# CARBON SEQUESTRATION IN NATURAL AND CREATED WETLANDS

Dissertation

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By

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# ABSTRACT

Wetland ecosystems are significant carbon sinks. Their high productivity and presence of water gives them the ability to efficiently sequester carbon in the soil, serving as a potential tool to mitigate the net greenhouse effect of carbon emissions to the atmosphere and abate climate change. We explored the efficiency of freshwater wetlands sequestering carbon under different climates, wetland types, and vegetation communities, in order to assess the conditions that favor carbon accumulation. We also studied the ability of created freshwater wetlands to sequester carbon and the effect of their vegetation communities on this task.

We found significant differences on carbon sequestration between wetland types in temperate and tropical regions, being consistently higher in the studied forested wetlands (260 ± 58 gC $m^{-2}$ $y^{-1}$) than the riverine ones (113 ± 27 gC $m^{-2}$ $y^{-1}$), indicating the importance of wetland productivity and the type of organic matter entering the system. Our temperate wetlands were also consistently more efficient in sequestering carbon than similar tropical ones (233 ± 89 and 151 ± 57 gC $m^{-2}$ $y^{-1}$, respectively), suggesting that higher temperatures in tropical climates may hinder carbon sequestration by intensifying organic matter decomposition rates. Within the tropical climates, we found that the tropical humid wetland sites had

significantly higher carbon sequestration rates (306 ± 77 gC $m^{-2}$ $y^{-1}$) than those located in the tropical dry regions, were there is a marked wet and dry season (63 ± 10 gC $m^{-2}$ $y^{-1}$ on average).

Our comparison between vegetation communities show that wetland productivity and permanent anaerobic conditions are key in enhancing soil carbon sequestration, being 214 ± 54 gC $m^{-2}$ $y^{-1}$ in the open water sites (with prolonged anaerobic conditions) and 184 ± 72 gC $m^{-2}$ $y^{-1}$ in the edges (typically more productive due to their fluctuating water levels). In the tropics, where temperature might to be a limiting factor for carbon sequestration in wetland soils, the presence of water seems to be of critical importance to enhance carbon sequestration rate and thus carbon sequestration rates were highest in the tropical permanently flooded wetland sites (306 ± 77 gC $m^{-2}$ $y^{-1}$). In temperate regions, where temperature is unlikely to limit carbon sequestration in wetland soils, permanent anaerobic conditions seem to be the most favorable condition for carbon accumulation. However, when organic matter inputs are high or recalcitrant, their role in favoring soil carbon sequestration is as important or more than the permanent presence of water (carbon sequestration rates in the temperate forested sites were, on average, 317 ± 93 gC $m^{-2}$ $y^{-1}$, while in the permanently flooded riverine ones were 160 gC $m^{-2}$ $y^{-1}$ in the natural wetland and 267 ± 21 gC $m^{-2}$ $y^{-1}$ in the created ones).

In the temperate created riverine wetlands carbon sequestration was strongly correlated with aboveground productivity. These two similar created wetlands in central Ohio differed only in their initial planting (one was planted and

the other one was colonized naturally), but otherwise have had the same hydrologic features and nutrient loadings. After 15 years, the originally planted wetland had lower carbon sequestration rate (219 ± 15 gC m$^{-2}$ y$^{-1}$) than the naturally colonized one (267 ± 17 gC m$^{-2}$ y$^{-1}$), which had also higher aboveground net primary productivity. Soil carbon sequestration had no correlation with the nitrogen concentrations in the water, suggesting that nutrient rich waters do not necessarily favor carbon accumulation in the soil directly, even though they enhance biomass productivity and thus soil carbon inputs. On average, these temperate created wetlands sequestered 243 ± 24 gC m$^{-2}$ y$^{-1}$ after 15 years since creation, 26 % more than the rate after 10 years (190 ± 7 gC m$^{-2}$ y$^{-1}$) and 55 % more than the similar natural wetland in the same region (140 ± 16 gC m$^{-2}$ y$^{-1}$), implying that once created wetlands are fully functional and structured they can successfully sequester carbon, especially in their early years.

# DEDICATION

This dissertation is dedicated to all those who think that an idea can change the world, and to all those with enough courage to try.

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# PUBLICATIONS

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## FIELD OF STUDY

**Major Field:** Environment and Natural Resources

**Area of Specialization:** Wetland biogeochemistry

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# CHAPTER 1

# INTRODUCTION

Wetland ecosystems are characterized by the presence of standing water during at least part of the year. This allows the development of specialized soils (i.e., hydric soils) and vegetation (i.e., hydrophytes) adapted to the presence of water and to the saturation of the soil (Mitsch and Gosselink 2007; Reddy and DeLaune 2008). The slow flow of water in the wetland allows the deposition of sediments suspended in the water column and of the organic matter that is produced within the wetland or brought in by inflowing. Wetland water levels usually fluctuate, creating different zones in the wetland according to the frequency of inundation, going from the permanently flooded and deeper site to the shallower area and to the edge, where the soil is exposed more frequently due to this "pulsing hydrology" (Mitsch and Gosselink 2007). This gradient allows different vegetation communities to develop (Boutin and Keddy 1993). Wetlands are known to be very productive ecosystems, due to their pulsing hydrology and their often nutrient rich waters (Odum et al. 1995; Cronk and Fennessy 2001; Mitsch and Gosselink 2007); consequently they introduce large amounts of organic matter into their soil. This soil organic matter accumulates in different decompositions stages for long periods of time because the saturation of the soil creates anoxic conditions that retard organic matter decomposition (Collins and Kuehl 2001; Mitsch and Gosselink 2007; Reddy and DeLaune 2008). When the organic matter is decomposed under anaerobic conditions it produces methane, a carbon-based gas that can be released back to the atmosphere or oxidized to carbon dioxide (which eventually reaches the atmosphere as well). The wetland carbon cycle has been extensively studied (Collins and Kuehl 2001; Mitsch and Gosselink 2007; Reddy and DeLaune 2008) and it is shown in detail in Figure 1.1. Slow decomposition rates and large organic matter accumulation makes wetlands soils significant sinks of carbon, at a rate that depends on the environmental (e.g., climate) and hydrogeomorphic (e.g., hydrology and setting in the landscape) features of the wetland.

