

Assessing the importance of seagrass habitat restoration to “blue carbon” sequestration in shallow coastal waters

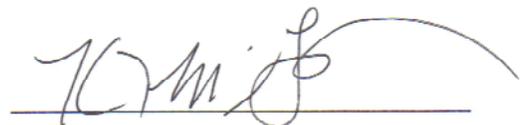
Jill Thompson Greiner
Norwalk, Connecticut

B.S., Cornell University, 2009

A Thesis Presented to the Graduate Faculty of the University of Virginia in Candidacy
for the Degree of Master of Science

Department of Environmental Sciences

University of Virginia
May, 2013

A handwritten signature in black ink, appearing to read "Michael L. Pace", written over a horizontal red line.

Michael L. Pace

Matt Reidubal

Abstract

Carbon sequestration is accelerated by the presence of seagrass in coastal habitats as the vegetation promotes the accumulation of carbon-rich sediment. Typically, measurements of carbon sequestration are conducted in mature seagrass meadows. Due to global seagrass decline, seagrass restoration efforts have increased to mitigate the continual loss of seagrass. However, it is unclear to what extent and at what level of maturity restored seagrass meadows effectively accumulate carbon.

The objective of this thesis is to quantify the carbon sequestration potential of the restored seagrass habitat at the Virginia Coast Reserve Long Term Ecological Research Site (VCR-LTER). Restoration sites of different ages, 0-, 4-, and 10-years (time since seeding) were used in this study. Seagrass age and density were expected to be the main drivers of carbon accumulation in the restored meadows. Results indicated that carbon accumulation increased with seagrass age and was linked to seagrass density. The oldest restored seagrass meadow sampled (10-years, seeded in 2001) had the largest carbon accumulation rate of $36.7 \text{ g C m}^{-2} \text{ yr}^{-1}$, and is projected to be accumulating carbon within measured ranges of natural seagrasses habitats within 12 years of seeding.

Carbon accumulating in seagrass sediment was from both seagrass and non-seagrass sources. The results of a three-source Bayesian mixing model indicated that the restored seagrass meadows were accumulating carbon from seagrass and non-seagrass sources, specifically benthic microalgae, in relatively equal amounts. The 10-year restored seagrass meadow accumulated seagrass carbon at a rate of $15.41 \text{ g C m}^{-2} \text{ yr}^{-1}$ and $20.86 \text{ g C m}^{-2} \text{ yr}^{-1}$ of non-seagrass materials.

These findings suggest that with time, restored seagrass habitats can accumulate carbon at the same rate as natural meadows, and from sources other than surrounding seagrass vegetation. In addition, this thesis provides the first measurement of “blue carbon” sequestration for a restored seagrass habitat and identifies the carbon sources that comprise the accumulating carbon pool in a restored meadow. These results support restoration and conservation efforts by providing quantitative evidence of potential atmospheric carbon dioxide emission mitigation and the ecosystem services provided by restored seagrass meadows in the form of carbon sequestration.

Acknowledgements

I give my utmost gratitude to all those that provided me guidance and support throughout my research. First I'd like to thank Karen McGlathery, whom has provided continuous support and has been instrumental in my development and scientific success. She has provided me with numerous opportunities that have allowed me to develop my scientific career tremendously. I would also like to thank my committee members: Michael Pace and Matthew Reidenbach for their involvement and advice along the way. In addition, I would like to thank Patricia Wiberg as a stand in committee member, and providing feedback throughout this process.

I was fortunate to have the help of collaborators from University of North Carolina, Chapel Hill as well as others outside my committee. John Gunnell demonstrated and explained sediment dating methods and processed my samples. Brent McKee provided essential advice and guidance in developing my first chapter. In addition, Grace Wilkinson has been instrumental by providing countless time, support, modeling expertise, and her amazing knowledge to my research and thesis. She has been an amazing friend and colleague throughout this process, and I could never have done any of this without her.

The completion of my field and lab work would not have been possible without the help of many colleagues. Kyle Emery participated in my entire first year of field sampling and lab work, and made many hours of work go by in no time. Jennie Rheuban was integral in research support by providing advice and help in thesis development. I owe a big thank you to Meg Miller, Carlos Disla, Kendall Combs, Andrew Lewis, Cy

Clemo, Gavin Bruno, Kelly Hondula, and the entire McGlathery Lab. Also thank you to the entire ABCRC staff, Art Schwarzschild, Donna Fauber, David Boyd, Chris Buck, and especially Brooke Rodgers, all of whom provided logistical, navigational, and scientific support essential in completing my research.

Finally, I would like to thank my remaining friends and family. I owe endless gratitude to Christian Wakeman, who provided support and encouragement during my research following me to Charlottesville and allowing me to pursue my desired career path. Thank you to all my friends who supported me and dealt with my absence due to field work and long work nights. Lastly, thank you to my family, who all supported me through this chapter in my life even when they did not completely understand what I was studying.

Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	vi
Table of Tables	ix
Table of Figures	x
Introduction	xiv
Background	xiv
Study Site	xvii
Chapter Summaries	xviii
Literature Cited	xix
Chapter 1 Seagrass Restoration Enhances “Blue Carbon” Sequestration in Coastal Waters	1
Abstract	2
Introduction	3
Methods	6
Results	10

Discussion	12
Literature Cited	18
Figures	23
Tables	26
Appendix	27
Chapter 2 Identifying carbon source contributors to restored seagrass sediment.....	30
Abstract	31
Introduction	32
Methods	36
Site Description	36
Sediment Sampling Methods.....	37
Carbon Source Methods	38
Mixing Model Methods.....	39
Statistical Analyses.....	42
Results	42
Sediment Profiles.....	42
Isotope Ratios	43
Mixing Model Results	44
Discussion	48

Literature Cited	57
Tables	65
Figures	69
Appendix	78
Appendix 2.1 Literature Cited:	83
Conclusion	99
Additional Appendix.....	103

Table of Tables

Chapter 1

Table 1.1: Seagrass density and sediment characteristics of different age treatments in top 10 cm of sediment.	26
---	----

Chapter 2

Table 2.1: Carbon and nitrogen source and sediment averages (mean), standard deviations (\pm SD), and sample size (n) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values used in 3-source, 2-tracer VCR-LTER Bayesian mixing model.	65
---	----

Table 2.2: Percent differences of carbon sources for multiple mixing models on each sediment treatment and individual depth interval.	66
--	----

Table 2.3: Percent differences of carbon sources for mixing models using 3-source, 2-tracer models with varying sources on each sediment treatment and individual depth interval.	68
--	----

Table of Figures

Introduction

Figure 0.1: Conceptual diagram from McGlathery et al. (2012) of seagrass increasing over time in the VCR-LTER..... xv

Figure 0.2: Study site map of the VCR-LTER on the Delmarva Peninsula in Virginia. xvii

Chapter 1

Figure 1.1: Vertical average down-core profiles of sediment characteristics in the top 10 cm..... 23

Figure 1.2: Total down core ^{210}Pb activity for all treatments. 24

Figure 1.3: Record of sediment accretion rate, percent organic carbon, and carbon burial rate in 10-year treatment. 25

Chapter 2

Figure 2.1: Vertical down-core profiles of sediment characteristics. 69

Figure 2.2: $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ ratio comparison between average sources (Z, BMA, and MA) and average sediment treatments. 70

Figure 2.3: $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ ratio comparison between average sources (Z, BMA/Seston, MA) and average sediment treatments. 71

Figure 2.4: Box plots showing quartile 5th, 25th, 50th, 75th, and 95th percentile of 3-source, 2-tracer VCR-LTER Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment). 72

Figure 2.5: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).	73
Figure 2.6: Three-source, two-tracer VCR-LTER and literature value Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).....	74
Figure 2.7: Four-source, three-tracer LTER-VCR and literature value Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).....	75
Figure 2.8: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals. Carbon sources are <i>Zostera marina</i> (Z), BMA and Seston (BMA/Seston), and macroalgae (MA) from the VCR-LTER.....	76
Figure 2.9: Box plots showing 3-source, 2-tracer VCR-LTER Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment). Carbon sources are <i>Zostera marina</i> (<i>Zostera</i>), BMA and seston (BMA/Seston), and macroalgae (MA).	77
Chapter 1 Appendix	
Appendix 1.1: Vertical average profiles of sediment characteristics in the top 10 cm of sediment in each age treatment (HI Unveg, 4-year, SB Unveg, 10-year).....	27
Appendix 1.2: Total down core ²¹⁰ Pb activity in all sediment treatments (HI Unveg, 4-year, SB Unveg, 10-year) to 20 cm depth in 1-cm intervals.....	29
Chapter 2 Appendix	

Appendix 2.1: VCR-LTER and literature review of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and molar C/N ratios for carbon sources used in Bayesian mixing model.....	78
Appendix 2.2: Carbon source and sediment age treatment averages (mean), standard deviations ($\pm\text{SD}$), and sample size (n) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N values used in Bayesian 4-source, 3-tracer mixing model.	83
Appendix 2.3: Vertical down-core profile data of sediment characteristics $\delta^{13}\text{C}$ (‰), $\delta^{15}\text{N}$ (‰), %C, and molar C/N.	85
Appendix 2.4: Isotope and C/N ratio comparisons between average sources and average sediment treatments.	88
Appendix 2.5: Box plots showing quartile 5th, 25th, 50th, 75th, and 95th percentile of carbon source for 4-source, 3-tracer Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment).	90
Appendix 2.6: Four-source, three-tracer Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.	91
Appendix 2.7: Four-source, three-tracer Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.	92
Appendix 2.8: Three-source, two-tracer VCR-LTER and literature value Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.	93

Appendix 2.9: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.....	94
Appendix 2.10: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.	95
Appendix 2.11: Carbon source, standard deviations (\pm SD), and sample size (n) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ used in Bayesian 3-source, 2-tracer VCR-LTER mixing model.....	96
Appendix 2.12: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contribution results in quartiles for each sediment treatment for the interval depths.	97
Appendix 2.13: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.	98
Additional Appendix	
Additional Appendix.1: Mean vertical 10 cm down-core profile data of sediment percent carbon (%C) in 1-cm intervals (depth) for sediment treatments 4-year and 10-year for sampling occurring in 2011 and 2012 at the same locations within the treatments.	103
Additional Appendix.2: Percent organic carbon (%C) for 4-year (2011), 5-year (2012), 10-year (2011), and 11-year (2012) in top 10 cm of sediment from sampling in 2011 and 2012.....	104

Introduction

Background

Quantifying carbon sequestration in ecosystems is important as carbon dioxide (CO₂) concentrations in the earth's atmosphere continue to rise beyond natural variations due to the existence of humans; however, carbon sequestration in both terrestrial and aquatic habitats helps offset the increasing CO₂ concentrations in the atmosphere with varying effectiveness based on ecosystem type. Terrestrial systems are widely cited as important sites of carbon accumulation via living biomass and soil organic matter, but can lose the accumulated carbon very quickly due to disturbances such as fire (Schlesinger and Lichter 2001; Mcleod et al. 2011) and leaching (Lovett et al. 2006). When compared to terrestrial ecosystems, aquatic systems, specifically in coastal environments, accumulate over 10 times as much carbon per year on an aerial basis (Mcleod et al. 2011). As the importance of the contribution of aquatic ecosystems to the global carbon sink has become more recognized, the term "blue carbon" has been coined to differentiate the aquatic carbon sink from the terrestrial ecosystem sink (Nellemann et al. 2009).

Aquatic habitats are effective at accumulating blue carbon in salt marshes, mangroves, and seagrass habitats, where each accumulates an average of over 100 g C m⁻² yr⁻¹ (Mcleod et al. 2011). These carbon sinks provide storage on decadal to millennial time scales due to fast accumulation rates and sediment carbon stocks that remain generally undisturbed, unlike terrestrial ecosystems (Mateo et al. 1997; Orem et al. 1999).

The vegetation canopy in these marine systems helps filter out suspended particles from the water column and stabilize the sediment with complex root systems that promotes the accumulation of carbon-rich particulates in the sediment (Gacia and Duarte 2001; Mcleod et al. 2011). The carbon accumulating in sediment comes from a variety of sources such as seagrass, macroalgae, benthic microalgae, marsh grass, mangroves, and phytoplankton (Gonneea et al. 2004; Kennedy et al. 2010). Differentiating the sources of the carbon stored in the sediments is important, as carbon accumulates from both in situ production or imports to the system.

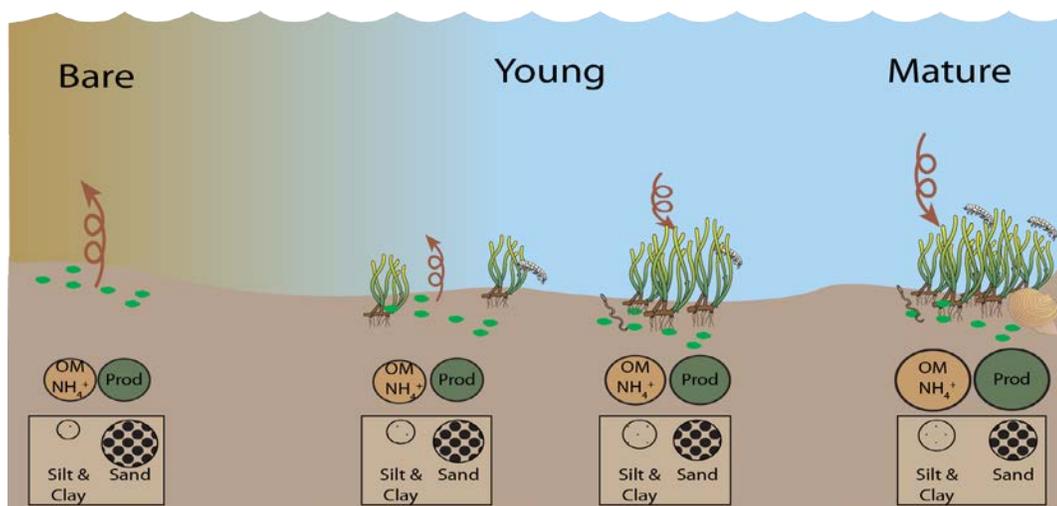


Figure 0.1: Conceptual diagram from McGlathery et al. (2012) of seagrass increasing over time in the VCR-LTER

and the resulting changes in ecosystem functioning that are associated and observed from yearly synoptic sampling. Here arrows convey sediment suspension and deposition of carbon and nutrients, where there is more sediment deposition occurring in mature seagrass meadows versus bare sediment.

Although seagrass ecosystems are important for carbon sequestration, the extent of seagrass habitat has been declining globally at a rate of 5% each year (Waycott et al. 2009). In order to offset this loss of seagrass habitat, conservation and restoration efforts are essential especially given estimated carbon accumulation rates of an average of $138 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Mcleod et al. 2011). However, there has not been any research on the ability of restored seagrass to accumulate carbon, at what age in their development this happens, and where the sources of the carbon are coming from.

The objective of this thesis is to test that seagrass restoration is effectively providing the ecosystem service of carbon accumulation with increases in age, and to establish at what point restored seagrass habitat meets carbon accumulation rates of natural seagrass meadows (Figure 0.1). This thesis examines carbon accumulation in a restored seagrass meadows, and establishes the first carbon accumulation rate for a restored seagrass habitat. Additionally, the carbon sources contributing to the seagrass sediment were identified. This thesis includes an introduction to thesis topic, two chapters written as manuscripts for submission to peer-reviewed journals, and a conclusion summarizing important findings and application of the results.

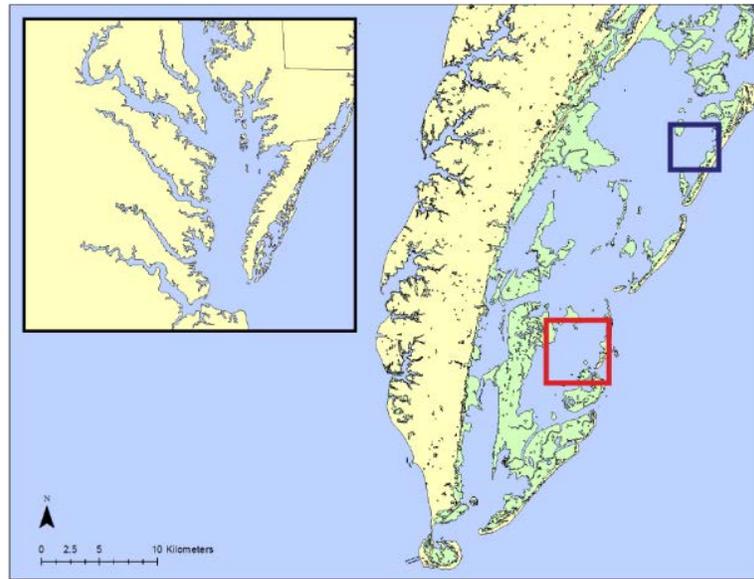


Figure 0.2: Study site map of the VCR-LTER on the Delmarva Peninsula in Virginia.

Red box notes the 10-year (seeded in 2001) and neighboring unvegetated treatment in South Bay. The blue box notes the 4-year (seeded in 2007) and neighboring unvegetated treatment in Hog Island Bay.

Study Site

All research for this thesis was conducted in the Virginia Coast Reserve Long Term Ecological Research Site (VCR-LTER) located on the ocean side of the Delmarva Peninsula in Virginia (Figure 0.2). The VCR-LTER is the site of a large-scale seagrass (*Zostera marina*) restoration via broadcast seeding with seagrass meadows of varying ages (time since seeding from 2001 – 2008). This restoration has begun to return parts of the system to a seagrass-dominated state where previously the seagrass was destroyed before 1933 by a wasting disease and a hurricane (Cottam 1934; Rasmussen 1977). The restoration effort has resulted in over 1700 ha of restored seagrass habitat (McGlathery et

al. 2012; Orth et al. 2012). The restored seagrass meadows used in this study were seeded in two different years, creating beds of different ages for comparison. The restored seagrass sites are in South Bay (10-year age treatment, seeded in 2001) and Hog Island Bay (4-year age treatment, seeded in 2007) with unvegetated treatments adjacent to the restored seagrass beds in each bay (Figure 0.2).

Chapter Summaries

In Chapter 1, carbon accumulation rates for a restored seagrass meadow are reported for the first time. Additionally, detailed sediment carbon analyses are used to compare accumulation among different aged seagrass treatments in the Virginia coastal bays with the expectation that the highest carbon accumulation would be in the 10-year treatment, little to no carbon accumulation in the 4-year treatment, and no carbon accumulation in the unvegetated treatments. We expected the 4-year treatment to accumulate some carbon due to the presence of seagrass; however, based on a previously observed 4-year lag in seagrass density (McGlathery et al. 2012), we expected carbon accumulation rates to be minimal. Combining sedimentation rates with carbon content measured at each treatment, carbon accumulation rates were calculated for the 10-year treatment. This chapter was submitted to PLoS ONE journal in early March, 2013.

Chapter 2 also reports for the first time carbon source contributions to restored seagrass sediments of different aged treatments in the Virginia coastal bays. Important carbon sources identified in the VCR-LTER included seagrass (*Zostera marina*), benthic microalgae, and macroalgae (*Gracilaria vermicuphylla*, *Ulva lactuca*, *Codium fragile*, and *Agardhiella subulata*). Stable isotopes of carbon and nitrogen were used in a

Bayesian mixing model to quantify the contribution of each source to the sediment organic matter mixture in all different aged treatments (Unvegetated Hog Island, 4-year, Unvegetated South Bay, and 10-year). It was hypothesized that seagrass would be the main contributor of carbon to the sediment mixture especially in the 4-year treatment as this site would be less effective at trapping particles in the water column than the denser 10-year seagrass meadow. In addition, benthic microalgae and macroalgae were expected to contribute a significant amount of carbon, particularly benthic microalgae, which are abundant on all sediment surfaces in the shallow lagoons. In addition, sediment dating from Chapter 1 was used for the 10-year treatment to determine how carbon sources change over time and the rate at which each source accumulated in the sediment. This chapter will be submitted to a peer-reviewed journal in May, 2013.

Literature Cited

- Cottam, C. 1934. Past periods of eelgrass scarcity. *Rhodora* **36**: 261 – 264.
- Gacia, E., and C. M. Duarte. 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuar Coast Shelf Sci.* **52**: 505 – 514.
- Gonneea, M. E., A. Paytan, and J. A. Herrera-Silveira. 2004. Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. *Estuar. Coast. Shelf Sci.* **61**: 211 – 227.
- Kennedy, H., J. Beggins, C. M. Duarte, J. W. Fourqurean, M. Holmer, N. Marba, and J. J. Middelburg. 2010. Seagrass sediments as a global carbon sink: isotopic constraints. *Glob. Biogeochem. Cycles.* **24**: 1 – 8, doi:10.1029/2010GB003848

- Lovett, G. M., J. J. Cole, and M. L. Pace. 2006. Is net ecosystem production equal to carbon storage? *Ecosystems*. **9**: 152-155.
- Mateo, M. A., J. Romero, M. Perez, M. M. Littler, and D. S. Littler. 1997. Dynamics of millenary organic deposits resulting from the growth of the Mediterranean seagrass *Posidonia oceanic*. *Estuar Coast Shelf Sci*. **44**: 103 – 110.
- McGlathery, K. J., L. K. Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, and A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Mar. Ecol. Prog. Ser.* **448**: 209 – 221.
- McLeod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Bjork, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. Silliman. 2011. A blueprint for blue carbon: toward an improvement understanding of the role of vegetated coastal habitats in sequestering CO₂. *Frontiers in Ecological Environments*. **9**: 552 – 560, doi: 10.1890/110004
- Nellemann, C., E. Corcoran, C. M. Duarte, L. Valdes, C. De Young, L. Fonseca, and G. Grimsditch. 2009. Blue Carbon: The Role of Healthy Oceans in Binding Carbon. A Rapid Response Assessment. United Nations Environment Programme, GRID, Arendal.
- Orem, W. H., C. W. Holmes, C. Kendall C, H. E. Lerch, A. L. Bates, S. R. Silva, A. Boylan, M. Corum, M. Marot, and C. Hedgman. 1999. Geochemistry of Florida Bay sediments: nutrient history at five sites in eastern and central Florida Bay. *Journal of Coastal Research*. **15**: 1055 – 1071.

- Orth, R. J., K. A. Moore, S. R. Marion, D. J. Wilcox, and D. B. Parrish. 2012. Seed addition facilitates eelgrass recovery in a coastal bay system. *Mar Ecol Prog Ser.* **448**: 177 – 195.
- Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on the environmental factors and fauna. In: McRoy, C.P., and C. Helfferich, editors. *Seagrass Ecosystems – a scientific perspective*. New York: Marcel Dekker. pp. 1 – 15.
- Schlesinger, W. H., and J. Lichter. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature.* **411**: 466 – 469.
- Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck Jr., A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrass across the globe threatens coastal ecosystems. *PNAS.* **106**: 12377 – 12381.

Chapter 1 Seagrass Restoration Enhances “Blue Carbon” Sequestration in Coastal Waters

This chapter is in review at PLOSOne as Greiner, J. T., K. J. McGlathery, J. Gunnell, and B. A. McKee. Seagrass restoration enhances “blue carbon” sequestration in coastal waters. PLoS ONE. In review.

Abstract

Seagrass meadows are highly productive habitats that provide important ecosystem services in the coastal zone, including carbon and nutrient sequestration. Organic carbon in seagrass sediment, known as “blue carbon,” accumulates from both in situ production and sedimentation of particulate carbon from the water column. Using a large-scale restoration (>1700 ha) in the Virginia coastal bays as a model system, we evaluated the role of seagrass restoration in carbon storage in sediments of shallow coastal ecosystems. Sediments of replicate seagrass meadows representing different age treatments (as time since seeding: 0, 4, and 10 years), were analyzed for % carbon, % nitrogen, bulk density, organic matter content, and ^{210}Pb for dating at 1-cm increments to a depth of 10 cm. The % carbon, % nitrogen, organic matter content, and carbon accumulation rates were higher in the 10-year restored seagrass meadow relative to the 4-year and unvegetated sediments. These differences were consistent with higher shoot density in the older meadow. Carbon accumulation rates determined for the 10-year restored seagrass meadows were between $36.7 (\pm 2.8) \text{ g C m}^{-2} \text{ yr}^{-1}$. Within 12 years of seeding, the restored seagrass meadows are expected to accumulate carbon at a rate that is comparable to measured ranges in natural seagrass meadows. This the first study to provide evidence of the potential of seagrass habitat restoration to enhance carbon sequestration in the coastal zone.

