C accumulation in salt marshes. Results from Chapter 2 suggests elevated OC densities. These further steps could be significant as AMF are beneficial for nutrient acquisition for their hosts, particularly in such harsh, saline environments. The growth and overall health of marsh vegetation is essential as they are responsible for inorganic C conversion, further research similar to Adame et al. (2012) should be completed to fully understand organic contribution to sediment OC. This research found extremely high AMF colonization rates exemplifying that AMF may be contributing to autochthonous C storage as they contain C in many of their anatomical structures. Furthermore, using elemental analysis, this research demonstrated accurate equations of OC densities for better estimates in Bay of Fundy salt marshes.

References

Abbott, K.M., Elsey-Quirk, T., and DeLaune, R.D. 2019. Factors influencing blue carbon accumulation across a 32-year chronosequence of created coastal marshes. Ecosphere 10: e02828. https://doi.org/10.1002/ecs2.2828

Adame, M.F., Wright, S.F., Grinham, A., Lobb, K., Reymond, C.E., and Lovelock, C.E. 2012. Terrestrial-marine connectivity: Patterns of terrestrial soil carbon deposition in coastal sediments determined by analysis of glomalin related soil protein. Limnology and Oceanography, 57: 1492-1502.

Anderson, R.C., Ebbers, B.C., and Liberta, A.E. 1986. Soil moisture influences colonization of Prairie Cordgrass (*Spartina pectinata* Lind.) by vesicular-arbuscular mycorrhizal fungi. New Phytologist, 102: 523-527. https://doi.org/10.1111/j.1469-8137.1986.tb00828.x

Bonfante, P. and Genre, A. 2010. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature Communications, 1:48. https://doi.org/10.1038/ncomms1046

Bowron, T., Neatt, N., van Proosdij, D., Lundholm, J., and Graham, J. 2011. Macro-tidal salt marsh ecosystem response to culvert expansion. Restoration Ecology, 19: 307-322. https://doi.org/10.1111/j.1526-100X.2009.00602.x

Bowron, T., Neatt, N., Graham, J., van Proosdij, D., Lundholm, J., and Lemieux, B. 2013. Post restoration monitoring (Year 7) of the Cheverie creek salt marsh restoration project. CB Wetlands & Environmental Specialists, Publication No. 33. Halifax, Nova Scotia.

Bowron, T.M., Graham, J., Kickbush, J., Ellis, K., McFadden, C., Poirier, E., Rabinowitz, T., Lewis, S., Elliott, M., Rogers, E., and van Proosdij, D. 2020. Post-restoration (Year 3) of the Converse salt marsh restoration (NS044)- 2020-2021 Technical Report. CB Wetlands & Environmental Specialists, Publication No. 65. Halifax, Nova Scotia.

Bowron, T.M., Graham, J., Kickbush, J., Matheson, G., Ellis, K., van Proosdij, D., and Lundholm, J. 2019. Pre-restoration monitoring (baseline) of the Truro-Onslow realignment & tidal wetland restoration projecy. CB Wetlands & Environmental Specialists, Publication No. 52. Halifax, Nova Scotia.

Bowron, T.M., Neatt, N.C., Graham, J.M., van Proosdij, D., and Lundholm, J. 2015a. Post restoration monitoring (Year 5) of the St. Croix River high salt marsh & tidal wetland restoration project. CB Wetlands & Environmental Specialists, Publication No. 41. Halifax, Nova Scotia.

Bowron, T.M., Neatt, N.C., Graham, J.M., van Proosdij, D., and Lundholm, J. 2015b. Post restoration monitoring (Year 5) of the Cogmagun river salt marsh restoration project. CB Wetlands & Environmental Specialists, Publication No. 42. Halifax, Nova Scotia.

Bulseco, A.N., Giblin, A.E., Tucker, J., Murphy, A.E., Sanderman, J., Hiller-Bittrolff, K., and Bowen, J.L. 2019. Nitrate addition stimulates microbial decomposition of organic matter in salt marsh sediments. Global Change Biology, 25: 3224-3241. https://doi.org/10.1111/gcb.14726

Burcham, A.K., Merino, J.H., Michot, T.C., and Nyman, J.A. 2012. Arbuscular mycorrhizae occur in common *Spartina* species. Gulf of Mexico Science, 2012: 14–19. https://doi.org/10.18785/goms.3001.03

Burden, A., Garbutt, A., and Evans, C.D. 2019. Effect of restoration on salt marsh carbon accumulation in Eastern England. Biology Letters 15: 20180773. https://doi.org/10.1098/rsbl.2018.0773

Burke, D.J., Hamerlynck, E.P., and Hahn, D. 2003. Interactions between the salt marsh grass *Spartina patens*, arbuscular mycorrhizal fungi and sediment bacteria during the growing season. Soil Biology & Biochemistry, 35: 501-511. https://doi.org/10.1016/S00380717(03)00004-X

Broome, S.W., Seneca, E.D., and Woodhouse Jr, W.W. 1988. Tidal marsh restoration. Aquatic Botany 32: 1-22. https://doi.org/10.1016/0304-3770(88)90085-X

Byers, S.E., and Chmura, G.L. 2007. Salt marsh vegetation recovery on the Bay of Fundy. Estuaries Coasts, 30: 869-877.

Cahoon, D. R., Lynch, J.C., Perez, B.C., Segura, B., Holland, R., Stelly, C., Stephenson, G., and Hensel, P. 2002. A device for high precision measurement of wetland sediment elevation: II. The rod surface elevation table. Journal of Sedimentary Research 72: 734-739.

CB Wetlands and Environmental Specialists. 2020. Living Shorelines and Natural Infrastructure. Accessed from: https://www.cbwes.com/livingshorelines-and-naturalinfrastucture

Chmura, G.L., Anisfeld, S.C., Cahoon, D.R., and Lynch, J.C. 2003. Global carbon sequestration in tidal, saline wetland soils. Global Biogeochemical Cycles, 17. https://doi.org/10.1029/2002GB001917

Chmura, G.L., Coffey, A., and Crago, R. 2001. Variation in surface sediment deposition on salt marshes in the Bay of Fundy. Journal of Coastal Research, 17: 221-227.

