

ABSTRACT

Title of Dissertation: HYDROLOGY, SOIL REDOX, AND PORE-WATER IRON REGULATE CARBON CYCLING IN NATURAL AND RESTORED TIDAL FRESHWATER WETLANDS IN THE CHESAPEAKE BAY, MARYLAND, USA

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Tidal freshwater wetlands are key sites for carbon (C) sequestration and main component in the global C budget. The overall research objective of my dissertation was to examine the physical and biogeochemical processes that impact C cycling in tidal freshwater wetlands. One natural and one restored tidal freshwater wetland (salinity < 0.3 ppt) were selected in Maryland, USA along the Patuxent River. Data logging water recorders were installed in wells at each habitat in February 2014 for monitoring water level at 10-minutes interval and for two years. Soil organic matter and C stocks were estimated and a novel soil C bioassay (CARBIO) was developed and tested to assess C stability (change of soil organic matter concentration over time) and decomposition rates in both sites. A total of 162 CARBIO units were deployed in the natural and restored sites, and 81 were retrieved after 1 year while the others were retrieved after 2 years. Static chambers were used to quantify methane (CH₄) and carbon dioxide (CO₂) flux

rates during day and nighttime. My results indicated that the natural wetland had significantly higher soil C stocks than the restored site (14.8 ± 0.50 and 8.9 ± 0.99 kg C m⁻², respectively, $P < 0.0001$). The swamp habitat had the highest soil organic matter (36.8%), while restored mudflat has the lowest (2.8%). Higher soil organic matter was partially correlated with shallower groundwater level relative to soil surface. Soil redox data with soil pH indicated that the soil of the natural wetland habitats was more reducing than the soil at the restored habitats. Based on CARBIO index, the soils in CARBIO units that were deployed in the natural wetland was significantly higher in C sequestration rate than the restored wetland (535 ± 291.5 and -1095 ± 429.4 g C m⁻² year⁻¹, respectively, $P_{\text{site}} < 0.05$). Under the current hydrological conditions, the restored wetland habitats were not able to accumulate C inside the CARBIO units after 1 or 2 years from deployment. In-situ CARBIO units can be employed in the newly constructed wetlands as in-situ sensors that reflect the C biogeochemical processes in the ambient soil to help better understanding C stability. The restored wetland had significantly higher annual CH₄ emission rates than the natural wetland (1372.1 ± 35.89 and 880.7 ± 144.73 g CH₄ m⁻² y⁻¹, respectively, $P < 0.05$) and the log CH₄ flux rate had a significant and strong negative correlation with the pore-water total available iron. Nighttime CH₄ fluxes had very low concentration (< 3650 μmole m⁻² h⁻¹). Future restoration efforts should focus on soil properties that will help increase C accumulation in newly constructed wetlands, but even more important every effort should be made to conserve the natural wetlands so that ecosystem function and services including wildlife habitat, water quality improvement, and offsetting the greenhouse gas emissions are maintained.

HYDROLOGY, SOIL REDOX, AND PORE-WATER IRON REGULATE
CARBON CYCLING IN NATURAL AND RESTORED TIDAL FRESHWATER
WETLANDS IN THE CHESAPEAKE BAY, MARYLAND, USA

by

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Dedication

I dedicate this dissertation to my parents for their love, endless support, and without whom none of my goals would be possible. I would also love to dedicate this dissertation for my wife for her encouragement and patience. Thank you for your support and unending love. Moreover, I would like to dedicate this dissertation to my son. You have made me stronger, better, more optimistic than I could have ever imagined.

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1 Introduction

Wetlands are transitional systems that occur intermediately between terrestrial ecosystems and aquatic ones, and occur in areas where soils are artificially or naturally inundated or saturated with ground or surface water during part or all of the year (Mitsch and Gosselink, 2000; Mitsch and Gosselink, 2007). Wetlands deliver a variety of ecological services and functions including C sequestration, storm water storage, and waste water treatment (Mitsch and Gosselink, 2007). Wetlands have a unique geographical setting as transitional ecosystems between aquatic and terrestrial ecosystems, and have been described as the “kidneys of the landscape” because of their role in improving water quality. Five to seven % of the world surface area is covered with wetlands and thus wetlands are key sites for global C budget. In the last centuries, many wetlands have been lost as a result of human impacts including converting large areas from wetlands to agricultural lands and urban areas. Large portions of coastal wetlands have been lost due to sea level rise (Nicholls, 2004). Wetlands have been and are still being drained in some parts of the world, however, wetlands are increasingly being restored, conserved, and protected.

At the upper end of the estuary, freshwater wetlands are located and they are less impacted with saltwater intrusion. Tidal freshwater wetlands are among the highest wetland ecosystems in plant productivity and plant species richness compared with brackish and saltmarshes (Baldwin et al., 2009). Horizontal vegetation zonation is a unique ecosystem characteristic for the tidal freshwater wetlands, which reflected in the existence of low and high marsh that are highly diverse in plant communities (Odum et al., 1984). Tidal fluctuations and the sedimentations are the two major physical processes

that control the development and the existence of the tidal freshwater wetlands (Baldwin et al., 2009; Barendregt et al., 2009; Odum et al., 1984).

The optimal goal, with no doubt, for restoring a wetland ecosystem is to make it capable of providing ecosystem functions and services. Water quality improvement, C accumulation, and wildlife habitats are amongst the top priorities for wetland restoration. Wetland restoration involves vegetation establishment, hydrology, and hydric soils re-enhancement. Construction approaches in newly constructed wetlands include soil surface excavation, adding dredged sediment, and re-establishment of the hydrology (Baldwin, 2009). Climate change makes the future of wetland restoration more complex and hence, wetland restoration faces many challenges (Erwin, 2009), as wetlands are vulnerable to change in their hydrological regime (Ferrati et al., 2005). Coastal wetlands will be significantly impacted by climate change (Poff and Hart, 2002), including changes in rainfall and major storms from hurricanes.

Anaerobiosis in wetland soils is considered the fundamental factor slowing or resulting in incomplete decomposition of dead plant materials, resulting in accumulation of organic matter (Kayranli et al., 2010). According to the enzymatic latch hypothesis (Freeman et al., 2001; Romanowicz et al., 2015), soil organic matter accumulates in wetlands for two main reasons: 1) microbial anaerobic decomposition pathways yield less energy than aerobic pathways, and 2) extracellular enzyme activity is reduced when oxygen is lacking. Because wetlands have the ability to sequester C from the atmosphere (Han et al., 2010; Xiaonan et al., 2008), enhancing C sequestration through wetland protection and restoration may offset accumulation of greenhouse gases in the

atmosphere. Wetlands store more C per unit area than upland soils, meaning that wetland conservation and restoration may offset the accumulation of greenhouse gases.

Globally, terrestrial and atmospheric C are stored inside the oceans in various forms of organic and inorganic C (IPCC, 2013). Soils store significantly more C than the atmosphere and plants combined (Schlesinger, 1991; Sommer and de Pauw, 2011). Soil C includes the C stored in forests, peatlands, permafrost, and wetlands. Soil C stocks represent a significant pool for the global C cycle. Those stocks result from the balance between the inputs and outputs of C. Soil C inputs are mainly from dead plant materials and other root detritus, while soil C outputs include gas fluxes (like carbon dioxide (CO₂) and methane (CH₄)) and leaching of the dissolved organic C into deep and shallow groundwater (Davidson and Janssens, 2006; Davidson et al., 2000). Wetlands store 975 Pg C (Bridgham et al., 2006; Maltby and Immirzi, 1993). Thus, wetlands can be considered an important C sink. Wetlands have been recognized for their capability to store C in their soils and they are high in productivity as well (Mitsch and Gosselink, 2007). Globally, soil C pools for current wetlands are estimated at 513 Pg C, including 215 Pg C for North American wetlands (Bridgham et al., 2006). The sequestered C inside wetland soils may be released back to the atmosphere quickly if these wetlands encountered disturbance in their hydrology regime or under high temperature scenarios (IPCC, 2007).

Soil C sequestration is the net accumulation of plant-fixed C as soil organic matter (Lal, 2004; Lal, 2008). One of the environmental issues that has gathered attention recently is promoting new techniques to offset the emission of CO₂ to the atmosphere that would otherwise contribute to global warming. Implementation of

measures to sequester atmospheric CO₂ may contribute fundamentally to meet the Kyoto Protocol (Schlesinger, 1999), an international agreement to reduce greenhouse gas emissions. Recently, the term “blue C”, coined by the United Nations Environmental Program (UNEP) in 2009, has been widely used to describe CO₂ sequestration in coastal systems. Living organisms store C as biomass in oceans and coastal ecosystems including mangroves, salt marshes, coastal wetlands, and seagrass beds (Kuwae et al., 2016; McLeod et al., 2011; Pendleton et al., 2012; Siikamaki et al., 2013).

The overall research objective of my dissertation was to examine the physical and biogeochemical processes that impact C cycling in tidal freshwater wetlands. This was achieved through three related studies presented in chapters 2, 3, and 4. Chapter two was an observational study where I investigated the differences in the hydroperiod (variation of surface and ground water level relative to soil surface over time) between different habitats in the natural and the restored wetland habitats. The natural site has five habitats (mudflat, low marsh, high marsh, swamp, and upland), while the restored site has similar habitats to the natural site but without the swamp habitat. Moreover, I quantified C stocks in both sites and investigated the correlation between the hydroperiod and C stocks, where the impact of hydrology on soil organic matter was investigated. Chapter three describes an experimental study with an ultimate goal to develop a novel method to assess the C stability and decomposition rate between the natural and the restored wetland habitats. In the study presented in Chapter three, I developed and tested a novel field C bioassay (CARBIO) to estimate C sequestration rate across different habitats in the natural and the restored site. Chapter four describes an observational study where I quantified the flux rate of CH₄ and CO₂ during day and nighttime from low marsh, high

marsh, and the swamp habitat in the natural site, while flux rates were estimated in low and high marsh for the restored site. Moreover, I examined the effect of pore-water iron on the flux rate of CH₄ and CO₂ for the natural and restored habitats.

2 Soil Redox and Hydropattern control Soil Carbon Stocks across different habitats in Tidal Freshwater Wetlands in a Sub-estuary of the Chesapeake Bay

Abstract

Wetlands contain spatial and temporal variations in hydrology that affect vegetation and soil processes. In this study different wetland habitats were identified in both a natural and restored wetland site that varied in hydropattern (level of surface or ground water over time), with the goal of understanding how inundation impacts redox conditions and soil organic matter. Tidal freshwater wetlands were selected in Maryland, USA along the Patuxent River, a Chesapeake Bay tributary. Five habitats (mudflat, low marsh, high marsh, swamp, and adjacent upland) were selected at Patuxent Wetland Park, a natural wetland, and four habitats (mudflat, low marsh, high marsh, and adjacent upland) were selected at Wootons Landing Wetland Park, a restored wetland. Within each habitat three randomly located plots were established, and a data logging water level recorders were installed at one plot per habitat in February 2014 to monitor water level at 10-minute intervals. Water level depth was also measured manually in two additional observation wells within plots every two weeks for one year from February 2014 to March 2015. Soil cores to a depth of 50 cm were collected and soil C stocks were calculated based on soil bulk density and C percentage. Natural wetland habitats had shallower groundwater than their restored counterparts. Mudflats in both sites were most frequently flooded, followed by marsh and swamp habitats in the natural site. The restored high marsh that was dominated by *Phragmites australis* had the highest soil redox measurements at 12.5 and 40 cm soil depth (273 ± 27 and 252 ± 33 mv, respectively). Soil organic matter concentrations were significantly higher in the natural site compared

to the restored wetland. For example, the high marsh soils in the natural wetland had 31 % soil organic matter, but the high marsh at the restored wetlands had 4% soil organic matter. Soil C stocks were also significantly higher in the natural compared to the restored wetland (14.8 ± 0.50 and 8.9 ± 0.99 , respectively, $P < 0.0001$). Restored mudflat and marsh habitats had similar hydrological regime compared to the natural counterparts, but they had lower soil C stocks. Monitoring of hydrology and vegetation in similar habitats in restored and reference sites may help improve restoration success in achieving specific structural or functional outcomes. Promoting the accumulation of soil organic matter in the restored wetland is not only controlled by the hydropattern, but also by the soil redox conditions that are impacted by the invasion of *Phragmites australis*.

2.1 Introduction

Coastal wetlands provide fundamental ecological services and functions including carbon (C) sequestration, storm water storage, and waste water treatment (Mitsch and Gosselink, 2007). Wetlands cover about 5 to 7 % of the world's surface area and have high plant productivity and slow decomposition rates, and thus play a significant role in global C cycling (Neue et al., 1997a). Large wetland areas have been lost, however, and still more wetland loss is projected due to land conversion and sea level rise (Nicholls, 2004). Anaerobiosis in wetland soils is considered the fundamental factor in slowing rates and causing incomplete decomposition of dead plant materials, resulting in the accumulation of soil organic matter (SOM) (Kayranli et al., 2010). Because wetlands have the ability to sequester carbon dioxide (CO₂) from the atmosphere (Han et al., 2010), enhancing the C sequestration function through wetland protection and restoration could offset greenhouse gas (GHG) emissions.

Hydrology is considered the master variable for wetland ecosystems (Mitsch and Gosselink, 2007). First, flooding frequency and duration shapes plant communities by restricting plant growth to those species adapted for wet conditions. Secondly, hydrology shapes physical and chemical soil properties particularly bulk density, nutrient availability, and pH. Moreover, hydrology influences the soil biota including soil microbes which differ in composition compared to upland and aquatic ecosystems (Herbert et al., 2015).

Soil organic matter generally increases with more frequent inundation (Tanner et al., 1998). Soil C pools are the result of the balance between C inputs and outputs. Variables that affect these pools include the ratio of above and belowground biomass and C sedimentation rates (Marchio et al., 2016). The vertical distribution of SOM is associated with vegetation types (Jobbagy and Jackson, 2000) which is affected largely by the hydrological regimes. Root distributions through soil impact the vertical placement of C in the soil and shoot to root ratio allocation controls the amount of C that stored in the soil. Perennial plant species like *Phragmites australis* have higher belowground biomass than annual plants like *Polygonum arifolium* and that impact the soil C pools.

Often a goal in wetland creation and restoration is achieving the correct hydrology and vegetation. Wetland hydrology is achieved in one of three ways: 1) excavate the upland soils to make it at the level of nearby water level or reaching the groundwater level; 2) adding soils to elevate the soil surface to be at the same level of adjacent river or ditch; and 3) connecting the wetland to an existing source of water like a stream or a river (Baldwin, 2009). Other hydrologic restoration techniques include plugging the drainage channels or scraping the surface of the wetland soil (Covington et al., 2003). Removing

upper soil layers in particular can have negative impacts on the soil C stocks (Fenstermacher et al., 2016a; Stolt et al., 2000).

Higher organic matter and successful plant species richness are good indicators for successful wetland restoration. Unfortunately, many research studies have reported low SOM in restored wetlands compared to their paired natural wetlands; for example: 11.8% in restored vs 28.9 % in natural (Bruland and Richardson, 2006), 2.8% in restored vs 7.2% in natural (Campbell et al., 2002), and 5.8% in restored vs 9.8 % in natural (Shaffer and Ernst, 1999). Moreover, lower plant species biodiversity had been reported in restored wetlands compared to the reference wetlands (Sheldon et al., 2016)

Located in the upstream end of the estuary, tidal freshwater wetlands have higher species richness and receive higher rates of sediments and nutrients than downstream salt marshes. They also exhibit distinct vegetation zonation due to differences in flooding frequencies and surface elevation (Baldwin et al., 2009). These unique characteristics are key factors in developing different habitats including mudflat, low marsh, high marsh, and swamps. Unfortunately, due to their locations in the upper estuary many have been heavily impacted by human activities that lead into their degradation (Barendregt et al., 2009), although there are also extensive efforts in locations such as Washington DC, USA to restore these types of wetlands. My research objectives were to: 1) assess differences in hydropatterns (the variation of surface and ground water level relative to soil surface over time) and soil C stocks between different habitats in a natural and restored wetland and 2) investigate the relationship between hydropattern, soil redox, and SOM accumulation across the different habitats with different hydrological regimes. I hypothesized that the percentage of SOM would be correlated with the hydropattern

across different habitats within the wetland. We also hypothesized that the natural wetland habitats would have lower soil redox, higher soil C stocks, and increased plant species diversity compared to habitats within the restored site.

2.2 Methods

2.2.1 Study site description

The study sites were located along the Patuxent River, a sub-estuary of Chesapeake Bay that is located entirely in Maryland USA. The Patuxent River has a watershed of 2290 km² plus 120 km² for its estuarine tributary and is considered the sixth largest tributary of the Chesapeake Bay (Boynton et al., 2008; Seldomridge and Prestegard, 2014; Williams et al., 2006). The watershed received 10.8±7.60 cm monthly average precipitation during 2015 and 8.7±4.66 cm during 2016 (MEAN±STD) according to Baltimore weather station (NOAA, 2017). For the time frame of 1951-1990, the mean freshwater flow in Patuxent River was 11 m³ s⁻¹ according to the United States Geological Survey (USGS) gauging station located at Bowie, MD (Magnien et al., 1992). The Patuxent River classified as micro-tidal (tidal range of 0.5 m) (Monbet, 1992). Salinity in the study area ranges from 0.15 – 0.25 Practical Salinity Unit (PSU).

Tidal freshwater wetlands were selected along the Patuxent River. Five habitats (mudflat, low marsh, high marsh, swamp, and upland) were selected at Patuxent Wetland Park, a natural wetland, and four habitats (mudflat, low marsh, high marsh, and upland) were selected at Wootons Landing Wetland Park, a restored wetland (Figure 2.1). The Wootons Landing restoration was completed at 1998, where their hydrology was restored by excavating or scraping (Siciliano, 2013). The soils series at the restored site prior to

restoration were classified as Udorthents (reclaimed gravel pits). The soils series at natural site were classified as Mispillion and Transquaking (Loamy, mixed, euic, mesic Terric Sulphemists) in the mudflat, low marsh, and high marsh; and Evesboro-Galestown (Mesic, coated Lamellic Quartzipsamments) in the upland (Natural Resources Conservation Service, 2017).

At each habitat, three random plots were selected resulting for a total of 27 plots. Selection criteria for plots were: 1) not to be located in a disturbed area; 2) not to be located in a middle of a creek or a ditch; 3) to be at least 15 meters from adjacent plots; and 4) to be randomly selected. High and low marshes in the restored wetlands were primarily monocultures, with *Phragmites australis* and *Pontederia cordata*, respectively. In the natural site, the first dominant species was *Ceratophyllum demersum*; *Nuphar lutea*; and *Polygonum arifolium* in the mudflat, low marsh and high marsh, respectively. To access the sites, permission was granted by private landowner for the swamp habitat, while the rest of the habitats were accessed by permission of the Jug Bay Wetlands Sanctuary.

2.2.2 Hydrology

In February 2015, at each habitat, a slotted and screened PVC well (SCHEDULE 40: OD 8.9 cm diameter, 2.41 m length and 0.01 mm slot size) was installed 1 m below the soil surface. Each well had a water level data-logger (Odyssey Capacitance Water Level Logger, Dataflow Systems Limited, New Zealand) that took a measurement every 10-minutes for two years, from February 2015 to February 2017. Water level data were downloaded frequently from the data loggers at the field sites using Odyssey data logging software and a field laptop. In addition, at each habitat, two additional observation wells

(SCHEDULE 40: OD 5.08 cm diameter, 1.5 m length and 0.01 mm slot size) were installed 0.75 m below the soil surface for measuring the water level manually every two weeks for 12 month, from February 2015 to March 2016. Manual sampling was conducted as follows: at the observation wells, a stainless steel solid messenger (WILDSCO corporation, Yulee, Florida USA) attached to an electrical wire was dropped in the well. The electrical wire was connected to a multi-meter (Radioshack, Fort Worth, Texas) and the water level was recorded once the electric circuit was completed. Water levels relative to soil surface were combined with data logger values in a linear regression analysis (Appendix A.1). The cumulative percentage of water level relative to soil surface was calculated and four hydrological zones were identified: 1) mudflat; 2) low marsh; 3) high marsh and swamp; and 4) uplands. All the wells were covered with filter fabric to prevent the slots becoming clogged with soil, and were maintained for proper continuous performance.

2.2.3 Soil

In February 2016 at each habitat, three random 50-cm soil cores were collected using a peat sampler 52 mm in diameter and 50 cm long (Eijkelkamp Soil and Water Corporation, Netherlands). Soil samples were sectioned into 5 cm increments in plastic bags and stored under 4 °C to minimize microbial activity until analysis (Bernal and Mitsch, 2008).

At each plot, a pilot hole was made using a stainless-steel rod and three platinum (Pt) electrodes were installed at 12.5 cm and three more at 40 cm. The soil oxidation reduction (Eh) measurement was recorded using a multi-meter modified with high resistance in conjunction with a calomel reference (Rabenhorst, 2009; Rabenhorst et al.,

2009). Soil samples were dried at 40 °C to a constant weight to estimate the soil bulk density. Subsequently they were ground and total C analysis was performed (Marchio et al., 2016; Wilson et al., 2009). Soil bulk density was estimated for each 5 cm soil section (i) by the core method (Black and Hartge, 1986; Elliott et al., 1999; Wilke, 2005), dividing soil oven dry weight (g) by the volume of the 5 cm section (cm³) according to the following equation:

$$SBD_i = \frac{m_i}{v_i}$$

where SBD_i: soil bulk density (g/cm³), m_i: oven dry weight (g), and v_i: volume (cm³) of soil section (i). The soil was tested for having inorganic C by treating the soil with 10 % HCl and looking for CO₂ bubbles under a dissecting microscope (Balduff, 2007); no bubbles were detected. The total C concentration % for each section (i) was determined by CHN method using a LECO CHN-2000 analyzer (LECO Corporation, St. Joseph, Michigan). Total C mass for each section (i) was determined by multiplying soil bulk density, total C concentration, and volume (Liu et al., 2014; Zabowski et al., 2011) according to the following equation,

$$TCM_i = SBD_i \times TCC_i \times V_i$$

where TCM_i: total C mass (g C), SBD_i: soil bulk density (g/cm³), TCC_i: total C concentration (% C g dry weight of soil), and V_i: volume (cm³) for section (i). Total C Stock to a depth of 50 cm (TCS) (g C) was determined according to the following equation (Han et al., 2010; Liu et al., 2014),

$$TCS = \sum_{i=1}^n TCM_i$$

where TCS: total C stock (g C) of the soil to a depth of 50 cm, TCMi: and total C mass (g C) for section (i).

2.2.4 Vegetation and above-ground biomass

Nomenclature for plant species followed United States Department of Agriculture Plants Database and was accessed on August 16, 2015 (USDA, 2017). Plant herbarium sheets were prepared (Smith, 1971) for each plant species and placed in the laboratory herbarium of A.H. Baldwin at University of Maryland, College Park, USA. In August 2015 at each habitat, three plots were randomly selected and six quadrats (0.25 m²), two per each plot, were established for aboveground biomass harvesting. All the plant vegetation biomass per quadrat was clipped at the ground level and separated by plant species. All the plant materials were oven-dried (40 – 60 °C) to a constant weight so total biomass could be calculated (Little, 2013). In the swamp and upland, six burlap leaf litter traps were installed in each habitat to reflect the biomass influence from leaf fall. Leaves were collected from leaf litter traps biweekly from August 2015 to January 2016.

2.2.5 Statistical analysis

Repeated three-way analysis of variance (3-way ANOVA) was used to test the main effects and interactions of site, habitat, and depth on SOM and water level. Soil organic matter data were normally distributed and not transformed, but water level relative to soil surface data were log transformed. Arithmetic means and standard error (MEAN±SE) were used for presentation. Two-way ANOVA was used to examine the main effects and interactions of site and habitat on soil C stocks, followed by least significant difference between the means of soil C stocks according to Duncan test.

Linear regression was used for the relationship between automatic recorded and manually water level relative to the soil surface. Also, linear regression was applied to examine the relationship between observed and recorded water level. Pearson correlation coefficient analyses were conducted to examine the correlation between cumulative percentage of water level relative to soil surface and soil organic matter percentage. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina).

2.3 Results

2.3.1 Hydrology

Linear regression analysis showed that a strong correlation between the observed and recorded water levels for natural ($R^2 = 0.917$, $P < 0.0001$) and restored wetlands ($R^2 = 0.965$, $P < 0.0001$) (Appendix A.1). The different hydrological zones reflected surface elevation differences between habitats (Appendix A.2). In the natural wetland, swamp and high marsh were about the same ground surface elevation and they fell within the same hydrological zone 3 and they were 70 cm relatively higher than mudflat. In restored wetlands low marsh was 15 cm higher than mudflat.

The natural wetland had a shallower ground water level compared to the restored wetland (Appendix A.3 & Figure 2.2). The highest water level was 140 cm above the soil surface of the mudflat, while the upland had the lowest (-75 cm for natural and -90 cm for restored wetland, Appendix A.3). Cumulative percentage for water level relative to soil surface indicated that the mudflats in the natural and restored sites were the most frequently flooded habitat, followed by the swamp and marsh habitats; upland habitats were never flooded (Figure 2.2). Ninety percent of the time, the swamp and high marsh

habitats in the natural wetland had water levels relative to soil surface of 5 cm or below, while mudflat had 90 cm or below in natural wetland and 40 cm or below in restored wetland (Figure 2.2). Two years of continuous water level data are presented in appendix A.4. There were significant differences between sites, habitats, and depth (Table 2.1). The swamp habitat and high marsh had less water level daily fluctuation compared with mudflat and low marsh in the natural wetland (Appendix A.5).

2.3.2 Soil C stocks and redox measurements

Statistical analyses for soil organic matter indicated significant differences among sites, habitats, and depth (Table 2.1). Soil organic matter was higher in natural compared to the restored counterparts (Appendix A.6). Soil organic matter in the natural site was highest in swamp and decreased in the order high marsh > low marsh > mudflat > upland (36.8, 30.8, 21.5, 17.8, and 5.6 % respectively). For restored wetland habitats soil organic matter was highest in low marsh and decreased in the order high marsh > upland > mudflat (9.0, 4.0, 2.9, and 2.8 % respectively). Total soil C decreased with depth for the majority of habitats, e.g. total soil C for swamp habitat had 19.9% in the top 5 cm and decreased to 11.4% at 50 cm soil depth (Figure 2.3). The high marsh at the restored wetland, dominated by *Phragmites australis*, had higher total soil C than low marsh, dominated by *Pontederia cordata* within the first 15-20 cm only (Figure 2.3). Soil bulk density for natural wetland habitats was lower than soil bulk density in the restored counterparts (Appendix A.6). Soil bulk density for natural wetland habitats was highest in upland sites (1.053 g cm⁻³) and lowest in the swamp (0.202 g cm⁻³). At the restored wetland the upland was also greatest (1.215 g cm⁻³). Soil bulk density increased with depth for the majority of habitats (Appendix A.7).

Total C stocks were significantly higher in the natural wetland than in the restored wetland (14.8 and 8.9 kg C m⁻², respectively, P site < 0.0001) (Figure 2.4). Total C stocks for low and high marsh in the natural wetland (16.0 and 16.1 kg C m⁻², respectively) were significantly higher than their counterparts habitats in restored wetland (12.3 and 5.7 kg C m⁻², respectively) (P habitat = 0.0474). Total C stocks varied by depth, with most C stocks increasing to a depth of 20-25 cm and then decreasing (Appendix A.8). Soil organic matter positively correlated with flooding percentage (r = 0.509 for natural wetland and r = 0.229 for restored wetland at P<0.05) according to Pearson correlation coefficient analyses.

The natural wetland soils were more reducing than the restored wetland soils for both 12.5 and 40 cm soil depth (Figure 2.5). The high marsh at the restored wetland which was dominated by *Phragmites australis* had the highest soil redox measurements at both 12.5 and 40 cm soil depth (273±27 and 252±33, respectively). At the natural wetland, soils had lower redox measurements at 40 cm than 12.5 cm soil depth for low and high marsh, although for the swamp habitat and the restored low marsh soils had higher redox measurements at 40 cm than 12.5 cm soil depth.

2.3.3 Above-ground biomass and vegetation composition

Above-ground biomass for the restored wetland was significantly higher than the natural wetland (1002.3±421.33 and 288.3±79.85 g dry weight m⁻², respectively, P < 0.0001, figure 2.6). The restored high marsh which was dominated by *Phragmites australis* had the highest above-ground biomass (3099.1±925.80 g dry weight m⁻²), while the restored mudflat had the lowest (41.3±15.45 g dry weight m⁻²). The natural upland had higher leaf litter input than the restored upland (474.5±17.36 and 236.9±118.48 g dry

weight m^{-2} , respectively). The natural wetland had more plant species diversity than the restored (30 and 14 plant species, respectively) (Figure 2.7), with a total of 33 plant species identified at both sites (Appendix A.9). High marsh in the natural wetland had the largest number of plant species (11, figure 2.7), while low and high marsh in the restored wetland had only one plant species each (*Phragmites australis* and *Pontederia cordata*, respectively), indicating that most of the restored wetland habitats were a monoculture plant community. Mudflats, for the natural and restored wetland, were closest to the creek and hence were 100% flooded, supporting the colonization of submerged aquatic vegetation. In the natural wetland, *Ceratophyllum demersum* was the dominant submerged species in the mudflat, while *Hydrilla verticillata* was the dominant for the mudflat at the restored wetland.

2.4 Discussion

The tidal freshwater wetland habitats, located along the Patuxent River, differed significantly in many basic variables like geographical settings, plant community structure, and spatial settings (e.g. distance from the tidal creek). Both of the study sites (natural and restored) were affected by daily tidal fluctuations. Due to different geographical setting, habitats had different hydropatterns. In the swamp habitat, hollows and hummocks were a characteristic topographic setting that had a significant effect on the hydropattern. The swamp habitat was at higher elevation than the mudflat (based on data presented in Appendix A.2), however it was flooded 70% of the time, a condition that likely played a role in high SOM accumulation (36.8 %). Moreover, the swamp habitat has no slope and very low hydraulic conductivity, implying very slow movement

of water. The swamp habitat, located farther from the creek, received less sediment than mudflat, likely contributing to soil being more organic rather than mineral.

Soil redox at 12.5 cm at the natural site was the lowest in the swamp habitat compared to low and high marsh (118 ± 35 , 169 ± 22 , and 240 ± 13 , mV respectively). Increased flooding frequencies results in a lack of available oxygen diffused to the soils, slowing or resulting in incomplete decomposition of dead plant materials, resulting in accumulation of SOM (Kayranli et al., 2010). The swamp habitat was located 90 m from the creek and it was affected by daily tidal fluctuation (Appendix A.5). After the swamp habitat gets flooded, surface water travels back to the creek, while the subsurface water stays for a longer time captured in soil between hummocks as it takes a longer time to reach the creek by diffusion or flow-through (Jackson et al., 2014).

Observed variations in soil C stocks between different habitats in the natural and the restored sites may be explained by spatial differences in habitats geomorphological setting affecting sedimentation. The quantity of sediment that a habitat receives is a key role in soil bulk density. Mudflats, for both natural and restored wetland, had the highest soil bulk density (0.364 and 0.865 g cm⁻³, respectively) after the uplands, while the swamp habitat had the lowest soil bulk density as it is the farthest from the creek for receiving sediment. Higher soil bulk density increases the chances for more soil particles to be readily available for coating with soil organic matter and forming more soil aggregates for storing more soil C stocks. Mudflats and marshes were higher in soil C stocks than the swamp habitat. Soil bulk density is negatively correlated with soil organic matter ($r=-0.695$, $p<0.0001$). Other research studies reported similar findings (Chaudhari et al., 2013; Curtis and Post, 1964; Sakin et al., 2011).

In general, the restored site had less soil C stocks than the natural site. Other research studies reported similar findings (Table 2.2). Restoration techniques and short time since restoration occurred are the main factors that lead into less C stocks in the restored site. Excavating the soil results in the loss of the rich C layer at the topsoil and exposing the subsoil that is very low in C content. Plant species diversity is another key factor for more soil C inputs with high quality litter characterized by low C/N ratio and high nitrogen concentration. Restored habitats were monoculture plant community, where the natural habitats were more diverse. On average, the restored wetland had aboveground biomass higher than the natural wetland, and that was not promoting the SOM accumulation in the restored wetlands. Soil texture might explain the reason for lower SOM in the restored wetland. Soil texture in natural wetland was silty clay in most of the habitats (chapter 3), while the restored sites had a higher sand content. Sand grains have larger particle size than silt and clay particles, while clay has larger surface area and high electrostatic force for accommodating more C (Ding et al., 2013a) to be stored compared with sandy soil. Moreover, adding clay (2-5%) to an incubated soil of freshwater marsh significantly decreased CO₂ production (Dodla, 2009), implying more C preserved within clay particles.

At the restored site, the soil was excavated to increase the hydroperiod (Siciliano, 2013). Many research studies report a negative impact on soil C stocks for restored wetlands where their hydrology was restored by excavation (Fenstermacher et al., 2016a; Stolt et al., 2000). Restoring the wetland hydrology by scraping or excavating the soil surface may not be recommended for future wetland restoration as the soil become

exposed to aerobic conditions that accelerate soil organic matter decomposition rate and hence, less soil C stocks.

Current study results concluded that more flooding frequency may enhance soil organic matter accumulation in natural wetland but not necessarily in the restored wetland. My hypothesis was that the natural wetland habitats had higher SOM than the restored wetland habitats. In support of that hypothesis restored wetland habitat had significantly less SOM. Many variables impact the soil C stocks including sediment input, productivity, hydrology, and decomposition rate. Our results showed that both the natural and restored site has similar hydrology as they located very close in the same watershed, but hydrology alone is not the main driving force for SOM accumulation. Soil texture, redox conditions and vegetation compositions are confounding factors for promoting the SOM accumulation in our sites. The restored high marsh which was dominated by *Phragmites australis* had the highest above ground biomass, however it had significantly lower C stocks than the natural high marsh which had higher plant species diversity. So, higher saturation levels, plant species diversity, and clay content at the soil of the natural high marsh might be the reason for having higher C stocks at the natural high marsh than the restored high marsh.