Introduction

Seagrass meadows are essential coastal ecosystems that provide many ecosystem services such as improvements in water quality and light availability, increases in biodiversity and habitat, sediment stabilization, and carbon and nutrient accumulation (Hemminga 2000; Orth et al. 2006a; McGlathery et al. 2012). Recently, seagrass meadows have been acknowledged for their carbon storage potential and it has been estimated that globally as much as 19.9 Pg of organic carbon is stored in seagrass meadows (Fourqurean et al. 2012). Seagrass meadows cover only 0.1% area of the world's ocean floor, yet account for 10 – 18% of the total oceanic carbon burial, accumulating carbon at rates of 48 to 112 Tg C yr⁻¹ (Duarte et al. 2005; Kennedy et al. 2010; Mcleod et al. 2011). Globally, seagrass ecosystems are declining in area by about 5% per year due to anthropogenic stresses such as decreased water quality and increased water temperatures (Orth et al. 2006a; Waycott et al. 2009; Mcleod et al. 2011), and this decline could result in the release of large amounts of stored carbon (Duarte et al. 2005). In order to partially mitigate seagrass decline, restoration in areas with suitable habitat is an effective option that has the potential to reestablish lost carbon stores and sinks, as well as other important ecosystem services seagrass meadows provide.

Carbon accumulation in marine sediments provides long-term storage of organic carbon and has been referred to as “blue carbon” to distinguish it from carbon in terrestrial sinks (Nellemann et al. 2009). Unlike terrestrial systems that store organic carbon primarily in living biomass and soil organic matter, coastal vegetated systems store the majority of organic carbon in sediment (Duarte et al. 2005; Mcleod et al. 2011;

Fourqurean et al. 2012). In addition, terrestrial habitats lose carbon stocks to the atmosphere as CO₂ via decomposition or by disturbances such as fires (Schlesinger and Lichter 2001; Duarte et al. 2011). Because marine sediments are often anoxic and continually accumulate sediment (Kennedy et al. 2010), organic carbon can be preserved over decadal to even millennial time scales where it is subjected to some diagenesis, but on balance is still considered a carbon sink (Mateo et al. 1997; Orem et al. 1999; McKee et al. 2007). Fast accumulation rates, low oxygen, low sediment hydraulic conductivity, and slower microbial decomposition rates facilitate carbon burial and the accumulation of carbon stocks in these coastal sediments (Mateo et al. 2006; Duarte et al. 2010; Duarte et al. 2011).

The dense canopy of seagrass meadows reduces flow velocity (Gacia et al. 1999; Hansen and Reidenbach 2012), which promotes the deposition of sediment and particles from the water column (Gacia and Duarte 2001; Agawin and Duarte 2002; Gacia et al. 2002) and reduces sediment resuspension (Gacia and Duarte 2001). When sediments of seagrass meadows are compared to unvegetated sediments, there can be as much as a threefold difference in resuspension of fine-grained sediment (Gacia et al. 1999; Hansen and Reidenbach 2012; Gacia et al. 2001). The particles that are trapped and deposited in seagrass-vegetated sediments are often rich in organic matter (OM), averaging 4.1%. (Kennedy et al. 2010). However, this trapping effect is reduced with decreased seagrass density, which could be driven by natural and human stresses on seagrass meadows such as storm disturbance or eutrophication (Hemminga and Duarte 2002; Gacia and Duarte 2001).

Carbon accumulation rates for established seagrass meadows vary depending on the seagrass species, sediment characteristics, and depth range where the seagrass grows. From a global survey of 123 sites, average carbon burial rate was $138 \pm 38 \text{ g C m}^{-2} \text{ yr}^{-1}$ (mean \pm SE, range = 45 – 190), with the large range in rates reflecting variation in shallow and deep habitats for both tropical and temperate seagrass (McLeod et al. 2011). Currently, there are only a few measurements of carbon accumulation from different seagrass species such as *Posidonia oceanica*, *Cymodocea nodosa*, and *Zostera marina*, and no carbon accumulation measurements in restored seagrass meadows (Duarte et al. 2005; Duarte et al. 2011; McLeod et al. 2011). Many studies lack accurate estimates of carbon burial due to the absence of direct measurements of key variables such as sediment bulk density and sedimentation rates (Fourqurean et al. 2012). In addition, many studies do not specify whether roots are included in measurements of sediment carbon, which potentially results in variable estimates of carbon burial. Kennedy et al. (2010) estimated that seagrass carbon contributed about 50% to the sediment organic carbon pool in seagrass-vegetated sediments globally, but did not distinguish between root, rhizome, and leaf material. The relative contribution of root and rhizome material is also influenced by decomposition rate, which varies among species (Fourqurean et al. 2012). Clarifying whether measurements include roots or not is important when determining carbon stock or accumulation measurements in seagrass meadows (Duarte et al. 2011).

At the current rates of seagrass habitat decline, this annual loss in seagrass habitat could result in the release of previously stored carbon of up to 299 Tg C yr^{-1} (Fourqurean

et al. 2012). Seagrass habitat loss due to land-use change, based on the annual loss rate of 0.4 – 2.6% seagrass habitat globally, was estimated to release between 0.05 to 0.33 Pg CO₂ yr⁻¹ back into the atmosphere, which was similar to the annual rates of fossil fuel CO₂ emissions in many small countries (Pendleton et al. 2012). This large evasion of CO₂ to the atmosphere could result in an economic cost of \$ 1.9 – 13.7 billion yr⁻¹ (Pendleton et al. 2012). Restoration is one way to mitigate the continual loss of seagrass habitat and to prevent seagrass from becoming a significant carbon source.

Despite the recent recognition that seagrass meadows are important marine carbon stores, the potential of habitat restoration in increasing carbon stocks and sinks in coastal waters is unknown. The goal of this study was to assess carbon stores and the rate of carbon accumulation in a large-scale restoration in Virginia coastal bays as a model system where eelgrass, *Zostera marina*, was restored via broadcast seeding at several different times. Restored seagrass meadows (4 and 10 years old) and unvegetated areas were used to determine how carbon accumulation was promoted by restored seagrass. Carbon accumulation rate in the restored seagrass meadow was measured and differences assessed in carbon accumulation between unvegetated habitat and seagrass meadows of varying densities and ages.

Methods

This study was conducted at the Virginia Coast Reserve Long Term Ecological Research site (VCR-LTER) on the Eastern Shore of Virginia, on the ocean side of the Delmarva Peninsula. Dense meadows of *Zostera marina* (eelgrass) carpeted the seafloor

pre-1933 (Orth et al. 2006b), and supported a lucrative scallop fishery. The slime-mold (*Labarinthula* sp.) wasting disease and a severe hurricane in 1933 caused a local extinction of the seagrass (Cottam 1934; Rasmussen 1977; Orth et al. 2006b). The loss of seagrass habitat in the Virginia coastal bays led to decreased sediment stabilization and habitat reduction for many fauna, most notably the collapse of the scallop fishery (Orth et al. 2006a; Orth et al. 2006b). Starting in 2001, eelgrass was seeded over multiple years in South Bay and Hog Island Bay, creating a system of seagrass meadows of varying ages that comprised over 1700 ha in 2011 (Orth et al. 2012; McGlathery et al. 2012).

Two adjacent locations were used for this study, Hog Island Bay (HI) (37° 24' 47" N, 75° 43' 36" W) and South Bay (SB) (37° 15' 54" N, 75° 48' 50" W), both with a tidal range of approximately 1.2 m (McGlathery et al. 2001). Both SB and HI are located within the VCR LTER site, where all necessary permits were obtained for field methods conducted in this study. The seagrass restoration area was set aside for restoration and seagrass research by the Virginia Marine Resource Commission; through collaboration with the Virginia Institute of Marine Sciences, permission was given to use these sites in the VCR for research purposes. Previous research has determined that these two locations are similar in terms of bathymetry, water depth, sediment, water-column characteristics, and current speeds (McGlathery et al. 2012). In South Bay (SB) replicate 0.4 ha plots were seeded with 100,000 seeds ha⁻¹ in 2001 and in Hog Island Bay (HI) replicate 0.2- and 0.4-ha plots were seeded with 100,000 seeds ha⁻¹ in 2007. These sites were used as a 10-year age treatment (SB) and a 4-year age treatment (HI), respectively. Previous analysis and monitoring found that there were no significant differences in sediment and

plant parameters as a result of different plot sizes or initial seed density, allowing for sites of different plot size (0.2- and 0.4-ha) to be pooled (McGlathery et al. 2012). Seagrass shoot densities increased with time since seeding, with an initial 4-year lag followed by a rapid linear increase in shoot density as a function of age (McGlathery et al. 2012). In addition, surrounding unvegetated sediment was sampled at both sites (SB and HI) to represent the 0-year age, or un-restored, reference treatment. For each age treatment, 6 plots were selected for sediment core sampling, except in the South Bay unvegetated treatment, where 4 plots were sampled.

Sampling was conducted during the summer of 2011 beginning in June and continuing through August. Additional cores were taken in October 2011 at the SB unvegetated plots. Depth profiles of sediment characteristics were not likely to be different between June through October in the unvegetated plots because these represented sediment accumulated over annual to decadal time scales. In the 10-yr and 4-yr seagrass plots, a 50-m (for 0.4-ha plots) and 25-m (for 0.2-ha plots) transect was placed parallel to the current and aligned with the center of each plot. Four 20-cm deep, 10-cm diameter cores were taken equidistant along each transect and processed the same day. Seagrass densities were measured by counting shoots in ten 0.25-m² quadrats at regular intervals along each transect, resulting in 60 measurements per treatment.

Extruded cores were divided into 1.0-cm intervals with large shells, rocks, and large rhizomes removed, and the wet weight of each core interval was measured. Subsamples from each interval were taken to measure % water content of the sediment, loss on ignition (%OM), percent carbon (%C) and percent nitrogen (%N). Percent water

content was used to calculate the bulk density (BD) of each core interval. Sediments were dried at 60°C for 48 h, and then placed in a muffle furnace at 500°C for 6 h to determine %OM using the loss on ignition method. A portion of the subsamples was dried at 60°C for 48 h, homogenized with grinding, and measured for %C and %N with a Carlo Erba Elemental Analyzer (Lakewood, New Jersey) using a helium gas carrier in a 1020°C combustion tube and 650°C reduction tube.

One core from each age treatment was used to establish ^{210}Pb profiles to determine sediment accretion rates. ^{210}Pb content was analyzed using isotope-dilution alpha spectrometry for the ^{210}Pb granddaughter isotope polonium (^{210}Po), because these radioisotopes are in secular equilibrium (El-Daoushy et al. 1999). Because the seagrass restoration occurred in the past 10 years and seagrass habitat was once present in the system 80 years ago, the half-life of ^{210}Pb radio isotope was the appropriate dating method for the sediment (El-Daoushy et al. 1999). A ^{209}Po spike was added to each sample. Sediment samples were digested in a microwave with concentrated nitric acid. Then, hydrogen peroxide and heat were used to digest the solution and extract the tracer from organic compounds (de Vleeschouwer et al. 2010). Polonium was spontaneously electroplated onto stainless steel planchets and $^{209}\text{Po}/^{210}\text{Po}$ activities were measured via alpha spectrometry using silicon surface barrier detectors linked to a multi-channel analyzer (Matthews et al. 2007). To determine sediment accretion rates, a constant rate of tracer supply model (CRS) of non-steady state sediment accretion was applied to the derived excess ^{210}Pb values as they changed versus mass-depth (Appleby 2001; Kloker et al. 2009). Sediment accretion rates were taken for each 1 cm interval using the specific

date, and carbon accumulation rates were calculated by multiplying carbon density and sediment accretion rate.

For carbon, nitrogen, organic matter content, and bulk density measurements, data from each variable were averaged from the top 10 cm of each age treatment (SB unvegetated $n = 80$, HI unvegetated $n = 230$, 4- and 10-year $n = 240$). The top 10 cm of sediment was used for analysis because the ^{210}Pb results did not show sediment accumulation below 10 cm depth indicating the sediment at this depth was most likely not influenced by the restoration. Additionally, the top 10 cm of sediment was within the depth range influenced by root growth.

Significant differences between age treatments in %C, %N, %OM, and BD were analyzed with a 2-way nested analysis of variance (ANOVA) to determine within group variance among each treatment and variance among the different treatments using SAS software (Version 9.2 of the SAS Systems for Windows, 2008, SAS Institute Inc.). Post hoc Ryan's Q tests were used to determine significant difference between each treatment. Seagrass densities were analyzed with an ANOVA using SAS software to determine differences in seagrass density between the 10- and 4-year treatments.

Results

Seagrass shoot densities increased significantly with bed age, where 4-year treatments averaged to 123.2 shoots m^{-2} and 10-year treatment averaged to 428.7 shoots m^{-2} ($F_{60,60} = 135.89$; $p < .0001$ (Table 1.1)). %OM profiles of sediment cores indicated that the 10-year treatment had significantly different %OM only in the top 6 cm of

sediment compared to all other age treatments, with a large increase in %OM concentrations from 3 to 6 cm (2.19 to 2.41 %OM) (Figure 1.1A). There was no significant difference in average %OM between HI unvegetated and 4-year treatments, but there was a significant difference in average %OM between SB unvegetated and 10-year treatments ($F_{230,240,80,240} = 35.20$; $p < 0.0001$) (Table 1.1). Bulk density (BD) of sediment cores decreased significantly with age treatment, with SB unvegetated treatment at 1.61 g cm^{-3} having the highest density compared to the 10-year treatment at 1.30 g cm^{-3} ($F_{240,80,240,230} = 60.59$; $p < 0.0001$) (Table 1.1).

The carbon content of each age treatment varied throughout the core. Carbon concentration in the 10-year treatment was significantly higher than the neighboring SB unvegetated sediment and treatments in Hog Island, and with a large increase in %C in between 3- and 6-cm depths ($F_{240,80,240,230} = 37.47$; $p < 0.0001$) (Table 1.1, Figure 1.1B). There was no significant difference between the 4-year treatment and the neighboring HI unvegetated sediment, with HI unvegetated sediment showing on average slightly higher %C values (Table 1.1, Figure 1.1B). Though occurring at very low concentrations, nitrogen depth profiles showed similar patterns to both %OM and %C profiles. Nitrogen concentrations were significantly higher in the 10-year treatments averaging 0.05 %N, and were highest between 3- and 6-cm depth ($F_{240,80,240,230} = 108.63$; $p < 0.0001$) (Table 1.1, Figure 1.1C). For the other age treatments (0 and 4 years) there were no significant differences throughout the core, all with average concentration of 0.02 %N (Table 1.1, Figure 1.1C).

The vertical profile of the 10-year treatment had higher levels of excess ^{210}Pb compared to the SB unvegetated treatment (Figure 1.2B). ^{210}Pb profiles from the 10-year and neighboring SB unvegetated treatments convey a background supported ^{210}Pb value below 10 cm depth where both the 0- and 10-year treatment values were the same, representing sediment before restoration (Figure 1.2B). Above 10 cm depth in the 10-year treatment, there was an excess in ^{210}Pb , indicating some accumulation of sediment over time allowing for a sediment accretion and carbon accumulation rate to be calculated as a result of the seagrass restoration (Figure 1.2B) (Appleby 2001). However, vertical core profiles showed low and background levels of ^{210}Pb activity in the 4-year and unvegetated treatments (Figure 1.2A). From the 10-year ^{210}Pb profiles, sediment accretion rates were approximately 0.66 cm yr^{-1} (Figure 1.3). Carbon accumulation rates increased over time following the seeding, with a rapid acceleration in accretion rates starting 5 years following the seeding as the seagrass density increased. For the 10-year treatment, the seagrass accumulated approximately $36.7 (\pm 2.8) \text{ g C m}^{-2} \text{ yr}^{-1}$ (Figure 1.3).

Discussion

Based on previous research at this site, restored seagrass meadows had double in OM concentration 9 years after seeding. In addition, there were three times more carbon and four times more nitrogen, and the meadows had accumulated and retained finer particles in the top 5 cm of sediments than bare, unvegetated areas (McGlathery et al. 2012). In this study, we quantify for the first time carbon accumulation rates in restored seagrass meadows and provide evidence for the potential of seagrass habitat restoration to

enhance carbon sequestration in the coastal zone.

Radioactive ^{210}Pb sediment dating showed a clear pattern in the 10-year seagrass site, providing the first measurement of restored seagrass sediment accumulation rate. ^{210}Pb profiles for the 10-year treatments showed both sediment that has been affected by the seagrass restoration above 10 cm depth, and sediment unaffected by restoration below 10 cm depth (Figure 1.2B). However, ^{210}Pb profiles from the 4-year and neighboring HI unvegetated treatment had a signature of low activity representing only the background supported ^{210}Pb , indicating an insignificant impact on sediment accumulation during the first 4 years of meadow development (Figure 1.2A). This profile would only be seen if there was sediment older than 100 years, or more likely, the sediment profile was compromised mainly from resuspension and/or bioturbation causing a dilution effect and shallow mixing. The low ^{210}Pb values was not the result of deep mixing because the carbon profiles for these sites were not homogenous; as a result, sedimentation and subsequent carbon accumulation rates for the 4-year treatment could not be determined (McGlathery et al. 2012).

Increases in seagrass shoot density over time in the restored seagrass meadows influences water flow and causes a shift from an erosional to a depositional environment (Hansen and Reidenbach 2012). In addition, low seagrass densities such as those we observed in the 4-year treatment accelerate flow around individual shoots, create turbulence, and increase sediment suspension similar to areas without seagrass habitat (Kolker et al. 2009). This mechanism also can explain the lack of change in organic matter and carbon content with depth in 0- and 4-year treatments. Our results suggest that

shoot densities in the 4-year treatments (average 123.2 shoots m^{-2}) were insufficient to reduce resuspension and shallow mixing of sediment compared to bare sediments, and that by 10 years after seeding (average 428.7 shoots m^{-2}) the seagrass meadow stabilized and trapped sediment more effectively allowing for sediment accretion. These results are consistent with previous studies where a significant increase in sediment stabilization in dense seagrass meadows promotes sediment accumulation compared to unvegetated areas (Gacia et al. 1999; Hansen and Reidenbach 2012).

Sedimentation rates from measured cores do not take into account any mixing from organisms. However, as the sediment environment becomes anoxic (as is the case in this study), the abundance of bioturbating organisms decreases greatly (Townsend et al. 1998; Mateo et al. 2006). In addition, nutrient and sedimentation profiles exhibit trends that are inconsistent with the homogenization of sediments by bioturbation (Figure 1.2A and 1.2B). The 10-year treatment had consistent accreting profile, and bioturbation most likely had little to no effect on the accretion profiles (Figure 1.2B).

Sediment accretion rates and %C in the 10-year treatment showed a steady-state accretion rate before seeding, and then a significant increase in carbon burial rates 10 years after the seeding initiated seagrass meadow development (Figure 1.3A). However, following the seeding event, there was approximately a 5-year lag before there was a doubling in the carbon burial rate, compared to past trends. This can be attributed to changes in seagrass density at this site, where a large increase in seagrass density took approximately 4 years, which coincided with the dramatic increase in sediment accretion rates observed in the present study (McGlathery et al. 2012). The reproductive phenology

of *Zostera marina* in this region is such that seedlings typically flower and produce seeds in their second year, and thereafter, those seeds that germinate and survive then produce seeds again after 2 years, resulting in an approximately 4-year lag in the rate of shoot density increase. Given the hydrological similarities between sites (McGlathery et al. 2012), we anticipate that the 4-year treatment will follow the same trajectory as the 10-year treatment and have similar accretion rates in the future as shoot densities increase rapidly. In addition, the %C data during this initial lag period suggests that the organic carbon source remained consistent, but that after the 5-year lag period there was a significant decrease in %C, indicating that the carbon accumulating in the sediment consisted of different sources of material. This suggests that initially during the lag period, carbon accumulation in seagrass sediments was low but rich with organic matter, most likely seagrass detritus; however, once seagrass meadows became more dense, there was increased trapping of particles with low carbon concentrations, such as seston and other allochthonous sources, which corresponds to the increase in the carbon burial rate (Agawin and Duarte 2002).

The 10-year restored seagrass meadows facilitated the accumulation of $36.68 (\pm 2.8) \text{ g C m}^{-2} \text{ yr}^{-1}$, which falls just slightly below the range for carbon burial in natural seagrass meadows ($45 - 190 \text{ g C m}^{-2} \text{ yr}^{-1}$) estimated by Mcleod et al. (2011). Because the restored seagrass in this area was still expanding and increasing in density at the time of this study, the carbon accumulation rate for the restored seagrass meadows will likely continue to increase. If we assume that the annual carbon accumulation rate is related to seagrass density and that this density will increase until some steady-state density is

reached (McGlathery et al. 2012), we can calculate the near-term carbon accumulation rates for these restored seagrass meadows. Applying the short-term linear trend to the last 5 years of carbon accumulation rates, we estimate a rate of $47 \text{ g C m}^{-2} \text{ yr}^{-1}$ by 12 years after seeding (2 years beyond the sampling reported here) that is within the measured range of natural seagrass meadows reported by Mcleod et al. (2011). This projection is assuming that sedimentation rates will continue to increase at their current rate for another two years, which is a reasonable short term project. Long-term, the sedimentation and carbon accumulation rates are expected to reach some steady state as a result of seagrass densities also reaching a threshold amount remaining at some steady state.

Due to the inconsistencies of methods, there has been little uniformity in past studies on including roots and rhizomes in sediment carbon measurements, which could potentially lead to higher estimates of carbon accumulation if roots were included (Fourqurean et al. 2012). We addressed this issue by analyzing the bulk carbon concentration for two additional cores (one from each of the 4-year and 10-year treatments) in which roots were not removed. We found that the average belowground biomass carbon stock was lower for the 4-year treatment (3.19 g C m^{-2} , ($n = 6$, $SE = 2.36$) vs. 9.67 g C m^{-2} ($n = 6$, $SE = 6.65$) for the 10-year treatment), and bulk density was significantly lower with the presence of more roots in the 10-year treatment (Table 1.1). However, there was no significant difference for either the 4-year or 10-year treatment in bulk carbon measurements for the top 10 cm between cores with and without roots and rhizomes ($X^2 < 16.92$; $df = 9$). This indicates that the roots and rhizome contribution to the carbon stock for these developing seagrass meadows was minimal compared to that

of other particulate carbon in the sediments. Future studies should determine the relative contribution of seagrass root and rhizome carbon to estimated accumulation rates, as this may be important in older or more established meadows.

Seagrass ecosystems are lost each year through habitat destruction, eutrophication, and other anthropogenic stressors (Waycott et al. 2009). However, restoration, such as that occurring at the VCR-LTER, can help mitigate the loss of habitat and associated ecosystem services (McGlathery et al. 2012; Orth et al. 2012). Seagrass ecosystems have only recently received global recognition for their ability to sequester carbon (Fourqurean et al. 2012; Mcleod et al. 2011), and rates have only been measured for a few systems and species (Kennedy et al. 2010; Duarte et al. 2011). Until this study, there has not been any work on how or when restored seagrass systems promote the accumulation of carbon. Under current estimates of the economic cost of \$41 per ton of CO₂ (United States Government 2010) and 2011 estimates of restored seagrass coverage at the VCR of 1700 ha (McGlathery et al. 2012), the restored seagrass provides an estimated social cost of approximately \$7,000 yr⁻¹ or \$4.10 ha⁻¹ yr⁻¹ of carbon storage. These carbon accumulation rates will be useful for planners and policy makers in assessing the potential of restored seagrass ecosystems to sequester “blue carbon”.

Literature Cited

- Agawin, N. S. R., and C. M. Duarte. 2002. Evidence of direct particle trapping by a tropical seagrass meadow. *Estuaries*. **25**: 1205 – 1209.
- Appleby, P. G. 2001. Chronostratigraphic techniques in recent sediments. In: Last WM, Smol JP, editors. *Tracking Environmental Change Using Lake Sediments. Volume 1: Basin Analysis, Coring, and Chronological Techniques*. Netherlands: Springer. pp. 171 – 203.
- Cottam, C. 1934. Past periods of eelgrass scarcity. *Rhodora*. **36**: 261 – 264.
- de Vleeschouwer, F., J. Sikorski, and N. Fagel. 2010. Development of lead-210 measurement in peat using polonium extraction, a procedural comparison. *Geochronometria*. **36**: 1 – 8.
- Duarte, C. M., H. Kennedy, N. Marbe, and I. Hendricks .2011. Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. *Ocean Coast Manag.* **51**: 671 – 688.
- Duarte, C. M., N. Marba, E. Gacia, J. W. Fourqurean, J. Beggins, C. Barron, and E. T. Apostolaki. 2010. Seagrass community metabolism: assessing the carbon sink capacity of seagrass meadows. *Global Biogeochem Cycles*. **42**: GB4032.
- Duarte, C. M., J. J. Middelburg, and N. Caraco. 2005. Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*. **2**: 1 – 8.
- El-Daoushy, F., K. Olsson, and R. Garcia-Tenorio. 1991. Accuracies in Po-210 determination for lead-210 dating. *Hydrobiologia*. **214**: 43 – 52.