## 1.1. Research goals and objectives

The goal of this dissertation is to investigate the ability of natural and created freshwater wetlands to sequester carbon under different climates and hydrogeomorphic settings, as well as the conditions within the wetlands that could affect their soil carbon sequestration capacity. The specific objectives of this research are as follows:

(1) Evaluate the efficiency in sequestering carbon of six different wetland communities in Ohio (temperate humid climate) that belong to two distinct hydrogeomorphic types (isolated depressional connected to the

groundwater table, and riverine flow-through that receives water from an agricultural watershed), by estimating their carbon sequestration rates with radiometric dating (Chapter 2).

*Rationale* – Riverine wetlands can accumulate more carbon in the soil than other wetland types because the nutrient rich waters entering the wetland from the river enhance wetland productivity (Cronk and Fennessy 2001), potentially increasing the inputs of carbon to the soil. On the other hand, isolated wetlands allow the establishment of trees, whose plant debris are more recalcitrant than those from emergent wetland species and therefore harder to decompose (Collins and Kuehl 2001), potentially remaining longer in the soil.

(2) Explore differences in carbon sequestration rates, determined by radiometric dating, of twelve wetland communities from four tropical wetlands that are located in two distinct climatic regions: two in the humid tropic (Eastern Costa Rica) and two in the seasonal dry tropic (Western Costa Rica and Northern Botswana), to find the hydrogeomorphic settings and/or wetland features that could favor carbon accumulation in tropical wetland soils (Chapter 3).

*Rationale* – Tropical humid ecosystems are known to be among the most productive ecosystems in the world (Schlesinger 1997) and thus are expected to introduce large amounts of carbon into the soil. However, warm temperatures favor the decomposition of organic matter (Franzluebbers et al. 2001), increasing the carbon losses from the soil. This loss of carbon might be even more significant in seasonal wetlands located in climates that suffer marked wet and dry seasons (Miller et al. 2005), despite having a pulsing hydrology that enhances wetland productivity (Odum et al. 1995).

(3) Determine the ability to sequester carbon of two similar created wetlands in central Ohio that differ only in their initial planting, but otherwise have had the same hydrologic features and nutrient loadings. Soil carbon sequestration was determined 15 years after these wetlands were created and compared to the rates estimated 10 years after their creation. The relationship between carbon and nitrogen contents in the soil was also explored to evaluate if C:N ratios can predict soil carbon accumulation and compared with the C:N ratios in the inflowing water (Chapter 4).

*Rationale* – After 15 years these created wetlands are likely to have faster carbon sequestration rates than similar natural wetlands because of the high productivity of young ecosystems (Odum 1969), and this rate is also expected to be greater than the one determined after 10 years of creation (Anderson and Mitsch 2006) because and increase in wetland productivity over the years (Mitsch et al. 2012). While no studies in literature evaluate the relationship between carbon and nitrogen in wetland soils, upland studies report inconsistent and some times contradictory results, making it unclear if nitrogen favors carbon accumulation in the soil by means of stabilization of the organic matter (Cambardella and Elliott 1994) or if it accelerates its decomposition (Bragazza et al. 2006).

## 1.2. Background

The rapid increase of greenhouse gases (GHG) in the atmosphere in the last century and their correlation with changes in climatic trends has created a generalized concern for the potential consequences of maintaining this rate of GHG emissions. Estimations on how climate is going to change are limited by the fact that we cannot predict with accuracy the magnitude of the changes and establish clear cause and effect relationships (Bodansky 1991; Levin 1992; IPCC 2007). The best tool that we have so far is to look at current trends and estimate what the worst–case scenario could look like if those trends are maintained.

Two of the most important greenhouse gases are carbon dioxide ($CO_2$) and methane ($CH_4$). They are produced naturally and anthropogenically, but the rapid increase of atmospheric GHG concentrations is associated with fossil fuel combustion. Thus, GHG levels have been increasing since the times of industrial revolution, $CO_2$ from 280 ppm to 381 ppm and $CH_4$ from 700 ppb to 1750 ppb (WMO 2006; IPCC 2007). Awareness and concern on this issue has created a generalized interest on finding effective ways to reduce net GHG emissions. There are three different ways to accomplish this task (Schrag 2007; Lal 2008): reducing global energy use, using an alternative no-carbon source of energy, and sequestering carbon from point sources or from the atmosphere through natural and engineered systems. Given our current situation, we cannot make a significant reduction on our energy use; alternative energies are becoming more important and efficient every day, but we are not ready yet to switch effectively to them and abandon the use of traditional energy sources (i.e. fossil fuels); and engineering techniques to sequester carbon are still in the development and testing phase (IPCC 2005). The use of ecosystems that naturally capture and sequester carbon is, as of today, one of the most efficient and cost-effective approaches to counteract the GHG emissions (Hanley and Spash 2003; IPCC 2005; Stern 2007).