Chmura, G.L. 2013. What do we need to assess the sustainability of the tidal salt marsh carbon sink? Ocean & Coastal Management, 83: 25-31. https://doi.org/10.1016/j.ocecoaman.2011.09.006

Connor, R.F., Chmura, G.L., and Beecher, C.B. 2001. Carbon accumulation in Bay of Fundy salt marshes: Implications for restoration of reclaimed marshes. Global Biogeochemical Cycles 15: 943-954. https://doi.org/10.1029/2000GB001346

Cooke, J.C., Butler, R.H., and Madole, G. 1993. Some observations on the vertical distribution of vesicular arbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. Mycologia, 85:_547-550. https://doi.org/10.1080/00275514.1993.12026307

Cooke, J.C., and Lefor, M.W. 1990. Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, Connecticut, USA. Environmental Management, 14: 131-137. https://doi.org/10.1007/BF02394027

Craft, C.B, Seneca, E.D., and Broome, S.W. 1991. Loss on ignition and kjeldahl digestion for estimating organic carbon and total nitrogen in estuarine marsh soils: Calibration with dry combustion. Estuaries, 14: 175-179. https://doi.org/10.2307/1351691

Crain, C.M. 2007. Shifting nutrient and eutrophication effects in marsh vegetation across estuarine salinity gradients. Estuaries and Coasts, 30: 26-34. https://doi.org/10.1007/BF02782964

Daleo, P., Alberti, J., Canepuccia, A., Escapa, M., Fanjul, E., Silliman, B.R., Bertness, M.D., and Iribarne, O. 2008. Mycorrhizal fungi determine saltmarsh plant zonation depending on nutrient supply. Journal of Ecology 96: 431-437

d'Entremont, T.W., López-Gutiérrez, J.C., and Walker, A.K. 2018. Examining arbuscular mycorrhizal fungi in Salt marsh Hay (*Spartina patens*) and Smooth Cordgrass (*Spartina alterniflora*) in the Minas Basin, Nova Scotia. Northeastern Naturalist, 25: 72-86. https://doi.org/10.1656/045.025.0107

d'Entremont, T.W., López-Gutiérrez, J.C., and Walker, A.K. 2021. Inoculating rhizome-propagated *Sporobolus pumilus* with a native mycorrhizal fungus increases salt marsh plant growth and survival. FACETS, 6: 1134-1145. https://doi.org/10.1139/facets-2020-0104

Desplanque, C., and Mossman, D.J. 2004. Tides and their seminal impact on the geology, geography, history, and socio-economics of the Bay of Fundy, eastern Canada. Atlantic Geoscience, 40:1. https://doi.org/10.4138/729

Drexler, J.Z., Davis, M.J., Woo, I., and De La Cruz, S. 2020. Carbon sources in the sediments of a restoring vs. historically unaltered salt marsh. Estuaries and Coasts, 43: 1345-1360.

Elsey-Quirk, T., Seliskar, D.M., Sommerfield, C.K., and Gallagher, J.L. 2011. Salt marsh carbon pool distribution in a mid-atlantic lagoon, USA: sea level rise implications. Wetlands, 31: 87-99. https://doi.org/10.1007/s13157-010-0139-2

Evelin, H., Kapoor, R., and Giri, B. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annals of Botany 104: 1263-1280. https://doi.org/10.1093/aob/mcp251

French, P.W. 2006. Managed realignment- the developing story of a comparatively new approach to soft engineering. Estuarine, Coastal and Shelf Science, 67: 409-423.

Gailis, M., Kohfeld, K.E., Pellatt, M.G., and Carlson, D. 2021. Quantifying blue carbon for the largest salt marsh in southern British Columbia: implications for regional coastal management. Coastal Engineering Journal, 63: 275-309. https://doi.org/10.1080/21664250.2021.1894815

Gallant, K., Withey, P., Risk, D., van Kooten, G.C., and Spafford, L. 2020. Measurement and economic valuation of carbon sequestration in Nova Scotian wetlands. Ecological Economics 171: 106619. https://doi.org/10.1016/j.ecolecon.2020.106619

Gleixner, G., Poirier, N., Bol, R., and Balesdent, J. 2002. Molecular dynamics of organic matter in a cultivated soil. Organic Geochemistry, 33: 357-366.

Gonneea, M.E., Maio, C.V., Kroeger, K.D., Hawkes, A.D., Mora, J., Sullivan, R., Madsen, S., Buzard, R.M., Cahill, N., and Donnelly, J.P. 2019. Salt marsh ecosystem restructuring enhances elevation resilience and carbon storage during accelerating relative sea-level rise. Estuarine, Coastal and Shelf Science, 217: 56-68. https://doi.org/10.1016/j.ecss.2018.11.003

Graham, J., Bowron, T.M., van Proosdij, D., Lundholm, J., Poirier, E., Kickbush, J., Ellis, K., Lewis, S., Akyol, R., Rabinowitz, T., and Rogers, E. 2021. Post-Restoration Monitoring (Year 3) of the Belcher St. Tidal Wetland Restoration Project (NS091) – 2020-21 Summary Report. CB Wetlands & Environmental Specialists, Publication No. 66. Halifax, Nova Scotia.

Grimsditch, G., Alder, J., Nakamura, T., Kenchington, R., and Tamelander, J. 2013. The blue carbon special edition – Introduction and overview. Ocean and Coastal Management 83: 1-4. http://dx.doi.org/10.1016/j.ocecoaman.2012.04.020

Hammer, E.C. and Rillig, M.C. 2011. The influence of different stresses on glomalin levels in an arbuscular mycorrhizal fungus—Salinity Increases Glomalin Content. PLoS ONE, 6: e28426. https://doi.org/10.1371/journal.pone.0028426

Heiri, O., Lotter, A.F., and Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate sediments: reproducibility and comparability of results. Journal of Paleolimnology, 25: 101–110. https://doi.org/10.1023/A:1008119611481

Howard, J., Hoyt, S., Isensee, K., Pidgeon, E., Telszewski, M. (eds.) 2014. Coastal Blue Carbon: Methods for assessing carbon stocks and emissions factors in mangroves, tidal salt marshes, and seagrass meadows. Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation of Nature. Arlington, Virginia, USA.

