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## The Development of Ecological Functions in Created Forested Wetlands

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**The Development of Ecological Functions in Created Forested Wetlands**

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**A Thesis**

**Presented to**

**The Faculty of the School of Marine Science  
College of William and Mary**

**In Partial Fulfillment of the  
Requirements for the Degree of  
Masters of Science**

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**By**

**Sean P. Charles**

**December 3, 2013**

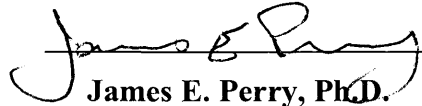
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**This thesis is submitted in partial fulfillment of  
The requirements for the degree of  
Master of Science**



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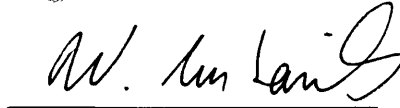
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## Abstract

Wetland mitigation has become a 2.4 billion dollar per year industry in the U.S. and in Virginia it leads to the replacement of 77 ha of palustrine forested wetlands (PFWs) per year with mitigation wetlands, including created forested wetlands (CFWs). Mitigation hinges on the idea that compensation wetlands lead to “no net loss of wetland function” when compared to impacted wetlands. We assessed the functions of provision of habitat and biogeochemical functions associated with production of biomass, the retention and removal of nutrients and the accumulation of soil C over 8 years in seven CFWs of approximately 11 and 20 years and compared them to natural reference wetlands (NRWs). CFW plant communities were similar to NRWs in all measured parameters in the herbaceous and shrub/sapling strata and in all strata combined. However, non-native dominance showed a significant positive linear relationship with CFW age. In the tree strata, 11 year old (yo) CFWs had lower richness than NRWs and both age classes of CFWs had lower FQI than NRWs. NRWs held 10 to 20 times more carbon in woody biomass than CFWs. Tree species composition was significantly different between CFWs and NRWs, however NRW trees were similar to CFW saplings. 11 yo CFWs held lower percentages of C, N and P and had higher  $D_b$  than NRWs in both the 0-10 and 10-20 cm depth. 20 yo CFWs developed similar levels of %C, %P, bulk density ( $D_b$ ), and nutrient ratios in the surface and displayed rapid increases in %C and %N over 8 years. However, CFWs offered 45% lower soil total soil C storage and 50% lower %N. Furthermore, all CFWs stored lower nutrient levels than NRWs in the 10-20 cm soil depth. We found that FQI correlated positively with total C accumulation rates in woody biomass and soil C, indicating that biogeochemical function and the provision of habitat can be complimentary in CFWs. Finally, 11 and 20 yo CFWs adhered to the regulatory performance standards established for Virginia in terms of stems per ha and wetland indicator status, but all wetlands (including NRWs) failed to achieve <5% non-native species cover.



## INTRODUCTION

By the mid-1980s, wetland acreage had been reduced by 53% in the lower 48 states and 42% in Virginia (Dahl 1990; Tiner and Finn 1986; USGS 1999). PFWs have borne the brunt of these losses, and continue to be the most displaced wetland type nationally and in Virginia, (Dahl 1990; Tiner and Finn 1986; USGS 1999; Dahl 2011, VADEQ 2012).

The army corps of engineers defines wetlands as “those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that normally do support, a prevalence of vegetation typically adapted for life in saturated soil conditions.” Similarly, delineating a wetland hinges on three factors: water, biota adapted to life in wetlands (often hydrophytic vegetation) and substrate formed under saturated conditions (often hydric soil) (Environmental Laboratory 1987; NRC 1995).

Due to their unique characteristics, wetlands are responsible for functions that effect the environment and provide value for society. These values were deemed to be of such a high priority to the common interest that they are now protected under the Federal Clean Water Act even when they occur on private property. Two of these functions can broadly be defined as providing habitat and effecting biogeochemical cycles (NRC 1995). For our purposes, habitat value is determined through the maintenance of a characteristic plant community, whereas biogeochemical cycling is concerned with the transformation, retention and removal of nutrients and the accumulation of organic carbon (NRC 1995).

Currently, wetland losses are regulated under section 404 of the Clean Water Act of 1977 (CWA), with a goal of “no net loss, with an adequate margin for safety” of wetland acreage and function (NRC 2001; USACOE 2002; 33 U.S.C. 1344). To achieve the no net loss policy in the face of development and land-use change, wetland losses are mitigated for through avoidance and minimization of wetland damage or compensating for the wetland impacts by protecting, enhancing, restoring or creating wetlands (CEQ 1978). For this paper we will be focused on created wetlands (CWs), wetlands developed where they did not previously exist.

The Army Corps of Engineers (USACE) oversees wetland impact. In conjunction with other members of the Interagency Review Team, they have developed performance standards to determine the success of CFWs in Virginia. To ensure that CFWs adequately account for lost wetland acres and function, sites are monitored for a ten-year period after creation (VADEQ 2012). Created forested wetlands (CFWs) are deemed successful replacement if they fulfill performance standards that include: wetland hydrology, hydric soil, hydrophytic vegetation, a minimum level of cover and woody stem density and a maximum invasive species ratio (Environmental Laboratory 1987; VADEQ 2012).

Many compensatory mitigation attempts have fallen short of their regulatory goals (Cole and Shafer 2002; NRC 2001; Brown and Veneman 2001), causing some to question if section 404 is preventing wetland losses as effectively as it should (Mitsch and Wilson 1996). After investigating the success of CWs, the National Research Council's Committee on Mitigating Wetland Losses found, that 50% of mitigation sites failed to meet prescribed criteria (NRC 2001). They concluded "the goal of no net loss of wetlands is not being met for wetland acres or functions by the mitigation program" (NRC 2001).

Furthermore, wetland hydrology, hydric soil (Mitsch et al. 2006), and hydrophytic vegetation (Erwin and Best 1985; Reinartz and Warne 1993; Mitsch et al. 1998; Brown 1999) often develop quickly and the inherent assumption of wetland creation is that once these criteria develop CWs become functionally similar to natural reference wetlands (NRWs). However, whether regulatory success leads to functional success remains dubious, as "successful" CWs often fail to function as NRWs (Mitsch and Wilson 1996; Sudol 1996; Balcombe 2005). The ecological functions of mature, NRWs often do not develop during short monitoring periods (currently 10 years in Virginia) and sometimes lack evidence of progress (Bischel-Machung et al. 1996; Zedler 1993), while some studies show that functional replacement may take considerable time (Ballantine and Schneider 2009) causing lag, that may lead to consistent and considerable wetland functional loss (Gutrich and Hitzhugen 2004; Bendor 2009).

In the mid-Atlantic region of the US, creating wetlands often entails removing vegetation and surface soil from uplands to allow lower surface elevations and allow

wetland hydrology to become established (Daniels and Whittecar 1999). Thus, young CWs often display ecological immaturity, in that they have lower organic carbon (Bischel-Machung et al. 1996; Campbell et al. 2002; Cole et al. 2001; Cummings 1999; Shaffer and Ernst 1999; Stolt et al. 2000; Whittecar and Daniels 1999), lower soil nutrient reserves (Stolt et al. 2000; Cummmings 1999; Bishel-Machung et al. 1996; Fennessy et al. 2008), higher bulk density (Atkinson et al. 1993; Bishel-Machung et al. 1996; Cummings 1999; Nair et al. 2001; Whittecar and Daniels 1999), different vegetation assemblages (Van der Valk 1981; DeBerry and Perry 2004) and low levels of woody biomass (Noon 1996; Mathews and Endress 2008; Atkinson et al. 2005; Fennessy et al. 2008) in comparison to NRWs.

In order to reach functional equivalency, CWs must develop over time (Reppert 1992; Noon 1996; Campbell et al. 2002; Johns et al. 2004). The success of this process has been called into question (Moy and Levin 1991; Simenstad and Thom 1996; Zedler 1996), and some have suggested that mitigation wetlands may tend toward alternative stable states (Hobbs et al. 2009; Suding et al. 2004). Even successful wetland creation or restoration often leads to a time lag before created or restored ecosystems provide the same level of function as the natural wetlands they were meant to replace (Craft et al. 1988; Sacco et al. 1994; Zedler 1999). Lag time leads to temporal functional losses, which accumulate until functions become equivalent (Gutrich and Hitzhusen 2004; Bendor 2009).

Ultimately we do not know whether CFWs reach functional equivalency with NRWs, or, if so, how long it takes them to develop. It is also possible that created sites trend toward alternative stable states (Scheffer et al. 2001; Moreno-Mateos 2012). Few studies have followed CFWs up to 20 years (Atkinson 2005), therefore, details of the development of older CFWs is valuable to determine how much time is necessary for them to mature to functional equivalency, or to determine if go down a different track (Mitsch and Wilson 1996).

## **Objectives**

This study has three goals: 1) to determine whether CFWs in Virginia mitigate lost ecological functions within an 11 and 20 year time frame 2) to determine how

CFWs develop over time and 3) to determine whether CFWs continue to fulfill regulatory performance standards 11 and 20 years after creation.

Seven CFWs from two age groups, 11 years old (yo) and 20 yo, were analyzed through a suite of soil and vegetation based parameters related to the functions of provision of habitat and biogeochemical cycling to determine progress and to assess whether they are functionally equivalent to four NRWs. Chosen age groups represent the end of the monitoring period (11 yo) and 10 years after the monitoring period ends (20 yo). To determine ecological success (functional equivalency), functions investigated included: 1) maintenance of a characteristic plant community 2) transformation, retention and removal of nutrients 3) accumulation of organic carbon and 3) (NRC 1995).

This thesis is divided into 3 chapters. Chapter one is a literature review to introduce readers to wetland losses, wetland functions, the mitigation process and past studies of CW ecosystem development. Chapter two focuses on investigating the development of biogeochemical functions associated with soil development in CFWs and comparing them to NRWs. Finally the third chapter assesses the levels of habitat provision and carbon storage provided by the vegetation communities of CFWs compared to NRWs.

## Chapter 1

### A Review of Wetland Loss, Mitigation policy and Ecosystem Development in Created Wetlands

#### Wetland Losses

Nationally, 53% of wetlands were lost from the 1780s to the mid 1980s (Dahl 1990). Wetlands occupy approximately four percent of Virginia's land mass (Dahl 1990). In the 1780's, wetlands covered about 748,263 ha (more than seven percent) of Virginia (Dahl 1990). By the mid-1980s, when permits began to be required for most impacts to wetlands, about 435,037 ha of wetlands remained in Virginia – a loss of about 42 percent in 200 years (Dahl 1990).

Approximately 72% of the wetlands in Virginia are in the Coastal Plain, with another 20% in the Piedmont and the remaining 9% in the other physiographic provinces. Vegetated palustrine wetlands cover 435,216 ha in Virginia compared to 76,890 ha of estuarine wetlands, 78 ha of lacustrine wetlands and 154 ha of riverine wetlands (Hershner et al. 2000). The most prominent wetlands in Virginia are non-tidal palustrine forested wetlands (PFWs) (Tiner and Finn 1986). PFWs are often associated with prime agricultural land due to high organic matter content and nutrient availability (Reddy and Gale 1994). The major causes of palustrine wetland loss are direct conversion to agriculture (45%), channelization and ditching (27%) and lake and pond creation (25%) (Tiner 1987).

#### Mitigation

In Virginia, wetlands are legally defined in the DEQ's Virginia Water Protection Permit (VWPP) regulation (9 VAC 25-210-10 et seq.) as "those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support and, under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions." This definition is in line with the federal definition of wetlands contained in Section 404 of the Clean Water Act.

Section 404 of the 1977 Federal Water Pollution Control Act (Clean Water Act) was created to "restore and maintain the chemical, physical, and biological integrity of the nation's waters". Section 404 granted the US Army Corps of Engineers (USACE)

permit authority for dredge and fill operations in jurisdictional wetlands (Gaddis and Cabbage 1998), and Congress eventually instructed the USACE to pursue the goal of “no net loss” of the nation’s remaining wetlands in Section 307 of the Water Resources Development Act. Wetlands are part of State Waters, defined as: “all water, on the surface and underground, wholly or partially within or bordering the Commonwealth” per section 62.1-44.3 of the Code of Virginia.

To accomplish this goal in the face of land use change, wetland losses are mitigated for. Mitigation is accomplished through avoidance and minimization of harm, and losses are compensated for by the creation of new wetlands or the restoration, enhancement or preservation of existing ones (CWA 1977; USACE and USEPA 1990). Compensatory mitigation enacted through the Clean Water Act requires replacement of wetland area and functions, most often through restoration, the process of restoring wetland hydrology, hydric soil and hydrophytic vegetation to an altered pre-existing wetland, or through wetland creation, altering hydrology to develop wetlands where they did not previously exist.

Wetland compensation has become a huge undertaking, accounting for 8,498 ha of annual wetland impacts and 17,624 ha of compensatory mitigation (Martin 2006). About 20% of wetland compensation is carried out through wetland creation (ELI 2006). Between 2008 and 2010, an average of 77 ha of PFWs were compensated each year (VADEQ 2012). Mitigation enacted through the Clean Water Act is estimated to cost 2.4 billion dollars per year (ELI 2007). In North America (Mexico, Canada, USA) \$70 billion dollars has been spent on wetland restoration and creation (Copeland 2010).

This study specifically examines wetland losses mitigated by wetland creation, the manipulation of physical, chemical or biological characteristics of a site to develop a wetland. Created wetlands in the mid-Atlantic (CWs) are most often created in former upland environments (Atkinson et al. 2005; Whittecer and Daniels 1999). Wetland creation is of great interest due to greater uncertainty over the return of wetland functions than restoration and the finite number of degraded wetlands to restore (Kusler and Kentula 1990; Spieles 2005).

In addition to national Clean Water Act resolutions to halt net loss of wetlands, Virginia has agreed to “achieve net wetland resource gain” within the Chesapeake Bay

Watershed (all study sites) in accordance with the Chesapeake Bay Agreement (Chesapeake Bay 2000 Agreement, Subsection 2.3).

### **Recent Losses**

Mitigation often requires a ratio of wetland creation to impact greater than one, which should significantly increase wetland area, yet wetland area decreased between 2004 and 2009 (Dahl 2011). Locally, a study of wetland trends in Southeastern Virginia showed a net loss of 850 ha (1.3%) between 1994 and 2000. The loss of palustrine wetlands was primarily due to conversion to uplands, while estuarine wetlands were lost through conversion to open water (Tiner et al. 2005).

The continuation of wetland area loss indicates lack of oversight or failure to properly mitigate wetland impacts. The National Research Council's Committee on Mitigating Wetland Losses determined that nationally 50% of surveyed mitigation sites failed to meet their prescribed criteria (NRC 2001). They concluded “the goal of no net loss of wetlands is not being met for wetland functions by the mitigation program, despite progress in the last 20 years” (NRC 2001). Continuation of overall loss and a failure to return function led the Government Accountability Office to declare that the Army Corps of Engineers lacks an effective oversight approach to make sure that compensatory mitigation is occurring properly (GAO 2005).

Areas with the highest mitigated impacts in Virginia have been located near the fastest growing urban centers in the Piedmont and Coastal Plain (VADEQ 2012), and is particularly concentrated in the coastal plain, where around 80% of wetland loss occurred from the 1980s to the late 1990s (VADEQ 2012). Northern Virginia, Greater Richmond, and Tidewater demonstrate high concentrations of issued permits, and are thus the focus of this project (figure 1.1). In Virginia, many mitigated wetland impacts are due to road building in association with the Virginia Department of Transportation (VDOT) (Atkinson et al. 1993).

### **Forested Wetland Impacts**

PFWs were the most impacted wetland type between the mid-1970s and mid-1980s, losing 1.4 million ha nationally (Dahl and Johnson 1991). They increased slightly

between 1998- 2004 (Dahl 2006), before dropping 1% between 2004 and 2009, marking the first overall decline since passage of major wetland regulations (Dahl 2011). Non-tidal wetlands with intermittent or seasonal flooding, such as PFWs (Cowardin 1979) are often the most at risk due to ease of conversion to upland through draining (Hershner 2000). Similarly, in recent decades Virginia's largest losses have been to PFWs, the most common wetland type in the state (Dahl 1990; Tiner and Finn 1986; USGS 1999; Dahl 2011).

Often impacts on PFWs are compensated for with other wetland types such as open water or emergent vegetation (Cole and Schafer 2002; Minkin and Ladd 2003). In Pennsylvania, a study of 23 section 404 permits from 1986 to 1999 showed that only 45% of the mitigation wetlands were the same type as the impact site, and that mitigation resulted in a shift from PFWs and scrub-shrub wetlands to open water ponds and uplands (Cole and Shaffer 2002). In Ohio, Porej (2003) found that 100% of mitigation wetlands were emergent, even though many impacts were to forested sites. PFWs are also more difficult to recreate than emergent wetlands due to the sensitivity of many tree species to hydrologic conditions, difficulty in creating a typical deep annual hydroperiod, and the long time period required for trees to reach maturity (Lewis et al. 1995; Michigan DEQ 2000, Daniels et al. 2000). In a study of 31 mitigation sites in Indiana, Robb (2001) found a failure rate of 71% in mitigation sites for PFWs, compared to 17% for shallow emergent systems and 4% for open water areas. Thus, even when impacts for PFWs are compensated for in-kind, mitigation might still lead to a shift from forested to emergent and open water wetlands.

### **Analysis of Successful Mitigation**

Ensuring that mitigation policy translates into the successful replacement of wetland functions is essential to achieving no net loss of function. The USACE Wetland Delineation Manual was created to define wetlands in a legal sense in order to protect them through the CWA (Environmental Laboratory 1987). The criteria outlined to define wetland hydrology, vegetation and soils still forms the basis for created wetland success.



Virginia's current performance standards are enumerated in the Mitigation Bank Template, created by an interagency review team chaired by the USACE, that applies to all current compensatory mitigation in Virginia (Steve Martin pers. comm.). Performance standards include vegetation, soil and hydrology indicators used to delineate jurisdictional wetlands as well as mandating a minimum of 80% cover of herbaceous plants, at least 400 woody stems per acre and less than 5% invasive species are achieved before a wetland is deemed successful compensation (Environmental Laboratory 1987; VADEQ 2010).

Numerous studies have shown that created wetlands (CWs) fail to reach their regulatory requirements. Balzano et al. (2002) found that 48% of New Jersey mitigation projects met their requirements. While Ambrose and Lee (2004) found 69%, Cole and Shaffer (2002) found 60%, Brown and Veneman (2001) found 43% and MDEQ (2001) found only 18%. Failure to meet regulatory criteria prompted the Government Accountability Office (2005) to review mitigation, and they concluded that there was little oversight into compensatory mitigation.

Ultimately, successful mitigation wetlands should exhibit characteristic levels of wetland structure and function. Questions still remain as to whether a created wetland deemed a regulatory success is in fact returning lost ecological function (Moy and Levin 1991; Simenstad and Thom 1996; Zedler 1996). Analyzing long-term data sets from 621 restoration projects, Moreno-Mateos (2012) found wetland restoration and creation sites from around the world do not develop equivalent biological structure or biogeochemical functions after a century. Though not specific to mitigation wetlands, this meta-analysis shows a general failing for all wetland creation and restoration sites globally in the last 100 years. The ability of mitigation wetlands to successfully restore function has been questioned for three decades (Race and Christie 1982; Race and Fonseca 1996; Zedler 1996; NRC 2001).

In order to evaluate the success of a wetland mitigation project, we must ask whether the constructed site functions in a similar fashion to a "natural wetland" (D'Avanzo 1987; Larson 1987; Kusler and Kentula 1989; Confer and Niering 1992; Malakoff 1998). Because extensive studies of all lost wetlands do not exist, functional equivalency must be based on a population of existing wetlands (Kentula et al. 1992).

The ultimate test is how well created wetlands resemble natural wetlands structurally and functionally (Galatowitsch and Van der Valk 1994; Brinson and Rheinhardt 1996; Whigham 1999).

Whether wetland regulations adequately address functional equivalency remains dubious, as some wetlands fulfill their permit requirements, but fail to resemble and function similarly to natural reference wetlands (Zedler and Langis 1999; Sudol 1996; Balcombe 2005; Mitch and Wilson 1996), causing some ecologists to oppose compensatory mitigation on the grounds that we don't have deep enough knowledge to successfully emulate natural systems (Roberts 1993). Zedler and Callaway (1999) outline 3 realities of wetland creation that must be considered: 1) compensation sites may never fully replace natural wetland functions, 2) the time to functional equivalency may exceed the usual monitoring period 3) long-term predictions of the time to functional equivalency may not be meaningful if they are based on short-term data from pulse-driven ecosystems. Similarly, Race and Fonseca (1996) argue that most monitoring of CWs occurs "too early in the developmental stages to demonstrate success." Kusler and Kentula (1990) state that short term revegetation does not guarantee a wetland will continue to function over time. The uncertainty of monitoring lead Zedler and Weller (1990) to conclude that whether CWs persist is one of the most important knowledge gaps in understanding the effects of mitigation on ecology.

### **Wetland Functions**

Wetland functions are all processes that occur in wetlands (NRC 1995). Many functions performed by wetlands are important for the greater environment. Wetlands serve as sinks for important elements such as nitrogen, phosphorus and carbon in the biosphere (Bowden, 1987; Mitsch and Gosselink 2007

Wetlands are also disproportionately important habitat, essential to 50% of America's endangered species (Mitsch and Gosselink 2007). Sixty- three species of plants and thirty-four species of animals that are endangered, threatened or candidates for listing live in Southern US PFWs (Harris and Gosselink 1990).

Wetland functions have been defined by the NRC (1995) as short and long term water storage, transformation and cycling of elements, retention and removal of

dissolved elements, accumulation of organic matter, accumulation of inorganic sediment, maintenance of characteristic plant community, and maintenance of characteristic energy flow. Those that I will be investigating are described below.

*Maintenance of a characteristic plant community:* A plant community similar to natural reference sites is established in terms of forest structure and biomass, and vegetation composition.

*Retention, removal of dissolved elements:* Reduction of elements between inflow and outflow of water from a wetland. Inflows of nutrients generally come from precipitation, groundwater flow, surface water flow, and (for nitrogen) through atmospheric deposition (Fisher and Oppenheimer 1991).

*Accumulation of organic carbon:* The ability of a system to accumulate organic matter from both autochthonous and allochthonous sources.

### **Maintenance of A Characteristic Plant Community**

Gleason (1925, 1926), stressed that for plants to survive in space and time they must be adapted to local and current environmental requirements. Hydrophytic plants have adaptations that allow them to establish, grow and persist in anaerobic soil conditions (Cronk and Fennessey 2001). Plants respond to, and reflect, physical, chemical, or biological disturbances and stressors, and are intricately linked to nutrient storage, release and cycling (Mitsch and Gosselink 2007). Plant communities and individual species are sensitive to disturbances, including sedimentation (Van der Valk 1981; Wardrop and Brooks 1998; Mahaney et al. 2004), nutrient enrichment (Pip 1984; Goldberg and Miller 1990; Kadlec and Bevis 1990; Hobbs and Huenneke 1992; Templer et al. 1998; Craft and Richardson 1998; Drohan et al. 2006), and hydrologic modification (Gosselink and Turner 1978; van der Valk 1981; Squires and van der Valk 1992). Plants respond to and reflect physical, chemical, and biological disturbances that are intricately linked to nutrient storage, release and cycling, making them great indicators of environmental conditions and wetland function (Bedford 1996; Mitsch and

Gosselink 2007). Parsons and Ware (1982) found soil chemistry and moisture to be the most important factors in influencing tree distribution in Coastal Plain swamps. Plants are therefore good indicators of ecosystem health and are the most commonly utilized wetland condition indicator (Mitsch and Gosselink 2007).

### **Vegetation Development and Succession**

Henry Cowles (1899, 1911) introduced the concept of plant succession, and revolutionized concepts of interactions between environmental conditions and plant assemblage. Ecological succession is the “unidirectional, sequential change in the relative dominance of species” within a vegetation assemblage (Smith 1990).

Succession can be seen from Clements’ perspective of the vegetation community as a “superorganism” that succeeds through its own inputs to a climax community. Alternatively, succession may be individualistic in that communities result from random processes of colonization, competition and replacement (Gleason 1927). In this concept, the population responds to the environment.

Van der Valk (1981) applied Gleason’s theory of succession directly to prairie wetlands, by enumerating the factors of wetlands that lead to changes in vegetation through the “environmental sieve” concept: 1) the destruction of existing vegetation 2) changes in physical or chemical properties of the environment 3) competition and 4) establishment. The environmental sieve concept provides a basis for predicting vegetation succession in wetlands as the combination of factors leads to the establishment and persistence of only those species with appropriate traits for the environment. However, this model was created only for emergent vegetation and in order to gain an understanding of the process of forested wetland succession, it is essential to integrate both the concepts of ecosystem development and forest succession (Leck 1989, DeBerry 2006).

Wetland creation is a unique undertaking in that it can be described as primary succession. The removal of soil and much of the seed bank means that much of the energy stored in the ecosystem and its ability to rebound have been removed. Part of the wetland creation process is an attempt to jumpstart the successional process, and to create conditions similar to secondary successional systems. Therefore, organic

amendments are added, and herbaceous plants and small saplings are generally planted to the sites.

#### Vegetation composition

As systems recover from disturbance (in this case the removal of upland vegetation and soil and the subsequent flooding of the system) richness, diversity, and hydrophytic indicator status often become similar to reference sites quite quickly (Confer and Niering 1992; Kentula et al. 1992; Brown 1999; Balcombe et al. 2005; Spieles et al. 2006; Brown and Veneman 2001), whereas indicators based on species composition tend not to reach equivalence with reference sites over the short term (Brown 1999; DeBerry and Perry 2004; Brooks et al. 2005; Spieles et al. 2006).

Colonizing species are mostly annuals, or facultative annuals that can persist under potentially stressful, low-nutrient conditions (van der Valk 1981; DeBerry and Perry 2004). As a system becomes more mature, environmental conditions change, allowing alternative species to pass through the “environmental sieve” (van der Valk 2003). This has been illustrated in that late successional species have low success in early created wetlands (McLeod et al. 2001), and pioneer species tend to develop first (Spencer et al. 2001), due to high acclimation potential, broad physiological responses and increased growth rate (Bazzaz 1979). Furthermore, Aronson and Galatowitsch (2008) found that while most common wetland species became established in restored wetlands, most rare species did not.

The intermediate disturbance hypothesis predicts that species richness and diversity often peak in partially disturbed rather than pristine ecosystems. This may be because in intermediate conditions, both ruderal and competitive species can coexist (Grime 1973, Connell 1978, MacArthur and Wilson 1967). Similarly, in forests, peaks may occur when shade tolerant and intolerant species overlap (Loucks 1920). Haussler et al (2004) found increased richness 5-12 years after logging.

#### **Floristic Quality Index**

Swink and Wilhelm (1979, 1994) developed the concept of Floristic Quality Index (FQI) as a way to evaluate the “quality” of a plant community. Different plant species have evolved varying tolerance levels to disturbance or environmental stress

(Odum 1985; Hobbs and Chapin 1991; Huenneke 1992), as well as varying degrees of fidelity to specific quality of habitats (Herman et al. 1997; Mushet et al. 2002). Tolerance and fidelity combine to create “species conservatism” (Swink and Wilhelm 1979). Species conservatism is expressed as a score from 0- 10 called a “coefficient of conservatism” (c-value). A low c-value indicates that a plant is rarely found in natural plant communities and is highly tolerant of disturbance, while a high c-value indicates that the plant usually exists in an undisturbed natural plant community (Matthews 2003). Most wetland species in Virginia have been assigned a c-value by a panel of botanists and wetland experts (VDEQ 2004).

FQI is often used as a tool for assessing natural wetland plant communities along a gradient of anthropogenic disturbance (Fennessey 1998; Mack et al. 2000; Wilcox et al. 2002). It has been shown to effectively measure disturbance and site conservation values spatially (Cohen et al. 2004; Miller and Wardrop 2006; Mack 2007). However, in created wetlands, distance from disturbance will be measured temporally. Site grading during construction creates a major disturbance and as sites age, they are less likely to continue to exhibit properties related to the disturbance (Odum 1969; Marks and Bormass 1972).

Chronosequence comparisons often find that older restorations have higher floristic values, implying an increase in Floristic Quality with time since restoration (Mushet et al. 2002; Balcombe et al. 2005). FQI has been shown to reflect soil conditions in CFWs in Virginia, but did not reflect wetland age (DeBerry 2006). Deviations in individual FQI successional trajectories have been shown to coincide with environmental damage such as invasive species invasion, potentially serving to identify problems (Spieles 2006; Mathews et al. 2009).

The FQI classically utilizes a presence absence approach to characterizing vegetation assemblages, however weighting the index to account for dominance can improve its sensitivity (Francis et al. 2000; Cohen et al. 2004; DeBerry 2006). Similarly, separating vegetation strata in PFW communities leads to increased detection of disturbance (Nichols et al. 2006). The tree layer contains the oldest individuals and often reflects historic conditions due to ecological inertia, whereas the herbaceous and shrub-sapling layers reflect current conditions (Huston and Smith 1987; Lopez et al.

2002). Nichols et al. (2006) found that the sapling layer was the most revealing strata for both buffer and watershed scale disturbances (Nichols et al. 2006), however, DeBerry and Perry (2012) found the shrub sapling layer to be an artifact to planting in CFWs in Virginia.

### **Floristic Parameters and Biogeochemical Function**

Cole et al. (2002) points out that some plant community parameters commonly used to assess ecosystem health fail to address the restoration of functions in wetlands. In other words, the level of herbaceous wetland plant cover has very little to do with functional replacement of lost functions. Furthermore, creating wetlands to achieve the dual roles of habitat provision (maintaining a characteristic plant community with reference levels of richness, diversity and floristic quality) may be at odds with creating a wetland that functions at a high level in terms of biogeochemistry (ie. carbon sequestration, nutrient uptake and cycling, etc). Achieving high function in both habitat provision and biogeochemical functioning was deemed unrealistic by both Ehrenfeld (2000) and Zedler (2000).

Some studies have attempted to determine how richness and diversity affect functions. In grasslands, a few studies have shown that increased richness is correlated with greater productivity and nutrient retention (Naeem et al. 1995; Tilman and Knops 1996; Symstad et al. 1998). Similarly, a study conducted on rooted submerged macrophytes in wetlands showed a 25% increase in overall productivity and a 30% increase in phosphorus retention (Engelhardt and Richie 2001). Richness indirectly improved functional performance by increasing the chance that crisped pondweed was present in the mix. This species was not very productive itself, but was associated with higher algal biomass and therefore increased nutrient uptake. In Alaskan riparian wetlands, Pollock et al. show that productivity was uni-modally associated with richness, also reflecting the disturbance regime (flooding) (Pollock 2001).

Richness can prove interesting because, as nutrient availability increases beyond some threshold, richness can be reduced and rare species correlate with species rich communities, therefore sites with high richness and diversity may be important for rare species (Bedford et al. 1999). Furthermore, indices of diversity may be particularly

revealing in the tree strata, as richness and diversity are significantly correlated with woody productivity in North American temperate forests (Paquette and Messier 2010), as well as promoting stability (Doak et al., 1998).

Evidence for high biogeochemical functioning from immature, low quality plant communities proliferates in both theoretical and experimental literature. Some ruderal species are known for their ability to colonize disturbed sites and to grow rapidly (Grime 1979). Similarly, Odum (1969) theorized that immature systems increase biomass rapidly, while mature systems exist in more of a steady state in his theory of ecosystem development. Furthermore, a number of low quality and invasive species exhibit high productivity. Wetlands dominated by *Typha* can be highly productive and efficient in the sequestration of carbon (Davey 2007). Windham (2001) found that the invasion of *Phragmites australis* into a brackish marsh dominated by native species lead to a doubling in biomass, thus *Phragmites australis* with a coefficient of conservatism of 0 leads to an increase in biogeochemical function indicating that maintenance of a high quality plant community and high levels of biogeochemical functioning may be at odds.

On the other hand, once ecosystems develop to have abundant resources (nutrients, OM), competitors dominate and devote significant resources to vegetative growth and long lived structures such as wood and roots (Grime 1977, 1979). Therefore, it may be that in immature systems, low quality ruderals are more productive, while once ecosystems develop, competitors become more productive.

### **Forest Succession**

Tree establishment is often the most difficult task in offsetting PFW impacts (Matthews and Endress 2008). Created CFWs are usually planted with saplings, but lack the structural complexity (over story, understory, groundcover and belowground biomass) and vertical dimensions of a mature forest. Noon (1996) noted that created wetlands are often in a state of arrested herbaceous perennial dominance due to the suppression of woody seedlings (Noon 1996), sometimes persisting even after 20 years (Atkinson et al. 2005). Failure to establish characteristic woody stem density may result from inadequate colonization from surrounding seed sources or through poor survival of planted woody vegetation (Robb 2002; Morgan and Roberts 2003; Spieles 2005). Poor



survival of trees results from inappropriate hydrology, low organic material and high bulk density (Campbell et al. 2002; Bruland and Richardson 2006; Daniels et al. 2005; Bailey et al. 2007). Bailey et al (2007) showed that woody species establishment was improved by organic matter additions.

Similarly, even when woody vegetation is successfully established, there are often differences in composition. Pioneer species such as *Salix nigra* tend to dominate in early successional wetlands (Spencer et al. 2001; Phillips 2002). Whereas later successional species rarely do well in young CFWs and need environmental conditions to develop before they can successfully establish (Mcleod et al. 2001). Early successional species such as *S. nigra* can serve as nurse species, allowing later successional species to develop under their canopy, where nurse species reduce competition with herbaceous species through reduction in light (Dulohery et al. 2000). Stands of *S. nigra* tend to persist for 30-40 years and then give way to other species (Mcleod 2001).

It is uncertain how long forests will take to develop, instigating long-range biomass modeling. Lack of knowledge has lead many to make speculation on the distant future, even leading Niswander and Mitsch (1995) to estimate total tree growth based on 2 years of data. Realistic estimates are in generational time frames (Mitsch and Gosselink 2007).

Chronosequence studies following forest cutting show that in maturing forested ecosystems, the vast majority of living biomass is woody. In a study of forested riparian areas in North Carolina, Rheinhardt et al. (2012) found even in regenerating forests that were clear-cut 5- 25 years ago, > 96% of aboveground biomass is in the trees and the percentage increased to over 99% for forests allowed to regenerate 25 years or more (Rheinhardt et al. 2012).

### **Ecosystem Development**

Odum's (1969) "Ecosystem development" theory posits that ecosystems mature as a whole. Immature ecosystems are characterized by high production to biomass ratios, high production to respiration ratios, simple linear grazing food chains, low species diversity, small organisms, simple life cycles and open mineral cycles. As

ecosystems mature, the ratios of production to biomass and production to respiration drop, grazing chains become more complex and detrital-based, diversity increases, nutrients become efficiently stored and recycled through the system and small simple organisms are replaced by complex large ones (trees) (Odum 1969). According to ecosystem development theory, individual species may come and go, but mature systems are able to resist short-term environmental fluctuation through the development of species diversity, nutrient storage, and recycling (Odum 1969).

Wetlands differ from Odum's model in that primary production tends to be very high, and respiration is often low thus, even mature systems often have a photosynthesis to respiration (P:R) ratio greater than one. Similarly, Odum used live biomass as his index of structure in an ecosystem, but wetland soil carbon can sometimes surpass above ground biomass (Mitsch and Gosselink 2007).

Created CFWs begin with low biomass, diversity, small organism size, a lack of detrital biomass, a high P:B ratio and high P:R ratios, and thus fit the definition of an immature ecosystem (Odum 1969). Reaching maturity can take centuries in forested systems and the benefit of ecosystem development is that changes over time can indicate trends (Mitsch and Gosselink 2007).

Clean deterministic succession to a climax (Clements 1936) has often been shown not to occur similarly to natural ecosystems in mitigation sites (Moreno-Mateos 2012). Often sites appear to trend toward alternative stable states or complex successional trajectories that result from construction practices and different environmental conditions (Hobbs and Norton 1996; Suding et al. 2004; Scheffer 2001; Walker et al. 2007; Moreno-Mateos 2012).

A site's surrounding can alter developmental trajectories. Proximity to propagule sources can influence dispersal, changing expected outcomes (Fastie 1995; del Moral 1998; Butaye et al. 2002; Holl and Crone 2004; Galatowitsch 2006; Brunet 2007). Similarly, local land use can influence nutrient supply, disturbance regimes, and thus lead to novel results (Cramer et al. 2008).

Wetlands can often be described as pulsed systems, with disturbances occurring during hydrologic events (for CFWs flooding and dry down). Rather than reaching a static climax community, wetlands eventually achieve pulsed stability (Odum et al.

1995). This may lead to systems with different species, diversity and composition, but similar functionality (Odum et al. 1995; Mitsch and Gosselink 2007).

The theory of “self-organization” suggests that “complex systems consisting of many parts tend to organize to achieve some sort of stable, pulsing state,” and that if the system is open to receiving new propagules, it will select the assemblage of plants, microbes and animals that are optimal to the current environmental conditions (Odum and Barrett 2005).

### **Forest Development and Biomass**

Sometimes vegetation communities in emergent wetlands can quickly achieve characteristic aboveground biomass (Whigham et al. 2002; DeBerry and Perry 2004). However, in forested sites, biomass accumulation to reference levels is a generational process, as it takes at least 50 years for trees to grow to reference levels (Niswander and Mitsch 1995; Mitsch and Gosselink 2007).