There are five natural carbon pools. From largest to lowest carbon stock, these pools are (Lal 2008): oceanic, fossil fuel, pedologic (soil), atmospheric, and biotic (mostly vegetation). These pools are interconnected through feedback loops and biogeochemical cycles (Schlesinger 1997). The carbon stock in these pools is usually transient, except for the oceanic and the pedological pools, which are more stable and permanent (IPCC 2005; Schrag 2007). Many scientists have considered using the oceans as carbon sinks, but techniques to successfully enhance and manage its carbon sequestration capacity are yet to be developed. The soil pool is therefore the most suitable carbon pool to manage and maximize in a cost–effective manner. Most carbon sequestration studies have been done in agricultural soils (Lal et al. 1997; Six et al. 2006) and boreal peatlands (Holden 2005; Roulet et al. 2007). Agricultural soils are easily manageable through adoption of agricultural practices, but their carbon sink capacity is limited and subject to crop production. In peatlands, like in every wetland ecosystem, the presence of standing water and the soil saturation retards the decomposition of organic materials, acting as significant sinks for carbon and nutrients (Chimner and Ewel 2005; Mitsch and Gosselink 2007). However, peatlands are one of the least productive types of wetlands (Gorham 1991; Mitsch and Gosselink 2007); wetlands in non–boreal regions are much more productive ecosystems due to the warmer temperatures and their hydrologic features and thus, introduce large amounts of organic matter (and therefore carbon) in the soil (Cronk and Fenessy 2001; Trettin and Jurgensen 2003; Chimner and Ewel 2005; Mitra et al. 2005; Mitsch and Gosselink 2007). The capacity of wetlands to sequester carbon under different conditions (wetland types and climatic regions, among others) is not fully understood yet, and more research in this area is needed to accurately quantify wetland's carbon pool and to define their role in global carbon cycles.

The increased wetland loss rates in the last century and the recognition of wetland values has lead to a "no net loss" policy in the United States (Dahl 2000; NRC 2001; Mitsch and Gosselink 2007), where wetlands are often created to replace natural ones that have been damaged or lost. The success in wetland creation and restoration has been extensively evaluated (Mitsch and Wilson 1996; Zedler and Calloway 1999; Kentula 2000; Campbell et al. 2002; Gutrich et al. 2009; among others) according to the ability of the new wetland to develop wetland structure (e.g., hydric soil and vegetation cover) and function (e.g., water quality improvement and wildlife habitat). The ability of created wetlands to efficiently accumulate and store organic matter in the soil has been less explored, until the recent concerns on carbon sequestration in natural systems have emerged (Bruland and Richardson 2005; Anderson et al. 2005; Anderson and Mitsch 2006; Fennessy et al. 2008; Hossler and Bouchard 2010). Waterlogged conditions and high productivity give created wetlands the potential to be carbon sequestering systems as effective as natural wetlands are; if created wetlands are proven to be efficient carbon sinks they could function as natural cost–effective mitigation tools to sequester carbon and abate the net greenhouse effect of carbon emissions.

### 1.3. Wetlands and climate change

The role that wetlands can play in climate change is defined mainly by their importance in global carbon budgets, since they are estimated to be a sink of 450 Pg C (about one-third of the total organic soil pool; Mitra et al. 2005; Lal 2008; Mitsch and Gosselink 2007) despite covering 6–8 % of the land (Lehner and Döll 2004; Mitra et al. 2005; Mitsch and Gosselink 2007), and they are also accounted responsible for 25 % of the yearly $CH_4$ emissions to the atmosphere (i.e., 60% of the naturally originated methane emitted each year; Bartlett and Harris 1993; Whalen 2005; IPCC 2007). In terms of a carbon balance, the carbon that wetlands emit as methane is only about 1–3 % of the net wetland biomass productivity (Whiting and Chanton 1993; Schlesinger 1997; Jokic et al*.* 2003; Melack et al. 2004), which in turn is greatly exceeded by the rate of carbon accumulation in the soil (Mitsch and Gosselink 2007; Saunders et al. 2007). For a full assessment of the net effect of a wetland as sink or source of carbon to the atmosphere, it is necessary to take into account the soil carbon sequestration rate and the two main fluxes of carbon (typically) of a wetland ecosystem, i.e. the $CO_2$ taken from the atmosphere (mainly through biomass productivity), and the $CO_2$ and $CH_4$ emitted through aerobic and anaerobic respiration. However, when comparing carbon species we have to consider their global warming potential (GWP, an estimation of how much the greenhouse gas contributes to global warming; IPCC 2007), and so every flux and stock of carbon in the ecosystem has to be transformed into "$CO_2$ equivalents" before balancing the ecosystem carbon budget. Since the GWP of $CH_4$ is much higher than the GWP of $CO_2$ (25 times the GWP of $CO_2$ in a 100 years time horizon), the net effect of the wetland as source or sink of GHG can be questioned when scientists claim that wetlands, and specially created wetlands, can sequester carbon. However, this controversy on wetlands functioning as sources or sinks of carbon to the atmosphere is something that can be managed in created freshwater wetlands by favoring the conditions that enhance carbon sequestration and reduce $CH_4$ emissions – for example, a pulsing hydrology (fluctuating water level) can keep $CH_4$ emissions low (Altor and Mitsch 2008) while it increases productivity (Odum et al. 1995) and thus carbon inputs to the soil.

The conservation of existing wetlands around the world and the creation of new ones is something that needs to be encouraged, not only because of their potential carbon sink capacity but also because of the numerous and valuable ecosystem services they provide (Folke 1991; Costanza et al. 1997; Söderquist et al. 2000; Mitsch and Gosselink 2007).