Howard, J., Sutton-Grier, A., Herr, D., Kleypas, J., Landis, E., McLeod, E., Pidgeon, E., and Simpson, S. 2017. Clarifying the role of coastal and marine systems in climate mitigation. Frontiers in Ecology and the Environment, 15: 42-50. https://doi.org/10.1002/fee.1451

IPCC (Intergovernmental Panel on Climate Change). 2021. Summary for Policymakers. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. World Meteorological Organization, Geneva, Switzerland.

John, J., Lundholm, J., and Kernaghan, G. 2014. Colonization of green root plants by mycorrhizal and root endophytic fungi. Ecological Engineering, 72: 651-659. https://doi.org/10.1016/j.ecoleng.2014.08.012

Kim, S., Rayburn, A.L., and Parrish A. 2012. Cytogeographic distribution and genome size variation in Prairie Cordgrass (*Spartina pectinata* Bosc ex Link). Plant Molecular Biology Reporter, 30: 1073-1079. https://doi.org/10.1007/s11105-012-0414-x

Lanfranco, L., Bonfante, P., and Genre, A. 2016. The mutualistic interaction between plants and arbuscular mycorrhizal fungi. Microbiology Spectrum, 4: 727-747. https://doi.org/10.1128/microbiolspec.FUNK-0012-2016

Leake, J., Johnson, D., Donnelly, D., Muckle, G., Boddy, L., and Read, D. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Canadian Journal of Botany, 82: 1016–1045.

Loder, A.L., Finkelstein, S.A. Carbon accumulation in freshwater marsh soils: a synthesis for temperate North America. Wetlands 40: 1173–1187. https://doi.org/10.1007/s13157 01901264-6

Lundholm, J., Rabinowitz, T.R.M., Greene, L., Glogowski, A.D., Bowron, T., and van Proosdij, D., 2021. Hitchhiking halophytes in wrack and sediment-laden ice blocks contribute to tidal marsh development in the upper Bay of Fundy. Wetlands Ecology and Management. https://doi.org/10.21203/rs.3.rs-1021544/v1

Maček, I. 2017. Arbuscular mycorrhizal fungi in hypoxic environments. ***In*** Mycorrhiza-Function, Diversity, State of the Art. 4th edition. Edited by A Varma, R Prasad, and N Tuteja. pp. 329-347.

Macreadie, P.I., Hughes, A.R., and Kimbro, D.L. 2013. Loss of ‘blue carbon’ from coastal salt marshes following habitat disturbance. PLoS One, 8: e69244.

Maricle, B.R., Cobos, D.R., and Campbell, C.S. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. Environmental and Experimental Botany, 60: 458-467.

McCoy, M., and Hughes, L. 2021. Nova Scotia's carbon sinks and 2050 net-zero scenarios. Accessed from: https://nslegislature.ca/sites/default/files/pdfs/committees/64_1_LACSubmissions/2021101/2021-11-01-057-006.pdf

McIntosh, C., and Wimp, G. 2022. Impacts of climate change on mycorrhizal fungi in salt marsh habitats. Georgetown Scientific Research Journal, 2: 14-23.

Mcleod, E., Chmura, G.L., Bouillon, S., Salm, R., Björk, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., and Silliman, B.R. 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Frontiers in Ecology and the Environment, 9: 552-560. https://doi.org/10.1890/110004

Millard, K., Redden, A.M., Webster, T., and Stewart, H. 2013. Use of GIS and high resolution LiDAR in salt marsh restoration suitability assessments in the upper Bay of Fundy, Canada. Wetlands Ecology and Management, 21: 243-262.

Miller, R.M. and Jastrow, J.D. 2000. Mycorrhizal fungi influence soil structure. In: Kapulnik Y, Douds D (eds) Arbuscular mycorrhizas: physiology and function. Kluwer Academic, Dordrecht, pp 4–18.

Miller, R.M., Reinhardt, D.R., and Jastrow, J.D. 1995. External hyphal production of vesicular arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. Oecologia, 103:17–23. https://doi.org/10.1007/BF00328420

Morton, J.B., Koske, R.E., Stürmer, S.L., and Bentivenga SP. 2004. Mutualistic arbuscular endomycorrhizal fungi. In Biodiversity of Fungi: Inventory and Monitoring Methods. Edited by GM Mueller, GF Bills, and MS Foster. pp. 317–336. https://doi.org/10.1016/B978-012509551-8/50018-0

Neatt, N.C., Bowron, T.M., Graham, J.M., van Proosdij, D., Lundholm, J., and Lemieux, B. 2013. Post-restoration monitoring (Year 7) of the Walton River salt marsh restoration project. CB Wetlands & Environmental Specialists, Publication No. 34. Halifax, Nova Scotia.

Olsson, P.A., Rahm, J., and Aliasgharzad, N. 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. FEMS Microbiology Ecology, 72: 125 131. https://doi.org/10.1111/j.1574-6941.2009.00833.x.