Biomass generation in CFWs is understudied. Wetland creation was seldom practiced until the Clean Water Act of 1977 created the legal framework and incentive. Therefore CWs have rarely been followed for 20 or more years (Atkinson et al. 2005). Noon (1996) describes a lack of vegetative perennials in his oldest sites, but they were only 11 years old, thus perhaps more patience is needed. Similarly, Atkinson et al. (2005) found a lack of woody species, but his sites were particularly disturbed due to mining activity. In the CFWs utilized for this study, there were negligible trees > 10 cm dbh after 12 years (DeBerry and Perry 2012). Trees increase height, diameter and volume but decrease in density, and biomass increases asymptotically, with mature trees devoting less energy to new growth (Wigley and Lancia 1998).

Trees seem particularly important as habitat for certain types of fauna. As trees become taller and forests more stratified, they are used by a higher diversity of forest dwelling birds (MacArthur and MacArthur 1961; Dickson et al. 1995) and small mammals (Ecke et al. 2002), basal area of trees was the best indicator of salamander habitat in riparian wetlands in West Virginia (Summers 2013) and black bears prefer large trees > 58cm DBH for their dens (Godfrey 1996).

As vegetation develops, it will be capable of adding more recalcitrant OM to the soil. Litter from woody species is generally higher in lignin and complex polysaccharides than herbaceous species, making it more difficult to degrade by microorganisms (Schlesinger, 1997; Wolf & Wagner, 2005; Berg & McClaugherty, 2008). Therefore it tends to decompose slower and persist longer in soils than rapidly degradable more labile compounds (Schlesinger, 1997; Trumbore, 1997; Wolf & Wagner, 2005). Due to increased input of longer lasting organic matter, as well as the reduction in temperature due to canopy development and shading, it makes sense that soil organic matter correlates significantly to tree biomass and stand age (Brinson et al. 2006).

Nutrient reduction in nearby water is correlated with CFW age. Due to increased detritus, chemical litter characteristics and increased complexity in the root zone, and forest biomass in riparian areas negatively correlates with nitrates in adjacent streams, implying that forest biomass leads to increased sequestration, cycling and removal of nitrate (Brinson et al 2006).

From an ecosystem development perspective, biomass accumulation in a young CFW would allow a net uptake of nutrients while mature forests would be more active in detaining nutrients through recycling mechanisms (Vitousek and Reiners 1975). However, in wetlands, P:R ratios often remain  $>1$ , indicating that nutrients are continually added to the system, rather than just recycling (Odum 1969; Mitsch and Gosselink 2007). Additionally, the root systems of older trees provide more complex microenvironments for denitrification than younger herbaceous vegetation or younger trees (Groffman et al. 1996). Furthermore, when microbes decompose organic material with a high C:N and C:P ratio, they assimilate inorganic nitrogen and phosphorus that might otherwise be exported downstream (Qualls 1984). Therefore, additions of high C:N tree materials (as opposed to herbaceous vegetation with low C:N generally), will lead to a reduction in downstream nitrates (Brinson et al 2006). Furthermore, litter with a low C:N ratio and high N content decomposes rapidly, releasing ammonium as nitrogenous compounds are metabolized for their carbon (Cadisch and Giller 1997; Segal et al. 1990).

### **Biogeochemical Function**

Soil provides the medium for plants to grow and the structure for the many biogeochemical functions we associate with wetland ecosystems (Mitsch and Gosselink 2007, Brinson 1993). Soils cycle nutrients, store pollutants, mediate groundwater, and provide habitat for microorganisms, invertebrates, and other more complex organisms (Richardson and Vepraskas 2001). Soil properties, reflect the wetland environment and are often used to examine and compare wetlands (Environmental Laboratory 1987; Bishel-Machung et al. 1996)

Hydric soils are soils that formed under conditions of saturation, ponding, or flooding long enough during the growing season to develop anaerobic conditions in the upper part of the soil (Federal Register 1994). Oxygen diffuses through saturated soils 10,000 times slower than through drained soil (Gambrell and Patrick 1998), causing inundated soils to become anaerobic when there is an adequate microbial population to consume newly available oxygen and a supply of organic matter for them to metabolize (Mitsch and Gosselink 2007). Once oxygen has been depleted, microbial activity shifts to less lucrative electron acceptors in order of energy yield, from nitrate, manganic manganese, Ferric iron, sulfate, and finally carbon dioxide (table 1.2). Heterotrophic microbes use these terminal electron acceptors based on hierarchical redox potentials (Wang and Patrick 2000; Megonigal et al. 2004) Redox reactions are integral to biogeochemical cycling in wetlands, influencing many biogeochemical processes (Mitsch and Gosselink 2007).

### **Organic Matter**

Organic matter (OM) is the portion of the soil derived from living organisms, the largest source usually being plants. Due to high productivity, and the reduced efficiency of anaerobic decomposition, wetlands tend to accumulate higher OM concentrations than other ecosystems (Ponnamperuma 1972; Ugolini and Edmonds 1983; Craft 2001).

OM contributes to important physical, biological and chemical properties of soil (McBride 1994, Stevenson 1994). Physically, OM increases infiltration, can holding up to 20 times its weight in water, reduces bulk density, and improves soil structure

(Boelter 1969; Bishel-Machung et al. 1996; Mitsch and Gosselink 2000; Nair et al. 2001). This led Reddy and Delaune (2008) to describe OM as the center of a development process that leads to increased gravimetric soil moisture, integral in the creation of hydric soil. Similarly in created wetlands, sufficient reduction may not occur in sites with very low OM contents, leading Vepraskas et al. (1995) to suggest a 3% OM content minimum for the development of hydric soil.

OM regulates many microbial processes (Groffman et al. 1996). OM is correlated to increases in respiration (Atkinson 2001), N mineralization (Fickbohm and Zhu 2006; Wolf 2011), and ammonification (Pinnay et al. 1995). Similarly, denitrification is highly correlated with OM content in wetlands (Reddy and D'Angelo 1997; Nair et al. 2001; Brady and Weil 2002; Sutton-Grier et al. 2009; Mitsch and Hernandez 2007; Ahn and Peralta 2012).

Plant roots generally cannot penetrate soil with a bulk density higher than 1.45-1.75 g/cm<sup>3</sup> depending on texture (Brady and Weil 2003). OM reduces bulk density via enhanced aggregation, stores nutrients and represents a major source of nutrients for plant growth making it essential for the establishment of a productive wetland plant community (Stauffer and Brooks 1997; Nair et al. 2001; Brady and Weil 2002). Similarly, OM has been shown to be an excellent indicator of other wetland functions such as heterotrophic activity and wetland soil development and reflects the level of disturbance at a site (Larson and Pierce 1991, Craft et al. 2003; Fennessy et al. 2004; Rokosch et al. 2009).

Since stable soil OM (humus) tends to be from 50-58% carbon (C), C is an excellent indicator of the previously mentioned benefits of OM (Nelson and Sommers 1982).

### **Created Wetlands and Soil Development**

Created wetlands are often formed in upland areas that lack characteristic wetland hydrology and soil structure. To create wetland hydrology, construction often requires the removal of surface soil in order to cause flooding or to intercept groundwater to create wetland hydrology (DeBerry and Perry 2004). In the mid-Atlantic, most wetland creation involves excavation and grading that leaves cut and

compacted B and C horizons coated with a thin layer of O and A horizon as the soil surface (Daniels and Whittecar 2011). The remaining soil is usually nutrient and organic matter depauperate and the construction process leads to soil being disturbed, uncovered and compacted, exacerbating soil organic matter loss and structural problems. Created wetlands commonly exhibit exaggerated dry and hot summer conditions due to high bulk density from grading and construction practices, lack of organic matter, and lack of insulating vegetation (Cummings 1999, Daniels and Whittecar 2004). These conditions can prevent soils from maintaining low redox soil conditions during the summer, thus limiting the competitiveness of wetland vegetation and allowing for oxidation of organic matter.

In CFW systems, soil organic matter is contributed by litterfall that forms an organic layer near the soil surface and roots that contribute biomass through turnover (Meronigal and Day 1988). Often, decomposition is slow and organic matter and nutrients from litter become buried in the soil where it represents a loss to the system (Conner and Day 1991). This leads to a thick O horizon and a thin A horizon, as most organic matter is stored on the forest floor (Brady and Weil 2008). Comparatively, herbaceous systems develop deeper organic layers due to higher biomass contribution from roots. (Mitsch and Gosselink 2007).

In an extensively studied created wetland in Ohio, the original (antecedent) soil surface did not change significantly in bulk density or percent organic matter 11 years after the wetland was created. It remained very distinguishable from the less dense sediment layer that accumulated above it. Anderson et al. found that the vast majority of accreted sediment material accumulated above the antecedent wetland surface (Anderson et al. 2005; Anderson and Mitsch 2006). Thus, much of the improvement of created wetland soils over time will come from autochthonous additions of organic matter to the wetland surface augmented with allochthonous additions of sediments and nutrients, and will remain distinguishable from the compacted mineral soil (Wetzel 2001; Anderson 2005; Anderson and Mitsch 2006).

Soils in mitigation wetlands often have much lower organic matter (Atkinson et al. 1993; Bishel- Machung et al. 1996; Campbell et al. 2001; Cole et al. 2001; Cummings 1999; Shaffer and Ernst 1999; Stolt et al., 2000; Whittecar & Daniels 1999)

and nutrients levels (Stolt et al 2000; Cummings 1999; Bishel-Machung 1996). Fennessy et al (2008) showed that N levels were 4 times higher and C levels were 5 times higher in natural sites than created ones. Developing hydric soils is often accomplished within the monitoring period (Vepraskas 1999; Cummings 1999), even within 2 years (Mitsch et al. 2006), or show a trend toward increasing hydric character with age (Atkinson et al. 1998). Organic matter is expected to increase as the ecosystem develops (Odum 1969). Organic matter has displayed increases with age in some studies (Campbell et al. 2002; Noon 1996; Johns et al. 2004; Nair et al. 2001; Craft et al. 1988), sometimes displaying faster accumulation than most natural reference sites (Craft 1997; Anderson and Mitsch 2006; Mitsch et al. 2012). At the Olengatany River Wetland Park, organic matter doubled after 10 years post creation and tripled in 15 (Mitsch et al. 2012). Optimistically, some created wetlands (especially salt marshes) can obtain reference levels of SOM as soon as 25 years post creation, but many take longer (Broome & Craft 1998; Craft et al. 1999).

A common problem in created wetlands is a lack of understanding for how long the development of organic matter should take. Some created wetland sites fail to show development of organic matter with wetland age (Bishel-Machung et al. 1996; Ernst and Shaffer 1999), sometimes even when biomass and hydrologic conditions are characteristic of reference wetlands (Cole 2001; Anderson and Cowell 2004). Ernst and Shaffer (1999) found that created marshes did not display change with age, and that there was no change when sites were resampled after 6 years. Bishel-Machung et al (1996) measured organic content at 5 and 20 cm to determine if organic matter accretion was occurring at the surface, but found no difference. Ballentine and Schneider (2009) found that soil organic matter in marshes didn't show development in a chronosequence until 30- 35 years, and sites up to 55 years old still had significantly less organic matter than NRWs. This study represents some of the oldest restored wetlands measured, and still they fall short of reference levels, illustrating that many sites continue to display dissimilarities in organic matter at the end of monitoring. This has led to modeling accumulation. Hossler and Bouchard (2009) modeled soil organic matter and concluded sites wouldn't reach reference levels for 300 years.



Organic matter has been shown to be an excellent indicator of other wetland functions such as heterotrophic activity and wetland soil development and reflects the level of disturbance at a site (Larson and Pierce 1991; Craft et al 2003; Fennessy et al. 2004; Rokosch et al. 2009). Similarly, lagging organic matter correlates with lower nutrient cycling (Hossler et al 2011), respiration (Atkinson 2001), denitrification (Wolf 2011; Ahn and Peralta 2012), water holding capacity, microbial biomass and P sorption (Bruland and Richardson 2004).

### **Wetlands as Sinks**

As soil develops through the accumulation of allochthonous and autochthonous materials, it is also functioning as a sink for carbon, nutrients and sediment. Young wetlands are accretionary in nature, and increase elevation at millimeters per year through the accretion of sediment, organic matter and nutrients (Craft and Richardson 1998). Wetlands often enhance water quality by accumulating nutrients, trapping sediments, and transforming a variety of substances (Mitsch et al. 1979; Lowrance et al. 1984; Whigham et al. 1988; Kuenzler 1989; Faulkner and Richardson 1989; Johnston 1991). In the Chesapeake Bay watershed, removal of nutrients and sediment from the watershed are central to the goals of the Chesapeake Bay Agreement, and one way to reduce nutrients in the watershed is to transform or sequester them in wetlands.

The IPCC recently reported that CO<sub>2</sub> in the atmosphere has increased 40% since 1750 (IPCC 2013). The inordinate carbon storage potential of wetlands is intriguing in the face of climate change. Despite only occupying 6-8% of the land surface, wetlands contain one-third of the global C pool (Mitsch and Gosselink 2007). Similarly, they are current net sinks of 830 Tg/year of C or 14% of the C released through the burning of fossil fuels globally (Mitsch et al. 2013). In combination with the carbon held in woody biomass, wetland soil represents a massive sink for C. It is therefore important to understand how mitigation with CWs alters wetland sink capacity.

### **Nitrogen**

Humans have doubled the amount of nitrogen that enters the land-based nitrogen cycle through fertilizer applications, fossil fuel burning and increasing use of nitrogen-

fixing crops (Vitousek et al 1997; Galloway et al. 2003). Excess nitrogen introduced into waterways as nitrate, creates eutrophication and hypoxia in coastal waters (NRC 2000). This is a particular problem in the Chesapeake Bay watershed, where population growth and a large watershed to estuary ratio makes excess nutrients a persistent problem, deemed deserving of enacting the multi-state Chesapeake Bay Agreement.

Excess nitrogen often enters wetlands as nitrate. From there it can be exported from the system, go through assimilatory nitrate reduction, in which it is utilized by plants or microbes and subsequently is transformed to an organic form, or it can undergo dissimilatory nitrogenous oxide reduction, which often takes the forms of reduction to ammonia or denitrification (Mitsch and Gosselink 2007). Denitrification and assimilatory nitrate reduction are the two major long-term removal mechanisms that wetlands exhibit for removing nitrogen from a system (Craft 1996). The largest pool of nitrogen in wetlands is in the sediment (Bowden 1984), where it mostly exists in organic matter (Craft et al. 1991). In young wetlands, Mitsch et al. (2012) demonstrated that organic nitrogen was a more common fate than denitrification by a factor of 10, but it increased with ecosystem development. However, in natural systems, denitrification often provides the highest level of nitrogen removal (Patrick and Tusneem 1972; Reddy and Patrick 1975).

In mature systems, nutrients are conservatively utilized and stored. Brinson et al. (1984) found that adding nitrogen to a floodplain swamp did not increase the soil nitrogen level, indicating that denitrification and vegetation uptake removed the nitrate. However, retention of external nutrient may be highest in young sites with aggrading stands (Vitousek and Reiners 1975).

Wetlands often have high productivity, high nutrient loading rates, and dynamic oxidation–reduction interfaces that facilitate nitrogen transformation and cycling (Mitsch and Gosselink 2007). In fact, wetlands are such important nutrient sinks that Mitsch et al. (2001) proposed restoring 250- 500 km<sup>2</sup> of wetlands in the heavily agricultural Mississippi River watershed in order to reduce N loading to the Gulf of Mexico and resolve the formation of the dead zone.

## **Carbon**

Soil is the largest terrestrial reservoir of carbon, accounting for 81% of Carbon in the terrestrial biosphere (WBGU 1998). Carbon accumulates in wetlands from both autochthonous and allochthonous sources. Autochthonous organic matter accumulation in wetland soils represents the balance between plant production and decomposition (Schlesinger 1991). As OM is accumulated and buried in wetlands, conditions shift from aerobic to anaerobic, limiting decomposition (Holden 2005). In forested soils, much of the organic matter accumulation that occurs is a product of leaves and woody detritus falling onto the soil surface as well as through root turnover (Richardson and Vepraskas 2000; Mitsch and Gosselink 2007). Atkinson et al (2001) found a positive correlation between litter accumulation and C:N ratios in young wetlands. Similarly, litter from woody species is higher in lignin and complex polysaccharides than herbaceous species, making it difficult to degrade by microorganisms (Schlesinger, 1997; Wolf & Wagner, 2005; Berg & McLaugherty, 2008). Therefore litter from woody species is capable of remaining longer in the soil than rapidly degradable labile compounds (Schlesinger, 1997; Trumbore, 1997; Wolf & Wagner, 2005).

Accumulation of large quantities of organic sediment occurs near the soil surface in CFWs (Richardson and Bigler 1984, Brady and Weil 2002, Anderson et al 2005). Similarly, most decomposition occurs near the soil surface, where fresh, labile, litter is often partially decomposed in months (Sherry et al 1998, Schlessenger 1997, Wolf and Wagner 2005). Thus, while surface deposition may represent the largest input of biomass, litter contribution to the soil organic matter pool is probably a similar amount to root contributions (Megonigal and Day 1988). Furthermore, significant amounts of soluble C leach from the surface organic matter into deeper soil. Thus, measuring accumulation of surface organic matter can give an indication of organic matter accumulation in wetlands, but will only account for a portion (Turunen et al. 1999).

### **Phosphorus**

Phosphorus accumulates in wetlands in association with deposited sediments, adsorption to minerals in the soil, and biological assimilation into organic matter (Khalid et al. 1977). A floodplain swamp subjected to nutrient loading showed P accumulation in the sediments and little evidence of loss from the system (Brinson et al. 1984).

However, Mitsch et al. (2012) experienced eventual phosphorus saturation and loss of sink capacity.

### **Sediment**

Sediment in surface waters increases turbidity, reducing the growth, reproduction and survival of aquatic organisms and rooted aquatic species both locally and with effects reaching the Chesapeake Bay (Henley et al. 2000; Bilotta and Brazier 2008; Bilotta et al. 2008). Furthermore, sediments transport nutrients and pesticides that can damage downstream waterways (Lockaby et al. 2005).

The Piedmont is the biggest modern source of sediment in Chesapeake Bay Watershed, with the lowest rates of background or geologic erosion rates (Gellis et al. 2008), while coastal plain wetlands can trap and store sediments and nutrients, representing one of the last places that can remove contaminants before they reach the Chesapeake Bay (USGS 2012; Hupp and Noe 2005). Sedimentation is also important for wetlands to counter subsidence (Conner 1993) and provide nutrients and non-compacted soil for roots to utilize (Aust et al 2006; McKee et al 2012).

Wetlands have proven to be effective sediment sinks, trapping up to 80-90 percent of the sediment from runoff water (Karr and Schlosser 1978; Johnston 1991, Gilliam 1994). Wetland factors that have been shown to influence sediment accumulation have included geomorphology (Hupp and Bazemore 1993; Johnston 2001), hydrology (Mitsch et al. 1995; Pasternack and Brush 1996; Olila et al. 1997; Craft et al. 2002), nutrient load (Richardson and Craft 1993; Brenner et al. 2001), and macrophyte cover and type (Pasternack and Brush 1996; Horppila and Nurminen 2001). The establishment of wetland vegetation reduces water velocity, making wetlands important for the deposition of suspended sediment and associated nutrients (Gurnell 1997).

Interestingly, young created wetlands can have high sedimentation rates (Fennessy et al. 1994, Braskerud 2001, Harter and Mitsch 2003) when compared to older created wetlands (Craft 2003) or natural wetlands (Johnston 1991, Peterjohn and Correll 1994, Craft and Casey 2000), indicating that unlike many of the other functions thus far discussed, sedimentation rate may diminish with ecosystem development.

Alternatively, it could be that in forested created wetlands, the establishment of woody vegetation will counter the previously reported reduction in sedimentation with age.

### **Soil Coring**

Grossman and Reinsch (2002) reported that the most suitable soil sampler for wet soils creates a vacuum to retain the soil sample in the tube. In order to obtain undisturbed sediment cores and avoid compaction and distortion of the core, the diameter of the sampler should be large (Rheinhardt and Cole 2000). When used properly, large corers have been shown to create negligible compaction, whereas smaller diameter corers often have substantial compaction (Crozier et al 1995; Rheinhardt and Cole 2000). Therefore in order to avoid distortion and compaction in soil cores, I will be utilizing a 12.7cm diameter steel corer with a removable valve to create a vacuum.

### **Cesium 137**

Radioactive Cesium-137 is the product of aboveground thermonuclear weapons testing. Cesium-137 deposition began in 1954 and peaked in 1964 (Ritchie and McHenry 1990). Once in the soil, Cesium-137 is strongly adsorbed to sediment, and remains stable making it a radionuclide widely used as a tracer in dating studies, especially in depositional environments such as wetlands where sediment accumulating above the peak is assumed to have accreted after 1964 (Ritchie and McHenry 1990, Craft and Richardson 1998, Bernal and Mitsch 2012). Thus the sediment accumulation rate can be estimated by dividing the sediment deposition by the time surpassed since 1964 (48) (Craft & Richardson, 1993; Craft & Casey, 2000; Graham et al., 2005; Stark et al., 2006). Cesium exhibits similar dating performance to lead-210 (Bernal and Mitsch 2012), and Aust et al. (2011) found cesium to compare favorably with both sediment pins and elevation surveys over multiple decades.

### **Chronosequence**

A chronosequence relies on selecting sites with similar environmental conditions, differing only with age. It is therefore possible to use sites of different ages as a surrogate for time, rather than to wait for sites to age. The problem is that because

of variable disturbance histories, differences among sites may be incorrectly attributed to ecosystem development, when it may actually be an artifact of variable disturbance history, creation methods or site differences (Pickett 1989). Therefore it is beneficial to strengthen chronosequence results with data following the progression of individual sites or groups of sites over time.

**Figure Captions**

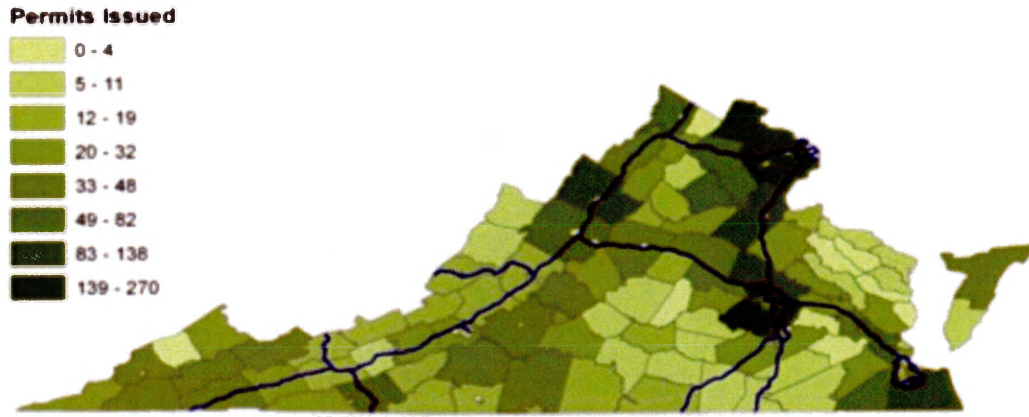
Figure 1.1: Cumulative total wetland and open water impacts for counties in Virginia permitted as reported by VDEQ (2001- 2009). (VADEQ 2012).

Figure 1.2: The redox hierarchy (Mitsch and Gosselink 2007).

Figures

Figure 1.1

VWP Permits Issued by County (2001-2009)





**Figure 1.2**

<i>Element</i>	<i>Oxidized Form</i>	<i>Reduced Form</i>	<i>Redox Potential (mV)</i>
Nitrogen	NO <sub>3</sub> <sup>-</sup> (nitrate)	N <sub>2</sub> O, N <sub>2</sub> , NH <sub>4</sub> <sup>+</sup> (nitrous oxide, nitrogen gas, and ammonium)	250
Manganese	Mn <sup>+4</sup> (manganic)	Mn <sup>+2</sup> (manganous)	225
Iron	Fe <sup>+3</sup> (ferric)	Fe <sup>+2</sup> (ferrous)	120
Sulfur	SO <sub>4</sub> <sup>-2</sup> (sulfate)	S <sup>-2</sup> (sulfide)	-75 to -150
Carbon	CO <sub>2</sub> (carbon dioxide)	CH <sub>4</sub> (methane)	-250 to -350

## Chapter 2

### Development of Vegetation Related Ecological Functions in 11 and 20 Year Old Created Forested Wetlands

#### Abstract

**Compensatory mitigation for** 77 hectares of palustrine forested wetlands (PFWs) occurs each year in Virginia. The loss of wetland area and function is often mitigated through the creation of new wetlands. Two important functions provided by PFWs are the maintenance of a characteristic plant community (an aspect of habitat provision) and the sequestration and storage of carbon in woody biomass (a biogeochemical function). Both of these functions are lacking at first, as the plant communities of young CFWs are different than natural reference wetlands (NRWs) and woody biomass is largely absent. It remains uncertain whether CFWs provide a functional replacement for lost NRWs, and if so how long temporal functional loss persists. Furthermore, the provision of high levels of biogeochemical functioning may be at odds with the development of a high quality plant community and vice versa. We utilized three CFWs that were approximately 11 years old (yo) and four CFWs that were approximately 20 yo that had all been sampled 8 years previously (2004) to investigate a suite of plant community parameters (Sorensen's similarity index, species richness, Shannon diversity index, floristic quality index, dominance by and percentage of non-native species and woody biomass) to determine changes over time and to compare them to a group of four NRWs to determine if functional replacement occurs within 20 years. There were no significant differences between CFWs and NRWs in terms of any vegetation parameters associated with combined strata or the herbaceous or shrub sapling strata. We did find significant differences in tree species richness, FQI and basal area, and species composition through analysis of similarity. The carbon stored in woody biomass was 10 times lower in 20 yo CFWs than NRWs and nearly 20 times lower in 11 yo CFWs. We did not find any significant linear relationships between vegetation parameters and CFW age. However, when analyzing data from 2012, there was a significant trend toward increased dominance by non-native species in the herbaceous strata of CFWs, indicating that CFWs may be susceptible to invasion by non-natives after being declared successful and released from monitoring. Our study suggests that CFWs can provide a characteristic plant community in the understory within 11 and 20 years, but not in the tree strata, which contains the vast majority of the biomass. We found that the provision of a characteristic plant community (in terms of FQI) is significantly positively correlated with carbon sequestration, indicating that habitat provision and biogeochemistry can be complementary in CFWs.

## Introduction

By the mid-1980s, wetland area had been reduced by 53% in the lower 48 states and 42% in Virginia (Tiner and Finn 1986; Dahl 1990; Dahl and Johnson 1991; USGS 1999). Palustrine forested wetlands (PFWs) have borne the brunt of these losses, and continue to be the most displaced wetland type nationally and in Virginia (Dahl 1990, Tiner and Finn 1986; USGS 1999; Dahl 2011).

Concern about the loss of wetlands prompted the national implementation of a policy to conserve remaining wetland area and function (NRC 2001, § 62.1-44.15:21 B, Code of Virginia). To avoid incurring net loss of wetland area and function despite 8,500 hectares of annual wetland impacts nationally (Martin et al. 2006), wetland impacts are often compensated for through the creation of new wetlands (National Research Council 2001; USACE 2002).

Dahl (2011) noted that since 1950, 7.7 million hectares of PFWs have been lost compared to 2.8 million hectares of emergent wetlands (Martin 2006). In Virginia PFW wetlands continue to make up the majority of wetland impacts (VADEQ 2010). Furthermore, mitigation has often led to PFW impacts being compensated for by other wetland types such as emergent wetlands and open water ponds (Allen 1996; Mack and Micacchion 2006; Brady et al. 2008; Kettlewell et al. 2008). Because of high losses of PFWs and their vulnerability to the mitigation process, it is especially important to understand the development of ecological functions in created PFWs.

Forested created wetlands (CFW)s are often created by removing vegetation and soil from uplands to allow for wetland hydrology to develop (Atkinson et al. 1993; Brown and Lant, 1999; Daniels and Whittaker 2004). Plant communities of young CFWs often differ from natural wetlands in that they have different vegetation assemblages (van der Valk 1981; DeBerry and Perry 2004), low levels of woody biomass (Noon 1996; Mathews and Endress 2008; Atkinson et al. 2005; Fennessy et al. 2008), and lower floristic quality (Mushet et al. 2002; Balcombe et al. 2005). These parameters relate to the functions of habitat provision and biogeochemical functioning, therefore, in order to reach functional equivalency with natural sites, CFWs must develop over time (Odum 1969; Reppert 1992; Noon 1996; Campbell et al. 2002; Johns et al. 2004).

Plant communities are intricately linked to nutrient storage, release and cycling,

making them great indicators of wetland function (Bedford 1996; Lopez and Fennessy 2002; Miller et al 2006; Miller and Wardrop 2006; Mitsch and Gosselink 2007; Veselka et al. 2010). Woody biomass offers both a direct and indirect indicator of carbon sequestration occurring in the landscape. Rheinhardt et al. (2012) found that 96% of the aboveground biomass of 5-25 yo regenerating riparian forests was held in trees, making them the most important sink for carbon in terms of vegetation. Furthermore, litter from woody species is higher in lignin and carbon to nitrogen ratio (C:N) than herbaceous plants making it resistant to microbial breakdown (Schlesser 1997; Richardson 2004; Wolf and Wagner 2005; Berg and McClaugherty 2008), thus soil carbon correlates significantly with tree biomass (Brinson et al. 2006).

Studies have demonstrated that as systems recover from disturbance (in this case the creation of the wetland) richness, diversity, and hydrophytic indicator status often become similar to natural reference wetlands (NRWs) within a typical 10 year monitoring period (Confer and Niering 1992; Kentula et al. 1992; Brown 1999; Balcombe et al. 2005; Spieles et al. 2006; Brown and Veneman 2001), whereas indicators based on species composition may not reach equivalence with NRWs over the short term (Brown 1999; DeBerry and Perry 2004; Brooks et al. 2005; Spieles et al. 2006). Similarly, while colonization and succession in marsh communities can occur quickly, (Landin and Webb 1986), tree establishment can be the more difficult aspect of offsetting PFW impacts (Robb 2002; Morgan and Roberts 2003; Spieles 2005; Matthews and Endress 2008), and even when successful, may take decades to begin to resemble natural systems (Kusler 1986; Mitsch and Gosselink 2007).

If CWs develop wetland hydrology, hydric soils and a hydrophytic plant community as well as fulfill additional performance standards specific to Virginia, they are deemed “successful” mitigation for wetland impacts (VADEQ 2010), inherently assuming that once a jurisdictional wetland is established, wetland functions will also be restored, however, this may not be true (Moy and Levin 1991; Simenstad and Thom 1996; Zedler 1996). The ability of mitigation wetlands to successfully restore functions has been questioned for three decades (Race and Christie 1982; Race and Fonseca 1996; Zedler 1996; NRC 2001).

While many studies have analyzed wetland vegetation development in young CWs very few have measured those up to 20 years old (Atkinson et al 2005). Race and Fonseca (1996) argue that most monitoring of CWs occurs “too early in the developmental stages to demonstrate success.” Similarly, Kusler and Kentula (1990) argue that short-term revegetation does not guarantee a wetland will continue to function over time, and Zedler and Weller (1990) conclude that whether CWs persist through time is one of the most important knowledge gaps in understanding the effects of mitigation on ecology. Mitch and Wilson (1996) speculate that emergent wetlands can develop within 15-20 years, but if all CWs function at a lower level for 20 years, temporal losses of function can lead to significant overall loss (Bendor 2009). Furthermore, time to functional equivalency is expected to take much longer in forested wetlands (Kusler 1986; Mitsch and Gosselink 2007). Moreno-Mateos (2012) utilized a meta-analysis to show that restored wetlands lag behind natural levels of function even after close to a century.

To determine whether CWs are ecologically successful, the ultimate test is whether CWs function as natural wetlands (Van der Valk; Brinson and Rheinhardt 1996; Rheinhardt et al. 1999; Whigham 1999). We will be focused on two functions: 1) The habitat provision function of the “maintenance of a characteristic plant community,” and 2) carbon storage, part of biogeochemical functioning, represented as carbon stored in above ground woody biomass (NRC 1995). Additionally, both Ehrenfeld (2000) and Zedler (2000) called into question the possibility of creating wetlands with both high quality plant communities and high levels of biogeochemical functioning, therefore we will also investigate how the provision of our two functions interact in CFWs.

The goals of this study were: 1) to determine if created palustrine forested wetlands (CFWs) in Virginia replace lost functions through the maintenance of a characteristic plant community and storage of carbon in woody biomass by 11 and 20 years post-creation 2) to understand how CFWs develop over time 3) to compare indices of plant community composition to the sequestration of carbon to determine if the goals of high levels of biogeochemical function and maintenance of a characteristic plant community are mutually attainable. Finally, we assess whether regulatory performance standards continue to be met 10 years after the cessation of monitoring.

## Methods

### Created Wetlands

Seven CFWs that were constructed to mitigate for wetland impacts to PFWs associated with the Virginia Department of Transportation (VDOT) were selected from two age classes. Three CFWs around 11 years old (11 yo CFWs) and four around 20 yo (20 yo CFWs) were selected from Virginia's Coastal Plain (4 sites) and Piedmont (3 sites) provinces.

Each site met the following criteria:

- (1) created as compensatory mitigation for impacts regulated under Section 404 of the Clean Water Act to replace impacted PFWs
- (2) at least one hectare
- (3) has satisfied the US Army Corps of Engineers criteria of wetland hydrology (Environmental Laboratory 1987). Furthermore, none of these sites was subjected to prolonged periods of ponding.
- (4) data is available from 2004 (DeBerry and Perry 2012) with which to compare current conditions
- (5) was approximately 11 or 20 years old (yo)
- (6) has not been drastically physically altered since construction

### 20 Year Old CFW Group (average age 20.3 ± 0.9 SE)

This group includes four of the older created mitigation wetlands associated with the Virginia Department of Transportation (Leo Sneed personal comm). They were deemed successful by regulatory agencies approximately ten years ago and allow ecological study of "successful" CFWs through their second decade of development and their first decade of independence.

**Sleeter Lake** is 1.4 ha (3ac) in size and was constructed in 1989. It was 23 yo when sampled for this study.

It is located in Loudon Co. in the Northern Piedmont physiographic province of Virginia. The surrounding landscape contains a mixture of agriculture and moderate-

density residential development. The hydrologic regime is contributed by overbank subsidies from Catoctin Creek as well as through groundwater discharge along the floodplain valley and on side-slopes. Regolith and coarse materials high in the soil profile indicate shallow bedrock. This site is located in the town of Purcellville.

**Bower's Hill** is a 10.9 ha wetland constructed in 1993. It was 19 yo when sampled for this study.

This site is located in Chesapeake Co. in the southeastern Coastal Plain physiographic province of Virginia. The underlying soil is characterized by mineral soils of maritime origin and relatively level topography. The surrounding landscape includes moderate-density residential communities, agriculture, and forested land. The hydrologic regime is surface capture of precipitation and groundwater discharge. This site is located in the City of Chesapeake, near the Interstate 664/ U.S. Route 58 interchange. It is in an abandoned surface mine near Goose Creek, northeast of Joliff road.

**Springfield Bypass (Route 7)** is a 2.5 ha wetland constructed in 1992. It was 20 yo when sampled for this study.

This site is in Fairfax Co. in the northern Piedmont physiographic province of Virginia. The surrounding landscape is composed of high-density residential neighborhoods and commercial properties. The hydrologic regime is a product of overbank flooding from Sugarland Run, groundwater discharge, and surface capture. This site is located near Herndon, Virginia, and lies in the floodplain of Sugarland Run.

**Courtland Bypass** is a 4.1 ha wetland constructed in 1993. It was 19 yo when sampled for this study.

This site is in Southampton County in the Coastal Plain physiographic province. Low topographic relief, predominance of sand and bottomland hardwood vegetation underlain by silty clay loam and high organic matter characterize the landscape. The surrounding landscape is mostly agriculture, but also contains a significant level of forested land. Water is contributed from toe slope groundwater discharge, through runoff and from small streams.

**11 Year Old CFW Group (Average age 10.7 ± 0.9)**

This group of CFWs represents wetlands within two years of completing their monitoring period. While Mattaponi is still being monitored, Manassas and Mount Stirling have recently been deemed “successful” by the regulatory agencies and have been relieved of monitoring. Sites were chosen that did not have major corrective management over their monitoring period such as grading or hydrologic alterations.

**Mount Stirling** is an 8.5 ha wetland constructed in 2001. It was 11 yo when sampled for this study.

This site is in Charles City County in the Coastal Plain physiographic province. The soils are mostly sandy with low-relief. However, falling within the floodplain of the Chickahominy River means that the soil also contains silty clay loam soils and accumulated organics. The surrounding landscape is predominantly forested, with some agriculture and surface mining in the region. Site hydrology is the product of floodwaters from the Chickahominy River, surface capture, and groundwater discharge from the toe of the primary Chickahominy scarp to the south.

**Mattaponi** is a 4.3 ha wetland constructed in 2003. It was 9 years old when sampled for this study.

This site is in Caroline County located in the Coastal Plain’s upland subprovince, very close to the piedmont. The surrounding landscape consists of low-density residential, agriculture, and forested lands. The hydrologic regime is groundwater discharge, surface subsidies from Mattaponi River flooding, and precipitation.

**Manassas** is a 7.2 ha wetland constructed in 2000. It was 12 years old when sampled for this study.

This site is in Prince William County, located in the northern Piedmont physiographic province, in the Mesozoic Lowlands subprovince. This subprovince is known for modest relief and is underlain by Triassic sedimentary igneous rocks. The surrounding



landscape consists of forested, agricultural, and suburban residential development. The hydrologic regime is contributed by surface flow from small tributaries of Broad Run.