### 1.4. Methodology overview

The lack of standard methodology for sampling and analysis for carbon sequestration in wetland soils makes difficult the comparison between studies and challenges the gain of a general idea of how carbon sequestration varies within wetlands and/or between wetlands. A method that is becoming more common in the last decade is the use of $^{137}Cs$ and $^{210}Pb$ radionuclides for estimating accumulation rates in natural wetlands. This method measures the activity of these independent radioisotopes in the wetland soil. These radioisotopes deposit atmospherically and bind strongly to the clay particle of the sediment, being affected by the same variables that would affect sedimentation on soils. In wetlands, being depositional environments, these sediments accumulate relatively undisturbed due to the little erosion that these soils suffer (compared to upland soils). These sediments containing $^{137}$Cs and $^{210}$Pb are brought in by runoff or suspended in the inflowing water, and once in the wetland they settle, accumulating in the bottom. Thus, estimation on rate of accumulation of these radioisotopes serves as an estimation of accumulation of sediments in the wetland over time. In created wetlands the estimation of sedimentation rates since creation date is easily determined by estimating the amount of soil accumulated over the underlying parent material since the wetland was created.

Once we know the amount of soil that the wetland accumulates over time we can calculate the rate of carbon accumulation by measuring carbon content of that soil. When we estimate carbon accumulation rates we are measuring the carbon that is staying in the soil under the given conditions, after the carbon is brought in (by plant biomass produced in the wetland, inflowing waters, or suspended sediments, among others) and after the carbon is consumed and has left the soil (mainly by organic matter oxidation and anaerobic decomposition). Therefore, the carbon sequestration rate we calculate is the net accumulation of carbon in the soil in the long term, regardless of its origin and after processes of carbon inputs and outputs take place.

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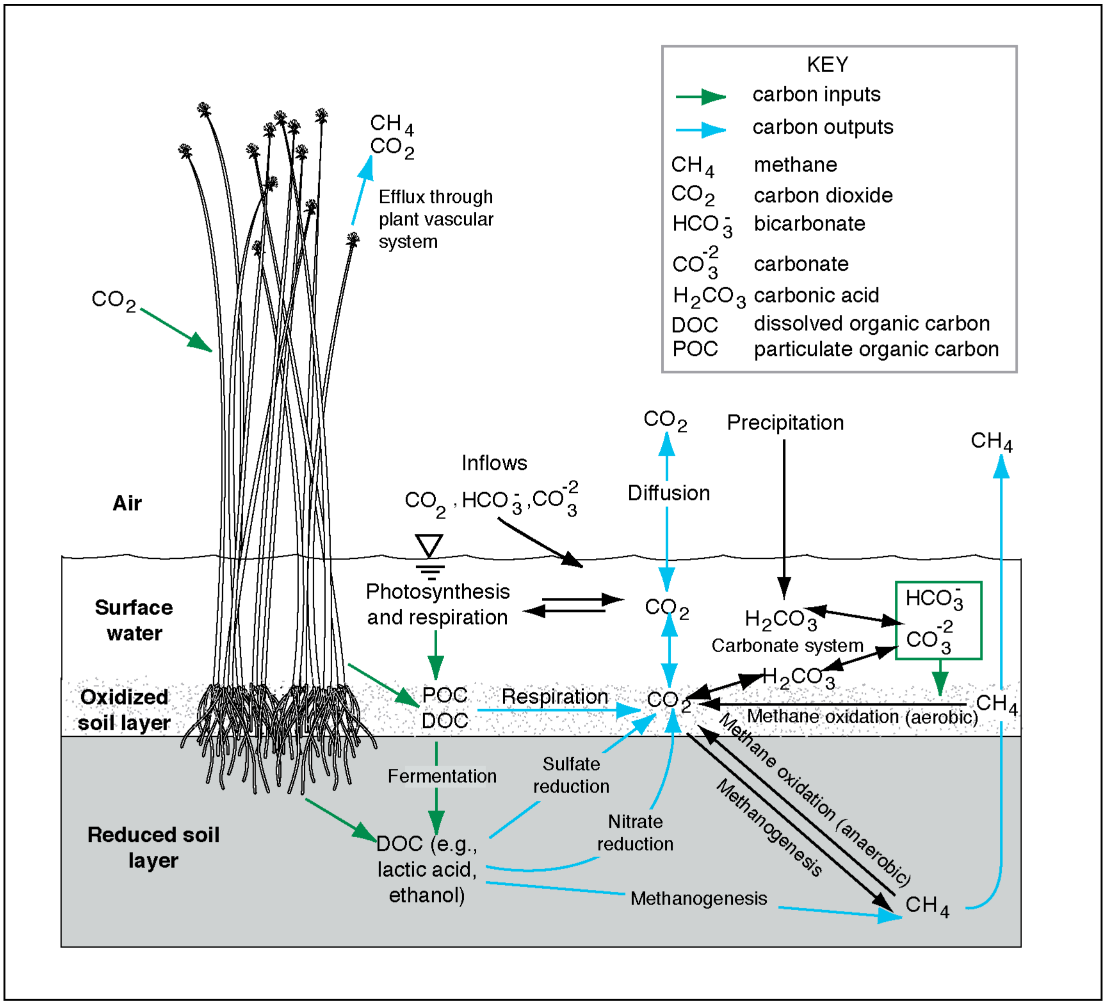

Figure 1.1. Carbon cycle in wetlands, adapted from Mitsch and Gosselink 2007.