Owers, C.J., Rogers, K., Mazumder, D., and Woodroffe, C.D. 2020. Temperate coastal wetland near surface carbon storage: Spatial patterns and variability. Estuarine, Coastal and Shelf Science, 235: 106584. https://doi.org/10.1016/j.ecss.2020.106584

Parihar, P., and Bora, M. 2019. Plants growing under salinity stress can be eased through mycorrhizal association. In Salt Stress, Microbes, and Plant Interactions: Causes and Solution. Edited by MS Akhtar. pp. 237-248. https://doi.org/10.1007/978-981-13-8801-9_11

Porter, C., Lundholm, J., Bowron, T., Lemieux, B., van Proosdij, D., Neatt, N., and Graham, J. 2015. Classification and environmental correlates of tidal wetland vegetation in Nova Scotia, Canada. Botany, 93: 825-841. https://doi.org/10.1139/cjb-2015-0066

ResNet. 2022. Research. Accessed from: https://www.nsercresnet.ca/overview.html

Saintilan, N., Rogers, K., Mazumder, D., Woodroffe, C. 2013. Allochthonous and autochthonous contributions to carbon accumulation and carbon store in southeastern Australian coastal wetlands. Estuarine, Coastal and Shelf Science, 128: 84-92. http://dx.doi.org/10.1016/j.ecss.2013.05.010

Solaiman, Z.M. 2014. Contribution of arbuscular mycorrhizal fungi to soil carbon sequestration. In Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration. Edited by Z.M. Solaiman, L.K. Abbott, and A. Varma. pp. 287-296.

Smith, S., and Read, D. (2010). Mycorrhizal Symbiosis. 3rd edition. Academic Press, San Diego. 800 p.

Teal, J. M. 2001. Salt marshes and mud flats. Encyclopedia of Ocean Sciences 5: 2490-2495. Academic Press, San Diego. https://doi.org/10.1016/B978-012374473-9.00087-4

Thirkell, T. J., Pastok, D., and Field, K. J. 2019. Carbon for nutrient exchange between arbuscular mycorrhizal fungi and wheat varies according to cultivar and changes in atmospheric carbon dioxide concentration. Global Change Biology, 26: 1725-1738. https://doi.org/10.1111/gcb.14851

Van de Broek, M., Temmerman, S., Merckx, R., and Govers, G. 2016. Controls on soil organic carbon stocks in tidal marshes along an estuarine salinity gradient. Biogeosciences, 13: 6611-6624. https://doi.org/10.5194/bg-13-6611-2016

van Proosdij, D., Davidson-Arnott, R.G.D., and Ollerhead, J. 2006. Controls on the spatial patterns of sediment deposition across a macro-tidal salt marsh over single tidal cycles. Estuarine, Coastal and Shelf Science, 69: 64-86. https://doi.org/10.1016/j.ecss.2006.04.022

van Proosdij, D., Lundholm, J., Neatt, N., Bowron, T., and Graham, J. 2010. Ecological re-engineering of a freshwater impoundment for salt marsh restoration in a hypertidal system. Ecological Engineering, 36: 1314-1332. https://doi.org/10.1016/j.ecoleng.2010.06.008

Veldhuis, E.R., Schrama, M., Staal, M., and Elzenga, J.T.M. 2019. Plant stress-tolerance traits predict salt marsh vegetation patterning. Frontiers in Marine Science 5: 501. https://doi.org/10.3389/fmars.2018.00501

Vierheilig, H., Coughlan, A.P., Wyss, U., and Piché, Y. 1998. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Applied and Environmental Microbiology, 64: 5004–5007. https://doi.org/10.1128/AEM.64.12.5004-5007.1998

Waltham, N.J., Alcott, C., Barbeau, M.A., Cebrian, J., Connolly, R.M., Deegan, L.A., Dodds, K., Goodridge Gaines, L.A., Gilby, B.L., Henderson, C.J., McLuckie, C.M., Minello, T.J., Norris, G.S., Ollerhead, J., Pahl, J., Reinhardt, J.F., Rezek, R.J., Simenstad C.A., Smith, J.A.M., Sparks, E.L., Staver, L.W., Ziegler, S.L., and Weinstein, M.P. 2021. Tidal marsh restoration optimism in a changing climate and urbanizing seascape. Estuaries and Coasts 2021. https://doi.org/10.1007/s12237-020-00875-1

Welsh, A.K., Burke, D.J., Hamerlynck, E.P., and Hahn, D. 2010. Seasonal analyses of arbuscular mycorrhizae, nitrogen-fixing bacteria and growth performance of the salt marsh grass *Spartina patens*. Plant and Soil, 330: 251-266. https://doi.org/10.1007/s11104-009-0197-5

Wężowicz, K., Rozpądek, P., and Turnau, K. 2017. Interactions of arbuscular mycorrhizae and endophytic fungi improve seedling survival and growth in post-mining waste. Mycorrhiza, 27: 499-511. https://doi.org/10.1007/s00572-017-0768-x

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., and Hartnett, D.C. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters, 12: 452-461. https://doi.org/10.1111/j.1461-0248.2009.01303.x

Wollenberg, J.T., Ollerhead, J., and Chmura, G.L. 2018. Rapid carbon accumulation following managed realignment on the Bay of Fundy. PLoS ONE, 13: e0193930. https://doi.org/10.1371/journal.pone.0193930

Wright, S.F. and Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science, 161: 575-586.

Wu, Y., Chaffey, J., Greenberg, D.A., Colbo, K., and Smith, P.C. 2011. Tidally-imported sediment transport patterns in the upper Bay of Fundy: A numerical study. Continental Shelf Research, 13: 2041-2053. https://doi.org/10.1016/j.csr.2011.10.009

Zhu, Y.G., and Miller, R.M. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. TRENDS in Plant Science, 8: 407-409. https://doi.org/10.1016/S1360-1385(03)00184-5

Zinke, L. 2020. The colours of carbon. National Reviews Earth & Environment, 1: 141. https://doi.org/10.1038/s43017-020-0037-y

Appendix A. Soil properties for 2020 study sample locations including arbuscular mycorrhizal colonization rates (%), organic carbon densities using Craft et al. (1991) equation, elemental analysis, and regression equation (separate regression for Cogmagun Reference). Organic carbon EA represents measurements from elemental analysis.