### **Natural Reference Wetlands (Average age $82 \pm 3.2$ )**

Four natural reference wetlands (NRWs) were sampled to make comparisons to the CFWs. NRWs were forested wetlands that have not been subject to clearing or extreme disturbance since at least 1964 (in order to allow for Cesium dating of the soil) and were located in close proximity to one of the created sites. Average NRW time since major disturbance was estimated by dating 1 tree from the dominant size class of each plot. Site age was the average of all 5 plots within each site, and was carried out in 2004 by DeBerry (2006). A 36 cm Suunto increment borer with a 0.5 cm cutting radius was used to take increment cores, which were processed using the dating methods specified in Forestry Suppliers (2004) and Husch et al. (1972). Dominant size class was utilized to identify trees from the oldest functional tree guild (Keddy 2000; Lopez et al. 2002). NRWs were chosen based on proximity to one of the sites (2 in the Piedmont and 2 in the Coastal Plain) and in all cases except for in Manassas were within 1 kilometer of the CFW site. These sites would not be considered pristine, but “minimally impaired” (U.S. EPA 2002), acknowledging that most impacted wetlands being mitigated for were likely to have been disturbed for timber harvesting or agriculture at an earlier date.

### **Age Class Comparisons**

The vegetation communities of seven CFWs from two age groups (11 and 20 years post creation) were sampled in fall 2012 for a number of parameters. Many of the same parameters were also collected from the same group of sites in 2004 (DeBerry and Perry 2012), when the current 11 yo and 20 yo CFWs were ~3 and ~12 respectively. When the relevant parameters were collected at both time periods, we compared all four age classes (2 groups at 2 different times) to one another and to a group of NRWs. This allowed us to compare NRWs to 4 different ages of CFWs and allowed us to look at changes in CFWs over the course of 8 years. When relevant data was not available from 2004 we compared the two CFW groups from 2012 to one another and to the NRWs, allowing us to determine if groups of 11 yo and 20 yo CFWs are similar to one another

and to NRWs.

### **Sampling Protocol**

At each CFW and NRW, a 1-ha study site was chosen based on relatively homogenous representative plant composition and age (Parsons and Ware 1982; Glascock and Ware 1979) and lack of major corrective action that may have altered functional succession. Within 1-ha sites established in DeBerry and Perry (2012), five study plots were established on a stratified random basis (Mueller-Dombois and Ellenberg 1974). A baseline was established along the site perimeter. This was divided into 30 m segments to stratify transects. Transect center points were determined by point of entry within the segment (1-30m) and distance into the site (1-66m), each determined by drawing a random number. The resultant point became the center for all transects (DeBerry and Perry 2012).

At each CFW and NW, five 11.3 m radius circular tree species plots were established representing an overall area in each CFW of .04 ha (Johnson 2000; DeBerry and Perry 2012). Cover and diameter at breast height (dbh), defined as 1.4 m above the ground, was measured for all trees greater than 10 cm dbh within plots. Importance value (IV) of each species was calculated as the combination of relative basal area and relative density within all plots of a site (Perry and Atkinson 1997; DeBerry and Perry 2012).

Similarly five 5-meter radius circular shrub/sapling plots were established in each CFW and NW. Modified cover scale was estimated for each species and density of saplings, shrubs, and woody vines (greater than 1m in height but less than 10 cm dbh) were recorded in each plot (Mueller-Dumbois and Ellenberg 1974). IV was calculated as the combination of relative cover and density (DeBerry and Perry 2012).

Additionally, in woody plots, dbh of all trees and saplings greater than 2.5 cm dbh was recorded for biomass estimates.

Cover of herbaceous species was measured in three 1m<sup>2</sup> plots, randomly located within the tree plot using a randomly drawn azimuth and distance from center point of the plot. Cover was estimated for each species (Mueller-Dumbois and Ellenberg 1974) and density per species was determined in 0.25 m<sup>2</sup> quadrats (DeBerry and Perry 2012).

Relative IV, which combines relative cover, relative density and relative frequency, was then calculated for all species (Perry and Atkinson 1997).

Vegetation was sampled in September because this period represents peak growing season for created sites in the area (DeBerry and Perry 2004). All plants were identified to species level using The Flora of Virginia (Weakley et al. 2012).

Importance value (IV) was calculated in each strata. In the herbaceous layer, species IV was calculated as the average of relative cover, relative density and relative frequency (Perry and Atkinson 1997). For trees and shrubs, IV was calculated as the average of relative cover (based on estimated cover class for shrub/ saplings and basal area for trees) and relative density, as relative frequency tends to overestimate rare species in woody strata (DeBerry and Perry 2012).

Dominant species were calculated across age classes for each strata according to the 50:20 rule, which identifies dominant species as the sum of species with the highest IV scores that represent 50% of the relative dominance as well as any species that makes up 20% or more (Tiner 1999).

### **Vegetation Indices**

Species composition was compared between CFW groups overall and within strata. CFW age classes were compared to one another and to NRWs through Sørensen similarity index (SSI) (Mueller-Dombois and Ellenberg 1974) using the formula:

$$2c/(a+b)$$

where c is the number of shared species between age classes, a is the number of species in the first class and b is the number in the second class.

Species richness (SR) was calculated as the total number of species identified in a site (SR) (Magurran 1988). We report SR as a sum of plots from all strata, as well as for individual strata (herbaceous, shrub/sapling, and tree) at each site.

Shannon diversity index is a measure of species diversity based on the proportion of the site represented by each species, i.e. a combination of SR and species evenness. This index is derived from information theory, and for a given sample is maximized when all species are equally abundant.

SDI is calculated as:

$$H' = - \sum P_i \ln P_i$$

Where  $P_i$  is the proportion of individuals from the overall population found in the  $i$ th species (Pielou 1975). This index is dependent on abundance data, and was therefore calculated from individual strata at each site.

Percent non-native species (%NN) was calculated as the proportion of species richness represented by non-native species (NN) to overall SR. This parameter was only calculated as a total for all strata.

%NN was calculated as:

$$\%NN = (NN/SR) * 100$$

Dominance of NN (DNN) was used to make comparisons within strata. DNN was calculated as the sum of relative IV for all nonnative species within a site. Because of dependence on IV, this was calculated within strata. DNN was calculated as:

$$DNN = \sum [NN (IV)]$$

The overall Floristic Quality Index ( $FQI_{all}$ ) was determined using presence absence data from all vegetation strata in all plots within the sites (Swink and Wilhelm 1979). FQI recognizes that different plant species have evolved varying tolerance levels to disturbance or environmental stress (Odum 1985, Hobbs and Chapin 1991, Huenneke 1992), as well as varying degrees of fidelity to specific quality of habitats (Herman et al. 1997, Mushet et al. 2002). Tolerance and fidelity combine to create “species conservatism” (Swink and Wilhelm 1979) and is expressed as a score from 0 - 10 called a “coefficient of conservatism” (c-value). A low c-value indicates that a plant is rarely found in natural plant communities and is highly tolerant of disturbance, while a high c-value indicates that the plant usually exists in an undisturbed natural plant community (Matthews 2003). In this study, c-values were obtained from the Virginia Department of Environmental Quality (VDEQ 2004).

Overall FQI was calculated as:

$$FQI_{all} = C' (\sqrt{SR})$$

A modified FQI that incorporates plot based data, rather than the classic walk-through method was used to enable comparison with previous data and because no significant differences were found between the two techniques (DeBerry 2006). The modified FQI divides vegetation into herbaceous, shrub-sapling and tree stratum

because strata reflect different ecological characteristics and disturbances. Long-lived species indicate chronic ecosystem stresses, while the annual plant guild indicate acute stress (Chapin 1991). Trees usually represent the oldest individuals at a site, and therefore woody species exhibit ecological inertia because of longer response times tied to life history strategies. Herbaceous species tend to more accurately reflect current conditions (Lopez et al. 2002). Saplings and shrubs represent short-term, yearly or decadal conditions. Saplings, however, also point to potential woody dominants of the future (DeBerry and Perry 2012). Therefore, modified FQI will be reported for the herbaceous strata (FQI<sub>H</sub>), the shrub sapling strata (FQI<sub>S</sub>) and the tree strata (FQI<sub>T</sub>) independently. The modified FQI also uses species IV in the equation to incorporate dominance rather than just presence/absence data. FQI<sub>mod</sub> has shown increased sensitivity to detect the effects of disturbance in natural wetlands (Nichols et al. 2006, DeBerry and Perry 2012) and to reflect environmental conditions in CFWs in Virginia (DeBerry and Perry 2012). The equation is the sum of the C value of each plant multiplied by its importance value, multiplied by the square root of native species (DeBerry 2006, DeBerry and Perry 2012):

$$FQI_{(H,S,T)} = [\sum C_i(IV_i/100)](\sqrt{N})$$

### Woody Species Composition

Due to lack of tree strata data from 2004, indices specific to trees were only compared within the two 2012 age groups of CFWs and NRWs, while all other vegetation indices were compared among all four CFW age classes and NRWs.

Woody vegetation assemblages were compared with Analysis of Similarity (ANOSIM) to test for statistically significant differences between the vegetation composition of groups of sites. It utilizes a dissimilarity matrix based on the species IV. Like non-metric multidimensional scaling, this method uses the rank order of dissimilarity values. If age classes are different, composition dissimilarities will be larger between groups than within them. The following equation represents the difference of mean ranks between groups ( $r_B$ ) and within groups ( $r_W$ ).

$$R = (r_B - r_W) / (N(N-1) / 4)$$

ANOSIM was used to compare the composition of the tree assemblages between 11 and 20 yo CFWs and between both ~11 and 20 yo CFWs and NRWs. It was also used to compare the sapling community compositions of the three groups and finally the sapling community of CFWs was compared to the tree community of NRWs to analyze whether the tree strata is likely to develop to be similar in CFWs with time.

### **Woody Biomass**

To determine biomass, trees >2.5cm dbh were measured. Regression equations, separating trees into 10 groups of species were used to translate dbh to biomass (Jenkins et al. 2003). Tree species were separated into groups based on taxonomic relationships, wood specific gravity and diameter-to-aboveground biomass relationships (Jenkins et al. 2003). Tree species not overtly classified by Jenkins et al. (2003) were placed in the group that most closely resembled their growth habit and wood structure (Rheinhardt et al. 2012). Woody biomass per hectare was calculated independently for trees and saplings as well as combined for overall woody biomass. Because biomass is approximately 50% carbon (Cairns et al. 2003; de Gier 2003; Brown 1997; IPCC 2003) biomass was used to calculate aboveground carbon storage by multiplying biomass per hectare by 0.5. Since tree data was not collected in 2004, biomass data was only compared among age classes sampled in 2012. Finally, to compare the rate of development of woody biomass among groups, biomass was divided by age of wetland to develop a rate of woody biomass development per year.

### **Correlation Between FQI and Carbon Storage**

FQI<sub>ALL</sub> in each CFW was compared to the percent change in soil carbon over 8 years determined by a related study in the same sites. It was also compared to current woody biomass present on the sites. Finally, it was compared to total carbon accumulation rate in above ground biomass and the soil. The accumulation of woody biomass is explained above. Carbon sequestration in the soil can be calculated by first estimating carbon stock according to the equation:

$$C_t = BD \times C_c \% \times D$$

Where  $C_t$  = total carbon stock, BD is bulk density and D is depth (Mann 1986; Guo and Gifford 2002).

Next carbon stocks can be compared at different times to develop a rate of change in carbon storage:

$$(C_{t2} - C_{t1}) / \Delta T = \text{rate of change in carbon storage}$$

Where  $C_{t2}$  is carbon stock at time 2 and  $C_{t1}$  is carbon stock at time 1 (Post and Kwon 2000; Guo and Gifford 2002).

We did not have data for soil bulk density from 2004. However, in a related study of the same sites, we took 47 soil samples (see ch. 3). Based on these 47 soil samples, a strong statistically significant relationship exists between % carbon and bulk density. Linear regression revealed an  $R^2$  value of -0.665 ( $p < 0.0001$ ), while Pearson's correlation yielded a correlation index of -0.815. Based on the strong relationship, I estimated previous bulk density based on the linear equation:

$$B_d = -0.0425c + 1.4064$$

Where  $B_d$  is the bulk density and c is the percent carbon.

From this stage, it was possible to compare estimated standing stocks of carbon in 2004 to standing stocks of carbon in 2012, yielding an estimated accretion rate. Though this rate is probably too rough of an estimate to report independently, we felt comfortable utilizing this estimate to determine a relationship between carbon sequestration and FQI in our CFWs.

### **Regulatory Wetland Success**

CFWs were evaluated according to the methods established in the Virginia Mitigation Banking Template, which “establishes guidelines and responsibilities for the establishment, use, operation and maintenance“ of wetland mitigation banks as well as compensatory mitigation sites (VDEQ 2010; Steve Martin personal communication). The template mandates adherence to performance criteria in order to compensate for wetland losses.

Data was used to determine if 11 and 20 yo CFWs comply with the performance criteria related to vegetation assemblage. Sites that comply will be considered to have achieved regulatory success.

The site will be deemed to fulfill vegetation performance standards if:

- 1) More than 50% of all dominant species are facultative “FAC” or wetter using the 50:20 rule to determine dominants (Tiner 1999).
- 2) The density of native, wetland (FAC or greater) woody species is at least 400 per acre, or the canopy coverage is greater than 30%.
- 3) No more than 5% aerial cover of non-native species.
- 4) Native herbaceous plant cover is at least 80%.

### **Statistical Comparisons**

We evaluated data sets using Cochran’s test for homogeneity of variance (Fried 1976; Cochran 1941) and found the assumption of homoscedasticity was often violated. Therefore we used the separate variances t-test, or Welch’s T-test to analyze differences between groups (Logan 2010). When the Welch’s T-test detected group differences, post-hoc pairwise T-test was used to determine where differences occurred. All statistics were performed using R version 1.12.1. P-values <.05 were considered to be significantly different.

Individual CFW site ages were then used to make linear regressions with vegetation indices as the dependent variable to determine if vegetation indices develop predictably with time. Significant relationships were determined to be those with p-values of <.05.

To investigate the relationships between FQI and biogeochemical functioning, spearman’s rank correlation coefficients were generated to calculate the product moment correlation coefficient because of a lack of linearity and normality in the data (Logan 2010).

## **Results**

### **All Strata Vegetation indices**

We identified 184 species in CFWs and NRWs combined. In CFWs we identified 18 tree species, 37 shrub species and 128 herbaceous species, for a total of 143 distinct species. In NRWs we found 26 tree species, 48 shrub/sapling species and 63 herbaceous species for a total of 103 distinct species. Of these species, 62 were shared between



NRWs and CFWs, yielding an SSI of 0.504. SSI over all strata was 0.34 between CFW age classes, 0.36 between 11 yo CFWs and NRWs and 0.39 between 20 yo CFWs and NRWs.

SR of the 11 yo CFWs increased from  $29.6 \pm 7.69$  ( $\pm$ SE) in 2004 to an average of  $30.25 \pm 3.06$  species per site. The 20 yo CFWs showed a slightly higher average of  $35.5 \pm 4.33$  than the 11 yo, but decreased slightly from  $37.5 \pm 4.33$  in 2004. The NRWs fell in between the CFW at  $33 \pm 4.3$  native species (table 2.2, figure 2.1). There were no significant differences among CFW age classes, over time in wetlands or between CFWs and NRWs ( $p=0.67$ ). There was no significant relationship between CFW age and overall SR ( $r^2=0.04$ ,  $p=0.23$ ) (table 2.6).

The average %NN in 11 yo sites increased slightly over 8 years from  $9\% \pm 5$  in 2004 to  $11.1\% \pm 2.15$  (range of 6.9 to 14 in 2012). 20 yo CFWs increased from  $7.3\% \pm 3.75$  in 2004 to  $14.83\% \pm 2.29$  in 2012. NRWs were  $9.52\% \pm 1.11$  (table 2.2, figure 2.2). As with SR, none of the differences were significant ( $p=0.399$ ). There was no significant relationship between %NN and CFW age ( $r^2=0.13$ ,  $p=0.12$ ).

FQI<sub>all</sub> showed the 11 yo CFWs increase in FQI from  $18.56 \pm 4$  to  $23.64 \pm 1.7$ . The older CFWs increased slightly from  $22.37 \pm .52$  to  $23.09 \pm 3.17$  in 2012. NRWs had the highest average floristic quality at  $26.9 \pm 1.41$  (table 2.2, figure 2.2). None of these differences were significant ( $p=0.227$ ). There was no significant relationship between FQI<sub>all</sub> and CFW age ( $r^2=0.048$ ,  $p=0.22$ ).

### Shrub Sapling Strata

In the shrub/sapling strata, 11 yo CFWs were dominated by *Fraxinus pensylvanica* (27.7), *Acer rubrum* (20.3) and *Salix nigra* (18.8). The 20 yo CFWs were dominated by *Salix nigra* (23.6), *Liquidambar styraciflua* (12.6), *Morella cerifera* (12.6) and *Cephalanthus occidentalis* (8.2)

NRWs were dominated by *Asimina triloba* (22), *Lindera benzoin* (8.4), *Ulmus Americana* (8.4), *Cornus amomum* (7.8), and *Ilex verticillata* (7.3).

SSI between CFW age classes was 0.64, 0.35 between 11 yo CFWs and NRWs and 0.33 between 20 yo CFWs and NRWs.

SR in the 11 yo CFW shrub/sapling strata increased from  $9.67 \pm 1.67$  in 2004 to

11.3 ± 1.2 in 2012, while the 20 yo CFWs decreased from 10.25 ± 2.75 in 2004 to 9.25 ± 3.35 in 2012. NRWs had an average shrub/sapling SR of 10.75 ± 1.11 (table 2.3, figure 2.4). None of the difference among groups was statistically significant (p=0.905). There was no significant relationship between SR in the shrub/sapling strata and CFW age ( $r^2=-0.07$ , p=0.69).

The Shannon diversity of the shrub/sapling strata of 11 yo sites decreased from 1.67 ± .167 in 2004 to 1.29 ± 0.37 in 2012. Similarly, Shannon diversity decreased from 1.73 ± .26 to 1.65 ± .26. NRWs exhibited the highest average diversity of 2.71 ± .95 (table 2.3, figure 2.5). None of the groups were significantly different in terms of diversity (p=0.751). There was no significant relationship between Shannon diversity of the shrub/sapling layer and CFW age ( $r^2=-0.08$ , p=0.83).

DNN of the shrub/sapling layer decreased from 9.5 ± 9.5 in 2004 to 0 in 2012 in 11 yo CFWs. 20 yo CFWs increased DNN from 1.2 ± 1.2 in 2004 to 6.3 ± 5.9 in 2012 and NRWs had 6.2 ± 5.9 DNN (table 2.3, figure 2.6). There were no significant differences among groups (p=0.65). There was no significant relationship between DNN in the shrub/sapling layer and CFW age ( $r^2=-0.07$ , p=0.80).

Average FQI<sub>s</sub> for 11 yo sites increased from 10.9 ± 2.57 in 2004 to 14.91 ± 3.19 in 2012. 20 yo sites decreased from 13.55 ± 1.12 to 10.64 ± 2.63 (table 2.3, figure 2.7). NRWs showed the highest average FQI of 15 ± 1.75. There were no significant differences among groups (p=0.65). There was no significant relationship between FQI<sub>s</sub> and CFW age ( $r^2=-0.06$ , p=0.61).

### Herbaceous Strata

The Herbaceous strata in the 11 yo sites was dominated by *Juncus effuses* (IV=14.8), *Persicaria hydropiperoides* (10.9), *Symphotrichum racemosum* (7.1), *Lespedeza cunneata* (5.3), *Scirpus cyperinus* (4.2), *Ludwigia palustris* (3.5), *Symphotrichum lateriflorus* (2.8), *Echinocloa crus-gali* (2.7). The dominant species in the 20 yo herbaceous strata were *Microstegium vimineum* (15.2), *Juncus effuses* (6.4), *Arthraxon hispidus* (5.6), *Chasmanthium laxum* (5.0), *Persicaria hydropiperoides* (4.0), *Typha angustifolia* (3.2), *Scirpus cyperinus* (3.2), *Mikania scandens* (2.8), *Persicaria perfoliata* (2.6), *Phragmites australis* (2.1). NRWs were dominated by *Microstegium*

*vimineum* (11.0), *Woodwardia areolata* (10.0), *Murdania keisak* (7.0), *Saururus cernuus* (5.1), *Carex stricta* (5.1), *Boehmeria cylindrica* (4.8) and *Arundinaria gigantea* (3.7).

SSI was 0.51 between CFW age classes, 0.29 between 11 yo CFWs and NRWs and 0.41 between 20 yo CFWs and NRWs.

Average SR in the herbaceous layer decreased from  $25 \pm 8.6$  to  $22.5 \pm 2.48$  in the younger age class and from  $33.25 \pm 2.3$  to  $27.5 \pm 2.9$  in the 20 yo sites, while the NRWs had the lowest average richness, with  $21 \pm 2.4$  (table 2.4, figure 2.8). None of the changes over time, differences among age classes, or differences between CFWs and NRWs were significant ( $p=0.083$ ). There was no significant relationship between SR in the herbaceous layer and CFW age ( $r^2 = -0.03$ ,  $p=0.43$ ).

Average herbaceous strata SDI in the 11 yo sites showed a slight increase from 2.37 to 2.78, while the 20 yo increased from 2.75 to 2.9 over an 8 year interval while NRWs averaged 2.7 (table 2.4, figure 2.9). There were no significant changes in wetland age classes over 8 years, nor were there differences between 11 and 20 yo age CFWs or between created and NRWs ( $p=0.688$ ). There was no significant relationship between SDI in the herbaceous strata and CFW age ( $r^2=0.18$ ,  $p=0.07$ ).

DNN in CFWs decreased from an average of  $25.5 \pm 12.9$  to  $15.9 \pm 2$  over the course of 8 years in the 11 yo sites. It showed an increase from  $7.8 \pm 4.9$  to  $34 \pm 7.4$  in the 20 yo sites, while NRWs averaged  $21 \pm 3.7$  (table 2.4, figure 2.10). There were no differences among groups ( $p=0.239$ ). There was no significant relationship between DNN in the herbaceous strata and CFW age ( $r^2=0.008$ ,  $p=0.31$ ). However, when only examining the 2012 data there was a significant relationship between CFW age and DNN ( $r^2 = 0.526$   $p=0.0396$ ).

FQI<sub>H</sub> in the 11 yo CFW age class increased slightly from  $13.61 \pm 2.7$  to  $14.1 \pm 1$ , while the 20 yo sites decreased in average FQI<sub>H</sub> over the same period from  $17.3 \pm 2.2$  to  $13.59 \pm 2.9$ . NRWs averaged  $16.38 \pm .7$  (table 2.4, figure 2.11). Sites showed no significant difference over 8 years, no differences between current age classes, and no difference between created and NRWs ( $p=0.3126$ ). There was no significant relationship between FQI<sub>H</sub> and CFW age ( $r^2=-0.07$ ,  $p=0.7$ ).

### **Tree Strata**

The 11 yo CFWs were dominated by *Salix nigra* in the tree strata, in which it was responsible for 70.4% of the relative dominance while 20 yo sites were dominated by both *Salix nigra* (37.3) and *Pinus taeda* (21.9). In the NRWs, which averaged 82 years since major disturbance, tree strata was dominated by *Acer rubrum* (35.5) and *Fraxinus pennsylvanica* (16.9).

SSI was 0.56 between CFW age classes, 0.48 between 11 yo CFWs and NRWs and 0.42 between 20 yo CFWs and NRWs.

Average tree strata SR for 11 yo CFWs averaged  $3.33 \pm 1.45$ , 20 yo CFWs  $6.5 \pm 1.71$ , and  $10.5 \pm 0.96$  for the NRWs (table 2.5, figure 2.12). CFW age classes were similar to one another ( $p=0.32$ ), while NRWs had statistically higher SR than 11 yo CFWs ( $p=0.02$ ), but similar SR to 20 yo sites ( $p=0.15$ ). There was no significant relationship between tree strata SR and site age ( $r^2=0.04$ ,  $p=0.23$ ).

Tree strata Shannon diversity is the lowest and most varied in the 11 yo CFWs, averaging  $0.83 \pm 0.46$ . The 20 yo CFWs had an average diversity of  $1.17 \pm 0.29$  and NRWs had the highest SDI of  $1.75 \pm 0.12$  (table 2.5, figure 2.13). None of these differences were statistically significant ( $p=0.26$ ).

DNN was zero for all groups in the tree strata.

Average  $FQI_T$  scores were lowest in 11 yo ( $7.25 \pm 2.68$ ), followed by 20 yo sites ( $9.14 \pm 1.86$ ) and the NRWs had the highest scores ( $15.75 \pm 2.21$ ) (table 2.5, figure 2.14). FQI showed significant differences between the 11 yo and the 20 yo CFW age classes ( $p=0.026$ ), as well as between the 11 yo sites and NRWs ( $p<0.0001$ ), and the 20 yo CFWs and NRWs ( $p=0.0001$ ).

Tree BA averaged  $1.05 \pm 0.395$  m<sup>2</sup>/ha in 11 yo sites,  $5.98 \pm 1.819$  m<sup>2</sup>/ha in 20 yo CFWs and  $35.83 \pm 4.823$  in NRWs (table 2.5, figure 2.15). Differences between 11 and 20 yo CFWs were not significant ( $p=0.057$ ), while NRWs had significantly more BA than either 11 yo sites ( $p=0.0005$ ) or 20 yo sites ( $p=0.002$ ).

### Woody species Composition

Analysis of similarities (ANOSIM) of the tree strata (>10 cm dbh) showed that ~11 and 20 yo CFWs had similar compositions ( $p=0.27$ ). The composition of NRWs was not similar to either 11 yo CFWs ( $p=0.034$ ) or 20 yo CFWs ( $p=0.032$ ). However,

when the sapling composition in the CFWs (trees <10 cm dbh) were compared to the composition of the tree strata (>10 cm dbh) in the NRWs, NRWs were similar to both 11 (p=0.165) and 20 yo CFWs (p=0.175).

### **Biomass Carbon**

Overall woody biomass in 11 yo CFWs averaged  $14.54 \pm 6.97$  Mg/ha, while 20 yo sites averaged nearly double at  $27.06 \pm 9.52$  Mg/ha and NRWs averaged over an order of magnitude higher with  $285.77 \pm 45.32$  Mg/ha. CFWs were statistically similar to each other (p=0.338), while NRWs had more biomass than either 11 yo CFWs (p=0.0015) and 20 yo CFWs (p=0.0015). This biomass translated to an overall standing stock of C in woody biomass >2.5 cm of  $7.28 \pm 3.5$  Mg/ha for 11 yo CFWs, while 20 yo sites hold almost twice as much, averaging  $13.53 \pm 4.8$  Mg/ha. NRWs hold over an order of magnitude more C than the 20 yo sites with  $143.92 \pm 22.7$  Mg/ha (figure 2.16). NRWs held statistically more C than both 11 yo (p=0.0015) and 20 yo sites (p=0.0017), which were similar to one another (p=0.338).

When biomass is separated by strata, tree strata woody biomass in 11 yo sites averaged  $4.64 \pm 1.7$  Mg/ha of biomass (or  $2.32 \pm 1.35$  Mg C/ha), while 20 yo sites averaged 5 times more, with  $23.47 \pm 8.675$  ( $11.74 \pm 4.34$  Mg C/ha), and NRWs averaged an order of magnitude higher with  $285.77 \pm 45.32$  Mg/ha ( $142.89 \pm 22.66$  Mg C/ha) (figure 2.17). NRWs had significantly more biomass in the tree strata than either 11 yo sites (p=0.0015) or 20 yo sites (0.0015). The 11 yo and 20 yo CFW biomass were not significantly different from each other (p=0.22).

Sapling biomass was highest in the youngest class of CFWs, averaging  $9.9 \pm 6.48$  Mg/ha ( $4.95 \pm 3.24$  Mg C/ha), followed by 20 yo CFWs with  $3.56 \pm 1.5$  Mg/ha ( $1.78 \pm .75$  Mg C/ha) and NRWs had the lowest sapling biomass with  $2.06 \pm 0.83$  Mg/ha ( $1.03 \pm 0.42$  Mg C/ha) (figure 2.18). In the sapling layer, there were no significant differences in biomass between CFW classes (p=0.70) or between CFWs and NRWs (p=0.54) or 20 yo CFWs and NRWs (p=0.70).

When the woody biomass of these wetlands is divided by the age of the wetland, NRWs sequester more than 2.5 times more C than CFWs per year. NRWs averaged a rate of  $1.822 \pm 0.4$  Mg/ha per year, which was statistically higher than the  $0.686 \pm 0.3$

Mg C/ ha/ year ( $p=0.048$ ) sequestered by 11 yo CFWs and the  $0.668 \pm 0.3$  Mg carbon/ha/year ( $p=0.05$ ) sequestered by 20 yo CFWs.

### **Correlation Between FQI and Carbon Dynamics**

In CFWs, Floristic Quality correlated positively but insignificantly with the change in % soil C between 2004 and 2012 ( $\rho=0.6995$ ,  $p=0.08$ ). FQI correlated with woody biomass ( $\rho = 0.64$ ,  $p\text{-value}=0.139$ ). Finally, the total amount of C accumulated per year, as measured by total C sequestration in both aboveground woody biomass and the top 10 cm of the soil showed significant correlation with a  $\rho$  of 0.821 and a  $p$ -value of 0.034.

### **Regulatory Wetland Success**

Woody stem density of at least 990 woody stems per hectare of wetland species was met by all 11 yo CFWs and  $\frac{1}{2}$  of 20 yo CFWs. 11 yo CFWs averaged  $5317 \pm 2050$ , while 20 yo CFWs averaged  $1228 \pm 528.5$  woody stems per hectare. Two sites were under 990 stems per hectare. Rte. 7 Springfield had 494 stems per acre and Sleeter Lake had 750 stems per hectare. In the mitigation guidelines for Virginia, CFWs no longer need to reach the minimum stem requirement if canopy cover is greater than 30%. In Sleeter Lake, the site was over 30% canopy closure, and thus fulfills the guideline, however, Rte 7 did not have 30% closure, and would therefore fail to meet the Ecological performance standards and would be deemed a failed wetland.

All sites had more than 50% of dominant species facultative or wetter. For the 20 yo age class, all sites showed 100 percent of dominants were facultative or wetter, while the 11 yo CFWs averaged  $82 \pm 5.7\%$ . These values were statistically similar ( $p=0.21$ ).

In CFWs all sites had less than 5 % coverage of non-native species in the tree strata. In the shrub/sapling strata, two 20 yo CFWs were above the limit, with 5.5% and 19.7% non-native in Rte. 7 and Sleeter Lake respectively. However, in the herbaceous strata, no CFW was successful in limiting non-native species to 5%. Courtland and Mt. Stirling are both fractionally over the recommended limit with 5.83 and 5.33 percent respectively. The 11 yo sites averaged  $7.71 \pm 5.2\%$  with a range of 3.03 to 13.3, while the 20 yo sites average  $21.28 \pm 12.4\%$  with a range of 5.83 and 38.83.

## Discussion

### Understory development

Since CFWs begin devoid of vegetation and a viable wetland seedbank, they can provide the unique opportunity to study CFWs as templates for early wetland succession (Noon 1996; Odland 1997; DeBerry and Perry 2004) and as a way to understand progression toward the functional replacement of a characteristic habitat (NRC 1995; Spieles 2005).

The intermediate disturbance hypothesis (Connell 1978) predicts that species richness and diversity often peak in partially disturbed rather than pristine or severely disturbed ecosystems (Odum 1963; Connell 1978; Fox 1979; Huston 1979; Sousa 1979; Ward and Stanford 1983), exhibiting uni-modal distribution along a disturbance gradient (Grime 1973). Other studies have shown that diversity increases with disturbance (Tilman 1983; Kneidel 1984; Kaczor and Hartnett 1990; Pollock et al. 1998), or decreases with increased disturbance (Robinson and Minshall 1986; Bailey 1988; Brown and Brussock 1991; Wilson and Tilman 1991).

Our data agrees with the intermediate disturbance hypothesis when strata are combined, but illustrates different responses across strata. While statistically similar across age groups, overall data showed a trend of increasing richness from age 3 to 11 yo and a decrease from 12 to 20 yo. The trend in overall richness is underlain by a significant increase in tree strata richness with age and an insignificant decrease in herbaceous richness with age, potentially indicating that at intermediate time since disturbance, opportunistic ruderal species (primarily in the herbaceous strata) can coexist with competitive species (composed of perennials in all strata, particularly tree species), (Grime 1973; Connell 1978; MacArthur and Wilson 1967) and shade tolerant and intolerant species overlap (Loucks 1920; Haeussler et al 2004). Many early successional species are r-selected species adapted to stressful conditions and full light (van der Valk 1981; Bazzaz 1979; DeBerry and Perry 2004; DeBerry and Perry 2012) and as competitive k-selected species become dominant, and light becomes more limiting, many species are removed. However, as succession proceeds, a variety of environmental factors are altered leading to the availability of additional niches in which

other species can become established (Ecke et al. 2002; Ishii et al. 2004). Van der Valk's (1981) "environmental sieve" concept indicates that changing environmental factors alter community composition by only allowing those species with the appropriate traits to persist in an environment at a certain time. Changing of species over time through successional development may mean that while richness remains similar over time, the composition and quality of the vegetation community may shift without being detected as a change in richness or diversity.

Other studies have indicated that species richness is often high early in the monitoring period for CFWs, but begins to decline with age (Fennessy and Roehrs 1997; Campbell et al. 2002; Balcombe et al. 2005; Gutrich et al., 2009). Stefanik and Mitch (2012) found that in their study, marshes tended to reach maximum species richness between the ages of 4 and 7, while our data suggests a potential richness peak between 12 and 20 years in CFWs.

SR and diversity often become similar to NRWs quickly (Confer and Niering 1992; Kentula et al. 1992; Brown 1999; Balcombe et al. 2005; Spieles et al. 2006; Brown and Veneman 2001), whereas indicators based on species composition tend not to reach equivalence with NRWs over the short term (Brown 1999; DeBerry and Perry 2004; Brooks et al. 2005; Spieles et al. 2006). We found no significant changes over time in CFWs and no differences between any CFW age classes and NRWs in herbaceous, shrub sapling or overall richness or diversity. Lack of any difference between NRWs of 82 years and CFWs as young as 3 indicates that using richness and diversity to determine if CFWs provide a "characteristic plant community" may not be useful.

Sørensen similarity index showed that overall and all individual strata, CFW age classes were more like one another than NRWs. Progression toward similarity with NRWs showed mixed results, as 20 yo CFWs were more like NRWs than 11 yo CFWs in the herbaceous strata and overall, but 20 yo CFWs were more similar to NRWs in the shrub and tree strata.

Interestingly, none of the vegetation parameters measured showed a positive linear relationship with CFW age. While mitigation monitoring often relies on predictable development of vegetation parameters with time, often development is non-linear and



Mathews (2008) found that only 48- 76% of restored wetlands could be relatively well described ( $r^2 > 0.5$ ) with non-linear models.

Created wetlands resemble natural sites in their level of non-native species throughout their establishment. Interestingly, none of the sites adhered to the policy maximum of <5% non-native species, and neither did the reference sites. In this study, DNN in the herbaceous strata was high at first (3 years), then decreased to a low point between 10 and 12 years post creation, and then increased on average by a factor of 3 from the age of 12 to 20. High DNN is expected at first, as ruderal species invade. What is of greater interest is the increase in non-natives in 20 yo sites. Most CFWs in Virginia are monitored and managed until 10 years of age or so, at which point they are deemed successful and left to their own devices (VADEQ 2010). It may be that created wetland management keeps invasive species artificially low, and when it ends, non-natives spread.

Average FQI displayed trends of increasing over time in our CFWs, but they fell short of NRWs, indicating potential as a tool for monitoring wetland development. However, like richness and diversity, there were no significant differences in overall, shrub sapling or herbaceous FQI scores, percent non-native species or IV of non-native species amongst CFW age classes, indicating that CFWs were similar to NRWs in all but the tree strata after 11 and 20 years. These results agree with a number of studies that indicate non-forested CFWs reach equilibrium and become similar to NRWs in terms of vegetation within a 20 year period (Atkinson et al. 2005; Balcombe et al. 2005; Gutrich et al 2009; Spieles 2005; Stefanik 2012). However, this finding goes against the international trend reported by Moreno-Mateos et al. (2012), which indicates that plants in created and restored wetlands (from a variety of wetland types) converged with NRWs statistically after 30 years, but remained below absolute reference levels for 100 years.

### **Forest Development**

In the 7 CFWs used for this study, negligible trees > 10 cm were found in 2004 (DeBerry 2006), a condition which has been noted before in CFWs on particularly disturbed sites, and may last up to 20 years (Noon 1996; Atkinson et al. 2005).

Interestingly in our study, 8 years later trees exist in every site. Perhaps this indicates progression toward better wetland construction practices, as 12 yo CFWs in 2004 lacked trees, while 11 yo CFWs in 2012 held 4.6 Mg/ha of biomass in the tree strata.