- Fourqurean, J. W., C. M. Duarte, H. Kennedy, N. Marba, M. Holmer, M. A. Mateo, E. T. Apostolaki, G. A. Kendrick, D. Krause-Jensen, K. J. McGlathery, and O. Serrano. 2012. Seagrass ecosystems as a globally significant carbon stock. *Nat Geosci.* doi:10.1038/NGEO1477.
- Gacia, E., and C. M. Duarte. 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuar Coast Shelf Sci.* **52**: 505 – 514.
- Gacia, E., C. M. Duarte, and J. J. Middelburg. 2002. Carbon and nutrients deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. *Limnol Oceanogr.* **47**: 23 – 32.
- Gacia, E., T. C. Granata, and C. M. Duarte. 1999. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquat Bot.* **65**: 255 – 268.
- Hansen, J. C. R., and M. A. Reidenbach. 2012. Wave and tidally driven flows within *Zostera marina* seagrass beds and their impact on sediment suspension. *Mar Ecol Prog Ser.* **448**: 271 – 287.
- Hemminga, M. A., and C. M. Duarte. 2000. *Seagrass Ecology*. Cambridge: Cambridge University Press.
- Kennedy, H., J. Beggins, C. M. Duarte, J. W. Fourqurean, M. Holmer, N. Marba, and J. J. Middelburg. 2010. Seagrass sediments as a global carbon sink: isotopic constraints. *Global Biogeochem Cycles.* **24**: 1 – 8.

- Kolker, A. S., S. L. Jr. Goodbred, S. Hameed, and J. K. Cochran. 2009. High-resolution records of the response of coastal wetland systems to long-term and short-term sea-level variability. *Estuar Coast Shelf Sci.* **84**: 493 – 508.
- Mateo, M. A., J. Cebrain, K. Dunton, and T. Mutchler. 2006. Carbon flux in seagrass ecosystems. In: Larkum A. W., Orth R. J., and Duarte C. M., editors. *Seagrasses: biology, ecology and conservation*. Netherlands: Springer. pp. 159 – 192.
- Mateo, M. A., J. Romero, M. Perez, M. M. Littler, and D. S. Littler. 1997. Dynamics of millenary organic deposits resulting from the growth of the Mediterranean seagrass *Posidonia oceanica*. *Estuar Coast Shelf Sci.* **44**: 103 – 110.
- Matthews, K. M., C-K. Kim, and P. Martin. 2007. Determination of ^{210}Po in environmental materials: a review of analytical methodology. *Applied Radiation and Isotopes.* **65**: 267 – 279.
- McLeod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Bjork, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. R. Silliman. 2011. A blueprint for blue carbon: toward an improvement understanding of the role of vegetated coastal habitats in sequestering CO_2 . *Front Ecol Environ.* **9**: 552 – 560.
- McGlathery, K. J., I. C. Anderson, and A. C. Tyler. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. *Mar Ecol Prog Ser.* **216**: 1 – 15.
- McGlathery, K. J., L. W. Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, and A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Mar Ecol Prog Ser.* **448**: 209 – 221.

- McKee, L. K., D. R. Cahoon, and I. C. Feller. 2007. Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Glob Ecol and Biogeogr.* **16**: 545 – 556.
- Nellemann, C., E. Corcoran, C. M. Duarte, L. De Valdes , C. Young, L. Fonseca, and G. Grimsditch. 2009. Blue Carbon: The Role of Healthy Oceans in Binding Carbon. A Rapid Response Assessment. United Nations Environment Programme, GRID, Arendal.
- Orem, W. H., C. W. Holmes, C. Kendall, H. E. Lerch, A. L. Bates, S. R. Silva, A. Boylan, M. Corum, M. Marot, and C. Hedgman. 1999. Geochemistry of Florida Bay sediments: nutrient history at five sites in eastern and central Florida Bay. *Journal of Coastal Research.* **15**: 1055 – 1071.
- Orth R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr., A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarink, F. T. Short, M. Waycott, and S. L. Williams. 2006a. A global crisis for seagrass ecosystems. *BioScience.* **56**: 987 – 996.
- Orth R. J., M. L. Luckenbach, S. R. Marion, K. A. Moore, and D. J. Wilcox. 2006b. Seagrass recovery in the Delmarva Coastal Bays, USA. *Aquat Bot.* **84**: 26 – 36.
- Orth R. J., K. A. Moore, S. R. Marion, D. J. Wilcox, and D. B. Parrish. 2012. Seed addition facilitates eelgrass recovery in a coastal bay system. *Mar Ecol Prog Ser.* **448**: 177 – 195.
- Pendleton L., D. C. Donato, B. C. Murray, S. Crooks, W. A. Jenkins, S. Silfleet, C. Craft, J. W. Fourqurean, J. B. Kauffman, N. Marba, P. Megonigla, E. Pidgeon, D. Herr,

- D. Gordon, and A. Balderal. 2012. Estimating global “blue carbon” emissions from conversion and degradation of vegetated coastal ecosystems. *PLoS ONE*. **7**: e43542.
- Rasmussen E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on the environmental factors and fauna. In: McRoy C. P., and Helfferich C., editors. *Seagrass Ecosystems – a scientific perspective*. New York: Marcel Dekker. pp. 1 – 15.
- Schlesinger W. H., and J. Lichter. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature*. **411**: 466 – 469.
- Townsend C. E., and M. S. Fonseca. 1998. Bioturbation as a potential mechanism influencing spatial heterogeneity of North Carolina seagrass beds. *Mar Ecol Prog Ser*. **169**: 123 – 132.
- United States Government. 2010. Technical Support Document: Social cost of carbon for regulatory impact analysis under executive order 12866. United States of Environmental Protection Agency website. Available at: <http://www.epa.gov/otaq/climate/regulations/scc-tsd.pdf>. Accessed 2013 Feb 1.
- Waycott M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck Jr., A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrass across the globe threatens coastal ecosystems. *PNAS*. **106**: 12377 – 12381.

Figures

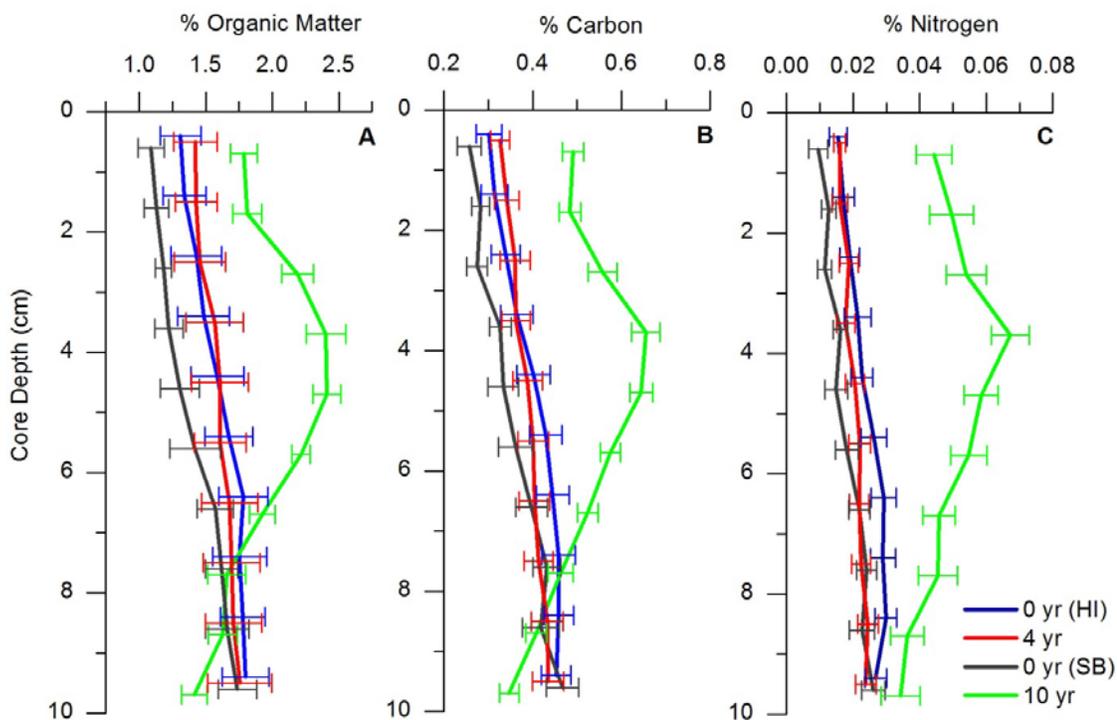


Figure 1.1: Vertical average down-core profiles of sediment characteristics in the top 10 cm.

A) Percent organic matter (%OM); **B)** Percent organic carbon (%C); **C)** Percent nitrogen (%N) for 4 different age treatments (0- (HI), 4-, 0- (SB), and 10-year) in top 10 cm of sediment, where error bars indicate standard error. Averages for each variable were calculated in 1-cm intervals until 10 cm depth.

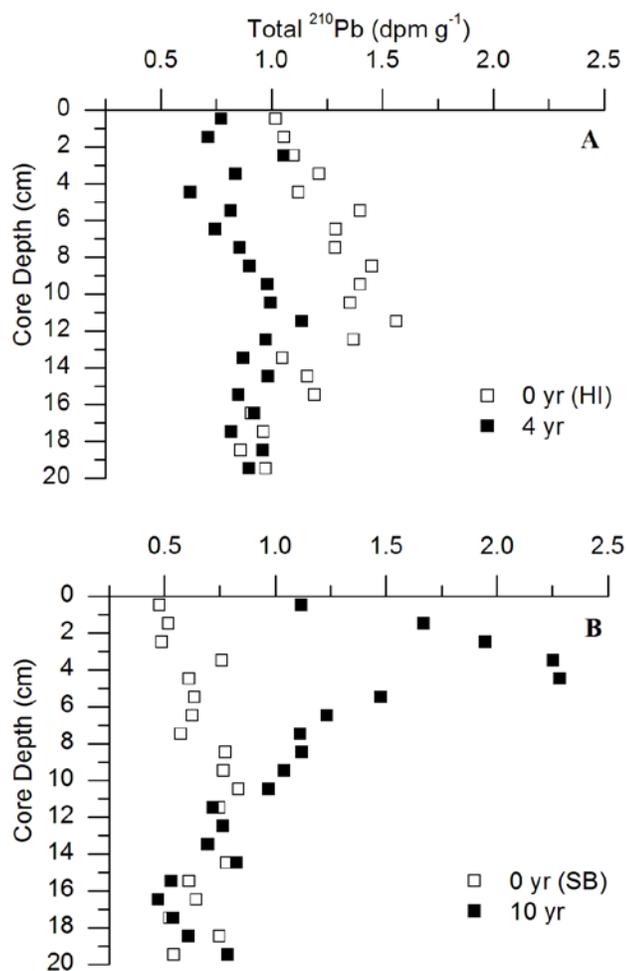


Figure 1.2: Total down core ^{210}Pb activity for all treatments.

A) Total down core ^{210}Pb activity in Hog Island treatments, 0-year (HI) and 4-year. There is no significant ^{210}Pb activity to determine sedimentation rate throughout the core. **B)** Total down core ^{210}Pb activity in South Bay treatments, 0-year (SB) and 10-year. There is significant ^{210}Pb activity in the 10-year treatment above 10 cm depth, allowing for the determination of a sedimentation rate. Error in each measurement was not significant due to low instrumental error.

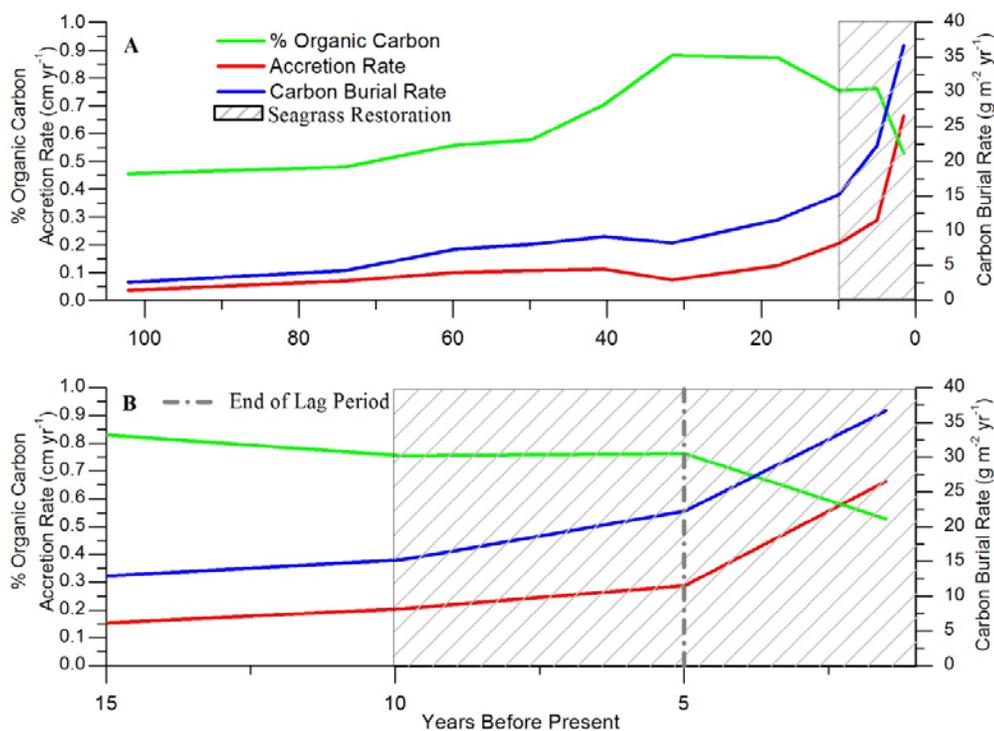


Figure 1.3: Record of sediment accretion rate, percent organic carbon, and carbon burial rate in 10-year treatment.

A) Historical record in the 10-year treatment (SB) of sediment accretion rate, percent organic carbon, and carbon burial rate with years before present starting in 2011 (=0 on x-axis). **B)** Recent record in the 10-year treatment of sediment accretion rate, % organic carbon, and carbon burial rate with years before present starting in 2011. Time influenced by seagrass restoration (10 years) is enclosed in box with grey diagonal lines. The vertical, grey hyphenated line at 5 years before present indicates the end of the 5-year lag period, where before there was little change in carbon burial rates due to low seagrass density.

Tables

Table 1.1: Seagrass density and sediment characteristics of different age treatments in top 10 cm of sediment.

Mean and standard error (SE) for 4 different treatments (HI Unveg, HI, SB Unveg, and SB) in the top 10 cm of sediment cores for percent carbon (%C), percent nitrogen (%N), percent organic matter (%OM), and bulk density from n number of samples. Age is the number of years since seagrass was seeded, and seagrass density is the number of shoots in a square meter. SB is significantly different from all other treatments for all measured variables ($p < .0001$).

Site	Age (yr)	Seagrass Density (Shoots m ⁻²)		% Carbon		% Nitrogen		% Organic Matter		Bulk Density (g cm ⁻³)		n
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
HI Unveg	0	0	0	0.40	0.011	0.02	0.001	1.61	0.03	1.53	0.02	230
HI	4	123.2	39.63	0.39	0.010	0.02	0.001	1.59	0.03	1.44	0.01	240
SB Unveg	0	0	0	0.36	0.012	0.02	0.001	1.39	0.04	1.61	0.03	80
SB	10	428.7	30.19	0.52	0.010	0.05	0.002	1.94	0.03	1.30	0.01	240

Appendix

Appendix 1.1: Vertical average profiles of sediment characteristics in the top 10 cm of sediment in each age treatment (HI Unveg, 4-year, SB Unveg, 10-year).

Sediment characteristics include organic matter (%OM), carbon (%C), and nitrogen (%N) content in 1-cm intervals. Standard errors (\pm SE) are reported along with each variable's sample size (n) per treatment.

Depth (cm)	%OM Mean	%OM \pm SE	%C Mean	%C \pm SE	%N Mean	%N \pm SE
HI Unveg ($n = 23$)						
1	1.31	0.15	0.30	0.03	0.02	2.65E-03
2	1.34	0.16	0.32	0.03	0.02	3.21E-03
3	1.43	0.19	0.34	0.03	0.02	2.97E-03
4	1.48	0.19	0.37	0.04	0.02	4.01E-03
5	1.59	0.20	0.41	0.04	0.02	3.28E-03
6	1.67	0.18	0.43	0.04	0.03	3.79E-03
7	1.78	0.19	0.45	0.04	0.03	3.78E-03
8	1.75	0.20	0.47	0.04	0.03	3.74E-03
9	1.78	0.17	0.46	0.03	0.03	3.29E-03
10	1.80	0.18	0.46	0.03	0.03	3.29E-03
4-year ($n = 24$)						
1	1.42	0.16	0.33	0.02	0.02	1.79E-03
2	1.43	0.16	0.34	0.03	0.02	2.34E-03
3	1.45	0.19	0.36	0.03	0.02	2.89E-03
4	1.57	0.22	0.36	0.03	0.02	2.74E-03
5	1.61	0.21	0.39	0.03	0.02	2.84E-03
6	1.61	0.20	0.40	0.03	0.02	3.28E-03
7	1.68	0.21	0.40	0.03	0.02	2.92E-03
8	1.69	0.21	0.41	0.03	0.02	2.89E-03
9	1.71	0.21	0.43	0.04	0.02	3.10E-03
10	1.76	0.24	0.43	0.03	0.02	3.09E-03
SB Unveg ($n = 8$)						
1	1.09	0.10	0.26	0.03	0.01	2.87E-03
2	1.13	0.09	0.28	0.02	0.01	2.17E-03
3	1.18	0.06	0.27	0.02	0.01	2.11E-03
4	1.22	0.11	0.33	0.02	0.02	2.12E-03

5	1.30	0.15	0.33	0.03	0.01	3.42E-03
6	1.42	0.19	0.36	0.04	0.02	3.43E-03
7	1.57	0.14	0.40	0.04	0.02	3.22E-03
8	1.62	0.12	0.43	0.03	0.02	3.08E-03
9	1.66	0.16	0.42	0.04	0.02	3.79E-03
10	1.74	0.15	0.47	0.04	0.03	3.53E-03
10-year ($n = 24$)						
1	1.79	0.10	0.49	0.02	0.04	0.01
2	1.81	0.11	0.48	0.02	0.05	0.01
3	2.19	0.12	0.56	0.03	0.05	0.01
4	2.40	0.15	0.66	0.03	0.07	0.01
5	2.41	0.10	0.64	0.03	0.06	0.01
6	2.22	0.07	0.58	0.02	0.05	0.01
7	1.92	0.10	0.52	0.02	0.05	4.80E-03
8	1.66	0.14	0.46	0.03	0.05	0.01
9	1.63	0.11	0.41	0.02	0.04	0.01
10	1.42	0.10	0.35	0.02	0.03	0.01

Appendix 1.2: Total down core ^{210}Pb activity in all sediment treatments (HI Unveg, 4- year, SB Unveg, 10-year) to 20 cm depth in 1-cm intervals.

Error in each measurement was not significant due to low instrument error.

Depth (cm)	Total ^{210}Pb (dpm g ⁻¹)			
	HI Unveg	4-year	SB Unveg	10-year
1	1.02	0.77	0.48	1.12
2	1.06	0.71	0.52	1.67
3	1.10	1.05	0.49	1.95
4	1.22	0.84	0.76	2.25
5	1.12	0.63	0.61	2.28
6	1.40	0.82	0.64	1.48
7	1.29	0.75	0.62	1.23
8	1.29	0.86	0.57	1.11
9	1.45	0.90	0.78	1.12
10	1.40	0.98	0.77	1.04
11	1.35	1.00	0.83	0.97
12	1.56	1.14	0.75	0.72
13	1.37	0.97	0.76	0.76
14	1.05	0.87	0.70	0.69
15	1.16	0.98	0.78	0.83
16	1.19	0.85	0.61	0.53
17	0.91	0.92	0.64	0.47
18	0.96	0.82	0.52	0.54
19	0.86	0.96	0.75	0.61
20	0.97	0.90	0.54	0.79

Chapter 2 Identifying carbon source contributors to restored seagrass sediment

This chapter will be submitted as Greiner, J. T., K. J. McGlathery, and G. M. Wilkinson.

Identifying carbon source contributors to restored seagrass sediment.

Abstract

Seagrass meadows accumulate carbon in sediments as a result of in situ production and sedimentation of particulate carbon. This carbon originates from many sources including the seagrass and non-seagrass sources such as benthic microalgae and macroalgae. We quantified organic matter source material in sediments of a restored seagrass meadows of two ages and of bare sediments. This large scale restoration (>1700 ha) in the Virginia coastal bays provided a model system to evaluate, the sources of carbon accumulation in restored seagrass meadows. Organic matter sources were identified using carbon and nitrogen stable isotopes of seagrass (*Zostera marina*), benthic microalgae, and multiple macroalgae species from the Virginia coastal bays. A three-source, two-isotope Bayesian mixing model was used to determine the contribution of each source to the sediment carbon in seagrass meadows of different age treatments (at time since seeding: 0-, 4-, and 10- years). Seagrass, benthic microalgae, and macroalgae contributed approximately 42.3%, 55.1%, and 1.4% respectively to the carbon pool in the top 10 cm of sediment in all age treatments. Combined with carbon accumulation rates ($37 \text{ g C m}^{-2} \text{ yr}^{-1}$), these results indicate that after 10 years since seeding, restored seagrass meadows accumulated seagrass carbon at a rate of $15 \text{ g C m}^{-2} \text{ yr}^{-1}$ and non-seagrass carbon (benthic microalgae and macroalgae) at a rate of $21 \text{ g C m}^{-2} \text{ yr}^{-1}$.

Introduction

Seagrass ecosystems are socially and ecologically important because they provide ecosystem services such as increased water quality, biodiversity, habitat, sediment stabilization, and carbon and nutrient accumulation (Hemminga and Duarte 2000; Orth et al. 2006b; Kennedy et al. 2010; McGlathery et al. 2012). Recently, it has been estimated that seagrass meadows accumulate between 48 and 112 Tg C yr⁻¹ globally based on the current estimates of global seagrass cover of 177,000 to 600,000 km² (Duarte et al. 2005a; Duarte et al. 2011; Mcleod et al. 2011). However, 5% of seagrass habitat is lost each year as a result of human influence such as coastal development and eutrophication (Orth et al. 2006b; Waycott et al. 2009; Mcleod et al. 2011). This loss of seagrass habitat is comparable to releasing 299 Tg C yr⁻¹ of previously stored carbon (Fourqurean et al. 2012) or 0.05 to 0.33 Pg CO₂ yr⁻¹ back into the atmosphere (Pendleton et al. 2012). Currently, the annual loss of seagrass habitat (using a conservative estimate of 1.5% yr⁻¹) has potential economic costs of \$1.9 – 13.7 billion yr⁻¹ from CO₂ emissions (Pendleton et al. 2012); therefore, the preservation and restoration of seagrass habitats is both economically and ecologically important.

The carbon accumulating in seagrass meadows is comprised of seagrass materials (leaves, roots, and rhizomes) that are deposited in situ and non-seagrass materials (marsh grass, macroalgae, benthic microalgae, phytoplankton, and seston), that are filtered out of the water column by the seagrass and deposited in the sediment (Gacia and Duarte 2001; Gacia et al. 2002; Bouillon and Boschker 2006; Kennedy et al. 2010). Once carbon accumulates in the seagrass sediments, it can be preserved on decadal to century

timescales (Orem et al. 1999), in part because the roots and seagrass canopy prevent resuspension of sediment, and sediment anoxia slows down microbial decomposition (Gacia et al. 2002; Mateo et al. 2006; Duarte et al. 2011). Seagrass meadows are highly productive systems producing large amounts of organic carbon; however, the amount and rate of organic carbon accumulating in the seagrass sediment exceeds the amount of carbon produced by seagrass, so outside inputs must be contributing to carbon stocks in seagrass habitats (Duarte and Cebrian 1996; Gacia et al. 2002; Duarte et al. 2005b; Duarte et al. 2010).

Stable isotopes can be used to determine different carbon source contributions to a mixture. Depending on the fraction of different contributing variables, each of these organic matter sources can potentially be identifiable from their stable isotope ratio (Fry et al. 1977; Fry and Sherr 1984; Peterson et al. 1985). Organic matter sources can have unique stable isotope values because there is variability among sources in their natural abundance of the stable isotope ratios, specifically $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, as well as different fractionation and uptake rates of each isotope (Fry et al. 1977; Fry 2006; Thayer et al. 1987). Because many of the potential organic matter sources have unique photosynthetic pathways, each carbon source contributor and the amount of carbon contributed can potentially be identified using naturally occurring stable isotopes (McConnaughey and McRoy 1979). However, since plant species can have analogous photosynthetic pathways resulting in similar fractionation patterns and carbon isotopic values, using multiple stable isotopes is often necessary to determine multiple source contributions. Additionally, in a multiple source model there needs to be n different

tracers used for $n+1$ contributing sources in order to determine a clear partitioning of each source (Phillips and Gregg 2003). Therefore, tracers such as ^{13}C and ^{15}N stable isotopes can be used to distinguish unique sources or end members, in an algebraic mixing model. The C/N ratio can also be used as a tracer to determine source partitioning as the ratio value can be source specific (Middelburg and Nieuwenhuize 1998; Gonnee et al. 2004).