Sample	Vegetation Type	Site	AMF colonization (%)	Organic Matter (%)	Organic Carbon (%)	Bulk Density (g·cm^{-3})	Organic Carbon (g·C·cm^{-3}) (Craft et al., 1991)	Organic Carbon (g·C·cm^{-3})
BELT1S4	*S. alterniflorus*	Belcher Restoration	0	0.0422	1.6881	1.1094	0.0188	0.0165
BELT4S4	*S. alterniflorus*	Belcher Restoration	34	0.0503	2.0103	1.1793	0.0239	0.0206
BELK2	*S. alterniflorus*	Belcher Restoration	7	0.0652	2.6098	0.8512	0.0224	0.0189
BELK3	*S. michauxianus*	Belcher Restoration	20	0.0497	1.9897	1.3785	0.0276	0.0238
BELT2S7	*S. michauxianus*	Belcher Restoration	74	0.0473	1.8925	1.4128	0.0269	0.0233
BELT2S6	*S. michauxianus*	Belcher Restoration	0	0.0656	2.6246	1.082	0.0287	0.0241
CONK1	*S. alterniflorus*	Converse Restoration	22	0.4626	18.518	1.2515	0.2493	0.071
CONK2	*S. alterniflorus*	Converse Restoration	0	0.0529	2.1171	1.0697	0.0228	0.0196
CONK3	*S. michauxianus*	Converse Restoration	24	0.076	3.0402	0.8531	0.0262	0.0217
CONK4	*S. alterniflorus*	Converse Restoration	32	0.1597	6.3889	0.9078	0.0595	0.0421
CONT1S2	*S. michauxianus*	Converse Restoration	56	0.0716	2.8642	0.8467	0.0245	0.0204
CONT2S3	*S. michauxianus*	Converse Restoration	68	0.0669	2.6751	1.3152	0.0356	0.0297
DUCK1	*S. michauxianus*	DUC Belcher Restoration	38	0.0495	1.9816	0.9948	0.0199	0.0171
DUCK2	*S. michauxianus*	DUC Belcher Restoration	42	0.066	2.639	0.5965	0.0159	0.0134
DUCK3	*S. pumilus*	DUC Belcher Restoration	48	0.0613	2.4538	0.7728	0.0191	0.0162
DUCK4	*S. pumilus*	DUC Belcher Restoration	52	0.0761	3.0443	0.6789	0.0209	0.0173
DUCK5	*S. alterniflorus*	DUC Belcher Restoration	37	0.0951	3.8035	1.0855	0.0421	0.0319
DUCK6	*S. alterniflorus*	DUC Belcher Restoration	0	0.0348	1.3924	1.3246	0.0185	0.0164
DUCK7	*S. alterniflorus*	DUC Belcher Restoration	60	0.0659	2.6346	0.9658	0.0257	0.0216
DUCK8	*S. michauxianus*	DUC Belcher Restoration	48	0.064	2.5601	0.8739	0.0226	0.019
SCWK2	*S. michauxianus*	St. Croix West Restoration	47	0.0308	1.2335	1.2192	0.0151	0.0134
SCWK3	*S. michauxianus*	St. Croix West Restoration	82	0.0418	1.6715	0.9828	0.0165	0.0144
SCWK4	*S. michauxianus*	St. Croix West Restoration	21	0.0461	1.8428	0.9181	0.017	0.0148
COGK1	*S. pumilus*	Cogmagun Restoration	24	0.0469	1.8747	1.2878	0.0243	0.0258
COGK2	*S. alterniflorus*	Cogmagun Restoration	20	0.0519	2.0761	0.7124	0.0149	0.0156
COGK3	*S. pumilus*	Cogmagun Restoration	57	0.0707	2.8275	0.753	0.0215	0.0215
COGK4	*S. michauxianus*	Cogmagun Restoration	82	0.0686	2.7449	0.9821	0.0272	0.0274
COGK5	*S. alterniflorus*	Cogmagun Restoration	6	0.3234	12.9429	0.7766	0.1058	0.0346
COGK6	*S. pumilus*	Cogmagun Restoration	52	0.1415	5.6594	0.7077	0.041	0.0325
COGK7	*S. alterniflorus*	Cogmagun Restoration	12	0.0539	2.1574	0.7017	0.0153	0.0159
COGK8	*S. michauxianus*	Cogmagun Restoration	26	0.0894	3.5761	0.8215	0.0298	0.0283
CHVK1	*S. michauxianus*	Cheverie Restoration	94	0.1452	7.2901	0.6374	0.0379	0.0277
CHVK2	*S. michauxianus*	Cheverie Restoration	88	0.1277	5.108	0.7997	0.0417	0.0314
CHVK3	*S. pumilus*	Cheverie Restoration	30	0.1062	4.2475	0.7824	0.0338	0.0265
CHVK4	*S. alterniflorus*	Cheverie Restoration	25	0.0959	3.8383	0.9381	0.0365	0.0292
CHVK5	*S. pumilus*	Cheverie Restoration	23	0.0969	3.8752	0.7229	0.0284	0.0227
CHVK6	*S. pumilus*	Cheverie Restoration	32	0.0709	2.8353	0.7845	0.0225	0.0187
CHVK7	*S. alterniflorus*	Cheverie Restoration	41	0.052	2.0817	0.8334	0.0175	0.015
CHVK8	*S. alterniflorus*	Cheverie Restoration	10	0.0757	3.0291	0.7096	0.0217	0.018
CHVK9	*S. michauxianus*	Cheverie Restoration	41	0.2292	9.1705	0.4247	0.0403	0.0249
WALK1	*S. pumilus*	Walton Restoration	0	0.0735	2.9412	0.5007	0.0149	0.0122
WALK3	*S. alterniflorus*	Walton Restoration	15	0.122	4.8802	0.6608	0.0329	0.025
WALK4	*S. pumilus*	Walton Restoration	4	0.1458	5.8323	0.6047	0.0361	0.0263
WALK5	*S. alterniflorus*	Walton Restoration	52	0.1075	4.2997	0.6526	0.0285	0.0222
WALK6	*S. alterniflorus*	Walton Restoration	22	0.1187	4.7472	0.8365	0.0404	0.0309
WALK7	*S. michauxianus*	Walton Restoration	70	0.1731	6.9254	0.7006	0.0498	0.0345
WALK8	*S. michauxianus*	Walton Restoration	46	0.1466	5.8655	0.8784	0.0527	0.0382
CORK1	*S. michauxianus*	Cogmagun Reference	53	0.1574	6.2958	0.5515	0.0356	0.0254
CORK2	*S. pumilus*	Cogmagun Reference	54	0.0647	2.5877	0.6492	0.017	0.0142
CORK3	*S. alterniflorus*	Cogmagun Reference	6	0.0548	2.1922	1.1822	0.0261	0.0224
CORK4	*S. pumilus*	Cogmagun Reference	88	0.0606	2.4253	1.0058	0.0246	0.0209
CORK5	*S. pumilus*	Cogmagun Reference	77	0.0968	3.8709	0.8643	0.034	0.0269
CORK6	*S. alterniflorus*	Cogmagun Reference	0	0.0556	2.2238	1.2946	0.029	0.0248
CORK7	*S. alterniflorus*	Cogmagun Reference	2	0.0608	2.4334	1.0844	0.0266	0.0226
CORK8	*S. michauxianus*	Cogmagun Reference	92	0.12	4.8018	0.5053	0.0247	0.0189
CORK9	*S. michauxianus*	Cogmagun Reference	30	0.1225	4.8995	0.9835	0.0491	0.0373
CHVRK1	*S. michauxianus*	Cheverie Reference	75	0.1428	5.7146	0.6358	0.0371	0.0272
CHVRK2	*S. pumilus*	Cheverie Reference	24	0.0689	2.7554	1.1299	0.0315	0.0263