11 yo CFWs were lower than NRWs in tree SR, while 20 yo CFWs were similar to NRWs potentially showing development with age. Richness may be of interest in CFWs because SR is significantly correlated with woody productivity in North American temperate forests (Paquette and Messier 2010), as well as promoting stability (Doak et al. 1998). Both CFW age classes were different in terms of BA and biomass and CFWs displayed different composition from NRWs in terms of ANOSIM. Unlike in the understory layer, the tree strata of 11 yo CFWs were lower than 20 yo CFWs in terms of FQI. Tree establishment is often the most difficult task in offsetting PFW impacts (Matthews and Endress 2008) and its development takes time (Niswander and Mitsch 1995). In 11 yo CFWs, the tree strata is dominated by *Salix nigra*, and the 20 yo sites are dominated by *S. nigra* and *Pinus taeda*. These species are pioneer species that often become established before other species (Spencer et al. 2001; Phillips 2002). Late successional species rarely do well in young CFWs (McLeod et al. 2001), and need conditions to improve before they can become established and thrive. *S. nigra* in particular is thought to be a nurse species because a canopy of *S. nigra* does not reduce survival or growth of secondary successional tree species (McLeod 2001), and may potentially improve conditions by reducing herbaceous competition (Dulohery et al. 2000). Willow stands are known to persist for 30-40 years before giving way to other woody species (McLeod 2001). In this study, *S. nigra* was the dominant tree in both 11 and 20 yo wetlands but did not exist in any of the NRWs. In many logged sites, as is probably the case for most NRWs, the majority of saplings sprout from coppice (Messina et al. 1997). Spencer and others (2001) found that young logged bottomland hardwood forests fell into separate categories, those dominated by willow appeared in areas with little resprout, while a more typical bottomland hardwood community arose in sites with high percentage of saplings from coppice. *S. nigra* dominated sites with little coppice sprouting for 10- 15 years, but in some of the oldest willow dominated sites (20 years), willow begins to stop reproducing and reaches the end of its life span, due to closed canopy conditions. In these sites, there were more ecologically desirable

species in the understory (Spencer et al. 2001). ANOSIM results from this study show that the tree species composition is different between CFWs and NRWs, but the sapling layer of CFWs do resemble the tree strata of NRWs, indicating that pioneer species that comprise the current tree strata may give way to more characteristic trees. The implication of these findings is that CFWs may provide a similar plant community in the tree strata with time, but it may take more than 20 years.

In the few studies directly measuring habitat usage by fauna in CFWs, it appears that CFWs may not be functionally equivalent to NRWs and most provide poorer habitat than NRWs (Johnson et al. 2002; Ambrose and Lee 2004). Furthermore, it is known that much of the provision of habitat is dependent on the tree strata in particular and that ecologically mature PFWs generally provide better habitat for fauna. As trees become taller and forests more stratified, they are used by a higher diversity of forest dwelling birds (MacArthur and MacArthur 1961; Dickson et al. 1995), and more niches for other animals develop (Ecke et al. 2002), basal area of trees was the best indicator of salamander habitat in riparian wetlands in West Virginia (Summers 2013) and black bears prefer large trees > 58cm for their dens (Godfrey 1996). In a study in arid environments, the majority of differences in bird dynamics were controlled by floristics, with a smaller amount varying with structure (Rotenberry 1985).

Until the tree strata in CFWs converge with those of NRWs, it seems as though the function of “maintenance of a characteristic plant community” may not be met by mitigation wetlands (NRC 1995). Since trees represent the vast majority of biomass in forested systems (Rheinhardt et al. 2012), and CFWs do not resemble them in terms of composition or biomass, it seems unlikely that these CFWs are restoring the habitat support functions of impacted wetlands.

### **Biomass**

Wetlands serve an important role as a carbon sink, sequestering 14% of global carbon released by the burning of fossil fuels every year (Mitsch et al. 2013). Similarly, forests sequester 16% of released carbon (Smith et al. 2009) (overlap exists between these two numbers), much of which is sequestered in woody biomass (Rheinhardt et al. 2012). Tree BA in riparian wetlands is also negatively correlated with nitrate in nearby

streams (Brinson et al. 2006) indicating that the function of nutrient cycling and retention is highly dependent on vegetation and woody biomass is a good indicator. In our study, overall woody biomass and stored carbon were 10 and 20 times lower in 20 and 11 yo CFWs respectively than in NRWs, indicating large differences in carbon storage and biogeochemical functioning between CFWs and NRWs.

While it is to be expected that NRWs would have higher biomass on site, as forest development is a process that proceeds on generational time scales, the magnitude of difference is a bit surprising. Furthermore, when the amount of biomass is divided by the age of the oldest trees of each age class, CFWs show lower rates of accumulation, indicating that created sites may not be catching up to NRWs. Biomass develops asymptotically and mature trees devote less energy to new growth, implying that younger forests should be adding biomass at a greater rate than mature forests (Wigley and Lancia 1998). NRWs accumulating biomass faster than created sites could be a product of time-scale, in that very young sites develop woody biomass slowly. It could also be a product of site preparation. NRWs were most likely logged somewhere between 60 and 85 years ago, and as a result trees may have sprouted from root stock, allowing them to grow more quickly (Spencer et al. 2001). On the other hand, it could be that poor soil conditions in our CFWs retarded the growth of trees. Particularly problematic may be the finding that soil bulk density in the 10- 20 cm depth range averages  $1.40 \text{ g/cm}^3$  (see chap. 3). Root limiting bulk densities in soils range from  $1.45 \text{ g/cm}^3$  for fine textures to  $1.75 \text{ g/cm}^3$  for coarse loamy textures (Brady and Weil 2008). In this study, a third of all soil samples from 11 yo CFWs were root limiting in the 10-20 cm soil section, indicating that the soil may be stunting biomass accumulation in these sites (see chap. 3). None of the soil samples from 20 yo sites were root limiting, potentially indicating an alleviation of stress with time, but NRWs displayed significantly lower bulk density at depth than either CFW age group. Similarly, soils in CFWs displayed >50% lower N after 20 years (see chap. 3). N is the major limiting nutrient to forest productivity globally (Kimmins 1987) and in North American PFWs (Bedfore et al. 1999), and may be contributing to slow biomass accumulation in our CFWs.

In terms of the storage of carbon, mitigation is almost certainly leading to temporal

loss of functions. Our sites showed that CFWs held 10 to 20 times less carbon in biomass, with an average difference between 20 yo CFWs and NRWs of 130 Mg/ha. Even if mitigation enforces a 2:1 ratio of wetland creation to impact, the mitigation of 77 ha of PFWs per year in Virginia (VADEQ 2012) leads to a loss from wetlands of 5,005 Mg of standing biomass carbon each year. Furthermore, there is a 19 to 20 Mg/ha difference in C stored in the top 20 cm of soil as well (see chap. 3), bringing the total difference to 150 Mg/ha (figure 2.19). When differences between both soil and biomass C are taken into account, the creation of wetlands leads to a loss of 5,775 Mg C/year. If all of this carbon were converted to CO<sub>2</sub>, it would release the equivalent of the annual CO<sub>2</sub> emissions of 4,411 cars per year (EPA Greenhouse Gas Equivalencies calculator).

### **Correlation between FQI and Carbon Dynamics**

Cole and others (2002) point out that some plant community parameters commonly measured to assess wetland health fail to address the restoration of functions (Cole et al 2001; Cole et al 2002). Creating or restoring wetlands to achieve the dual roles of supporting a diversity of species while also performing ecosystem services has been deemed unrealistic by some (Ehrenfeld 2000; Zedler 2000). In fact, there is some evidence that floristic quality and biogeochemical cycling might be negatively correlated, in that plants ranked highly in the floristic quality index are seldom found in areas with high nutrient loading, because ruderal species are able to grow rapidly in disturbed and immature ecosystems (Grime 1973; Swink and Wilhelm 1979). Immature systems are often more active in the sequestration of carbon, while mature systems exist closer to steady state (Odum 1969), indicating that high quality plant communities associated with mature ecosystems may function differently than young CFWs. In Alaskan riparian wetlands, Pollock and others (2001) show that productivity was unimodally associated with the disturbance regime, indicating communities at intermediate maturity tend to provide higher levels of biogeochemical function than totally mature sites. Primary productivity is dependent on the type of species present, and some species with very low FQI scores may be capable of fixing large amounts of carbon. For example, invasion of *Phragmites australis*, which is weedy and invasive, with a C value of 0 lead to a doubling of biomass in a brackish marsh (Windham 2001),

and high productivity is linked to wetlands dominated by *Typha* and invasive species (Davey 2007).

In this study, FQI correlated positively with measurements of carbon sequestration in both the above and belowground carbon pool, and showed significant correlation with the total carbon sequestration rate in the combined above and belowground pool. The positive correlation may be because as wetlands mature and increase in soil carbon and woody biomass, more conservative species can become established. Lopez and Fennessy (2002) found that FQI correlated with total organic soil carbon ( $p=0.01$ ). This study did not find a correlation between soil carbon and FQI, but did find a correlation between the increase in carbon and FQI score. While Lopez and Fennessy found that soils higher in organic carbon (potentially as an artifact of site preparation) tend to support vegetation communities with higher floristic quality, our study shows that higher quality plant communities and the ongoing sequestration of carbon are correlated in CFWs, regardless of initial site conditions, and thus the functions of habitat provision and carbon sequestration are mutually attainable.

### **Reference Wetlands**

NRWs would be better referred to as natural comparison sites. With an average FQI score of 26.9, these sites fall far behind the 45-60 range of “reference standard” identified by Miller and Wardrop (2006). This may be because their vegetation is still maturing at an average of 82 years. It may also reflect that wetland functions can be impaired not only by impacts to the wetland itself, but also by adjacent land use (Moss 1984; Burbridge 1994; Detenbeck et al. 1996). Houlahan et al. found that adjacent land use of wetlands altered species richness (Houlahan et al. 2006). The impact of road building, which necessitated the creation of wetlands that we are now studying, the environment was highly impacted in close proximity to the current NRWs. For example, the NRW associated with Courtland Bypass is directly adjacent to the created mitigation wetland, and receives water from it. During the road building impact that completely removed one wetland, tertiary impacts may have caused less perceptible impacts to other local environments. Evidence to that effect could be seen in the soil profile for Courtlands NRW, in that half-way above the cesium peak of 1964, an

otherwise highly organic soil profile is interrupted by a 3 centimeter sand lens that was likely the result of localized changes in hydrology associated with the building of a created wetland in 1991 (See chap. 3).

## Conclusions

Our findings imply that compensatory wetland creation may lead to temporal functional losses of both the provision of habitat and the storage of carbon. Though CFWs are similar to NRWs in overall and in the understory strata, the tree strata did not develop within the ten-year monitoring time frame or even 10 years beyond. Saplings in CFWs show promise of developing similar canopy communities in the future and tree biomass has increased since 2004. Lack of development within a 20 year time frame illustrates the need for more research to determine when sites may provide similar tree strata habitat (Niswander and Mitsch 1995; Mitsch and Gosselink 2007). Furthermore, more monitoring is needed to determine if CFWs will become increasingly dominated by non-native species. Non-native species increased in our 20 yo CFWs over the course of 8 years to a point of near significant difference when compared with the same sites 8 years earlier ( $p=0.07$ ). Similarly, a simple linear regression including just the data from 2012 showed that a significant positive relationship exists between created wetland age and dominance of non-native herbaceous plants ( $r^2= 0.526$   $p=0.0396$ ), indicating an increase in non-native herbaceous dominance after the monitoring period. Out of 10 dominant species in the 20 yo age class, 4 are invasive, including the most dominant species, *Microstegium vimineum* (15%). On the positive side, there is no difference between CFWs and NRWs in terms of vegetation indices in the shrub sapling or herbaceous strata. However, based on the lag time to tree habitat replacement, perhaps more emphasis should be placed on preserving PFWs rather than attempting to recreate them.

## Table Captions

Table 2.1: Virginia Wetland Plants C-value List ranking criteria (Virginia Department of Environmental Quality 2004) These criteria are used in the Floristic Quality Index.

Table 2.2: Results for overall vegetation parameters for combined strata from both CFWs and NRWs in terms of Species Richness (SR), Floristic Quality Index (FQI) and Percent Non-native species (%NN). For all

Table 2.3: Results for vegetation parameters in the shrub/sapling strata from both CFWs and NRWs in terms of Species Richness (SR), Floristic Quality Index (FQI), Shannon Diversity Index (SDI) and Dominance by non-native species in term so of Importance Value (IV).

Table 2.4: Results for vegetation parameters in the herbaceous strata from both CFWs and NRWs in terms of Species Richness (SR), Floristic Quality Index (FQI), Shannon Diversity Index (SDI) and Dominance by non-native species in term so of Importance Value (IV)..

Table 2.5: Results for vegetation parameters in the tree strata from both CFWs and NRWs in terms of Species Richness (SR), Floristic Quality Index (FQI), Shannon Diversity Index (SDI) and Basal Area (BA).

Table 2.6: Results from linear regression of vegetation parameters based on the age of each individual CFW, including data from 2004 and 2012.

Table 2.7: Results from linear regression of vegetation parameters based on each individual CFW age, including data from only 2012.



## Figure Captions

**For all figures below, Figures are comparing age classes of Created forested wetlands (CFWs) to natural reference wetlands (NRWs). The X axis defines the groups by Age (how old they were currently when sampled) and year sampled, in the form: Age, Year Sampled (10, '12).**

Figure 2.1: Results of Species Richness (SR) by age class for all strata combined. There were no significant differences among CFW groups or NRWs.

Figure 2.2: Results of Percent non-native (% NN) for all strata combined. There were no significant differences among CFW groups or NRWs.

Figure 2.3: Results of Floristic Quality Index (FQI) for all strata combined. There were no significant differences among CFW groups or NRWs.

Figure 2.4: Results for SR in the shrub/sapling strata. There were no significant differences among CFW groups or NRWs.

Figure 2.5: Results for Shannon Diversity Index (SDI) in the shrub/sapling strata. There were no significant differences among CFW groups or NRWs.

Figure 2.6: Results for Dominance by Non-native Species (DNN) in the shrub/sapling strata. There were no significant differences among CFW groups or NRWs.

Figure 2.7: Results for FQI in the shrub/sapling strata. There were no significant differences among CFW groups or NRWs.

Figure 2.8: Results for SR in the herbaceous strata. There were no significant differences among CFW groups or NRWs.

Figure 2.9: Results for SDI in the herbaceous strata. There were no significant differences among CFW groups or NRWs.

Figure 2.10: Results for DNN in the herbaceous strata. There were no significant differences among CFW groups or NRWs.

Figure 2.11: Results for FQI in the herbaceous strata. There were no significant differences among CFW groups or NRWs.

Figure 2.12: Results for SR in the tree strata. There was a significant difference between 11 yo CFWs and NRWs, while 20 yo CFWs were similar to both 11 yo CFWs and NRWs. Note that for tree strata, there was no available data for 2004, thus tree figures will only display three groups.

Figure 2.13: Results for SDI in the tree strata. There were no significant differences between groups of CFWs or NRWs. Note that for tree strata, there was no available data for 2004, thus tree figures will only display three groups.

Figure 2.14: Results for FQI in the tree strata. There were significant differences among all groups. Note that for tree strata, there was no available data for 2004, thus tree figures will only display three groups.

Figure 2.15: Results for BA for the tree strata. There were significant differences between both age groups of CFWs and NRWs. CFWs were similar to each other. Note that for tree strata, there was no available data for 2004, thus tree figures will only display three groups.

Figure 2.16: Results for total biomass C in Mg/ha. There were significant differences between both age groups of CFWs and NRWs. CFWs were similar to each other. Note, there was no available biomass data for 2004, thus we only compare three groups.

Figure 2.17: Results for biomass C in trees in Mg/ha. There were significant differences between both age groups of CFWs and NRWs. CFWs were similar to each other. Note there was no available biomass data for 2004, thus we only compare three groups.

Figure 2.18: Results for biomass C in saplings in Mg/ha. There was no significant differences among CFW age groups or NRWs. Note there was no available biomass data for 2004, thus we only compare three groups.

Figure 2.19: Results for the combination of biomass C in saplings and trees and C stored in the top 20 cm of the soil (calculated in ch. 3) in Mg/ha. NRWs were significant different from both CFW age classes, which were similar to each other. Noter there was no available biomass or soil carbon stock data from 2004, thus we only compare three groups.

## Tables

Table 2.1

C-Value Range	Ranking Criteria
0	Non-native species
1-3	"Weedy", opportunistic, disturbance-tolerant species with a characteristically broad ecological amplitude. Due to natural or human disturbances, these species are often opportunistic invaders of natural areas.
4-7	Plants with an intermediate range of ecological tolerances. These taxa typify a stable phase of some native community, but persist under minor disturbances.
8-10	Disturbance intolerant, localized, and/or edaphically restricted species with a characteristically narrow ecological amplitude. These species generally exhibit relatively high degrees of fidelity to a narrow range of synecological parameters.

Table 2.2

Overall Vegetation Parameters						
Age class	Year Sampled	Information	Age	SR	FQI	%NN
CFW ~3	2004	Average	2.7	29.7	18.6	9.0
		SE	0.9	7.7	4.0	5.0
CFW ~11	2012	Average	10.7	33.0	23.7	11.1
		SE	0.9	3.1	1.7	2.2
CFW ~12	2004	Average	12.3	37.5	22.4	7.3
		SE	0.9	1.7	0.5	3.8
CFW ~20	2012	Average	20.3	35.5	23.1	14.8
		SE	0.9	4.3	3.2	2.3
RW ~ 82	2012	Average	81.5	33.0	26.9	9.5
		SE	3.2	4.3	1.4	1.1

Table 2.3

Shrub/Sapling Strata Vegetation Parameters							
Age class	Year Sampled	Data	Age	SR	FQI	SDI	DNN
CFW ~3	2004	Average	2.7	9.7	10.9	1.7	9.5
		SE	0.9	1.7	2.6	0.2	9.5
CFW ~11	2012	Average	10.7	11.3	14.9	1.3	0.0
		SE	0.9	1.2	3.2	0.4	0.0
CFW ~12	2004	Average	12.3	10.3	13.6	1.7	1.2
		SE	0.9	2.8	1.1	0.3	1.2
CFW ~20	2012	Average	20.3	9.3	10.7	1.7	6.3
		SE	0.9	3.4	2.6	0.3	4.7
RW ~ 82	2012	Average	81.5	10.8	15.0	2.7	6.2
		SE	3.2	1.1	1.8	0.9	5.9

Table 2.4

Herbaceous Strata Vegetation Parameters							
Age class	Year Sampled	Data	Age	SR	FQI	SDI	DNN
CFW ~3	2004	Average	2.7	25.0	10.5	2.4	25.5
		SE	0.9	7.0	2.2	0.3	12.9
CFW ~11	2012	Average	10.7	16.0	6.4	1.3	19.2
		SE	0.9	9.0	4.1	1.0	6.3
CFW ~12	2004	Average	12.3	33.3	17.3	2.8	7.8
		SE	0.9	2.3	2.2	0.1	4.9
CFW ~20	2012	Average	20.3	27.5	12.5	2.9	34.2
		SE	0.9	2.9	2.9	0.1	7.3
RW ~ 82	2012	Average	81.5	20.8	15.8	2.7	21.1
		SE	3.2	2.5	0.6	0.1	3.7

Table 2.5

Tree Strata Vegetation Parameters								
Age class	Year Sampled	Data	Age	SR	FQI	SDI	DNN	BA
CFW ~11	2012	Average	10.7	3.3	7.2	0.8	0.0	1.0
		SE	0.9	1.5	2.7	0.5	0.0	0.4
CFW ~20	2012	Average	20.3	6.5	9.2	1.2	0.0	6.0
		SE	0.9	1.7	1.9	0.3	0.0	1.8
RW ~ 82	2012	Average	81.5	10.5	15.8	1.7	0.2	35.9
		SE	3.2	1.0	2.2	0.1	0.2	4.8

Table 2.6

Linear Regression CFW Vegetation parameters based on Age (2004 and 2012 data)		
Vegetation parameter	R <sup>2</sup>	P
All strata		
Richness	0.0439	0.23
FQI	0.0479	0.223
%NN	0.126	0.115
Herbaceous Strata		
Richness	-0.07	0.702
Diversity	0.18	0.072
FQI	-0.069	0.702
DNN	0.008	0.31
Shrub/ Sapling Strata		
Richness	-0.0686	0.692
Diversity	-0.079	0.83
FQI	-0.059	0.609
DNN	-0.002	0.76
Tree Strata		
Richness	-0.089	0.51
Diversity	-0.03	0.41
FQI	-0.89	0.508
DNN	NA	NA
Basal Area	0.325	0.105



Table 2.7

Linear Regression of CFW Vegetation parameters based on Age (2012 data only)		
Vegetation parameter	R <sup>2</sup>	P
All Strata		
Richness	0.044	0.23
FQI	-0.19	0.848
%NN	0.1311	0.23
Herbaceous Strata		
Richness	0.04	0.314
Diversity	-0.144	0.642
FQI	-0.05	0.44
DNN	0.526	0.039
Shrub/Sapling Strata		
Richness	0.12	0.235
Diversity	0.023	0.334
FQI	0.099	0.254
DNN	0.276	0.129

## Figures

Figure 2.1

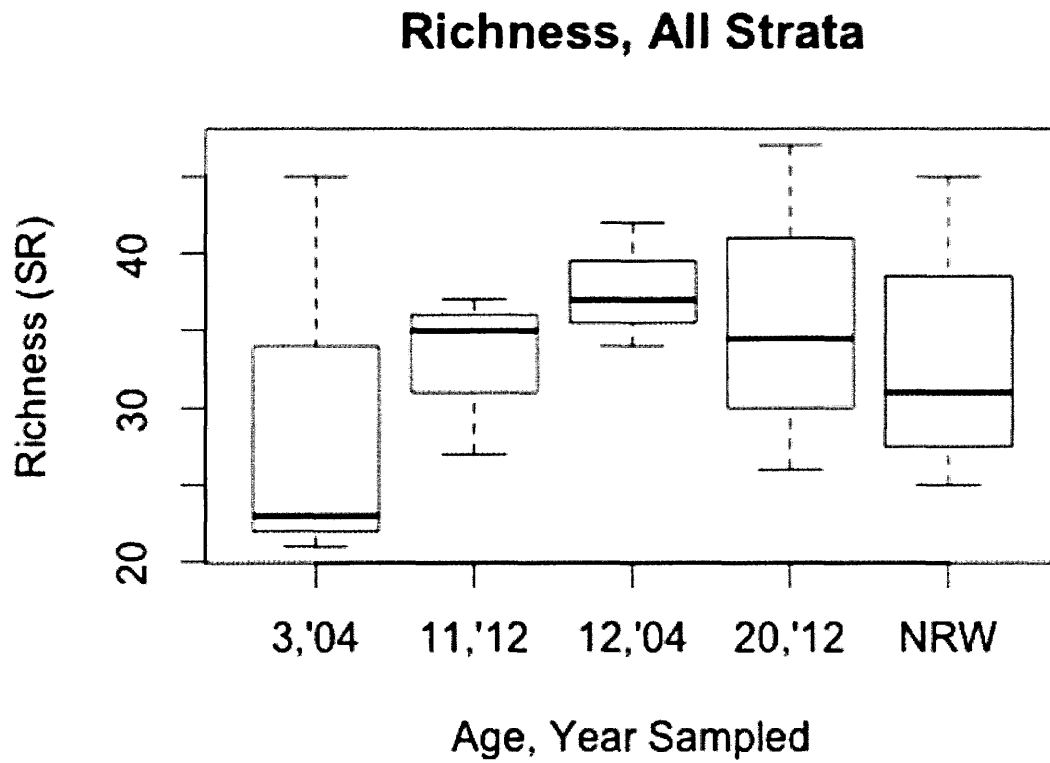


Figure 2.2

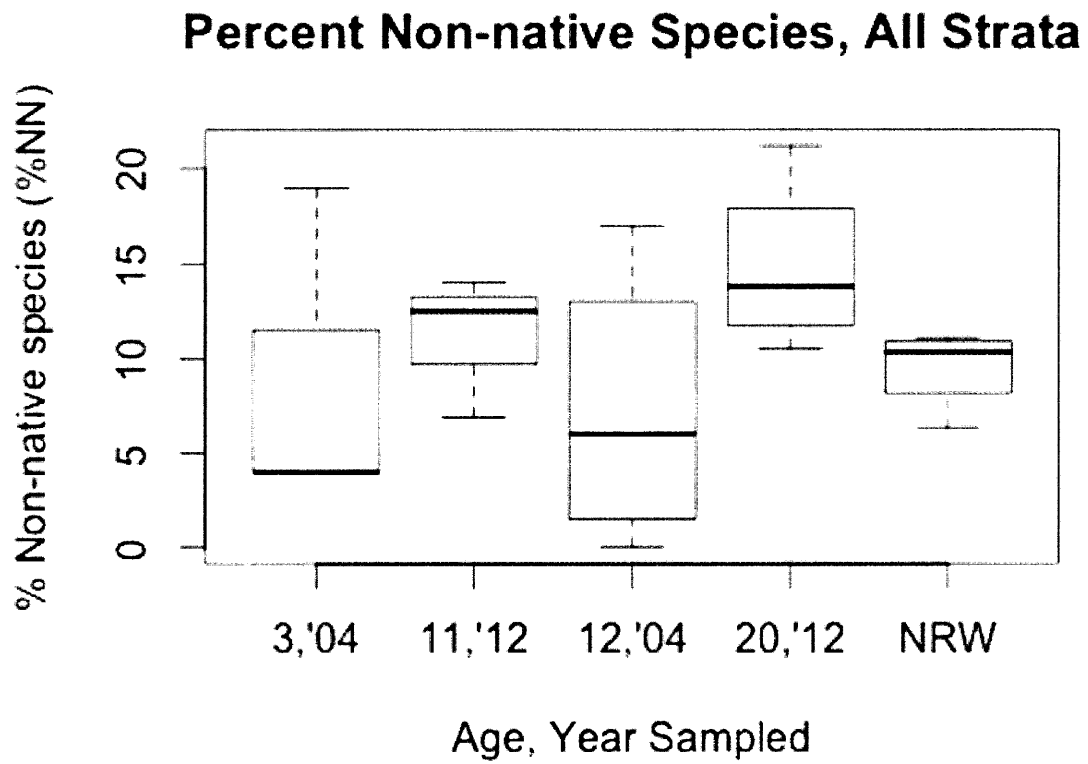


Figure 2.3

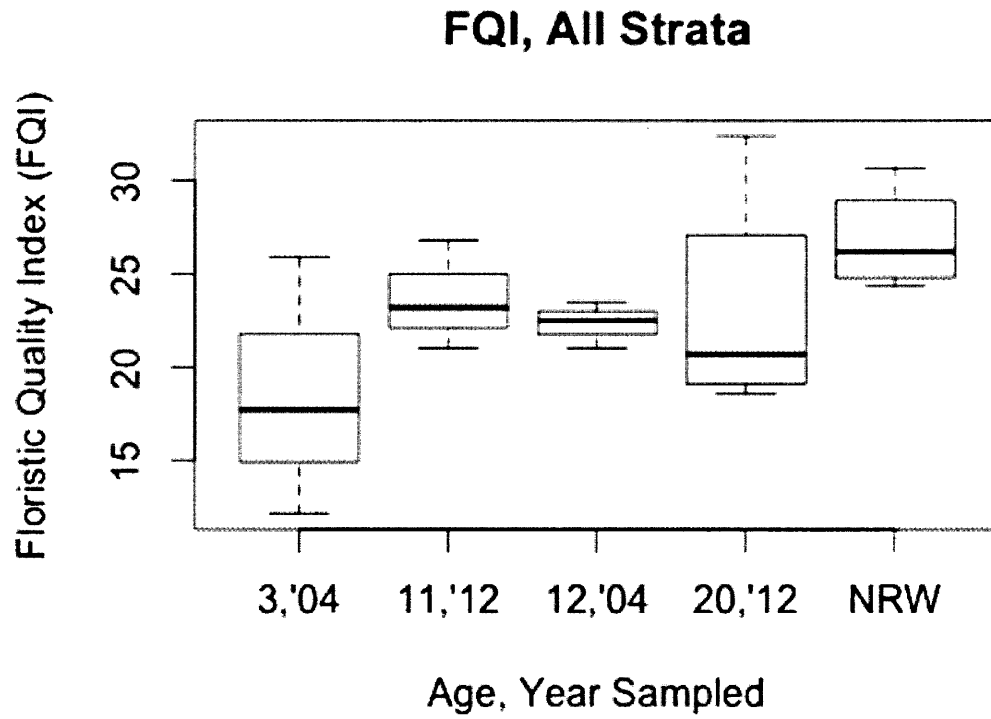


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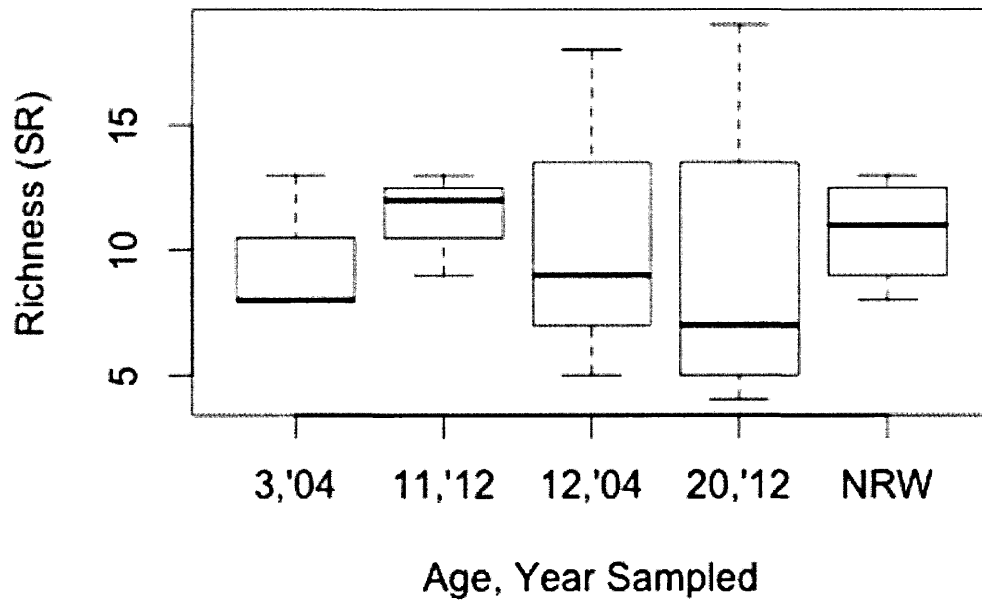
**Species Richness, Shrub/sapling Strata**

Figure 2.5

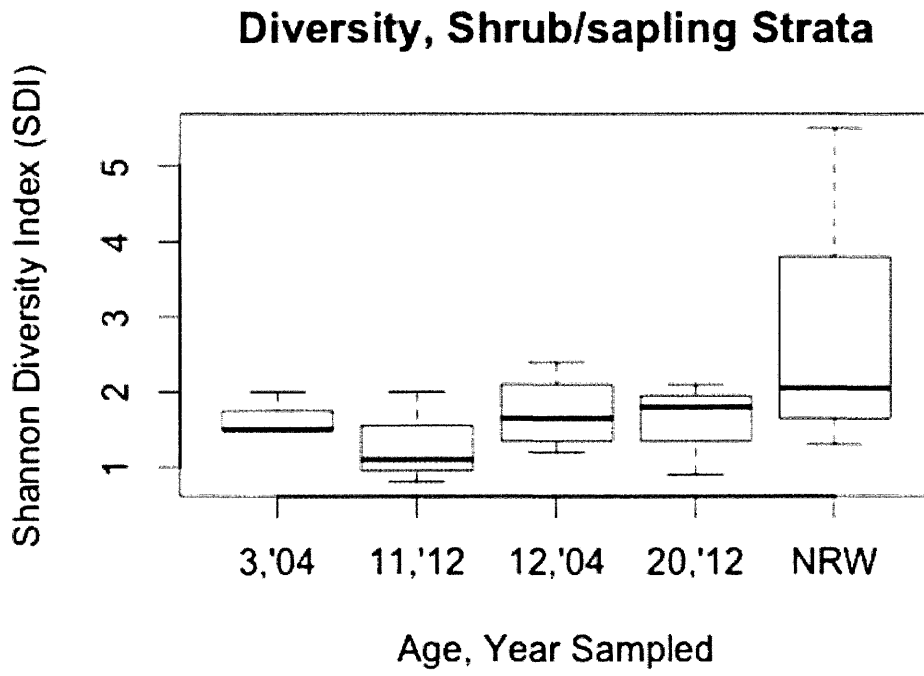


Figure 2.6

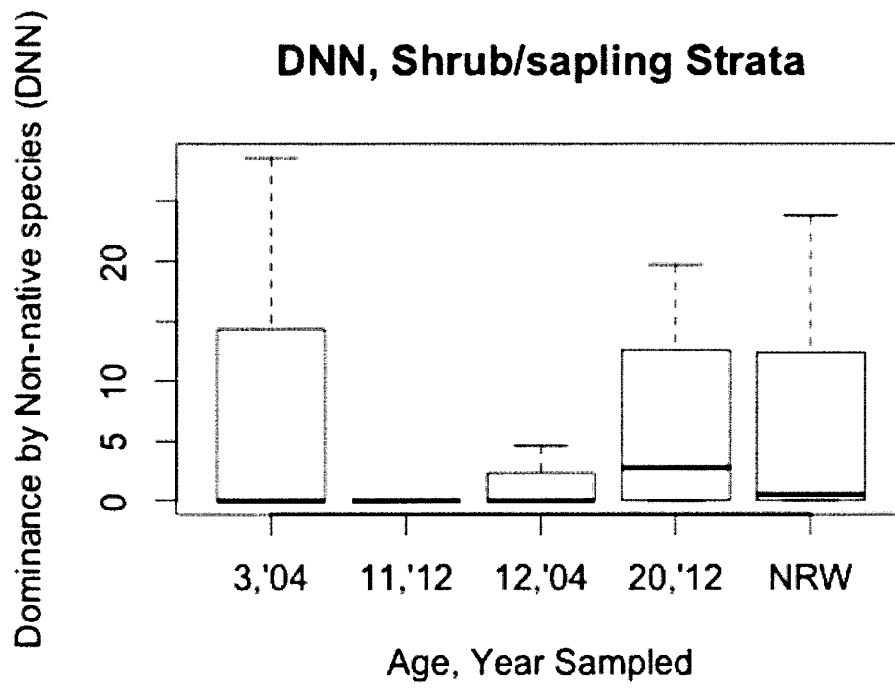


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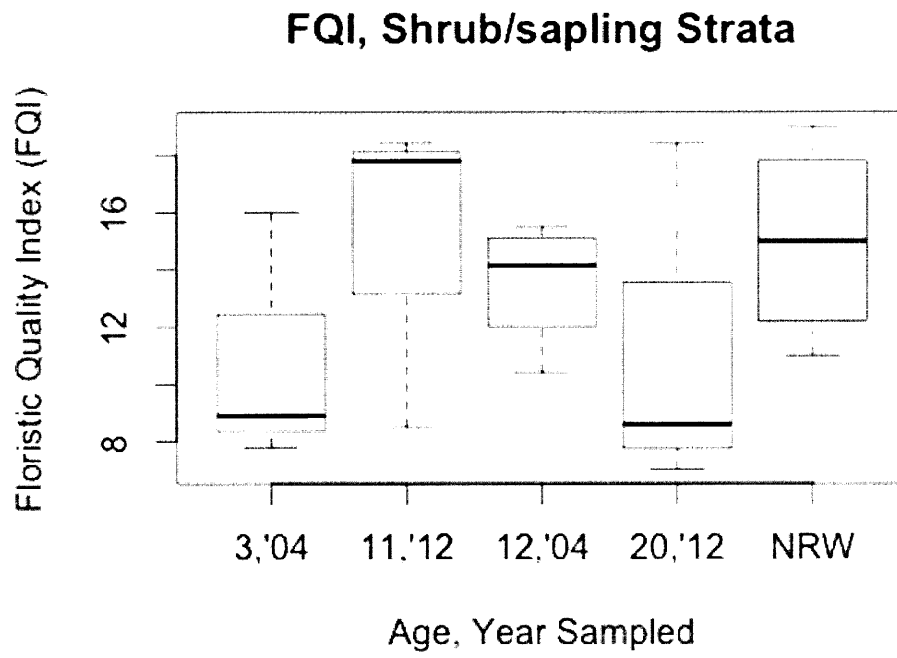