# CHAPTER 2

## COMPARING CARBON SEQUESTRATION IN TEMPERATE FRESHWATER WETLAND COMMUNITIES [1]

### 2.1. Abstract

High productivity and waterlogged conditions make many freshwater wetlands significant carbon sinks. Most wetland carbon studies focus on boreal peatlands, however, with less attention paid to other climates and to the effects of hydrogeomorphic settings and the importance of wetland vegetation communities on carbon sequestration. This study compares six temperate wetland communities in Ohio that belong to two distinct hydrogeomorphic types: an isolated depressional wetland site connected to the groundwater table, and a riverine flow-through wetland site that receives water from an agricultural watershed. Three cores were extracted in each community and analyzed for total carbon content to determine the soil carbon pool. Sequestration rates were determined by radiometric dating with $^{137}$Cs and $^{210}$Pb on a set of composite cores extracted in each of the six communities.

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Cores were also extracted in uplands adjacent to the wetlands at each site. Wetland communities had accretion rates ranging from 3.0 to 6.2 mm y$^{-1}$. The depressional wetland sites had higher ($P < 0.001$) organic content (146 ± 4.2 gC kg$^{-1}$) and lower ($P < 0.001$) bulk density (0.55 ± 0.01 Mg m$^{-3}$) than the riverine ones (50.1 ± 6.9 gC kg$^{-1}$ and 0.74 ± 0.06 Mg m$^{-3}$). The soil carbon was 98–99 % organic in the isolated depressional wetland communities and 85–98 % organic in the riverine ones. The depressional wetland communities sequestered 317 ± 93 gC m$^{-2}$ y$^{-1}$, more ($P < 0.01$) than the riverine communities that sequestered 140 ± 16 gC m$^{-2}$ y$^{-1}$. The highest sequestration rate was found in the *Quercus palustris* forested wetland community (473 gC m$^{-2}$ y$^{-1}$), while the wetland community dominated by water lotus (*Nelumbo lutea*) was the most efficient of the riverine communities, sequestering 160 gC m$^{-2}$ y$^{-1}$. These differences in sequestration suggest the importance of addressing wetland types and communities in more detail when assessing the role of wetlands as carbon sequestering systems in global carbon budgets.

## 2.2. Introduction

Wetlands have an important role in global carbon cycles. They are highly productive ecosystems that accumulate large amounts of organic matter in the soil, functioning as significant carbon sinks (Odum et al. 1995; Chmura et al. 2003; Mitra et al. 2005). Despite covering 6–8% of the freshwater surface, wetlands are estimated to account for one-third of the world's organic soil carbon pool (Mitra et al., 2005; Lal, 2008; Mitsch and Gosselink 2007). Part of the carbon that the wetland produces is released to the atmosphere as methane (about 3% of the net wetland production; Schlesinger 1997; Jokic et al. 2003), a powerful greenhouse gas. Wetlands are estimated to be responsible for 25 % of the current yearly methane emissions to the atmosphere, representing 60 % of the naturally originated methane emitted each year (Bartlett and Harris 1993; Whalen 2005; IPCC 2007).

The amount of carbon that a wetland stores and emits every year depends greatly on the hydrogeochemical characteristics of the ecosystem, which, in turn, determine the wetland vegetation communities. Therefore, to estimate with precision a wetland's carbon pool and carbon sequestration capacity, it would be more accurate to differentiate between wetland types, especially if wetlands are to be used as a carbon–sequestering systems to reduce net greenhouse gas emissions (Stern 2007). Despite the known importance of wetlands in global carbon budgets, the lack of systematic studies and adequate models, and the limited information on their carbon turnover rates and temporal dynamics, has probably led to an underestimation of their relevance to global and regional levels, to the point that they are typically omitted from large-scale assessments (Trenttin and Jurgensen 2003).

Wetlands are ecosystems defined by the presence of standing water and/or saturated soil during at least part of the year, a condition that is subsequently responsible for the development of specialized vegetation (hydrophytes) and hydric soil (Mitsch and Gosselink 2007). These factors (hydrology, vegetation, and soil) and their interaction create the signature characteristics of wetland ecosystems and communities, and can be used to differentiate and classify wetland types. The U.S. Fish and Wildlife Service recognizes five wetland systems (marine, estuarine, riverine, lacustrine, and palustrine; Cowardin et al. 1979) while the U.S. Army Corps of Engineers uses a wetland classification system developed by Brinson (1993) based on wetland hydrogeomorphology (HGM classification), that recognizes four geomorphic settings (depressional, riverine, fringe, and extensive peatlands), three water sources (precipitation, groundwater discharge, and surface inflow), and three hydrodynamics (vertical fluctuation, unidirectional flow, and bidirectional flow). Much of the rest of the world uses the 32 Ramsar wetland classes identified mainly for wildlife and biodiversity (*http://www.ramsar.org*). Wetland plant communities (plant associations generally described by the dominant plant species) adapt to the conditions of particular wetland zones and often reflect an environmental gradient as they change through the wetland from deep water to the upland (Boutin and Keddy 1993; Mitsch and Gosselink 2007). Recognition of a wetland community can be used to identify not only the plant species, but also hydrogeomorphic features that define the wetland site.