Freshwater marshes are among the highest ecosystems for net primary productivity (Keddy, 2010; Mitsch and Gosselink, 2007), as they are receiving higher rates of sediment than non-tidal wetlands, the main source of nutrients, and they have high rates of C use efficiency (Rocha and Goulden, 2009). At the natural wetland, low marsh are closer to the creek and receives more sediment than the swamp habitat, but the swamp habitat had a shallower groundwater compared to the marsh. That might partly

explain why the swamp habitat had higher SOM than low and high marsh. Moreover, the swamp habitat had lower soil redox measurement than the low marsh at 12.5 cm soil depth, and meaning that decomposition rates are slower at the swamp habitat. Previous research findings revealed valuable ecosystem services and functions for natural wetlands that include, but not limited to, plant species diversity, C sequestration, and higher soil C pools (Mitsch and Gosselink, 2007; Ricaurte et al., 2017). Not only do natural wetlands require conservation and protection, but also should be considered as a valuable model for more efforts for wetland restoration.

Common reed or *Phragmites australis* was the only plant species at the high marsh at the restored site. It is a perennial grass that occur in brackish and freshwater wetlands, and it has a huge belowground biomass as rhizomes from which new sprouts comes out as new shoots at the beginning of the growing season. It has a significant amount of aerenchyma, plant tissue that is responsible for the gas exchange, in stems, rhizomes, and roots. Those plant tissues as a kind of structural adaptation and other mechanical adaptations that prevent or inhibit the growth of other plant species make the common reed a very powerful invasive plant species and able to colonize easily in new habitats. Plant species richness in habitats dominated by common reed is very low compared to their counterparts. The unique and extensive aerenchyma for the common reed play a significant role in driving more oxygen down to the roots zone and that make the oxygen available for soil microbes that can be used to oxidize SOM. It therefore, makes sense that I observed less SOM percentage. Also, that gas exchange system through the stems-roots-rhizome-leaves make the pathway for any gases like CO₂ or CH₄

to be released to the atmosphere (Bernal et al., 2016), and again makes sense given that the restored high marsh had lower soil C stocks.

Wetland restoration is a key technique for wetland mitigations to secure potential wildlife habitats, wastewater treatment, and C sequestration. Future research should involve multiple restored sites that span different geohydrological settings and where hydrology has been restored with different restoration techniques. That will enable rigorously extrapolation of wetland hydrology restoration on soil C stocks. Moreover, invasion of common reed, *Phragmites australis*, to marshes should be addressed and taken in consideration as the common reed induces the soil organic matter priming by radial oxygen loss by roots and more aerobic condition and that will negatively impact the soil C stocks.

2.5 Conclusion

The natural wetland habitats had significantly higher SOM than their corresponding restored habitats. The swamp habitat had the highest soil organic matter (36.8%), while mudflat at the restored wetland had the lowest (2.8 %). Soil C stocks were significantly higher in natural wetland than the restored (14.8 and 8.9 kg C m⁻², respectively, P site < 0.0001). The swamp habitat at the natural wetland had the lowest soil redox at 12.5 cm soil depth and shallower ground water level that makes it the highest in soil organic matter, however, it had lower C stocks compared to the natural marsh. The restored wetland had lower soil organic matter and C stocks than the natural wetland in all four habitats and that might be a result of the way that the hydrology was restored. The natural wetlands had soils with more reducing conditions than the restored wetland soils at both 12.5 and 40 cm soil depth, and that is a key factor for making the

restored wetland lose more C with higher decomposition rates. Moreover, the loss of rich C layer at the topsoil as a result of the excavation of the restored site and exposing the poor C subsoil to the surface had a significant impact on lower C stocks at the restored wetland. Wetland restoration practice should take into account vegetation diversity, above-ground biomass, and establishment of reducing conditions in the soil.

Table 2.1: Results of repeated three-factor ANOVAs summarizing the effects of Site (Natural vs. Restored), Habitat (mudflat, low marsh, high marsh, swamp, and upland), and Depth (5 cm increment up to 50 cm soil depth) on the (a) soil organic matter % and (b) log flooding %.

| Source | Type 3 Tests of Fixed Effects | | | |
|-------------------------------------|-------------------------------|--------|---------|--------|
| | Num DF | Den DF | F Value | Pr > F |
| a) Soil organic matter % | | | | |
| Site | 1 | 17.7 | 55.48 | <.0001 |
| Habitat | 6 | 17.8 | 67.11 | <.0001 |
| Site*Habitat | 1 | 17.7 | 26.45 | <.0001 |
| Depth | 9 | 8.76 | 8.2 | 0.0024 |
| Site*Depth | 9 | 6.83 | 0.3 | 0.9507 |
| Habitat*Depth | 54 | 11.2 | 3.54 | 0.0126 |
| Site*Habitat*Depth | 9 | 6.83 | 0.22 | 0.979 |
| b) Log flooding percentage % | | | | |
| Site | 1 | 14 | 36.18 | <.0001 |
| Habitat | 6 | 12.9 | 44.53 | <.0001 |
| Site*Habitat | 1 | 14 | 35.63 | <.0001 |
| Depth | 9 | 137 | 8.63 | <.0001 |
| Site*Depth | 9 | 138 | 5.76 | <.0001 |
| Habitat*Depth | 54 | 137 | 5.2 | <.0001 |
| Site*Habitat*Depth | 9 | 138 | 6.13 | <.0001 |

Table 2.2: Comparison of soil C stocks (kg C m⁻²) in wetlands around the world.

| Country/Region | Nature of wetland ¹ | Wetland ¹ | Soil C stocks (kg C m ⁻²) | Core depth (cm) |
|-----------------------------|-----------------------------------|-------------------------|--|-----------------------|
| Natural wetlands | | | | |
| USA, Ohio | Nature preserve | Riverine - marsh | 1.1 | 35e |
| USA, Ohio | Nature preserve | Riverine - mudflat | 1.3 | 30e |
| Costa Rica | National protected zone | Riverine - flow through | 6.8 | 24a |
| Australia | Undisturbed | Mangrove | 9.4 | 20b |
| Mexico, Punta Gorda | Biosphere reserve | Coastal brackish marsh | 9.5 | 30c |
| Costa Rica | Nature preserve | Isolated - forested | 9.9 | 6a |
| Australia | Undisturbed | Saltmarsh | 13.0 | 20b |
| USA, Ohio | Nature preserve | Riverine - flow through | 14.3 | 36a |
| Costa Rica | National protected zone | Slow-flowing - slough | 15.3 | 54a |
| Belgium-Netherlands | Nature preserve | Freshwater - low marsh | 16.4 | 60f |
| Belgium-Netherlands | Nature preserve | Brackish - low marsh | 18.6 | 60f |
| Belgium-Netherlands | Nature preserve | Brackish - high marsh | 19.6 | 60f |
| China | Lake - water reserve | Freshwater Swamp | 19.7 | 30d |
| USA, Ohio | Nature preserve | Isolated - forested | 21.0 | 36a |
| USA, Virginia | Natural | Depressional wetland | 21.5 | 100g |
| Belgium-Netherlands | Nature preserve | Freshwater - high marsh | 21.7 | 60f |
| Mexico, Vigia Chico | Biosphere reserve | Coastal brackish marsh | 23.8 | 100c |
| Other than natural wetlands | | | | |
| USA, Delaware | Restored by scraping ² | Depressional wetland | 2.7 | 100g |
| Australia | Disturbed | Mangrove | 5.7 | 20b |
| Australia | Disturbed | Saltmarsh | 6.0 | 20b |
| USA, Maryland | Restored by plugging ³ | Depressional wetland | 6.1 | 100g |
| USA, Delaware | Prior converted cropland | Depressional wetland | 8.0 | 100g |

1: Nature of wetland, wetland type, and hydrology regime were quoted as it was described from the citation; 2: surface excavation; and 3: plug for the draining system.

a: (Bernal and Mitsch, 2008), b: (Howe et al., 2009), c: (Adame et al., 2013), d: (Huang et al., 2012), e: (Bernal and Mitsch, 2012), f: (Van de Broek et al., 2016), and g: (Fenstermacher et al., 2016b)

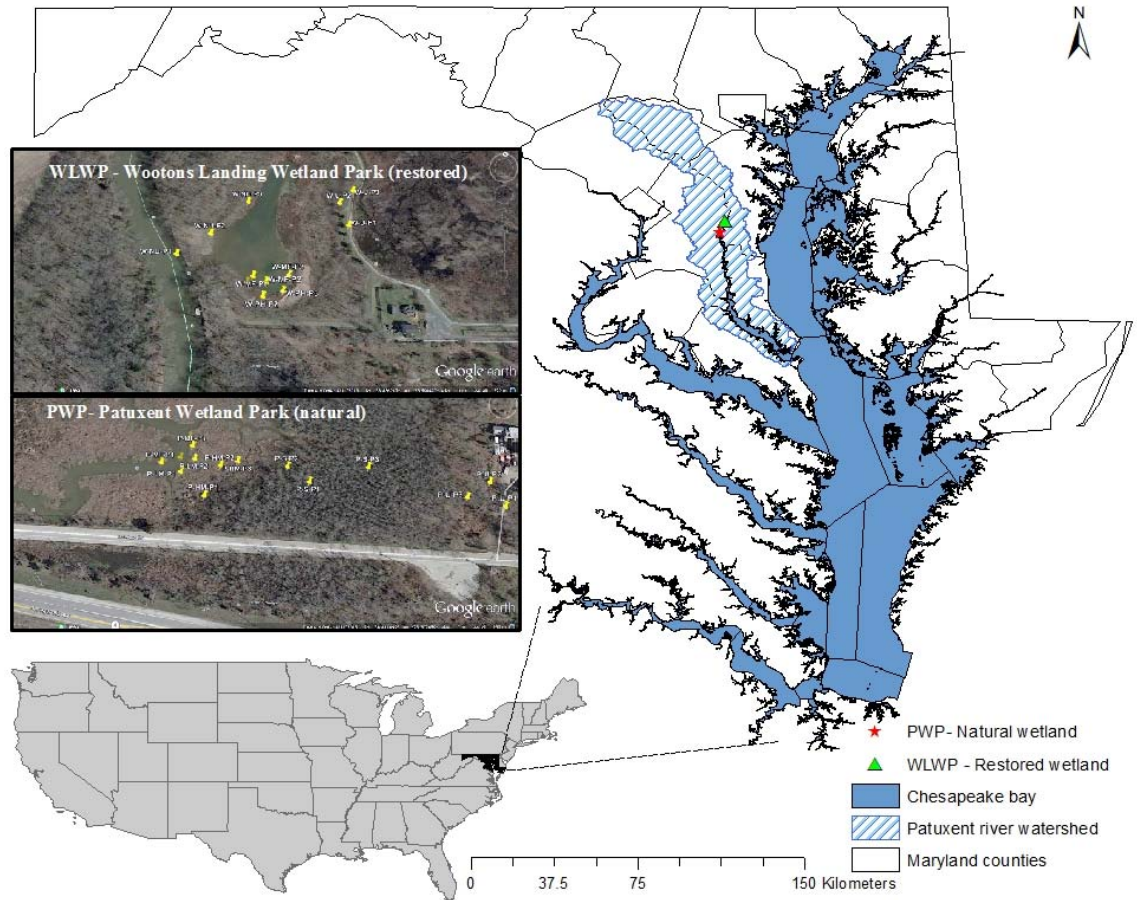


Figure 2.1: Study sites map showing Patuxent Wetland Park (PWP), a natural wetland and Wootons Landing Wetland Park (WLWP), a restored wetland. Maryland counties, Chesapeake Bay, Patuxent river, and Patuxent watershed were generated by ArcGIS 10.4 (Law and Collins, 2015), while site images were obtained from Google Earth (May 2017).

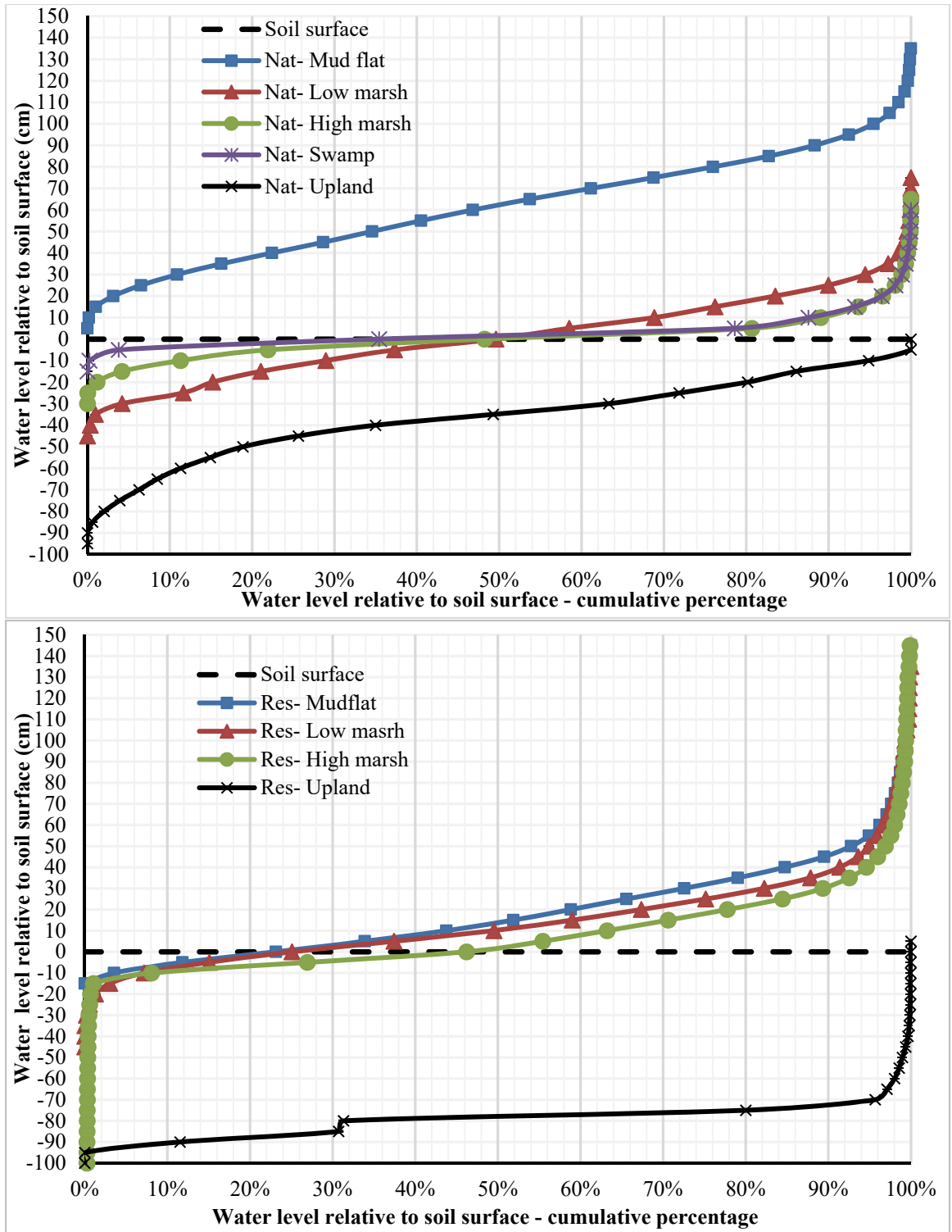


Figure 2.2: Cumulative percentage for water level relative to soil surface (MEAN±SE) for natural (top) and restored (bottom) tidal fresh water wetlands for two years (February 2015 to February 2017). MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland.

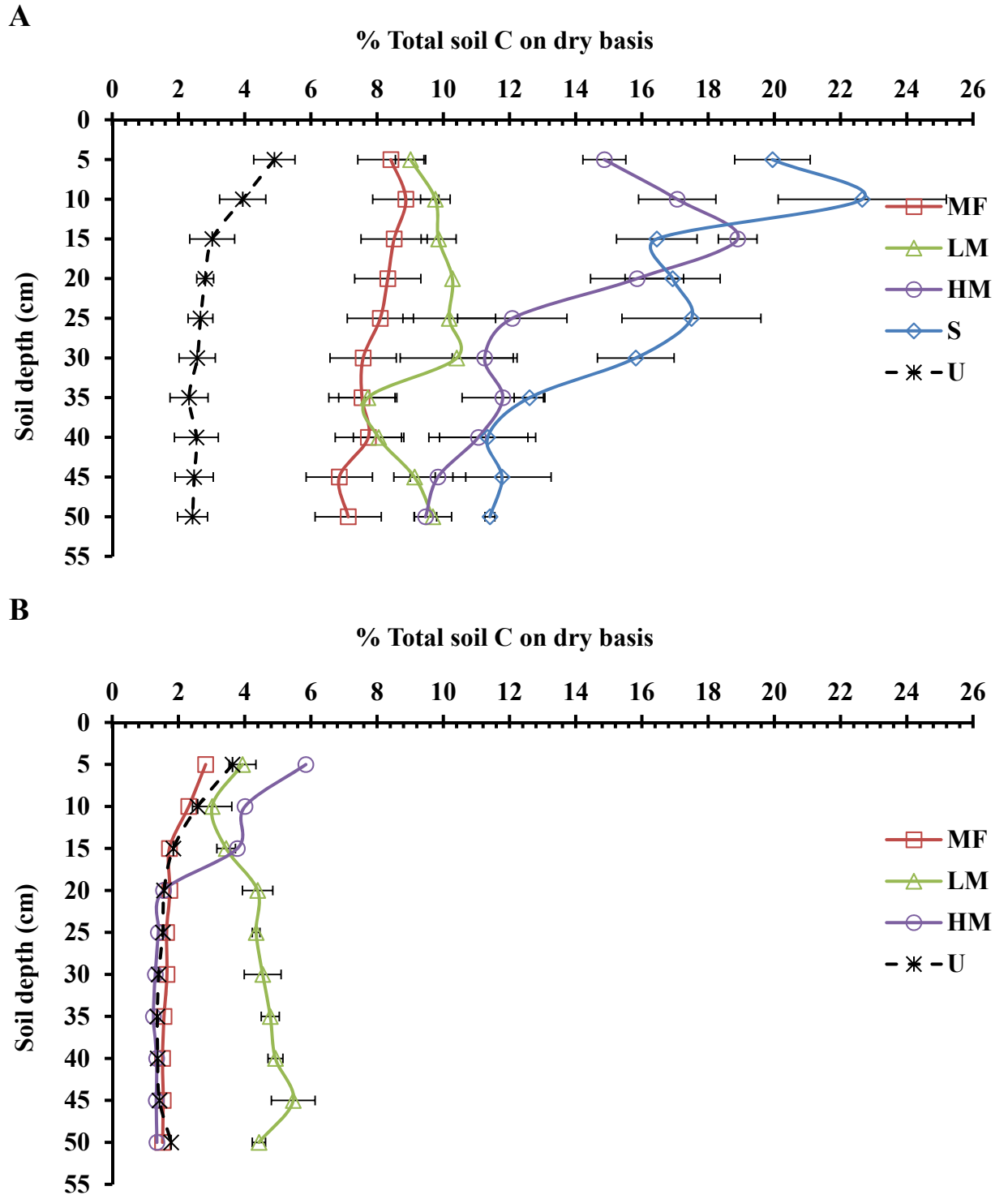


Figure 2.3: Distribution of total soil C % (mean \pm SE) over 50 cm soil depth in natural (A) and restored (B) tidal fresh water wetlands. MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland. Each data point represents 3 samples.

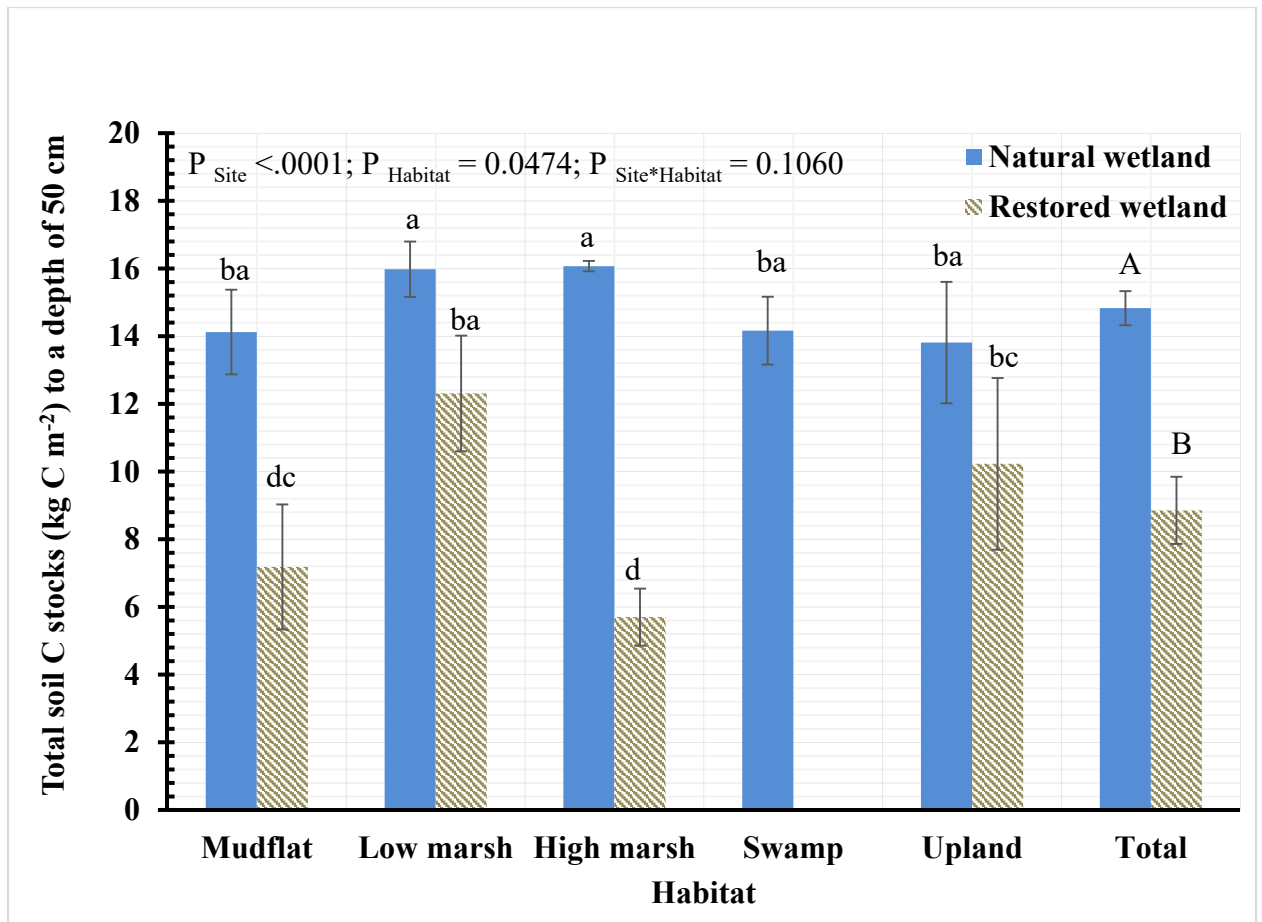


Figure 2.4: Total C stocks (mean \pm SE) kg C m⁻² up to 50 cm soil depth for natural and restored tidal fresh water wetlands. Means with different letters are significantly different at $P < 0.05$ after Duncan test. P values represent the results of two-factor ANOVAS summarizing the effects of Site (Natural vs. Restored) and Habitat (mudflat, low marsh, high marsh, swamp, and upland) on total C stocks. Each bar represents 30 samples.

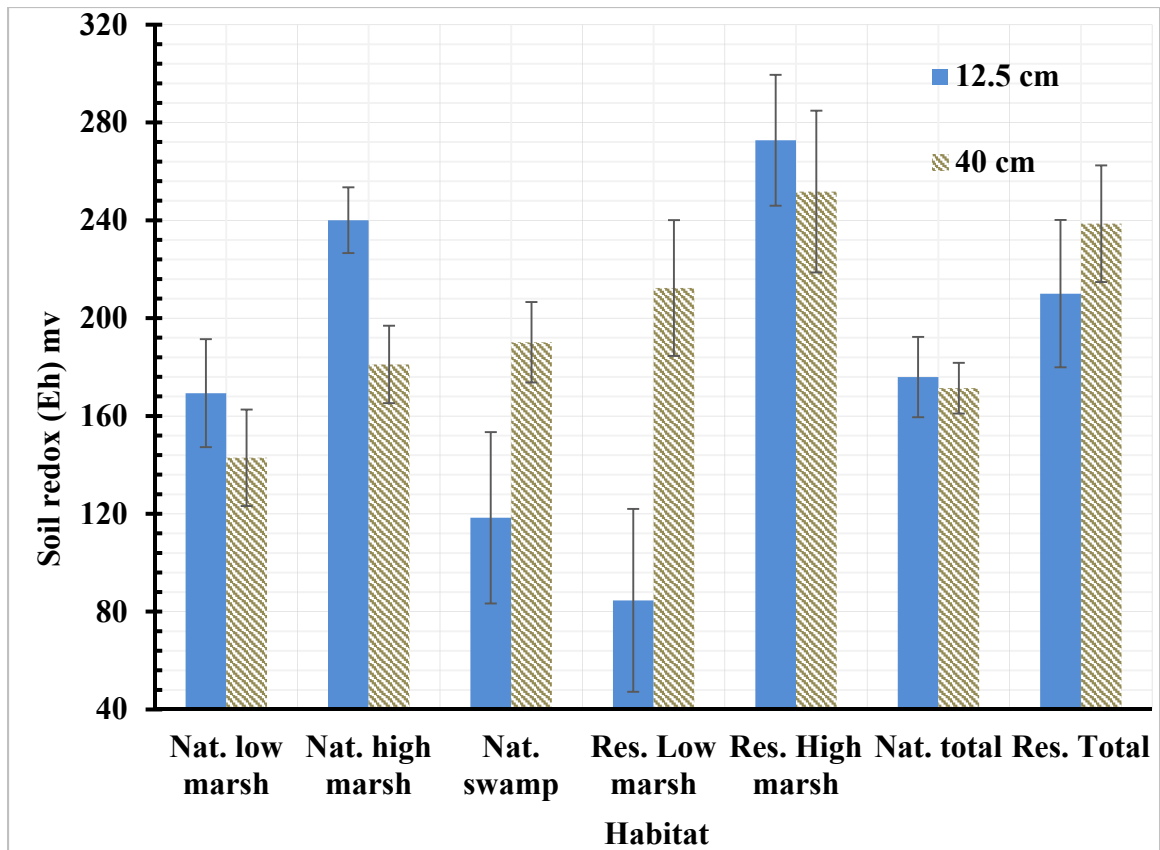


Figure 2.5: Soil redox (mv) (mean \pm SE) for 12.5 and 40 cm soil depth for natural and restored tidal fresh water wetlands. Each bar represents 9 samples.

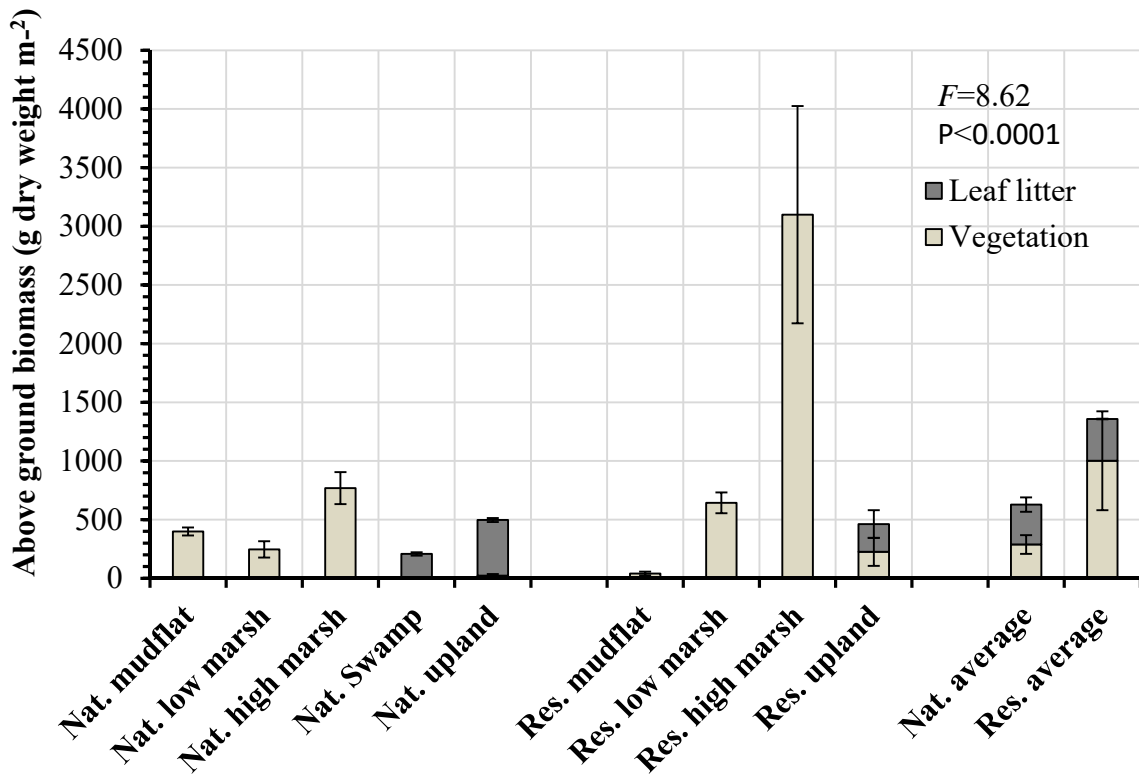


Figure 2.6: Above-ground biomass and leaf litter input (g dry weight m⁻²) (mean ± SE) for the natural (Nat.) and restored (Res.) wetlands. Each bar represents 6 samples.

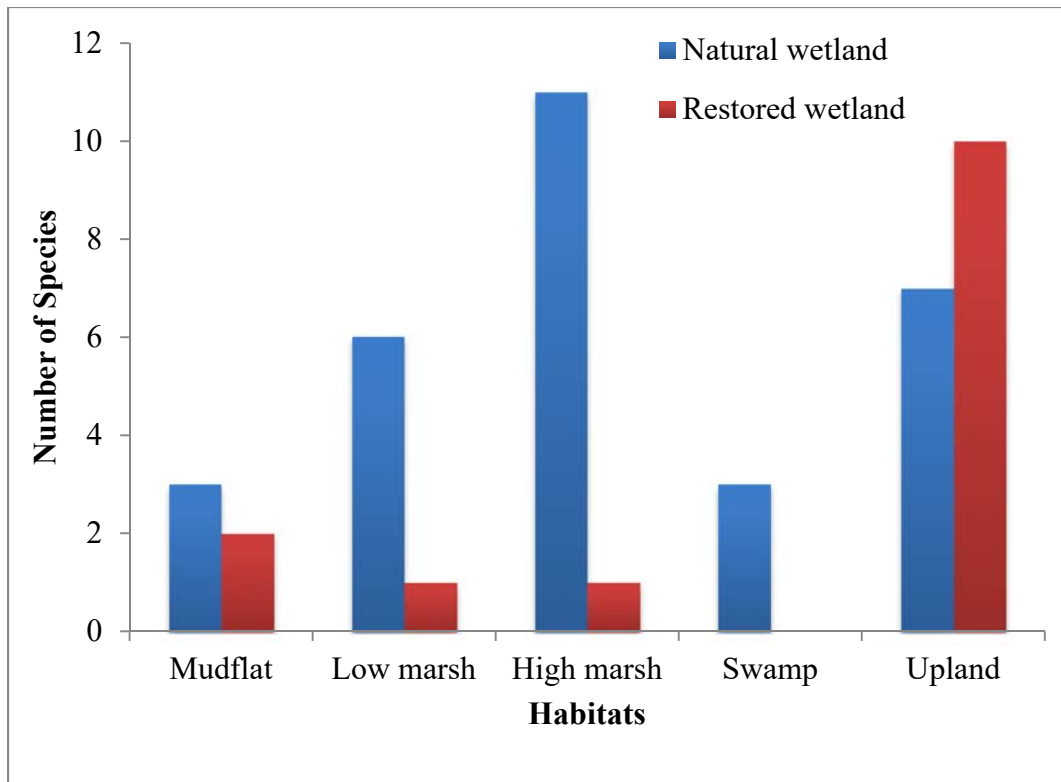


Figure 2.7: Number of plant species in each habitat for the natural and restored wetlands.