Figure 2.8

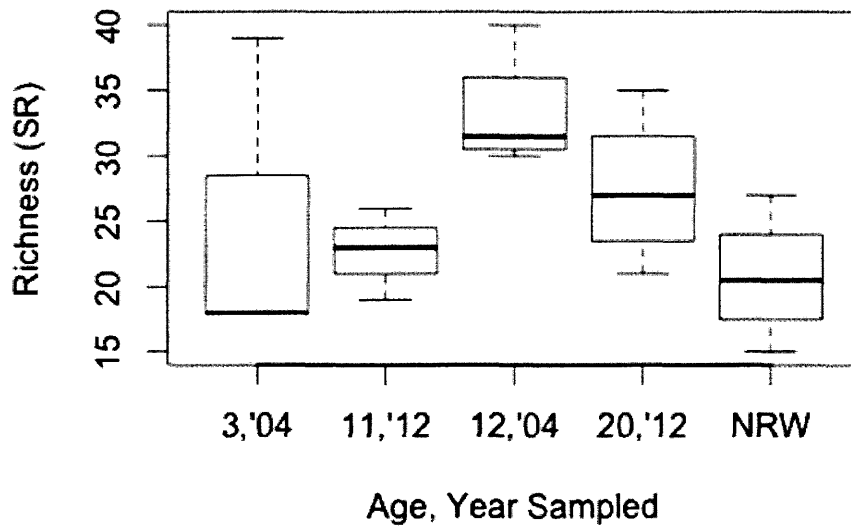
**Species Richness, Herbaceous Strata**

Figure 2.9

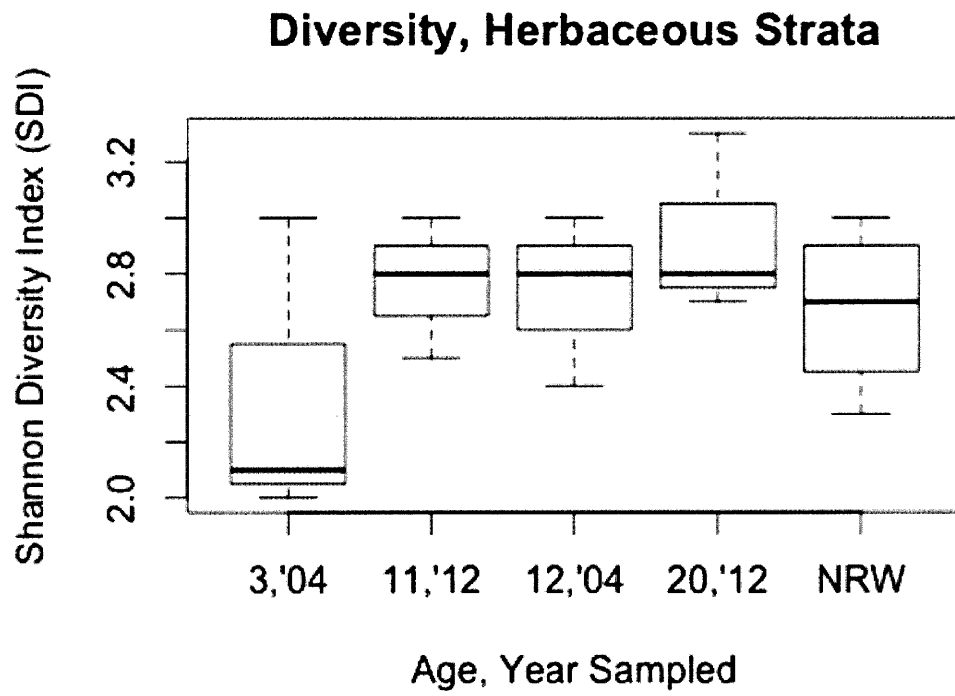


Figure 2.10

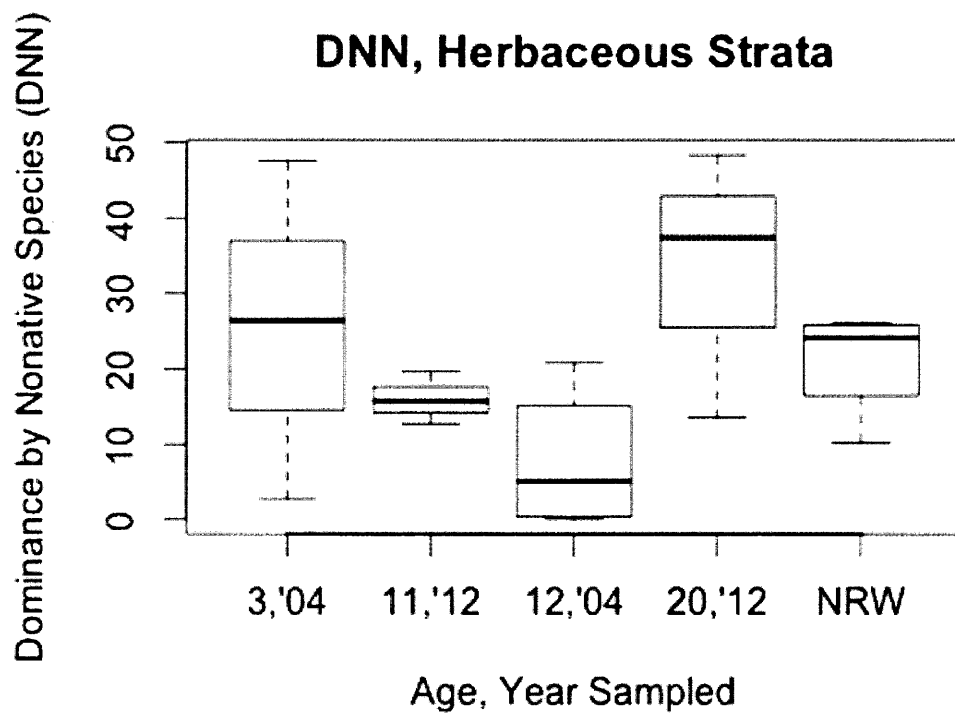


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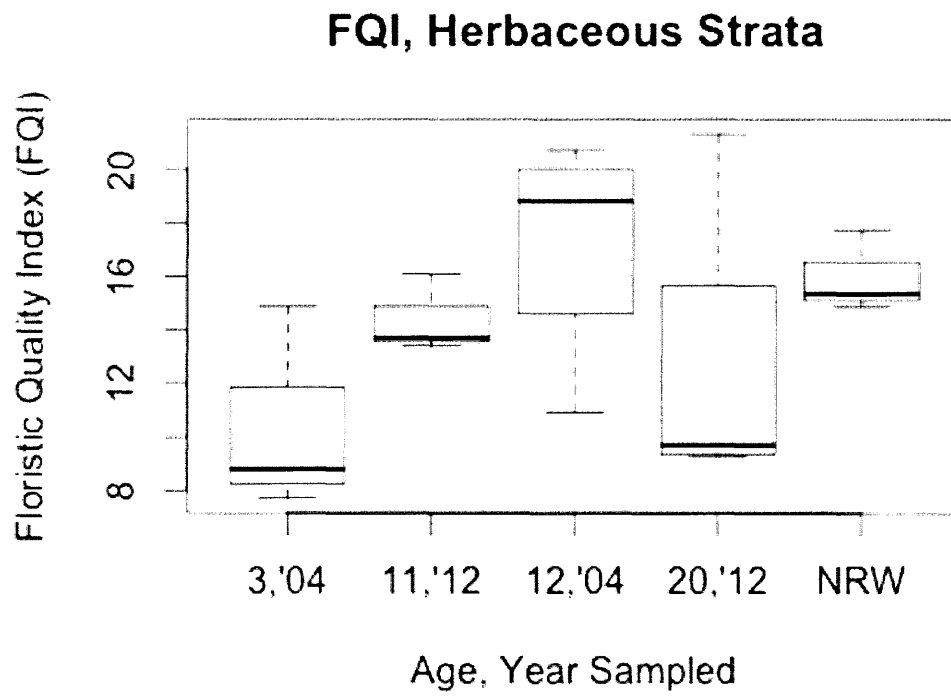


Figure 2.12

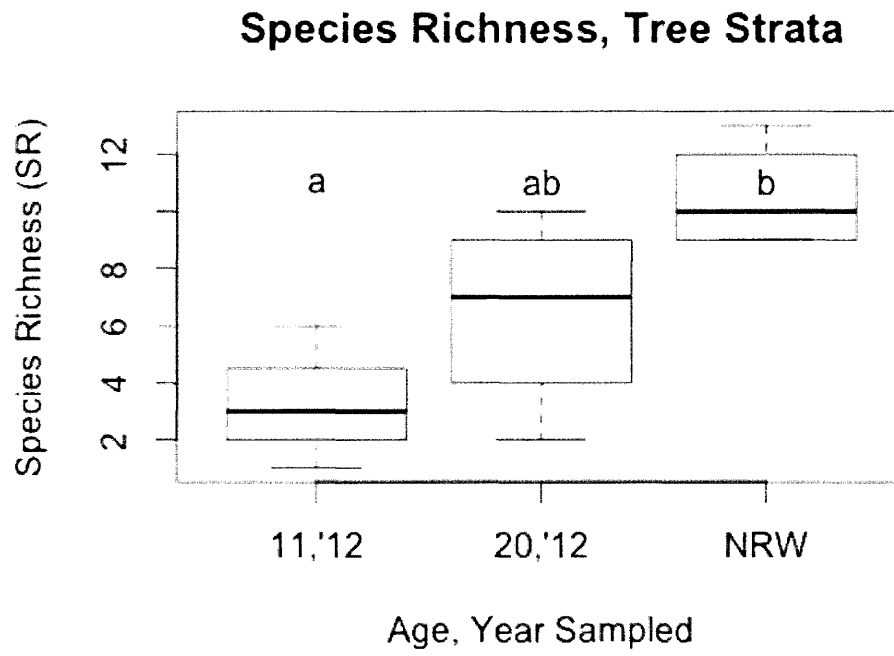


Figure 2.13

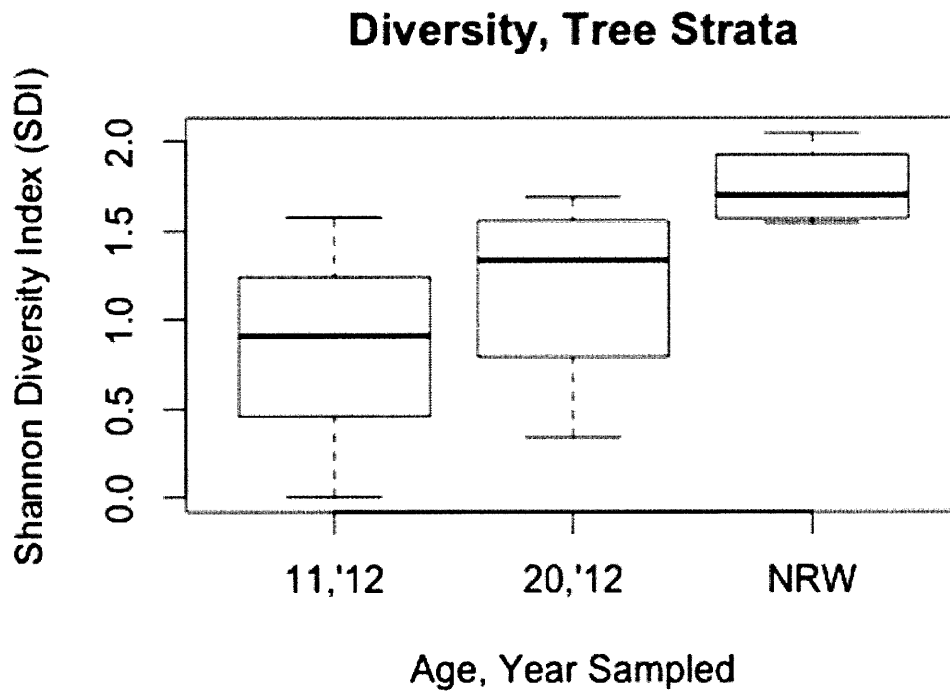


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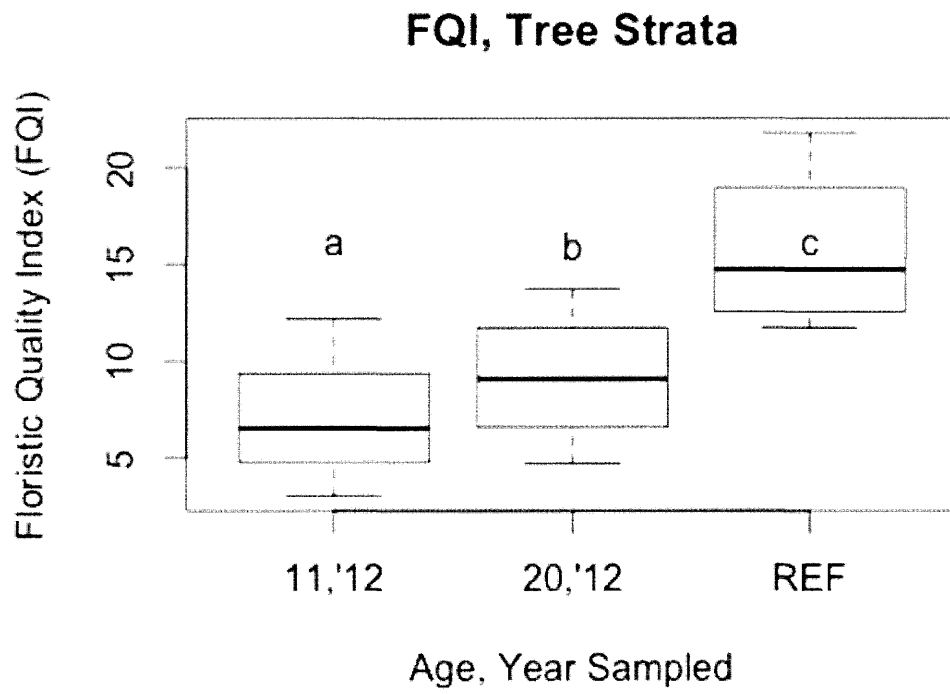


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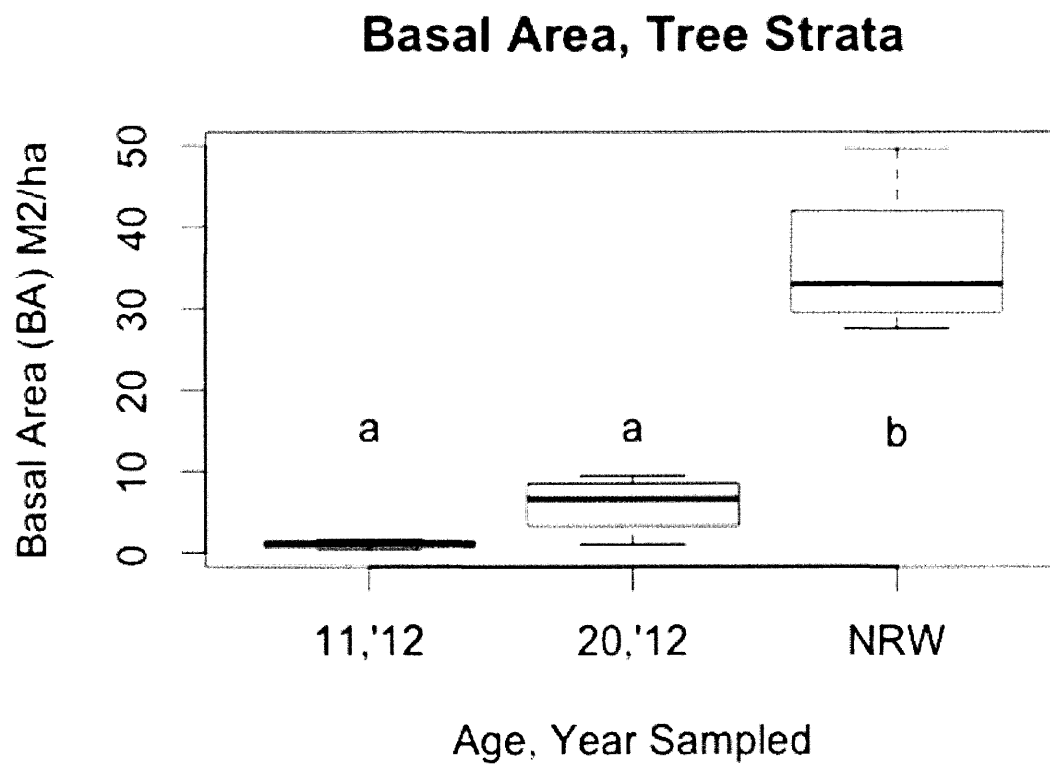




Figure 2.16

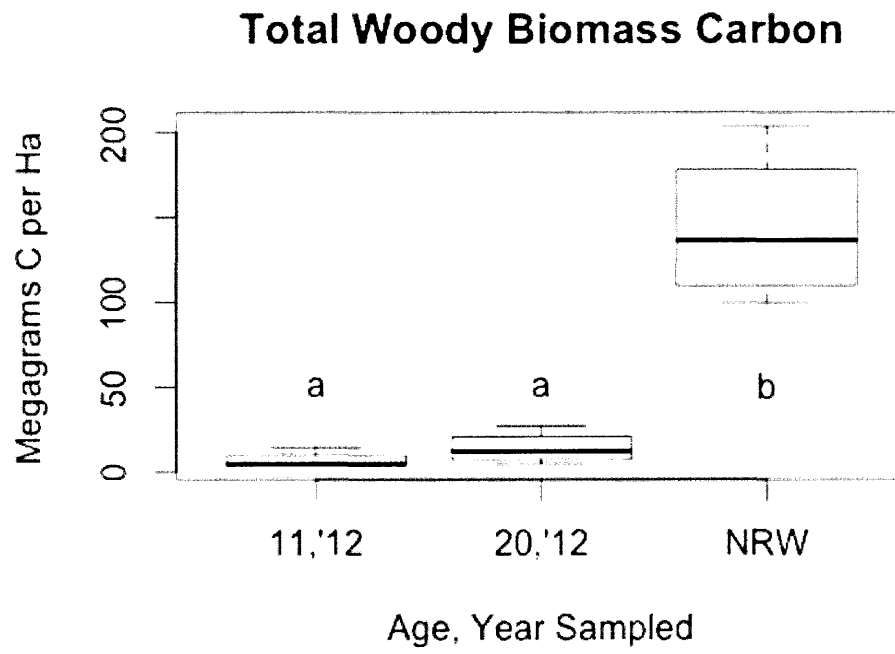


Figure 2.17

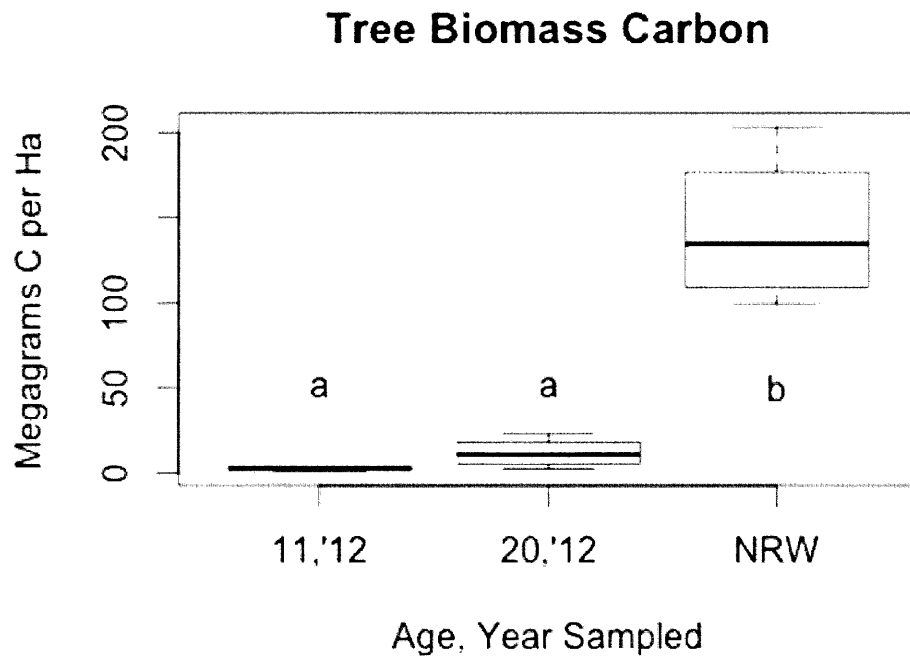


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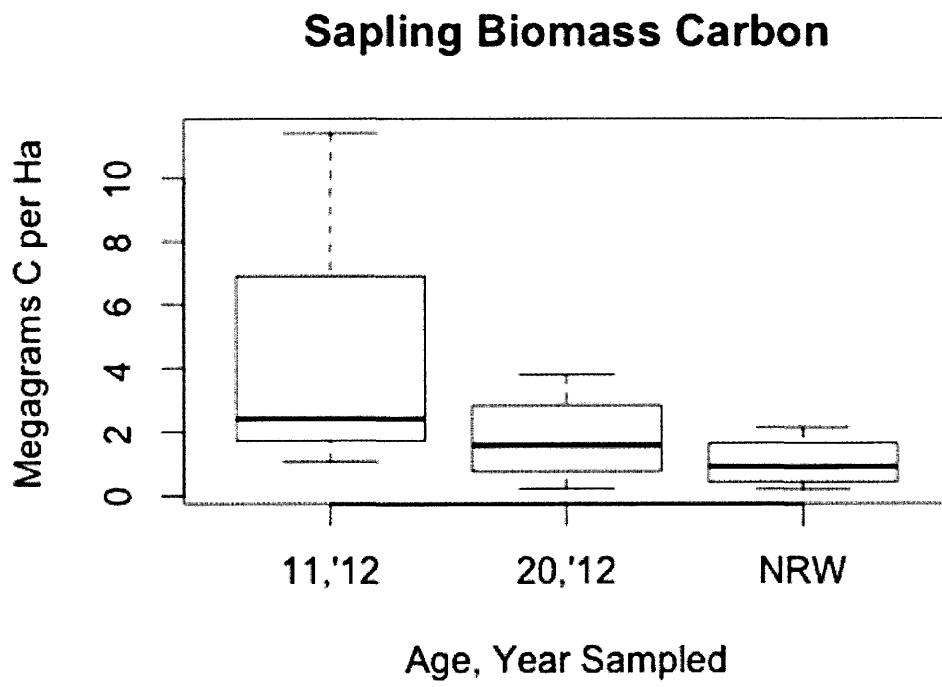
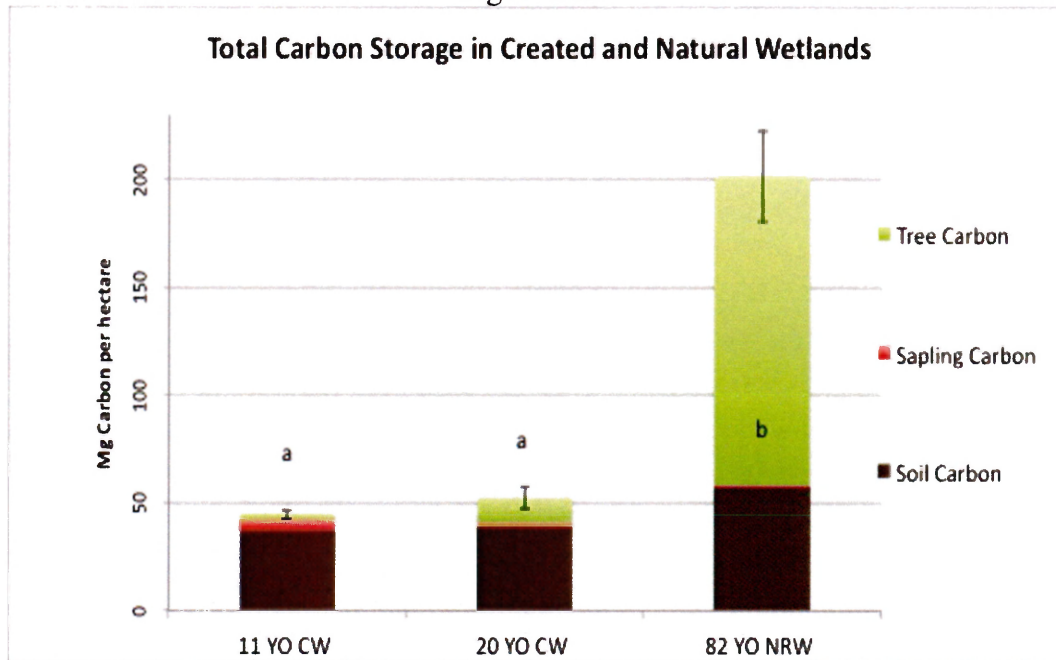


Figure 2.19



### Chapter 3

#### Soil Development and Functional Replacement in 11 and 20 year old Created Palustrine Forested Wetlands

##### Abstract

Palustrine forested wetlands (PFWs) are both the most prevalent and most impacted wetland type nationally and in Virginia. Many impacts to PFWs are mitigated for through the creation of new wetlands in former upland environments. These wetlands are often different than natural reference wetlands (NRWs) and must develop with time to become functional replacements for impacted wetlands. If and when this is likely to occur is the subject of debate. Our study utilizes two age classes of created forested wetlands (CFWs); a group of three wetlands averaging 11 years post creation (11 yo CFWs) and a group of four 20 yo CFWs to determine if CFWs become similar to NRWs in the functions of retention and removal of nutrients and the accumulation of carbon (NRC 1995). Additionally, data collected from the same sites 8 years previously were compared to current data to determine if CFWs are progressing toward functional equivalency. Soil % C %P, bulk density ( $D_b$ ), and C:N, C:P and N:P ratios in the top 10 cm of CFWs developed to statistically similar levels to NRWs within 20 years, indicating that, CFWs in Virginia begin to replace some aspects of wetland functions within 20 years. However, whole soil C storage and % N remained significantly lower in CFWs than NRWs after 20 years. CFWs were also dissimilar to NRWs in all measured parameters except C:N after 11 years and both CFW classes were dissimilar to NRWs at depth in all but nutrient ratios. At 0-10 cm depth, %N, %C, bulk density and C:N ratios showed significant trends toward equivalency with NRWs with age, while %P, total carbon, N:P, and C:P ratios did not. At 10-20 cm depth, CFW %N, N:P, and total C showed trends toward NRW conditions with age, while %C, %P, bulk density, C:N and C:P did not. The total carbon stock of the soil did not show a relationship with CFW age. Our findings indicate positive progression toward natural wetland functions, but highlight continuing dissimilarities and a temporal lag in functional replacement inherent in the mitigation system that likely creates “consistent and considerable” functional loss, thus, the creation of CFWs in Virginia does not necessarily lead to “no net loss” of function within 20 years.

## Introduction

Wetland area in the United States was reduced by 53% from European colonization to the 1980s (Dahl 1990, Tiner and Finn 1986, USGS 1999, Dahl 2011). To protect remaining wetland resources, impacts to wetlands are now regulated under Section 404 of the Clean Water Act of 1977 (33 U.S.C. s/s 1251 et seq.) with a goal of providing “no net loss, with an adequate margin for safety” of wetland area and function (US EPA 1990; NRC 2001; USACOE 2002). Compensatory mitigation of wetland impacts is meant to replace the area and ecological functions lost from impacted wetlands (US EPA 1990), however, many studies have cast doubt on whether these created wetlands are functionally equivalent to lost wetlands (Race and Fonseca 1996; Zedler 1996; Zedler 2000; NRC 2001; Brooks et al. 2005; Ambrose et al. 2006; Hoeltje and Cole 2007), and in a national review, the National Research Council (2001) concluded that the goal of “no net loss” is not being met by the mitigation program.

Palustrine forested wetlands (PFW) have borne the brunt of both historical wetland loss and current impacts both nationally (Tiner and Finn 1986; USGS 1999; Dahl 2011) and in Virginia (VADEQ 2010). Dahl (2011) noted that since 1950, 7.7 million hectares of PFW wetlands have been lost, compared to 2.8 million hectares of emergent wetlands (Dahl 2011), and often impacts to PFWs are mitigated for with emergent or open water wetlands (Robb 2001; Balzano et al. 2002; Cole and Shaffer 2002).

In the Mid-Atlantic region wetlands are often created by removing upland surface soil in order to facilitate wetland hydrology. This process alters bulk density ( $D_b$ ) and commonly results in compaction, the exposure of low nutrient subsoil and a lack of organic matter (Atkinson et al. 1993; Brinson and Rheinhardt 1995; Whittecar and Daniels 1999; Bruland and Richardson 2004; Atkinson et al. 2005).

Bulk density tends to be high in young created wetlands (CFWs) because of the removal of surface soil and compaction by machinery (Atkinson et al. 1993; Bishel Machung et al. 1996; Cummings 1999; Nair et al. 2001; Whittecar and Daniels 1999). High bulk density in soils leads to increased erosion and surface water flow and reduced porosity, gas exchange, hydraulic conductivity, infiltration, and denitrification enzyme activity (Montgomery et al., 2001; Ahn and Peralta 2012). Furthermore bulk densities

above 1.45- 1.75 g/cm<sup>3</sup> for fine and coarse textured soil respectively limits root penetration, limiting productivity and potentially altering the plant community (Brady and Weil 2008; NRC 1995).

Young CWs often have soils with lower carbon (C) than natural wetlands (Atkinson et al. 1993; Bishel-Machung et al. 1996; Campbell et al. 2001; Cole et al. 2001; Cummings 1999; Shaffer and Ernst 1999; Stolt et al. 2000; Whittecar and Daniels 1999). Sufficient soil C is needed for proper nutrient cycling, and the establishment of plant and microbial communities (Duncan and Groffman 1994; Hossler et al. 2011; Dee and Ahn 2012). Insufficient labile soil C limits soil reduction (Vepraskas et al. 1995) and reduces rates of respiration (Howard and Howard 1993; Atkinson 2001), denitrification (Brettar and Hofle 2002; Wolf 2011; Ahn and Peralta 2012), and P sorption (Axt and Walbridge 1999). Furthermore, wetland soils store disproportionate levels of C for their area (Mitsch and Gosselink 2007; Lal 2007), all of which makes soil C an excellent indicator of wetland functions (Fennessy et al. 2004; Rokosch et al. 2009; Craft et al. 2003).

Young CWs often contain low levels of soil N and P (Craft et al. 2002; Anderson et al. 2005; Fennessy et al. 2008; Cummings 1999; Bruland et al. 2009), both of which are essential to the proper establishment and development of plants and microbial communities (Moser et al. 2009; Dee and Ahn 2012). Like C, the removal and storage of N and P by wetlands can alleviate environmental problems, decreasing potential eutrophication in accordance with the Chesapeake Bay Agreement (Chesapeake Bay 2000 Agreement, Subsection 2.3).

Soil C:N ratio, an important parameter for plant and microbe mediated processes is often low in CWs (Nair et al. 2001). Bacterial community structure is related to C:N ratio in a number of soils (Hartman et al. 2008; Ahn and Peralta 2009; Lauber et al. 2009), including created mitigation wetlands in Virginia (Peralta et al. 2013).

Wetland soils are expected to develop with time as soil C and nutrient stores accumulate through autochthonous and allochthonous inputs, alleviating high bulk density and altering stoichiometric ratios (Odum 1969; Stevens and Walker 1970; Mausbach and Richardson 1994; Wolf et al., 2011). In CWs, a number of studies have indicated soil development with time (Campbell et al. 2002; Noon 1996; Johns et al.

2004; Nair et al. 2001; Craft et al. 1988; Mitsch et al. 2012; Broome and Craft 1998; Craft et al. 1999 ), while other studies failed to find evidence of soil development (Bruland and Richardson 2004; Bischel-Machung et al. 1996; Cole 2001; Anderson and Cowell 2004), or development proceeded on long time scales (Ballentine and Schneider 2009; Hossler and Bouchard 2009).

Mitigation is meant to replace ecological functions lost because of wetland impacts (US EPA 1990), but because pre-impact monitoring of damaged wetlands rarely occurs, natural adjacent wetlands are used as a proxy for comparison of maturation of functions (LePage 2011). The ultimate test of ecological success (the replacement of functions) in CWs is how well they resemble natural reference wetlands (NRWs) in structure and function (Van der Valk 1994; Brinson and Rheinhardt 1996; Rheinhardt et al. 1999; Whigham 1999), but whether regulatory success leads to functional success remains dubious, as “successful” CWs often fail to function as NRWs (Zedler and Langis 1999; Mitsch and Wilson 1996; Sudol 1996; Balcombe 2005).

Moreno-Mateos (2012) determined that wetland creation and restoration do not generally reach the level of biogeochemical functioning of natural ecosystems even after a century. Uncertainty exists as to whether CWs reach functional equivalency with natural NRWs or if they trend toward alternative stable states (Scheffer et al. 2001). Furthermore, if equivalency is reached, it is important to understand the lag time between CW creation and functional equivalency. Consistent temporal loss of functions may lead to considerable wetland functional loss overall (Gutrich and Hitzhugen 2004; Bendor 2009).

Until CFWs are comparable to natural NRWs, it is unlikely that the mitigation process is leading to “no net loss” of wetland function. This study focuses on comparing created and natural wetlands in terms of soil structure and the functions of the retention and removal of elements (in this case nutrients) and the accumulation of organic matter. To that end, the goals of this study are to determine the biogeochemical success of CFWs by: 1) determining whether soils in CFWs are comparable to NRWs after 11 and 20 years in terms of % C, N, and P, nutrient ratios and bulk density 2) determining how CFW soils developed over 8 years and 3) analyzing differences in total C storage between 11 and 20 yo CFWs and NRWs..



## Methods

### Created Forested Wetlands

Seven created palustrine forested wetlands (CFWs) constructed to mitigate for wetland impacts to forested wetlands associated with the Virginia Department of Transportation (VDOT) were selected from two age classes. Three wetlands that average 11 year old (11 yo CFWs) sites and four sites that average 20 years old (20 yo CFWs) sites were selected from Virginia's Coastal Plain (4 sites) and Piedmont (3 sites) provinces.

Each site met the following criteria:

- (1) created as compensatory mitigation for impacts regulated under Section 404 of the Clean Water Act to replace impacted PFWs
- (2) at least one hectare in size
- (3) has satisfied the US Army Corps of Engineers criteria of wetland hydrology (Environmental Laboratory 1987). Furthermore, none of these sites was subjected to prolonged periods of ponding.
- (4) Soil nutrient data is available from 2004 (DeBerry and Perry 2012) with which to compare current conditions
- (5) site has not been physically altered since construction

### **11 year old age group (11 yo CFWs): average age $10.7 \pm 0.9$**

These CFWs represent CFWs that are nearing the end or have just progressed from their 10 year mandatory monitoring period (VA DEQ 2010). These sites represent 11 years of soil development as well as the final stage in the mitigation process. After this stage, wetland trajectories proceed without regulatory interference.

### **20 year old age group (20 yo CFWs): average age $20.3 \pm 0.9$**

This group of CFWs was chosen since they are some of the oldest CFWs related to VDOT (Leo Sneed personal comm), and because they all graduated from their monitoring period approximately ten years ago. They represent a picture of the

progression of “successful” CFWs through their second decade of development and their first decade of independence.

### **Natural Reference Wetlands (NRWs): average Age $82 \pm 3.2$**

Four natural reference wetlands (NRWs) were sampled to compare to CFWs. NRWs were palustrine forested wetlands that were located in close proximity to one of the created sites. The NRWs had not been subject to clearing or extreme disturbance since at least 1964 (in order to allow for Cesium dating of the soil). NRW time since major disturbance was estimated by dating 1 tree from the dominant size class of each plot. Site age was the average of all 5 plots within each site, and was carried out in 2004 by DeBerry (2006). A 36 cm Suunto increment borer with a 0.5 cm cutting radius was used to take increment cores, which were processed using the dating methods specified in Forestry Suppliers (2004) and Husch et al. (1972). Dominant size class was used to identify trees from the oldest functional tree guild (Lopez et al. 2002; Keddy 2000). NRWs were chosen based on proximity to one of the CFWs and 2 were located in the Piedmont and 2 in the Coastal Plain. Three of the NRW were located within 1 kilometer of a CFW while the fourth was located approximately 6.3 km Northeast of the Manassas CFW. None of these NRWs would be considered pristine, but “minimally impaired” (U.S. EPA 2002).

### **Sampling protocol**

At each CFW and NRW a 1-ha sampling site was identified to match the sampling methods of DeBerry and Perry (2012). These sites were originally chosen based on homogenous stand composition and age (Parsons and Ware 1982; Glascock and Ware 1979). Vegetation plots were created in a stratified random pattern originating from a wetland baseline and extending into the wetland in distances determined by random numbers draw (Mueller-Dumbois and Ellenberg 1974; Tiner 1999).

One soil sample was extracted from near the center of each plot to a depth of 20 cm for CFWs. In NRWs three out of the five plots were randomly chosen for soil sampling and a 25 cm soil sample was extracted. Each sample was taken from near the center, but exact location was chosen based on representative plant community and

elevation (ie, not hummocks or tree tip pits). Vegetation and the O<sub>i</sub> soil layer were first removed (DeBerry 2006) and then cores were taken with 12.7 cm diameter aluminum corers to reduce compaction (Reinhardt et al. 2000). Once the corer was in the soil, compaction was measured by change of elevation inside and outside of the corer. If compaction was detected, the core was discarded and an alternative acquired. An airtight seal was created to extract cores, which were then capped in the field, and kept below 5 C until analysis (Reinhardt and Cole 2000; Bernal and Mitsch 2012).

### **Laboratory Analysis**

CFW soil was sectioned into 0-10 cm and 10- 20 cm sections. Bulk density was determined on intact soil cores that were dried at 60C (Blake and Hartge 1986). Sections were then homogenized with a mortar and pestle and a subsample was analyzed for percent Nitrogen (%N) and %C using a Perkin-Elmer 2400 elemental analyzer, while another subsample was used to measure total phosphorus with a modified ashing/acid extraction process followed by colorimetric analysis (Chambers and Fourqurean 1991).

### **Nutrient Comparisons**

%C and %N and C:N molar and mass ratios were compared among 11 and 20 yo CFWs sampled in 2012 and the same sites sampled in 2004 when they were ~3 and ~12 yo respectively to determine CFW progression through time. CFWs were then compared to NRW soils to determine progress toward functional equivalency. This method provides for both analysis of current condition and progress within groups of sites over time.

%P, N:P, and C:P molar and mass ratios and bulk density were compared among 11 and 20 yo CFWs and NRWs. Data from 2004 were lacking or incompatible for these criteria so could not be used for determination of progress of these functions.

Standing stocks of C in the top 20 centimeters of the soil were calculated as

$$C_t = D_b \times C_c \% \times D$$

Where C<sub>t</sub>= total C stock, D<sub>b</sub> is bulk density and D is depth (Mann 1986; Guo and Gifford 2002).

C stock was compared among 11 and 20 yo CFWs and NRWs. These data were combined with C stocks from woody biomass developed in chapter 2 to report the C stock of woody biomass and the top 20 cm of the soil.

Finally, soil parameters were compared to age of individual wetland with linear regression.