One potential problem with using stable isotopes to determine carbon sources in sediment is that carbon is subjected to early diagenesis primarily from microbial metabolism, which could change stable isotope values (Freudenthal et al. 2001). In anoxic conditions, there is typically a decrease in sediment $\delta^{13}\text{C}$ as a result of degradation and metabolism, and there are conflicting results about change in sediment $\delta^{15}\text{N}$ (Freudenthal et al. 2001). Fourqurean and Schrlau (2003) showed that seagrass (*Thalassia testudinum*) leaf decomposition resulted in a depletion of 2‰ in $\delta^{13}\text{C}$ in the initial year, but little to no change was seen in seagrass rhizomes. There was a similar initial change in seagrass leaves in $\delta^{15}\text{N}$ of 2‰, and after a week, decomposition of the seagrass leaves caused the $\delta^{15}\text{N}$ to continue to decline, which caused potential problems for using $\delta^{15}\text{N}$ as an indicator for organic matter sources (Fourqurean and Schrlau 2003). In contrast, Zieman et al. (1984) found that there was minimal change in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (-2‰ $\delta^{13}\text{C}$ and -1‰ $\delta^{15}\text{N}$ respectively) when seagrass (*Halodule wrightii* and *Thalassia testudinum*) decomposed, but did find a significant decrease in C/N ratios in decomposed seagrass leaves. The significant change (13.8 – 19.8% in C/N ratios) in fresh seagrass materials was primarily the result of changes in %N by microbial activity (Zieman et al. 1984). The changes associated with $\delta^{15}\text{N}$ isotope values have the largest potential for

variation and least consensus on the direction or amount of change from diagenesis; due largely to deamination and substrate composition (Macko and Estep, 1984). However, Seagrass tissue and especially rhizomes typically decompose slowly (range of <1% dry weight d^{-1} ; Harrison 1989); as a result, past studies often assume little to no change in the $\delta^{13}C$ of the seagrass tissue during decomposition when compared to spatial and source variability (Kennedy et al. 2010; Papadimitriou et al. 2005a).

The sources of carbon accumulating in seagrass meadows can vary due to human and climatic changes as these forces often dictate the growth and survival of different vegetation and impact isotope values. Based on a review of over 200 samples from 88 locations, Kennedy et al. (2010) found that on average seagrass contributed about 50% (33% and 62% are 25th and 75th percentiles respectively) to the accumulating carbon in sediments ($41 - 66 \text{ g C m}^{-2} \text{ yr}^{-1}$), and non-seagrass sources, such as phytoplankton, terrestrial plants, and algae contributed the other half. Gonneea et al. (2004) measured carbon source contributions to sediment and found that phytoplankton and seston contributed variable amounts to accumulated carbon (range 20 – 80%), seagrass (species included *Halodule wrightii*, *Ruppia* sp., *Thalassia testudinum*) contributed about 30%, and the remaining large range of contribution (20 to 70%) came from terrestrial sources such as mangroves. Variation of the different carbon source contributions within sampled sites was attributed to anthropogenic influences (such as coastal development) and climatic fluctuations, which all had a negative impact on carbon accumulation in the sediment as they directly impact vegetation health and distribution, and sedimentary processes (Gonneea et al. 2004). For example, in the healthiest seagrass environment,

seagrass contribution was the largest compared to other sites experiencing anthropogenic impact (Gonneea et al. 2004)

The sources of carbon accumulating in seagrass meadows are dictated by the environment, potential carbon sources, and seagrass density (Gacia et al. 1999; Gacia and Duarte 2001; Hansen and Reidenbach 2012). Nevertheless, there is currently no published literature regarding the sources of carbon accumulating in restored seagrass meadows and how the sources might change as the seagrass meadows age. Restored seagrasses accumulate carbon at a rate of $36.68 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the 10 years since seeding, and are projected to accumulate carbon in the 12 years since seeding at a rate of $47.06 \text{ g C m}^{-2} \text{ yr}^{-1}$ which is a similar rate to natural seagrass meadows (Greiner et al. in review). The goal of this study was to identify potential contributions from seagrass and non-seagrass carbon sources to a restored seagrass meadow, and to determine if the contribution concentrations change with seagrass meadow age and shoot density. Carbon sources in seagrass sediments were distinguished using ^{13}C and ^{15}N stable isotopes in a large-scale seagrass restoration in the Virginia coastal bays.

Methods

Site Description

Carbon source analyses were conducted at the Virginia Coast Reserve Long Term Ecological Research site (VCR-LTER) on the Eastern Shore of Virginia, located on the ocean side of the Delmarva Peninsula. Before the 1930's the VCR-LTER supported expansive seagrass meadows in the coastal bays; however, a wasting disease and

hurricane in 1933 led to the local extinction of seagrass in the region (Cottam 1934; Rasmussen 1977; Orth et al. 2006a). Now, the VCR-LTER is the location of a successful, large-scale restoration of *Zostera marina* (eelgrass) that was seeded via broadcasting over multiple years starting in 2001, with over 1700 ha of seagrass habitat restored by 2012 (McGlathery et al. 2012; Orth et al. 2012).

Sediment Sampling Methods

Sediment samples were collected from two adjacent locations in the VCR-LTER, Hog Island Bay (HI) (37° 24' 47" N, 75° 43' 36" W) and South Bay (SB) (37°, 15' 54" N, 75° 48' 50" W), both of which are shallow coastal bays with an approximate tidal range of 1.2 m (McGlathery et al. 2001; McGlathery et al. 2012). Past monitoring of these two coastal bays has determined that bathymetry, sediment, and water characteristics were all similar (McGlathery et al. 2012). In both sites, restoration was initiated by broadcasting seeds in replicate 0.4 ha plots with 100,000 seeds ha⁻¹. Sites were seeded in SB in 2001 and HI in 2007, representing 10-year and 4-year age treatments, respectively. Nearby unvegetated sediments were also sampled close to the restored seagrass as the 0-year age, or un-restored, reference treatments (SB Unveg and HI Unveg).

Sediment sampling occurred during the summer of 2011 between June and August, with the exception of a core taken in October 2011 at the SB unvegetated plot; differences in sediment characteristics between these sampling times are unlikely. Previous analyses of the sediment showed no significant difference in organic matter, carbon, and nitrogen content within 24 replicate cores at each age treatment (8 replicates

at SB Unveg; Greiner et al. in review). One 20-cm deep, 10-cm diameter core was taken for each age treatment and processed the same day. Each core was chosen from an analysis of sediment characteristics from multiple cores from the different age treatments in Greiner et al. (in review) to encompass the average trend of that age treatment.

Sediment cores were extruded and divided into 1.0 cm intervals, and large shells, rocks, and rhizomes were removed, keeping fine roots in the sediment. Subsamples were dried at 60 °C for 48 h, ground to homogenize, and analyzed for stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and %C, and %N. Samples were measured using an isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (Flagstaff, AZ). The carbon accumulation rate and sediment age of the 10-year treatment was determined using ^{210}Pb profiles and is reported in Greiner et al. (in review).

Carbon Source Methods

Vegetation samples were collected in the VCR-LTER and categorized into 3 different groups: seagrass, benthic microalgae, and macroalgae. The only seagrass species found at this location was eelgrass (Z), *Zostera marina*. Benthic microalgae (BMA) samples were comprised primarily of benthic diatoms from the top layer (1 cm) of sediment. Macroalgae (MA) samples were comprised of *Gracilaria vermicuphylla* ($n=8$), *Ulva lactuca* ($n=10$), *Codium fragile* ($n=4$), *Agardhiella subulata* ($n=3$), *Ectocarpus siliculosus* ($n=1$), *Enteromorpha flexuosa* ($n=2$), *Fucus vesiculosus* ($n=1$), *Polysiphonia nigrescens* ($n=2$), and *Scytosiphon lomentaria* ($n=2$). Grouping of MA samples was to provide variation in isotope values as a variety of these MA species could impact the VCR-LTER sediment.

Vegetation samples from the VCR-LTER were collected in November 2010, and February, April, June, July, and September in 2011 (Hondula 2012). Samples were processed by rinsing, drying at 60°C for 48 hours, and grinding the sample to homogenize. Subsamples were analyzed for stable isotopes at the Colorado Plateau Stable Isotope Laboratory (Flagstaff, AZ). Stable isotope ratios are reported as standard del (‰) notation relative to the international standards Peedee Belemnite (¹³C) and atmospheric N₂ (for ¹⁵N), and expressed as δ¹³C and δ¹⁵N so that,

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \times 10^3 \quad (1)$$

where X is ¹³C or ¹⁵N, and R is ¹³C/¹²C or ¹⁵N/¹⁴N of the sample or stand.

Mean and variance were calculated for the isotope ratios of each source (Z, BMA, and MA) from the VCR-LTER (Table 1). In addition, mean and variance of isotope ratios of each sediment treatment from the VCR-LTER (HI Unveg, 4-yr, SB Unveg, and 10-yr) were calculated for the top 10 cm, 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm of sediment (Table 1).

Mixing Model Methods

Using the stable isotope ratios, a 3-source (Z, BMA, and MA) 2-tracer (¹³C and ¹⁵N) Bayesian mixing model was used to quantify the fractional contribution of each source of organic matter to the sediment mixture in each sediment treatment (HI Unveg, 4-yr, SB Unveg, and 10-yr). Additional model runs were performed including *Spartina alterniflora*, the dominant marsh grass at this site, as another source but did not improve

the model results (see below). In addition, model runs were performed using the average of isotope values for BMA and seston samples measured at the VCR LTER (see below). Bayesian models allow the inclusion of multiple sources of uncertainty combined with prior information to determine the maximum likelihood of a solution, expressed as a posterior distribution (Moore and Semmens 2008; Semmens et al. 2009). Source end-member contributions to the sediment mixture were estimated in a Bayesian mixing model framework using the following equations:

$$\delta^{13}C_{sed} = (\phi_Z \times \delta^{13}C_Z) + (\phi_{BMA} \times \delta^{13}C_{BMA}) + (\phi_{MA} \times \delta^{13}C_{MA}) \quad (2)$$

$$\delta^{15}N_{sed} = (\phi_Z \times \delta^{15}N_Z) + (\phi_{BMA} \times \delta^{15}N_{BMA}) + (\phi_{MA} \times \delta^{15}N_{MA}) \quad (3)$$

$$1 = \phi_Z + \phi_{BMA} + \phi_{MA} \quad (4)$$

in which $\delta^{13}C$ and $\delta^{15}N$, were the measured stable isotope (^{13}C and ^{15}N) of the sediment mixture treatment (sed) and the isotope ratios of the sources (Z, BMA, and MA). ϕ denotes the proportional contribution of each source to the sediment mixture.

The mean and variance of source isotope ratios were gathered from the VCR-LTER values (described above) and used as informed priors in the mixing model. Sediment mixture isotope ratios were analyzed by combining the top 10 cm, or separately considering 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm depths. Source portion priors were center-log transformed (Semmens et al. 2009) and all calculations were done in JAGS (MCMC JAGS Project 2012) and R (R Development Core Team 2012).

Several additional Bayesian mixing models were run to confirm fractional source contribution results as well as test assumptions about potential contributing carbon sources other than the 3 sources considered. A 3-source, 2-tracer mixing model was run using both VCR-LTER alone (described above) and VCR-LTER and literature values averaged together (Appendix 2.1). In addition, a 4-source, 3-tracer Bayesian mixing model was run using the dominant marsh grass (SP), *Spartina alterniflora*, as an additional source, and all source C/N ratios were incorporated into the model as end members. For the 4-source, 3-tracer model and 3-source, 2-tracer model using literature isotope and C/N ratios, literature values were averaged with VCR-LTER data. Additional mixing model equations were applied to include SP source in stable isotope equations 2 and 3 and with the additional equations:

$$\begin{aligned} \left(\frac{C}{N}\right)_{sed} = & \left(\phi_Z \times \left(\frac{C}{N}\right)_Z\right) + \left(\phi_{SP} \times \left(\frac{C}{N}\right)_{SP}\right) + \left(\phi_{BMA} \times \left(\frac{C}{N}\right)_{BMA}\right) + \\ & \left(\phi_{MA} \times \left(\frac{C}{N}\right)_{MA}\right) \end{aligned} \quad (5)$$

$$1 = \phi_Z + \phi_{SP} + \phi_{BMA} + \phi_{MA} \quad (6)$$

where variables were the same as in previous mixing model and C/N ratio of the sediment mixture and of the sources (Z, SP, BMA, and MA). Lastly, a 3-source, 2-tracer Bayesian mixing model was performed for sources Z, MA, and an average mixture of seston particles and BMA (BMA/Seston). Sources were all collected from the VCR-LTER and analyzed using ^{13}C and ^{15}N stable isotopes. Results were analyzed using the same method as the previous 3-source, 2-tracer model. Isotope and C/N ratios for all

sediment treatments were determined for the top 10 cm, 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm depths; this is the depth range that previous work has determined to have been influenced by the seagrass restoration (Greiner et al. in review).

Statistical Analyses

Stable isotope values of different sediment treatments were analyzed with a 2-way analysis of variance (ANOVA) to determine within group variance and variance among the different treatments using SAS software (Version 9.2 of the SAS System for Windows, 2008, SAS Institute Inc.). Post hoc Ryan's Q tests were used to determine significant differences between each sediment mixture treatment (HI Unveg, 4-yr, SB Unveg, 10-yr) at different depth intervals (top 10 cm, 0 – 3 cm, 3 – 6 cm, 6 – 10 cm). In addition, mixing model carbon source portion posterior distributions were reported using median and quartile values (5th, 25th, 75th, and 95th). Percent differences of carbon source fraction were calculated between all mixing model source iterations using median values from each depth interval (0 – 3 cm, 3 – 6 cm, 6 – 10 cm).

Results

Sediment Profiles

Depth profiles of stable isotopes and C/N values in different sediment treatments had varying patterns. Both unvegetated sites (HI Unveg and SB Unveg) had significantly different $\delta^{13}\text{C}$ profiles, with the unvegetated HI site as the most depleted in $\delta^{13}\text{C}$ and with the largest differences between the two profiles found in the top 10 cm of sediment (Figure 2.1A). The 10-year age treatment was more depleted in ^{13}C relative to the 4-year

treatment to approximately 10 cm depth (Figure 2.1A). In the top 10 cm of sediment, there was a significant difference in average $\delta^{13}\text{C}$ (top 10 cm) between the seagrass sites and each neighboring unvegetated site ($F_{10,10,10,10} = 19.65$, $p < .0001$), but no significant difference between the 10-year and 4-year treatments (Table 2.1). Similar $\delta^{15}\text{N}$ down-core profiles (Figure 2.1C) resulted in only a significant difference in average $\delta^{15}\text{N}$ (top 10 cm) of vegetated and unvegetated treatments between South Bay and Hog Island Bay ($F_{10,10,10,10} = 11.16$, $p < .0001$) (Table 2.1).

Carbon content (%C) profiles were consistent, with the 10-year treatment having more %C in the top 6 cm of sediment, but dropping lower than the nearby unvegetated site below 10 cm depth (Figure 2.1D). Interestingly, the HI unvegetated treatment had more %C than the 4-year neighboring vegetated site throughout most of the core (Figure 2.1D). The %N profiles showed very similar patterns to the %C profiles, although applied as a ratio (C/N) there was larger variation and no pattern (Figure 2.1B), with only a significant difference in the average C/N (top 10 cm of sediment) between the 4-year treatment and all the other age treatments ($F_{10,10,10,10} = 15.42$, $p < .0001$) (Appendix 2.1).

Isotope Ratios

Ratio comparisons of the two tracers, $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, showed potential carbon source contributions to each sediment age treatment. Sediment treatments all had variable $\delta^{13}\text{C}$ values that covered a similar $\delta^{13}\text{C}$ range as the macroalgae (MA) sources (Figure 2.2). However, the $\delta^{15}\text{N}$ values for sediment were all very similar and showed sediment dominated by the seagrass (Z) and BMA (Figure 2.2). When the two tracers were combined, the values for the sediment mixtures were consistent with a large input of

carbon from Z and BMA, and less contribution from MA (Figure 2.2). None of the source isotope ratios overlap for both of the isotopes; therefore, there is a clear separation between Z, BMA, and MA with $\delta^{13}\text{C}$ and a clear separation of MA with $\delta^{15}\text{N}$.

In addition, when the BMA source were averaged with seston as a contributing carbon source, the $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ isotope comparison showed similar results but with more distinction between Z, BMA/Seston, and MA. The BMA/seston source isotope values were more depleted in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, resulting in the full inclusion of each sediment treatment within the expected bounds of the end-member sources (Figure 2.3). From these results, seagrass and seston were still the main contributors to the sediment carbon, but with more contribution from seagrass.

Mixing Model Results

In the top 10 cm of sediment, the seagrass contribution to the sediment carbon was 25% in the HI Unveg treatment (20% and 31% as 25th and 75th percentile respectively), 48% in the 4-year treatment (43% and 53% as 25th and 75th percentile respectively), 57% in the SB Unveg (51% and 63% as 25th and 75th percentile respectively), and 38% in the 10-year (33% and 43% as 25th and 75th percentile respectively) (Figure 2.4). BMA was also an important contributor to sediment carbon in each treatment with 71% in HI Unveg (65% and 77% as 25th and 75th percentile respectively), 49% in the 4-year (44% and 54% as 25th and 75th percentile respectively), 41% in SB Unveg (35% and 47% as 25th and 75th percentile respectively), and 60% in the 10-year (55% and 65% as 25th and 75th percentile respectively) (Figure 2.4). The

remaining contributing carbon source material was macroalgae, which was less than 2.5% in all sediment treatments (Figure 2.4).

When cores were divided into intervals of the top 3 cm, 3 – 6 cm, and 6 – 10 cm of each sediment age treatment, seagrass contributed the most to the top 3 cm of sediment and the relative contribution decreased with depth (Figure 2.5). By dividing the sediment into intervals, most of the sediment stable isotope ratios were significantly different among each treatment (Table 2.1). Interestingly, the SB Unveg treatment had the highest contribution of seagrass (81%) in the top 3 cm of sediment (Figure 2.5C). The HI Unveg treatment had the lowest contribution of seagrass (35%) in the top 3 cm of sediment as expected (Figure 2.5A). BMA carbon contributions typically increased with core depth; whereas macroalgae contributions were consistently low though out the core (Figure 2.5).

A 3-source, 2-tracer Bayesian mixing model was also run using literature values of carbon sources to complement the VCR-LTER data (Appendix 2.1). Differences in source values were minimal when comparing VCR-LTER specific data to VCR-LTER and literature combined data. Differences in mean VCR-LTER versus VCR-LTER combined with literature value for Z were the same with -10.07‰ and -10.06‰ $\delta^{13}\text{C}$ and 6.55‰ and 6.12‰ $\delta^{15}\text{N}$, BMA were -20.82‰ and -22.90‰ $\delta^{13}\text{C}$ and 6.04‰ and 7.12‰ $\delta^{15}\text{N}$ (VCR-LTER versus VCR-LTER combined with literature data respectively (Table 2.1 and Appendix 2.2). Average carbon source values for MA were -17.40‰ and -16.62‰ $\delta^{13}\text{C}$, and 8.53‰ and 9.47‰ $\delta^{15}\text{N}$ (VCR-LTER versus VCR-LTER combined with literature data respectively (Table 2.1 and Appendix 2.2). When literature values were added to VCR-LTER data, isotope value distributions increased; however, most

variances were still small except in $\delta^{15}\text{N}$ variances, which did not change or benefit informed priors for the model runs (Table 2.1 and Appendix 2.2). Variances were on the same order of magnitude expect for Z $\delta^{15}\text{N}$ (0.47 and 5.32 for VCR-LTER only and VCR-LTER and literature data respectively), and BMA $\delta^{15}\text{N}$ (0.48 and 2.90 for VCR-LTER only and VCR-LTER and literature data respectively) (Table 2.1 and Appendix 2.2).

There were minimal differences between the two 3-source, 2-tracer models comparing VCR-LTER values to literature and VCR-LTER values in terms of patterns down core (Table 2.2). The largest differences were seen when comparing MA source contribution, where in all 0 – 3 cm depth profiles there was a 100% increase when literature values were used; however, the contribution of MA was still minimal (2.5 – 5%) compared to the overall source contributions of Z and BMA (Table 2.2). There were also some changes in fraction contribution patterns particularly in the HI Unveg treatment where Z contributions remained similar when literature values were used instead of decreasing in depth when just VCR-LTER values were used (Figure 2.5 and Figure 2.6). In HI Unveg, this changed Z source contribution from 34.7% to 45% (VCR-LTER data and VCR-LTER and literature data respectively); however, both values fall within each other's 25th and 75th percentile (Appendix 2.8 – 2.10).

In addition, a 4-source, 3-tracer Bayesian mixing model was applied using VCR-LTER data with the supported literature values (Appendix 2.1). Marsh grass (SP) was added to the model as the additional source, and C/N ratio for the additional tracer. The 4-source, 3-tracer model and the 3-source, 2-tracer model using both VCR-LTER and

literature values showed very similar patterns down core with minimal changes in fraction contribution (Table 2.2, Figure 2.6, and Figure 2.7). Percent differences of MA between these two models at HI Unveg were 26%, 21%, and 7% (interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm respectively), 4-year were 43%, 29%, and 20% (interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm respectively), SB Unveg were 20%, 17%, and 1% (interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm respectively), and 10-year treatment were 15%, 10%, and 32% (interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm respectively) (Table 2.2). Although there were differences in MA, the source contributions of MA to the sediment carbon were all very low between less than 1% to 8% contributions (Table 2.2). In addition, there were few differences and similar patterns between the 4-source, 3-tracer model versus the 3-source, 2-tracer model using just VCR-LTER data (Table 2.2, Figure 2.5, and Figure 2.7). There was little change in seagrass contribution especially in the top 3cm as Z contribution differences were 17%, 3%, 3%, and 12% (HI Unveg, 4-year, SB Unveg, and 10-year respectively) (Table 2.2). Differences were seen in MA contribution and BMA especially in the top 3cm as BMA contribution differences were 26%, 29%, 55%, and 27% (HI Unveg, 4-year, SB Unveg, and 10-year respectively) (Table 2.2).

Lastly, when the 3-source, 2-tracer mixing models from the VCR-LTER specific for Z, BMA, and MA sources were compared to the model with Z, BMA/Seston, and MA sources, patterns of carbon source contributions were very similar. When BMA and seston were combined as a source, the fraction carbon contribution of seagrass increased throughout all treatments and depths. Percent differences between these two models in

the top 3 cm of sediment for seagrass between the two model runs were 29%, 14%, 3%, and 22% (HI Unveg, 4-year, SB Unveg, and 10-year respectively), where there was always an increase in seagrass contribution to the sediment in the BMA/Seston model (Table 2.3). Percent differences in BMA and BMA/Seston contributions were consistently high and for the top 3 cm of sediment between the two models runs were 22%, 23%, 15%, and 21% (HI Unveg, 4-year, SB Unveg, and 10-year respectively) where there was always a decrease in BMA or BMA/Seston contribution to the sediment in the BMA/Seston model (Table 2.3). Lastly, percent differences between the two models in terms of MA carbon contribution to the top 3 cm of sediment were 24%, 40%, 5%, and 7% (HI Unveg, 4-year, SB Unveg, and 10-year respectively) where a small decrease in MA contribution occurred in the BMA/Seston model except in the 4-year treatment (Table 2.3); however, MA contributions to the sediment carbon were minimal in both model runs.

Discussion

Natural seagrass meadows accumulate carbon effectively and remain in the seagrass sediment for long periods of time. Previous research at the VCR-LTER has shown that the 10-year restored seagrass treatment accumulated carbon at a rate of $36.7 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Greiner et al. in review), and that by 12 years after initial seeding the meadow was projected to accumulate carbon at rates similar to other natural seagrass meadows ($45 - 190 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Mcleod et al. 2011)). In this study, we identify the main contributing carbon sources to the restored seagrass sediment and document for the first

time that seagrass and benthic macroalgae together contributed greater than 95% of sediment carbon.

Seagrass and benthic microalgae were the dominant contributors to sediment carbon in all age treatments. In the top 10 cm of sediment from each age treatment, seagrass contributed between 25 to 57%, with the remaining carbon contribution coming from primarily BMA which contributed between 41 to 71% (Figure 2.4). These ranges of the contribution of seagrass to sediment carbon were similar to reported literature values of 29% (Thayer et al. 1987) and 50% (Kennedy et al. 2010). In addition, BMA has been observed as a main carbon source contributor in food web analyses in seagrass systems (Moncreiff and Sullivan 2001). Benthic microalgae contribution to sediment can increase with seagrass presence as a result of the seagrass canopy trapping BMA from outside the system (seston) and depositing that material on the benthic floor (Gacia and Duarte 2001; Mateo et al. 2006). The production of BMA carbon sources can also originate in situ of both seagrass and unvegetated habitats, which explains why BMA contribution in seagrass treatments were similar to unvegetated treatments (Middelburg et al. 2000; Mateo et al. 2006). In addition, the small contribution of macroalgae to sediment carbon across all age treatments (2.2% to 0.8%) suggests that there was a consistent impact of macroalgae into the seagrass meadows or neighboring unvegetated sediment.