3 A Novel Method to Assess Soil Organic Matter Decomposition and Carbon Stability in Natural and Restored Wetlands

Abstract

One of the main objectives of wetland restoration is enhancing and promoting soil organic matter (SOM) accumulation, but it might take few decades for newly constructed or restored wetlands to build SOM as fast as the natural wetlands. The change of SOM concentration over time (referred to here as C stability) impacts soil C release, and consequently change Carbon dioxide (CO₂) concentration in the atmosphere. My goal was to develop a novel method and test an in-situ field bioassay using soil C bioassay cores (hereafter, CARBIO) as an index for C stability in restored and natural wetlands. I first created three types of bags (3.5 cm x 50 cm) by filling them with native wetland soil materials that were well homogenized and sieved. The pore size was 3.5 mm for the first bag and 1 mm for the second and the third bag, while the third bag was inside a slotted (0.01 mm) PVC pipe to maintain a proper hydrology. Five habitats (mudflat, low marsh, high marsh, swamp and upland) were selected at Patuxent Wetland Park, a natural wetland, and four habitats (mudflat, low marsh, high marsh and upland) were selected at Wootons Landing Wetland Park, a restored wetland. Both study sites are tidal freshwater wetlands (salinity < 0.3 ppt) located at the Patuxent River, a Chesapeake Bay tributary in Maryland, USA. One hundred and sixty-two CARBIO units were deployed in the different habitats for natural and restored wetlands in December 2014, with 81 retrieved after one year and processed to evaluate C sequestration rate and the second set retrieved after two years (February 2017). CARBIO cores were sectioned into 5 cm increment then

soil bulk density and percent of soil C were estimated. Based on the CARBIO index, the soil inside the bags that were deployed in the natural wetland sequestered $535 \pm 291.5 \text{ g C m}^{-2} \text{ year}^{-1}$, while the soil inside the bags that were deployed in the restored wetlands lost $1095 \pm 429.4 \text{ g C m}^{-2} \text{ year}^{-1}$ ($P_{\text{site}} < 0.05$). According to CARBIO index, the SOM decomposition rates varied between wetland habitats and between the natural and restored site. The results also show the importance of conserving the natural wetlands that play an important role in offsetting GHGs emissions. Under the current environmental and hydrological regimes for both sites and according to the CARBIO index, the soils inside the CARBIO that were deployed in the natural wetlands were able to accumulate C, but the ones that were deployed in the restored wetlands did not accumulate C, instead C content decreased in the CARBIO units.

3.1 Introduction

Wetlands have a significant role in global C cycle, but there are many challenges in C models: e.g. lack of the direct impact of the microbial activity on soil C pools (Allison et al., 2010; Treseder et al., 2012; Wieder et al., 2013), contradictory results between lab and field experiments (Conant et al., 2011), and uncertainty of the ability of current C models to predict responses of C pools to the warming climate (Friedlingstein et al., 2006). Integrating different aspects of C dynamics will help to better understand and model the global C cycle. Soil organic matter decomposition rate (Mueller et al., 2016), C use efficiency (Rocha and Goulden, 2009), net primary productivity (Reddy and DeLaune, 2008), and C sequestration rates (Belyea and Malmer, 2004; Bernal and Mitsch, 2012) are crucial components for modeling the global C cycle. The change of SOM concentration overtime (hereafter soil C stability) has a significant role in global C

cycle. Many research studies had recognized the importance of wetlands in the global C budget (Bridgham et al., 2006; Keller, 2011; Meng et al., 2016); however, the long-term experimental studies that investigate the differences in the C stability between natural and restored wetlands have less attention from researchers.

Wetlands have high primary productivity (Mitsch and Gosselink, 2007) and are key sites for C sequestration (Adame and Fry, 2016; Bernal and Mitsch, 2012; IPCC, 2007; McKee et al., 2007). Carbon accumulation in wetlands is temporally and spatially variable, however. These variations are controlled by nutrient inputs (Morris and Bradley, 1999), microbial biomass (Wooller et al., 2003), temperature (Kirwan and Mudd, 2012), sea level rise (Kirwan et al., 2013), rainfall (Adeolu et al., 2015), net primary productivity (Sjögersten et al., 2014), SOM decomposition rates (Malmer et al., 2005; Philippot et al., 2009), vegetation composition (Thormann et al., 1999), and salinity (Baustian et al., 2017; Morrissey et al., 2014; Neubauer et al., 2013). Tidal freshwater wetlands are located at the upperstream of the estuary with less impact from saltwater, but receive high amounts of nutrients and thus, play a key role in the global C cycle. Tidal freshwater wetlands have higher C pools than brackish and saltmarshes (Bridgham et al., 2006; Craft, 2007; Loomis and Craft, 2010; Reddy and DeLaune, 2008; Van de Broek et al., 2016). Many research studies had investigated C sequestration rates in tidal freshwater wetlands (Adame et al., 2015; Bernal and Mitsch, 2012; Drexler et al., 2013; Reddy et al., 1993), but the mechanism governing C stability in natural and restored wetlands are poorly understood.

The majority of the methods in use for evaluating and assessing C sequestration rate in wetlands are based on ^{137}Cs or ^{210}Pb radionuclide dating (Bernal and Mitsch,

2013a; Craft and Casey, 2000; Marchio et al., 2016; Preston et al., 2013; Teasdale et al., 2011; Ward et al., 2014; White et al., 2002). Radionuclide dating using ^{137}Cs is based on the assumption that there has been constant sedimentation rates since 1964, which might be problematic especially in coastal marshes. Other methods in use to evaluate C sequestration rate are based on assessing the soil C density and the sediment accumulation rate using surface elevation tables (Cahoon et al., 2002; Callaway et al., 2013; Marion et al., 2009). This method requires the verification of no vertical motion in the benchmark itself, an assumption which usually made, and does not account for C concentration directly. Calculation C concentrations and mass balancing C inputs and outputs is another way to estimate C sequestration rate (Kayranli et al., 2010; Mander et al., 2008; Mander et al., 2005), while some other studies used chambers to report C sequestration rates based on CO_2 exchange rate (Whiting and Chanton, 2001), which doesn't sufficiently reflect major biochemical process that involved in the C budget.

Leaf litter bags and decomposition strips are well known methods to assess relative decomposition rate (Ballantine and Schneider, 2009; Benfield, 1996; de Neiff et al., 2006; Hayes et al., 2017). They estimate the decomposition of labile C only (fresh litter inputs), which is not a sufficient representation for the wetland soils as the large proportion of C stored in the soil. A common C sequestration rate bioassay method uses leaf litter bags (Emery and Perry, 1996; Keuskamp et al., 2013; Lee and Bukaveckas, 2002) or peat (Kirwan et al., 2013), so, there was a need to develop an in-situ field bioassay that better represent and reflect the C of native soil materials. The soil materials that were used to fill the soil cores were native wetland soils from the study sites which is

more realistic than leaf litters or peat for assessing the ability of stored C to be retained or lost from a location.

There is a well-established relationship between mineral surface area and the soil organic matter (Mayer and Xing, 2001), and that is a crucial factor impacting the C accumulation rates. Mineral deposition can be important in C accretion rates (McCarty et al., 2009), where coating of the mineral surface areas might stabilize C compounds during SOM accumulation (Mayer and Xing, 2001; Sollins et al., 1996). The mesh size of decomposition bags is therefore important (Agoston-Szabo et al., 2016; Benfield, 1996; de Neiff et al., 2006; Gingerich et al., 2015), because some mesh will allow mineral deposits and others will not. Also important to consider for mesh size is the ability of roots to grow into cores. My research objective was to use a novel field bioassay to estimate C sequestration and decomposition rate for habitats within a natural and restored tidal freshwater wetland. My hypotheses were: 1) CARBIO units placed in mudflats, low and high marshes and swamp will accumulate C, but those in adjacent uplands will lose C; and 2) mesh bag size have a significant impact on C accumulation or loss in both natural and restored wetlands by excluding interactions with plant roots and excluding mineral inputs.

3.2 Materials and methods

3.2.1 Study site description and making CARBIO units

Tidal freshwater wetlands were selected in Maryland, USA along the Patuxent River, a Chesapeake Bay tributary. For a detailed site description, please refer to chapter 2 (Figure 2.1). For making the CARBIO units (Figure 3.1), soil samples were collected

from five tidal freshwater wetlands, one from Choptank River (A), four from Patuxent River (B, C, D, & E). Soil samples were collected using a peat sampler that is 52 mm in diameter and 50 cm long (Eijkelkamp Soil and Water Corporation, Netherlands). Preliminary analyses, including organic matter, particle size, and pH, were performed to select the most convenient starting soil that will be used to build the CARBIO units. Percentage of SOM was determined for the five wetland soils by combustion for two hours at 550 °C according to the loss-on-ignition method (Wilke, 2005) (Table 3.1). My criteria for selecting the most appropriate starting soil to make CARBIO units were: 1) to have soil C within this range (9-12 %), and 2) the SOM does not change significantly with grinding and sieving (Appendix B.1). The main reason to estimate the soil organic matter for the five wetland soils after applying grinding and different sieving sizes was to assess the most constant soil organic matter: e.g., soil organic matter from site E did not change under the different grinding and sieving practices (Appendix B.1). As a consequence, soil from site E has intermediate soil organic matter content (22.34 %) and did not significantly change with different estimation techniques, which lead us to decide that soil was the most convenient soil to start with. I collected 14 more 19 L buckets from site E and soils were stored at 4 °C to minimize microbial activity until analysis (Bernal and Mitsch, 2008).

For soil C concentration, I removed living roots and rhizomes, and screened out dead plant parts. I tested several different sieving sizes processes for soils from site E (wet sieving through 1, 5, and 12.5 mm) before I started particle-size analysis for the soil. Particle size analysis was conducted according to the hydrometer Bouyoucos method (Gee and Bauder, 1986). The texture class (silty-clay) for E didn't significantly change

under different sieving conditions (Appendix B.2). Sieving the soil through 1 mm using tap water was not practical and resulted in a change in the pH of soils from site E from 5.02 to 5.69 ($P = 0.0049$). I prepared wet soils from site E by pressing it through a 5 mm sieve by hand to eliminate coarse roots and large dead parts (dead leaves and stems). Soil from site E was then spread in a pan and remaining roots were picked by hand with the exception of very fine roots, which were difficult to remove. Soil was then homogenized using a cement mixer for 7 minutes (Sharpe and Baldwin, 2013). For achieving soil homogeneity, I divided the soil into three main groups and for every group, half of a bucket was been selected randomly and placed to the mixer then the other half was added randomly then mixing with each other for 7 minutes and placed again to the empty ones randomly. Then, the soil was divided into four groups and every group was blended as stated above. The homogeneity of the blended soil was tested using a Carbon Hydrogen Nitrogen (CHN) analyzer and no significant differences were detected.

Three types of mesh bags were in use to make the CARBIO units. Multiple measurements were taken for the mesh pores using a ruler with reporting the average mesh size for each bag type. The first mesh bag has large mesh size (average size 3.5 mm) that allows roots to penetrate through. The second has fine mesh size (1 mm) that does not allow root penetration, except for very fine roots. The third has fine mesh (as the second bag), but was placed inside slotted (0.01 mm) PVC pipes that prevent any root penetration but allows water exchange and maintain hydrology regime. The mesh bags dimensions were 8.89 cm wide (flattened) and 50.80 cm in length. Each mesh bag was installed inside unslotted PVC pipes (except the third mesh bag type) to keep the mesh bag open and easy to fill with the soil, after that about 1.1 kg of wet soil has been added

to mesh bag. For the filling process, first I started to add small amount of soil and tamped inside the mesh bag. I used the same amount of wet soil (1.1 kg) for all the mesh bags.

3.2.2 Total C Concentration, stocks, and sequestration rates

In the natural and restored sites, three replicate plots were established in each of mudflat, low and high marsh, swamp and upland in a randomized block design. Criteria for selecting plots were: 1) randomly selected; 2) not to be located in a disturbed area or in a ditch; and 3) to be at least 15 meters from adjacent plots. A total of 162 CARBIO units were deployed in December 2014; 81 were retrieved in February 2016 and the other 81 in February 2017. Before deploying the CARBIO units, I selected 9 CRABIO units randomly (3 for large mesh, 3 for fine mesh and 3 for fine mesh-SPVC) to test for the initial C concentration. Each CARBIO unit was divided into 10 section (5 cm for each), then soil bulk density was determined for each 5 cm soil section (i) by the core method (Black and Hartge, 1986; Elliott et al., 1999; Wilke, 2005) by dividing soil oven dry weight (g) by volume (cm³) of the 5 cm section according to the following equation:

$$SBD_i = \frac{m_i}{v_i}$$

Where: SBD_i : Soil bulk density (g/cm³) for each section (i) in the mesh bag, m_i : oven dry weight (g), v_i : volume (cm³). Soils were dried at 40 °C until constant weight to estimate the SBD and subsequently CHN analysis without any effect at C content (Wilson et al., 2009).

For Total C concentration (% C in dry weight of soil), soil first was tested for inorganic C by treating the soil with 10 % HCL and looking for CO₂ bubbles under a

dissecting microscope (Balduff, 2007). No bubbles were detected, indicating no detectable inorganic C was present. Total C Concentration for each section of the 5 cm soil was determined by CHN method using LECO CHN-2000 analyzer, LECO Corporation, St. Joseph, Michigan. Total C mass (g C) for each section was determined by multiplying soil bulk density, total C concentration, and volume of the section (Liu et al., 2014; Zabowski et al., 2011) according to the following equation:

$$TCM_i = SBD_i \times TCC_i \times V_i$$

Where: TCM_i : Total C mass (g C) for each section (i), SBD_i : Soil bulk density (g/cm³) for each section (i), TCC_i : Total C concentration (% C g dry weight of soil) for each section (i), and V_i : Volume (cm³) for each section (i). Total C stock (g C bag⁻¹) was determined according to the following equation (Han et al., 2010; Liu et al., 2014)

$$TCS = \sum_{i=1}^n TCM_i$$

Where: TCS : Total C stock (g C bag⁻¹) of the soil inside each mesh bag as initial C stock, and TCM_i : Total C mass (g C) for each section (i). Total C stocks were estimated in the same manner for the CARBIO units that were retrieved after 15 and 26 month to be compared with the initial total C stocks and the difference in the C mass was used to estimate C sequestration rate.

3.2.3 Below-ground biomass productivity

Below-ground biomass productivity inside the CARBIO units was estimated to quantify live root production after one year from deployment. Each CARBIO core was sectioned to a 5 cm increment and live roots were handpicked. Live roots were categorized in three main categories based root diameter: 1) fine roots < 1mm; 2) lateral

roots 1-2 mm; and rhizome >2mm. The process of handpicking of the roots was time-consuming (current study) and the root ingrowth method has a lot of limitations and shortcomings concerning accurate estimation of below-ground biomass productivity (Eissenstat and Yanai, 2002; Graham and Mendelsohn, 2016; Hendricks et al., 2006). However, the below-ground biomass productivity data were helpful to compare root production below the soil surface within the different wetland habitats and to assess the mesh bag size impact on the root production and C accumulation rates. To avoid thermal decomposition of plant root organic materials and reduction for dry weight (Campbell and Plank, 1992; Campbell and Plank, 1998; Jones Jr et al., 1991), plant roots materials were oven-dried at 60 °C to remove moisture content until they reached a constant weight. Plant root materials were ground using a ceramic mortar and pestle to pass a 1mm screen and the C percentage on a dry basis was determined by the CHN method using LECO CHN-2000 analyzer, LECO Corporation, St. Joseph, Michigan.

3.2.4 Statistical analyses

Analysis of variance (ANOVA) was used to test the main effects and interactions of site, habitat, and mesh bag type on C stocks and sequestration rate. Carbon stocks and sequestration rates data were tested and found to be normally distributed with homogeneous variances. One-way ANOVA was used to examine the main effect of mesh bag type on soil C stocks before deployment, then followed by examination of least significant difference between the means of soil C stocks according to Duncan test. Two-way ANOVA was used to test the main effects of depth and mesh bag type on the initial C concentration inside the CARBIO units. Repeated measure ANOVA was used to test the main effects of site, habitat, and mesh bag type, and depth on the below-ground

biomass productivity. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

3.3 Results

3.3.1 Total soil C concentration

In the natural wetland, soil total C concentration percentage for mudflat and the swamp habitat increased largely, with regard to initial total soil C concentration, in the first 15 cm and continued to increase to the depth of 50 cm for all three types of the CARBIO units (Figure 3.2). In the restored mudflat, soil total C concentration decreased, with regard to initial total C concentration, largely in the first 10 cm and continued to decrease to the deeper sections for large and fine mesh bags, while fine mesh-SPVC showed slight decrease over depth. Change in soil total C concentration percentage in natural and restored upland indicated that both fine and fine mesh-SPVC showed slight increase in soil total C concentration for most of the soil depths, while large mesh showed no change in soil total C concentration for the natural site with large decrease in the last 15 cm. The initial (t_0) total soil C concentration % was not significantly different per soil depth ($P = 0.9667$, Appendix B.3) and that indicated all soil sections had the same concentration for the initial soil C at the start of the experiment (t_0). After 15 month (t_{15}) from CARBIO units deployment, average total C concentration % increased for most of the natural wetland habitats and decreased for most of their counterparts in restored wetlands (Appendix B.4). For example, total C concentration for the swamp habitat soils had increased by 10% for large mesh, 8% for fine mesh, and 7% for fine mesh-SPVC (Appendix B.4). Conversely, CARBIO units deployed in the restored wetland lost soil C

with time. For example, total C concentration for restored mudflat soils had largely decreased by 28% for large mesh, 27 for fine mesh, and 9 for fine mesh-SPVC (Appendix B.4). More changes were noticed in the first 15-20 cm from the soil surface for most of the natural and restored habitats, while deeper soil sections didn't change significantly (Figure 3.2).

After 26 months (t_{26}) from CARBIO units deployment and with regard to t_0 soil total C concentration %, all the natural wetland habitats showed large increase in soil total C concentration % in the first 15 cm and continued to slightly increase to the end of the CARBIO cores for the three mesh bag types (Figure 3.3). Conversely, the three mesh bag types deployed in the restored mudflat showed large decrease in soil total C concentration % especially in the first 5-15 cm soil depth and continued to show slight decrease for the deeper soil sections. The restored low marsh showed large decrease in soil total C concentration % in the first 10 cm for all mesh bags, while both fine and fine mesh-SPVC showed slight increase in the deeper soil sections (20-50 cm). Restored high marsh was dominated by *Phragmites australis* where soil total C concentration % decreased within most of the soil sections for the large mesh bag, while fine mesh-SPVC showed increase in soil total C concentration % for all soil depths except the first 5 cm. Change in soil total C concentration % showed the same pattern in natural and restored upland where both fine and fine mesh-SPVC showed slight increase in soil total C concentration % for most of the soil depths, while large mesh showed large decrease in soil total C concentration % for most of the soil depths.

3.3.2 Change in soil C stocks after 15 and 26 month from deploying

Total C stock for CARBIO (g C bag^{-1}) indicated no significant difference between the three types of mesh bags before deploying them (t_0) ($P = 0.3619$) (Table 3.2), implying all the CARBIO units had the same amount of C to start with. After 15 month, total C stocks (g C bag^{-1}) inside the fine mesh-SPVC increased in the top 30 cm for most of the natural wetland habitats, and decreased for the all restored wetland habitats (Figure 3.4). Total C stocks estimated inside the large mesh decreased for all natural and restored habitats except the swamp habitat, which increased by 11%. Total C stocks for the fine mesh deployed in natural wetlands increased by 23, 17, 14, and 7% for low marsh, high marsh, swamp, and upland respectively. Total C stocks for the fine mesh deployed in the restored wetlands increased by 7, 1, 5, and 1% for mudflat, low marsh, high marsh, and upland respectively (Figure 3.4).

After 26 month (t_{26}) from CARBIO units deployment, total C stocks (g C bag^{-1}) inside the fine mesh-SPVC largely increased for natural mudflat and swamp, and decreased for the all the restored wetland habitats (Figure 3.5). Total C stocks estimated inside the large mesh decreased for all natural and restored habitats including uplands. Total C stocks for the fine mesh deployed in natural wetlands increased by 35, 19, 12, and 3% for swamp, low marsh, high marsh, and mudflat respectively. Total C stocks for fine mesh deployed in restored wetlands increased by 12 and 10% for high marsh and low marsh respectively (Figure 3.5), while decreased by 18% in the restored mudflat.

3.3.3 Carbon sequestration rates

Based on the CARBIO index and after one year from deployment, the soil inside the bags that were deployed in the natural wetland sequestered $535 \pm 291.5 \text{ g C m}^{-2} \text{ year}^{-1}$,

while the soil inside the bags that were deployed in the restored wetlands lost $1095 \pm 429.4 \text{ g C m}^{-2} \text{ year}^{-1}$ ($P_{\text{site}} < 0.05$) (Table 3.3). In the natural wetland, the CARBIO units that deployed in the swamp had the highest CSR ($1377 \pm 601.0 \text{ g C m}^{-2} \text{ year}^{-1}$) followed by the high marsh then the low marsh (1043 ± 759.1 and $929 \pm 724.1 \text{ g C m}^{-2} \text{ year}^{-1}$, respectively, $P_{\text{habitat}} < 0.05$). All the CARBIO units that were deployed in the restored wetland habitats lost C where mudflat was the highest followed by low marsh and then high marsh (1903 ± 1474.4 , 1573 ± 799.4 , and $780 \pm 558.5 \text{ g C m}^{-2} \text{ year}^{-1}$, respectively, $P_{\text{habitat}} < 0.05$) (Table 3).

After two years from CARBIO units deployment, the soil in the CARBIO units that were deployed in the natural wetland continued to sequester C, while restored wetland continued to lose C but with lower rates compared with one year deploying. The soil in the CARBIO units that were deployed in the natural wetland sequestered $38 \pm 550.5 \text{ g C m}^{-2} \text{ 2year}^{-1}$, while restored wetlands lost $633 \pm 463.9 \text{ g C m}^{-2} \text{ 2year}^{-1}$ (Table 3.3). In natural wetlands, the swamp and mudflat CARBIO units were the only habitats that were able to continuing the C accumulation (2504 ± 1377.7 and $473 \pm 924.5 \text{ g C m}^{-2} \text{ 2year}^{-1}$, respectively). Similar to what happened in the restored habitats after one year from CARBIO units deployment, the soil in the CARBIO that were deployed in the restored habitats continued to lose C after 2 year from deployment where mudflat was the highest followed low and high marsh (2119 ± 1009.2 , 846 ± 974.1 , and $209 \pm 859.7 \text{ g C m}^{-2} \text{ 2year}^{-1}$, respectively) (Table 3.3).

3.3.4 Below-ground biomass productivity

The type of mesh bag of the CARBIO units had a significant effect on the below-ground biomass productivity ($P = 0.0064$, figure 3.6). The restored and the natural upland

habitats had the highest below-ground biomass productivity inside the large mesh (1243.7±299.88 and 499.7±114.03 g dry weight m⁻³ y⁻¹, respectively), while the restored mudflat had the lowest 22.8±4.56 g dry weight m⁻³ y⁻¹. Inside the fine mesh bag, the restored high marsh which was dominated by *Phragmites australis* had higher below-ground biomass productivity than the natural high marsh (592.1±173.45 and 433.8±147.27 g dry weight m⁻³ y⁻¹, respectively, P < 0.0001). The productivity was significantly different between habitats and through depth, as well (P < 0.0001).

3.4 Discussion

C cycling in coastal wetlands is a key component in the global C cycle (Smith et al., 2005). Wetlands with different geographical setting can be sources or sinks for C (Mitsch and Gosselink, 2007) and that has implications for understanding and predicting global environmental change. Many factors can control the potential for a wetland to be considered either a C source or a C sink; these factors include hydrology, geographical setting, nutrient dynamics, vegetation composition, and soil biogeochemical processes. The balance between C inputs and outputs determines whether a wetland ecosystem is a C source or a sink. Since wetland soil is anaerobic, soil organic matter decomposition rate is very slow compared with upland and other terrestrial ecosystems. My results revealed that C turnover in CARBIO units is more stable in the natural site (535±291.5 g-C m⁻² year⁻¹) than the restored site (-1095±429.4 g-C m⁻² year⁻¹). The natural wetland can be considered a C sink, and this observation supports my first hypothesis. On the other hand, the C turnover rate is relatively high at the restored site under the current hydrological and soil redox conditions (Chapter 2). That might be due to differences in soil texture, vegetation composition, hydrology, soil redox, sediment dynamics, geographical setting,

and soil microbe community. Results from chapter two indicated that the natural site had a shallower water level relative to soil surface, while the water level at the restored site is at deeper soil depths. Saturation negatively impact the abundance of oxygen, and that is a key factor in lowering the decomposition rate of soil organic matter as oxygen diffusion in water is very slow. Moreover, soil redox at the natural site is lower than the restored site (Figure 2.5), implying more reducing condition for natural wetland soils than the restored. Having more reducing conditions at the natural site than the restored might explain the C accumulation at the natural site and higher C decomposition rate at the restored site. Other research studies reported similar results for lower C sequestration rates in restored than natural wetlands (Waddington and Warner, 2001). Other research studies reported that restored wetlands have less SOM (Ballantine and Schneider, 2009; Bernal and Mitsch, 2013b; Bruland and Richardson, 2006; Campbell et al., 2002; Shaffer and Ernst, 1999), and lower C pools compared to natural wetlands (Drexler et al., 2013; Fenstermacher et al., 2016a; Howe et al., 2009).

Soil C sequestration rates are extremely variable as indicated in Table 3.4. Carbon sequestration could be as low as $21 \text{ g-C m}^{-2} \text{ y}^{-1}$ in tidal marine dominated marshes (Craft, 2007), or in restored tidal freshwater wetlands could be losing $1095 \text{ g C m}^{-2} \text{ y}^{-1}$ (current study). Other research studies reported higher rates in natural and impounded saltmarsh (Bryant and Chabreck, 1998; Chmura et al., 2003); and constructed wetland (Kayranli et al., 2010; Mander et al., 2008; Mander et al., 2005) ($714, 1713, 1850 \text{ g C m}^{-2} \text{ y}^{-1}$, respectively). Based on the CARBIO index in the current study, C sequestration rates fall within the same range of the C sequestration rates reported from some constructed wetlands (Kayranli et al., 2010; Mander et al., 2008; Mander et al., 2005), but were

higher compared with other studies for natural and restored sites (Anderson and Mitsch, 2006; Mitsch et al., 2012) (Table 3.3 and 3.4). While the natural habitats are accumulating C and restored are not maintain the same function, other research studies reported the opposite that is restored and created wetland ecosystems were accumulating higher C than their natural references wetlands: for example restored prairie potholes wetlands that had been restored for more than a decade (Euliss et al., 2006) were able to accumulate C that is 3.7 times faster than their natural wetland references; created riverine wetlands in Midwestern USA that were 15-years-old (Bernal and Mitsch, 2013b) were able to accumulate C that is 70% higher in a similar natural wetland within the same region; impounded tidal saltmarsh (Bryant and Chabreck, 1998; Chmura et al., 2003) were able to accumulate C that is 58% higher in natural tidal saltmarsh located in the same region. Extreme variations between those wetlands in sedimentation rates, primary productivity, hydrology inputs, soil redox conditions, and vegetation composition might explain their differences in C sequestration rates. Given that C sequestration is among priorities for wetland restoration, it is important to acknowledge that it might take years for a restored wetland to reach same level of C accumulation of a natural coastal marshes (Craft et al., 2002).

This study, to my knowledge, is the first experimental study to investigate the impact of mesh size on C stability in natural and restored wetlands, and is also unique as native soil material from the site were used to make the CARBIO units. In my study, the mesh bag type of the CARBIO units had a significant effect ($P < 0.05$) on the below-ground biomass productivity, and that might impact C accumulation rates in natural and restored wetland habitats. Mesh size of the leaf litter bags ranges from 1 mm and using

plant litters (Ballantine and Schneider, 2009) to 5 μm and using peat (Kirwan et al., 2013) and that is a key factor for the interaction between inside materials and the surrounding soil. In my study, all CARBIO cores were filled with native soil material, where large mesh bags had an average pore size of 3.5 mm, while fine mesh bags had a pore size of 1 mm, similar to most of leaf litter bags in use. After 15 months from CARBIO units deployment, the large mesh bag inside the swamp habitat was the only bag that demonstrated 11 % increase of total C stocks. Hydrology and soil redox data presented in chapter 2 (Figures 2.2 and 2.5) might be key factors in those results. Seventy % from the time, water level relative to soil surface in the swamp habitat was either at 0 cm or above soil surface, and that is a key factor for retarding the diffusion of oxygen to the soil, and hence lower soil organic matter decomposition rate. The swamp habitat had the lowest soil redox measurement at 12.5 cm soil depth, implying more reducing conditions. Moreover, the swamp habitat was the farthest from the stream and receiving less sediment compared to the marsh and mudflat, and this helps explain why the swamp has high levels of organic materials and less mineral sediments. Total C stocks for the fine mesh deployed in natural wetland habitats largely increased by 23, 17, and 14 % for low marsh, high marsh, and the swamp habitat respectively, while slightly increased in the restored wetland habitats by 7, 1, and 5% for mudflat, low marsh, and high marsh respectively (Figure 3.4). Those fine mesh bags had a pore size of 1 mm, and hence less impacted by the surrounding environmental and hydrological conditions. Soil C stocks for fine mesh-SPVC had increased in most of the natural wetland habitats, and decreased for all the restored wetland habitats. My second hypothesis was that mesh bag size have a significant impact on C stability in both natural and restored wetlands. In natural and

restored wetlands, C stocks for the three mesh bag types and the differences in their C stability are impacted by the mesh size, in support of the hypothesis. Restored wetland habitats are not able to accumulate or reserve C in the same level as natural wetland habitats, regardless the mesh bag size, and those results are similar to other research findings (Waddington and Warner, 2001).

Soil texture at the natural site was silty-clay with 36.5% clay, while soil at the restored site classified as reclaimed gravel pits that is high in sand and glauconite. Clay is fine particles that has very light density, but higher surface area. More clay particles are a key component for higher exchange potential for different soil minerals and higher organic matter accumulation (Ding et al., 2013b). Dissolved oxygen is another key factor in regulating the decomposition rates for soil organic matter (Greenwood, 1961). Higher concentration of dissolved oxygen available in soil pores will lead into higher decomposition rates for soil organic matter. Wetlands are anaerobic systems and hence Oxygen diffusion is very slow and as a result of that decomposition rate for soil organic matter is very low (McLatchey and Reddy, 1998; Megonigal et al., 2004; Mitsch and Gosselink, 2007). Restored wetland habitats were dominant by vegetation that rich with aerenchyma tissue (*Pontederia cordata* for low marsh and *Phragmites australis* for the high marsh) and that promote the diffusion of oxygen through the plant stem-root pathway to deeper soil and hence, more aerobic condition. That might be the reason that makes the soil at the restored site was less anaerobic and soil organic matter decomposition rate was higher, and that might be explain why C accumulation is very low in the restored habitats.

Both natural and restored sites are tidal freshwater wetlands and the main hydrologic control was the Patuxent River. The hydrology at the restored site was restored by scraping the surface of the old marsh (Siciliano, 2013) and building a rim of rocks at the inlet of water to help trap the sediment from escaping back to the river during lower tides cycles. The natural site was located at a similar elevation, however spatial variation existed between mudflats, marshes, and swamps. In the natural site, high marsh and the swamp habitat were about the same elevation (appendix A.2) and both located at higher elevation from the low marsh. Based on my research findings, both marsh and the swamp habitat are accumulating C, while mudflat and uplands are not (Table 3.3). That might be due to difference in elevation, sediment dynamics, plant species richness, and spatial distance from the creek (source of nutrients and surface water). Since, marshes and swamp are receiving more silt and clay compared to the mudflat that is receiving more sand, and that might be the reason for making marshes and the swamp habitat are accumulating C as they have more clay content. Moreover, uplands at both natural and restored sites are not accumulating C (-210 and -212 g-C m⁻² year⁻¹, respectively table 3.3) and that might be because they are receiving less sediment, have less primary productivity, and more aerobic soil conditions compared to wetlands. C sequestration is a key component in the global C cycle as it addresses how efficient an ecosystem is in terms of C cycling and can be estimated by variety of methods. Radionuclide dating is the most expensive and cannot be afforded by all the research facilities, while C sequestration rate can be calculated using the CARBIO (current study) which is inexpensive, based on field experiment, and according to long-term study.

CARBIO index can be useful for comparing the SOM stability between different habitats and could be beneficial to use by wetland restoration practitioners in monitoring the newly constructed and restored wetlands. Extrapolating the research finding from the current study should be carried out with considering the differences in the site, vegetation, and hydro-geomorphological settings. Further investigation and future research should be expanding to include multiple restored and newly constructed wetlands with different hydro-morphological settings to apply the CARBIO methodology. I recommend to conserve the natural wetland ecosystems and more restoration efforts for newly constructed and restored wetlands to have them building organic matter rather than losing their C. In-situ CARBIO units can be viewed as in-situ sensors that reflect the C biogeochemical processes in the ambient soil to help better understanding C stability and mineralization in the wetland soils

3.5 Conclusion

I conclude that CARBIO is an in-situ sensors that can be employed to compare C stability in natural and restored wetlands. Under the current environmental and hydrological conditions at both a natural and a restored site, marshes and the swamp habitat were able to accumulate C, while all other habitats at the restored site were neither able to accumulate C nor preserve the C that was inside the CARBIO units. My results recommend the necessity for improving hydrology restoration at the newly constructed or created wetlands. Moreover, I am not recommending the excavating techniques in restoring the hydrology of the coastal marshes. Since, natural wetland habitats have the capability of accumulating C, conserving them should be at the highest level of priority

for keeping their ecosystem functions and services including C sequestration, water quality improvement, and wildlife habitats.

Table 3.1: Mean \pm standard error for soil moisture content (%) and organic matter (%) for the five wetland soils (n=3) from Choptank (A) and Patuxent Rivers (B, C, D, E). Each soil sample was a composite sample that was collected using a peat sampler (52 mm in diameter and 50 cm long).

| Site | Moisture Content % | Organic Matter % |
|----------|--|--|
| A | 40.37 ^B \pm 0.13 | 35.70 ^B \pm 0.19 |
| B | 20.43 ^C \pm 0.13 | 13.86 ^D \pm 0.07 |
| C | 2.51 ^D \pm 0.04 | 2.83 ^E \pm 0.10 |
| D | 45.39 ^A \pm 0.14 | 37.40 ^A \pm 0.04 |
| E | 20.10^C\pm0.14 | 22.34^C\pm0.19 |
| | P <.0001 | P <.0001 |

P values represent analysis of variance 1- way (ANOVA) (n=3). Means in the same column followed by different letters are significantly different at P < 0.05 according to Duncan multiple range test.