### **Reference Wetland Carbon Accretion**

To determine C accretion rates in NRWs, three 25 cm samples were extracted within each site. These samples were divided into 3.3 cm sections, which were dried at 60 C and weighed for bulk density (Blake and Hartge 1986). Subsamples were removed for C and nutrient analysis, as well as analyzed using gamma ray spectroscopy to determine the depth of the 1963 peak in Cesium-137 (Ritchie and McHenry 1990). The 1963 Cesium-137 peak was determined by counting dried, ground samples for 90,000 seconds. Specific activity was determined from the peak intensity at 661.66 keV with detector efficiency derived from a commercial Cesium-137 standard. Cesium peaks were identified as the centerpoint of the 3.3 cm soil section in which the peak occurred. Due to Cesium-137's stability within sediment, it can be assumed that soil above the Cesium-137 peak, has accumulated since 1963 (Yeager and Santschi 2003; Stark et al. 2006; Ritchie and McHenry 1990; Craft and Richardson 1998; Bernal and Mitsch 2012). Thus, C accumulation rates can be determined as the standing stock of C above the 1963 peak, divided by the 49 years that passed between 1963 and 2012 (Craft and Richardson 1993; Goodbred and Kuehl 1998; Craft and Casey 2000; Graham et al. 2005; Stark et al. 2006).

### **Statistical Analyses**

We tested for differences between groups with the Kruskal-Wallis test since many data sets violated the assumption of normality because of positive skew and exhibited outliers (McCune and Mefford 1999). We used the Kruskal-Wallis test to test for significant differences at the 95% confidence interval since it functions properly in situations with non-normal data and outliers (Logan 2010). Wilcoxon paired-samples signed-rank test was used to determine where differences occurred in combination with

Bonferroni correction, which multiplies p-values by the number of comparisons to control family-wise error (Logan 2010). The Wilcoxon signed-rank test uses sums of positive and negative rank difference between paired observations to test whether two populations are similar (Logan 2010). All statistical tests were performed in R statistical software (version 2.13.1).

## Results

### Bulk Density

$D_b$  at 0-10 cm depth averaged  $1.20 \pm 0.05$  g/cm<sup>3</sup> for 11 yo CFWs, and  $0.97 \pm 0.05$  g/cm<sup>3</sup> in 20 yo CFWs. NRWs had an average  $D_b$  of  $0.80 \pm 0.09$ . 11 yo CFWs had significantly higher  $D_b$  than 20 yo CFWs ( $p=0.019$ ) and NRWs ( $p=0.0066$ ), however 20 yo CFWs were similar to NRWs ( $p=0.402$ ) (figure 3.1). There was a significant but weak relationship in CFWs between  $D_b$  and age in the upper 10 cm ( $r^2=0.16$ ,  $p=0.01$ ) (table 3.1).

In the 10-20 cm soil depth, 11 yo CFWs'  $D_b$  averaged  $1.43 \pm 0.07$  g/cm<sup>3</sup>, the 20 yo CFWs averaged  $1.37 \pm 0.08$  and NRWs averaged  $1.02 \pm 0.11$ . CFW age classes were statistically similar, while NRWs had lower  $D_b$  than either 11 yo CFWs ( $p=0.015$ ) or 20 yo CFWs ( $p=0.032$ ) (figure 3.2). There was no significant relationship between  $D_b$  and age ( $r^2=0.016$ ,  $p=0.22$ ) (table 3.1).

### Percent Carbon

Surface (0-10cm) carbon percent in the 11 yo CFW class increased significantly from  $1.10 \pm 0.06$  to  $2.20 \pm 0.23$  ( $p=0.0012$ ), while %C in 20 yo sites increased from  $1.97 \pm 0.21$  to  $2.65 \pm 0.23$ , but did not represent a significant difference ( $p=0.07$ ). NRWs had an average %C of  $5.62 \pm 1.46$ , which was statistically higher than 11 yo CFWs ( $p=0.049$ ), but similar to 20 yo sites ( $p=0.098$ ) (figure 3.3). Linear regression showed that %C was influenced by age of created wetlands ( $r^2=0.28$ ,  $p < 0.0001$ ) (table 3.1).

In 10 to 20 cm deep soil samples, 11 yo CFWs averaged  $0.79 \pm 0.07$  %C, 20 yo wetlands averaged  $1.24 \pm 0.19$  and NRWs averaged  $3.71 \pm 1.40$ . CFW age classes were statistically similar ( $p=0.231$ ), while NRWs had a significantly higher %C than both 11

yo CFWs ( $p=0.0002$ ) and ~21 yo CFWs ( $p=0.0162$ ) (figure 3.4). Linear regression showed a weak but significant relationship between %C and age ( $r^2=0.14$ ,  $p=0.016$ ) (table 3.1).

### Percent Nitrogen

Average %N in the top 10 cm of the soil increased 263% in the 11 yo age class from  $0.081 \pm 0.005$  in 2004 to  $0.213 \pm 0.021$  in 2012 showing a significant difference ( $p<0.0001$ ). Similarly, the ~21 yo CFWs increased 190% from  $0.133 \pm 0.017$  to  $0.253 \pm 0.02$  ( $p=0.0005$ ). Both age classes of CFWs were statistically similar ( $p=0.069$ ). NRWs averaged  $0.51 \pm 0.093$ , double that of CFWs and had a significantly greater %N than both 11 ( $p=0.0022$ ) and 20 ( $p=0.0008$ ) yo CFWs (figure 3.5). Linear regression showed that %N was influenced by site age ( $r^2=0.37$ ,  $p < 0.0001$ ) (table 3.1).

%N in the 10 to 20 cm section of the soil averaged  $0.101 \pm 0.007$  in the 11 yo CFWs,  $0.159 \pm 0.018$  in the 20 yo CFWs and  $0.341 \pm 0.104$  in the NRWs. Percent N was significantly higher in 20 yo CFWs than 11 yo sites ( $p=.0035$ ), and NRWs had a higher percentage than 11 yo CFW ( $p<.0001$ ) and 20 yo CFWs ( $p=0.027$ ) (figure 3.6). Linear regression showed a significant positive relationship between %N and site age ( $r^2=0.24$ ,  $p=0.0019$ ) (table 3.1).

### Percent Total Phosphorus

In the top 10 centimeters of the soil column, %P was  $0.03 \pm 0.0036$  % for 11 yo sites,  $0.037 \pm 0.0065$ % in 20 yo sites, and  $0.056 \pm 0.0065$  in NRWs. %P was similar between created wetland age classes ( $p=0.257$ ) in the top 10 cm of the soil. NRWs had higher 0-10 cm depth %P than 11 yo site ( $p=0.0022$ ) but were statistically similar to 20 yo CFWs ( $p=0.0817$ ) (figure 3.7). There was no linear relationship between %P and CFW age ( $r^2=0.068$ ,  $p=0.074$ ) (table 3.1).

In the 10- 20 cm soil section, 11 yo sites averaged  $0.024 \pm 0.003$  %P, 20 yo wetlands averaged  $0.023 \pm 0.003$  and NRWs averaged  $0.04 \pm 0.006$ . Created wetland age classes were statistically similar ( $p=0.949$ ), while NRWs had higher percentages than 11 yo created sites ( $p=0.0086$ ) and 20 yo created sites ( $p=0.038$ ) (figure 3.8). Linear regression showed no significant relationship between percent P and wetland age

( $r^2=-0.026$ ,  $p=0.69$ ) (table 3.1).

### **Carbon to Nitrogen Ratio**

**The mass ratio of C:N in the top 10 cm of the soil decreased over 8 years in both the 11 yo age class from  $13.88 \pm 0.72$  to  $10.29 \pm 0.34$  and in the 20 yo CFWs from  $15.63 \pm 0.69$  to  $10.42 \pm 0.30$ . NRWs averaged  $9.94 \pm 0.68$ . C:N decreased significantly as CFWs aged from 3 to 11 ( $p<0.0001$ ) and as CFWs aged from 12 to 20 ( $p<0.0001$ ). While 2004 CFW soils had higher C:N than NRWs at age 3 ( $p=0.006$ ) and age 12 ( $p<0.0001$ ), CFW soils in 2012 were similar to NRWs ( $p=0.98$ ) (figure 3.9). Linear regression showed a relationship between C:N ratio and site age ( $r^2=0.07$ ,  $p=0.014$ ) (table 3.1) .**

Between 10 and 20 cm, C:N mass ratios averaged  $7.74 \pm 0.52$  in 11 yo CFWs,  $7.68 \pm 0.52$  in 20 yo CFWs and  $9.55 \pm 0.57$  in NRWs. There were no significant differences between CFW age classes or NRWs and CFWs ( $p=0.051$ ) (figure 3.10). There was no significant relationship between C:N ratio and CFW age ( $r^2=-0.03$ ,  $p=0.7$ ) (table 3.1).

### **Carbon to Phosphorus Ratio**

In 0-10 cm depth soil samples, C:P mass ratios averaged  $82.37 \pm 11.98$  in 11 yo CFWs,  $94.67 \pm 13.56$  in 20 yo CFWs and  $95.91 \pm 16.60$  in NRWs. There were no significant differences between the CFW age classes or the NRWs ( $p=0.83$ ) (Figure 3.11). There was no significant relationship between C:P and site age in the 10-20 cm depth ( $r^2=-0.0312$ ,  $p=0.983$ ) (table 3.1).

In the 10 to 20 cm soil samples C:P mass ratio averaged  $35.70 \pm 3.27$  in 11 yo CFWs,  $71.49 \pm 13.04$  in 20 yo CFWs and  $78.61 \pm 17.66$  in NRWs. CFW classes were similar ( $p=0.15$ ), while NRWs had higher C:P ratios than 11 yo CFWs ( $p=0.002$ ), but similar to 20 yo CFWs ( $p=0.77$ ) (figure 3.12). There was no significant relationship between C:P ratio and CFW age ( $r^2=0.089$ ,  $p=0.983$ ) (table 3.1).

### **Nitrogen to Phosphorus Ratio**

In the 0 to 10 cm depth mass N:P ratio averaged  $7.87 \pm 0.93$  in 11 yo CFWs,  $8.74 \pm 1.09$  in 20 yo CFWs and  $9.31 \pm 1.11$  in NRWs. There were no significant differences

between CFW age classes or NRWs ( $p=0.63$ ) (figure 3.13). There was no significant relationship between N:P and age ( $r^2=-0.031$ ,  $p=0.993$ ) (table 3.1).

In the 10- 20 cm soil depth, N:P mass ratios averaged  $4.80 \pm 0.45$  in 11 yo CFWs,  $8.49 \pm 1.05$  in 20 yo CFWs and  $7.77 \pm 1.22$  in NRWs. CFW age classes were significantly different with N:P ratios higher in the 20 yo CFWs ( $p=0.021$ ). NRWs had higher N:P mass ratios than 11 yo CFWs ( $p=0.016$ ), but were similar to 20 yo CFWs ( $p=0.95$ ) (figure 3.14). There was a significant relationship between N:P ratio and site age ( $r^2=0.103$ ,  $p=0.036$ ) (table 3.1).

### Stored Whole Soil Carbon

Overall C stored in the top 20 cm of the soil profile was  $37.25 \pm 3.47$  Mg/ha in 11 yo CFWs, with  $26.72 \pm 3.71$  Mg/ha (72%) of the C in the top 10 cm and  $11.36 \pm 3.30$  Mg/ha (28%) in the 10-20 cm soil section. 20 yo CFWs held  $38.87 \pm 2.29$  Mg/ha in their soil, of which  $24.22 \pm 1.39$  Mg/ha (64%) in the top 10 cm and  $14.5 \pm 1.1$  Mg/ha (36%) in the 10-20 cm soil depth. NRWs held  $57.497 \pm 9.762$  Mg/ha in the top 20 cm of the soil, with  $33.5 \pm 3.3$  Mg/ha (57%) at the surface and  $24.04 \pm 3.3$  Mg/ha (43%) in the 10-20 cm layer of the soil (table 3.2).

In the 0-10 cm depth, C stocks were similar between 11 yo and 20 yo CFWs ( $p=0.691$ ). NRWs held more C than 20 yo CFWs ( $p=0.016$ ), but were similar to 11 yo CFWs ( $p=0.071$ ) (figure 3.15). In the 10-20 cm soil depth, CFWs had similar C stocks ( $p=0.167$ ). NRWs held more C than either 11 yo ( $p=0.0001$ ) and 20 yo ( $p=0.014$ ) (figure 3.16). Overall stored C in the top 20 cm of the soil profile was similar between both ages of CFWs ( $p=0.71$ ) but NRWs held more C than both 11 yo ( $p=0.038$ ) and 20 yo CFWs ( $p=0.013$ ) (figure 3.17). There was no significant relationship between C stored in the surface soil of CFWs and wetland age ( $r^2=-0.027$ ,  $p=0.72$ ) or total C in the combined 0-20 cm soil depth ( $r^2=-0.096$ ,  $p=0.52$ ). However there was a significant relationship between stored C in the 10-20 cm soil depth and CFW age ( $r^2=0.115$ ,  $p=0.028$ ) (table 3.1).

### Overall Carbon Storage

Overall C stored in both aboveground woody biomass (calculated in chap. 2) and the top 20 cm of the soil were similar in 11 ( $44.52 \pm 1.89$  Mg/ha) and 20 ( $52.4 \pm 5.26$



Mg/ha) yo CFWs ( $p=0.396$ ), but the NRWs held significantly (4 times) more C ( $201.41 \pm 20.855$  Mg/ha) than the 11 yo ( $p=0.0024$ ) and the 20 yo sites ( $p=0.0017$ ) (figure 3.18).

### Reference Wetland Soil Carbon Sequestration

**While the top 10 cm of the soil did not change in %C ( $p=0.14$ ) or %N ( $p=0.90$ ),** NRWs accreted  $10.3 \pm 2.3$  cm vertically between 1963 and 2012, or  $0.22 \pm 0.05$  cm per year (Appendix 1). The C sequestered in the accreted surface soil was  $3077.7 \pm 785.30$  grams/ $m^2$ , or  $64.1 \pm 16.4$  grams/ $m^2$ /yr. When extrapolated to the site level, NRWs sequestered an average of  $30.77 \pm 7.85$  Mg/ha since 1963, or  $0.641 \pm 0.164$  Mg/ha/yr. This equates to an addition of 1.1% of the average C stored in the top 20 cm of the soil per year (table 3.3).

The average C sequestration for the 2 sites in the Coastal Plain province was  $4762.6 \pm 35.76$  grams  $c/m^2$  since 1963 while the 2 sites for the Piedmont averaged  $1428.8 \pm 68.5$ , representing a significant difference between rates in each province ( $p=0.0498$ ) (table 3.4).

### Regional Differences in CFWs

There were no differences between CFWs in the Piedmont and Coastal Plain in terms of overall C stocks ( $p=0.248$ ) or soil C stocks ( $p=0.07$ ). There was also no difference between bulk density ( $p=0.118$ ), N:P ratio ( $p=0.49$ ), or C:P ratios ( $p=0.26$ ) between regions. However, the Piedmont tended to have higher percentages of C ( $p=0.013$ ), higher percentages of N ( $p=0.0007$ ), a higher percentage of P ( $p=0.0008$ ), and lower C:N ratios ( $p=0.042$ ).

## Discussion

### Soil Development

Soils of the CFWs showed an overall pattern of positive soil development towards mimicking those of NRWs. Trends of progress toward reference characteristics existed

in all measured parameters, however the level of progress and whether equivalency was achieved within 20 years differed. Soil development as seen through increased organic matter (OM) and nutrient reserves, lower bulk density and increased importance of detritus is expected to advance with age (Odum 1969; Marks and Bormann 1972; Odum 1985; Chadwick and Graham 2000). Soil-forming processes such as accumulation of organics and soil oxidation/reduction are time dependent, linked to the development of colonizing vegetation, the depletion of soil oxygen and the availability of palatable C resources (Craft 2001; Mitsch and Gosselink 2000; Megonigal et al. 2004).

### **Carbon**

Averaging 2.19%- 2.6% for 11 yo and 20 yo CFWs respectively, %C in the 0-10 cm soil depth was higher than the 1.5% organic C minimum that Vepraskas et al (1995) found was necessary for the production of hydric soils. % C in our study was higher than some studies of created wetlands in which soil C was <1% (Stauffer and Brooks 1997; Whittecar and Daniels 1999), but was slightly below a number of other studies which ranged from 3.1 to 5.9 %C in surficial soil (Bruland and Richardson 2005, Bischel-Machung et al. 1996, Shaffer and Ernst 1999), indicating that our CFWs are unlikely to be exceptional in terms of C content.

In the top 10 cm of the soil, our results support the ability of CFWs to increase %C rapidly. % C doubled between year 3 and 11 to reach an average of 2.19% C and increased 35% between 12 and 20 years to reach 2.65% C. Similarly, a significant linear relationship exists between %C in the upper 10 cm and wetland age. This finding reflects those of Mitsch et al. (2012) who demonstrated a doubling of C from 0-10 years post construction and a tripling from year 0-15. Our data show that CFWs in Virginia can reach reference levels of % C in the top 10 cm of the soil within a 20-year time frame, even faster than the 25 years outlined by Craft and colleagues (1999) for salt marshes. The speed at which CFWs reached equivalent C percentage reflects the findings of Bernal and Mitsch (2012) that immature forested depressional wetlands sequestered C at the highest rate of all surveyed wetlands.

Our results differ from a number of studies that demonstrate a problematic lack of C development with CW age (Shaffer and Ernst, 1999, Bruland and Richardson 2005,

2006). Fenessy et al. (2008) found that C levels were 5 times lower in young CWs than reference sites, while Ballentine and Schneider (2009) found that marsh sites did not show increased organic C until 30-35 years post-restoration and soil C lagged behind NRWs after 55 years. CW soils in Virginia have shown lack of %C development with age (Bruland and Richardson 2004) and in the same CWs over 5 years (Cummings 1999), which was ascribed to poor hydrology, high bulk density, a lack of organic matter and insufficient insulating vegetation in the latter study. However even when biomass and hydrologic conditions are characteristic of NRWs, they sometimes fail to progress toward NRW C levels (Cole 2001; Anderson and Cowell 2004). Similarly, Bischel-Machung and others (1996) found that while NRWs had higher organic matter near the surface than at 20 cm, the created wetlands they studied had similar levels throughout the soil profile, indicating that organic matter was not accumulating at the surface. However in this study CFW %C at the surface was similar to NRWs by 20 years, while the deeper soil level remains significantly lower, indicating accretion at the surface.

In the 10-20 cm layer, 20 yo CFWs had insignificantly higher C than 11 yo CFWs, both groups had less than 1/3 the %C of NRWs at depth, indicating that organic matter tends to develop from the surface in PFWs from litter fall (Megonigal and Day 1988) and may indicate a lack of carbon input through root penetration at depth due to root limiting bulk density in 1/3 of 11 yo wetlands.

Total C stock in the upper 20 cm of the soil in NRWs was 19 Mg/ha higher than 20 yo CFWs and 20.2 Mg/ha higher than 11 yo CFWs. Furthermore, there was no significant relationship between total C and CFW age, indicating a lack of progress. If all 77 ha of created CFWs in Virginia (VADEQ 2012), have similar outcomes, it would create a loss of 11,550 Mg of soil C per year. To sequester a similar level of C in the top 20 cm of the soil, CFWs would have to increase their current soil C by 48-54%.

Wetlands are responsible for one-third of the global C pool (Mitsch and Gosselink 2007; Lal 2007) despite only occupying 6-8% of the land surface (Roulet 2000; Mitsch and Gosselink 2007) and are currently net sinks of 830 Tg/year of C or 14% of C released globally through the burning of fossil fuels (Mitsch et al. 2013). If this C sink is to be maintained, both wetland area and C stores must be maintained through the mitigation program.

In our study, NRW held 43% of their overall C in surface soil, compared to 72% in 10 yo CFWs and 64% in 20 yo CFWs. This may be because soil C accumulation is mostly the product of litterfall to the surface and root turnover (Megonigal and Day 1988). Anderson and Mitsch (2006) found that the majority of C accumulation in an experimental wetland occurred above the antecedent soil surface, while lower layers of soil remained unchanged (Anderson et al. 2005). Vertical accretion in our NRW was 0.2 cm /yr, leading to gradual increases in soil carbon, while the %C in the top 10 cm did not change. Our CFWs may accrete faster, as Bernal and Mitsch 2012 found a young forested wetland in Ohio that averaged 0.6 cm/yr of soil accretion. If CFWs sequester C mostly through vertical accretion, like our NRWs, the development of soil C stores at depth may take a long time. Similarly, Hossler and Bouchard (2009) modeled C sequestration and estimated that CFWs wouldn't reach NRW levels for 300 years. Though modeling of time to equivalency was not part of this study, lack of a relationship between C storage and CFW age coupled with finding similar soil C stocks in 11 and 20 yo CFWs indicates that soil development to NRW conditions could be a lengthy process.

Though our study indicates rapid increases in %C in the surface soil to equal NRW levels in 20 years and the development of similar C stocks to NRW in the surface soil within 11 years, CFWs have lower soil C stocks in the upper 20 cm of the soil after 20 years. CFWs stored 45% less C in the top 20 cm of the soil than NRW, reflecting an international average of 50% less carbon stored in restored or created wetlands after 20 years reported in Moreno-Mateos et al. (2012). If wetlands are mitigated at a 2:1 ratio of wetland creation to wetland impact, then the mitigation process may lead to an increase in soil C storage after 20 years. However, when aboveground biomass is combined with soil C, CFWs offer only a quarter of the C storage provided by NRW after 20 years. Soil C is an excellent indicator of wetland functions (Fennessy et al. 2004; Rokosch et al. 2009; Craft et al. 2003). In CFWs in Virginia, soil C correlates with water holding capacity, P sorption, microbial biomass (Bruland and Richardson 2004), respiration (Atkinson 2001) and denitrification (Wolf 2011; Ahn and Peralta 2012). Therefore, while %C is trending toward NRW levels, until levels of %C reach the levels of NRW, CFWs may not be restoring wetland function. On the other hand, rapid increases in %C and parity with NRW in the surface soil within 20 years are positive

indications of the development of ecosystem functions.

### **Reference Carbon**

While there was no significant change in %C in the upper 10 cm over 8 years, NRWs continued to sequester C through soil accretion at an average rate of  $0.621 \pm 0.204$  Mg/ha/yr. Odum (1969) indicated that ecosystems tend to reach equilibrium with maturity, however, studies conducted since have indicated that older forests continue to sequester C in their soils (Luyssaert et al., 2008). Our sequestration rates fell on the lower end of the spectrum of PFW sequestration rates in the Eastern United States. At the high end, Bernal and Mitsch (2012) recorded rates 7.6 times higher than ours in a PFW in Ohio. Studies found 1.1-1.3 Mg/ha/yr (1.5-2 times the rate of our study) in East Coast PFWs in Virginia, Florida (Craft et al. 2008), Georgia (Craft and Casey 2000) and North Carolina (Bridgham and Richardson 1993). However, our NRWs sequester carbon at a higher rate than boreal peatlands, which averaged 0.15-0.26 Mg/ha/yr (Turumen et al. 2002) or North American peatlands, which averaged 0.29 Mg/ha/yr (Gorham 1991).

### **Bulk Density**

In this study 20 yo CFWs had significantly lower  $D_b$  than 11 yo, indicating potential reduction with time and 20 yos were equivalent to NRWs in the surface soil. Thus our results indicate that CFWs can become equivalent to NRWs in 20 years, but not 11 years. Created wetlands often exhibit higher  $D_b$  than natural wetlands (Atkinson et al. 1993, Bishel- Machung et al. 1996, Cummings 1999, Nair et al. 2001, Whittecar and Daniels 1999, Campbell 2002), as was the case with our 11yo CFWs. However, few studies follow CFWs for 20 years, and in our study, 20 yo CFWs reached NRW levels and  $D_b$  showed a significant negative linear relationship with CFW age, indicating that at the surface, time proved the remedy to high bulk density. However, in the 10-20 cm section, CFWs had significantly higher  $D_b$  than NRWs at both ages, and the relationship between  $D_b$  and CFW age was negative but not significant.

In our study in the 10-20 cm soil depth, 1/3 of soil samples in 11 yo CFWs had root limiting bulk density, while no samples in the 20 yo sites exceeded root limiting bulk density, defined as:  $D_b$  above 1.45- 1.75 g/cm<sup>3</sup> for fine and coarse textured soil

respectively (Brady and Weil 2003). Our  $D_b$  findings have implications for functional capacity in CFWs. Root limiting soil can prevent vegetation establishment and reduce growth and productivity.

In comparison, % soil C displays a significantly negatively relationship with  $D_b$  in our study ( $R^2 = -0.665$ ,  $p < 0.0001$ ). The major ways that C accumulates in the soil is from the accumulation of litter on the surface and root biomass turnover in the soil (Megonigal and Day 1988) coupled by mixing by soil fauna. All of these input mechanisms can be retarded when  $D_b$  is in excess. When roots can't penetrate dense soil, they can't loosen soil or deposit C, and lack of sufficient root penetration may alter plant productivity and thus litter deposition (Brady and Weil 2003).

### **Nitrogen and Phosphorus**

In both the 0-10 cm and 10-20 cm soil layers, CFWs in Virginia have <50% the %N of NRWs, indicating major differences between CFWs and NRWs. N is documented as the major limiting nutrient to forest productivity globally (Kimmins 1987) and in temperate North America, most swamps are N limited (Bedford et al. 1999). Furthermore, N limitation has been shown to reduce productivity and therefore C storage in soils (van Groenigen et al. 2006) including in restored wetlands (Knops and Tilman 2000). This may have a pronounced effect in created wetlands, which often have low levels of N (Craft et al. 2002; Anderson et al. 2005; Fennessy et al. 2008; Cummings 1999; Bruland et al. 2009; Moser et al. 2009; Dee and Ahn 2012). Fennessy et al. (2008) found that nitrogen levels were 4 times lower in young created wetlands than reference sites. Humans have doubled the amount of N in the biosphere causing the eutrophication of coastal waters (Galloway et al. 2003; NRC 2000). In the Chesapeake Bay watershed, sequestering nutrients in wetlands is of particular importance in reducing eutrophication in accordance with the Chesapeake Bay Agreement (Chesapeake Bay 2000 Agreement, Subsection 2.3

However, our CFWs increased %N in the 0-10 cm soil by 260% and 190% in 11 yo and 20 yo CFWs respectively over 8 years. Similarly, in the 10-20 cm depth %N was significantly higher in the 20 yo CFWs, potentially indicated development.

In many young wetlands, the most common removal mechanism for nitrogen is

assimilatory reduction (Mitsch et al. 2012), where it is stored in soil organic matter (Bowden 1984, Craft et al. 1991). This process is likely occurring rapidly in our CFWs, and our findings differ drastically from the findings of Cummings (1999) who found that the low levels found in her sites did not increase over 5 years of development. Moreno-Mateos et al. (2012) found that N increased slowly, but continuously in wetlands in their meta-analysis, but remained below reference levels. Another interesting finding was that 11 yo CFWs now have significantly higher %N than their 12 yo counterparts from 2004. This may indicate that construction practices are improving in CFWs in Virginia, and that soil construction best management practices have significantly improved in the storage of nitrogen.

In the surface 10 cm, 20 yo CFWs had similar %P to the NRWs, while 11 yo CFWs had significantly lower %P, indicating that our CFWs accumulated surface P at a pace rapid enough to become similar to natural wetlands within 20 years. Moreno-Mateos et al. (2012) found that P was relatively similar to NRWs almost immediately, a finding they attributed to P's lack of cycling with the atmosphere (Smil 2000). An insignificant decrease in surface N:P ratios despite rapid %N increase bolsters this theory. However, at 10-20 cm, %P was significantly lower in both age groups and N:P increased with age, indicating that P increases may be limited to surface deposition since P accumulates in wetlands in association with deposited sediments, adsorption to minerals in the soil, and biological assimilation into OM (Khalid et al 1977). Indications of P increase agree with the idea that CFWs often have higher sedimentation rates than natural wetlands (Johnston 1991, Peterjohn and Correll 1994, Craft and Casey 2000), therefore our CFWs were able to catch up to NRW levels of %P within 20 years.

### **Nutrient Ratios**

In our study, current 11 and 20 yo CFWs had statistically similar average C:N ratios to NRWs. Each group of wetlands were dissimilar to NRWs in 2004, but over 8 years they became similar to NRWs through significant decreases in C:N ratio from 2004 to 2012. The older CFWs had significantly higher C:N than NRWs when measured in 2004, but became statistically similar over 8 years. Similarly, there was a weak but significant negative relationship between CFW age and C:N ratio. This finding differs

from Nair et al. (2001) that found young created wetlands to have low C:N ratios.

Interestingly, at 10-20 cm depth, the pattern is switched, with C:N ratios insignificantly lower in the CFWs than NRW, potentially because of low %C and the leaching of surface N. Similarly, CN displayed a weak but significant relationship with age in the top 10 cm, but not at depth.

C:N ratio has important implications for the palatability of organic material to consumers, the ability for plants to take up nutrients and the decomposition of soil (Schlesinger, 1997; Wolf & Wagner, 2005; Berg & McLaugherty, 2008). Bacterial community structure is related to C:N ratio in a number of soils (Hartman et al., 2008, Ahn and Peralta, 2009 and Lauber et al., 2009), including created mitigation wetlands in Virginia (Peralta et al. 2013), thus the lack of significant difference between CFWs and NRWs and the pattern toward becoming more similar are positive findings for the replacement of function in CFWs.

There was no significant relationship between age of CFW and C:P or N:P ratio from 0-10 cm depth, nor were there differences between CFW age classes and NRWs in the 0-10 cm soil depth. However there was a difference in C:P and N:P ratios in the 10-20 cm soil depth between 11 yo CFWs and NRWs, but not between 20 yo CFWs and NRWs. These findings indicate that CFWs start out with dissimilar nutrient ratios at depth that equilibrate with NRWs with time. Similarly, there is a significant positive relationship between N:P ratio and CFW age at depth. Young soils tend to have little N, and during early soil development they tend to experience rapid addition of N and C (Sterner and Elser 2002; Mitsch and Gosselink 2007). On the other hand, N:P is (insignificantly) negatively related to age at the surface, perhaps indicating that while %N is increasing rapidly at the surface, P is being deposited at a faster rate, potentially due to the high deposition rate noted for young CWs. (Fennessy et al. 1994; Braskerud 2001; Harter and Mitsch 2003; Craft 2003; Johnston 1991; Peterjohn and Correll 1994; Craft and Casey 2000). This trend is not reflected at depth, probably because most P deposition occurs near the surface, and P is not mobile in these systems.

## **Conclusions**

11 yo CFWs held a lower percentage of C, N, and P than NRWs in both the 0-10



and 10-20 cm depths, displaying that CFWs may not provide functional replacement after 11 years. Soil %C %P,  $D_b$ , and C:N, C:P and N:P ratios in the top 10 cm of CFWs developed to statistically similar levels to natural wetlands within 20 years, indicating that, forested CFWs in Virginia can begin to replace some aspects of wetland functions within 20 years. However, total C storage and %N remained significantly lower in CFWs than NRWs after 20 years. In the 10-20 cm soil depth, both age groups of CFWs were dissimilar in %C, %N, %P and  $D_b$ , while 11 yo CFWs were dissimilar in C:P and N:P as well.

C storage in the top 20 cm of the soil was lower in CFWs than NRWs, and when the C stored in woody biomass is incorporated into the comparison, CFWs held less C than their natural counterparts after 20 years, even when a 2:1 mitigation ratio is incorporated. Thus at best, every time the wetland mitigation process occurs, the net C storage of wetlands in Virginia may be reduced for more than 20 years, potentially leading to a consistent loss of wetland function (Bendor 2009), and at worst, created wetlands in Virginia may never reach C equivalency.

These findings indicate positive progression toward natural wetland functions, but also indicate that soils remain dissimilar after 20 years. More research is needed to determine if these differences between CFWs and NRWs indicate the creation of wetlands leading to alternative stable states (Hobbs et al. 2009; Suding and Hobbs 2009), or if CFWs will become like NRWs with time. At the least, these findings illustrate the temporal lag in functional replacement inherent in the mitigation system that likely creates “consistent and considerable” functional loss (Bendor 2009). Soil development inherently takes time as incorporation of organic matter, soil oxidation-reduction, and weathering are time dependent processes (Jenny 1941; Stevens and Walker 1970; Mausbach and Richardson 1994), thus, the creation of CFWs in Virginia does not necessarily lead to “no net loss” of function within 20 years.

**Table Captions**

Table 3.1: Results of Linear regression in created forested wetlands (CFWs) of Soil parameters of percent carbon (%C), percent nitrogen (%N), percent phosphorus (%P), Bulk Density ( $D_b$ ) based on age of individual CFW.

Table 3.2: Soil carbon storage based on %C and  $D_b$ , expressed in Mg/ha in each soil depth 0-10 cm and 10-20 cm.

Table 3.3: Natural reference wetland (NRW) soil accretion and carbon sequestration.

Table 3.4: Carbon sequestration in NRWs by province.

**Figure Captions**

Figure 3.1: Results for bulk density among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) groups in the 0-10 cm soil depth.

Figure 3.2: Results for bulk density among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) groups in the 10-20 cm soil depth.

Figure 3.3: Results for percent carbon among 3 yo CFWs from 2004 (3,'04), the same sites 8 years later in 2012 (11,'12), 12 yo CFWs from 2004 (12, '04), the same sites 8 years later in 2012, when they are 20 years old (20,'12) in the 0-10 cm soil depth.

Figure 3.4: Results for percent carbon among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.5: Results for percent nitrogen among 3 yo CFWs from 2004 (3,'04), the same sites 8 years later in 2012 (11,'12), 12 yo CFWs from 2004 (12, '04), the same sites 8 years later in 2012, when they are 20 years old (20,'12) in the 0-10 cm soil depth.

Figure 3.6: Results for percent nitrogen among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.7: Results for percent total phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 0-10 cm soil depth.

Figure 3.8: Results for percent total phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.9: Results for mass ratio of carbon to nitrogen among 3 yo CFWs from 2004 (3,'04), the same sites 8 years later in 2012 (11,'12), 12 yo CFWs from 2004 (12, '04), the same sites 8 years later in 2012, when they are 20 years old (20,'12) in the 0-10 cm soil depth.

Figure 3.10: Results for mass ratio of carbon to nitrogen among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.11: Results for mass ratio of carbon to phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 0-10 cm soil depth.

Figure 3.12: Results for mass ratio of carbon to phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.13: Results for mass ratio of nitrogen to phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 0-10 cm soil depth.

Figure 3.14: Results for mass ratio of nitrogen to phosphorus among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil depth.

Figure 3.15: Results for total carbon stock in Mg/ha among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 0-10 cm soil.

Figure 3.16: Results for total carbon stock in Mg/ha among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 10-20 cm soil.

Figure 3.17: Results for total carbon stock in Mg/ha among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the 0-20 cm soil.

Figure 3.18: Results for total carbon stock in Mg/ha among current 11 year old (yo) created forested wetlands (CFWs) (11,'12), current 20 yo CFWs (20,'12) and Natural Reference Wetlands (NRWs) in the soil and in woody biomass combined in a stacked bar graph.