CHVRK3	*S. alterniflorus*	Cheverie Reference	52	0.0593	2.3727	1.0383	0.0249	0.0211
CHVRK4	*S. alterniflorus*	Cheverie Reference	72	0.1771	7.0874	0.6111	0.0446	0.0298
CHVRK5	*S. pumilus*	Cheverie Reference	38	0.1181	4.7256	0.6823	0.0328	0.0252
CHVRK6	*S. alterniflorus*	Cheverie Reference	41	0.068	2.7207	0.8316	0.0229	0.0191
CHVRK7	*S. michauxianus*	Cheverie Reference	84	0.2474	9.8994	0.4021	0.0414	0.0243
CHVRK8	*S. pumilus*	Cheverie Reference	16	0.0949	3.7949	0.8989	0.0346	0.0277
CHVRK9	*S. michauxianus*	Cheverie Reference	42	0.0499	1.9947	1.2373	0.0249	0.0214
WALRK1	*S. pumilus*	Walton Reference	58	0.0579	2.3164	0.8663	0.0202	0.0172
WALRK2	*S. alterniflorus*	Walton Reference	8	0.0918	3.6729	0.9866	0.0368	0.0295
WALRK3	*S. alterniflorus*	Walton Reference	10	0.0536	2.1429	1.012	0.0219	0.0187
WALRK4	*S. pumilus*	Walton Reference	39	0.0887	3.549	0.6106	0.022	0.0177
WALRK5	*S. michauxianus*	Walton Reference	80	0.0547	2.1886	1.1075	0.0244	0.0209
WALRK6	*S. alterniflorus*	Walton Reference	6	0.0681	2.7257	1.0574	0.0291	0.0243
WALRK7	*S. pumilus*	Walton Reference	63	0.0578	2.3115	1.0038	0.0234	0.0199
WALRK8	*S. michauxianus*	Walton Reference	87	0.0622	2.4864	1.0397	0.0261	0.022

Appendix B. Soil properties for 2021 study sample locations including organic carbon densities using Craft et al. (1991) equation, elemental analysis, and regression equation (separate regression for Cogmagun Reference). Organic carbon EA represents measurements from elemental analysis.