Seagrass and BMA carbon contributions to lagoon sediment were important in both seagrass and unvegetated habitats. The comparison of Hog Island unvegetated and vegetated (4-yr) treatments were consistent with expectations that the seagrass contribution would be less in unvegetated versus vegetated sediments (Figure 2.5A and

2.5B). The carbon from unvegetated sediments came primarily from BMA, which typically inhabit the surface and down-core sediment. The BMA profiles often increased with depth, which was likely the result of mixing within the sediment as well as BMA within the sediment (living and dead), as BMA has been measured at depths up to 10 cm depth as a result of light attenuation (living BMA) and shallow mixing (dead BMA) (Middleburg et al. 2000; Rheuban 2013). Therefore, there is no way to determine if BMA, and also MA carbon, was produced in situ or transported to the seagrass meadow via seston or export (Middleburg et al. 2000; Mateo et al. 2006). In South Bay, the unvegetated site had a greater carbon contribution from seagrass than the 10-year vegetated site (Figure 2.5C and 2.5D). This may be the result of seagrass exported via wrack to nearby unvegetated areas, from seagrass die off of the dense and expanding 10-year seagrass meadows (McGlathery et al. 2012; Orth et al. 2012). Seagrass wrack exports from meadows can vary as much as 0 to 100% of total production, especially with slow seagrass decomposition (Hemminga and Duarte 2000; Mateo et al. 2006). However, organic matter and carbon content of the top 10 cm of the unvegetated sediment was significantly different from the 10-year treatment (1.39% and 1.94% organic matter, and 0.36% and 0.52% carbon in SB Unveg and 10-year respectively; Greiner et al. in review), which does not support the hypothesis of a large seagrass wrack input.

Restored seagrass took 4 years before rapid increases in seagrass density occurred (McGlathery et al. 2012), and about 5 years before the seagrass accumulated carbon (Greiner et al. in review). Seagrass contribution to sediment carbon was higher in the 4-

year treatment compared to the 10-year treatment throughout the entire core (Figure 2.5B and 2.5D). The increased seagrass carbon contribution in the 4-year treatment was consistent with dating results indicating that 5 years after restoration, an increase in carbon accumulation was from a low carbon source (Greiner et al. in review). It was hypothesized that the seagrass meadows changed carbon sources from carbon rich (enriched $\delta^{13}\text{C}$) seagrass leaves to low carbon (depleted $\delta^{13}\text{C}$) BMA sources (Table 2.1; Greiner et al. in review). The ability for the 10-year meadows to filter particles from the water column is greater because the seagrass densities for the two vegetated treatments (4-year and 10-year) were significantly different with the 4-year meadows having an average of 123.2 shoots m^{-2} and the 10-year meadows with an average of 428.7 shoots m^{-2} (Greiner et al. in review). Therefore, the 10-year meadows were more likely to have the sediment carbon comprised of BMA (in the seston) trapped by the seagrass.

Carbon source analysis, combined with sedimentation rates, provides a historical timeline of how the sediment carbon changed over time. Based on previous dating of VCR-LTER sediment, the top 3 cm of the sediment reflected the 10 years since the seeding initiated seagrass colonization at this site (Greiner et al. in review) (Figure 2.5D). The next interval of 3 – 6 cm covers 10 – 40 years and the bottom 4 cm (6 – 10 cm) is associated with approximately 40 – 100 years pre-restoration (Greiner et al. in review) (Figure 2.5D). There was a slight increase in seagrass carbon contribution from the middle 3 – 6 cm depth to the top 3 cm, potentially showing some impact in the surface sediment from the seagrass restoration (Figure 2.5). The effects of seagrass restoration on sediment carbon can be identified by combining source contributions and sedimentation

rates. Although results indicated that from the 10-year treatment there was little change in source contributions over time (Figure 2.5D), the lack of change with depth could have been the result of previous surface mixing that occurred in the sediment at deeper depths when seagrass was not present.

Based on the similar patterns across all models, VCR specific data was most informative for source analysis because of this unique restoration study. Therefore, there was validation that carbon sources were attributed appropriately by adding sources, tracers, and variable data, because the contribution of marsh grass to the sediment carbon was negligible resulting in little change in results when omitted. To determine if carbon source isotope variation impacted Bayesian mixing model results, models were run using VCR-LTER specific results and VCR-LTER and reported literature values of the same species. Literature values along with VCR-LTER results were used in a 3-source, 2-tracer and 4-source, 3-tracer Bayesian mixing model, where carbon contribution patterns were similar to the 3-source, 2-tracer VCR-LTER model (Figure 2.5 – 2.7). Carbon contribution patterns concerning the dominant source contributor were similar between all models; except for HI Unveg, which switched from seagrass contribution decreasing with depth to it remaining more constant down core when literature values were included both in the 4-source, 3-tracer model and 3-source, 2-tracer model (Figure 2.5 – 2.7). There were differences in contributions to sediment carbon by MA; however, because MA contributions were small (<5%), changes in MA contributions had little impact on the remaining source contributions. In addition, marsh grass (SP) was omitted from analyses due to minimal source contribution (median 0.01 - 0.02%) to the sediment in all

treatments as confirmed in the 4-source, 3-tracer mixing model using both VCR-LTER and literature values (Figure 2.7). C/N ratios were highly variable within sediment cores (Figure 2.1B) resulting in little differentiation between sediment C/N ratios. Marsh grass was identified using 2 isotope comparisons as a small contributor to sediment carbon as C/N ratios significantly separated it from all sediment treatments (Appendix 2.4B and 2.4C). Similar patterns concerning marsh grass contributions have been seen as a result of the sediment treatments being too far away from the marshes, or because the marsh grass rarely would get mixed in the shallow lagoon sediment (Gonneea et al. 2004; Hemminga et al. 1994).

Isotope ratios of each sediment treatment were very similar to each other when compared to end-member sources. As a result, both end-member isotopes were important for determining source contributions. Using more tracers does allow for more prior information to be incorporated into the mixing model; however, they can overestimate source contributions when included in a model and are insignificant. Another potential carbon source that may be important in shallow coastal system is phytoplankton, but we chose not to consider this source as phytoplankton concentrations estimated by water column chlorophyll-*a* concentrations are very low at the VCR-LTER (McGlathery et al. 2007). However, a final Bayesian mixing model was performed to assess the importance of seston, which included phytoplankton as well as suspended benthic microalgae, to the sediment carbon the restored seagrass. Mixing model results indicated very little change in patterns of source contribution to all treatments down core (Table 2.3, Figure 2.8). When BMA and seston was used as a source, more of the carbon in the sediment was

indicated to come from seagrass over BMA/Seston; however the changes were small and no larger than increasing or decreasing by 12% contribution between model runs for seagrass and BMA/Seston (Table 2.3, Figure 2.8). In addition, there was little variation around median values of the fraction of contributions for each source (Figure 2.9). The minimal differences in BMA and seston contributions indicate that most of the carbon in the sediment coming from microalgae sources was from benthic microalgae.

The seagrass isotopic carbon and nitrogen value, as well as values of other vegetative sources, can be variable depending on which parts (epiphytes, leaves, roots, or rhizomes) of the plant are analyzed and when the vegetation sample was taken. There has been some debate over whether to include epiphytes in seagrass isotope analysis. Epiphytes typically enriched the isotopic value for $\delta^{13}\text{C}$ by about 2‰ (Kennedy et al. 2010), yet others found that epiphytes were more depleted in $\delta^{13}\text{C}$ by about 4.5‰ (Moncreiff and Sullivan 2001) or 4.2‰ (Thayer et al. 1987). Seagrass roots and rhizomes are also important source material that can potentially result in changes to the seagrass isotopic value attributed to slower decompositions rates of rhizome material (Fourqurean and Schlau 2003). However, Padadimitriou et al. (2005a) found no significant difference between the leaves and rhizomes of seagrass in stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ because site variation was insignificant compared to spatial variations between samples. Variation in carbon source contributions can also be the result of isotopic variation due to seasonal and temporal changes in the source materials. Seasonal differences in isotopic values of source materials vary from -10.6‰ $\delta^{13}\text{C}$ in the fall and winter to -9.6‰ $\delta^{13}\text{C}$ in the spring and summer (Thayer et al. 1978). However, temporal variations were significantly

different than spatial variations in isotope and C/N ratios measured in *Zostera marina* (Papadimitriou et al. 2005b). Intra-annual variation was 3.6‰ $\delta^{13}\text{C}$ in *Thalassia testudinum* and 4.1‰ $\delta^{13}\text{C}$ in *Zostera noltii*; however, these differences were not significant enough to influence end-member source contributions to the evaluated mixtures (Fourqurean et al. 2005). Carbon sources should be measured year around to include seasonal differences, and changes between belowground and aboveground biomass for seagrass (Orth and Moore 1986; Gacia et al. 2002; Kennedy et al. 2010).

In addition, stable isotope values down core can be impacted to some extent by diagenesis. The different conclusions on the impact of diagenesis on stable isotopes in marine sediments are due to variations in substrate, anoxic/oxic sediment, and source materials (Freudenthal et al. 2001). Due to the low organic matter content in the VCR-LTER, previous studies have indicated that stable isotope ratios do not change significantly as a result of diagenesis (Meyers and Ishiwatari, 1993; Gonnee et al. 2004). In addition, the largest changes in stable isotope values due to diagenesis have been measured for $\delta^{15}\text{N}$ in sediment as a result of deamination (Macko and Estep, 1984). However, down-core profiles of $\delta^{15}\text{N}$ show small variation of 1.5‰ (Figure 2.1C). This was consistent with other studies that measured small variation in $\delta^{15}\text{N}$ down-core and attributed this to diagenic changes occurring to the organic matter before sedimentation (Mayers and Ishiwatari, 1993). Finally, any diagenic influence on organic matter would have resulted in large variations in stable isotope values. However, when literature values, which increase stable isotope values variation, were included in Bayesian mixing

model runs, there were small changes in source contribution patterns to the sediment carbon.

Combining carbon accumulation rates with carbon source partitioning provides essential information on coastal carbon cycling processes. From a previous study, the carbon accumulation rate in the 10-year meadows in the VCR-LTER was estimated at $36.7 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Greiner et al. in review). For the first time, by combining the carbon accumulation rate with carbon source contributions in the top 3 cm of sediment, the restored 10-year seagrass sediment accumulated about $15.41 \text{ g C m}^{-2} \text{ yr}^{-1}$ from seagrass and the remaining $20.86 \text{ g C m}^{-2} \text{ yr}^{-1}$ from non-seagrass sources (primarily BMA). Therefore, after 10 years the restored seagrass accumulated carbon from both seagrass and non-seagrass materials confirming that restored seagrass meadows are an important carbon sink for the system as a whole.

Literature Cited

- Bouillon, S., and H. T. S. Boschker. 2006. Bacterial carbon sources in coastal sediments: a cross-system analysis based on stable isotope data of biomarkers. *Biogeosciences*. **3**: 175 – 185.
- Cottam, C. 1934. Past periods of eelgrass scarcity. *Rhodora*. **36**: 261 – 264.
- Duarte, C. M., H. Kennedy, N. Marbe, and I. Hendricks. 2011. Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. *Ocean Coast. Manage.* **51**: 671 – 688, doi:10.1016/j.ocecoaman.2011.09.001
- Duarte, C. M., J. Borum, F. T. Short, and D. I. Walker. 2005a. Seagrass ecosystems: their global status and prospects, p. 281 – 294. *In* N.V.C. Polunin (Ed.), *Aquatic Ecosystems: Trends and Global Prospects*.
- Duarte, C. M., and J. Cebrian. 1996. The fate of marine autotrophic production. *Limnology and Oceanography*. **41**: 1758 – 1766.
- Duarte, C. M., J. J. Middelburg, and N. Caraco. 2005b. Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*. **2**: 1 – 8, doi:1726-4189/bg/2005-2-1
- Duarte, C. M., N. Marba, E. Gacia, J. W. Fourqurean, J. Beggins, C. Barron, and E. T. Apostolaki. 2010. Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles*. **24**: GB4032, doi:10.1029/2010GB003793
- Fourqurean, J. W., and J. E. Schlau. 2003. Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and mangrove leaves along

a nutrient availability gradient in Florida Bay, USA. *Chemistry and Ecology*. **19**: 373 – 390.

Fourqurean, J. W., S. P. Escorcia, W. T. Anderson, and J. C. Ziemann. 2005. Spatial and seasonal variability in elemental content, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of *Thalassia testudinum* from South Florida and its implications for ecosystem studies. *Estuaries*. **28**: 447 – 461.

Fourqurean, J. W., C. M. Duarte, H. Kennedy, N. Marba, M. Holmer, M. A. Mateo, E. T. Apostolaki, G. A. Kendrick, D. Krause-Jensen, K. J. McGlathery, and O. Serrano. 2012. Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*. doi:10.1038/NGEO1477

Freudenthal, T., T. Wagner, F. Wenzhofer, M. Zabel, and G. Wefer. 2001. Early diagenesis of organic matter from sediments of the eastern subtropical Atlantic: evidence from stable nitrogen and carbon isotopes. *Geochimica et Cosmochimica Acta*. **65**: 1795 – 1808.

Fry, B. 1977. Stable carbon isotope evidence for two sources of organic matter in coastal sediments: seagrasses and plankton. *Geochimica et Cosmochimica Acta*. **41**: 1875.

Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York, NY.

Fry, B. and E. B. Sherr. 1984. $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science*. **27**: 13 – 48.

- Gacia, E., C. M. Duarte, and J. J. Middelburg. 2002. Carbon and nutrients deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. *Limnol. Oceanogr.* **47**: 23 – 32.
- Gacia, E., and C. M. Duarte. 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuar. Coast. Shelf Sci.* **52**: 505 – 514.
- Gacia, E., T. C. Granata, and C. M. Duarte. 1999. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquat. Bot.* **65**: 255 – 268.
- Greiner, J. T., K. J. McGlathery, J. Gunnell, and B. A. McKee. Seagrass restoration enhances “blue carbon” sequestration in coastal waters. *PLoS ONE*. In review.
- Gonneea, M. E., A. Paytan, and J. A. Herrera-Silveira. 2004. Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. *Estuar. Coast. Shelf Sci.* **61**: 211 – 227.
- Hansen, J. C. R., and M. A. Reidenbach. 2012. Wave and tidally driven flows within *Zostera marina* seagrass beds and their impact on sediment suspension. *Mar. Ecol. Prog. Ser.* **448**: 271 – 287, doi:10.3354/meps09225
- Harrison, P. G. 1989. Detrital processing in seagrass systems: a review of factors affecting decay rates, remineralization and detritivory. *Aquatic Botany.* **23**: 263 – 288.
- Hemminga, M. A., and C. M. Duarte. 2000. *Seagrass Ecology*. Cambridge, United Kingdom. Cambridge University Press.

- Hemming, M. A., F. J. Slim, J. Kazungu, G. M. Ganssen, J. Nieuwenhuize, and N. M. Kruyt. 1994. Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). *Mar Ecol Prog Ser.* **106**: 291 – 301.
- Hondula, K. L., and M. L. Pace. 2012. Macroalgal support of cultured hard clams in a low nitrogen coastal lagoon. *Mar Ecol Prog Ser.* In review.
- Kennedy, H., J. Beggins, C. M. Duarte, J. W. Fourqurean, M. Holmer, N. Marba, and J. J. Middelburg. 2010. Seagrass sediments as a global carbon sink: isotopic constraints. *Glob. Biogeochem. Cycles.* **24**: 1 – 8, doi:10.1029/2010GB003848
- Macko, S. A., and M. L. F. Estep. 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Org. Geochem.* **6**: 787 – 790.
- Mateo, M. A., J. Cebrain, K. Dunton, and T. Mutchler. 2006. Carbon flux in seagrass ecosystems, p. 159 – 192. *In* A. W. Larkum, R. J. Orth, and C. M. Duarte [eds.], *Seagrasses: biology, ecology and conservation.*
- McConnaughey, T., and C. P. McRoy. 1979. ^{13}C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. *Marine Biology.* **53**: 263 – 269.
- McGlathery, K. J., L. K. Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, and A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Mar. Ecol. Prog. Ser.* **448**: 209 – 221.
- McGlathery, K. J., K. Sundback, and I. C. Anderson. 2007. Eutrophication in the shallow coastal bays and lagoons: the role of plants in the coastal filter. *Mar. Ecol. Prog. Ser.* **348**: 1 – 18, doi:10.3354/meps07132

- McGlathery, K. J., I. C. Anderson, and A. C. Tyler. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. *Mar. Ecol. Prog. Ser.* **216**: 1 – 15.
- Mcleod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Bjork, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. Silliman. 2011. A blueprint for blue carbon: toward an improvement understanding of the role of vegetated coastal habitats in sequestering CO₂. *Frontiers in Ecological Environments.* **9**: 552 – 560, doi: 10.1890/110004
- Meyers, P. A., and R. Ishiwatari. 1993. Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* **20**: 867 – 900.
- Middelburg, J. J., C. Barranguet, H. T. S. Boschker, P. M. J. Herman, T. Moens, and C. H. R. Heip. 2000. The fate of intertidal microphytobenthos carbon: an in situ ¹³C-labeling study. *Limnol. Oceanogr.* **45**: 1224 – 1234.
- Middelburg, J. J., and J. Nieuwenhuize. 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. *Marine Chemistry.* **60**: 217 – 225.
- Moncreiff, C. A., and M. J. Sullivan. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Mar Ecol Prog Ser.* **215**: 93 – 106.
- Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters.* **11**: 470 – 480.

- Orem, W. H., C. W. Holmes, C. Kendall, H. E. Lerch, A. L. Bates, S. R. Silva, A. Boylan, M. Corum, M. Marot, and C. Hegman. 1999. Geochemistry of Florida Bay sediments: nutrient history at five sites in eastern and central Florida Bay. *Journal of Coastal Research*. **15**: 1055 – 1071.
- Orth, R. J., and K. A. Moore. 1986. Seasonal and year-to-year variations in the growth of *Zostera marina* L. (eelgrass) in the lower Chesapeake Bay. *Aquat. Bot.* **24**: 335 – 341.
- Orth, R. J., K. A. Moore, S. R. Marion, D. J. Wilcox, and D. B. Parrish. 2012. Seed addition facilitates eelgrass recovery in a coastal bay system. *Mar. Ecol. Prog. Ser.* **448**: 177 – 195, doi: 10.3354/meps09522
- Orth, R. J., M. L. Luckenbach, S. R. Marion, K. A. Moore, and D. J. Wilcox. 2006a. Seagrass recovery in the Delmarva Coastal Bays, USA. *Aquatic Botany*. **84**: 26 – 36.
- Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr., A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. L. Williams. 2006b. A global crisis for seagrass ecosystems. *BioScience*. **56**: 987 – 996.
- Papadimitriou, S., H. Kennedy, D. P. Kennedy, C. M. Duarte, and N. Marba. 2005a. Sources of organic matter in seagrass-colonized sediments: a stable isotope study of the silt and clay fraction from *Posidonia oceanica* meadows in the western Mediterranean. *Organic Geochemistry*. **36**: 949 – 961.

- Papadimitriou, S., H. Kennedy, D. P. Kennedy, and J. Borum. 2005b. Seasonal and spatial variation in the organic carbon and nitrogen concentration and their stable isotopic composition in *Zostera marina* (Denmark). *Limnology and Oceanography*. **50**: 1084 – 1095.
- Pendleton, L., D. C. Donato, B. C. Murray, S. Crooks, W. A. Jenkins, S. Sifleet, C. Craft, J. W. Fourqurean, J. B. Kauffman, N. Marba, P. Megonigal, E. Pidgeon, D. Herr, D. Gordon, and A. Baldera. 2012. Estimating global “blue carbon” emissions from conversion and degradation of vegetated coastal ecosystems. *PLoS ONE*. **7**: e43542. doi:10.1371/journal.pone.0043542
- Peterson, B. J., R. W. Howarth, and R. H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science*. **227**: 1361 – 1363.
- Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia*. **136**: 261 – 269.
- Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on the environmental factors and fauna p. 1 - 15. *In* C. P. McRoy and C. Helfferich [eds.], *Seagrass Ecosystems – a scientific perspective*. Marcel Dekker, New York, NY.
- Rheuban, J. E. 2013. Oxygen metabolism in restored eelgrass (*Zostera marina* L.) meadows measured by eddy correlation. M.S. thesis. University of Virginia.

- Semmens, B. X., E. J. Ward, J. W. Moore, and C. T. Darimont. 2009. Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. *PLoS One*. **4**: e6187.
- Thayer, G. W., P. L. Parker, M. W. LaCroix, and B. Fry. 1978. The stable carbon isotope ratio of some components of an eelgrass, *Zostera marina*, bed. *Oecologia*. **35**: 1 – 12.
- Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, Jr., A. R. Hughes, G. A. Kendrick, W. J. Kensworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrass across the globe threatens coastal ecosystems. *PNAS*. **106**: 12377 – 12381.
- Zieman, J. C., S. A. Macko, and A. L. Mills. 1984. Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bulletin of Marine Science*. **35**: 380 – 392.

Tables

Table 2.1: Carbon and nitrogen source and sediment averages (mean), standard deviations (\pm SD), and sample size (n) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values used in 3-source, 2-tracer VCR-LTER Bayesian mixing model

Source values were for samples collected at the VCR-LTER. Each sediment treatment (HI Unveg, 4-year, SB Unveg, and 10-year) was averaged for the top 10 cm, 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm of sediment. Carbon sources include *Zostera marina* (Z), benthic microalgae (BMA), and multiple macroalgae species (MA).

Source	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	\pm SD	Mean	\pm SD
Z	11	-10.07	0.43	6.55	0.68
BMA	9	-20.82	1.45	6.04	0.7
MA	33	-17.4	3.28	9.47	1.26
0 - 10 cm		Sediment			
HI Unveg	10	-17.74	0.76	5.98	0.31
4-year	10	-15.44	0.64	5.9	0.22
SB Unveg	10	-14.41	1.66	5.49	0.18
10-year	10	-16.49	0.64	5.57	0.16
0 - 3 cm		Sediment			
HI Unveg	3	-16.81	0.635	5.75	0.08
4-year	3	-15.09	0.24	6.09	0.31
SB Unveg	3	-12.2	0.75	5.54	0.23
10-year	3	-16.02	0.89	5.63	0.15
3 - 6 cm		Sediment			
HI Unveg	3	-17.82	0.22	5.84	0.08
4-year	3	-15.07	0.44	5.74	0.04
SB Unveg	3	-14.83	0.52	5.50	0.18
10-year	3	-17.01	0.43	5.63	0.09
6 - 10 cm		Sediment			
HI Unveg	4	-18.37	0.18	6.25	0.34
4-year	4	-15.98	0.62	5.87	0.15
SB Unveg	4	-15.75	0.45	5.44	0.19
10-year	4	-16.45	0.32	5.49	0.21

Table 2.2: Percent differences of carbon sources for multiple mixing models on each sediment treatment and individual depth interval.

Mixing model runs include the 3-source, 2-tracer model using just VCR-LTER data (3, 2 VCR), a 3-source, 2-trace model using both VCR-LTER data and literature values (3, 2 VCR + Lit), and a 4-source, 3-tracer model using both VCR-LTER data and literature values (4, 3 VCR + Lit). Depth intervals include 0 to 3 cm, 3 to 6 cm, and 6 to 10 cm for all sediment treatments (HI Unveg, 4-year, SB Unveg, 10-year). Carbon sources include *Zostera marina* (Z), benthic microalgae (BMA), and multiple macroalgae species (MA). Literature values are for same species found in VCR-LTER (Appendix 2.1).

Mixing Model Run	HI Unveg			4-year			SB Unveg			10-year		
	Z	BMA	MA	Z	BMA	MA	Z	BMA	MA	Z	BMA	MA
Depth: 0 cm to 3 cm; Percent Difference (%)												
3, 2 VCR vs. 3, 2 VCR + Lit	25	26	108	7	31	131	2	64	133	19	28	101
3, 2 VCR vs. 4, 3 VCR + Lit	17	26	88	3	29	102	3	55	122	12	27	90
3, 2 VCR + Lit vs. 4, 3 VCR + Lit	8	0	26	4	3	43	4	10	20	8	1	15
Depth: 3 cm to 6 cm; Percent Difference (%)												
3, 2 VCR vs. 3, 2 VCR + Lit	36	25	103	11	32	145	12	31	125	29	25	67
3, 2 VCR vs. 4, 3 VCR + Lit												76
3, 2 VCR + Lit vs. 4, 3 VCR + Lit	25	26	87	5	31	130	6	30	114	20	26	

3, 2 VCR + Lit vs. 4, 3 VCR + Lit	11	1	21	5	1	29	66	1	17	9	1	10
Depth: 6 cm to 10 cm; Percent Difference (%)												
3, 2 VCR vs. 3, 2 VCR + Lit	49	23	55	16	26	106	18	26	97	22	26	120
3, 2 VCR vs. 4, 3 VCR + Lit	34	25	49	6	29	120	11	27	98	15	26	98
3, 2 VCR + Lit vs. 4, 3 VCR + Lit	16	1	7	10	3	20	7	1	1	6	0	32

Table 2.3: Percent differences of carbon sources for mixing models using 3-source, 2-tracer models with varying sources on each sediment treatment and individual depth interval.