## Tables

**Table 3.1**

Linear Regression CFW Soil Parameters based on Age		
Soil Parameter	R <sup>2</sup>	P
0-10 cm		
%N	0.37	<0.0001
%P	0.068	0.074
%C	0.28	0.0001
Bulk Density	-0.16	0.01
CN	-0.07	0.014
CP	-0.0312	0.983
NP	-0.031	0.993
Total C	-0.0283	0.7663
10-20 cm		
%N	0.1416	0.0162
%P	-0.03	0.851
%C	0.076	0.063
Bulk Density	-0.021	0.579
CN	-0.031	0.93
CP	0.089	0.054
NP	0.103	0.036
Total C	0.124	0.023

**Table 3.2**

Soil Carbon storage Mg/ha			
	11 yo CFW	20 yo CFW	NRW
Soil C storage 0-10cm	26.73	24.22	33.46
SE	3.71	1.39	3.28
Soil C storage 10-20 cm	11.36	14.50	24.04
SE	3.30	1.10	3.30
Soil C Storage 0-20 cm	37.25	38.87	57.50
SE	3.47	2.29	9.76

**Table 3.3**

NRW Accretion						
	Accretion since 1948	Accretion per year	Accreted grams C/ m <sup>2</sup> since 1964	Accreted grams C/ m <sup>2</sup> / year	Accreted C Mg/ha since 1964	Accreted C Mg/ ha/ yr
Mean	10.33	0.22	3077.72	64.12	30.77	0.64
SE	2.29	0.05	785.30	16.36	7.85	0.16

**Table 3.4**

Comparison of Carbon sequestration by province Mg/ha		
	Coastal Plain	Piedmont
Average since 1964	47.27	14.29
SE	0.36	0.68



## Figures

Figure 3.1

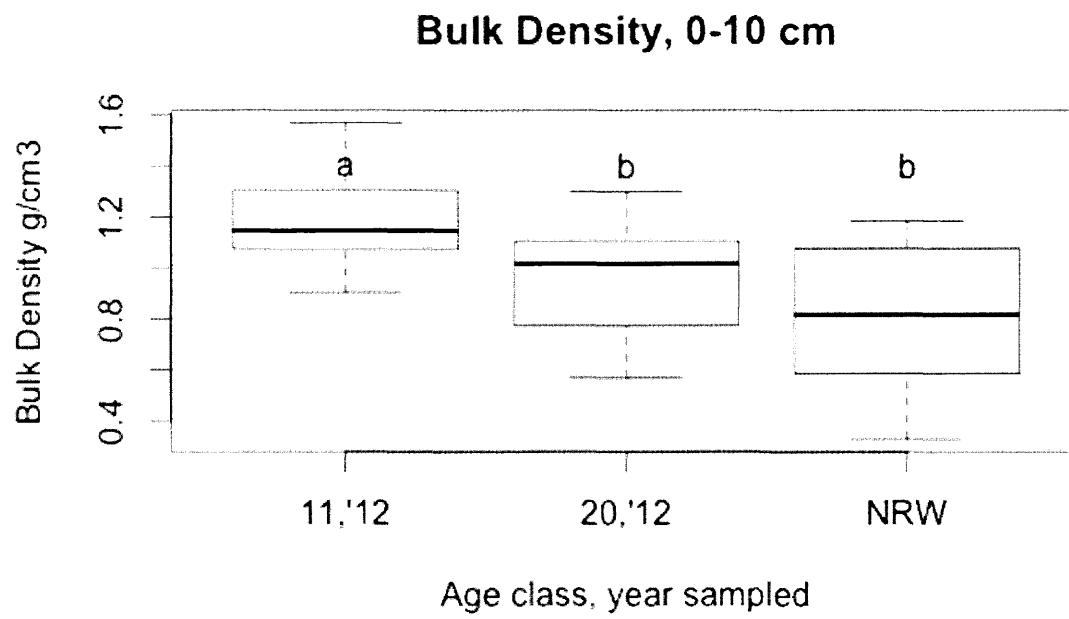


Figure 3.2

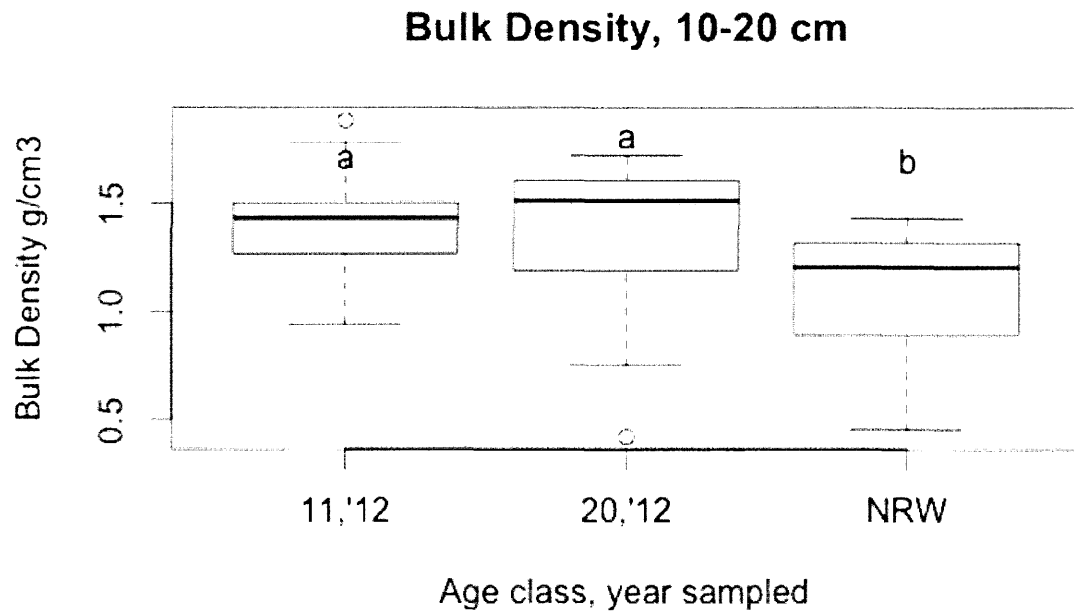


Figure 3.3

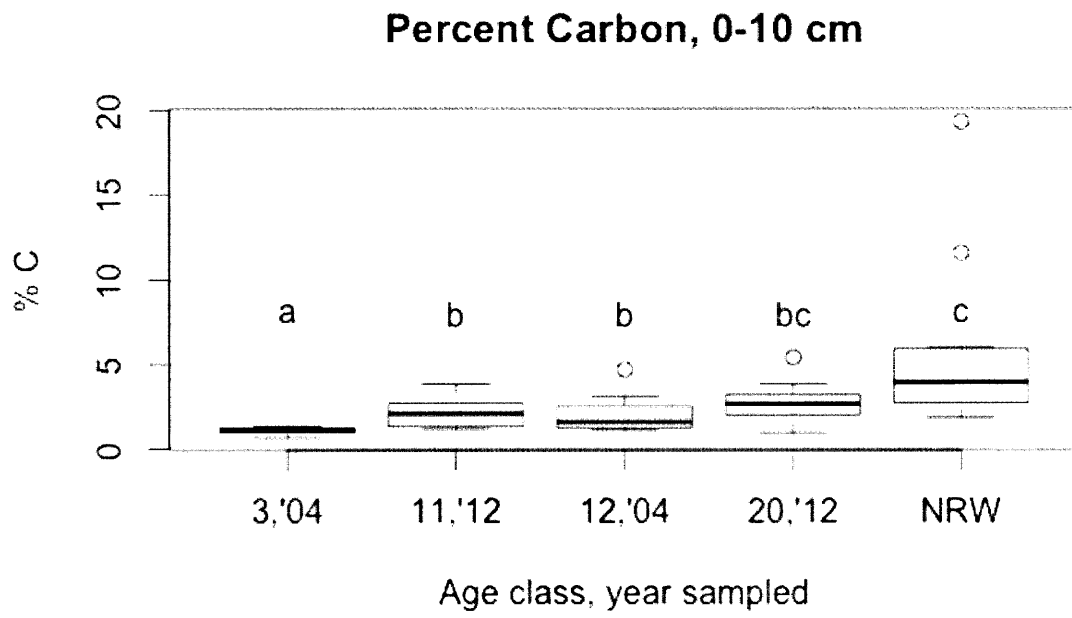


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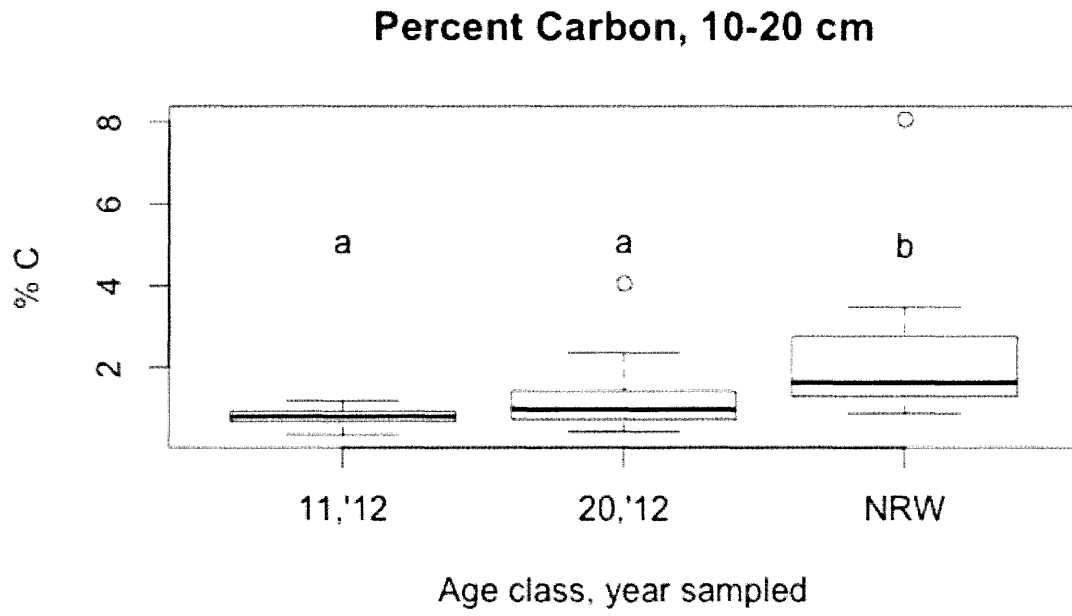


Figure 3.5

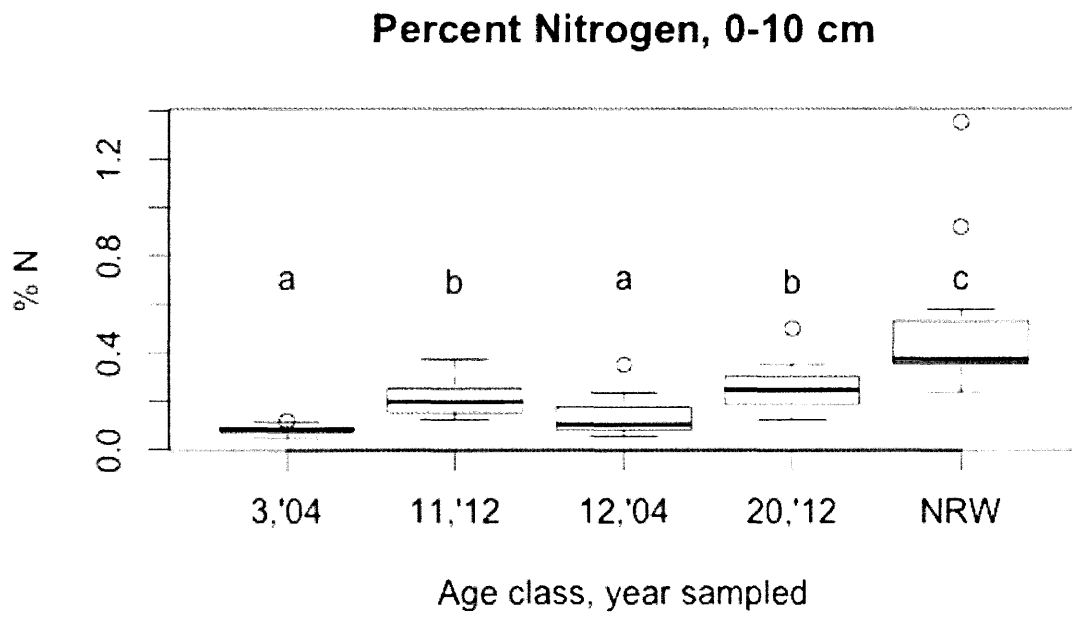


Figure 3.6

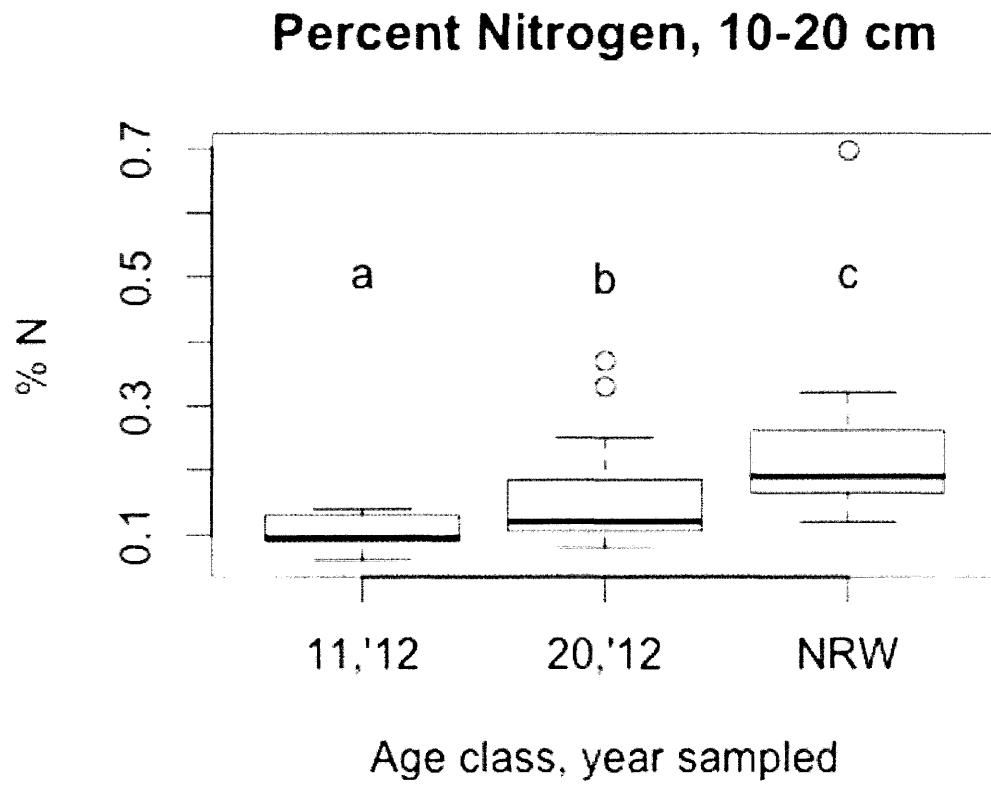


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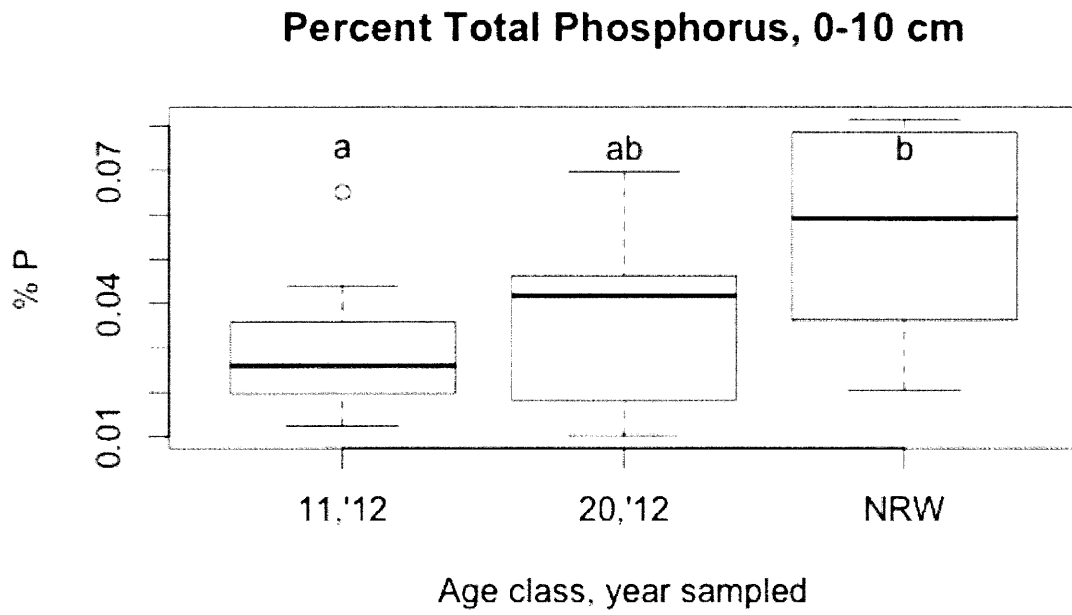


Figure 3.8

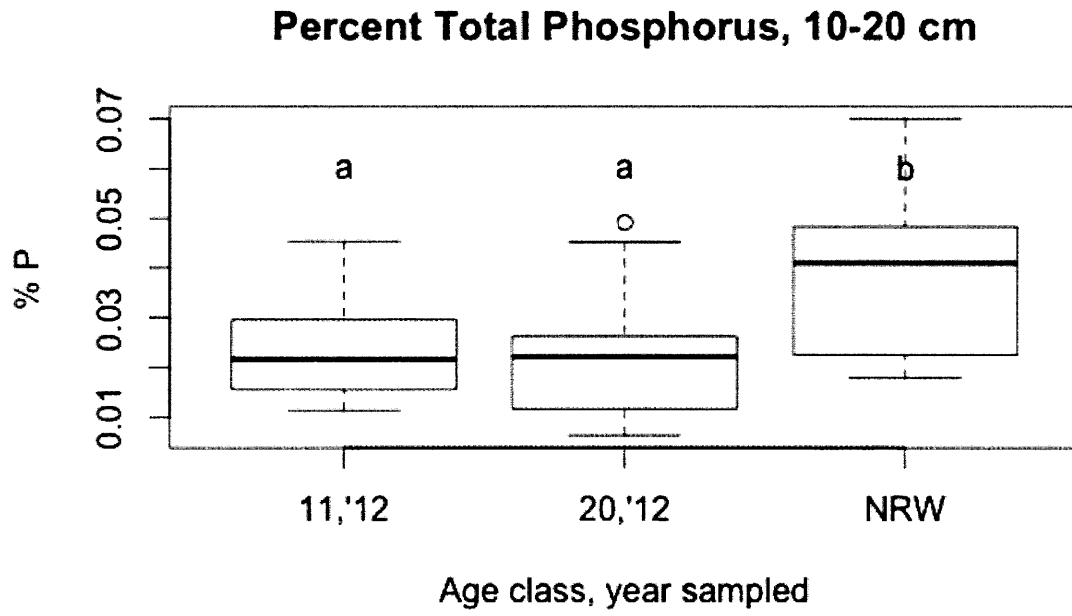




Figure 3.9

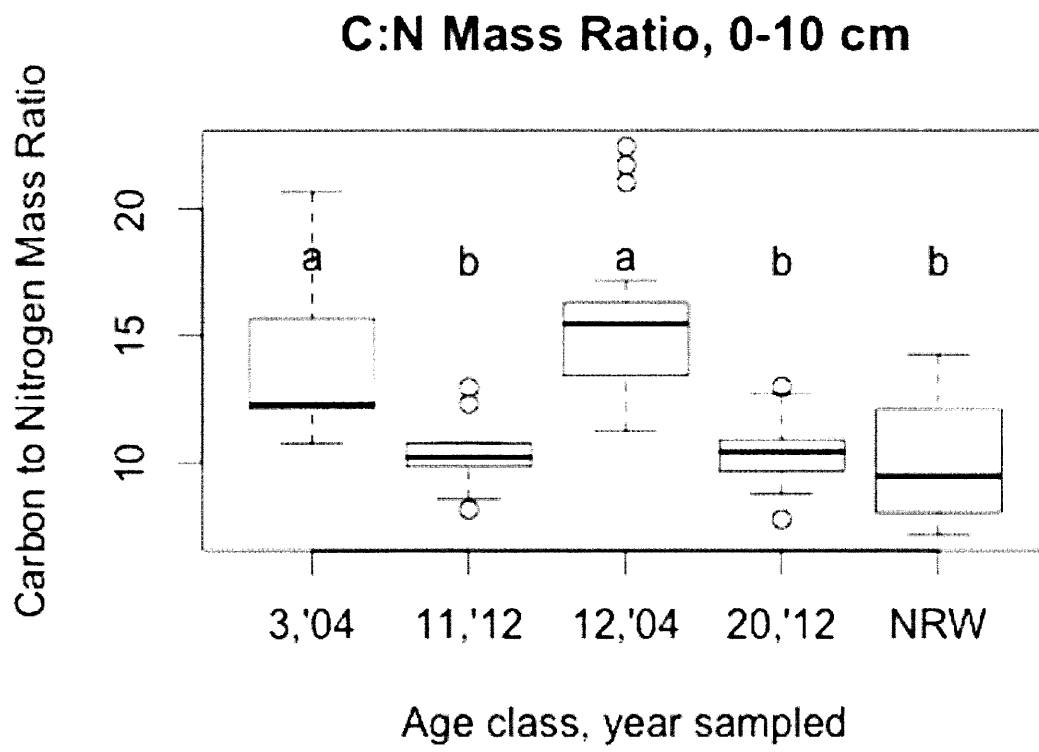


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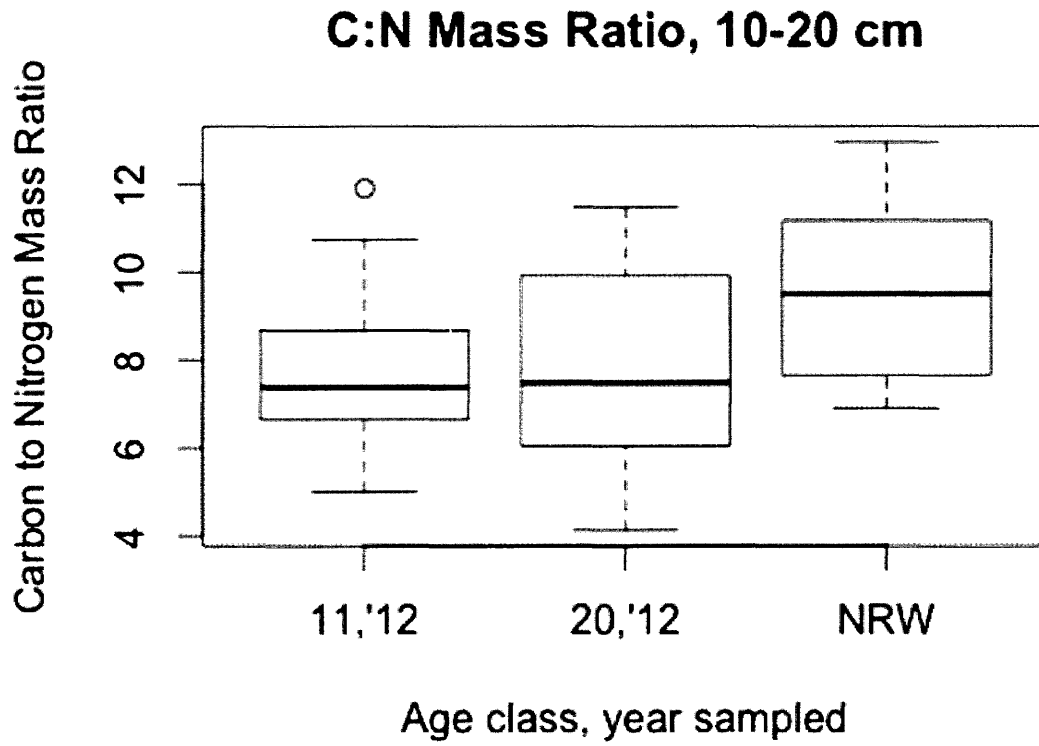


Figure 3.11

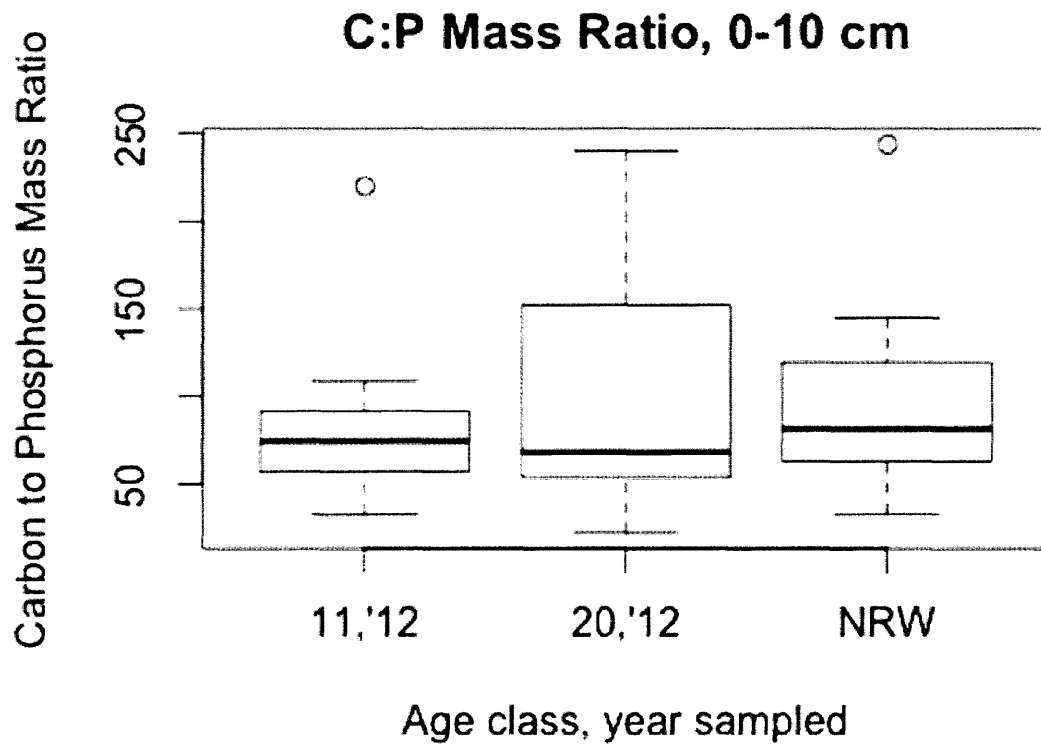


Figure 3.12

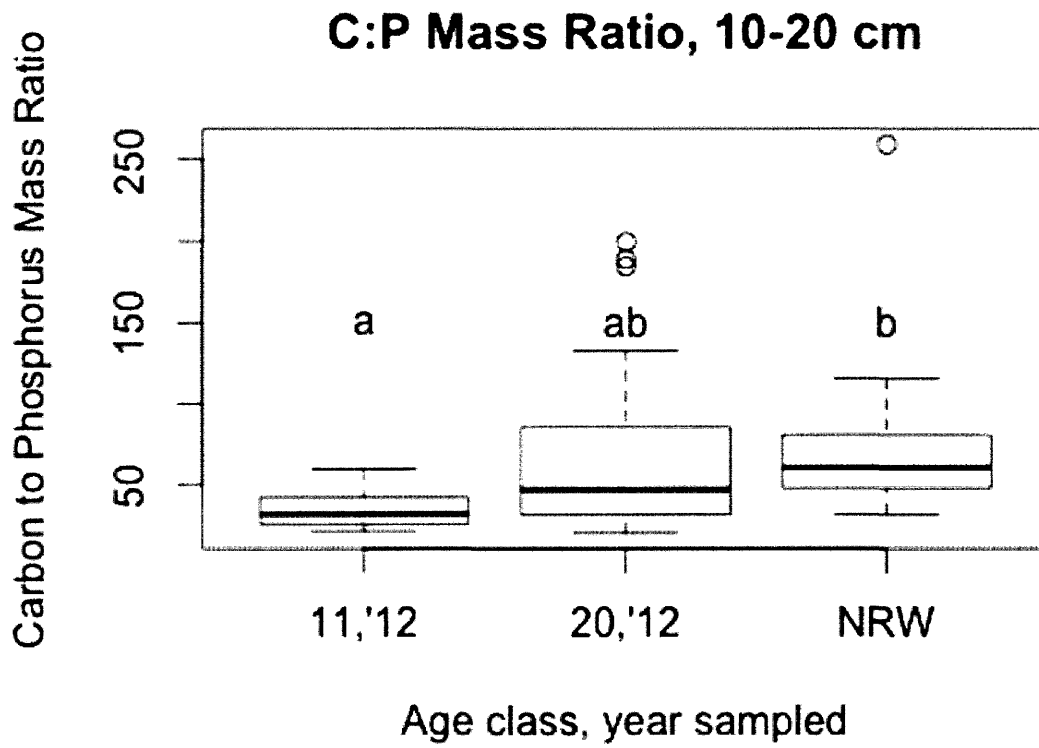


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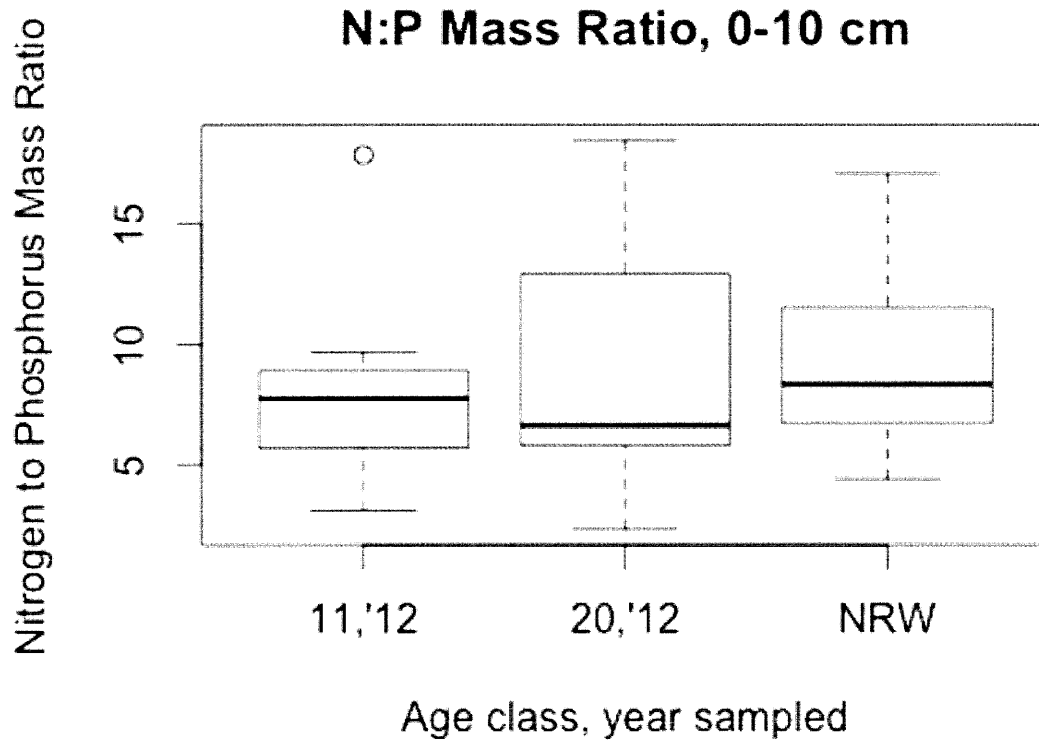


Figure 3.14

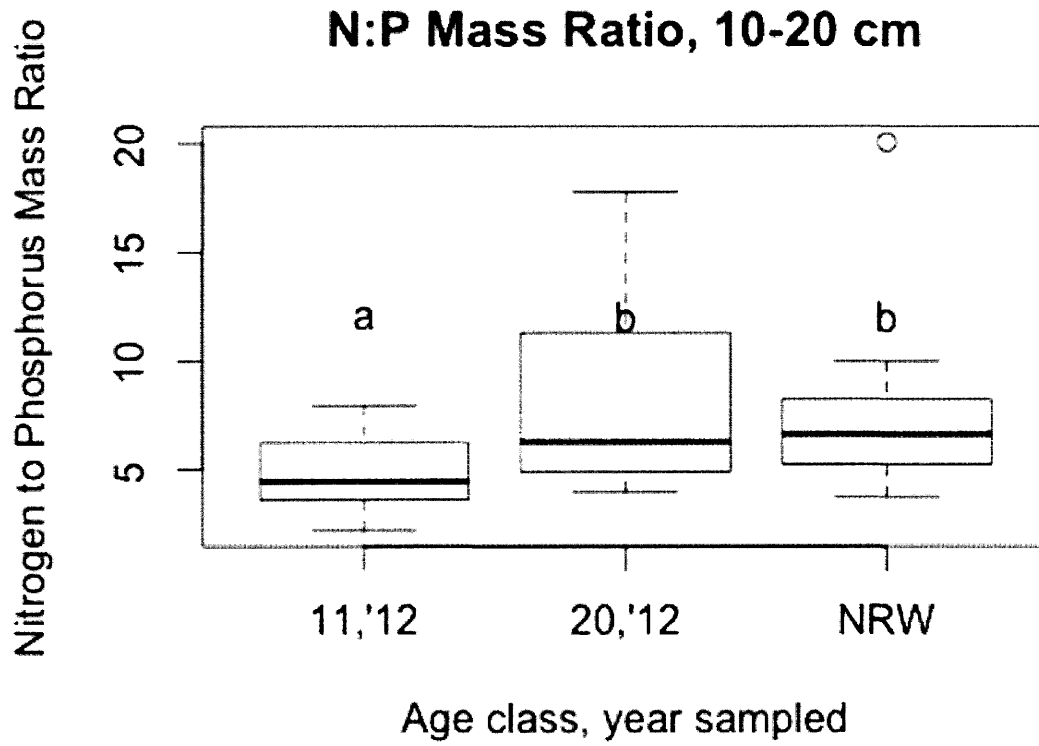


Figure 3.15

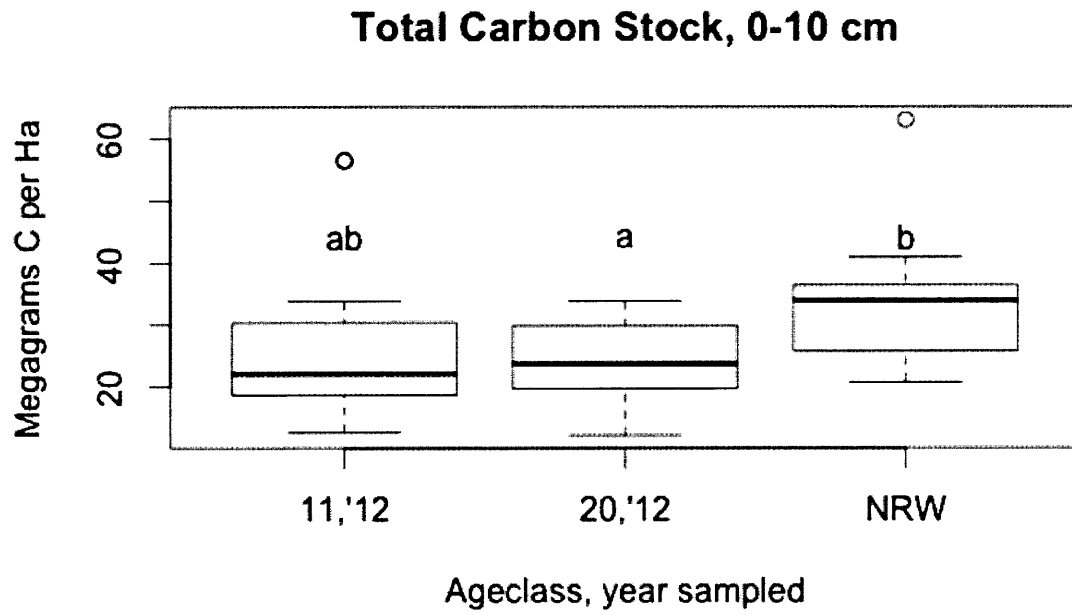


Figure 3.16

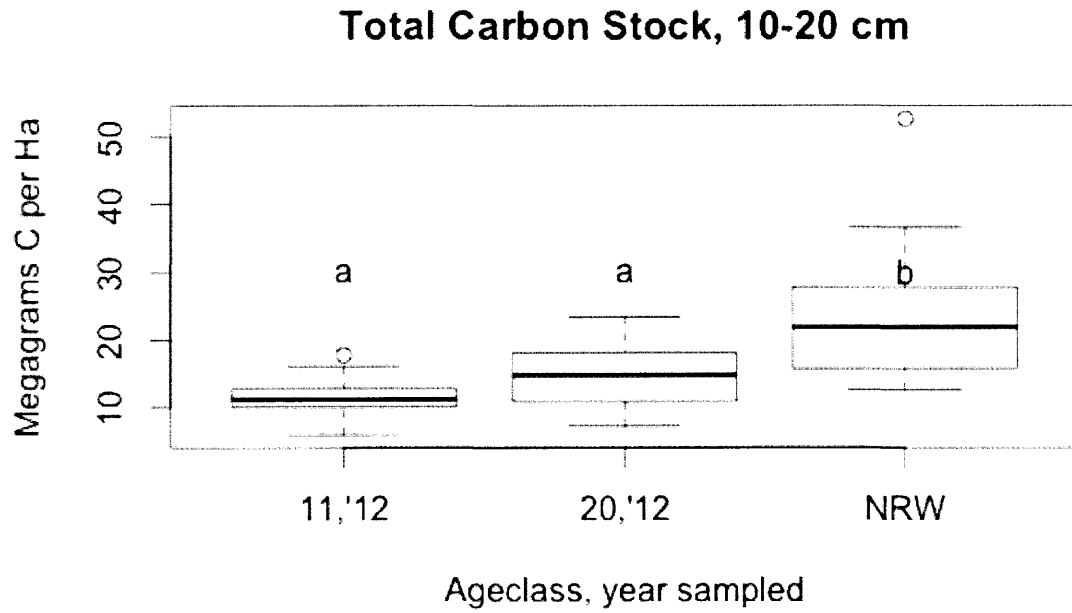




Figure 3.17

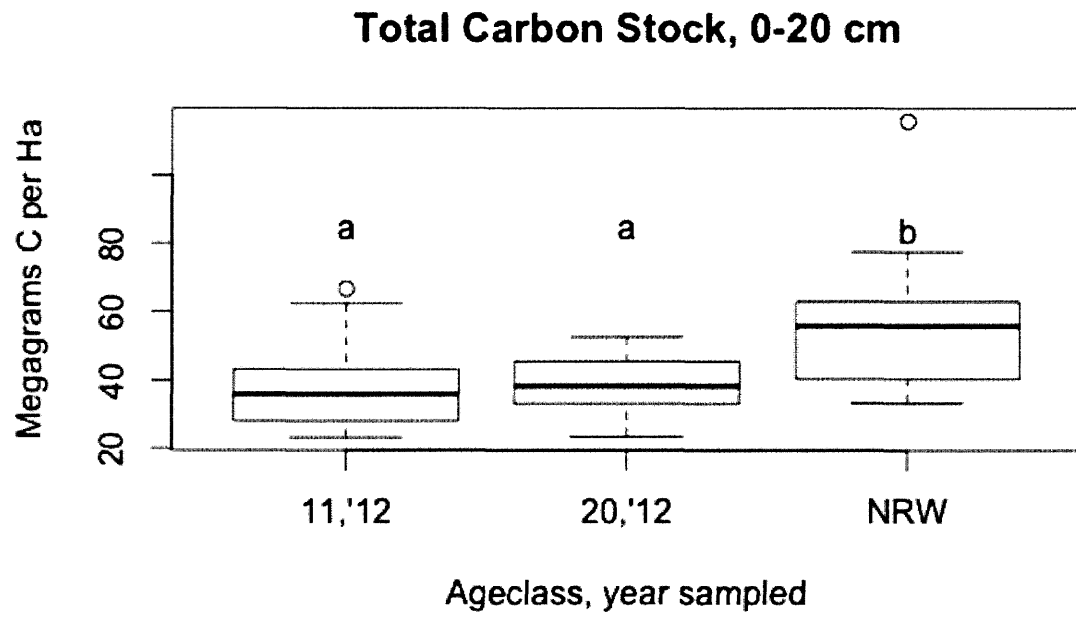
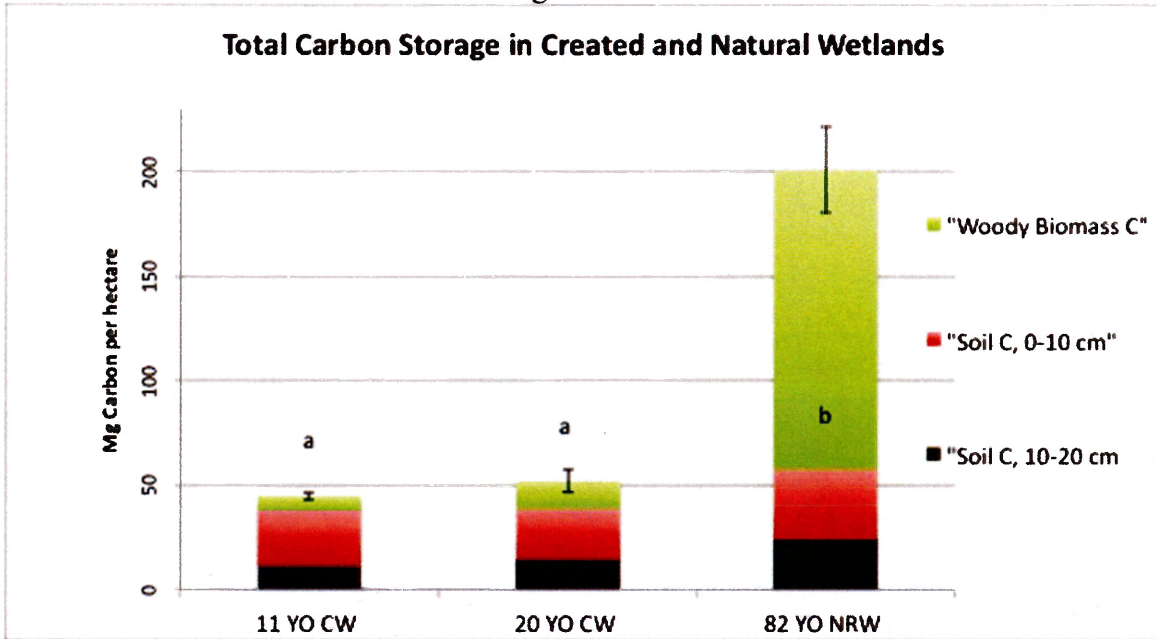


Figure 3.18



### Summary

Mitigation of wetland impacts through the Clean Water Act has become a huge undertaking in the United States, costing 2.4 billion dollars per year (ELI 2007) and leads to the replacement of 77 hectares of palustrine forested wetlands (PFWs) per year with mitigation wetlands, many of which are created forested wetlands (CFWs) (VADEQ 2012). The mitigation practice hinges on the idea that CFWs (and other wetland mitigation efforts) lead to “no net loss of area or function” when compared to the wetland that was impacted (NRC 2001; USACOE 2002; 33 U.S.C. 1344). Because pre-impact monitoring rarely occurs, natural adjacent wetlands are used as a proxy for comparison (LePage 2011). Therefore, the ultimate test of ecological success in CFWs is whether they function as natural wetlands (van der Valk 1994; Brinson and Rheinhardt 1996; Rheinhardt et al. 1999; Whigham 1999).