In this study, we compare the ability of wetlands to accumulate carbon in two hydrogeomorphic conditions and several vegetation communities by estimating their soil carbon pool and sediment accretion rate. Given the depositional nature of wetlands, a frequently used technique to estimate accretion rates is radiometric dating with $^{137}$Cs and $^{210}$Pb, two independent atmospherically deposited radionuclides of similar half-life that bind strongly to the sediments and accumulate in wetlands, functioning as a reference to date the soil (Appleby and Oldfield 1978; Craft and Richardson 1993; Graham et al. 2005; Stark et al. 2006). We determine carbon pools and sequestration rates of six different wetland communities in two different hydrogeomorphic settings of temperate humid Ohio – one flow-through riverine, inland deltaic wetland with surface inflow and directional flow (Ramsar classes L and M) and one isolated palustrine and depressional wetland, with vertical fluctuation of the water table mainly fed by groundwater and precipitation (Ramsar classes Ts and Xf). The communities associated with the riverine system would be likely to have high total carbon accumulation because of additional external organic inputs and nutrients in the inflow water, while wetland communities on depressional and isolated sites are usually less productive than flow-through or slow-flow ones (Mitsch and Ewel 1979; Mitsch et al. 1991; Conner and Day 1992; Watt and Golladay 1999; Cronk and Fennessy 2001; Wilson et al. 2005). On the other hand, we might expect to find high carbon accumulation in a forested wetland community that is intermittently flooded because of the recalcitrant nature of the organic matter produced there (high in lignin and cellulose, two organic compounds that are harder to degrade by microbes due to their complexity; Dalva and Moore 1991; Schlesinger 1997), and because of the protection from direct wind and sun exposure that the tree canopy provides, potentially retarding plant litter decomposition rates (Kirschbaum 1995; Fierer et al. 2005; Bernal and Mitsch 2008).

## 2.3. Materials and methods

### *2.3.1. Site descriptions*

Our depressional forested wetland communities are located in a suburban area in central Ohio (40°0′ N, 82°50′ W). These woodland pools are mainly fed by groundwater and precipitation (Korfel et al. 2010), and their surface and groundwater water tables fluctuate with precipitation events. Gamble and Mitsch (2009) and Korfel et al. (2010) described the seasonality of these swamp pools as depressional bodies of water that remain saturated all year-round, with permanent standing water in some of the deeper pools that fluctuates vertically, remaining frozen from December to March and drying down for the most part between June and July. The deepest pools reach up to 40 cm of water depth and saturation under driest conditions is found at 5 cm deep in the soil (Korfel et al. 2010). All the pools are isolated and their water remains stagnant rather than flowing. Hydric soils (Pewamo and Carlisle muck) dominate these wetland areas (Natural Resources Conservation Service, U.S. Department of Agriculture, National Cooperative Soil Survey 2010).

The wetland communities studied for soil carbon accumulation at this site (Table 2.1) include a permanent pool where cattail grows (*Typha* spp.), a semipermanently flooded forest site with pin oak (*Quercus palustris*), and an edge site of buttonbush (*Cephalanthus occidentalis*). Upland soil samples were also collected in a beech–maple (*Fagus* spp.–*Acer* spp.) forest adjacent to the wetland area.

Our flow-through wetland communities are located in the Old Woman Creek State Natural Preserve (41°22’ N, 82°31’ W), in the southwestern shores of Lake Erie. The site is a 230-ha park that connects a 69-km$^2$ agricultural watershed (75 % cropland) with the lake through a 56-ha wetland that comprises the lower 3 km of the Old Woman Creek (Mitsch and Reeder 1991; Francko and Whyte 1999;

Herdendorf et al. 2006). They receive water from the watershed (unidirectional flow) and from occasional wind-driven seiches when the outlet mouth is not blocked by the formation of a small barrier beach (bidirectional flow). The barrier is present almost half of the time due to lake wave action, and is usually broken by storm flows from the watershed, giving these wetland communities a pulsing hydrology and an ability to exchange nutrients and sediments with the watershed and the lake. Water depths range between 0.3 and 1.6 m at the mouth, with most of the wetland basin at about 0.5 ± 0.1–0.2 m deep daily (Herdendorf 1990; Herdendorf et al. 2006). The soils in the wetland are hydric (Fluvaquents and Adrian muck; Natural Resources Conservation Service, U.S. Department of Agriculture, National Cooperative Soil Survey 2010). In the deepest areas of the wetland, floating beds of *Nelumbo lutea* (American water lotus) dominate the deep water zones that are guarded from the wind (Klarer and Millie 1992; Whyte et al. 2003; Herdendorf et al. 2006). Emergent plants such as *Phragmites australis* (common reed) and *Typha angustifolia* (narrow-leaved cattail), and to a lesser extent *Schoenoplectus tabernamontani* (softstem bulrush) and *Scirpus fluviatilis* (river bulrush) grow in saturated or shallowly submerged soils, up to 50 cm of standing water (Klarer and Millie 1992; Whyte et al. 2003; Herdendorf et al. 2006). The mudflats on the shallow part of the wetland (< 10 cm of standing water) are characterized by *S. fluviatilis* (river bulrush) and dense stands of *Leersia oryzoides* (rice cutgrass), as described by Whyte et al. (2003) and Herdendorf et al. (2006). The upland areas surrounding the wetland are a mixed hardwood forest dominated by red oak (*Quercus rubra*), pin oak (*Q. palustris*), white ash (*Fraxinus americana*), and buttonbush (*C. occidentalis*).

Samples were collected in the three wetland communities at this riverine site: the deep water floating beds of *N. lutea*, the emergent vegetation marshes dominated by *P. australis* and *S. fluviatilis*, and the mudflats dominated by *L. oryzoides* (Table 2.1). The upland forest adjacent to the wetland and creek bank of the stream that connects the wetland to the watershed were also sampled.

### *2.3.2. Soil sampling and samples preparation*

Two sets of samples were collected in each of the six wetland communities (units of stratified sampling), one for radiometric analysis and the other for bulk density and carbon content determination. For radiometric analysis, a composite sample consisting of three 7-cm diameter sediment cores, up to 36 cm deep. The three cores for a composite sample were spaced within 40 cm to include variation of deposition in the sample area (Isaksson et al. 2001; Stark et al. 2006) and, divided in situ into 2-cm-thick increments. Corresponding layers were pooled together into one sample per layer and packed in sealed containers (Bernal and Mitsch 2008). Triplicated soil samples (7 cm in diameter, 35 cm long) were extracted for the determination of bulk density and carbon content in each wetland community, divided in the field into 5 cm increments, and packed in sealed containers. One set of triplicated samples was also collected in the upland area adjacent to these wetlands. These upland samples were 10 cm diameter, 35 cm long, and were collected by the core method described by Grossman and Reinsch (2002). Upland samples were collected for carbon pool determination as a reference site for each wetland.