Sample ID	Site	Vegetation Species	Bulk Density ($g \cdot cm^{-3}$)	Organic Matter (%)	Organic Carbon ($g \cdot C \cdot cm^{-3}$) (Craft et al., 1991)	Organic Carbon EA ($g \cdot C \cdot cm^{-3}$)	Organic Carbon ($g \cdot C \cdot cm^{-3}$)
CONT1S2	Converse Restoration	*S. michauxianus*	1.0496	5.7336	0.0243	0.0136	0.0207
CONT2S3	Converse Restoration	*S. michauxianus*	1.2314	5.4783	0.0272	0.0129	0.0233
CONK1	Converse Restoration	*S. alterniflorus*	1.1155	26.305	0.1227	0.0601	0.0701
CONK2	Converse Restoration	*S. alterniflorus*	1.2918	13.1692	0.0699	0.0138	0.052
CONK3	Converse Restoration	*S. michauxianus*	1.0697	6.6471	0.0287	0.0124	0.0241
CONK4	Converse Restoration	*S. alterniflorus*	0.9104	6.5662	0.0242	0.0143	0.0203
CONK5	Converse Restoration	*S. alterniflorus*	0.9849	39.1725	0.1638	0.0081	0.0668
CONK6	Converse Restoration	*S. alterniflorus*	1.0486	4.9619	0.021	0.0113	0.0181
CONK7	Converse Restoration	*S. michauxianus*	1.1543	6.3173	0.0295	0.0118	0.0249
CONK9	Converse Restoration	*S. alterniflorus*	0.9405	6.1432	0.0233	0.0146	0.0197
CONK10	Converse Restoration	*S. michauxianus*	0.8624	7.2208	0.0252	0.0234	0.0209
CHVK1	Cheverie Restoration	*S. michauxianus*	0.6146	14.1291	0.0355	0.0235	0.0261
CHVK2	Cheverie Restoration	*S. michauxianus*	0.4493	9.4397	0.0172	0.0177	0.0138
CHVK3	Cheverie Restoration	*S. pumilus*	0.4506	15.5557	0.0287	0.0185	0.0206
CHVK4	Cheverie Restoration	*S. alterniflorus*	0.8054	7.1621	0.0233	0.0164	0.0194
CHVK5	Cheverie Restoration	*S. pumilus*	0.6468	24.5831	0.0662	0.0262	0.0394
CHVK6	Cheverie Restoration	*S. pumilus*	0.6826	9.7561	0.027	0.0199	0.0215
CHVK7	Cheverie Restoration	*S. alterniflorus*	1.0424	5.8372	0.0246	0.0123	0.0209
CHVK8	Cheverie Restoration	*S. alterniflorus*	0.7695	8.9119	0.0278	0.0169	0.0225
CHVK9	Cheverie Restoration	*S. michauxianus*	0.4093	20.326	0.0343	0.0334	0.0224
CHVK10	Cheverie Restoration	*S. pumilus*	0.371	13.1467	0.0199	0.0101	0.0149
CHVK11	Cheverie Restoration	*S. pumilus*	0.8613	28.2655	0.1017	0.0578	0.0557
CHVK12	Cheverie Restoration	*S. alterniflorus*	0.4421	39.0951	0.0734	0.0305	0.03
CHVK13	Cheverie Restoration	*S. michauxianus*	0.5282	28.6235	0.0632	0.0329	0.0343
CHVK14	Cheverie Restoration	*S. michauxianus*	0.4994	34.6026	0.073	0.0311	0.034
CHVK15	Cheverie Restoration	*S. michauxianus*	0.449	33.7852	0.0639	0.0449	0.0304
CHVK16	Cheverie Restoration	*S. alterniflorus*	0.4597	6.7011	0.0125	0.0074	0.0104
CHVK17	Cheverie Restoration	*S. alterniflorus*	0.5935	11.9682	0.0289	0.0129	0.0222
CHVK18	Cheverie Restoration	*S. pumilus*	0.7938	11.9623	0.0387	0.0461	0.0296
DUCK1	Belcher DUC Restoration	*S. michauxianus*	1.1967	5.5244	0.0267	0.0162	0.0228
DUCK2	Belcher DUC Restoration	*S. michauxianus*	1.0917	8.0552	0.0356	0.0188	0.0292
DUCK3	Belcher DUC Restoration	*S. pumilus*	1.0039	8.3687	0.034	0.0144	0.0278
DUCK4	Belcher DUC Restoration	*S. pumilus*	0.8444	2.2413	0.0076	0.0303	0.0068
DUCK6	Belcher DUC Restoration	*S. alterniflorus*	1.2508	5.4184	0.0273	0.0113	0.0234
DUCK7	Belcher DUC Restoration	*S. alterniflorus*	1.069	6.151	0.0266	0.0139	0.0225
DUCK8	Belcher DUC Restoration	*S. michauxianus*	1.2138	4.8973	0.024	0.0164	0.0207
DUCK9	Belcher DUC Restoration	*S. pumilus*	0.6849	8.1624	0.0226	0.0158	0.0185

DUCK10	Belcher DUC Restoration	*S. pumilus*	0.8147	9.1707	0.0303	0.021	0.0244
DUCK11	Belcher DUC Restoration	*S. pumilus*	0.6338	15.7483	0.0412	0.0144	0.0292
DUCK12	Belcher DUC Restoration	*S. pumilus*	0.8669	8.5375	0.03	0.0158	0.0244
DUCK13	Belcher DUC Restoration	*S. michauxianus*	1.0735	9.0278	0.0393	0.0211	0.0317
DUCK14	Belcher DUC Restoration	*S. michauxianus*	1.1669	6.0138	0.0283	0.018	0.024
DUCK15	Belcher DUC Restoration	*S. michauxianus*	1.106	5.264	0.0235	0.0222	0.0202
DUCK16	Belcher DUC Restoration	*S. alterniflorus*	0.9526	5.6064	0.0215	0.01	0.0184
DUCK17	Belcher DUC Restoration	*S. alterniflorus*	1.3785	5.2647	0.0293	0.0207	0.0251
CHVRK1	Cheverie Reference	*S. michauxianus*	0.661	13.2801	0.0359	0.0711	0.0268
CHVRK2	Cheverie Reference	*S. pumilus*	1.0025	7.4178	0.0301	0.0261	0.0249
CHVRK3	Cheverie Reference	*S. alterniflorus*	0.9151	6.8547	0.0254	0.0161	0.0212
CHVRK4	Cheverie Reference	*S. alterniflorus*	0.3315	28.3885	0.0394	0.0258	0.0215
CHVRK5	Cheverie Reference	*S. pumilus*	0.4713	20.6126	0.0401	0.0297	0.026
CHVRK6	Cheverie Reference	*S. alterniflorus*	0.9856	28.0587	0.1155	0.0616	0.0636
CHVRK7	Cheverie Reference	*S. michauxianus*	0.3792	5.8446	0.0089	0.037	0.0076
CHVRK8	Cheverie Reference	*S. pumilus*	0.735	12.957	0.0389	0.0278	0.0292
CHVRK9	Cheverie Reference	*S. michauxianus*	0.9169	6.9843	0.0259	0.0937	0.0216
CHVRK10	Cheverie Reference	*S. michauxianus*	1.2126	2.9062	0.0142	0.007	0.0126
CHVRK11	Cheverie Reference	*S. pumilus*	1.0089	7.7732	0.0318	0.0233	0.0261
CHVRK12	Cheverie Reference	*S. alterniflorus*	0.3241	24.8255	0.0334	0.0251	0.0198
CHVRK13	Cheverie Reference	*S. pumilus*	0.6545	22.8499	0.062	0.0441	0.0384
CHVRK14	Cheverie Reference	*S. pumilus*	0.5205	13.3172	0.0283	0.0278	0.0211
CHVRK15	Cheverie Reference	*S. alterniflorus*	0.9492	6.0559	0.0232	0.0182	0.0197
CHVRK16	Cheverie Reference	*S. michauxianus*	0.3404	30.1118	0.0429	0.0486	0.0225
CHVRK17	Cheverie Reference	*S. alterniflorus*	0.9165	6.5858	0.0244	0.0175	0.0205
CHVRK18	Cheverie Reference	*S. michauxianus*	0.8159	7.2286	0.0239	0.0301	0.0198
COGK1	Cogmagun Restoration	*S. pumilus*	0.8986	4.4412	0.0161	0.0118	0.014
COGK2	Cogmagun Restoration	*S. alterniflorus*	1.1017	5.196	0.0231	0.0139	0.0198
COGK3	Cogmagun Restoration	*S. pumilus*	0.7056	4.7796	0.0136	0.0114	0.0118
COGK4	Cogmagun Restoration	*S. michauxianus*	0.9346	25.3326	0.0996	0.0287	0.0577
COGK5	Cogmagun Restoration	*S. alterniflorus*	0.629	9.0603	0.0231	0.0164	0.0186
COGK6	Cogmagun Restoration	*S. pumilus*	0.539	8.3291	0.0182	0.0132	0.0148
COGK7	Cogmagun Restoration	*S. alterniflorus*	1.0156	5.5369	0.0227	0.0111	0.0194
COGK8	Cogmagun Restoration	*S. michauxianus*	0.723	10.5806	0.0311	0.027	0.0244
COGK9	Cogmagun Restoration	*S. pumilus*	0.8708	6.0808	0.0214	0.0154	0.0181
COGK10	Cogmagun Restoration	*S. michauxianus*	0.9536	10.6861	0.0414	0.0314	0.0325
COGK11	Cogmagun Restoration	*S. michauxianus*	0.7638	8.2147	0.0254	0.0186	0.0208
COGK12	Cogmagun Restoration	*S. pumilus*	0.7357	7.9567	0.0237	0.0143	0.0195
COGK13	Cogmagun Restoration	*S. alterniflorus*	1.2548	6.1636	0.0312	0.0153	0.0264
COGK14	Cogmagun Restoration	*S. pumilus*	0.7881	6.4841	0.0206	0.0099	0.0174