Mixing model runs include the 3-source, 2-tracer model using just VCR-LTER data for sources *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA); and a 3-source, 2-trace model using VCR-LTER data for sources Z, average of benthic microalgae and seston (BMA/Seston), and MA. Depth intervals include 0 to 3 cm, 3 to 6 cm, and 6 to 10 cm for all sediment treatments (HI Unveg, 4-year, SB Unveg, 10-year).

Depth	HI Unveg			4-year			SB Unveg			10-year		
	Z	BMA/ Seston	MA	Z	BMA/ Seston	MA	Z	BMA/ Seston	MA	Z	BMA/ Seston	MA
Percent Difference (%)												
0 - 3 cm	29	22	24	14	23	40	3	15	5	22	21	7
3 - 6 cm	44	23	19	15	21	11	14	21	20	32	21	13
6 - 10 cm	58	25	37	20	21	32	19	20	13	25	21	5

Figures

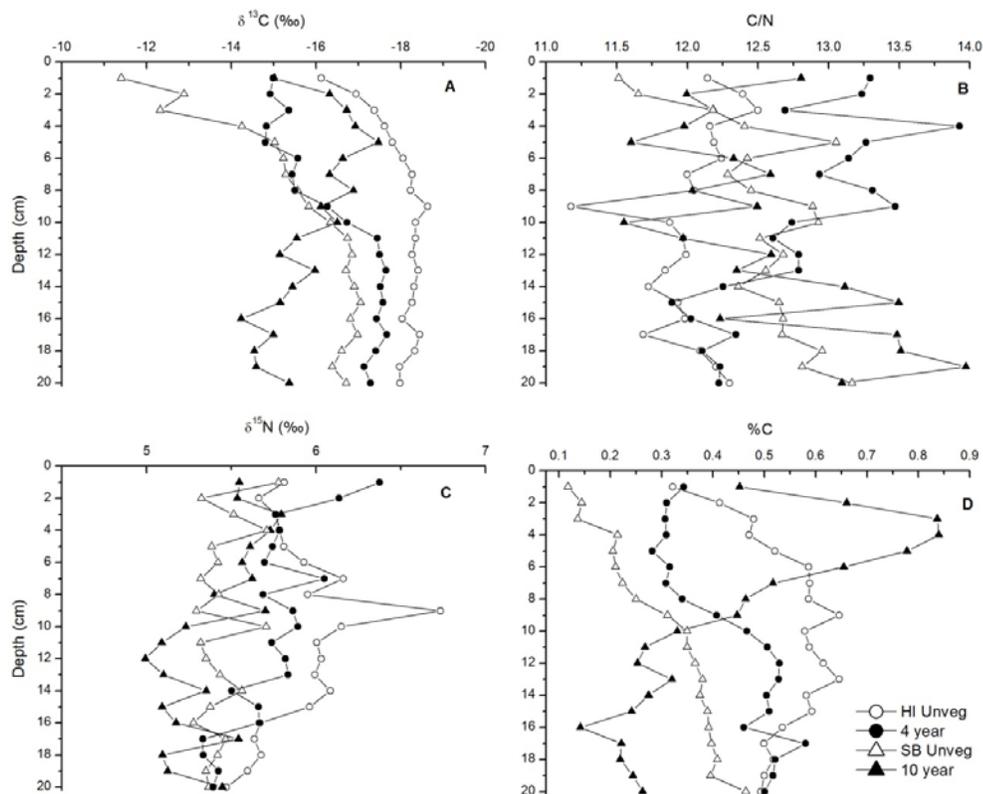


Figure 2.1: Vertical down-core profiles of sediment characteristics.

A) Stable isotope ^{13}C ($\delta^{13}\text{C}$ (‰)); **B)** ratio of molar carbon to nitrogen (C/N); **C)** stable isotope ^{15}N ($\delta^{15}\text{N}$ (‰)); **D)** percent organic carbon (%C), for 4 different age treatments (HI Unveg, 4 year, SB Unveg, and 10 year) in top 20 cm of sediment from 1-cm intervals.

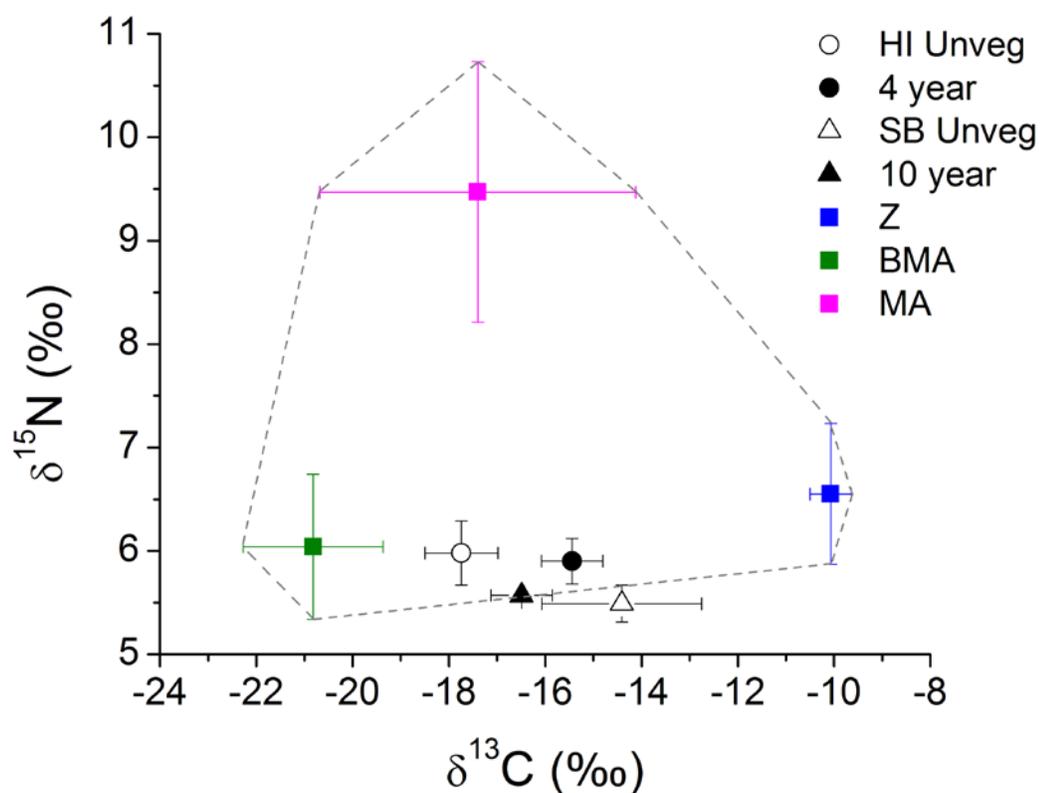


Figure 2.2: $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ ratio comparison between average sources (Z, BMA, and MA) and average sediment treatments.

Average end-member sources from VCR-LTER include *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA), noted as colored squares, and averages from the 4 different age treatments (HI Unveg, 4 year, SB Unveg, and 10 year) from the top 10 cm of sediment noted as filled (restored seagrass) and no filled (unvegetated) circles (HI) and triangles (SB). Error bars are standard deviations. Dashed grey line indicates end-member source bounds for sediment mixture, with expectations of finding sediment treatments within grey lines.

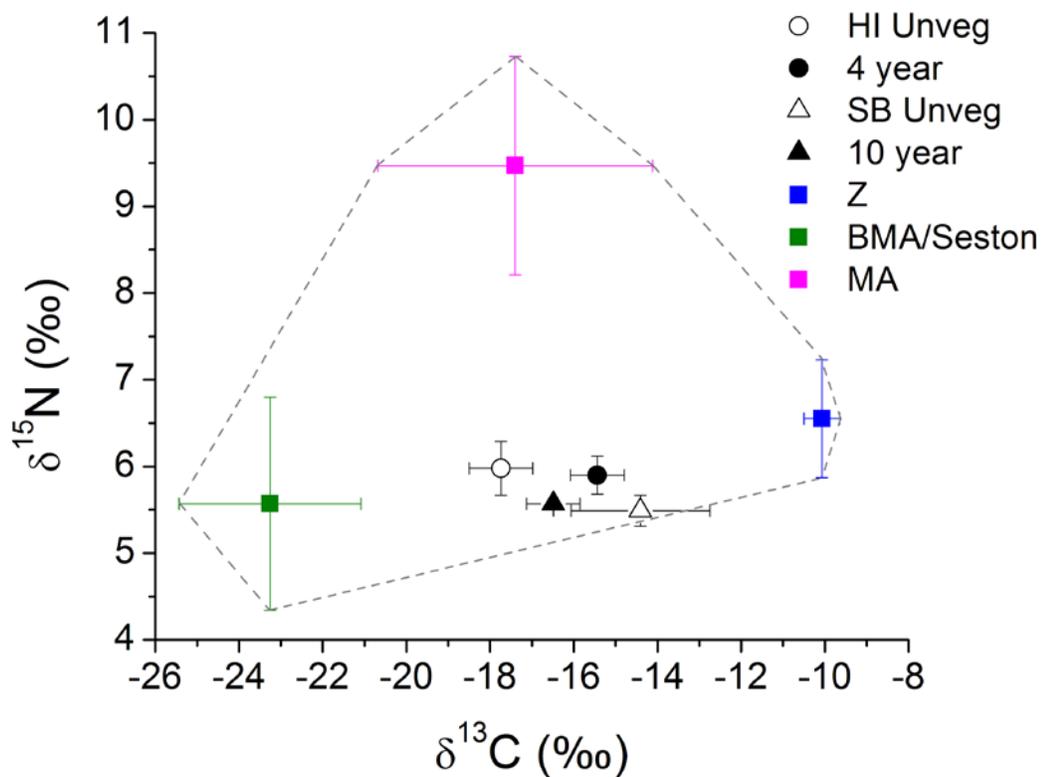


Figure 2.3: $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ ratio comparison between average sources (Z, BMA/Seston, MA) and average sediment treatments.

Average end-member sources from VCR-LTER include *Zostera marina* (Z), BMA and seston (BMA/Seston), and macroalgae (MA), noted as colored squares, and averages from the 4 different age treatments (HI Unveg, 4 year, SB Unveg, and 10 year) from the top 10 cm of sediment noted as filled (restored seagrass) and no filled (unvegetated) circles (HI) and triangles (SB). Error bars are standard deviations. Dashed grey line indicates end-member source bounds for sediment mixture, with expectations of finding sediment treatments within grey lines.

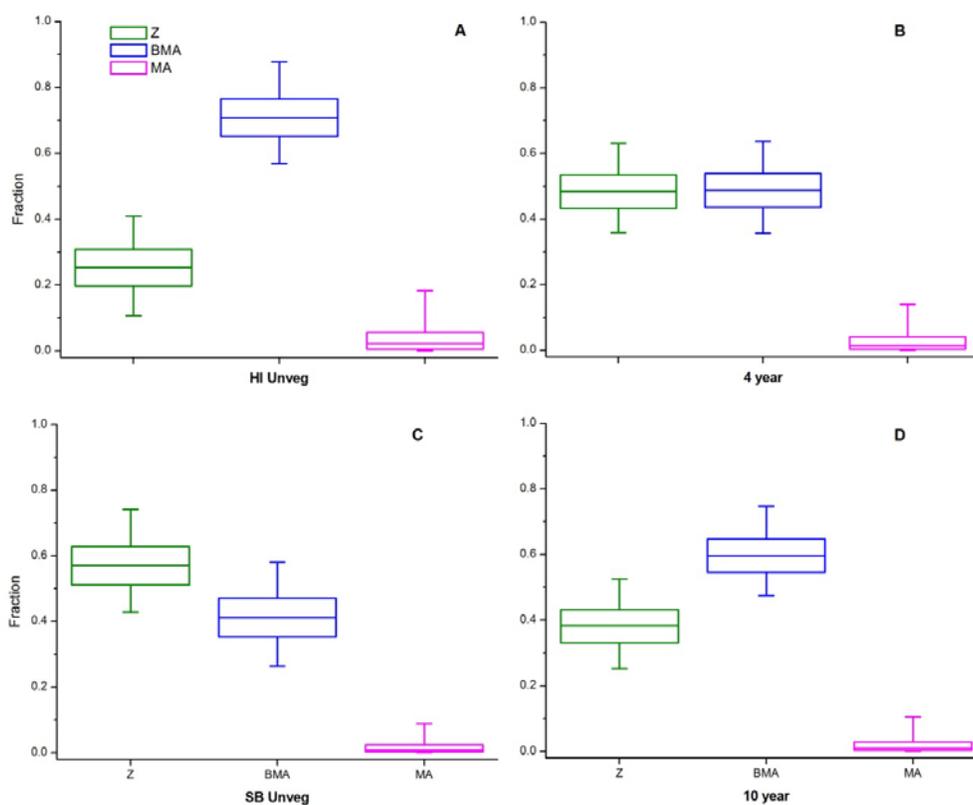


Figure 2.4: Box plots showing quartile 5th, 25th, 50th, 75th, and 95th percentile of 3-source, 2-tracer VCR-LTER Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment).

Carbon sources are *Zostera marina* (Zostera), benthic microalgae (BMA), and macroalgae (MA). **A:** unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year).

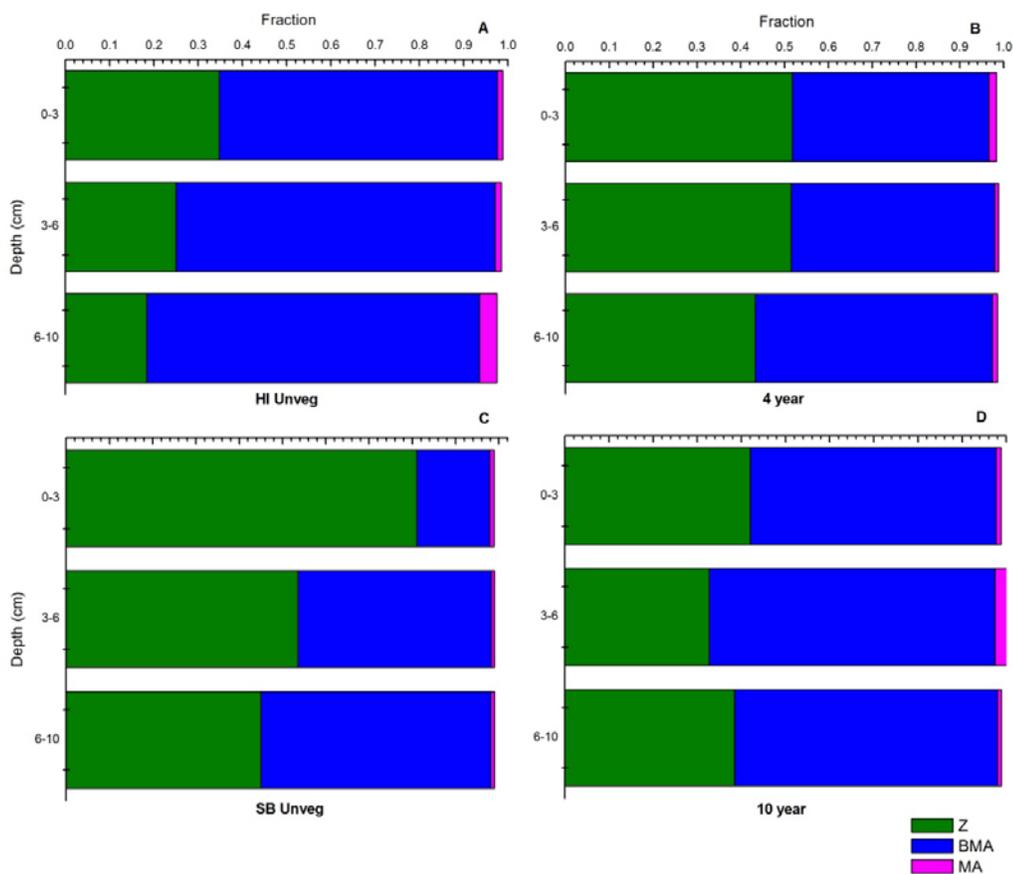


Figure 2.5: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).

Carbon sources are *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA) from the VCR-LTER. **A:** unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year).

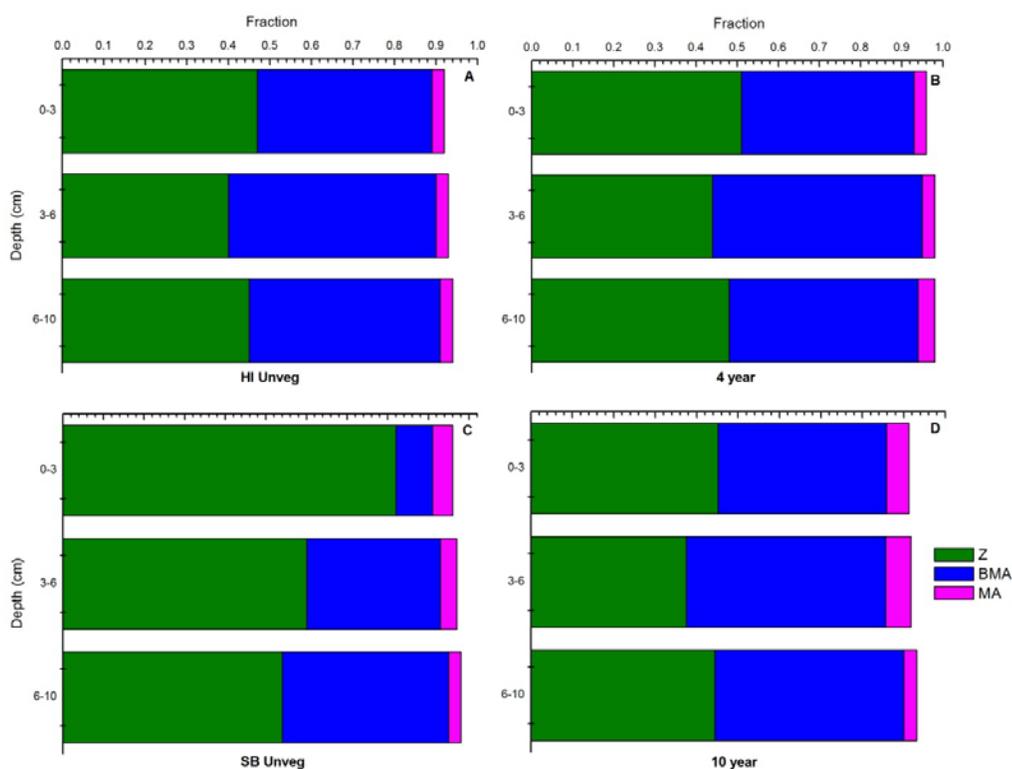


Figure 2.6: Three-source, two-tracer VCR-LTER and literature value Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).

Carbon sources are *Zostera marina* (*Z*), benthic microalgae (BMA), and macroalgae (MA) are from VCR-LTER and literature values (Appendix 2.1). **A:** unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year).

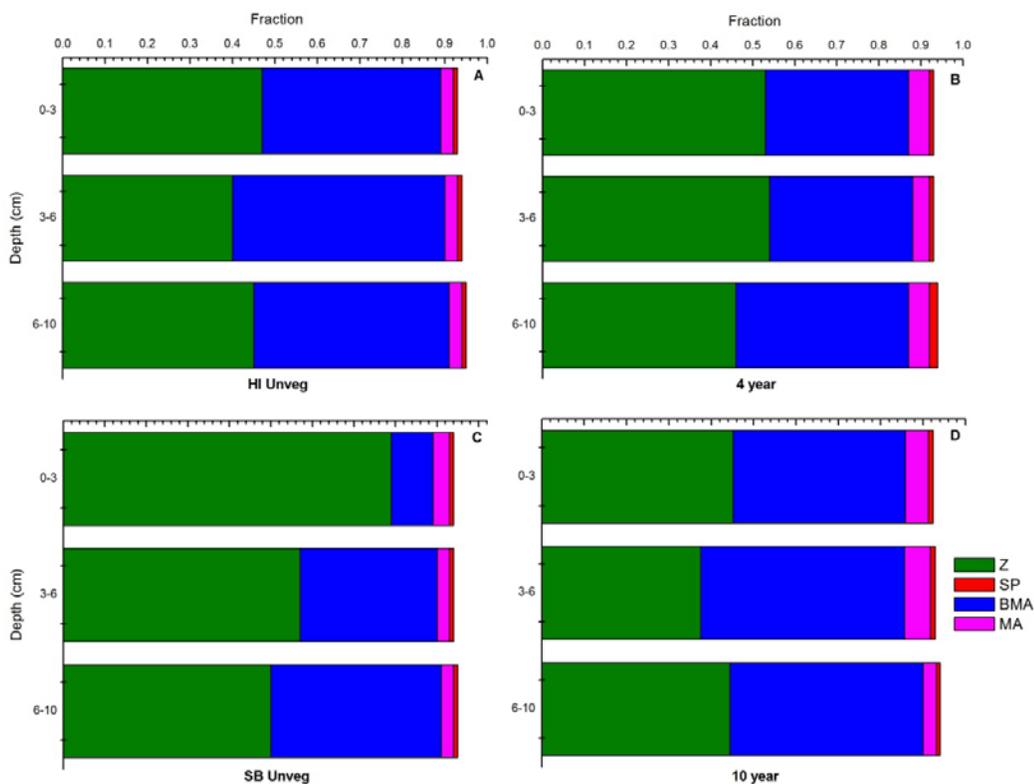


Figure 2.7: Four-source, three-tracer LTER-VCR and literature value Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals (0 – 3 cm, 3 – 6 cm, and 6 – 10 cm).

Carbon sources are *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), and macroalgae (MA) are from VCR-LTER and literature values (Appendix 2.1). **A:** unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year).

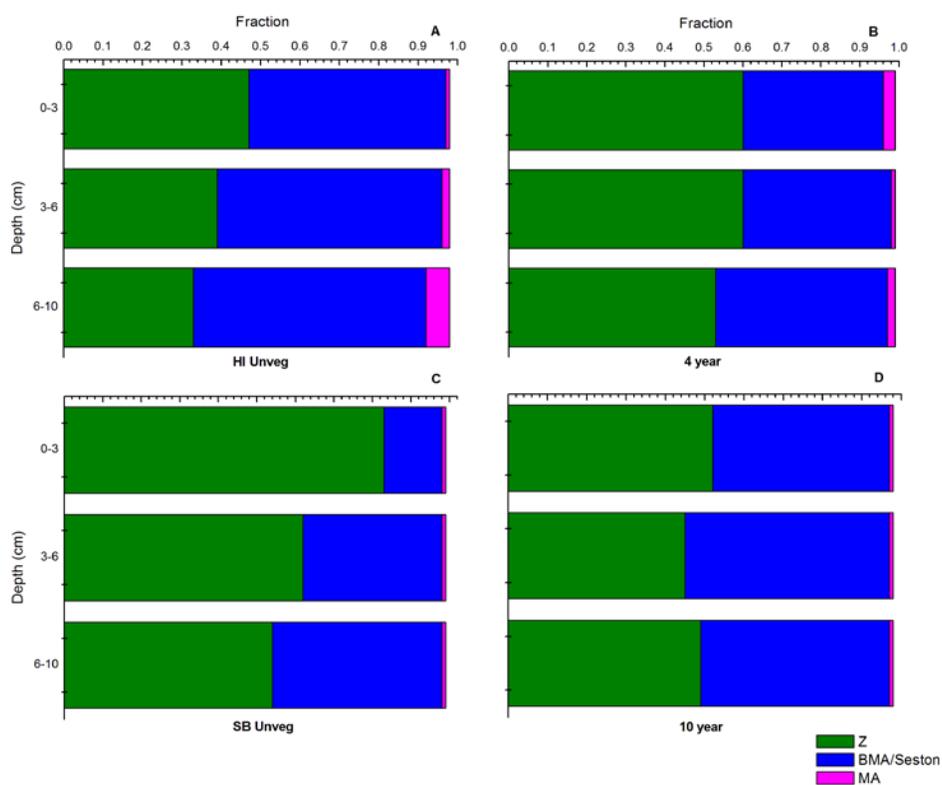


Figure 2.8: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contributions to each sediment treatment in down-core intervals. Carbon sources are *Zostera marina* (Z), BMA and Seston (BMA/Seston), and macroalgae (MA) from the VCR-LTER.