Another extra set was collected in the creek bank feeding the wetland of Old Woman Creek, right in the inflow of the wetland basin. This set of creek cores included samples for radiometric and carbon analysis, following the procedure described above. Every soil sample taken was stored under 4 °C until analysis to minimize losses from volatilization and microbial activity, ground and passed through a 2 mm sieve after been oven-dried, and homogenized (Bernal and Mitsch 2008). Samples collected from radiometric dating were dried at 105 °C until constant weight was reached (Craft and Richardson 1993; Grossman and Reinsch 2002), while the samples for carbon analysis were dried at 60 °C until constant weight to avoid potential oxidation of carbon in very rich organic soils (Grossman and Reinsch 2002).

### *2.3.3. Estimation of sediment accretion rates*

Accretion rates in the soil were determined non-destructively with $^{137}$Cs and $^{210}$Pb activity (pCi, $10^{-12}$ Ci) in each 2 cm increment soil sample by c spectrometry for 24 h (Craft and Richardson 1993; Smith et al. 2000; Graham et al. 2005) at 661.7 and 46.5 keV respectively, using a high efficiency germanium detector (Canberra, GL 2820R; Canberra Industries, Inc., Meriden, CT, USA). Radiocesium ($^{137}$Cs) is a man-made fallout radionuclide (30.1 years half-life) worldwide distributed as consequence of deposition from atmospheric nuclear weapon tests (Smith et al. 2000; Ilus and Saxén 2005). According to its depositional pattern, 1964 had the highest $^{137}$Cs deposition on the globe. Once in the soil, $^{137}$Cs binds strongly to the sediment and moves with it, remaining unaltered and making it a radionuclide widely used as tracer in dating studies, especially successful in depositional environments such as wetlands and floodplains (Yeager and Santschi 2003; Ilus and Saxén 2005; Stark et al. 2006). The identification of the layer in the soil profile with the peak in the activity is assumed to correspond to the year 1964. Thus, the sediment accumulated in the wetland since that year can be estimated, and the accretion rate calculated assuming constant sedimentation rate, unless evidence in the profile of the opposite (Craft and Richardson 1993; Craft and Casey 2000; Graham et al. 2005; Stark et al. 2006). In cases where $^{137}$Cs profiles are not conclusive to determine accretion rates, $^{210}$Pb in the core layers is often analyzed to corroborate the dating (Craft and Richardson 1993; He and Walling 1996; Faure and Mensing 2005; Graham et al. 2005), as they are two independent tracers that have the similar depositional pattern and behavior in the soil. $^{210}$Pb (22.3 years half-life) is a naturally occurring radionuclide of the $^{238}$U decay series that deposits atmospherically from the decay of $^{226}$Ra (Oldfield and Appleby 1984; Matisoff et al. 2002; Faure and Mensing 2005). The soil and the vertical accretion rate can be calculated estimating excess $^{210}$Pb activity in the core and using the constant activity model, described in Eqn. [1] (Appleby and Oldfield 1978; Oldfield and Appleby 1984; Bricker-Urso et al. 1989; Pfitzner et al. 2004; Faure and Mensing 2005; Graham et al. 2005):

$$A_d = A_0\ e^{-(\lambda d/s)} \qquad [1]$$

where $A_d$ is the $^{210}$Pb activity at depth $d$, $A_0$ is the $^{210}$Pb activity at the surface, $\lambda$ is the decay constant of $^{210}$Pb (0.0311 $y^{-1}$), $d$ is the depth (cm), and $s$ is the sediment accretion rate (cm $y^{-1}$).

### 2.3.4. *Determination of soil carbon content and carbon sequestration rate*

Triplicates (50 mg) of each soil sample were analyzed for total carbon content (sum of organic and inorganic carbon) using a Total Carbon Analyzer for Soil Samples (TOC-V series, SSM-5000A; Shimadzu Corporation, Kyoto, Japan). The soil carbon concentration ($\mathrm{Cconc}_{\mathrm{layer}}$) and pool ($\mathrm{Cpool}_{\mathrm{layer}}$) of each soil sample increment were calculated as described by Bernal and Mitsch (2008):

$$\mathrm{Cconc}_{\mathrm{layer}}\ (\mathrm{gC\ kg^{-1}}) = 10 \times TC_{\mathrm{layer}}\ (\%) \qquad [2]$$

$$TC_{\mathrm{layer}}\ (\mathrm{g}) = W_{\mathrm{layer}}\ (\mathrm{g}) \times TC_{\mathrm{layer}}\ (\%) \times 10^{-2} \qquad [3]$$

$$\mathrm{Cpool}_{\mathrm{layer}}\ (\mathrm{kgC\ m^{-2}}) = 10^{-3} \times TC_{\mathrm{layer}}\ (\mathrm{g}) / A_{\mathrm{core}}\ (\mathrm{m^2}) \qquad [4]$$

where $TC_{\mathrm{layer}}$ (%) $TC_{\mathrm{layer}}$ (g) are the percentage of total carbon in the layer, and the total mass of carbon in the layer in grams, respectively, $W_{\mathrm{layer}}$ is the dry weight of soil in grams, and $A_{\mathrm{core}}$ is the area of the sediment sampler in $\mathrm{m^2}$.