Table 3.2: Mean \pm standard error for the total C stock (g C bag⁻¹) before deploying them (to) for soils collected from site E for the three types mesh bag. P values represent analysis of variance 1- way (ANOVA) n=3.

| Mesh bag | Total C stocks (g C bag ⁻¹) |
|--------------------------------|---|
| Large mesh | 36.2 \pm 0.70 |
| Fine mesh | 34.1 \pm 1.25 |
| Fine mesh inside PVC | 36.0 \pm 1.22 |
| Total mean | 35.4 \pm 1.06 |
| P _{mesh bag} = 0.3619 | |

Table 3.3: C sequestration rate (mean \pm SE) after one year ($\text{g C m}^{-2} \text{ year}^{-1}$) and two years ($\text{g C m}^{-2} \text{ 2year}^{-1}$) from soil CARBIO deployment for natural and restored wetlands. All rates are to a depth of 30 cm. Means in the last two rows represents average of all rates across all habitats per site.

| Site - habitat | Large mesh | | Fine mesh | | Fine mesh - SPVC | | Total average | |
|------------------------|--------------------|--------------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | one year | two years | one year | two years | one year | two years | one year | two years |
| Natural - mudflat | -286 \pm 689.3 | -1117 \pm 900.2 | -543 \pm 532.3 | 329 \pm 751.2 | -562 \pm 1385.5 | 2209 \pm 2469.8 | -464 \pm 474.5 | 473 \pm 924.5 |
| Restored - mudflat | -5911 \pm 4591.5 | -2395 \pm . | 743 \pm 1936.0 | -2280 \pm 2042.2 | -1877 \pm 810.7 | -1867 \pm 1703.6 | -1903 \pm 1474.4 | -2119 \pm 1009.2 |
| Natural - low marsh | -452 \pm 459.9 | -3385 \pm 770.3 | 2491 \pm 1321.1 | 2411 \pm 1571.0 | 747 \pm 1464.7 | -243 \pm 2456.5 | 929 \pm 724.1 | -406 \pm 1208.1 |
| Restored - low marsh | -4170 \pm 722.1 | -2073 \pm 2323.6 | 140 \pm 1312.4 | 1252 \pm 904.4 | -689 \pm 442.1 | -1717 \pm 1355.1 | -1573 \pm 799.4 | -846 \pm 974.1 |
| Natural - high marsh | -873 \pm 1290.9 | -4464 \pm 1300.7 | 1851 \pm 1328.6 | 1522 \pm 3600.5 | 2151 \pm 841.6 | -197 \pm 1279.1 | 1043 \pm 759.1 | -1046 \pm 1466.1 |
| Restored - high marsh | -2106 \pm 545.8 | -1987 \pm 668.2 | 499 \pm 1219.4 | 1524 \pm 1702.0 | -735 \pm 510.0 | -165 \pm 1563.3 | -780 \pm 558.5 | -209 \pm 859.7 |
| Natural - swamp | 1178 \pm 1566.8 | -515 \pm 947.6 | 1564 \pm 1256.1 | 4420 \pm 1613.3 | 1388 \pm 513.5 | 3607 \pm 3503.3 | 1377 \pm 601.0 | 2504 \pm 1377.7 |
| Natural - upland | -239 \pm 484.2 | -2353 \pm 1176.7 | 767 \pm 920.2 | 849 \pm 834.9 | -1158 \pm 1334.2 | -2495 \pm 1927.0 | -210 \pm 561.9 | -1333 \pm 883.6 |
| Restored - upland | -170 \pm 517.0 | -1307 \pm 849.6 | 104 \pm 1040.9 | 805 \pm 1768.8 | -569 \pm 932.7 | 1437 \pm 1637.7 | -212 \pm 441.1 | 312 \pm 846.5 |
| Natural total average | -134 \pm 422.4 | -2367 \pm 550.1 | 1226 \pm 505.4 | 1906 \pm 836.0 | 513 \pm 548.8 | 576 \pm 1082.7 | 535 \pm 291.5 | 38 \pm 550.5 |
| Restored total average | -2833 \pm 933.5 | -1849 \pm 673.2 | 372 \pm 609.8 | 325 \pm 844.0 | -967 \pm 339.9 | -578 \pm 782.2 | -1095 \pm 429.4 | -633 \pm 463.9 |

Table 3.4: Comparison of C sequestration rates of different wetlands with different estimation methods

| Wetland type* | Natural vs restored (y) | CSR g C m ⁻² y ⁻¹ | Salinity** | SBD g cm ⁻³ | T. C % | Dominant Vegetation*** |
|---|-------------------------|--|------------|---------------------------|--------|---|
| Temperate regions | | | | | | |
| Natural flow through wetlands Ohio, USA ¹ | Natural | 142 [†] | NR | NR | NR | <i>Nelumbo lutea</i> , <i>Typha</i> spp., <i>Scirpus fluviatilis</i> , and <i>P. australis</i> |
| Created flow-through wetlands Ohio, USA ¹ | created (15) | 243 [†] | NR | NR | NR | macrophytes |
| Created temperate marshes Ohio, USA ² | created (10) | 187 ^{†††} | NR | 0.5 | 3.9 | macrophytes |
| Tidal freshwater wetlands Georgia, USA ³ | natural | 108 [†] | 0.15-16.5 | 0.3 | 10.2 | <i>S. alterniflora</i> , <i>S. cynosuroides</i> , <i>J. roemerianus</i> , <i>Zizaniopsis milaceae</i> |
| Tidal marine dominated marshes Georgia, USA ⁴ | natural | 21 [†] | 13.5-30 | 0.5 | 5.0 | <i>S. alterniflora</i> , <i>J. roemerianus</i> |
| Tidal marine dominated marshes Georgia, USA ⁴ | natural | 32 [†] | 13.5-30 | 0.6 | 4.2 | <i>Spartina alterniflora</i> |
| Marsh Ohio, USA ⁵ | natural | 105 [†] | NR | 0.8 | 2.7 | <i>Phragmites australis</i> , <i>Scirpus fluviatilis</i> |
| Mudflat Ohio, USA ⁵ | natural | 112 [†] | NR | 0.8 | 3.5 | <i>Leersia oryzoides</i> |
| Floating bed - Ohio, USA ⁵ | natural | 160 [†] | NR | 0.6 | 8.7 | <i>Nelumbo lutea</i> |
| marsh - Virginia, USA ⁶ | natural | 97 ^{††} | NR | NR | NR | <i>Peltandra virginica</i> |
| marsh - Virginia, USA ⁶ | natural | 75 ^{††} | NR | NR | NR | <i>Typha latifolia</i> |
| Horizontal subsurface flow constructed wetlands, Estonia ⁷ | constructed (7) | 1850 ^{†††} | NR | NR | NR | <i>Typha latifolia</i> |
| Semitropical | | | | | | |
| Tidal saltmarsh - Louisiana, USA ⁸ | natural | 714 [†] | NR | 0.5 | 35.0 | NR |
| Tidal saltmarsh - Louisiana, USA ⁸ | impounded | 1713 [†] | NR | 0.7 | 39.3 | NR |
| Tidal freshwater wetland - Natural site - Current study (Temperate) | | | | | | |
| Mudflat - Maryland, USA | natural | -464 | 0.15-0.25 | 0.36 | 7.9 | <i>Hydrilla verticillata</i> |
| Low marsh - Maryland, USA | natural | 929 | 0.15-0.25 | 0.36 | 9.4 | <i>Nuphar lutea</i> |
| High marsh - Maryland, USA | natural | 1043 | 0.15-0.25 | 0.27 | 13.2 | <i>Polygonum arifolium</i> |
| Swamp - Maryland, USA | natural | 1377 | 0.15-0.25 | 0.20 | 15.6 | <i>P. arifolium</i> , <i>Fraxinus</i> spp. |
| Tidal freshwater wetland - Restored site - Current study (Temperate) | | | | | | |
| Mudflat - Maryland, USA | restored (17) | -1903 | 0.15-0.25 | 0.86 | 1.8 | <i>Ceratophyllum demersum</i> |
| Low marsh - Maryland, USA | restored (17) | -1573 | 0.15-0.25 | 0.59 | 4.3 | <i>Pontederia cordata</i> |
| High marsh - Maryland, USA | restored (17) | -780 | 0.15-0.25 | 0.82 | 2.3 | <i>Phragmites australis</i> |

*: due to different wetland classification system used, name and type of wetland was quoted as was described in the cited study, **: Salinity was either not reported or was based on adjacent river as quoted from each cited study, *** as quoted from each cited study, NR: not reported
†: 137Cs - 210Pb, ††: CO₂ exchange rate - chamber based, †††: difference between C inputs and outputs, ††††: sediment rate and C content
1(Mitsch et al., 2012), 2(Anderson and Mitsch, 2006), 3(Craft, 2007; Craft et al., 2009), 4(Craft, 2007), 5(Bernal and Mitsch, 2012), 6(Whiting and Chanton, 2001), 7(Kayranli et al., 2010; Mander et al., 2008; Mander et al., 2005), 8(Bryant and Chabreck, 1998; Chmura et al., 2003)



Figure 3.1: Field photo showing the three types of CARBIO. From left to right: large mesh, fine mesh, and fine mesh inside the slotted PVC. Photo credit: Andrew Baldwin.

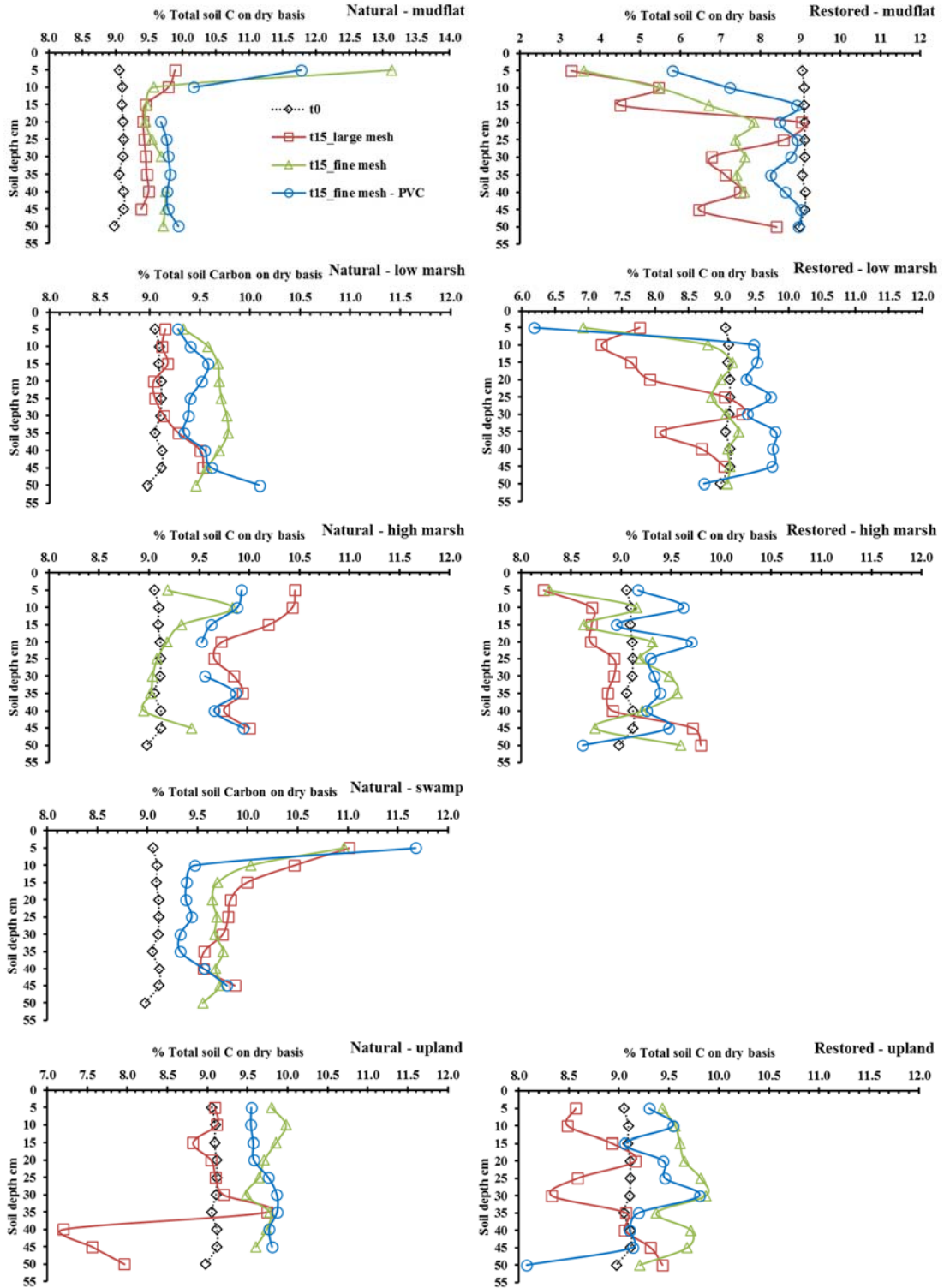


Figure 3.2: Distribution of total C concentration % (mean) over 50 cm soil depth for the mesh bags soil after 15 month from deploying in natural (left) and restored (right) wetlands. t_0 : initial time; t_{15} : 15 month after deploying. Plotted values are mean total C concentration (%) of $n=3$ in 5-cm long sections from the mesh bag cores; error bars were removed for clarity.

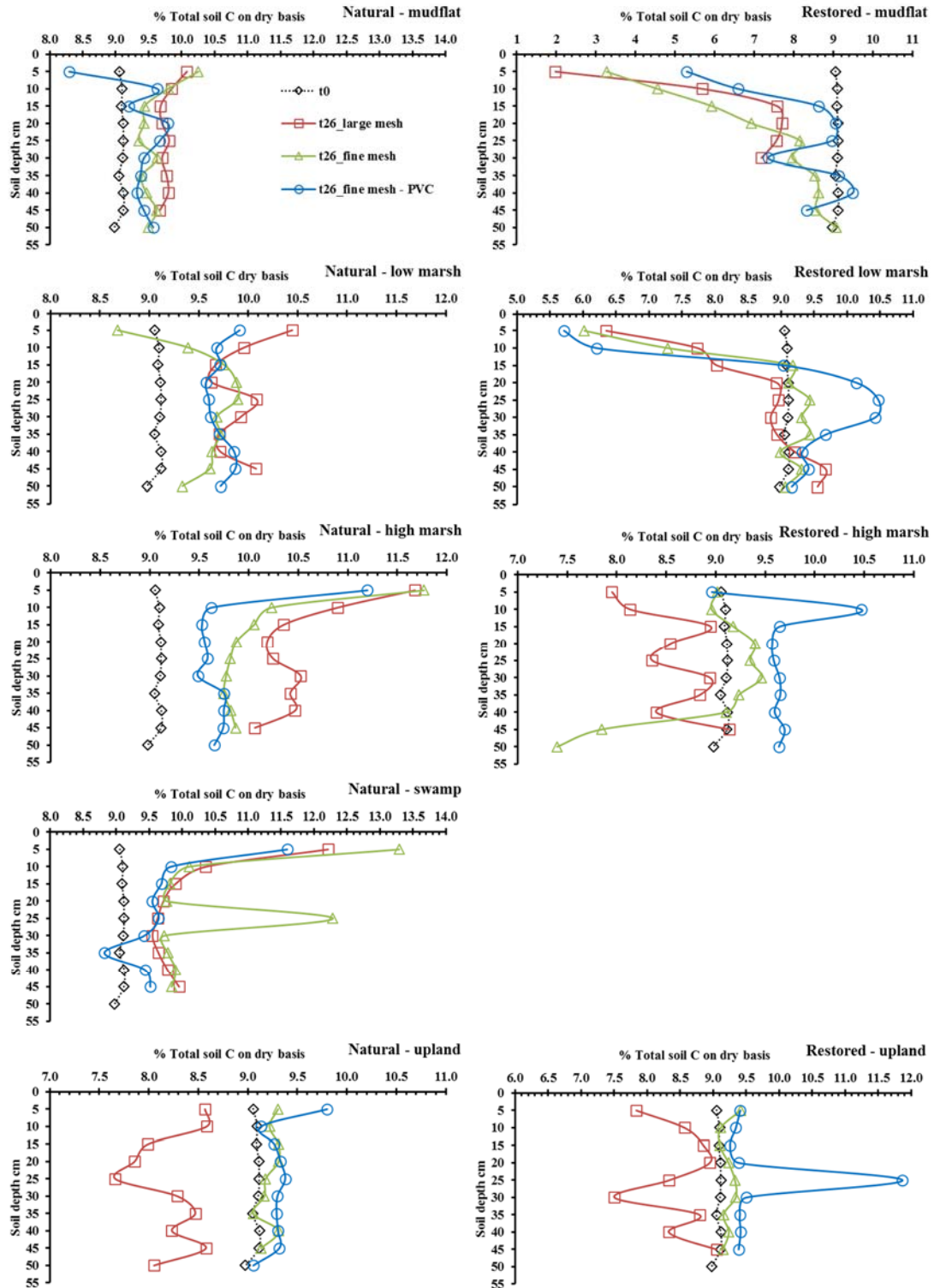


Figure 3.3: Distribution of total C concentration % (mean) over 50 cm soil depth for the mesh bags soil after 26 month from deploying in natural (left) and restored (right) wetlands. t_0 : initial time; t_{26} : 26 month after deploying. Plotted values are mean total C concentration (%) of $n=3$ in 5-cm long sections from the mesh bag cores; error bars were removed for clarity.

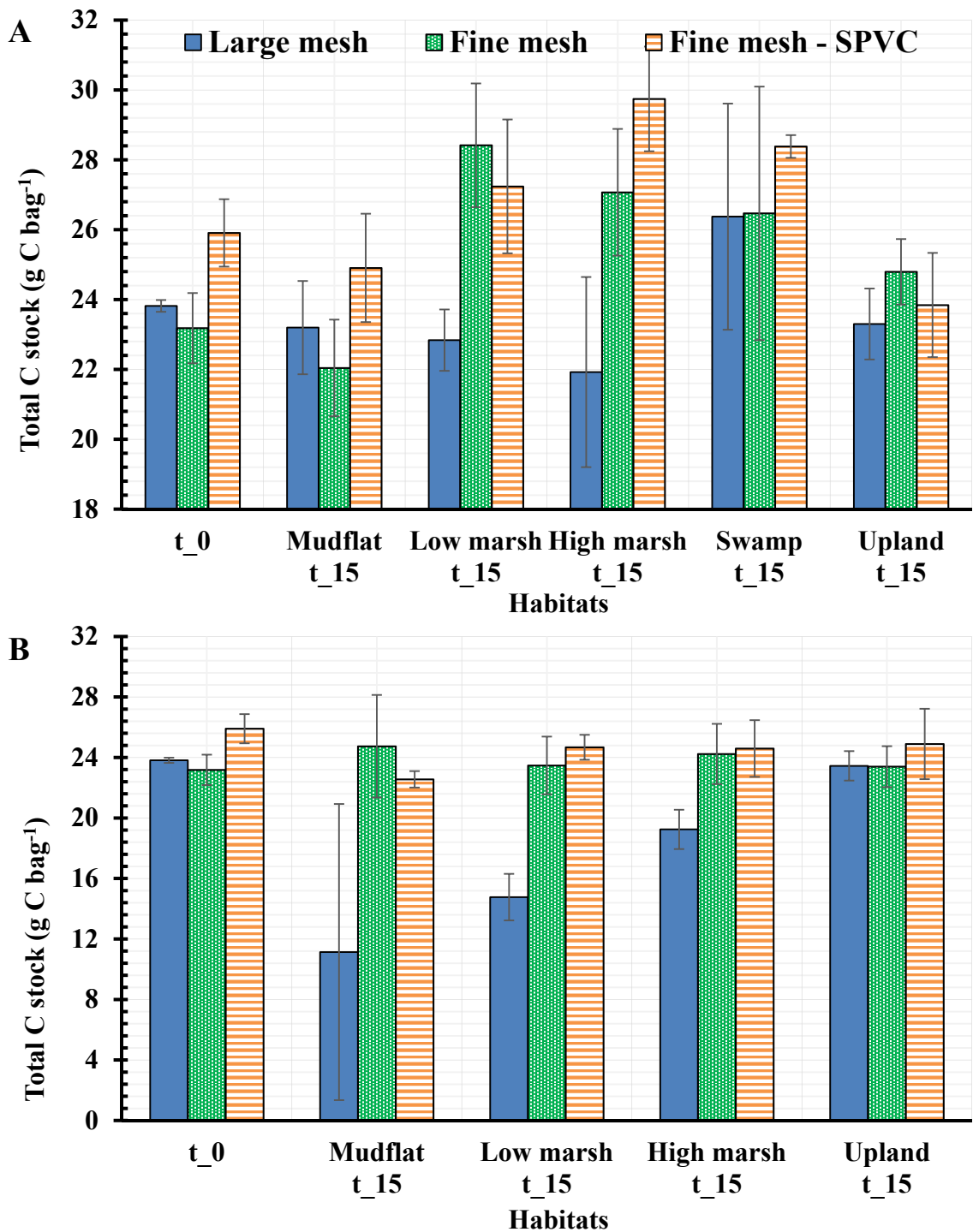


Figure 3.4: total C stocks g C bag^{-1} (mean \pm SE) within the first 30 cm for the mesh bags soil after 15 month from deploying in natural (A) and restored (B) wetlands. t_0 : initial time; t_{15} : 15 month after deploying. Each bar represents 18 samples.

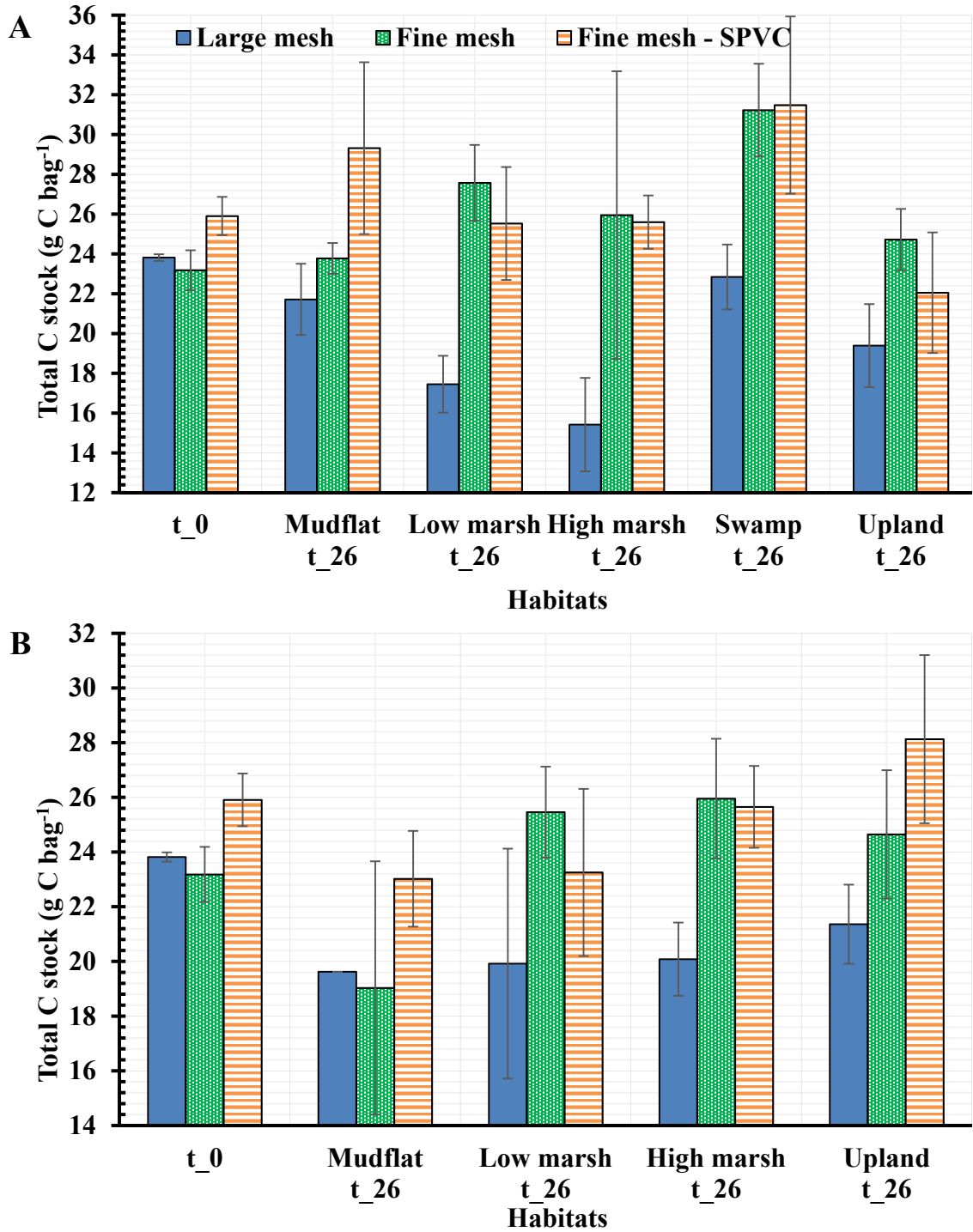


Figure 3.5: total C stocks g C bag^{-1} (mean \pm SE) within the first 30 cm for the mesh bags soil after 26 month from deploying in natural (A) and restored (B) wetlands. t_0 : initial time; t_{26} : 26 month after deploying. Each bar represents 18 samples.

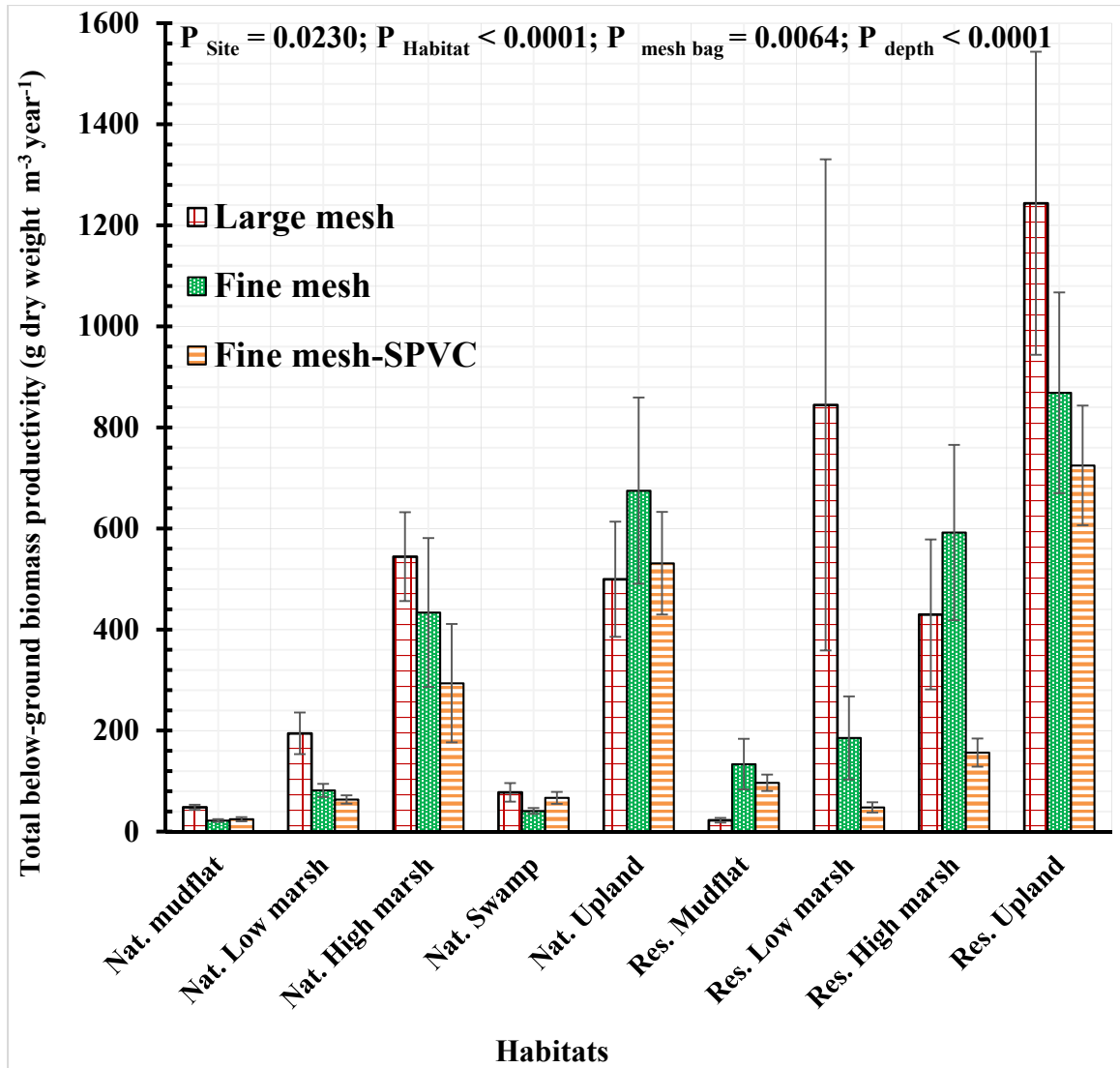


Figure 3.6: Total below-ground biomass productivity g dry weight m⁻³ y⁻¹ (mean ± SE) for the natural (Nat.) and the restored (Res.) wetland habitats after one year from CARBIO units deployment. Each bar represents 30 samples.

4 Diurnal Methane and Carbon Dioxide Fluxes from Natural and Restored Tidal Freshwater Wetlands in a Chesapeake Bay Tributary

Abstract

Most chamber-based studies of greenhouse gas emission in wetlands have measured methane (CH₄) fluxes during daytime only. To simulate nighttime conditions, the static chamber is often covered with a black sheet of cloth to mimic what is happening during the night, but this has the confounding effect of increased temperatures inside the chamber and the effect of plant roots outside the chamber. My goal was to quantify flux rates of CH₄ during day and night across different vegetation strata in natural and restored tidal freshwater wetlands. Three habitats (low marsh, high marsh, and swamp) were selected at Patuxent Wetland Park, a natural wetland, and two habitats (low and high marsh) were selected at Wootons Landing Wetland Park, a restored wetland. Both sites are tidal freshwater wetlands (salinity <0.3 ppt) located on the Patuxent River in Maryland, USA. Static chambers were used to quantify day and night variation in CH₄ flux rates once a month during day and nighttime from May to August 2016, soil pore-water CH₄ and total iron concentrations were measured on the same days as the flux rates. Restored wetland habitats had significantly higher annual CH₄ emission rates than the natural wetland habitats (1372.1±35.89 and 880.7±144.73 g CH₄ m⁻² y⁻¹, respectively, P <0.05). Restored wetland habitats were dominated by monoculture plant species *Phragmites australis* and *Typha latifolia*, respectively. Low marsh at the restored site had the highest total available iron concentration at 40 cm soil depth (12.8±4.18 mg

L⁻¹). There was a significant and strong negative correlation between total available iron concentration and the log CH₄ flux rate ($r = -0.64$ for the natural wetland at 12.5 cm soil depth and $r = -0.79$ for the restored site at 40 cm soil depth, $P < 0.05$). Pore-water CH₄ concentrations were higher at 12.5 than at 40 cm soil depth, and the low marsh habitat at the restored wetland had the highest pore-water CH₄ in August 2016 (9178.6 ± 4068.8 $\mu\text{mole CH}_4 \text{ L}^{-1}$). Since nighttime sampling detected a very low CH₄ concentrations ($< 3650 \mu\text{mole m}^{-2} \text{ h}^{-1}$), greenhouse gas models should take the daily variation of CH₄ fluxes and their differences between the natural and restored wetlands into consideration in order to better represent C budget in wetlands.

4.1 Introduction

Water vapor, carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are all greenhouse gases. The main human sources of greenhouse gas emissions are the excessive use of fossil fuel and deforestation, although some wetlands emit CH₄ and CO₂. Current atmospheric concentrations for greenhouse gases have reached unprecedented levels. Studies have shown that the globally averaged concentrations of CO₂, CH₄, and N₂O have increased since 1750 (40%, 150%, and 20% respectively) (IPCC, 2014). Total USA greenhouse gas emissions increased by 7.4 % from 1990 to 2014 and by 1 % from 2013 to 2014 (EPA, 2016), and that was related to increased usage of fossil fuels. Anthropogenic releases of these gases is predicted to result in atmospheric warming, which will have widespread impacts on human and natural systems (IPCC, 2014). CH₄ is a potent greenhouse gas and has a shorter lifetime in the atmosphere than CO₂, however CH₄ has a global warming potential of 25 times higher than CO₂ (IPCC, 2014). Wetlands are considered the main natural source for CH₄, while activities like leakage from natural

gas system and livestock are important anthropogenic sources of CH₄ (IPCC, 2007).

Wetlands emit, on average, 170.3 Tg CH₄ year⁻¹ (42.7 from northern bogs and 127.6 from tropical swamps), which constitutes 81.9 % of CH₄ emissions from all natural sources (EPA, 2010). That makes the wetlands a significant natural CH₄ source (Crawford et al., 2014; Dlugokencky et al., 2011; Walter et al., 2001) among other sources like uplands, riparian areas, oceans, rivers, permafrost, and lakes.

Environmental factors including temperature, water level relative to soil surface, organic matter content, and the C quality of litter (litter C and N concentrations) can all affect CH₄ emissions from wetlands (Yu et al., 2013). Furthermore, relatively small changes in these factors will affect the balance between the consumption and production of CH₄ by soil microbes. Soil microbes utilize available C and organic material as a C source under aerobic conditions. Under anaerobic conditions, as in wetland ecosystems, soil microbes seek alternative electron acceptors and a series of biogeochemical reactions are initiated. Those reactions are sequential and occur from aerobic (higher soil oxidation-reduction (Eh)) to anaerobic (low Eh). From high to low Eh, the main reactions are the reduction of nitrate NO₃⁻, manganese Mn (IV), iron Fe (III), sulfate SO₄²⁻ and CO₂ (Ponnamperuma, 1972; Reddy et al., 1989; Yu and Patrick, 2004). The presence of iron-reducing bacteria in the rhizosphere of aquatic macrophytes has a negative impact on the availability of the organic C for other heterotrophic micro-organisms including methanogens (King and Garey, 1999; Laanbroek, 2010), which has a biogeochemical implication that inhibit the CH₄ production (Meronigal et al., 2004; Roden and Wetzel, 2003; Van der Nat and Middelburg, 1998).