A: unvegetated sediment in Hog Island Bay (HI Unveg), B: 4-year restored seagrass treatment in Hog Island Bay (4 year), C: unvegetated sediment in South Bay (SB Unveg), D: 10-year restored seagrass treatment in South Bay (10 year). Down-core intervals are for 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm depth.

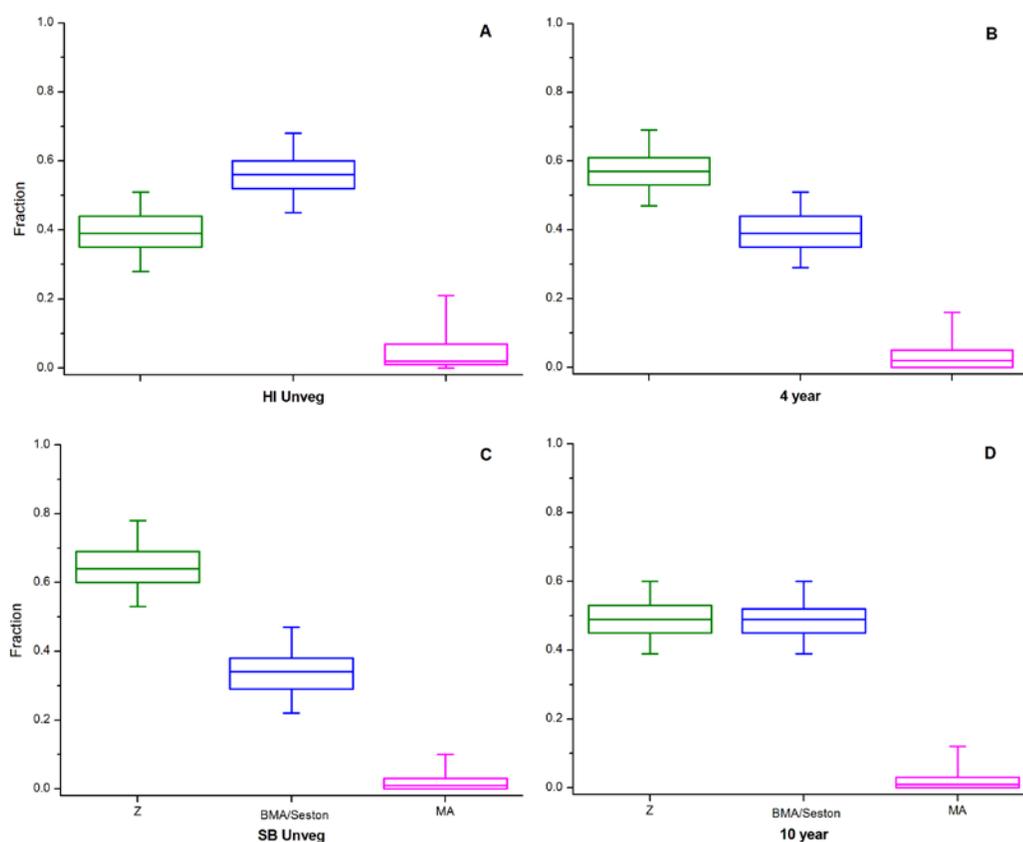


Figure 2.9: Box plots showing 3-source, 2-tracer VCR-LTER Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment).

Carbon sources are *Zostera marina* (Zostera), BMA and seston (BMA/Seston), and macroalgae (MA).

A: unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year). Box plots show quartile 5th, 25th, 50th, 75th, and 95th percentiles.

Appendix

Appendix 2.1: VCR-LTER and literature review of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and molar C/N ratios for carbon sources used in Bayesian mixing model.

VCR-LTER values from each carbon source were from Hondula 2012 only, where other samples taken at VCR-LTER were included as literature values. Literature values use all same vegetation species found at the VCR-LTER. In addition, Literature values were combined with values measured directly from the Virginia Coast Reserve Long Term Ecological Research Site (VCR-LTER), where average source values are reported in Table 2.1. Carbon source values include *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), *Agardhiella subulata* (MA), *Codium fragile* (MA), *Ectocarpus siliculosus* (MA), *Enteromorpha flexuosa* (MA), *Fucus vesiculosus* (MA), *Gracilaria vermiculophylla* (MA), *Polysiphonia nigrescens* (MA), *Scytosiphon lomentaria* (MA), *Ulva lactuca* (MA), *Gracilaria tikvahiae* (MA), *Codium fragile* (MA), *Fucus vesiculosus* (MA), and seston particles (Seston).

Source	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Molar C/N	Location	Citation
<i>Z. marina</i> (Z)	-9.96	6.14	21.70	VCR LTER (VA, USA)	Hondula 2012
	-9.92	6.26	21.94	VCR LTER (VA, USA)	Hondula 2012
	-10.13	6.20	22.57	VCR LTER (VA, USA)	Hondula 2012
	-9.91	6.58	21.77	VCR LTER (VA, USA)	Hondula 2012
	-10.63	5.77	23.67	VCR LTER (VA, USA)	Hondula 2012
	-10.26	5.76	22.50	VCR LTER (VA, USA)	Hondula 2012
	-10.38	7.08	18.69	VCR LTER (VA, USA)	Hondula 2012
	-9.63	6.60	27.06	VCR LTER (VA, USA)	Hondula 2012
	-10.01	7.66	22.65	VCR LTER (VA, USA)	Hondula 2012
	-10.68	7.77	16.93	VCR LTER (VA, USA)	Hondula 2012
	-9.22	6.30	32.99	VCR LTER (VA, USA)	Hondula 2012
	-7.70	1.20		Cape Code (MA, USA)	Olsen et al. 2011

	-10.90	1.90		Cape Code (MA, USA)	Olsen et al. 2011
	-11.20	3.50		Cape Code (MA, USA)	Olsen et al. 2011
	-10.20			Newport River Estuary (NC, USA)	Thayer et al. 1987
	-10.90			Ria Formosa, Portugal & Spain	Kennedy et al. 2010
	-9.60	9.70	22.97	Tomales Bay (CA, USA)	Fourqurean et al. 1997
	-9.90	9.50	23.32	Tomales Bay (CA, USA)	Fourqurean et al. 1997
<i>S. alterniflora</i> (SP)	-14.15	8.20		VCR LTER (VA, USA)	Hondula 2012
	-14.29	2.18	67.29	VCR LTER (VA, USA)	Hondula 2012
	-13.02	5.85	38.53	VCR LTER (VA, USA)	Hondula 2012
		8.68		VCR LTER (VA, USA)	Hondula 2012
		11.26		VCR LTER (VA, USA)	Hondula 2012
	-13.41	4.79		VCR LTER (VA, USA)	Hondula 2012
			48.30	VCR LTER (VA, USA)	Tyler 1997
			41.20	VCR LTER (VA, USA)	Tyler 1997
			50.10	VCR LTER (VA, USA)	Olcott 2011
			39.90	VCR LTER (VA, USA)	Mozdzer 2009
			44.20	VCR LTER (VA, USA)	Mozdzer 2009
	-12.20		74.20	MA, USA	Middelburg et al. 1997
	-12.50		69.80	MA, USA	Middelburg et al. 1997
	-12.80		55.30	MA, USA	Middelburg et al. 1997
	-13.10		37.30	MA, USA	Middelburg et al. 1997
	-13.00	5.30		Newport River Estuary (NC, USA)	Currin et al. 1995
	-12.60	6.60		GA, USA	Foegel et al. 1989
	-12.90	6.00		Sapelo Island (GA, USA)	Howarth, 1987
	-13.20	5.20		Graveline Bay Marsh (MS, USA)	Sullivan & Moncreiff, 1990
	-14.30	4.30		SC, USA	Couch, 1989
Benthic Microalgae (BMA)	-23.65	6.38		VCR LTER (VA, USA)	Hondula 2012
	-21.03	5.92		VCR LTER (VA, USA)	Hondula 2012
	-20.62	5.71		VCR LTER (VA, USA)	Hondula 2012
	-21.60	5.16		VCR LTER (VA, USA)	Hondula 2012
	-21.04	6.19		VCR LTER (VA, USA)	Hondula 2012
	-18.13	5.74		VCR LTER (VA, USA)	Hondula 2012

-20.12	6.94		VCR LTER (VA, USA)	Hondula 2012
-20.26	7.15		VCR LTER (VA, USA)	Hondula 2012
-20.97	5.20		VCR LTER (VA, USA)	Hondula 2012
-19.62	9.07	7.60	Tolay Creek (CA, USA)	Cloern et al. 2002
-20.08	9.08	7.90	Tolay Creek (CA, USA)	Cloern et al. 2002
-19.96	8.57	7.90	Tolay Creek (CA, USA)	Cloern et al. 2002
-21.29	7.95	8.10	Tolay Creek (CA, USA)	Cloern et al. 2002
-21.33	7.94	8.20	Tolay Creek (CA, USA)	Cloern et al. 2002
-20.79	8.31	8.20	Tolay Creek (CA, USA)	Cloern et al. 2002
-24.34	9.40	10.10	Pal Alton (CA, USA)	Cloern et al. 2002
-24.40	9.75	10.50	Pal Alton (CA, USA)	Cloern et al. 2002
-24.33	10.06	10.20	Pal Alton (CA, USA)	Cloern et al. 2002
-24.53	10.31	9.50	Pal Alton (CA, USA)	Cloern et al. 2002
-24.30	10.87	9.70	Pal Alton (CA, USA)	Cloern et al. 2002
-24.37	10.52	9.70	Pal Alton (CA, USA)	Cloern et al. 2002
-24.15	10.36	8.70	Pal Alton (CA, USA)	Cloern et al. 2002
-24.03	9.78	8.30	Pal Alton (CA, USA)	Cloern et al. 2002
-24.33	10.57	9.40	Pal Alton (CA, USA)	Cloern et al. 2002
-26.53	5.81	14.10	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.53	6.44	13.40	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.62	7.00	12.30	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.10	6.55	13.70	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.39	6.94	11.80	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.54	7.31	14.00	Tolay Creek (CA, USA)	Cloern et al. 2002
-27.43	8.01	14.70	Tolay Creek (CA, USA)	Cloern et al. 2002
-27.42	7.82	13.90	Tolay Creek (CA, USA)	Cloern et al. 2002
-27.34	7.97	13.30	Tolay Creek (CA, USA)	Cloern et al. 2002
-20.57	7.26	7.20	Tolay Creek (CA, USA)	Cloern et al. 2002
-20.62	7.13	7.10	Tolay Creek (CA, USA)	Cloern et al. 2002
-20.84	6.85	7.40	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.44	3.58	10.90	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.77	2.85	10.70	Tolay Creek (CA, USA)	Cloern et al. 2002
-26.81	4.52	11.00	Tolay Creek (CA, USA)	Cloern et al. 2002
-21.95	6.00	7.90	Tolay Creek (CA, USA)	Cloern et al. 2002
-21.75	6.02	7.70	Tolay Creek (CA, USA)	Cloern et al. 2002
-21.52	6.73	7.60	Tolay Creek (CA, USA)	Cloern et al. 2002
-23.76	7.52	8.60	Pal Alton (CA, USA)	Cloern et al. 2002
-23.78	6.30	8.60	Pal Alton (CA, USA)	Cloern et al. 2002
-23.56	6.28	8.40	Pal Alton (CA, USA)	Cloern et al. 2002
-23.87	5.38	8.40	Pal Alton (CA, USA)	Cloern et al. 2002
-24.14	6.43	8.80	Pal Alton (CA, USA)	Cloern et al. 2002

	-23.93	6.07	8.60	Pal Alton (CA, USA)	Cloern et al. 2002
	-24.71	5.76	8.10	Pal Alton (CA, USA)	Cloern et al. 2002
	-24.28	5.96	7.50	Pal Alton (CA, USA)	Cloern et al. 2002
	-24.54	5.91	7.90	Pal Alton (CA, USA)	Cloern et al. 2002
	-20.21	6.42	6.10	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-20.20	6.52	6.00	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-20.16	6.13	6.00	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.88	6.71	6.00	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.83	6.89	5.90	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.76	7.05	6.00	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.94	6.53	6.40	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.91	6.82	6.30	Grizzly Bay (CA, USA)	Cloern et al. 2002
	-19.91	6.82	6.30	Grizzly Bay (CA, USA)	Cloern et al. 2002
<i>A. subulata</i> (MA)	-19.73	9.79		VCR LTER (VA, USA)	Hondula 2012
<i>A. subulata</i> (MA)	-15.62	10.04		VCR LTER (VA, USA)	Hondula 2012
<i>A. subulata</i> (MA)	-21.18	8.27	13.89	VCR LTER (VA, USA)	Hondula 2012
<i>C. fragile</i> (MA)	-16.10	8.98		VCR LTER (VA, USA)	Hondula 2012
<i>C. fragile</i> (MA)	-13.90	10.01		VCR LTER (VA, USA)	Hondula 2012
<i>C. fragile</i> (MA)	-17.64	11.15	15.52	VCR LTER (VA, USA)	Hondula 2012
<i>C. fragile</i> (MA)	-16.01	9.98	16.49	VCR LTER (VA, USA)	Hondula 2012
<i>E. siliculosus</i> (MA)	-16.71	8.69	11.67	VCR LTER (VA, USA)	Hondula 2012
<i>E. flexuosa</i> (MA)	-19.81	9.77	10.12	VCR LTER (VA, USA)	Hondula 2012
<i>E. flexuosa</i> (MA)	-20.33	8.67	16.32	VCR LTER (VA, USA)	Hondula 2012
<i>F. vesiculosus</i> (MA)	-10.64	9.89	10.43	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-20.38	10.05		VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-14.48	11.59		VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-18.92	10.75	15.40	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-15.52	8.29	15.93	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-14.65	10.52	25.13	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-17.11	7.80	36.39	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-20.52	9.00	18.16	VCR LTER (VA, USA)	Hondula 2012
<i>G. vermiculophylla</i> (MA)	-17.64	10.95	17.37	VCR LTER (VA, USA)	Hondula 2012
<i>P. nigrescens</i> (MA)	-15.98	9.82		VCR LTER (VA, USA)	Hondula 2012

<i>P. nigrescens</i> (MA)	-15.88	9.96		VCR LTER (VA, USA)	Hondula 2012
<i>S. lomentaria</i> (MA)	-12.78	8.95	15.12	VCR LTER (VA, USA)	Hondula 2012
<i>S. lomentaria</i> (MA)	-15.44	7.78	18.04	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-19.47	12.45		VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-18.96	8.69		VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-14.06	9.99	22.46	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-10.91	10.45	12.47	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-16.96	10.41	17.03	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-16.76	9.18	30.86	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-22.82	7.92	26.29	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-19.96	8.37		VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-23.58	7.23	25.60	VCR LTER (VA, USA)	Hondula 2012
<i>U. lactuca</i> (MA)	-23.79	7.11	33.07	VCR LTER (VA, USA)	Hondula 2012
<i>G. tikvahiae</i> (MA)	-19.10	5.80		Cape Code (MA, USA)	Olsen et al. 2011
<i>G. tikvahiae</i> (MA)	-17.60	6.60		Cape Code (MA, USA)	Olsen et al. 2011
<i>C. fragille</i> (MA)	-11.30	5.90		Cape Code (MA, USA)	Olsen et al. 2011
<i>C. fragille</i> (MA)	-12.30	5.70		Cape Code (MA, USA)	Olsen et al. 2011
<i>C. fragille</i> (MA)	-14.00	5.90		Cape Code (MA, USA)	Olsen et al. 2011
<i>F. vesiculosus</i> (MA)	-12.70	4.70		Cape Code (MA, USA)	Olsen et al. 2011
<i>F. vesiculosus</i> (MA)	-14.20	5.80		Cape Code (MA, USA)	Olsen et al. 2011
<i>U. lactuca</i> (MA)	-12.50	4.30		Cape Code (MA, USA)	Olsen et al. 2011
<i>U. lactuca</i> (MA)	-14.60	5.10		Cape Code (MA, USA)	Olsen et al. 2011
<i>U. lactuca</i> (MA)	-12.70	5.70		Cape Code (MA, USA)	Olsen et al. 2011
Macroalgae (MA)	-16.00			Newport River Estuary (NC, USA)	Thayer et al. 1987
Seston	-23.75	6.81	7.71	VCR LTER (VA, USA)	Hondula 2012
	-23.47	8.32	8.36	VCR LTER (VA, USA)	Hondula 2012
	-24.49	6.46	8.04	VCR LTER (VA, USA)	Hondula 2012
	-24.31	6.35	8.21	VCR LTER (VA, USA)	Hondula 2012
	-23.65	6.38	8.16	VCR LTER (VA, USA)	Hondula 2012
	-24.27	6.28		VCR LTER (VA, USA)	Hondula 2012
	-26.30	3.30		VCR LTER (VA, USA)	Hondula 2012
	-25.18	4.18		VCR LTER (VA, USA)	Hondula 2012
	-25.17	4.78		VCR LTER (VA, USA)	Hondula 2012
	-26.12	4.00		VCR LTER (VA, USA)	Hondula 2012
	-26.16	5.66		VCR LTER (VA, USA)	Hondula 2012
	-24.79	4.28		VCR LTER (VA, USA)	Hondula 2012
	-23.94	5.87		VCR LTER (VA, USA)	Hondula 2012

-24.55	3.92	VCR LTER (VA, USA)	Hondula 2012
-23.73	4.23	VCR LTER (VA, USA)	Hondula 2012
-24.27	4.09	VCR LTER (VA, USA)	Hondula 2012

Appendix 2.1 Literature Cited:

- Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography*. **47**: 713 – 729. Web Appendix 1. Stable isotope ratios and C:N ratios of plants and high-Chl *a* seston samples collected in the San Francisco Bay estuarine system.
- Couch, C. A. 1989. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Estuarine, Coastal and Shelf Science*. **28**: 433 – 441.
- Currin, C. A., S. Y. Newell, and H. W. Paerl, 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: consideration based on multiple stable isotope analysis. *Marine Ecology Progress Series*. **121**: 99 – 116.
- Foegel, M. L., E. K. Sprague, A. P. Gize, and R. W. Frey. 1989. Diagenesis of organic matter in Georgia salt marshes. *Estuarine, Coastal and Shelf Science*. **28**: 211 – 230.
- Fourqurean, J. W., T. O. Moore, B. Fry, and J. T. Hollibaugh. 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. *Marine Ecology Progress Series*. **157**: 147 – 157.

- Hondula, K. L., and M. L. Pace. 2012. Using multiple stable isotopes including deuterium ($\delta^2\text{H}$) to trace organic matter in complex near-shore lagoon. M.S. thesis. University of Virginia.
- Kennedy, H., J. Beggins, C. M. Duarte, J. W. Fourqurean, M. Holmer, N. Marba, and J. J. Middelburg. 2010. Seagrass sediments as a global carbon sink: isotopic constraints. *Glob. Biogeochem. Cycles*. **24**: 1 – 8, doi:10.1029/2010GB003848
- Middelburg, J. J., J. Nieuwenhuize, R. K. Lubberts, O. van de Plassche. 1997. Organic carbon isotope systematic of coastal marshes. *Estuar. Coast. Shelf Sci.* **45**: 681 – 687.
- Mozdzer, T. J. 2009. Variation in the availability and utilization of dissolved organic nitrogen by the smooth cordgrass, *Spartina alterniflora*. Ph.D. thesis. University of Virginia.
- Olcott, C. A. 2011. Impacts of nitrogen addition on the monthly above- and belowground production of *Spartina alterniflora* in a Virginia marsh. Undergraduate thesis, University of Virginia.
- Olsen, Y. S., S. E. Fox, M. Teichberg, M. Otter, and I. Valiela. 2011. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass. *Marine Ecology Progress Series*. **421**: 83 – 96.
- Peterson, B. J. and R.W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography*. **32**: 1195 – 1213.

- Sullivan, M. J. and C. A. Moncreiff. 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series*. **62**: 149 – 159.
- Thayer, G. W., P. L. Parker, M. W. LaCroix, and B. Fry. 1978. The stable carbon isotope ratio of some components of an eelgrass, *Zostera marina*, bed. *Oecologia*. **35**: 1 – 12.
- Tyler, C. 1997. Geomorphological and hydrological controls on pattern and process in a developing barrier island salt marsh. M.S. thesis. University of Virginia.

Appendix 2.2: Carbon source and sediment age treatment averages (mean), standard deviations (\pm SD), and sample size (n) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N values used in Bayesian 4-source, 3-tracer mixing model.

Source values (*Zostera marina* (Z), marsh grass (SP), benthic microalgae (BMA), and macroalgae (MA) were from values collected at the VCR-LTER and literature values for the same species (Appendix 2.1). Each sediment treatment (HI Unveg, 4-year, SB Unveg, and 10-year) was averaged for the top 10 cm, 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm of sediment.

Source	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)			C/N		
	Mean	\pm SD	n	Mean	\pm SD	n	Mean	\pm SD	n
Z	-10.06	0.78	18	6.12	2.31	16	19.71	3.29	13
SP	-13.19	0.68	13	6.21	2.44	11	51.47	13.38	11
BMA	-22.90	2.60	60	7.12	1.70	60	9.07	2.47	51
MA	-16.62	3.36	44	8.56	2.03	43	19.26	7.34	22
0 cm – 10 cm									
					Sediment				
HI Unveg	-17.74	0.76	10	5.98	0.31	10	12.07	0.36	10
4-year	-15.44	0.64	10	5.90	0.22	10	13.20	0.36	10
SB Unveg	-14.41	1.66	10	5.49	0.18	10	12.38	0.51	10
10-year	-16.49	0.64	10	5.57	0.1	10	12.16	0.41	10
0 cm – 3 cm									
					Sediment				
HI Unveg	-16.81	0.64	3	5.75	0.08	3	12.34	0.18	3
4-year	-15.09	0.24	3	6.09	0.31	3	13.08	0.33	3
SB Unveg	-12.20	0.75	3	5.54	0.23	3	11.78	0.35	3
10-year	-16.02	0.89	3	5.63	0.15	3	12.33	0.42	3
3 cm - 6 cm									
					Sediment				
HI Unveg	-17.82	0.22	3	5.84	0.08	3	12.20	0.04	3
4-year	-15.07	0.44	3	5.74	0.04	3	13.44	0.42	3
SB Unveg	-14.83	0.52	3	5.50	0.18	3	12.63	0.37	3
10-year	-17.01	0.43	3	5.63	0.09	3	11.97	0.36	3
6 cm - 10 cm									
					Sediment				
HI Unveg	-18.37	0.18	4	6.25	0.34	4	11.77	0.41	4
4-year	-15.98	0.62	4	5.87	0.15	4	13.12	0.34	4

SB Unveg	-15.75	0.45	4	5.44	0.19	4	12.64	0.32	4
10-year	-16.45	0.32	4	5.49	0.21	4	12.17	0.48	4

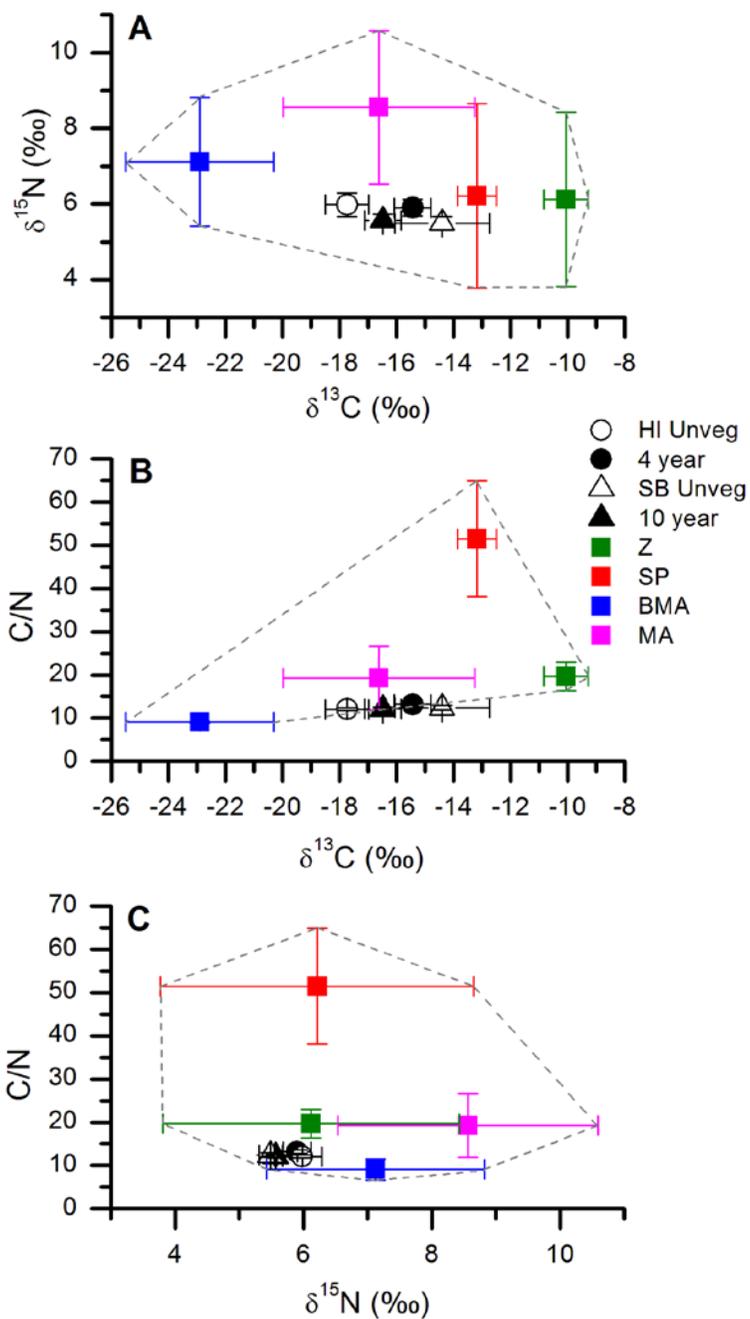
Appendix 2.3: Vertical down-core profile data of sediment characteristics $\delta^{13}\text{C}$ (‰), $\delta^{15}\text{N}$ (‰), %C, and molar C/N.