Functions were assessed through both soil and vegetation parameters and focused on the habitat function of “maintenance of a characteristic plant community” and the biogeochemical functions of: the production of biomass, the retention and removal of nutrients and the accumulation of soil C (NRC 1995).

In this study we utilized 7 CFWs (three 11 yo and four 20 yo) that had all been previously sampled 8 years previously in 2004) to compare to natural reference wetlands (NRWs) and to assess for development over time. Our goals were to 1) to determine whether created palustrine forested wetlands (CFWs) in Virginia mitigate lost ecological functions within an 11 and 20 year time frame 2) to determine how created wetlands change functionally over time and 3) to compare plant community indices to the sequestration of carbon to determine whether the goals of providing high levels of habitat function and biogeochemical function are mutually attainable. Finally we use our data to assess whether CFWs continue to fulfill regulatory performance standards 10 years after cessation of the monitoring period.

CFWs had similar plant communities to NRWs in the herbaceous and shrub/sapling layer in terms of species richness (SR), Shannon Diversity, %Non-Native species (%NN), Dominance of Non-Native species (DNN) or Floristic Quality Index (FQI), indicating that CFWs can replace habitat function in the herbaceous and shrub/sapling layers within 11 years. However, DNN showed a significant positive

linear relationship with age among 11 yo CFWs to 20 yo CFWs and should be further investigated to determine if CFWs trend toward increased dominance by non-natives with age. Furthermore, in the tree strata, 11 yo CFWs had lower SR than NRWs and both age classes of CFWs had lower FQI than NRWs. Major differences existed in woody biomass, as NRWs held 10 times more carbon than 20 yo CFWs and nearly 20 times more carbon than 11 yo CFWs. The tree species composition was significantly different between CFWs and NRWs according to ANOSIM, however when the Tree strata of NRWs was compared to the saplings in CFWs, both age groups of CFWs were similar to NRWs.

11 yo CFWs held a significantly lower percentage of C, N and P and had higher bulk density ( $D_b$ ) than NRWs in both the 0-10 and 10-20 cm depth. 11 yo CFWs also had significantly lower C:P and N:P ratios at 10-20 cm, though nutrient ratios were similar to NRWs at 0-10 cm. 20 yo CFWs developed similar levels of %C, %P,  $D_b$ , and nutrient ratios, but offered 45% lower soil C storage and 50% lower %N. Furthermore, both 11 and 20 yo CFWs lagged behind NRWs in %C, %N, and %P in the 10-20 cm soil depth, indicating that changes originate near the soil surface. Rapid improvement in soil function was noted in the 0-10 cm soil depth, as %N increased 190% to 260% in 11 and 20 yo CFWs over 8 years and %C increased 100% and 35% in 11 and 20 yo CFWs, indicating provision of some important biogeochemical functions, but CFWs remained functionally different than NRWs after 20 years.

We found that overall FQI correlated positively with the change in %C in the top 10 cm of the soil ( $r=0.7$ ) and with woody biomass ( $r=0.6$ ), but that neither of these correlations was significant. However, total C accumulation per year in the woody biomass and soil C was significantly correlated with FQI ( $r=0.8$ ), indicating that biogeochemical function and the provision of habitat can be complimentary in CFWs.

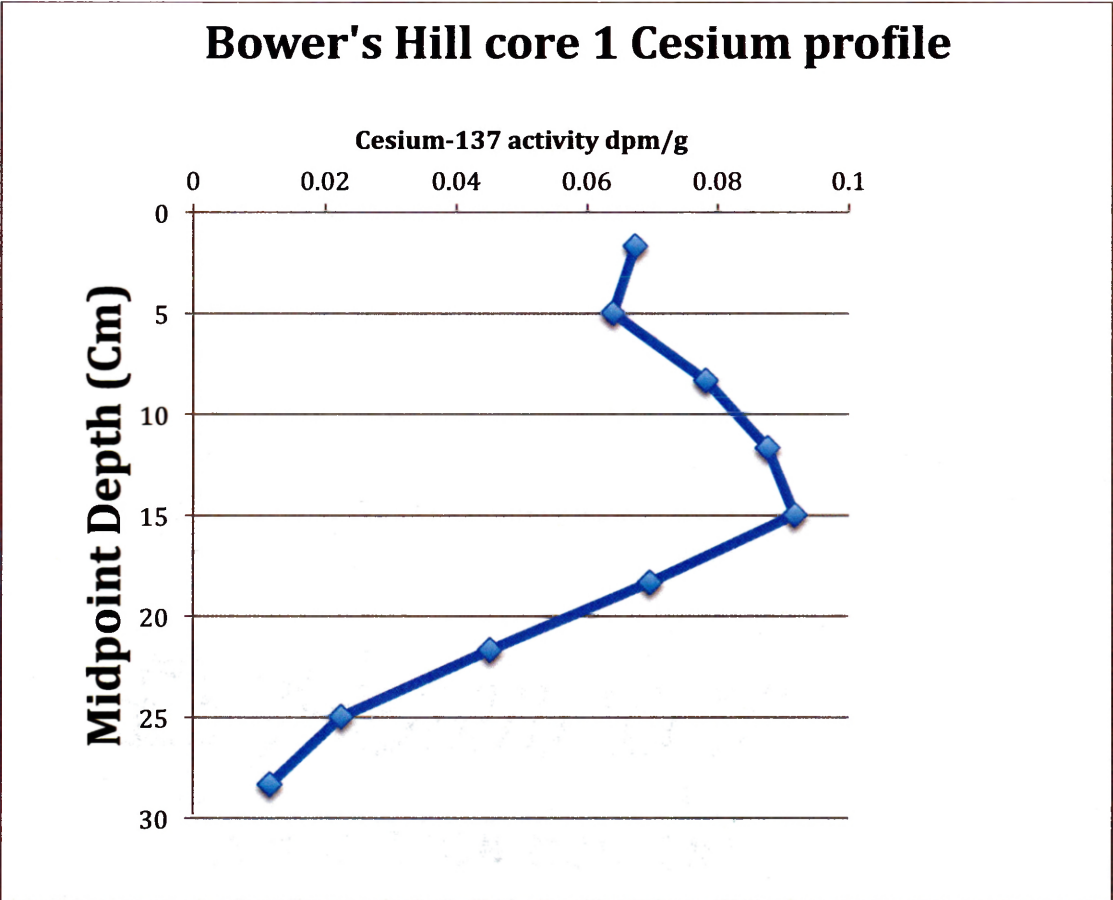
Finally, our data showed that 11 and 20 yo CFWs adhered to the regulatory performance standards established for Virginia in terms of stems per hectare and wetland indicator status, but all wetlands (including NRWs) failed to achieve <5% non-native species cover.

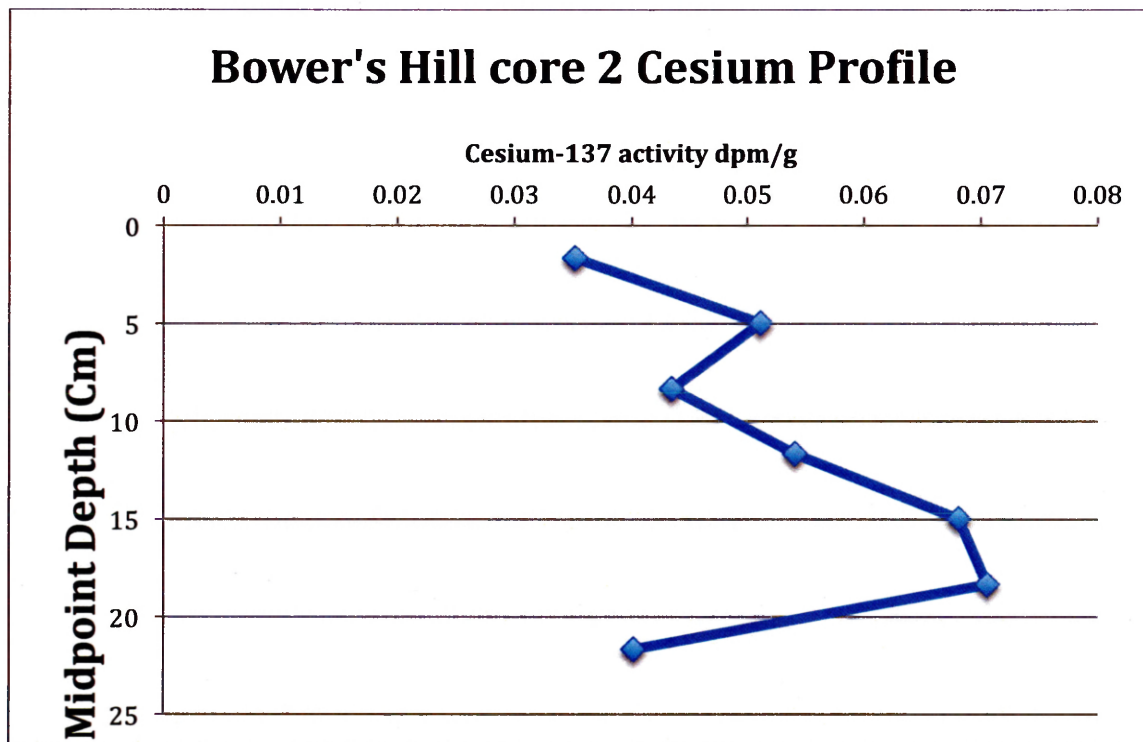
Our data indicate that CFWs provide lower levels of function than NRWs in terms of habitat provision and biogeochemical function after 20 years of development.

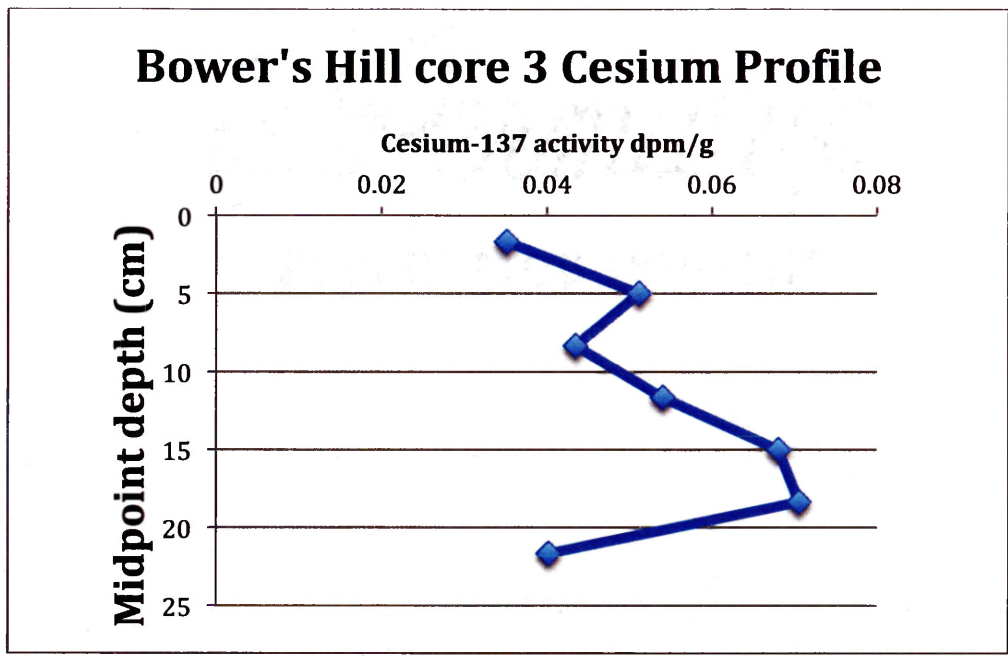
More research is needed to determine if CFWs trend toward an alternative stable state than NRWs or if they become similar to NRWs in time. Regardless, our study indicates that at the very least, the creation of CFWs in the mitigation process leads to a temporal lag in functional replacement that likely creates “consistent and considerable functional loss” on the landscape scale (Bendor 2009).

More research is needed to determine the fate of ecosystem functions of CFWs in the future. If and when CFWs restore the lost functions associated with habitat and biomass accumulation in NRWs was not answered through this study. Neither was how long the accumulation of C and N will take to reach reference levels. Our findings show that NRW functional levels were not reached in 20 years for some important parameters, and therefore researchers should follow CFWs for longer time periods. Furthermore, high variability among and even within CFWs provide research opportunities to learn how to improve construction and management practices as well as to answer ecological questions in manipulated conditions.

Appendix 1

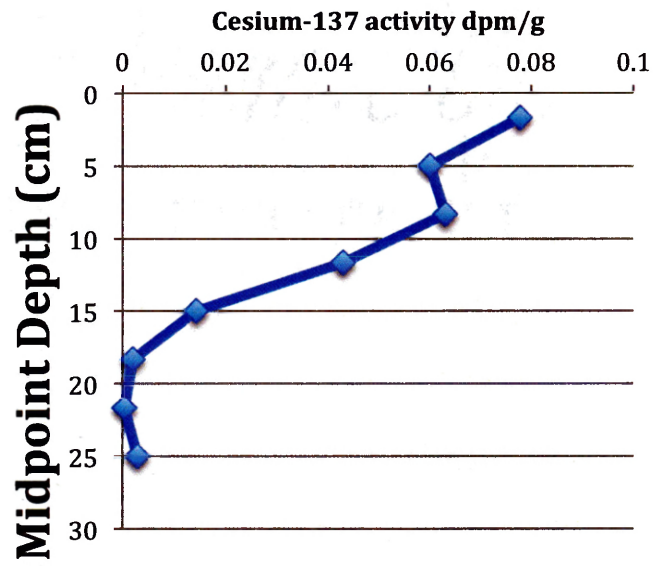


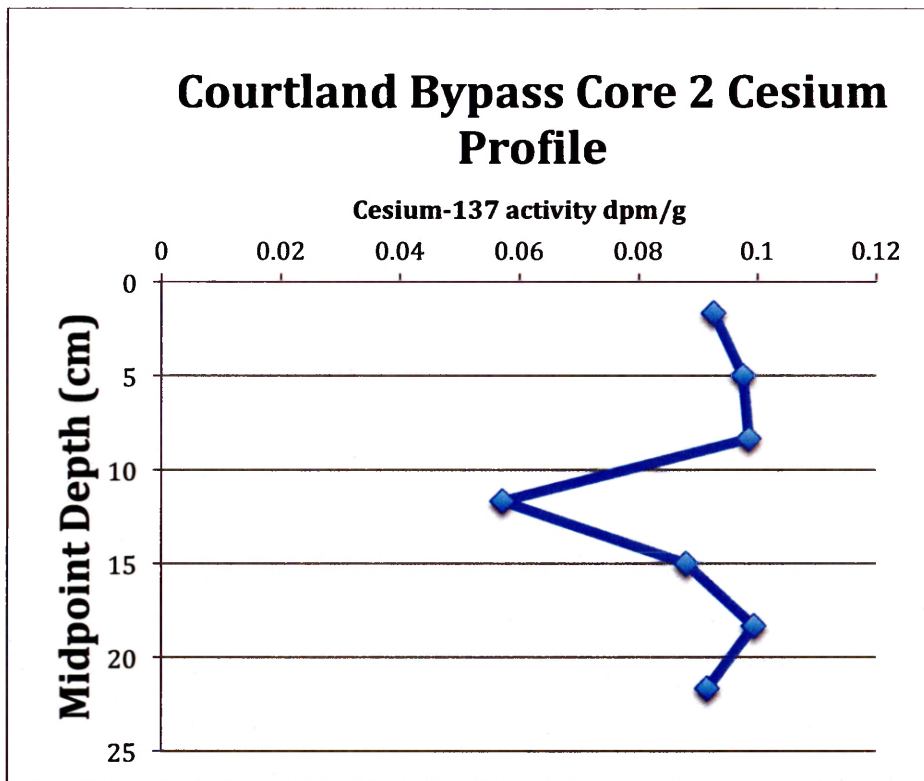


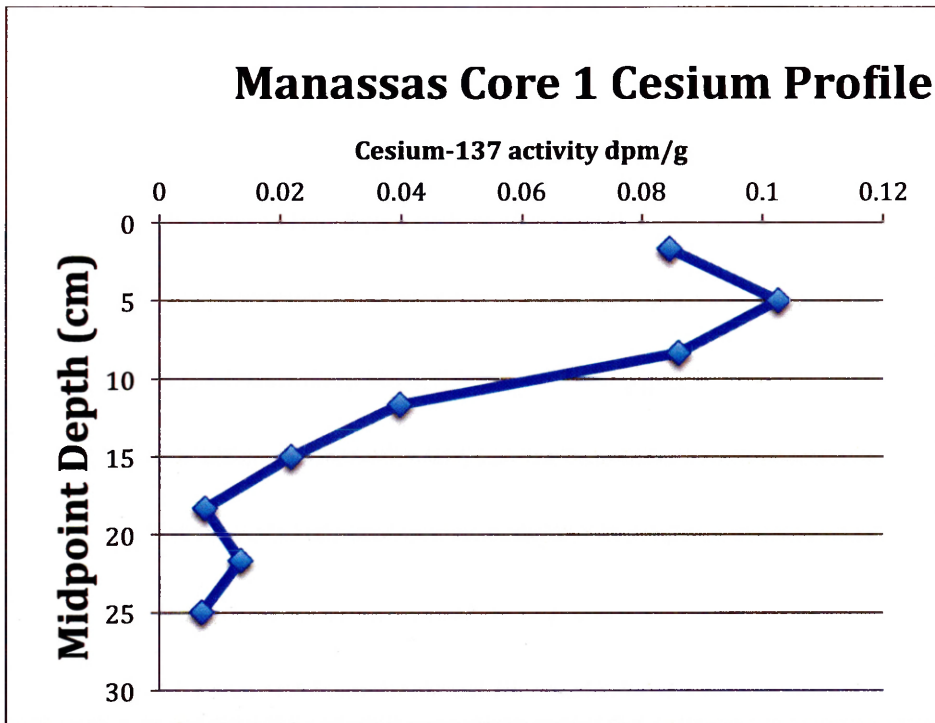


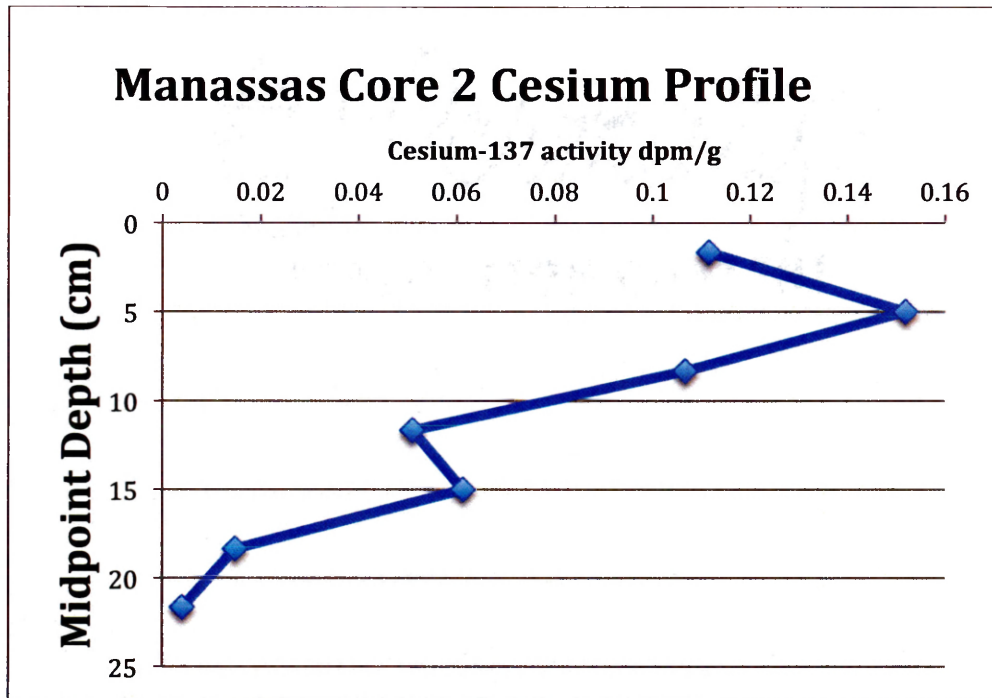


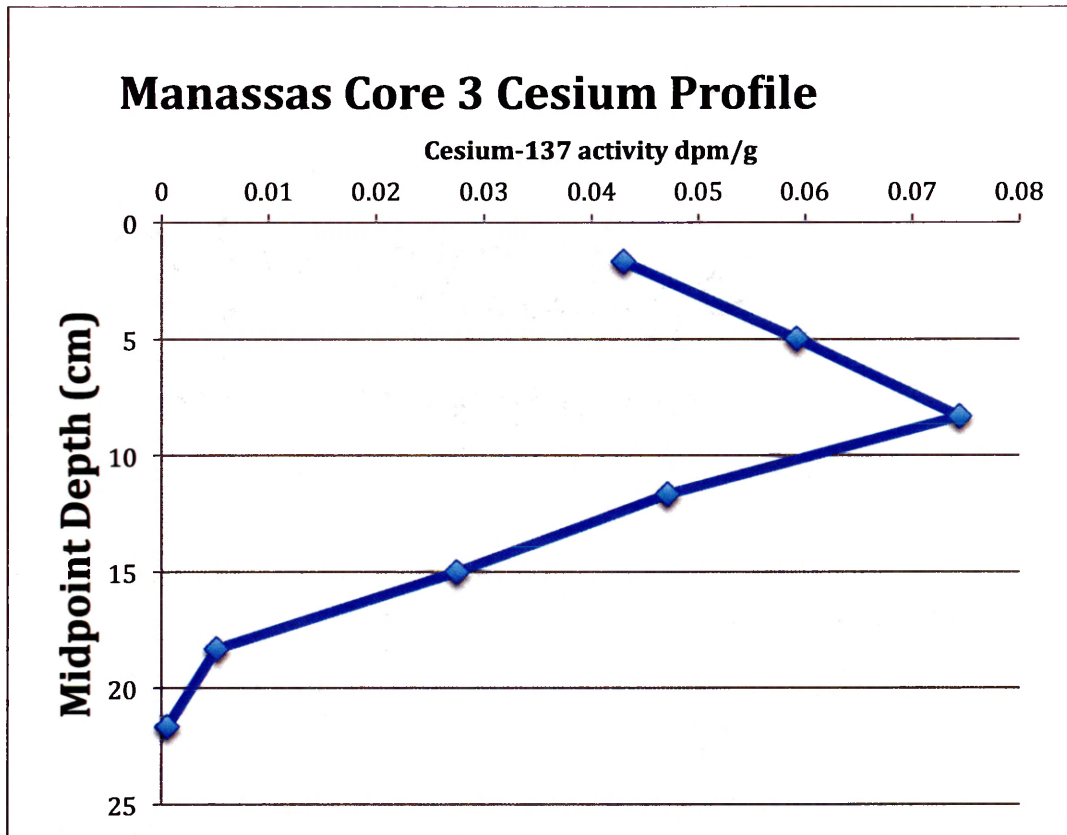
### Courtland Bypass 1 Total Activity (dpm/'g)

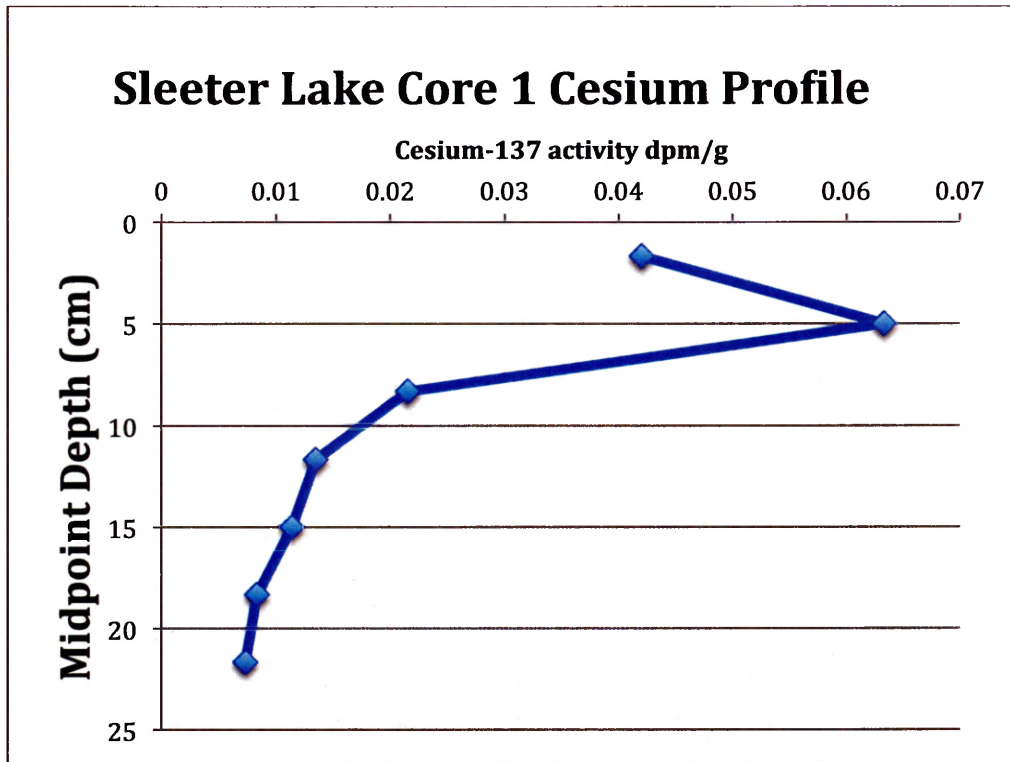


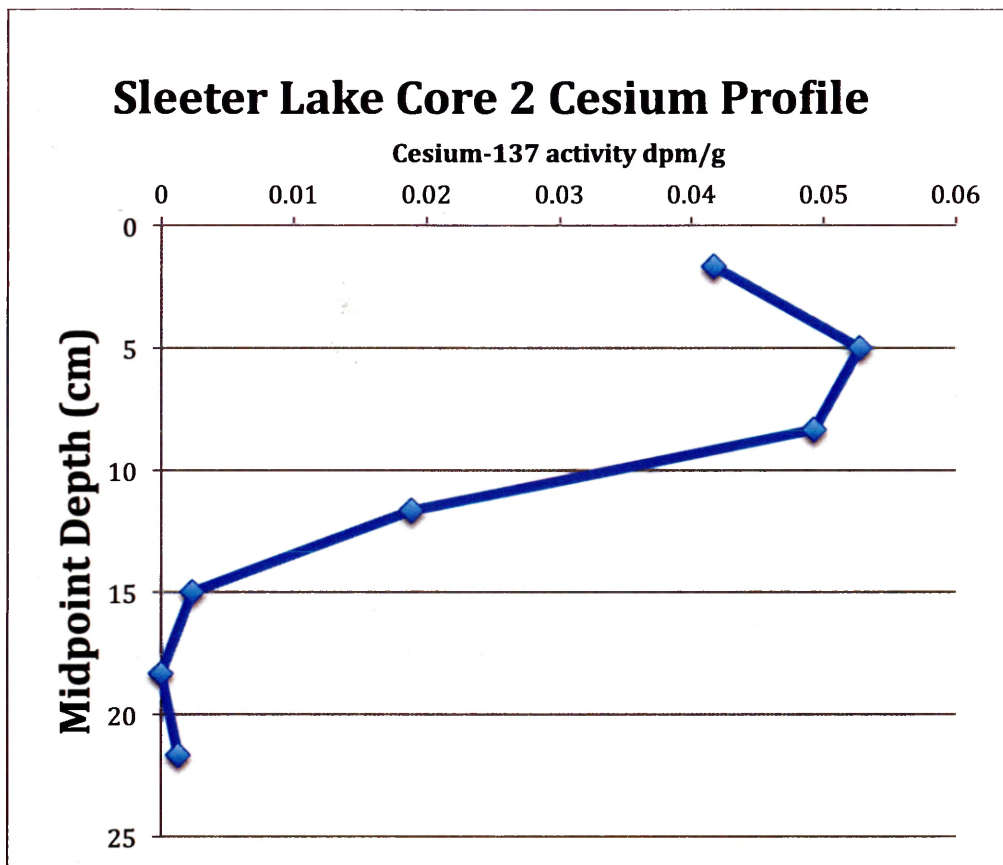












Appendix Table 1

Cesium Activity					
Site	Core#	Depth (cm)	Midpoint depth	Total Activity (dpm/'g)	Error (dpm/g)
Bower's Hill	1	0 to 3.33	1.66	0.067395687	0.003951887
Bower's Hill	1	3.33 to 6.66	4.99	0.063984845	0.003991257
Bower's Hill	1	6.66 to 9.99	8.33	0.078254481	0.003741676
Bower's Hill	1	9.99 to 13.33	11.66	0.087643021	0.00434677
Bower's Hill	1	13.33 to 16.66	14.99	0.091788377	0.00394991
Bower's Hill	1	16.66 to 20	18.33	0.069661702	0.004007934
Bower's Hill	1	20 to 23.3	21.66	0.045169941	0.003412441
Bower's Hill	1	23.33 to 26.66	24.99	0.022500009	0.002801206
Bower's Hill	1	26.66 to 30	28.32	0.011649935	0.00215444
Bower's Hill	2	0-3.33	1.66	0.035147545	0.002995029
Bower's Hill	2	3.33-6.66	4.99	0.051154486	0.00371513
Bower's Hill	2	6.66-10	8.33	0.043470303	0.002985085
Bower's Hill	2	10-13.33	11.66	0.054101602	0.003161195
Bower's Hill	2	13.33-16.66	14.99	0.068094138	0.003608894
Bower's Hill	2	16.66-20	18.33	0.070560995	0.003248971
Bower's Hill	2	20-23.3	21.66	0.040173345	0.002531247
Bower's Hill	3	0-3.33	1.66	0.051050243	0.003977941
Bower's Hill	3	3.33-6.66	4.99	0.046420736	0.00362662
Bower's Hill	3	6.66-10	8.33	0.0463184	0.003370362
Bower's Hill	3	10-13.33	11.66	0.057850745	0.003546018
Bower's Hill	3	13.33-16.66	14.99	0.06480137	0.003240068
Bower's Hill	3	16.66-20	18.33	0.06860266	0.00362367
Bower's Hill	3	20-23.3	21.66	0.069329034	0.003067656
Courtland	1	0-3.33	1.66	0.077808638	0.004446208
Courtland	1	3.33-6.66	4.99	0.060038822	0.004194852
courtland	1	6.66-10	8.33	0.063138169	0.003248266
courtland	1	10-13.33	11.66	0.042980959	0.002647378
courtland	1	13.33-16.66	14.99	0.014381813	0.00191346
Courtland	1	16.66-20	18.33	0.002106862	0.000963137
Courtland	1	20-23.3	21.66	0.000445183	0.000741971
Courtland	1	23.33-26.66	24.99	0.002972572	0.001251609
Courtland	2	0-3.33	1.66	0.092677665	0.004899877
Courtland	2	3.33-6.66	4.99	0.097668423	0.004782732
courtland	2	6.66-10	8.33	0.0986605	0.004657266
courtland	2	10-13.33	11.66	0.057174834	0.003827604
courtland	2	13.33-16.66	14.99	0.087997927	0.004725578
Courtland	2	16.66-20	18.33	0.099496349	0.004677605



courtland	2	20-23.3	21.66	0.091619635	0.004553875
Manassas	1	0-3.33	1.66	0.084576934	0.004188952
Manassas	1	3.33-6.66	4.99	0.102617784	0.004082936
Manassas	1	6.66-10	8.33	0.086097097	0.004142747
Manassas	1	10-13.33	11.66	0.03980365	0.003594009
Manassas	1	13.33-16.66	14.99	0.021773348	0.00260954
Manassas	1	16.66-20	18.33	0.007569475	0.001996345
Manassas	1	20-23.3	21.66	0.013494346	0.001999162
Manassas	1	23.33-26.66	24.99	0.00708155	0.001582048
Manassas	2	0-3.33	1.66	0.111627221	0.004441401
Manassas	2	3.33-6.66	4.99	0.151980718	0.004934439
Manassas	2	6.66-10	8.33	0.106708614	0.004372541
Manassas	2	10-13.33	11.66	0.051129468	0.003549325
Manassas	2	13.33-16.66	14.99	0.06134472	0.003159385
Manassas	2	16.66-20	18.33	0.014935535	0.002330979
Manassas	2	20-23.3	21.66	0.004047396	0.001722296
Manassas	3	0-3.33	1.66	0.043002117	0.003372715
Manassas	3	3.33-6.66	4.99	0.059227121	0.003093954
Manassas	3	6.66-10	8.33	0.074453871	0.004212383
Manassas	3	10-13.33	11.66	0.047103691	0.003254437
Manassas	3	13.33-16.66	14.99	0.027499289	0.002478134
Manassas	3	16.66-20	18.33	0.005172825	0.001293206
Manassas	3	20-23.3	21.66	0.000544401	0.000933259
Sleeter lake	1	0-3.33	1.66	0.042040548	0.00280821
Sleeter Lake	1	3.33-6.66	4.99	0.063339596	0.002838331
Sleeter Lake	1	6.66-10	8.33	0.021572597	0.002609588
Sleeter Lake	1	10-13.33	11.66	0.01348019	0.00215364
Sleeter Lake	1	13.33-16.66	14.99	0.011415033	0.001641888
Sleeter Lake	1	16.66-20	18.33	0.008386289	0.001661435
Sleeter Lake	1	20-23.3	21.66	0.007320521	0.001772337
Sleeter lake	2	0-3.33	1.66	0.041720529	0.003039938
Sleeter Lake	2	3.33-6.66	4.99	0.052764546	0.003029076
Sleeter Lake	2	6.66-10	8.33	0.049291919	0.003227966
Sleeter Lake	2	10-13.33	11.66	0.018922477	0.002481636
Sleeter Lake	2	13.33-16.66	14.99	0.002382855	0.001340356

Sleeter Lake	2	16.66-20	18.33	0	0
Sleeter Lake	2	20-23.3	21.66	0.001301629	0.000689098

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