Assessments of global CH₄ emissions are based on data collected from studies that are either bottom-up approaches (field scale) or top-down (atmospheric inverse technique). The global CH₄ budget, especially based on bottom-up approaches, shows the potential of uncertainty of CH₄ budget (Kirschke et al., 2013) and that might be due to unrealistic incorporation of ebullition from stream rivers (Crawford et al., 2014) as well as higher level of variability in field studies (Bridgham et al., 2013; Neubauer and Megonigal, 2015). Measurements of greenhouse gas emissions to the atmosphere have been based on either closed-chamber method (DeLaune et al., 2002; Krauss et al., 2016; Neubauer, 2013; Weston et al., 2014; Weston et al., 2011; Yu et al., 2013) or tower-based micrometeorological approaches (e.g. eddy covariance) (Baldocchi, 2003; Corbin et al., 2010; Crosson, 2008; Glenn et al., 2006; Hargreaves et al., 2001; Hsu et al., 2010; Lund et al., 2010; Rinne et al., 2007; Syed et al., 2006). Many studies have reported that data for CH₄ emissions based on either chambers or eddy covariance are typically highly variable temporally and spatially (Bridgham et al., 2013; Krauss et al., 2016; Neubauer and Megonigal, 2015).

Most recent chamber-based studies of greenhouse gas emission in wetlands focus on CH₄ fluxes during daytime only, or researchers would cover the static chamber with a black sheet of cloth to mimic what is happening during the night, neglecting the fact that covering the chamber with black cloth may create high temperatures inside the chamber, which in turn will impact the gas flux rate. Diurnal variations of CH₄ fluxes are highly variable in literature where nighttime CH₄ emission is higher than daytime fluxes (Godwin et al., 2013), while some other studies reported less CH₄ emission during the nighttime where it is correlated with soil temperature (Butterbach-Bahl et al., 2016; Neue

et al., 1997b). Moreover, existing data available for predicting CH₄ or CO₂ emissions from wetlands may be limited because they do not incorporate data on the daily variation in fluxes, primarily due to the difficulty of sampling gas fluxes at night in wetlands. My overall research goals were to 1) quantify flux rate of CH₄ and CO₂ during day and night across different vegetation strata, geomorphology, soils, and hydrology in natural and restored tidal freshwater wetlands; 2) determine a range for soil oxidation-reduction potential (Eh) for wetland soil with minimal CH₄ emissions; and 3) assess the role of pore-water iron (Fe) in controlling the flux rate of CH₄. I hypothesized that: 1) pore-water iron would be negatively correlated with CH₄ emissions and that differences in iron concentrations would help explain the variations in CH₄ emissions between restored and natural wetland habitats; 2) CH₄ concentration would be higher during the day than at nighttime; 3) CH₄ concentrations would be higher at 12.5 cm from soil surface compared with deeper soil surface (40 cm in my study); and 4) habitats dominated by perennial plant species would emit more CH₄ compared to those dominated by annual plant species.

4.2 Materials and methods

4.2.1 Site description

The study sites were located at Patuxent River, a sub-estuary of Chesapeake Bay, Maryland USA. For more details about the study sites, refer to chapter 2. Both of the study sites are tidal freshwater wetlands of the Patuxent River, a Chesapeake Bay tributary. Three habitats (low marsh, high marsh and swamp) were selected at Patuxent Wetland Park, a natural wetland, while two habitats (low marsh and high marsh) were

selected at Wootons Landing Wetland Park, a restored wetland (Figure 4.1). Restored and natural mudflat were excluded from the study sites for difficulties during the sampling as the mudflat soil surface never exposed from the river water, while upland habitats were excluded as well from gas flux sampling as no emission rates were detected at all during the first two months of the study. At each habitat, five random plots were selected except for the low marsh at the restored site, where only three plots were selected due to site habitat topography restrictions, resulting in 23 total plots. Selection criteria for plots were: 1) not to be located in a disturbed area; 2) not to be in the middle of a creek or a ditch; 3) to be at least 15 meters far from adjacent plots; and 4) to be randomly selected.

4.2.2 Gas sampling and analysis

At each plot, an aluminum frame (50x50 cm) was inserted in the marsh ground and a 3 meter boardwalk installed as a permanent sampling platform (USGS. Department of the Interior, 2010) in the front of the frame (Appendix C.1). The boardwalk and the frames were installed one month in advance and before the first sampling took place on May 2016 to prevent gas ebullition during the gas sampling. Gas sampling on the field was conducted using static chamber (Lovelock et al., 2014). For all habitats, only one chamber was mounted on the frame, with two exceptions: the low marsh at the restored site which was dominated by *Typha latifolia*, two chambers were mounted above each other to accommodate the plant height; in the restored high marsh three chambers were stacked to accommodate the *Phragmites australis* that was dominant (Appendix C.2). Gas sampling spanned three time intervals during the day: daytime or morning, “any time after 10 am until 2 pm;” evening time, “after 2 pm until sunset;” and nighttime, “from 4 am and before sunrise”. The gas flux rate were measured on monthly basis from May –

August 2016 and gas samples were taken at 0, 30, 60, 90, 120, 150, and 180 minute time interval using a 20 mL syringe. I started the gas sampling on May and June 2016 until 180 minutes, then reduced to 90 minutes in July and August 2016 as we did not see change in gas flux with tidal cycles. Therefore, only data for 90 minutes sampling are presented in the results. During May and June 2016, the gas sampling were collected twice a day, and collected once during daytime in July and August 2016 as we did not observe change in the gas flux between the morning and evening. Nighttime gas flux sampling were conducted during July and August 2016 on the swamp and high marsh for the natural wetland. Gas samples were injected to flushed and evacuated exetainers that are 12 mL and made from borosilicate glass with double wadded caps separate with a silicone layer made specifically to avoid any gas leak under multiple injections. One day before the field sampling, exetainers were evacuated and flushed with ultra-purity helium to prevent any air contamination. The flushing and evacuation protocol was as follows: evacuate for 30 seconds followed by flushing for 1.5 minutes with helium at 25 PSI. Then evacuate for 1.5 minutes, flush for 1.5 minutes with helium at 25 PSI, and do a final evacuation for 2 minutes. By doing so, the exetainers were safe to be stored for up to 2 weeks without being contaminated by the surrounding air (Ekeberg et al., 2004). All gas samples were analyzed for CH₄ and CO₂ using Gas Chromatography Agilent Technology (Agilent HP 7890A) connected with a thermal conductivity detector (TCD) having the following parameters: (1) an injection temperature of 250 °C; (2) a detector temperature of 250 °C; (3) an oven temperature of 60 °C; and (3) a carrier gas flow rate of 8.6 mL He/min. The column used was an HP-Plot Q capillary column (Agilent J&W; USA).

4.2.3 Gas flux rates

Gas flux rates were calculated in two different ways: 1) by assessing the change of gas concentration over time to establish a linear relationship between time and the gas concentration (Lovelock et al., 2014); and 2) by dividing the final gas concentration at 90 minutes over the total time to report the average gas emission. Gas composition was calculated according to the ideal gas law (Equation 1),

$$n = \frac{PV}{RT} \quad (1)$$

where n is the number of moles of total gas, P is the pressure of the air in the chamber (atm), v is the volume of the chamber (L), R is the gas constant ($L \text{ atm mole}^{-1} \text{ Kelvin}^{-1}$; $R = 0.0820$), and T is the air temperature inside the chamber. HOBO temperature data-loggers were installed inside the chambers to record air temperature inside the chambers every 5 minutes, and additional loggers were installed outside the chambers as well to compare the inside temperature with the ambient temperature surrounding the chamber.

4.2.4 Pore-water, soil, and vegetation analysis

At each plot, a sipper was used to collect pore-water samples at 12.5 and 40 cm soil depth. A tygon tube with holes at the end and connected with a syringe from the top was inserted to the specific depth, either 12.5 or 40 cm soil depth, and suction was applied to extract the pore-water. Five mL of pore-water were transferred immediately to 12 mL evacuated and flushed exetainers and shaken vigorously for 2 minutes to reach equilibrium with the headspace. Gas samples were drawn from the headspace to be analyzed for CH_4 and CO_2 on the gas chromatography as described above immediately the day after field sampling. At each plot, two soil thermometers were installed at 12.5 and 40 cm and soil temperature was recorded during the time of the gas sampling. In

addition, a pilot hole was made using a stainless-steel rod and 3 platinum (Pt) electrodes were installed at 12.5 cm and 3 others at 40 cm soil depth, and a soil oxidation reduction (Eh) measurement was recorded using a multi-meter adapted to be a high resistance in conjunction with a calomel reference (Rabenhorst et al., 2009). During each month of gas sampling and using a peat sampler (52 mm in diameter and 50 cm long, Eijkelkamp Soil and Water Corporation, Netherlands), soil cores to a depth of 50 cm were taken and soil was collected at a depth 12.5 and 40 cm for measuring soil pH in the field. Soil slurry was made into a 1:1 solution by adding distilled water to the soil and stirring the slurry for 2-3 minutes, then letting it settle for 10 more minutes before a pH meter was used to measure the soil pH at the site. Salinity at each plot was measured in the field using a portable meter (Model 30, YSI, Yellow Springs, OH) on the water filling the hole created by the soil core. Pore-water was analyzed for total available iron using inductively coupled plasma optic emission spectrometry (ICP-OES) after microwave digestion of the pore-water with Nitric acid according to EPA-3015 method. At each habitat, datalogging water level recorders were installed in wells to monitor water level relative to soil surface at 10-minute intervals. All the vegetation inside the frame was identified, plant vegetation cover in the field was estimated (Peet et al., 1998), and Shannon-Weaver diversity and evenness indices were calculated. Nomenclature for plant species were accessed from the United States Department of Agriculture Plants Database on August 17, 2015 (USDA, 2017). To access the sites, permission was granted by private landowner for the swamp habitat, while the rest of the habitats were accessed by permission of the Jug Bay Wetlands Sanctuary.

4.2.5 Statistical analysis

Repeated analysis of variance (ANOVA) was used to examine variation between habitats (low marsh, high marsh, and swamp for the natural wetland, and low and high marsh for the restored wetland) over time on the flux rate of CH₄ and CO₂. Due to high variations in the gas flux data, CH₄ flux rates were log-transformed in order to meet the criteria for ANOVA. Pearson correlation coefficient analyses were conducted to examine the correlation between the log CH₄ flux rates and the total iron concentration in pore-water for the whole dataset and for subset that represent only July and August 2016. Two-way ANOVA was used to test the effect of the site and habitat on plant species diversity, then followed by the investigation of least significant differences between the arithmetic means based on Duncan's test. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina).

4.3 Results

4.3.1 Diurnal flux rates

The restored wetland habitats had significantly higher annual average of CH₄ flux rate compared to the natural wetland habitats (1372.1±35.89 and 880.7±144.73 g CH₄ m⁻² y⁻¹, respectively, P<0.05). Data for the morning cumulative CH₄ flux rates show that the high marsh at the restored wetland had the highest rate in May 2016 (10296.9±6.8 μmole m⁻² h⁻¹); the low marsh at natural wetland had the highest rate in June and July 2016 (20791.8±12258.4 and 19563.7±3551.8 μmole m⁻² h⁻¹, respectively); and the natural high marsh had the highest rate in August 2016 (11387.3±8043.2 μmole m⁻² h⁻¹, Figure 4.2).

The majority of CO₂ concentrations were between 450 and 650 ppm, while the majority of CH₄ concentrations were below 200 ppm (Appendix C.3).

For the morning flux rate for CO₂: the high marsh at the restored wetland had the highest rate during May ($7404.4 \pm 3012.8 \mu\text{mole m}^{-2} \text{h}^{-1}$, appendix C.4); the high marsh at the natural wetland had the highest rate during June 2016 ($2849.8 \pm 2760.4 \mu\text{mole m}^{-2} \text{h}^{-1}$); the high marsh at the restored wetland had the highest rate during July 2016 ($4388.2 \pm 3134.5 \mu\text{mole m}^{-2} \text{h}^{-1}$); and the high marsh at the natural wetland had the highest rate during August 2016 ($15046.2 \pm 5000.23 \mu\text{mole m}^{-2} \text{h}^{-1}$) (Appendix C.4). In August 2016, the morning average CO₂ flux rate was highest at the high marsh of natural wetland ($43234.4 \pm 13409.9 \mu\text{mole m}^{-2} \text{h}^{-1}$), followed by the high marsh at the restored wetland ($33731.6 \pm 3279.6 \mu\text{mole m}^{-2} \text{h}^{-1}$).

Night CH₄ flux rates were significantly lower than the evening or morning CH₄ flux rate (Appendix C.4). For example, the swamp habitat at the natural wetland had a flux rate of $-0.4 \pm 0.6 \mu\text{mole m}^{-2} \text{h}^{-1}$ in July 2017 during nighttime, while the high marsh for the natural wetland had a flux rate of $-6.8 \pm 1.0 \mu\text{mole m}^{-2} \text{h}^{-1}$ in August 2016 during nighttime. In May 2016, the swamp habitat at the natural wetland had the highest CH₄ flux rate ($34804.3 \pm 23672.8 \mu\text{mole m}^{-2} \text{h}^{-1}$) during the morning, while the high marsh for the restored wetland had the highest rate during the evening ($302.4 \pm 41.7 \mu\text{mole m}^{-2} \text{h}^{-1}$). Moreover, the low marsh at the natural wetland had the highest morning flux rate for CH₄ emission during June and July 2016 (13644.6 ± 10176.2 and $9498.3 \pm 2751.9 \mu\text{mole m}^{-2} \text{h}^{-1}$ respectively).

4.3.2 Pore-water CO₂ and CH₄

Pore-water CO₂ and CH₄ concentrations at 12.5 and 40 cm soil depth are presented in figure 4.3. About 45% of the CO₂ pore-water concentrations were in the range of 1000-5000 μ mole CO₂ Liter⁻¹ (Appendix C.5). Pore-water CH₄ concentrations at 12.5 cm were higher than the concentrations at 40 cm soil depth, and about 30% of the CH₄ pore-water concentrations were in the range of 5-10 μ mole CH₄ L⁻¹ (Appendix C.5). In June, July and August 2016, pore-water CO₂ at 40 cm was higher than at 12.5 cm soil depth in all natural habitats, although pore-water CO₂ was higher at 12.5 than 40 cm soil depth in all restored habitats. Moreover, low marsh dominated by *Typha latifolia* at the restored wetlands had higher CO₂ pore-water concentrations over the whole timeframe of the study. Pore-water CH₄ concentrations were the highest in July 2016, and lowest in May 2016. Low marsh dominated by *Typha latifolia* at the restored wetlands had the highest CH₄ pore-water concentrations over the entire timeframe of the study.

4.3.3 Soil biogeochemistry

Soil redox measurement (Eh) in conjugation with soil pH revealed that over a four-month period neither the soil of the low marsh nor the high marsh at the restored wetland was reduced relative to the technical standard line for the hydric soil (Figure 4.4). On the other hand, the soils of all the habitats in the natural wetland were reduced during the 4-month period. Soil Eh at 40 cm soil depth was more reduced than soils at 12.5 cm soil depth, except for the swamp habitat at the natural wetland which was exactly the opposite (Appendix C.6). Moreover, soil Eh was the lowest during June and increased again by the end of August 2016. Soil pH decreased slightly over time for all habitats (Appendix C.7). Soil pH for the low and high marsh at the restored wetland were near

neutral (7.1 to 7.7), while the natural wetland soils were all below pH 7. Salinity ranged from 0.1 to 0.25 ppt and changed slightly over the course of the study (Appendix C.8). Soil temperature was warmer at 12.5 cm than 40 cm soil depth for all habitats, and increased from 14-18 °C at 12.5 cm during May to 22-27 °C in August 2016 (Appendix C.9).

Total available iron was higher at 40 cm soil depth than 12.5 cm for the restored habitats and the swamp habitat at the natural site, while its concentration at 12.5 cm was higher than 40 cm soil depth for both low and high marsh for the natural site (Figure 4.5). At 40 cm soil depth, low marsh at the restored site has the highest total available iron concentration, followed by the swamp habitat, then high marsh at the restored site (12.8±4.18, 11.0±6.68, and 7.6±3.50 mg L⁻¹ respectively). No correlation were detected between total iron in pore-water and log CH₄ flux rates ($r < 0.1$ & $P > 0.05$), however, a correlation analysis for a subset dataset that represents July and August revealed that total available iron concentration had a significant and a strong negative correlation with the log CH₄ flux rate ($r = -0.64$ at the natural wetland at 12.5 cm soil depth and $r = -0.79$ for the restored site at 40 cm soil depth, $P < 0.05$).

4.3.4 Vegetation structure

Vegetation composition analysis showed that high marsh at the natural wetland has the highest plant species diversity indices (Shannon-Weiner index H: 1.7±0.1 & Shannon evenness S: 0.8±0.0), indicating that the high marsh habitat was significantly ($P_{\text{site}} < 0.0001$; $P_{\text{habitat}} < 0.0001$) more diverse than the rest of the habitats in both the natural and restored sites (Tables 4.2 and 4.3). All the plant species identified in the study sites

are presented in appendix C.10. Plant vegetation at high marsh for the natural wetland has the highest plant species density (7.2 ± 0.4) and total cover (98.2 ± 9.3) (Appendix C.11).

4.4 Discussion

Both of the study sites are freshwater marshes (0.15-0.25 PSU), and they had annual CH₄ emission rates on average of 1372.1 ± 35.89 and 880.7 ± 144.73 g CH₄ m⁻² y⁻¹, for restored and natural, respectively. The annual flux rate of CH₄ emission in the natural site in our study (880.7 ± 144.73 g CH₄ m⁻² y⁻¹) is higher than other annual rates reported from freshwater marshes (213.3 g CH₄ m⁻² y⁻¹) dominated by *Panicum hemitomon* and brackish marshes (97.3 g CH₄ m⁻² y⁻¹) dominated by *Spartina patens* (Delaune et al., 1983), but similar to annual flux rates reported from saltmarshes (804.3 g CH₄ m⁻² y⁻¹) that dominated by *Carex rugulosa* and *Phragmites australis* (Hirota et al., 2007). These higher rates at our study sites could be due to the nature of the study site of being freshwater marshes and having lower SO₄²⁻ as both of the study sites had very low salinity. The negative correlation between salinity level and CH₄ emission rates (Poffenbarger et al., 2011) is in support of our findings about CH₄ emission rates from our tidal freshwater marshes and the presence of higher SO₄²⁻ are reducing the emission of CH₄ (Pennock et al., 2010). Our restored wetland site had significantly higher annual CH₄ emission rate than the natural wetland (1372.1 ± 35.89 and 880.7 ± 144.73 g CH₄ m⁻² y⁻¹, respectively, $P < 0.05$). Other research studies reported similar findings of higher CH₄ emission rates in restored wetlands compared with natural wetlands (Badiou et al., 2011; Richards and Craft, 2015). At the restored site, *Phragmites australis* and *Typha latifolia* were the only plant species colonized at the high and low marsh, respectively. These plants are perennial vegetation and they have extensive aerenchyma tissue that work as gas

exchange system through the stems-roots-rhizome-leaf and make the pathway for any gases like CO₂ or CH₄ to be released easily to the atmosphere, and hence higher methane emission.

Iron is a microelement that is available in minerals of hematite, magnetite, pyrite, and taconite (Eaton et al., 2005). Ferrihydrite, hematite, and goethite are common in most hydric soils, typical wetland soil, where 30-60% of iron in the hydric soils is available in the form of ferrihydrite mineral (Richardson and Hole, 1979). The average iron abundance in streams is 0.7 mg/L, in groundwater ranges from 0.1 to 10 mg/L, and in soils ranges from 0.5 to 4.3% (Eaton et al., 2005). From high to low Eh, the reduction of nitrate NO₃⁻, manganese Mn (IV), iron Fe (III), sulfate SO₄²⁻ and CO₂ are mediated by soil microbes (Ponnamperuma, 1972; Reddy et al., 1989; Yu and Patrick, 2004). Total available pore water iron was higher at 40 cm soil depth at the restored site than the natural site. The occurrence of iron in wetland soils and within pore-water will reduce the potential reduction of CO₂ to CH₄ and hence less CH₄ emission in the natural site, and that support my first hypothesis that pore-water iron was negatively correlated with CH₄ emissions. Moreover, my results indicated that pore-water iron had a significant and strong negative correlation with log CH₄ flux rate for July and August dataset, which also might be the reason for having lower CH₄ emission rates at the natural site. However, no correlation was detected between pore-water iron and log CH₄ flux rates for the whole dataset, implying that time and temperature might also influence correlation between pore-water iron and log CH₄ flux rates.

Spatial variability in sedimentation patterns and hydrology (chapter 2) between different habitat of the restored and the natural wetlands likely contributed to variation in

pore-water iron concentrations. The geospatial and geo-morphological setting are crucial factors for controlling differences in iron abundance between habitats. Low marsh at the natural site has a lower elevation and close to the creek, main source of water and sediment, while high marsh and the swamp habitat are farther and about the same elevation but higher than the low marsh. For the restored site, low marsh is closer to the river and has lower elevation than the high marsh. At 40 cm soil depth in the restored site, pore-water iron in low marsh is largely higher than the high marsh and that could be due to low marsh is closer to the river and receiving more sediment compared to the high marsh. Moreover, tidal fluctuation in the restored site could be another reason for making the iron more abundant in the low marsh. In my study, all the pore-water sampling had occurred during low tide at which surface water had moved quickly to the river, while pore-water especially in the soil macropores tends to discharge to the river as well but in slower rates compared to the surface water. The natural low and high marsh where pore-water iron concentrations are higher in low marsh settings, however the swamp habitat did not follow that pattern which could be because of the presence of hollow and hummocks settings. The swamp had the highest SOM (Chapter 2) and low minerals as the soil is more organic as a result of receiving less sediment from the river. The presence of the hummocks structure with the trees roots might impact the biogeochemistry of the soil and pore-water iron.

Soil Eh data in conjunction with soil pH data revealed that soils of the natural wetland habitats were more reducing than the restored wetland habitats. Labile C, oxygen levels, and water level relative to soil surface are crucial factors in soil Eh. Near to the soil surface, 12.5 cm soil depth in my study, fresh and labile C is available with higher

concentration of oxygen and that result in higher soil Eh, which implies more aerobic conditions. Deeper in the soil surface, 40 cm soil depth in my study, labile C is less available with lower concentration of oxygen and that result in lower soil Eh, which implies more anaerobic conditions. That might be the reason for having higher soil Eh at 12.5 cm soil depth in low and high marsh for both natural and restored sites (Appendix C.8). The swamp did not follow that pattern however, where soil Eh was lower at 12.5 cm and higher at 40 cm. That is exactly the opposite findings in marsh settings. The swamp habitat has trees and shrubs whose roots have a higher respiration rates and hence more air in soil a result of root respiration and more oxygen flow down to the roots under anaerobic conditions. Having higher concentration of oxygen deeper in the swamp habitat soil may be the reason for having higher soil Eh compared to the soil surface.

At the restored site, *Phragmites australis* is the only plant species colonized at the high marsh, which is generally the case of the newly restored wetland to be invaded by invasive plants. The natural wetlands did not have *Phragmites australis* in their vegetation composition. Common reed or *Phragmites australis*, is a perennial grass that occur in brackish and freshwater wetlands (e.g. was the only plant species at the high marsh at the restored site). It has a huge belowground biomass as rhizomes from which new sprout comes out as new shoots at the beginning of the growing season. It has a significant amount of aerenchyma, plant tissue that is responsible for the gas exchange, in stems, rhizomes, and roots. Those plant tissues as a kind of structural adaptation and other mechanical adaptations that prevent or inhibit the growth of other plant species make the common reed a very powerful invasive plant species and able to colonize easily in new habitats. The unique and extensive aerenchyma for the common reed play a

significant role in driving more oxygen down to the root zone and that make the oxygen available for soil microbes that can be used to oxidize soil organic matter, and hence less soil organic matter percentage. Also, that gas exchange system through the stems-roots-rhizome-leaf make the pathway for any gases like CO₂ or CH₄ to be released easily to the atmosphere, and hence higher methane emission and that make the common reed primes soil organic matter as had been founded by other research studies (Bernal et al., 2016)

At the natural site, low marsh was dominated by *Nuphar lutea*, while low and high marsh at the restored site was dominated by *Typha latifolia* and *Phragmites australis*, respectively. Not only are they perennial with huge underground root system, but also they are rich with aerenchyma tissues in their stems and roots. My fourth hypothesis was habitats that are dominant by perennial plant species emit more CH₄ compared with their counterparts that are dominated by annual plant species. Habitats that were dominated by perennial plant species had the highest CH₄ flux rate (2.9±0.08, 3.0±0.00, and 3.2±0.00 Log g CH₄ m⁻² y⁻¹, respectively), and that reject the null of the fourth hypothesis. That fact could be due to the presence of the air flow pathway through root-stem-leaf that made available by the aerenchyma tissues and as a result, any CH₄ that had been produced could find its way for external atmosphere and result in higher CH₄ flux rates. Based on the soil Eh-pH chart presented at figure 4.4, low and high marsh for the restored site were dominant by aerobic soil conditions, which makes the oxygen more readily available for soil microbes to use as an electron acceptor for oxidizing more soil organic matter, and hence prime the soil C stocks. Those findings were similar to other studies (Bernal et al., 2016).

Many field-based research studies report considerable variations CH₄ rates (Bridgham et al., 2013; Krauss et al., 2016; Neubauer and Megonigal, 2015). Those variations could be due to differences in soil microbe composition and quantity, geomorphological settings, vegetation, soil biogeochemistry, salinity, wetland types, water budget, and soil C stocks. My study reports both linear and average rates of CH₄, to assist future scientists in modeling wetland C budgets. Based on my findings, I highly recommend that other studies also report both rate calculations or to be more explicit about their calculations for CH₄ rates. Moreover, precautions should be taken during extrapolating CH₄ emission rates from wetland ecosystems that were based on field and small scale to a global scale. My results indicated lower CH₄ emission rates during the nighttime compared with daytime in support of my second hypothesis. Real nighttime CH₄ fluxes measurements should be substituted for methods that only mimic nighttime conditions. Perennial vegetarians like *Phragmites australis* and *Typha latifolia* that have aerenchyma tissues in their gas-exchange systems should be the focus of the future research as those plants might prime the soil C stocks by providing more oxygen flow down to the root zone through their stems.

4.5 Conclusion

Restored wetland habitats had significantly higher CH₄ rates than the natural wetland habitats (1372.1 ± 35.89 and 880.7 ± 144.73 g CH₄ m⁻² y⁻¹, respectively, P < 0.05). The log CH₄ flux rate had a significant and strong negative correlation with the total available iron in pore-water ($r = -0.64$ at the natural wetland at 12.5 cm soil depth and $r = -0.79$ for the restored site at 40 cm soil depth, P < 0.05). The freshwater natural and restored wetland habitats had higher pore-water iron concentrations than the normal

abundance in other similar freshwater wetlands. Natural high marsh habitat had the highest plant species diversity indices (Shannon-Weiner index $H: 1.7 \pm 0.1$ & Shannon evenness $S: 0.8 \pm 0.0$, $P < 0.05$), while low and high marsh at the restored wetland were monoculture plant communities (*Typha latifolia* and *Phragmites australis*, respectively). Data from soil Eh-pH chart indicated that all the natural wetland habitats had anaerobic soil conditions, while the restored wetland habitats demonstrated less anaerobic soil conditions.

Table 4.1: Results of repeated measures ANOVAs summarizing the effects of habitats (natural low marsh, natural high marsh, natural swamp, restored low marsh, and restored high marsh) and time (May, June, July, and August 2016) on the (a) log CH₄ flux rate and (b) log average CH₄ flux rate.

| Source of variation | Type 3 Tests of Fixed Effects | | | |
|---|-------------------------------|--------|---------|--------|
| a) CH ₄ flux rate Log μ mole CH ₄ m ⁻² h ⁻¹ | Num DF | Den DF | F Value | Pr > F |
| Habitat | 4 | 24.4 | 2.3 | 0.0876 |
| Time | 3 | 40.7 | 6.3 | 0.0013 |
| Habitat*time | 8 | 40 | 7.08 | <.0001 |
| b) average CH ₄ flux rate Log μ mole CH ₄ m ⁻² h ⁻¹ | Num DF | Den DF | F Value | Pr > F |
| Habitat | 4 | 25.5 | 22.04 | <.0001 |
| Time | 3 | 80.5 | 7.28 | 0.0002 |
| Habitat*time | 10 | 80.5 | 6.56 | <.0001 |

Table 4.2: the dominant plant species in each habitat for natural and the restored wetlands with their life duration indicated.

| Site | Habitat | species | Duration |
|------------------|------------|--|-----------|
| Natural wetland | Low marsh | <i>Nuphar lutea</i> (L.) Sm. | Perennial |
| | | <i>Peltandra virginica</i> (L.) Schott | Perennial |
| | High marsh | <i>Pilea pumila</i> (L.) A. Gray | Annual |
| | | <i>Impatiens capensis</i> Meerb. | Annual |
| | | <i>Polygonum arifolium</i> L. | Annual |
| | Swamp | <i>Murdannia keisak</i> (Hassk.) Hand.-Maz. | Perennial |
| | | <i>Bidens laevis</i> (L.) Britton, Sterns & Poggenb. | Annual |
| | | <i>Leersia oryzoides</i> (L.) Sw. | Perennial |
| Restored wetland | Low marsh | <i>Typha latifolia</i> L. | Perennial |
| | High marsh | <i>Phragmites australis</i> (Cav.) Trin. ex Steud. | Perennial |

Table 4.3: Plant species diversity indices for vegetation in natural and restored wetlands.

P values represent two way-ANOVAs. Values are significantly different when $P < 0.05$.

| Site | Habitat | Shannon-Weiner index (H') | Shannon evenness index (S) |
|------------------|------------|--|--|
| Natural wetland | Low marsh | 0.1 ^{CB} ±0.1 | 0.2 ^{CB} ±0.2 |
| | High marsh | 1.7 ^A ±0.1 | 0.8 ^A ±0.0 |
| | Swamp | 0.4 ^B ±0.1 | 0.4 ^B ±0.1 |
| Restored wetland | Low marsh | 0.0 ^C ±0.0 | 0.0 ^C ±0.0 |
| | High marsh | 0.0 ^C ±0.0 | 0.0 ^C ±0.0 |
| ANOVA | | $P_{\text{site}} < 0.0001$; $P_{\text{habitat}} < 0.0001$ | $P_{\text{site}} = 0.0004$; $P_{\text{habitat}} = 0.0004$ |

Means in the same column with different letters are significantly different at $P < 0.05$.

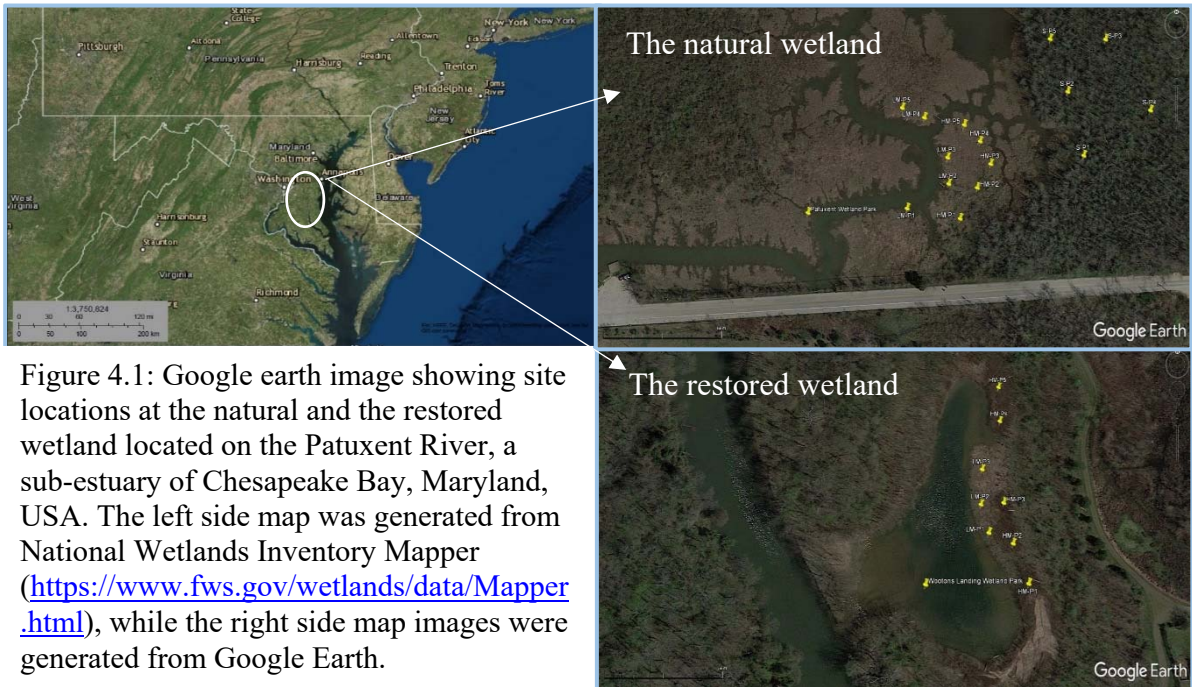


Figure 4.1: Google earth image showing site locations at the natural and the restored wetland located on the Patuxent River, a sub-estuary of Chesapeake Bay, Maryland, USA. The left side map was generated from National Wetlands Inventory Mapper (<https://www.fws.gov/wetlands/data/Mapper.html>), while the right side map images were generated from Google Earth.