Sediment treatments are HI Unveg, 4 year, SB Unveg, and 10 year in the top 20 cm of sediment in 1-cm intervals (depth).

Treatment	Depth (cm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	Molar C/N
HI Unveg	1	-16.12	5.81	0.32	12.14
HI Unveg	2	-16.94	5.66	0.41	12.39
HI Unveg	3	-17.37	5.76	0.48	12.50
HI Unveg	4	-17.61	5.79	0.47	12.16
HI Unveg	5	-17.80	5.81	0.52	12.19
HI Unveg	6	-18.05	5.93	0.59	12.24
HI Unveg	7	-18.27	6.16	0.59	12.00
HI Unveg	8	-18.23	5.95	0.59	12.05
HI Unveg	9	-18.63	6.73	0.65	11.18
HI Unveg	10	-18.35	6.15	0.58	11.88
HI Unveg	11	-18.34	6.00	0.59	11.97
HI Unveg	12	-18.27	6.03	0.61	11.99
HI Unveg	13	-18.41	5.99	0.65	11.84
HI Unveg	14	-18.31	6.08	0.58	11.73
HI Unveg	15	-18.27	5.96	0.59	11.94
HI Unveg	16	-18.03	5.67	0.54	11.98
HI Unveg	17	-18.44	5.63	0.50	11.69
HI Unveg	18	-18.33	5.67	0.52	12.09
HI Unveg	19	-17.97	5.60	0.50	12.20
HI Unveg	20	-17.98	5.47	0.49	12.30
4 year	1	-14.99	6.37	0.34	13.30
4 year	2	-14.92	6.14	0.31	13.24
4 year	3	-15.36	5.76	0.31	12.69
4 year	4	-14.83	5.78	0.31	13.93
4 year	5	-14.80	5.74	0.28	13.26
4 year	6	-15.57	5.70	0.32	13.14
4 year	7	-15.44	6.05	0.31	12.94
4 year	8	-15.50	5.69	0.34	13.31
4 year	9	-16.27	5.86	0.41	13.47
4 year	10	-16.73	5.89	0.47	12.74

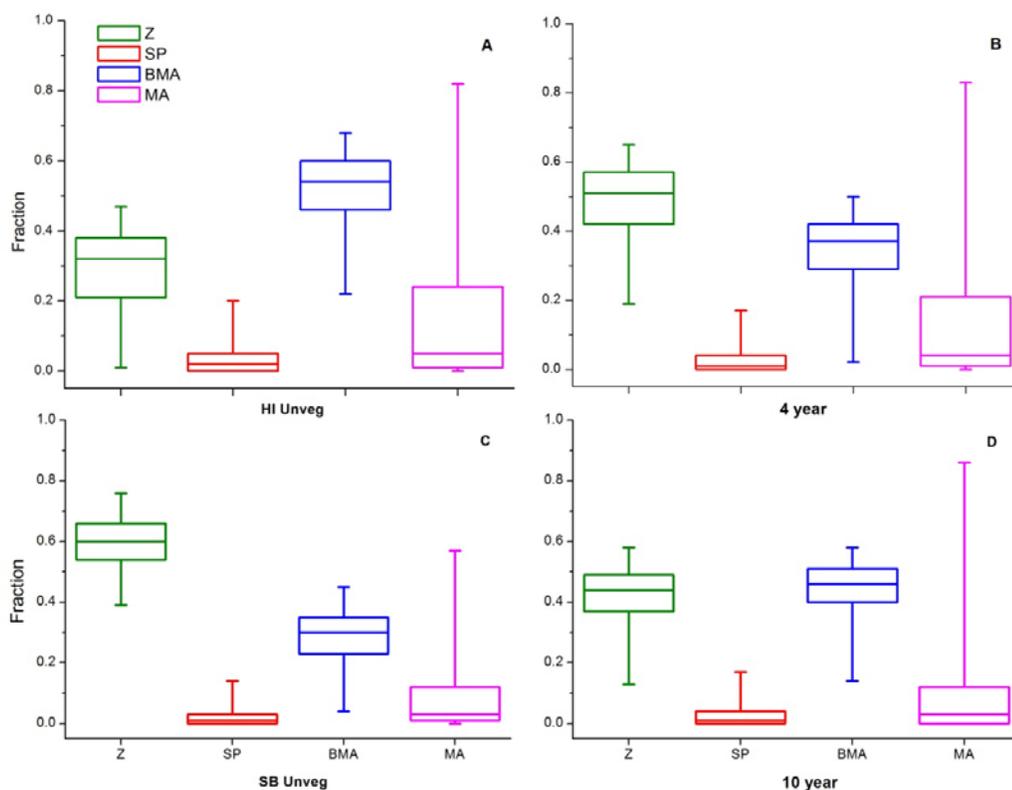
4 year	11	-17.45	5.74	0.51	12.61
4 year	12	-17.50	5.82	0.53	12.79
4 year	13	-17.65	5.83	0.53	12.79
4 year	14	-17.51	5.50	0.50	12.25
4 year	15	-17.58	5.66	0.51	11.89
4 year	16	-17.42	5.66	0.46	12.03
4 year	17	-17.67	5.33	0.58	12.34
4 year	18	-17.41	5.33	0.52	12.11
4 year	19	-17.13	5.42	0.52	12.23
4 year	20	-17.29	5.39	0.50	12.22
SB Unveg	1	-11.40	5.78	0.12	11.51
SB Unveg	2	-12.88	5.32	0.14	11.65
SB Unveg	3	-12.32	5.51	0.14	12.18
SB Unveg	4	-14.24	5.71	0.21	12.41
SB Unveg	5	-15.02	5.38	0.21	13.05
SB Unveg	6	-15.23	5.42	0.21	12.42
SB Unveg	7	-15.28	5.32	0.22	12.29
SB Unveg	8	-15.56	5.43	0.25	12.45
SB Unveg	9	-15.83	5.29	0.31	12.89
SB Unveg	10	-16.34	5.70	0.35	12.93
SB Unveg	11	-16.74	5.32	0.35	12.51
SB Unveg	12	-16.85	5.35	0.37	12.68
SB Unveg	13	-16.71	5.43	0.38	12.55
SB Unveg	14	-16.90	5.56	0.37	12.36
SB Unveg	15	-17.05	5.38	0.39	12.65
SB Unveg	16	-16.81	5.28	0.39	12.68
SB Unveg	17	-16.98	5.47	0.40	12.67
SB Unveg	18	-16.60	5.42	0.41	12.95
SB Unveg	19	-16.38	5.35	0.39	12.81
SB Unveg	20	-16.71	5.37	0.46	13.17
10 year	1	-15.02	5.55	0.45	12.81
10 year	2	-16.32	5.54	0.66	12.00
10 year	3	-16.72	5.79	0.84	12.18
10 year	4	-16.92	5.73	0.84	11.98
10 year	5	-17.47	5.61	0.78	11.60
10 year	6	-16.63	5.56	0.65	12.33
10 year	7	-16.31	5.62	0.52	12.59
10 year	8	-16.88	5.40	0.46	12.04
10 year	9	-16.12	5.70	0.45	12.49
10 year	10	-16.50	5.23	0.33	11.55
10 year	11	-15.55	5.09	0.27	11.97

10 year	12	-15.14	4.99	0.25	12.59
10 year	13	-15.97	5.10	0.32	12.35
10 year	14	-15.44	5.35	0.27	13.12
10 year	15	-15.15	5.09	0.24	13.50
10 year	16	-14.23	5.17	0.14	12.23
10 year	17	-14.99	5.54	0.22	13.48
10 year	18	-14.54	5.09	0.22	13.51
10 year	19	-14.59	5.12	0.24	13.97
10 year	20	-15.37	5.45	0.26	13.09



Appendix 2.4: Isotope and C/N ratio comparisons between average sources and average sediment treatments.

Average end-member sources from VCR-LTER and literature values include *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), and macroalgae (MA), noted as colored squares, and averages from the 4 different age treatments (HI Unveg, 4-year, SB Unveg, and 10-year) from the top 10 cm of sediment noted as filled (restored seagrass) and no filled (unvegetated) circles (HI) and triangles (SB). Standard deviations represent error bars. Dashed grey lines indicate end-member source bounds for sediment mixture, with expectations of finding sediment treatments within grey lines. **A:** $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$; **B:** $\delta^{13}\text{C}$ vs. C/N; **C:** $\delta^{15}\text{N}$ vs. C/N.



Appendix 2.5: Box plots showing quartile 5th, 25th, 50th, 75th, and 95th percentile of carbon source for 4-source, 3-tracer Bayesian mixing model fraction contributions to sediment treatments (top 10 cm of sediment).

Carbon sources from VCR-LTER and literature values are *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), and macroalgae (MA). **A:** unvegetated sediment in Hog Island Bay (HI Unveg), **B:** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C:** unvegetated sediment in South Bay (SB Unveg), **D:** 10-year restored seagrass treatment in South Bay (10 year).

Appendix 2.6: Four-source, three-tracer Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.

Carbon sources from VCR-LTER and literature values are *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), and macroalgae (MA). Quartiles are for the 5th, 25th, median, 75th, and 95th percentile.

Quartile	Z	SP	BMA	MA
HI Unveg				
0.05	0.01	0.00	0.22	0.00
0.25	0.21	0.00	0.46	0.01
0.50	0.32	0.02	0.54	0.05
0.75	0.38	0.05	0.60	0.24
0.95	0.47	0.20	0.68	0.82
4-year				
0.05	0.19	0.00	0.02	0.00
0.25	0.42	0.00	0.29	0.01
0.50	0.51	0.01	0.37	0.04
0.75	0.57	0.04	0.42	0.21
0.95	0.65	0.17	0.50	0.83
SB Unveg				
0.05	0.39	0.00	0.04	0.00
0.25	0.54	0.00	0.23	0.01
0.50	0.60	0.01	0.30	0.03
0.75	0.66	0.03	0.35	0.12
0.95	0.76	0.14	0.45	0.57
10-year				
0.05	0.13	0.00	0.14	0.00
0.25	0.37	0.00	0.40	0.00
0.50	0.44	0.01	0.46	0.03
0.75	0.49	0.04	0.51	0.12
0.95	0.58	0.17	0.58	0.86

Appendix 2.7: Four-source, three-tracer Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.

Carbon sources from VCR-LTER and literature sources are *Zostera marina* (Z), *Spartina alterniflora* (SP), benthic microalgae (BMA), and macroalgae (MA). Tracers used for model run are $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N ratios. Quartiles are for the 5th, 25th, median, 75th, and 95th percentile.

Treatment	Z	SP	BMA	MA
0 - 3 cm				
HI Unveg	0.41	0.01	0.48	0.03
4-year	0.53	0.01	0.34	0.05
SB Unveg	0.79	0.01	0.10	0.04
10-year	0.47	0.01	0.42	0.03
3 - 6 cm				
HI Unveg	0.32	0.02	0.56	0.04
4-year	0.54	0.01	0.34	0.04
SB Unveg	0.57	0.01	0.33	0.03
10-year	0.40	0.01	0.50	0.03
6 - 10 cm				
HI Unveg	0.26	0.02	0.59	0.06
4-year	0.46	0.02	0.41	0.05
SB Unveg	0.50	0.01	0.41	0.03
10-year	0.45	0.01	0.46	0.03

Appendix 2.8: Three-source, two-tracer VCR-LTER and literature value Bayesian mixing model fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.

Carbon sources from VCR-LTER and literature values are *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA). Tracers used for model run are $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes. Quartiles are for the 5th, 25th, median, 75th, and 95th percentile.

Treatment	Z	BMA	MA
0 - 3 cm			
HI Unveg	0.45	0.48	0.04
4-year	0.56	0.33	0.08
SB Unveg	0.82	0.09	0.05
10-year	0.51	0.42	0.03
3 - 6 cm			
HI Unveg	0.36	0.56	0.04
4-year	0.57	0.34	0.05
SB Unveg	0.60	0.33	0.04
10-year	0.44	0.51	0.03
6 - 10 cm			
HI Unveg	0.30	0.59	0.07
4-year	0.51	0.42	0.04
SB Unveg	0.54	0.41	0.03
10-year	0.48	0.46	0.04

Appendix 2.9: Three-source, two-tracer VCR-LTER Bayesian mixing model

fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.

Carbon sources from VCR-LTER values are *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA). Tracers used for model run are $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes.

Treatment	Z	BMA	MA
0 - 3 cm			
HI Unveg	0.35	0.63	0.01
4-year	0.52	0.45	0.02
SB Unveg	0.81	0.17	0.01
10-year	0.42	0.56	0.01
3 - 6 cm			
HI Unveg	0.25	0.72	0.01
4-year	0.51	0.46	0.01
SB Unveg	0.53	0.45	0.01
10-year	0.33	0.65	0.01
6 - 10 cm			
HI Unveg	0.18	0.75	0.04
4-year	0.43	0.54	0.01
SB Unveg	0.45	0.53	0.01
10-year	0.38	0.60	0.01

Appendix 2.10: Three-source, two-tracer VCR-LTER Bayesian mixing model

fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.

Carbon sources from VCR-LTER values are *Zostera marina* (Z), benthic microalgae (BMA), and macroalgae (MA). Quartiles are for the 5th, 25th, median, 75th, and 95th percentile.

Quartile	Z	BMA	MA
HI Unveg			
0.05	0.11	0.57	0.00
0.25	0.20	0.65	0.01
0.50	0.25	0.71	0.02
0.75	0.31	0.76	0.06
0.95	0.41	0.88	0.18
4 year			
0.05	0.36	0.36	0.00
0.25	0.43	0.44	0.00
0.50	0.48	0.49	0.01
0.75	0.53	0.54	0.04
0.95	0.63	0.64	0.14
SB Unveg			
0.05	0.43	0.26	0.00
0.25	0.51	0.35	0.00
0.50	0.57	0.41	0.01
0.75	0.63	0.47	0.02
0.95	0.74	0.58	0.09
10 year			
0.05	0.25	0.48	0.00
0.25	0.33	0.55	0.00
0.50	0.38	0.60	0.01
0.75	0.43	0.65	0.03
0.95	0.53	0.75	0.11

Appendix 2.11: Carbon source, standard deviations (\pm SD), and sample size (n) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ used in Bayesian 3-source, 2-tracer VCR-LTER mixing model.

Source values (*Zostera marina* (Z), benthic microalgae and seston (BMA/Seston), and macroalgae (MA) were from values collected at the VCR-LTER (Appendix 2.1).

Source	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	\pm SD	Mean	\pm SD
Z	11	-10.07	0.43	6.55	0.68
BMA/Seston	25	-23.26	2.17	5.57	1.51
MA	33	-17.4	3.28	9.47	1.26

Appendix 2.12: Three-source, two-tracer VCR-LTER Bayesian mixing model fraction contribution results in quartiles for each sediment treatment for the interval depths.

Carbon sources from VCR-LTER values are *Zostera marina* (Z), benthic microalgae and seston (BMA/Seston), and macroalgae (MA). Tracers used for model run are $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes. Sediment treatments were HI Unveg, 4-year, SB Unveg, and 10-year for the interval depths 0 – 3 cm, 3 – 6 cm, and 6 – 10 cm.

Treatment	Z	BMA/Seston	MA
0 - 3 cm			
HI Unveg	0.47	0.50	0.01
4-year	0.60	0.36	0.03
SB Unveg	0.83	0.15	0.01
10-year	0.52	0.45	0.01
3 - 6 cm			
HI Unveg	0.39	0.57	0.02
4-year	0.60	0.38	0.01
SB Unveg	0.62	0.36	0.01
10-year	0.45	0.52	0.01
6 - 10 cm			
HI Unveg	0.33	0.59	0.06
4-year	0.53	0.44	0.02
SB Unveg	0.54	0.44	0.01
10-year	0.49	0.48	0.01

Appendix 2.13: Three-source, two-tracer VCR-LTER Bayesian mixing model

fraction contribution results in quartiles for each sediment treatment (HI Unveg, 4-year, SB Unveg, 10-year) for the top 10 cm of sediment.

Carbon sources from VCR-LTER values are *Zostera marina* (Z), benthic microalgae and seston (BMA/Seston), and macroalgae (MA). Quartiles are for the 5th, 25th, median, 75th, and 95th percentile. Sediment treatments were HI Unveg, 4-year, SB Unveg, 10-year for the top 10 cm of sediment.

Quartile	Z	BMA/Seston	MA
HI Unveg			
0.05	0.28	0.45	0.00
0.25	0.35	0.52	0.01
0.50	0.39	0.56	0.02
0.75	0.44	0.60	0.07
0.95	0.51	0.68	0.21
4 year			
0.05	0.47	0.29	0.00
0.25	0.53	0.35	0.00
0.50	0.57	0.39	0.02
0.75	0.61	0.44	0.05
0.95	0.69	0.51	0.16
SB Unveg			
0.05	0.53	0.22	0.00
0.25	0.60	0.29	0.00
0.50	0.64	0.34	0.01
0.75	0.69	0.38	0.03
0.95	0.78	0.47	0.10
10 year			
0.05	0.39	0.39	0.00
0.25	0.45	0.45	0.00
0.50	0.49	0.49	0.01
0.75	0.53	0.52	0.03
0.95	0.60	0.60	0.12

Conclusion

The goal of this thesis was to quantify and understand carbon accumulation rates in a restored seagrass habitat. I found that the 10-year restored seagrass meadow accumulated carbon in the seagrass sediment; however, unvegetated and 4-year restored seagrass meadows had continual shallow mixing and resuspension of sediment that prevented consistent carbon accumulation. In addition, the carbon in the seagrass and neighboring unvegetated sediments was primarily a mixture of seagrass and benthic microalgal source carbon. This study gave the first measurements and values related to carbon sequestration in a restored seagrass habitat. In particular, the results from this study show the potential of seagrass restoration aiding in the offset of the increasing atmospheric CO₂ concentrations.

In Chapter 1, the carbon, nitrogen, and organic matter content were significantly different between the 10-year meadow and all other treatments. In addition, the first carbon accumulation rate using ²¹⁰Pb dating techniques was calculated for a restored seagrass meadow in the 10-year meadow as 36.68 (± 2.8) g C m⁻² yr⁻¹. However, no sediment accumulation and consequently carbon accumulation rates were calculated for unvegetated and 4-year meadow. This was the result of ²¹⁰Pb profiles showing no significant changes from background ²¹⁰Pb values because of continual shallow mixing and resuspension of sediment. The lack of sediment accumulation was expected in the unvegetated sediments as there was no vegetation to stabilize the sediment and prevent resuspension. The 4-year meadow was predicted to show some accumulation of carbon as a result of the presence (although at low densities) of seagrass at this meadow. However,

the lack of carbon accumulation in the 4-year meadow was attributed to the similar 4-year lag seen in changes in seagrass densities, which resulted in a delayed 5-year lag in carbon and sediment accumulation. Ultimately from the carbon accumulation rate in the 10-year meadow, it was estimated that within 12 years since seeding, the restored seagrass meadow was projected to accumulate carbon at rates within literature values for natural seagrass meadows. Lastly, if this carbon accumulation rate were applied to the entire current coverage of restored seagrass in the VCR-LTER (1700 ha), it would provide a social cost of approximately \$7,000 yr⁻¹ or \$4.10 ha⁻¹yr⁻¹ of carbon storage.

In Chapter 2, the first analysis of carbon source contributions was determined for a restored seagrass habitat, which showed the importance of both seagrass and non-seagrass sources contributing to the sediment carbon in the VCR-LTER. Carbon sources (seagrass, benthic microalgae, and macroalgae) were analyzed for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to determine source contributions to each age meadow. In all different age meadows, seagrass and benthic microalgae contributed the most (42.3% and 55.1% respectively) to the sediment carbon. Interestingly, both of the unvegetated sediment sites had a significant amount of seagrass carbon contributing to the sediment carbon pool, specifically in the unvegetated site neighboring the 10-year meadow (South Bay). This suggested the potential for seagrass carbon distributed throughout the system and incorporated in sediments outside the restored meadows. Also, it was expected that the 4-year meadow would have significantly higher seagrass contribution compared to the 10-year meadow as a result of primarily carbon-rich seagrass contributing to the sediment; however, seagrass source contributions were higher but not significantly in the 4-year

meadow, indicating that the 4-year meadow was not getting carbon just from seagrass. Lastly using Chapter 1 carbon accumulation rates, the seagrass carbon accumulation rate was $15.41 \text{ g C m}^{-2} \text{ yr}^{-1}$ and a non-seagrass carbon accumulation rate, which was primarily composed of benthic microalgae, was calculated at $20.86 \text{ g C m}^{-2} \text{ yr}^{-1}$.

As this was the first estimate of carbon accumulation in a restored seagrass habitat, further research is warranted to determine when exactly a restored seagrass meadow meets the range of natural carbon accumulation rates and at what point restored meadows start to accumulate carbon during the development of the seagrass. Using dating methods, we were able to determine that a significant change occurred in the sediment at approximately 5 years, where sedimentation rate increased and carbon concentration decreased. Due to shallow mixing in these shallow lagoons, the confirmation of the 5-year lag before the increased carbon accumulation and the 12-year projection indicating restored seagrass meadows accumulate carbon at similar rates to natural seagrass is important when assessing seagrass restoration benefits. Further research needs to be done to identify the impact of seagrass roots and rhizomes on the carbon stock. This study did not find a significant impact of roots on the carbon stock, but this was based on a small subset of sediment cores. Identifying the impact of roots and rhizomes on carbon stock is also necessary when determining sediment carbon stable isotope and C/N values. There is a large disagreement among researchers concerning root, rhizome, and epiphytes importance in the sediment and involvement in the contribution to sediment carbon. Further analyses of carbon source contribution to restored seagrass sediment needs to include greater replication of sediment treatments.

Blue carbon sequestration in coastal habitats may help offset the increasing CO₂ in the atmosphere. This thesis reports the first carbon accumulation rate and source partitioning for a restored seagrass meadow. Conclusions indicate the need for further studies measuring both carbon accumulation rates and carbon source contributions in both restored and natural seagrass meadows. These results will be useful for planners and policy makers in assessing the potential of seagrass ecosystems to sequester “blue carbon”.

Additional Appendix

Additional Appendix.1: Mean vertical 10 cm down-core profile data of sediment percent carbon (%C) in 1-cm intervals (depth) for sediment treatments 4-year and 10-year for sampling occurring in 2011 and 2012 at the same locations within the treatments.

Standard error (SE) is reported.

Depth (cm)	4-year (2011)		4-year (2012)		10-year (2011)		10-year (2012)	
	%C	±SE	%C	±SE	%C	±SE	%C	±SE
1	0.32	0.03	0.39	0.04	0.49	0.02	0.62	0.04
2	0.34	0.03	0.36	0.04	0.53	0.04	0.57	0.04
3	0.37	0.03	0.36	0.05	0.60	0.05	0.65	0.04
4	0.38	0.03	0.36	0.04	0.66	0.05	0.76	0.04
5	0.40	0.03	0.34	0.03	0.70	0.03	0.77	0.03
6	0.43	0.03	0.39	0.04	0.61	0.04	0.81	0.05
7	0.44	0.04	0.41	0.04	0.58	0.03	0.74	0.02
8	0.45	0.03	0.41	0.05	0.51	0.04	0.66	0.02
9	0.48	0.03	0.41	0.04	0.45	0.03	0.59	0.02
10	0.48	0.03	0.45	0.04	0.39	0.02	0.53	0.02

Additional Appendix.2: Percent organic carbon (%C) for 4-year (2011), 5-year (2012), 10-year (2011), and 11-year (2012) in top 10 cm of sediment from sampling in 2011 and 2012.

Averages for each variable were calculated in 1-cm intervals until 10 cm depth and error bars indicate standard error. 4-year and 5-year averages are from the same sites in Hog Island Bay. 10-year and 11-year averages are from the same sites in South Bay.