COGK15	Cogmagun Restoration	*S. alterniflorus*	1.1796	5.705	0.0272	0.0088	0.0231
COGK16	Cogmagun Restoration	*S. alterniflorus*	0.9262	11.9548	0.0452	0.0089	0.0345
COGK17	Cogmagun Restoration	*S. michauxianus*	0.8265	19.5386	0.0666	0.049	0.0441
CORK1	Cogmagun Reference	*S. michauxianus*	0.551	14.4502	0.0326	0.0395	0.0264
CORK2	Cogmagun Reference	*S. pumilus*	0.925	8.8432	0.0332	0.0217	0.0316
CORK3	Cogmagun Reference	*S. alterniflorus*	1.1335	6.0856	0.0279	0.0177	0.0285
CORK4	Cogmagun Reference	*S. pumilus*	1.0366	6.4436	0.027	0.0179	0.0274
CORK5	Cogmagun Reference	*S. pumilus*	0.5714	8.9723	0.0208	0.0154	0.0198
CORK6	Cogmagun Reference	*S. alterniflorus*	1.0919	5.7186	0.0252	0.0138	0.0261
CORK7	Cogmagun Reference	*S. alterniflorus*	1.0376	4.8294	0.0202	0.011	0.0214
CORK8	Cogmagun Reference	*S. michauxianus*	0.524	16.2666	0.035	0.051	0.0267
CORK9	Cogmagun Reference	*S. michauxianus*	0.6771	12.8895	0.0356	0.0272	0.0303
CORK10	Cogmagun Reference	*S. michauxianus*	0.5436	18.6716	0.0418	0.0281	0.0294
CORK11	Cogmagun Reference	*S. pumilus*	0.586	12.244	0.0293	0.0224	0.0253
CORK12	Cogmagun Reference	*S. alterniflorus*	1.02	5.7298	0.0236	0.0135	0.0244
CORK13	Cogmagun Reference	*S. alterniflorus*	1.084	22.6594	0.1038	0.0134	0.0614
CORK14	Cogmagun Reference	*S. pumilus*	1.0677	3.8251	0.0164	0.0074	0.0178
CORK15	Cogmagun Reference	*S. alterniflorus*	1.0765	4.5203	0.0196	0.0119	0.0209
CORK16	Cogmagun Reference	*S. pumilus*	0.8392	10.4682	0.0357	0.0258	0.0326
CORK17	Cogmagun Reference	*S. michauxianus*	0.4661	20.567	0.0396	0.0342	0.026
CORK18	Cogmagun Reference	*S. michauxianus*	0.4784	14.6578	0.0287	0.0334	0.0231
TRKS1	Onslow North River Restoration	*S. alterniflorus*	1.187	3.3933	0.0162	0.0075	0.0143
TRKS2	Onslow North River Restoration	*S. michauxianus*	0.9292	4.3356	0.0162	0.0063	0.0141
TRKS3	Onslow North River Restoration	*S. alterniflorus*	1.1198	3.4259	0.0165	0.0055	0.0136
TRKS4	Onslow North River Restoration	*S. michauxianus*	0.964	4.0581	0.0157	0.0089	0.0138
TRKS5	Onslow North River Restoration	*S. alterniflorus*	1.1653	2.5045	0.0117	0.007	0.0105
TRKS6	Onslow North River Restoration	*S. michauxianus*	1.197	3.9461	0.019	0.0066	0.0167
TRKS7	Onslow North River Restoration	*S. alterniflorus*	1.3454	2.4596	0.0133	0.0094	0.0119
TRKS8	Onslow North River Restoration	*S. michauxianus*	1.0109	4.7598	0.0194	0.0116	0.0168
TRKS9	Onslow North River Restoration	*S. alterniflorus*	1.2752	2.7748	0.0142	0.0062	0.0127
TRKS10	Onslow North River Restoration	*S. michauxianus*	1.0288	7.005	0.0291	0.0185	0.0243
TRKS11	Onslow North River Restoration	*S. alterniflorus*	1.3561	2.7281	0.0149	0.0073	0.0133
TRKS12	Onslow North River Restoration	*S. michauxianus*	0.9982	6.291	0.0254	0.0091	0.0214