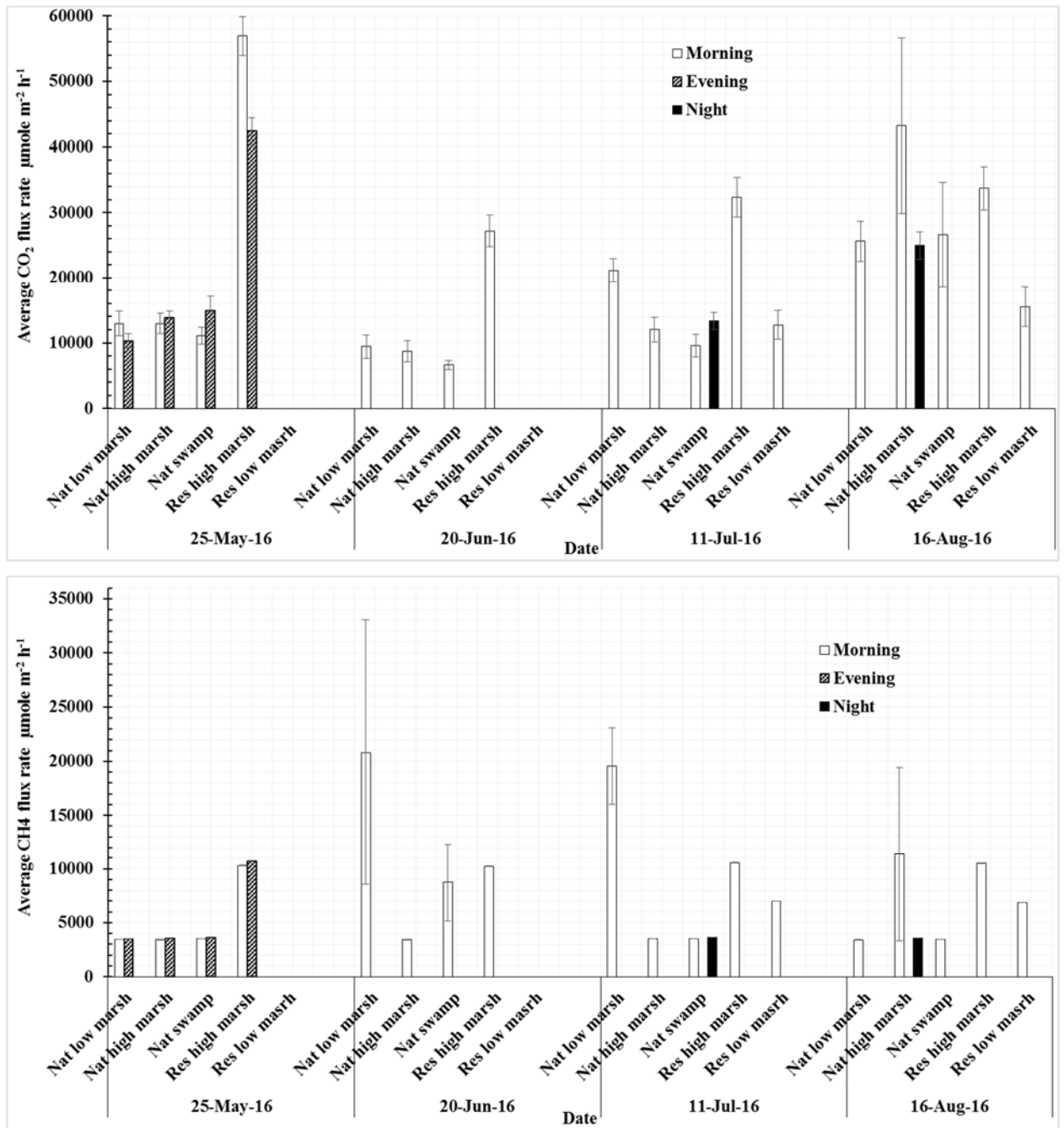


Figure 4.2: Monthly variation for diurnal average (MEAN±SE) CO₂ and CH₄ flux rate for the natural (Nat) and restored (Res) wetland habitats from May to August 2016. Each bar represents 5 samples.

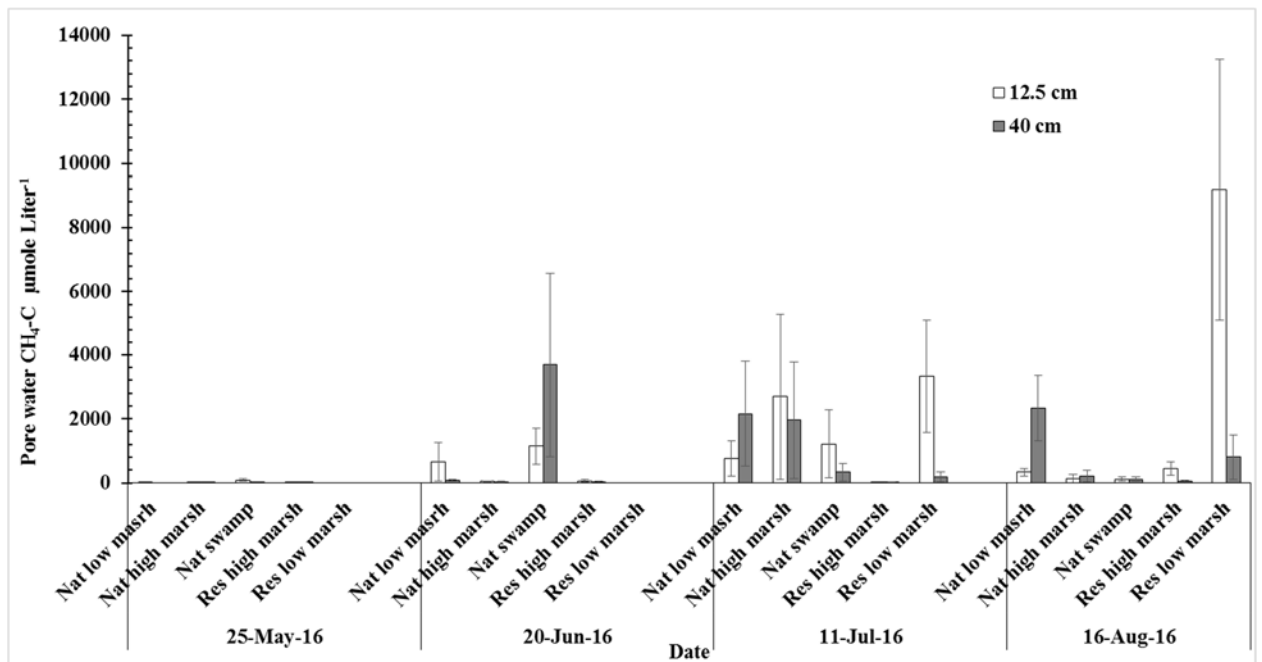
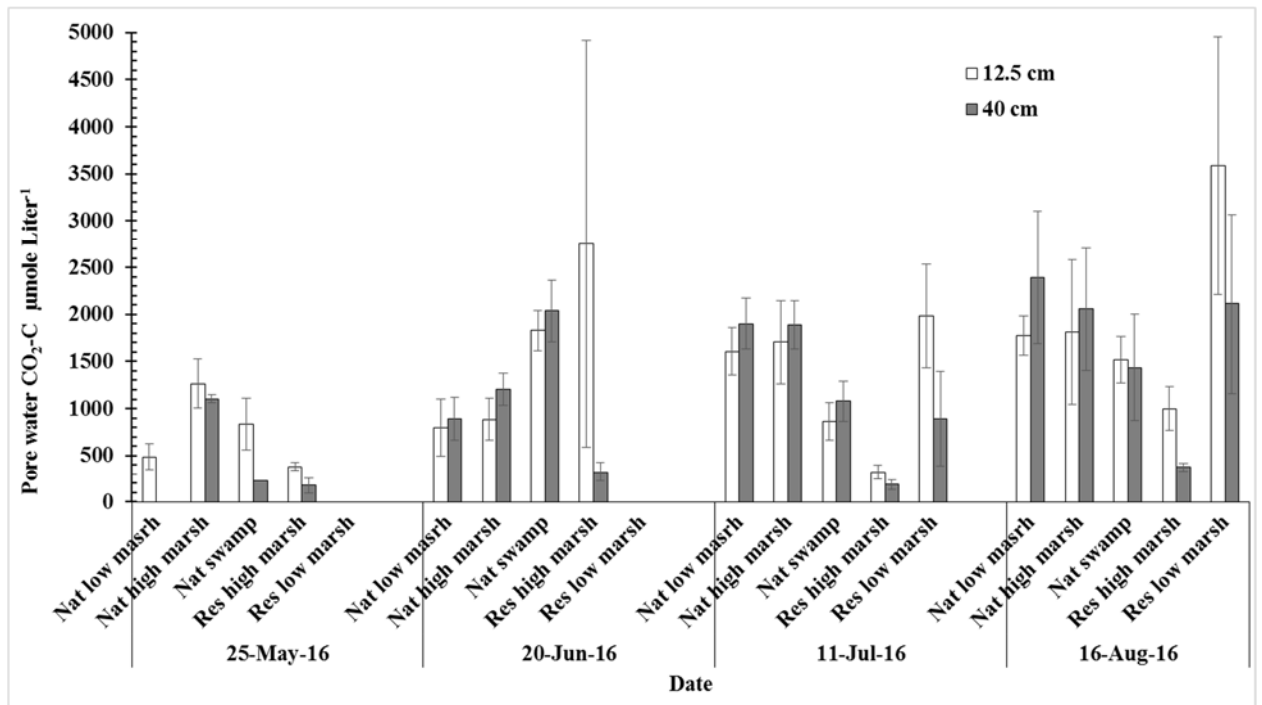


Figure 4.3: Monthly variation (MEAN±SE) in pore-water CO₂ and CH₄ concentrations at 12.5 and 40 cm soil depth for the natural (Nat) and restored (Res) wetland habitats from May to August 2016. Each bar represents 5 samples except the restored low marsh represents 3 samples.

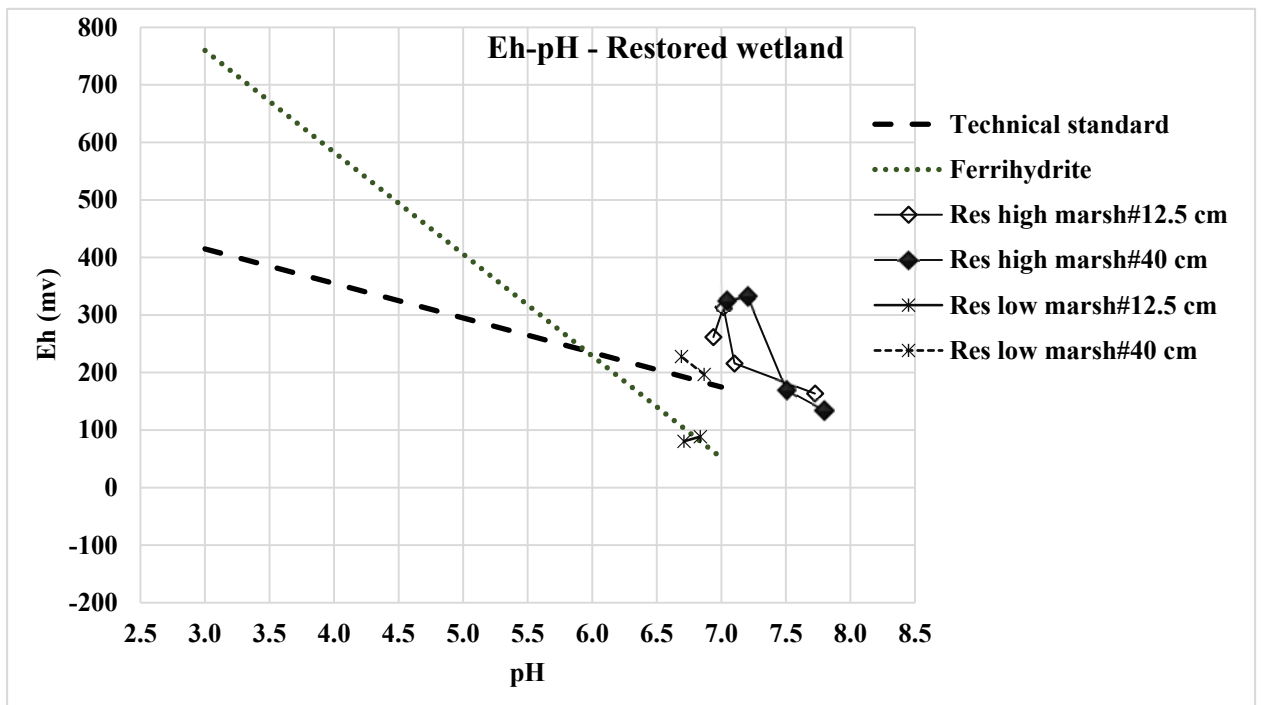
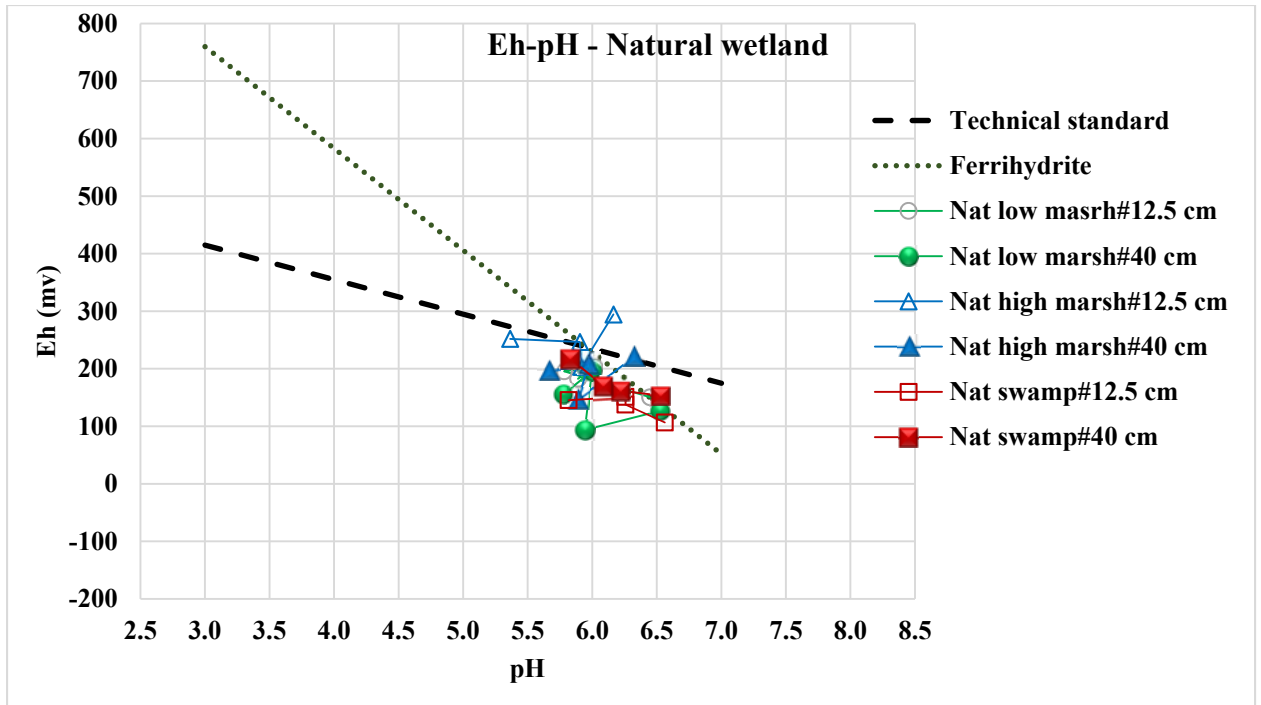


Figure 4.4: Soil oxidation-reduction (Eh) measurements at 12.5 and 40 cm soil depth for natural (Nat) and restored (Res) wetlands. Each bar represents 15 samples except the restored low marsh represents 9 samples.

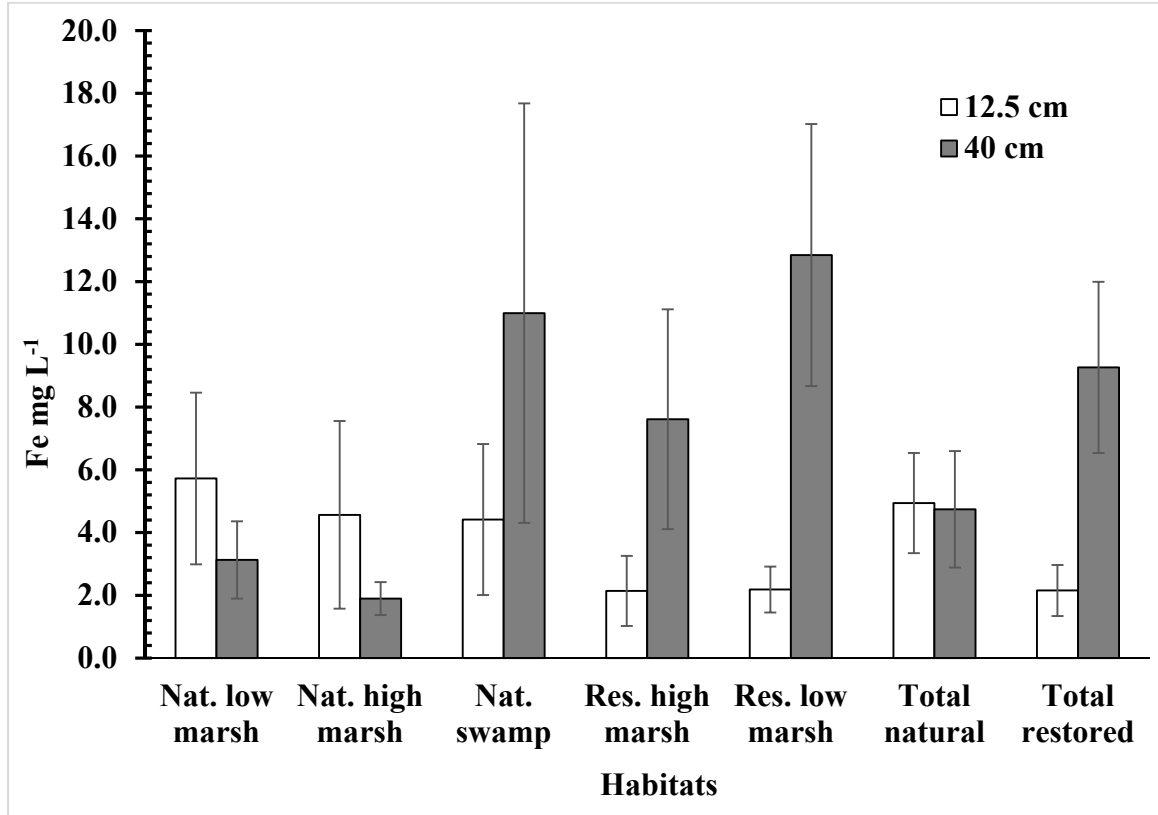


Figure 4.5: Total available iron in pore-water at 12.5 and 40 cm soil depth for natural (Nat.) and restored (Res.) wetlands. Each bar represents 20 samples except the restored low marsh represents 12 samples.

5 Conclusions

Tidal freshwater wetlands provide a variety of wetland ecosystem services and functions including wildlife habitats, water quality improvement, storm water storage, and C sequestration. Wetlands cover 5 to 7% from the world surface area and they are key components of the global C budget. Accumulation of C in wetlands is controlled by many physical factors and biogeochemical processes that are mediated by soil microbes. Net primary productivity, hydrology, decomposition rates, soil redox condition, sedimentation, vegetation composition, rainfall, temperature, and soil microbial communities are crucial factors in C accumulation in wetland ecosystem. Hydrology is the master variable that control the development of soil substrate that enhance the colonization and the development of hydrophytic vegetation that able to handle very low concentrations of oxygen.

In chapter 2, I assessed the differences in the hydro pattern between the natural and the restored wetland habitats. I conclude that higher soil organic matter in the wetland habitats could be partially correlated with shallower groundwater level relative to soil surface. The habitats of the natural wetland had soil organic matter that is significantly higher than their restored counterparts ($P < 0.05$). The highest soil organic matter content was at the swamp habitat (36.8%), while restored mudflat had the lowest (2.8%). The total soil C stock at the natural site was significantly higher than the restored site (14.8 and 8.9 kg C m⁻², respectively, $P \text{ site} < 0.05$). The hydrology at the restored site was restored by excavating the soil surface and that had a negative impact on soil C pools. Groundwater level relative to soil surface was at shallower depths in the natural site than the restored. The swamp habitat had the lowest soil redox measurement at 12.5 cm soil depth, while the restored low and high marsh had higher values for soil redox,

implying less anaerobic conditions at the restored site. So, based on my data, I conclude that C accumulation might be very slow at the wetland sites that were restored by excavation.

In chapter 3, the natural wetland habitats accumulated C, while the restored wetland habitats were neither able to accumulate C nor maintain the C inside the CARBIO. After one year from CARBIO units deployment, C sequestration rate for the soil inside the CARBIO deployed in the natural wetland was significantly higher than the restored wetlands (535 ± 291.5 and -1095 ± 429.4 g C m⁻² year⁻¹, respectively, $P_{\text{site}} < 0.05$). Mesh bag type had a significant effect ($P < 0.05$) on the below-ground biomass productivity, and that might affect C accumulation rates on the natural and the restored wetland habitats. However, no matter what was the CARBIO mesh bag size, the restored wetland habitats weren't accumulating C at the same level as the natural site, implying the necessity for improving the wetland restoration technique and conserving the natural wetlands.

In chapter 4, flux rates for CH₄ were assessed during day and nighttime for the natural and the restored wetland habitats. Annual CH₄ emission rates were significantly higher at the restored site than the natural (1372.1 ± 35.89 and 880.7 ± 144.73 g CH₄ m⁻² y⁻¹, respectively, $P < 0.05$). The log CH₄ flux rate had a significant and strong negative correlation with the pore-water total available iron ($r = -0.64$ at the natural wetland at 12.5 cm soil depth and $r = -0.79$ for the restored site at 40 cm soil depth, $P < 0.05$). Average of the total available iron in pore-water was higher than the normal abundance in similar freshwater wetlands, and pore water iron in the restored site was higher than the natural wetland, and that might be a key factor for having lower CH₄ fluxes the natural

wetlands. Soil redox data with the soil pH revealed that the natural wetland soils have more reducing conditions than the restored which demonstrated aerobic condition in the Eh-pH chart (Figure 4.4). Vegetation composition analysis indicated that the natural wetland habitats had significantly higher plant species diversity indices than the restored site, and the high marsh for the natural wetland was the most diverse (Shannon-Weiner index H: 1.7 ± 0.1 & Shannon evenness S: 0.8 ± 0.0 , $P < 0.05$). Both low and high marsh at the restored site were monoculture plant communities dominated by *Typha latifolia* and *Phragmites australis*, respectively. CH₄ nighttime fluxes had very low concentrations ($< 3650 \mu\text{mole m}^{-2} \text{ h}^{-1}$), implying that greenhouse gas emission models should take the daily variation of CH₄ fluxes into consideration in order to better represent global C budget in wetlands.

Future research should be extended by testing multiple restored, newly constructed, and disturbed wetlands along the salinity gradient. With applying CARBIO, C stability could be examined in fresh, brackish, and saltmarshes to study the effect of salinity on C decomposition rates. Future sampling sites should be more focused on habitats that are dominated by vegetation that are rich in the aerenchyma tissues in their stems and roots, as current results from chapter 4 indicating higher CH₄ flux rates from the habitats that were dominated by such species *Typha latifolia* and *Phragmites australis*. With no doubt, studying microbial activities that control CH₄ fluxes should be at top priority, as soil microbes mediate the biochemical processes that control the CH₄ emissions. Moreover, soil characterization for C and N should be investigated for the soil that in use for filling CARBIO before and after deployment as that will be deepening our understating for C accumulation in wetlands.

Appendices

Appendix A

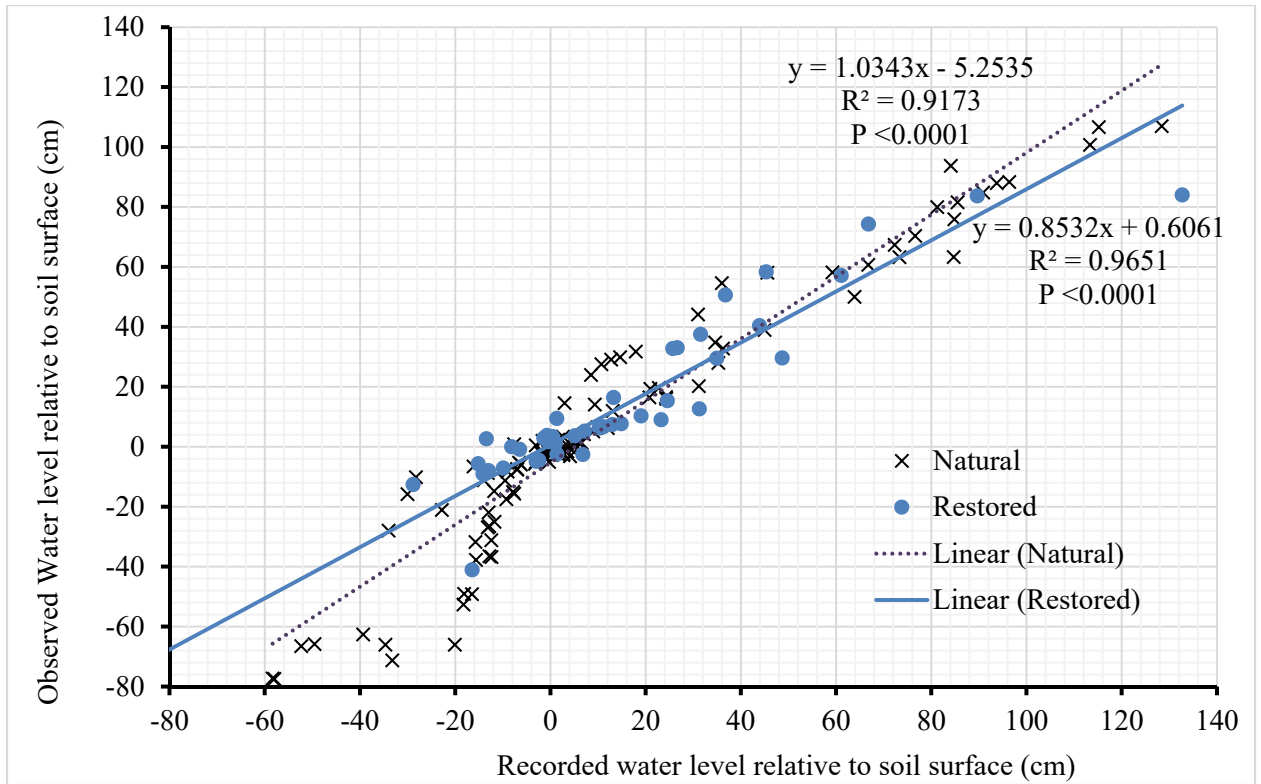


Figure A.1: Linear regression analysis of recorded vs observed water level for natural and restored sites.

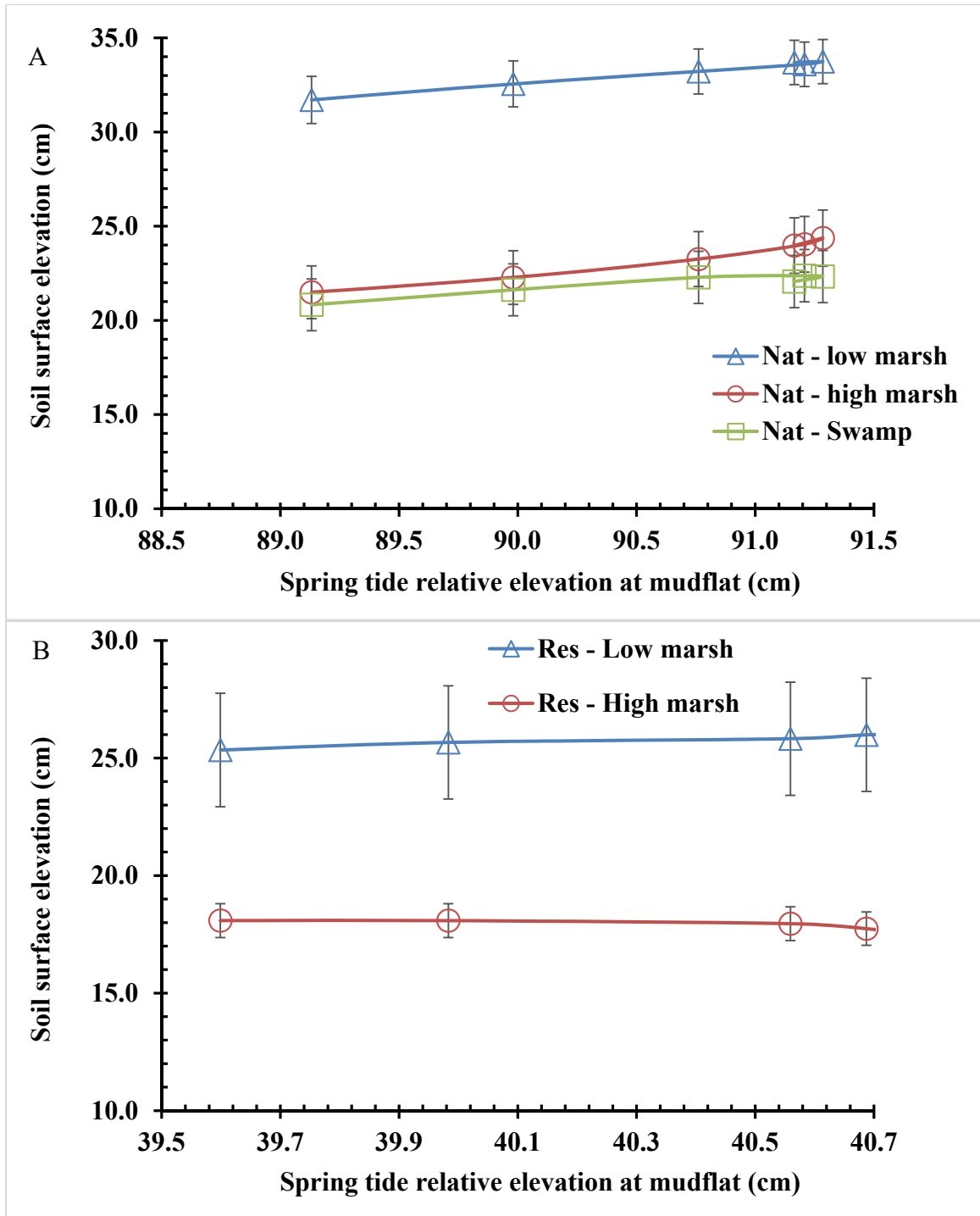


Figure A.2: Habitat soil surface elevation (mean \pm SE) relative to mudflat during spring tide in natural (A) and restored (B) tidal fresh water wetlands.

Table A.3: Summary for hydrological parameters for natural and restored Wetlands.

PWP: Patuxent Wetland Park; WLWP: Wootons Landing Wetland Park. MF: mudflat,

LM: low marsh, HM: high marsh, S: swamp, and U: upland.

| Hydrological parameter | Natural wetland PWP | | | | | Restored wetland WLWP | | | |
|--------------------------|---------------------|------|------|------|-----|-----------------------|-----|------|-----|
| | MF | LM | HM | S | U | MF | LM | HM | U |
| Flood frequency (%) | 100 | 46.5 | 62.5 | 75.5 | 0 | 100 | 68 | 60 | 0.1 |
| Highest water level (cm) | 140 | 75 | 65 | 65 | -5 | 120 | 140 | 55 | 15 |
| Lowest water level (cm) | 15 | -20 | -20 | -5 | -75 | 5 | -25 | -100 | -90 |

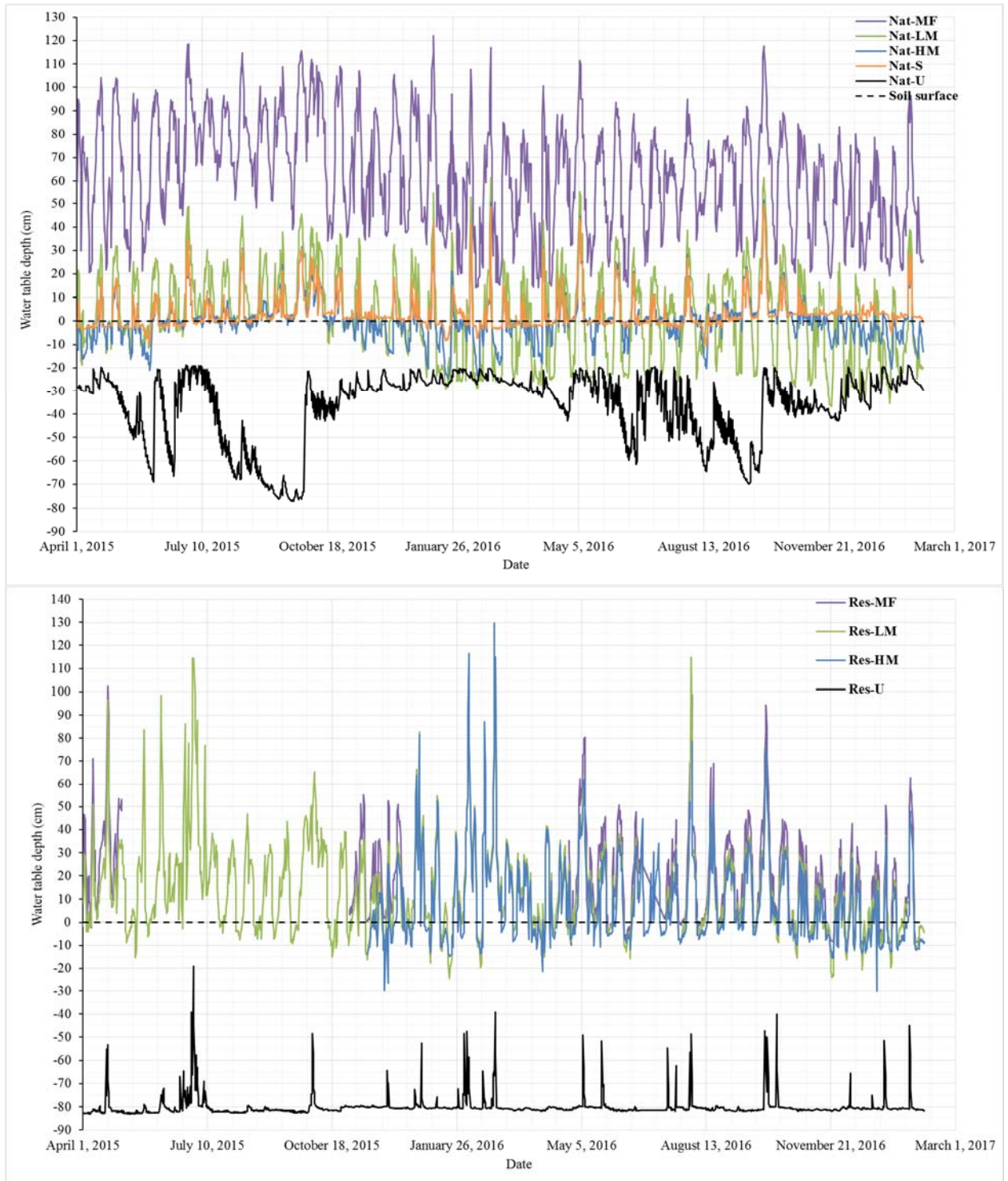


Figure A.4: Water level fluctuations (MEAN) for natural (top) and restored (bottom) tidal fresh water wetlands from April 2015 to March 2017. MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland. Error bars were removed for clarity.

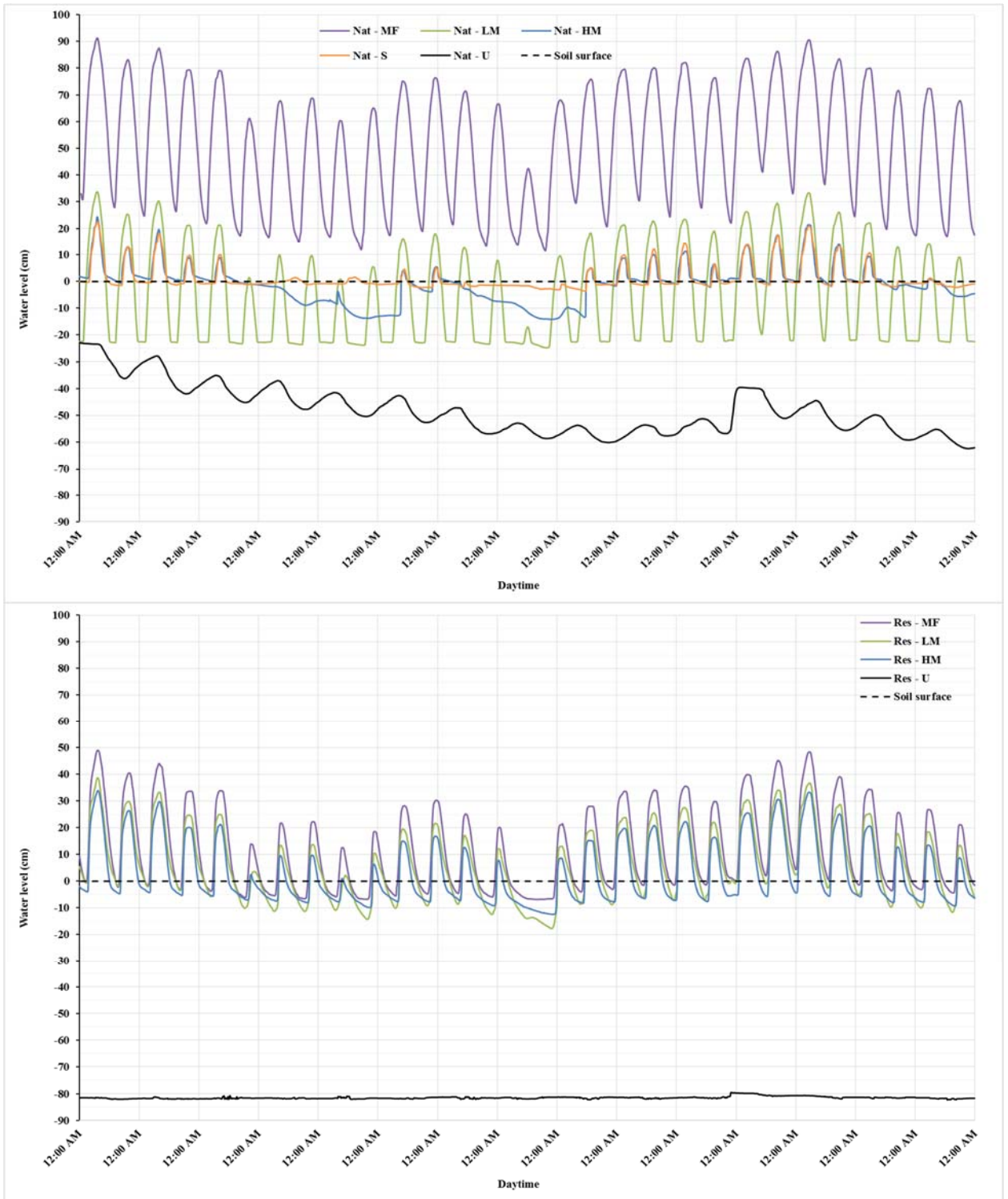


Figure A.5: Water level fluctuations for natural (top) and restored (bottom) tidal fresh water wetlands during June 6-20, 2016. MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland.

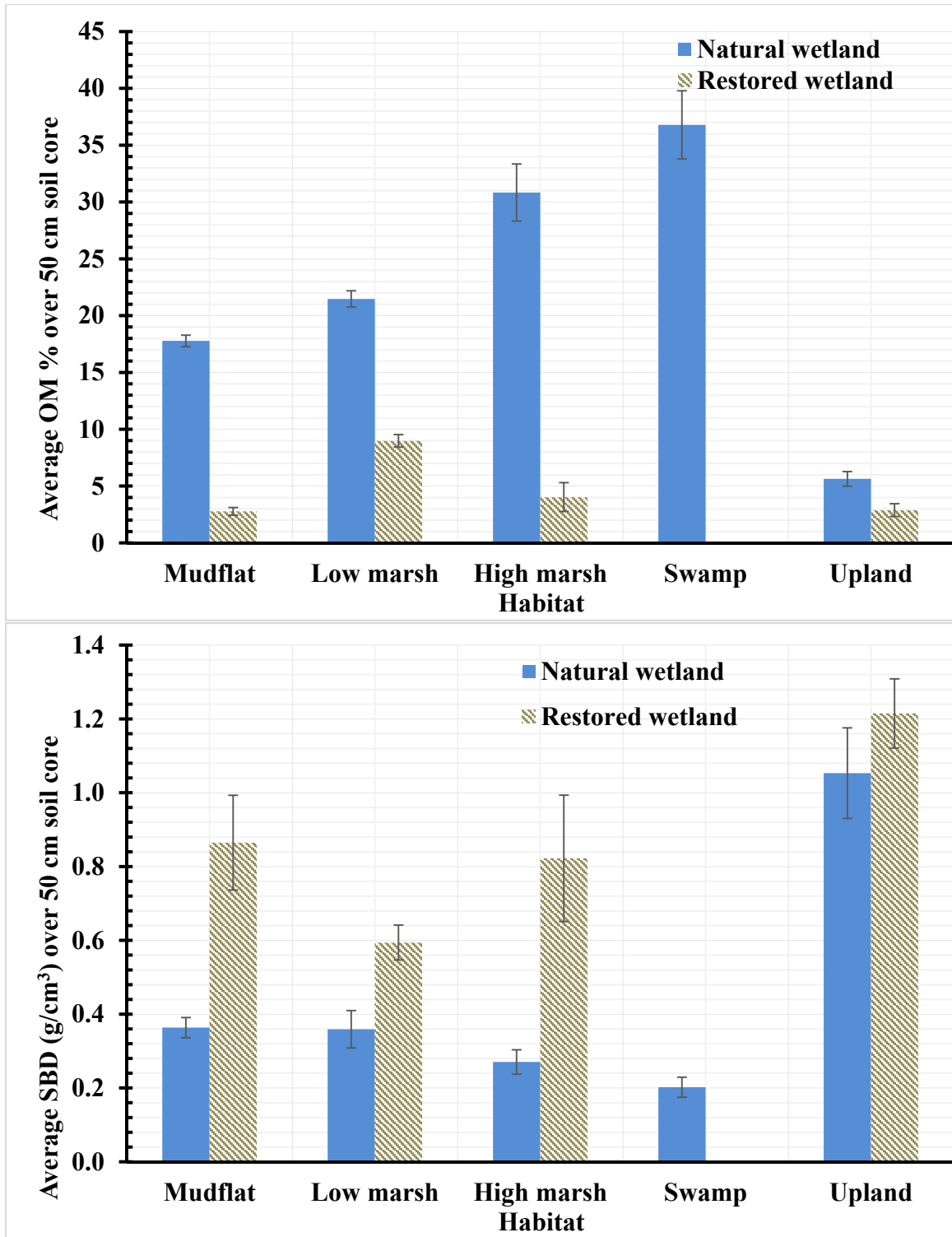


Figure A.6: Average soil organic matter % (top) and Soil bulk density g cm^{-3} (bottom) (mean \pm SE) over 50 cm soil depth in natural and restored tidal fresh water wetlands.

Table A.7: Distribution of soil bulk density (mean \pm SE) g cm^{-3} for 50 cm soil depth in natural (left) and restored (right) tidal fresh water wetlands. MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland.

| Habitat | Mudflat | | low marsh | | High marsh | | Swamp | Upland | |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Soil depth (cm) | Nat. | Res. | Nat. | Res. | Nat. | Res. | Nat. | Nat. | Res. |
| 00-05 | 0.20 \pm 0.06 | 0.15 \pm 0.07 | 0.21 \pm 0.04 | 0.23 \pm 0.11 | 0.12 \pm 0.02 | 0.11 \pm | 0.08 \pm 0.01 | 0.40 \pm 0.04 | 0.56 \pm 0.11 |
| 05-10 | 0.26 \pm 0.07 | 0.35 \pm 0.20 | 0.27 \pm 0.00 | 0.56 \pm 0.03 | 0.14 \pm 0.02 | 0.18 \pm 0.02 | 0.06 \pm 0.01 | 0.69 \pm 0.11 | 1.03 \pm 0.10 |
| 10-15 | 0.30 \pm 0.07 | 0.56 \pm 0.30 | 0.25 \pm 0.02 | 0.77 \pm 0.06 | 0.15 \pm 0.02 | 0.26 \pm 0.16 | 0.17 \pm 0.04 | 0.96 \pm 0.31 | 1.49 \pm 0.22 |
| 15-20 | 0.39 \pm 0.03 | 0.79 \pm 0.41 | 0.13 \pm 0.09 | 0.66 \pm 0.05 | 0.20 \pm 0.04 | 0.43 \pm 0.10 | 0.18 \pm 0.03 | 1.38 \pm 0.18 | 1.62 \pm 0.14 |
| 20-25 | 0.37 \pm 0.02 | 0.84 \pm 0.34 | 0.28 \pm 0.08 | 0.72 \pm 0.03 | 0.31 \pm 0.10 | 0.71 \pm 0.03 | 0.18 \pm 0.06 | 1.12 \pm 0.04 | 1.15 \pm 0.22 |
| 25-30 | 0.44 \pm 0.03 | 0.99 \pm 0.27 | 0.34 \pm 0.05 | 0.62 \pm 0.10 | 0.34 \pm 0.04 | 1.30 \pm 0.08 | 0.22 \pm 0.03 | 0.82 \pm 0.06 | 1.03 \pm 0.09 |
| 30-35 | 0.43 \pm 0.01 | 1.21 \pm 0.14 | 0.60 \pm 0.10 | 0.61 \pm 0.02 | 0.36 \pm 0.05 | 1.24 \pm 0.13 | 0.28 \pm 0.01 | 0.87 \pm 0.27 | 1.39 \pm 0.15 |
| 35-40 | 0.41 \pm 0.02 | 1.19 \pm 0.15 | 0.57 \pm 0.04 | 0.63 \pm 0.11 | 0.36 \pm 0.03 | 1.48 \pm 0.07 | 0.31 \pm 0.03 | 1.15 \pm 0.15 | 1.31 \pm 0.09 |
| 40-45 | 0.46 \pm 0.02 | 1.33 \pm 0.07 | 0.50 \pm 0.05 | 0.50 \pm 0.19 | 0.37 \pm 0.07 | 1.35 \pm 0.06 | 0.29 \pm 0.05 | 1.45 \pm 0.20 | 1.33 \pm 0.12 |
| 45-50 | 0.38 \pm 0.02 | 1.25 \pm 0.06 | 0.45 \pm 0.04 | 0.63 \pm 0.12 | 0.35 \pm 0.08 | 1.18 \pm 0.03 | 0.25 \pm 0.01 | 1.70 \pm 0.31 | 1.24 \pm 0.27 |

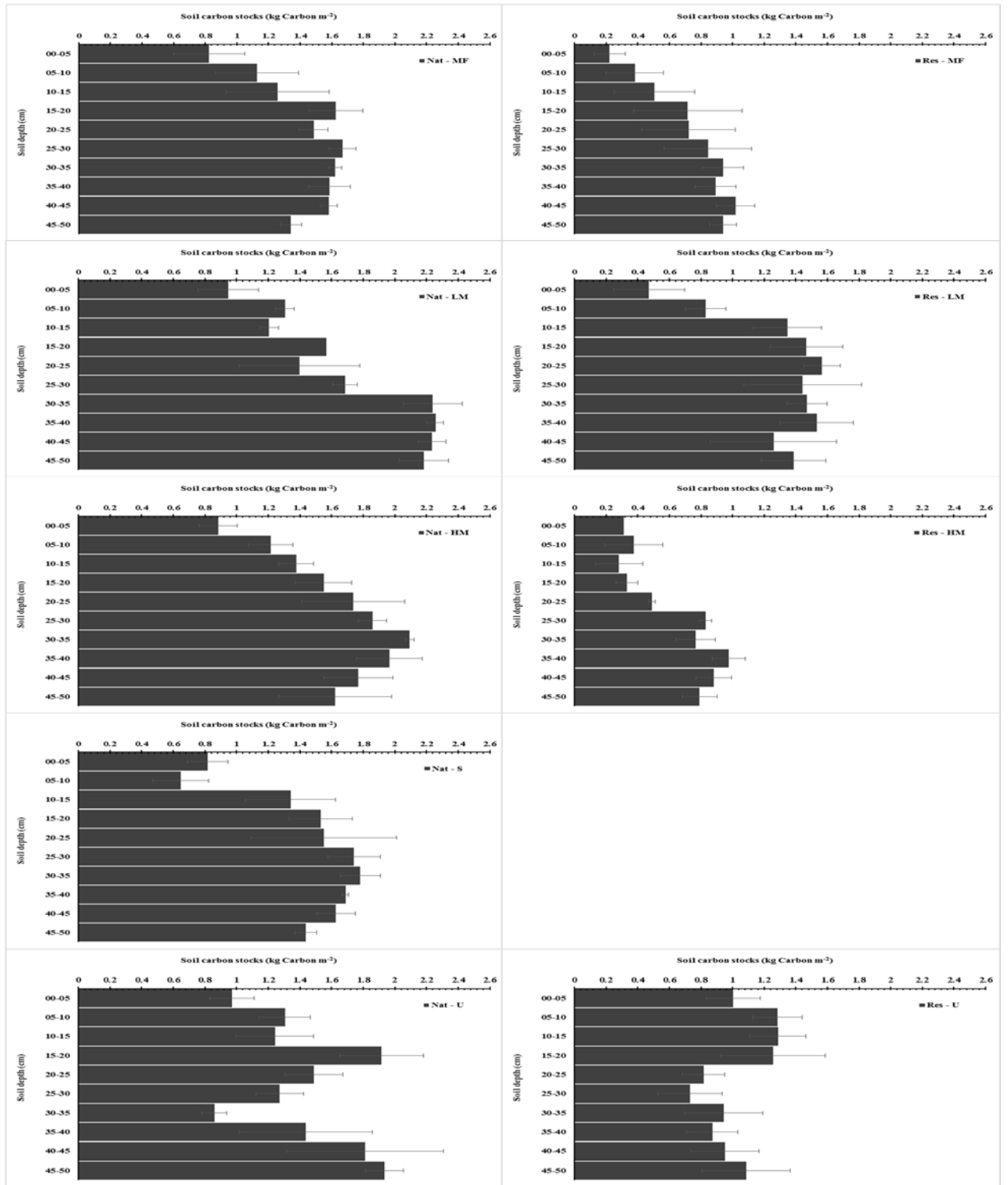


Figure A.8: Distribution of soil C stocks (mean \pm SE) kg C m⁻² over 50 cm soil depth in natural (left) and restored (right) tidal fresh water wetlands. MF: mudflat, LM: low marsh, HM: high marsh, S: swamp, U: upland.

Table A.9: List of plant species identified for the natural and restored wetlands. Habitats are MF: Mud Flat, LM: Low Marsh, HM: High Marsh, S: Swamp and U: Upland.

| Species | Natural wetland | | | | | Restored wetland | | | |
|--|-----------------|----|----|---|---|------------------|----|----|----|
| | MF | LM | HM | S | U | MF | LM | HM | U |
| <i>Ceratophyllum demersum</i> L. | + | + | | | | + | | | |
| <i>Hydrilla verticillata</i> (L. f.) Royle | + | + | | | | + | | | |
| <i>Pontederia cordata</i> L. | | | | | | | + | | |
| <i>Phragmites australis</i> (Cav.) Trin. ex Steud. | | | | | | | | + | |
| <i>Najas marina</i> L. | + | | | | | | | | |
| <i>Nuphar lutea</i> (L.) Sm. | | + | + | | | | | | |
| <i>Sagittaria latifolia</i> Willd. | | + | + | | | | | | |
| <i>Peltandra virginica</i> (L.) Schott | | + | + | | | | | | |
| <i>Polygonum punctatum</i> Elliott | | + | | | | | | | |
| <i>Polygonum arifolium</i> L. | | | + | + | | | | | |
| <i>Polygonum hydropiperoides</i> Michx. | | | | + | | | | | |
| <i>Impatiens capensis</i> Meerb. | | | + | | | | | | |
| <i>Pilea pumila</i> (L.) A. Gray | | | + | + | | | | | |
| <i>Mikania scandens</i> (L.) Willd. | | | + | | | | | | |
| <i>Cuscuta gronovii</i> (Willd. ex Schult). | | | + | | | | | | |
| <i>Bolboschoenus fluviatilis</i> (Torr.) Soják | | | + | | | | | | |
| <i>Cicuta maculata</i> L. | | | + | | | | | | |
| <i>Bidens cernua</i> L. | | | + | | | | | | |
| <i>Onoclea sensibilis</i> L. | | | | | + | | | | |
| <i>Rubus flagellaris</i> Willd. | | | | | + | | | | + |
| <i>Campsis radicans</i> (L.) Seem. ex Bureau | | | | | + | | | | |
| <i>Parthenocissus quinquefolia</i> (L.) Planch. | | | | | + | | | | + |
| <i>Hedera helix</i> L. | | | | | + | | | | |
| <i>Celastrus orbiculatus</i> Thunb. | | | | | + | | | | |
| <i>Ilex verticillata</i> (L.) A. Gray | | | | | + | | | | |
| <i>Lespedeza cuneata</i> (Dum. Cours.) G. Don | | | | | | | | | + |
| <i>Solidago</i> sp. | | | | | | | | | + |
| <i>Dichanthelium</i> sp. | | | | | | | | | + |
| <i>Ligustrum</i> sp. | | | | | | | | | + |
| <i>Toxicodendron radicans</i> (L.) Kuntze | | | | | | | | | + |
| <i>Geum canadense</i> Jacq. | | | | | | | | | + |
| <i>Rosa multiflora</i> Thunb. | | | | | | | | | + |
| <i>Solidago rugosa</i> Mill. | | | | | | | | | + |
| Total species = 33 | 3 | 6 | 11 | 3 | 7 | 2 | 1 | 1 | 10 |

Appendix B

Table B.1: Mean \pm standard error for soil organic matter % determined under different approaches with loss-on-ignition for 2 hours at 550 °C.

| Approaches for estimating soil organic matter | | | | |
|---|--------------------------|-------------------------------------|--|---|
| Site | Air dried Sieved 2 mm | Air dried Grinded Sieved 2 mm | Sieved 1 mm Air dried Grinded | Sieved 12.5 mm Air dried Grinded Sieved 2 mm |
| A | 35.70 \pm 0.19 | 34.16 \pm 0.05 | 31.33 \pm 0.05 | -- |
| B | 13.86 \pm 0.07 | 14.17 \pm 0.02 | -- | -- |
| C | 2.83 \pm 0.10 | 6.48 \pm 0.16 | -- | -- |
| D | 37.40 \pm 0.04 | 36.34 \pm 0.08 | 30.61 \pm 0.02 | -- |
| E | 22.34 \pm 0.19 | 22.23 \pm 0.08 | 21.29 \pm 0.18 | 22.58 \pm 0.05 |

Table B.2: Mean and standard error for the particle size analyses for site (E). P values represent analyses of variance 1- way (ANOVA) and n=3. Means in the same column followed by different letters are significantly different at P < 0.05, according to Duncan multiple range test.

| Soil from site E | | sand % | silt % | clay % | Fine clay % | *FC/TC | Texture Class |
|--|-----------|--------------------|--------------------|--------------------|--------------------|-------------------|---------------|
| Sieved through 1mm while wet, then air dried, then grinded | Mean | 11.18 ^A | 52.34 ^B | 36.47 ^A | 12.77 ^A | 0.35 ^A | Silty-Clay |
| | Std Error | 0.37 | 0.70 | 0.82 | 0.43 | 0.00 | |
| Air dried, then grinded, then sieved through 2 mm | Mean | 8.99 ^B | 54.76 ^A | 36.25 ^A | 13.01 ^A | 0.36 ^A | Silty-Clay |
| | Std Error | 0.61 | 0.45 | 0.26 | 0.25 | 0.00 | |
| P - value | | 0.0377 | 0.0433 | 0.8050 | 0.6631 | 0.2451 | |

* FC/TC: Fine clay/Total clay ratio.

Table B.3: Mean \pm standard error for the total C concentration (% C dry weight soil) for soils inside CARBIO before deploying (t_0). P values represent analysis of variance 2- way (ANOVA) n=3.

| Soil depth | Large mesh | Fine mesh | Fine mesh-SPVC |
|--|---------------------------|---------------------------|---------------------------|
| 0-5 | 9.05 \pm 0.05 | 9.01 \pm 0.03 | 9.11 \pm 0.09 |
| 5-10 | 8.97 \pm 0.02 | 9.06 \pm 0.11 | 9.26 \pm 0.14 |
| 10-15 | 9.01 \pm 0.04 | 9.06 \pm 0.06 | 9.19 \pm 0.09 |
| 15-20 | 9.04 \pm 0.01 | 9.12 \pm 0.04 | 9.18 \pm 0.13 |
| 20-25 | 9.08 \pm 0.04 | 9.08 \pm 0.03 | 9.19 \pm 0.06 |
| 25-30 | 9.06 \pm 0.05 | 9.13 \pm 0.05 | 9.13 \pm 0.03 |
| 30-35 | 9.00 \pm 0.01 | 9.02 \pm 0.04 | 9.13 \pm 0.07 |
| 35-40 | 9.07 \pm 0.07 | 9.16 \pm 0.03 | 9.13 \pm 0.10 |
| 40-45 | 9.29 \pm 0.24 | 9.02 \pm 0.05 | 9.04 \pm 0.07 |
| 45-50 | 8.98 \pm ----- | -- | -- |
| Total Mean | 9.06 ^B n=27 | 9.07 ^B n=27 | 9.15 ^A n=24 |
| P _{mesh bag} = 0.0410, P _{soil depth} = 0.9667, P _{mesh bag* soil depth} = 0.4657 | | | |

Means in the same row followed by different letters are significantly different at $P < 0.05$ according to Duncan multiple range test.

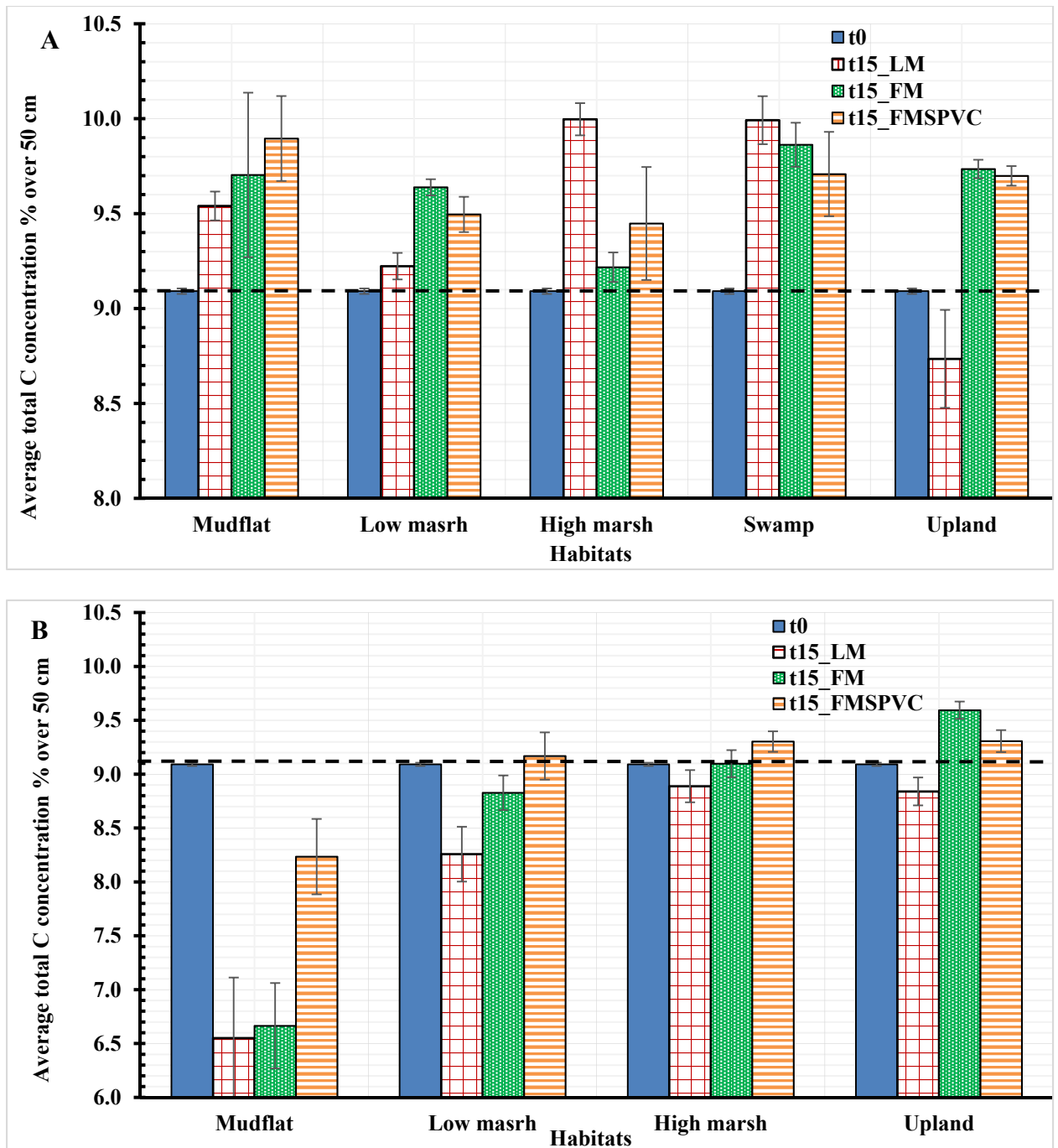


Figure B.4: Variation in total C concentration % (mean \pm SEM) for the mesh bags soil after 15 month from deployment in natural (A) and restored (B) wetlands. t₀: initial time; t₁₅: 15 month after deploying; LM: Large mesh; FM: fine mesh; FMSPVC: fine mesh inside the slotted PVC. Horizontal dashed line represent the total C concentration at t₀.

Appendix C



Figure C.1: Field photo showing the gas flux chamber setup.

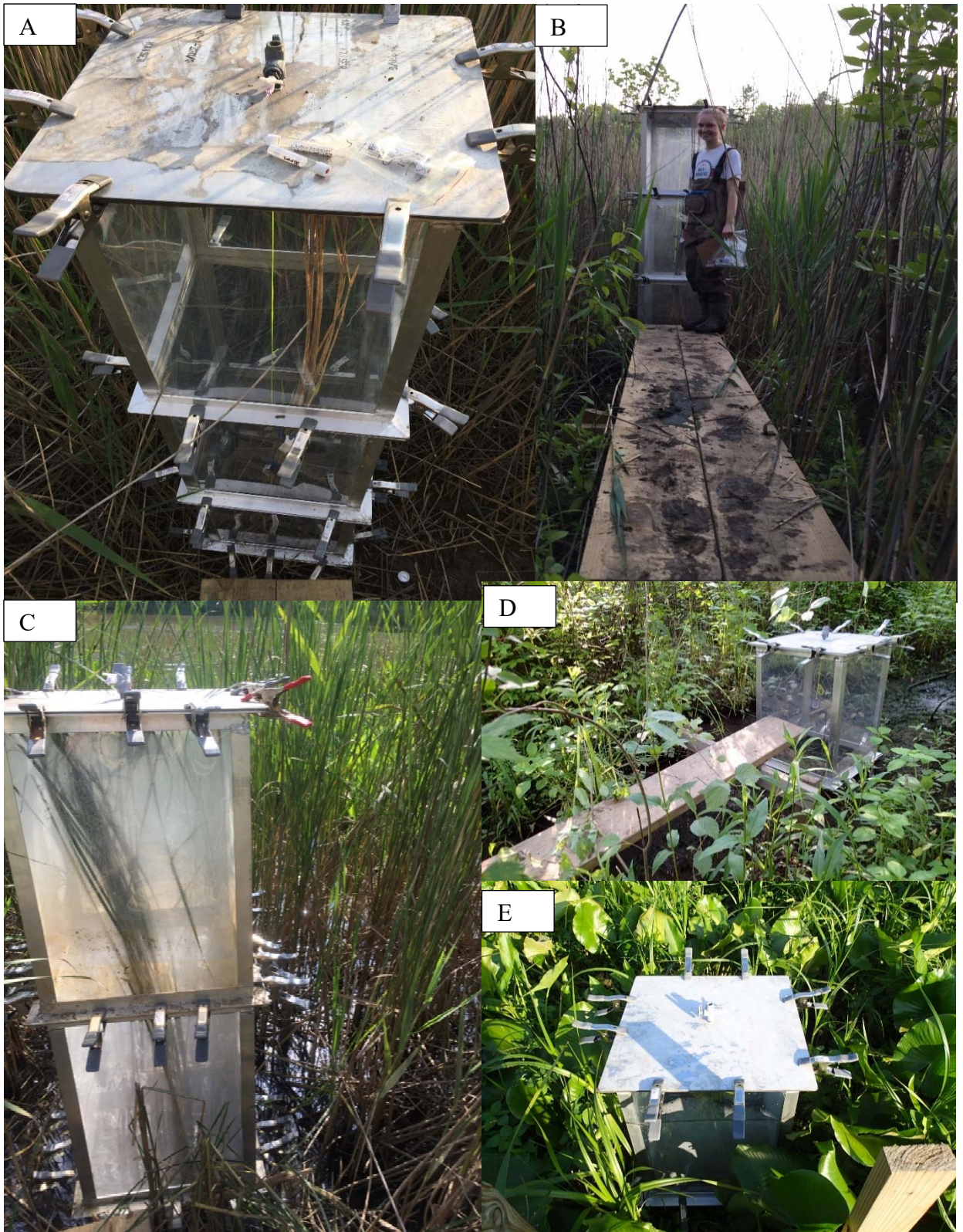


Figure C.2: Field photos showing three chambers stacked above each other to accommodate *Phragmites australis* (A & B), two chambers stacked to accommodate *Typha latifolia* (C), and one chamber with 3 meters boardwalk (D & E).

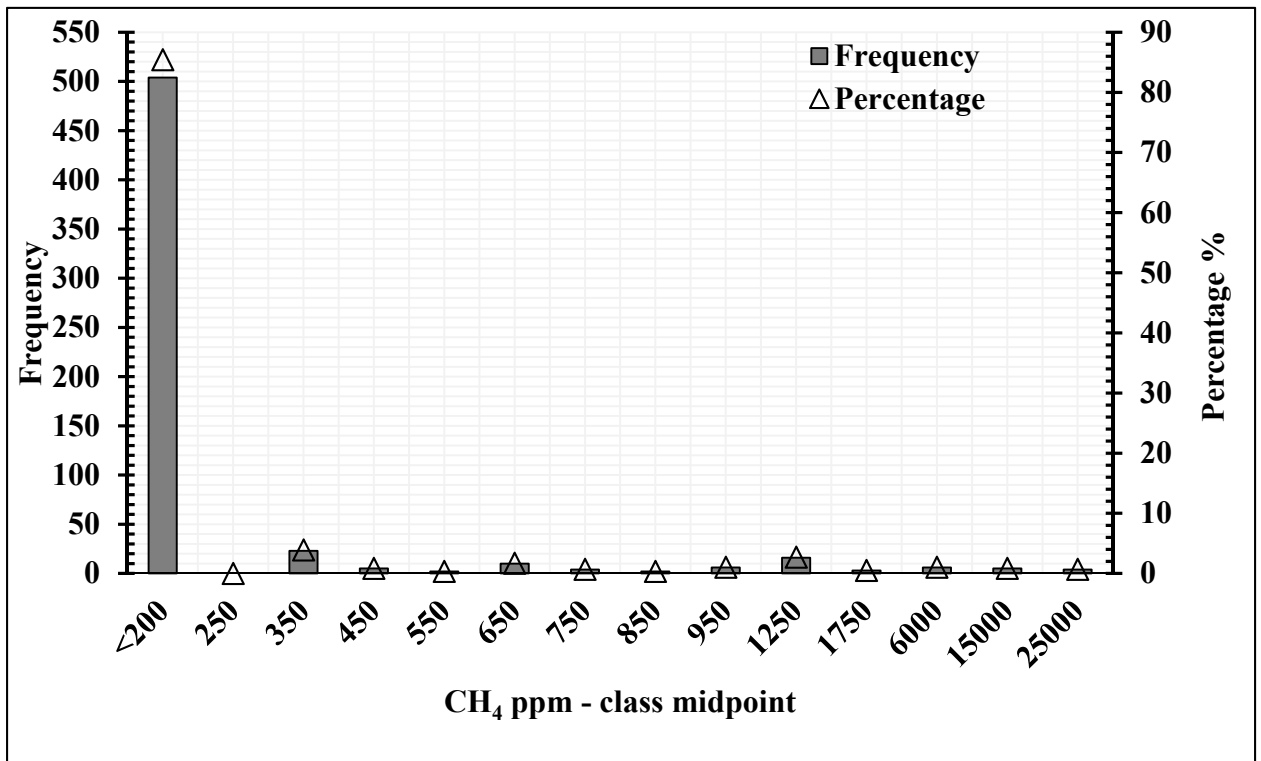
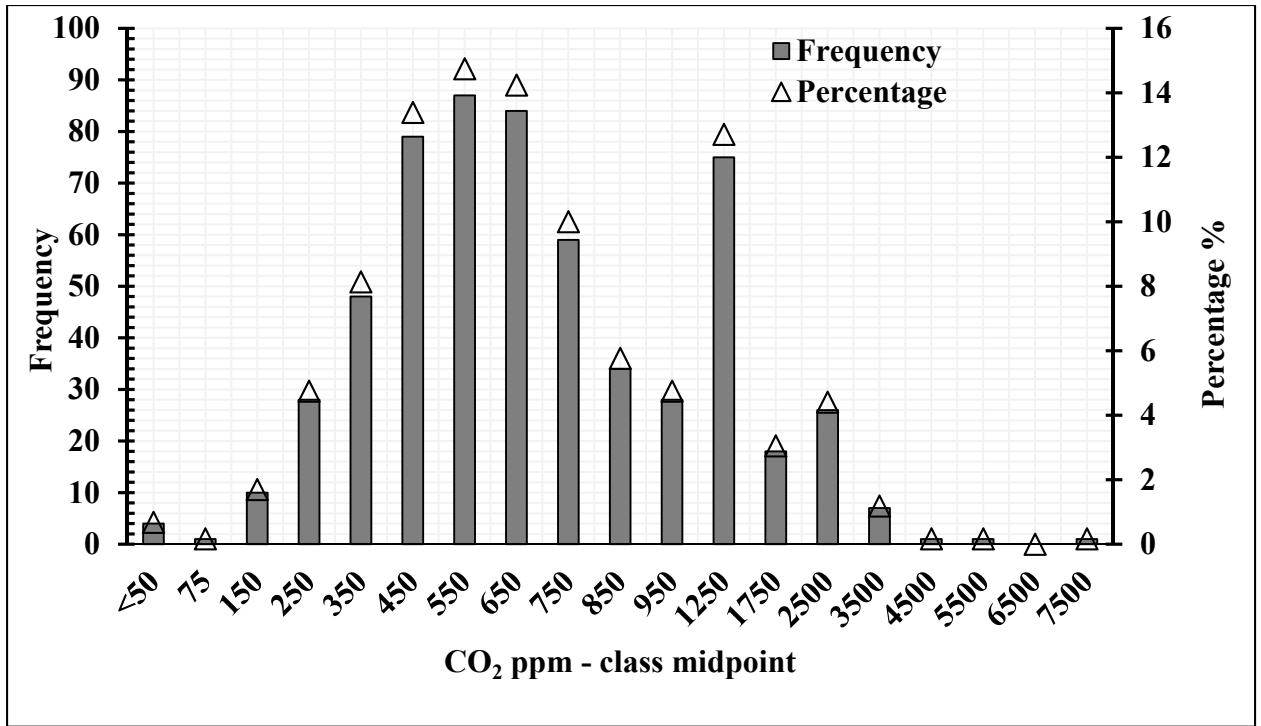


Figure C.3: Histogram showing the distribution of CH₄ and CO₂ concentrations emitted from all natural and restored wetland habitats from May to August 2016.

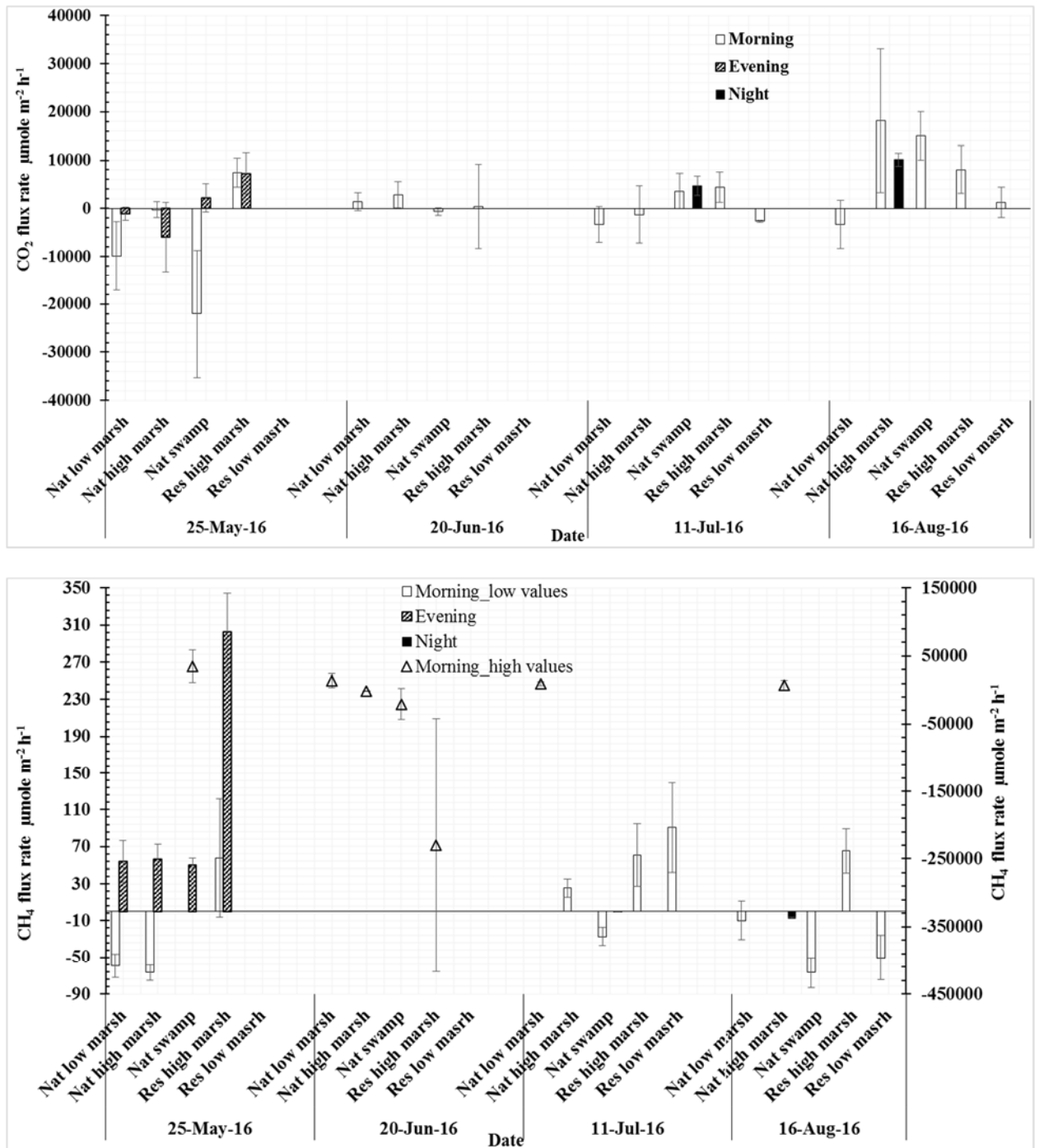


Figure C.4: Variation of diurnal CO₂ and CH₄ flux rate for natural (Nat) and restored (Res) wetlands from May to August 2016. Lower values presented at the left axis, while the higher values present at the right axis for the bottom chart.

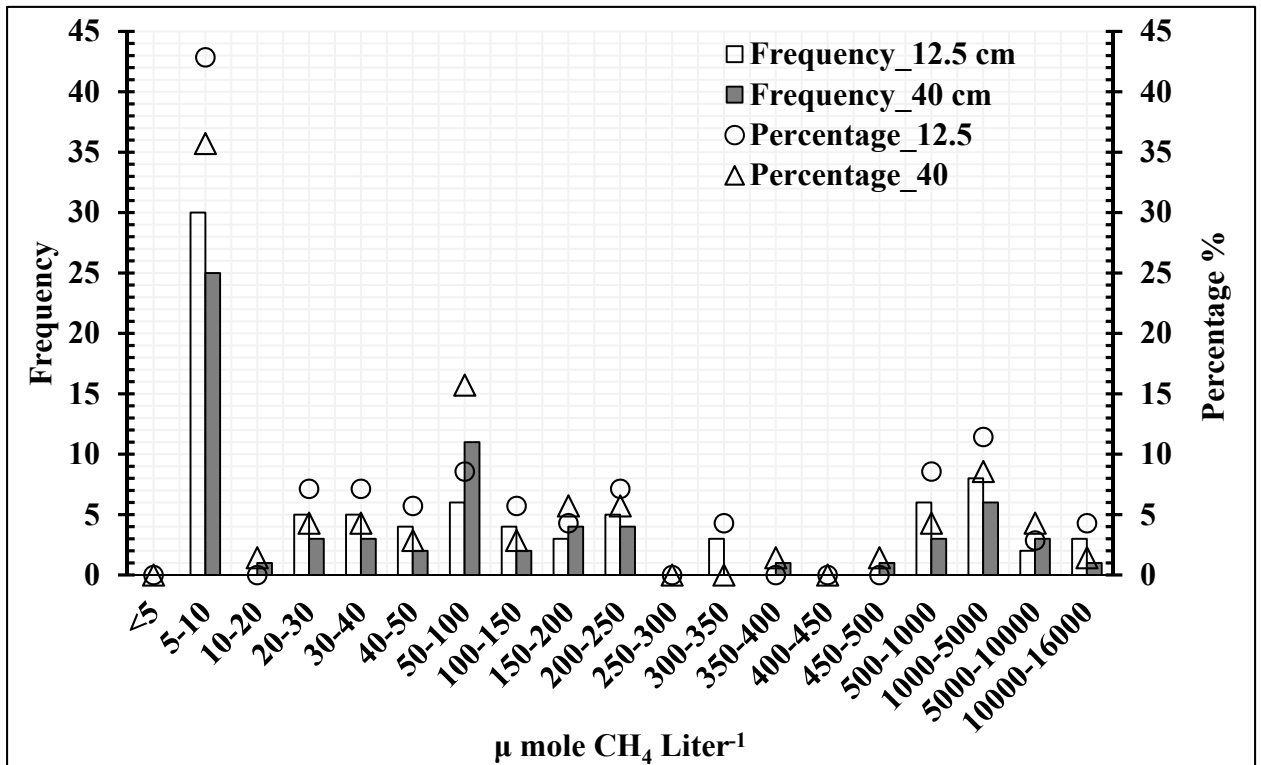
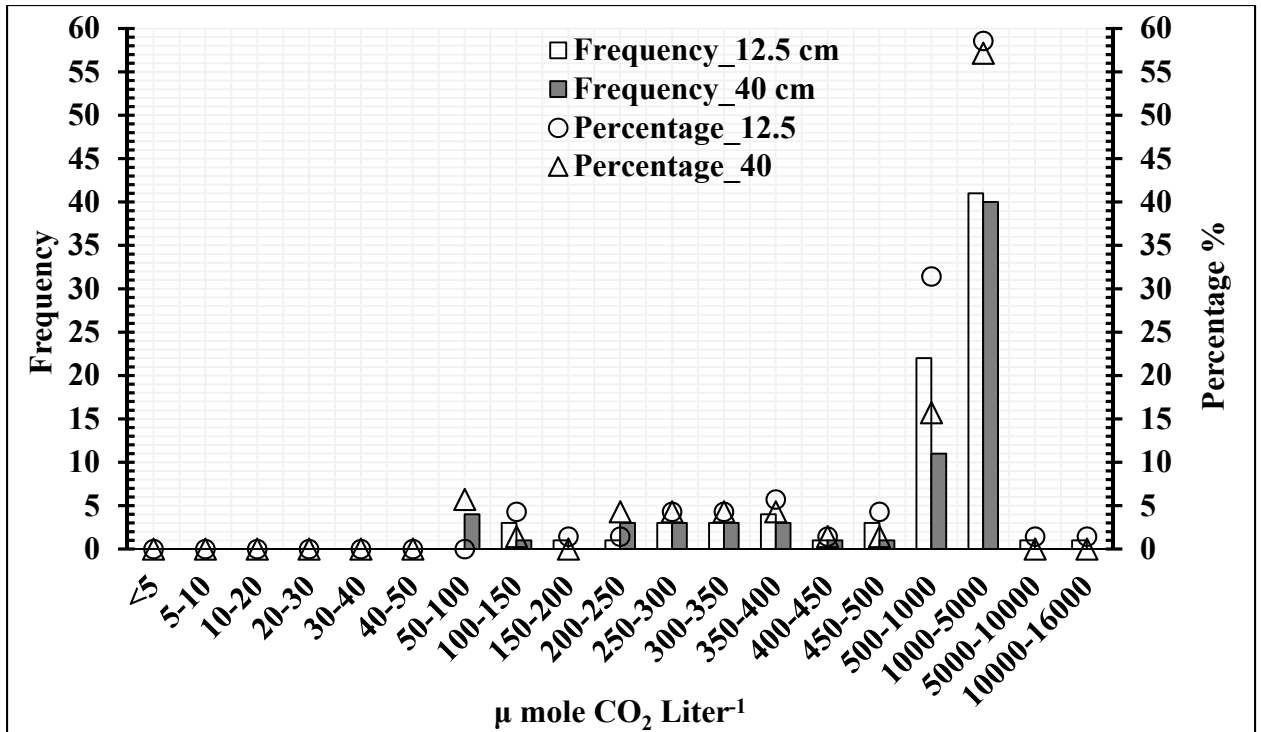


Figure C.5: Histogram showing distribution of pore-water CO₂ and CH₄ concentrations at 12.5 and 40 cm soil depth at all the natural and restored wetland habitats from May to August 2016.

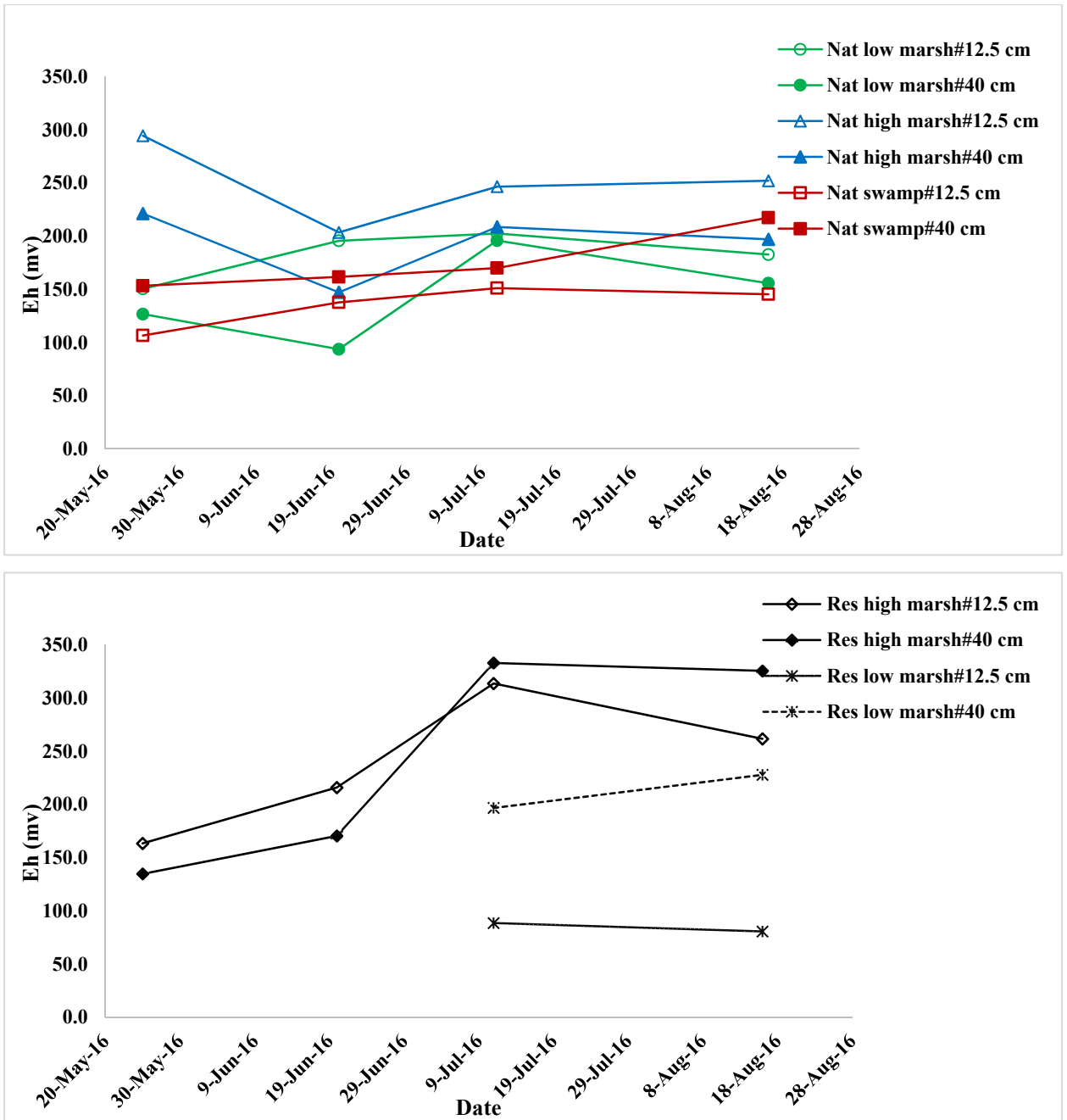


Figure C.6: Change of soil Eh over time at 12.5 and 40 cm soil depth for the natural (Nat) and restored (Res) wetlands from May to August 2016.

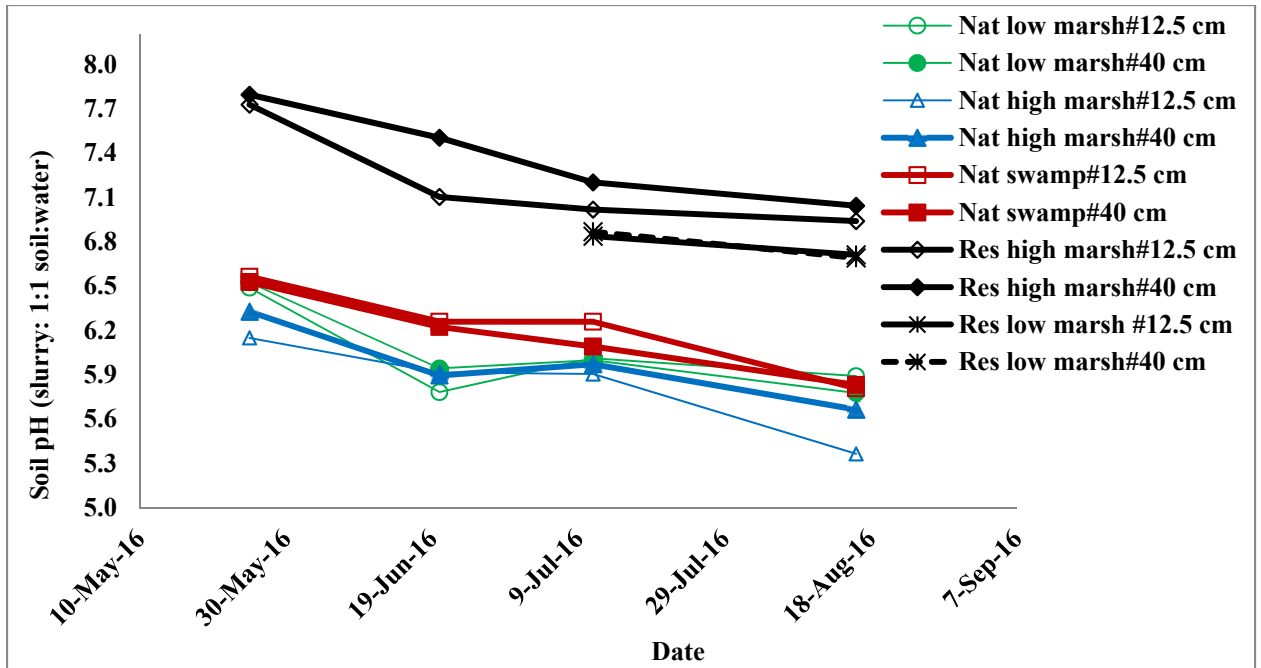


Figure C.7: Change in soil pH over time at 12.5 and 40 cm soil depth the natural (Nat) and restored (Res) wetland habitats from May to August 2016.

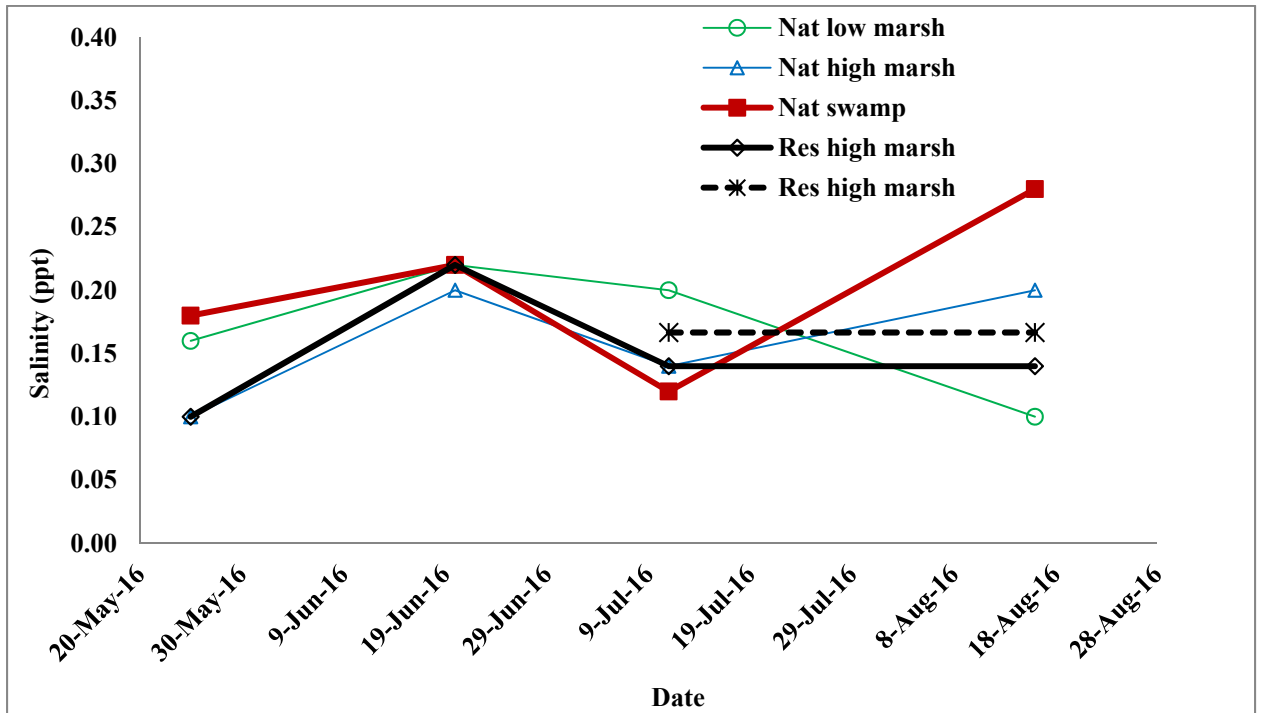


Figure C.8: Change in salinity for the natural (Nat) and restored (Res) wetland habitats from May to August 2016.

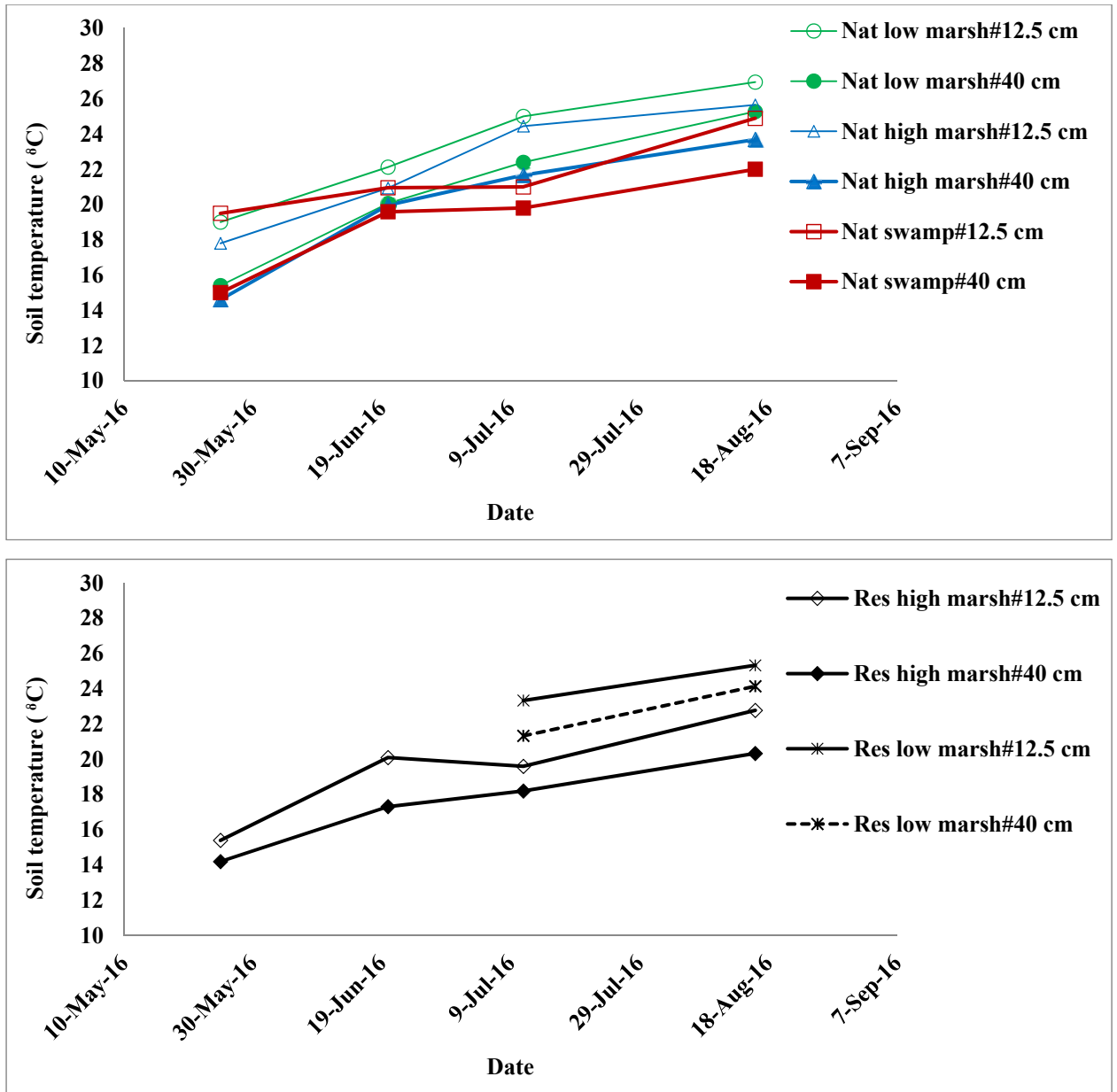


Figure C.9: Change in soil temperature over time at 12.5 and 40 cm soil depth for the natural (Nat) and restored (Res) wetlands from May to August 2016.

Table C.10: Plant species identified in the natural and restored wetland habitats.

| Species | Natural wetland | | Swamp | Restored wetland | |
|---|-----------------|------------|-------|------------------|------------|
| | low marsh | high marsh | | low marsh | high marsh |
| <i>Amaranthus cannabinus</i> (L.) Sauer | | + | | | |
| <i>Biden</i> sps. | | | + | | |
| <i>Bidens laevis</i> (L.) Britton, Sterns & Poggenb. | | + | + | | |
| <i>Carex lacustris</i> Willd. | | + | | | |
| <i>Cicuta maculata</i> L. | | + | + | | |
| <i>Cuscuta gronovii</i> Willd. ex Schult. | | + | | | |
| <i>Impatiens capensis</i> Meerb. | | + | | | |
| <i>Leersia oryzoides</i> (L.) Sw. | | + | + | | |
| <i>Murdannia keisak</i> (Hassk.) Hand.-Maz. | | + | + | | |
| <i>Nuphar lutea</i> (L.) Sm. | + | + | | | |
| <i>Peltandra virginica</i> (L.) Schott | + | + | | | |
| <i>Phragmites australis</i> (Cav.) Trin. ex Steud. | | | | | + |
| <i>Pilea pumila</i> (L.) A. Gray | | + | | | |
| <i>Polygonum arifolium</i> L. | | + | | | |
| <i>Polygonum punctatum</i> Elliott | | | + | | |
| <i>Polygonum sagittatum</i> L. | | + | | | |
| <i>Schoenoplectus fluviatilis</i> (Torr.) M.T. Strong | | + | | | |
| <i>Typha latifolia</i> L. | | + | | + | |
| <i>Zizania aquatica</i> L. | | | + | | |
| Total = 19 | 2 | 15 | 7 | 1 | 1 |

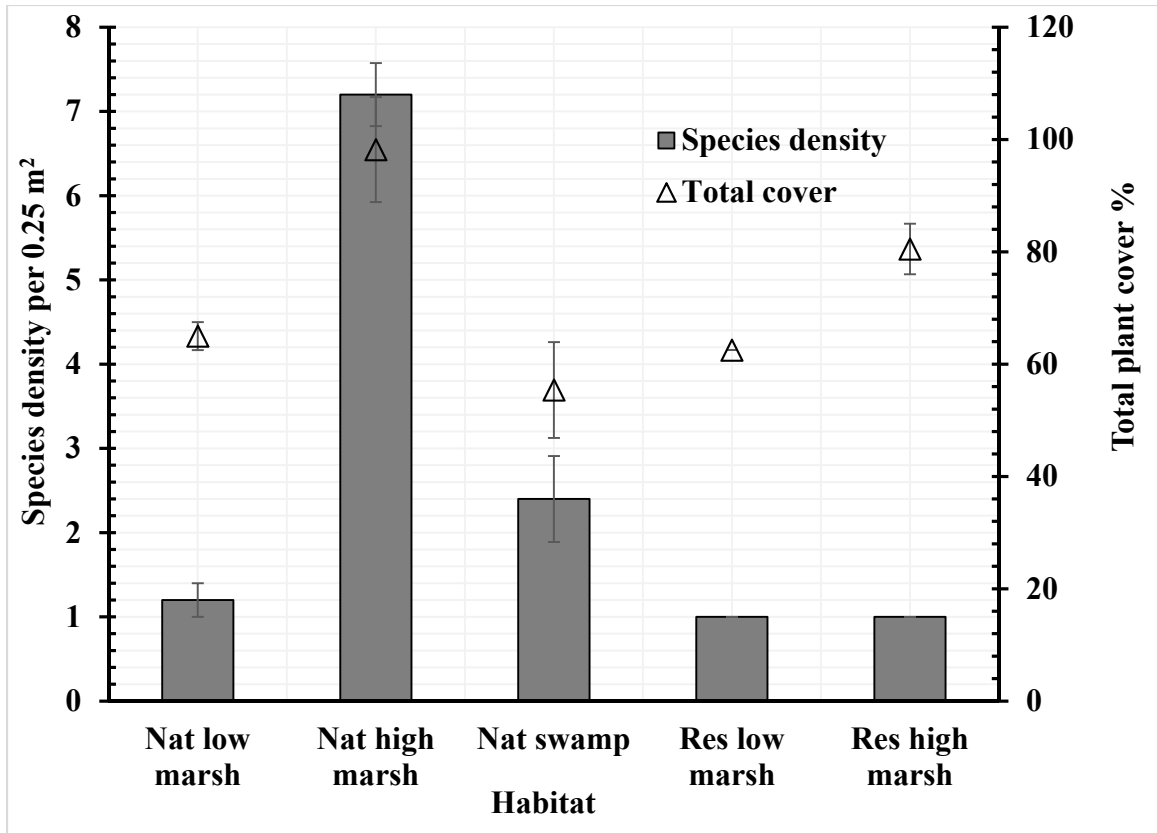


Figure C.11: Plant species density and plant cover per 0.25 m² plot for natural (Nat) and restored (Res) wetlands.

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