The carbon accumulation since 1964 is calculated by estimating the total soil carbon pool from the soil surface to the soil layer that, according to the radiometric profile, corresponds to the year 1964.

### 2.3.5. *Statistical analysis*

Statistical analyses were performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) for Macintosh. Student's *t*-test for independent samples at 95 % significant level was used to find differences between carbon concentrations and carbon pools of the six wetland communities, between the hydrogeomorphic wetland types, and between the wetland areas and their respective non-wetland sites (upland in Gahanna Woods, upland and creek bank in Old Woman Creek).

Carbon sequestration rates of each wetland were also compared using a *t*-test, at a significance level of 95 % and 90 %. Carbon sequestration was compared in every wetland community individually, and within each wetland hydrogeomorphic type, at these same significance levels. Significant differences indicate $P \leq 0.05$, $\leq 0.01$ for 95 % and 99 % confidence, respectively (Fowler et al. 2003).

## 2.4. Results

### *2.4.1. Soil carbon content*

The total carbon concentration in the depressional isolated wetland sites was, on average, about three times the average concentrations of the riverine flow-through sites (146 ± 4.2 and 50.1 ± 6.9 gC $kg^{-1}$, respectively; Table 2.2, $P < 0.001$). However, the concentrations of total carbon of the depressional sites were only 1.5–2 times greater than the floating bed community (water lotus). In every site, soil carbon was predominantly organic (98–99 % in the isolated depressional wetland communities, 85–98 % in the riverine ones). The carbon content increased with depth of soil in the riverine deep-water community dominated by floating beds of *N. lutea* and slightly in the mudflat, as well as in the forested community and the cattail marsh of the depressional wetland (Figure 2.1). Total carbon concentration in the upland site adjacent to the depressional wetlands was 16.5 ± 6.3 gC $kg^{-1}$, about 10 % of the carbon content in the wetland sites, while the upland at the riverine sites was 7.1 ± 2.9 gC $kg^{-1}$ (14 % of what the wetland area contains; Table 2.2). A wetland's carbon content was significantly higher than its adjacent uplands in both cases ($P < 0.001$). The creek feeding the riverine wetland had low carbon content as well (9.1 ±

1.1 gC kg$^{-1}$) compared to the wetland communities, but it was 30 % higher than the carbon at the upland of the riverine site. The carbon content of the creek bank was significantly different to the wetland content ($P < 0.001$) but not to the upland.

The bulk densities (Table 2.3) of the depressional wetland communities were very similar, and on average (0.57 ± 0.01 Mg m$^{-3}$) compared to the density of the riverine ones (0.77 ± 0.06 Mg m$^{-3}$). Soil bulk density was consistently lower in the wetland sites compared to their adjacent uplands (upland bulk densities ranged between 1.60 and 1.68 Mg m$^{-3}$). The creek, despite being a non-wetland site, had a bulk density similar to the wetlands (0.84 ± 0.01 Mg m$^{-3}$), but much lower carbon pool (0.38 ± 0.05 kgC m$^{-2}$). Differences in carbon pools between wetland and non-wetland sites are evident in these sites, being the non-wetland ones one-fourth to one-third lower than in their corresponding wetland sites (1.01 ± 0.32 kgC m$^{-2}$ *vs.* 4.18 ± 0.25 kgC m$^{-2}$ in the depressional sites, and 0.64 ± 0.22 kgC m$^{-2}$ *vs.* 1.50 ± 0.19 kgC m$^{-2}$ in the riverine ones, Table 2.3). The average total carbon pool (up to 35 cm, Table 2.3) of the depressional wetland category was significantly different ($P < 0.001$) to the average pool of the riverine ones. In all the wetland communities studied, except of the *Phragmites* – *Scirpus* marsh, soil carbon increases with depth (Figure 2.2).

### *2.4.2. Sediment accretion rates*

Every wetland core analyzed for $^{137}$Cs showed peaks of this radionuclide's activity (Figure 2.2), corresponding to 1964. On the contrary, the creek bank had no $^{137}$Cs activity detected. Old Woman Creek sites had peaks on $^{137}$Cs at very similar depths (18 cm deep in both shallow reed – bulrush sites and intermittently flooded mudflat, and 16 cm deep in the water lotus floating beds), resulting in accretion estimates of 4.3 mm $y^{-1}$ for the former, and 3.8 mm $y^{-1}$ in the latter (Table 2.4). Based on these accretion rates, we estimated the weighted average annual sediment accumulation of the entire riverine wetland area of Old Woman Creek to be 28 tons $ha^{-1}$ $y^{-1}$. A previous study by Wilson et al. (2005) in this same wetland area reported a peak on $^{137}Cs$ activity at 17.5 cm deep (with an accumulation rate of 4.3 mm $y^{-1}$), and estimated a sediment sink capacity for the entire Old Woman Creek wetland area of 47 %.

In the depressional wetland sites, the $^{137}Cs$ activity peaked at 16 cm deep in the buttonbush community and at 18 cm deep in the *Typha* marsh, both very similar to the depths obtained in the riverine wetland sites (Figure 2.2). The forested community dominated by pin oaks, however, had a much deeper peak at 28 cm. Due to the lack of consistency between these three wetland communities and because of the absence of accretion rates from the wetlands in Gahanna Woods reported in literature, we used $^{210}Pb$ to estimate accretion rates (Figure 2.3) and support the values obtained with $^{137}Cs$. The resulting accumulation of 14 cm since 1964 in the buttonbush community, 16 cm in the *Typha* marsh, and 29 cm in the forested site, are similar to the depths obtained by the $^{137}Cs$ method. Thus, these sites are accumulating 3.2, 3.6, and 6.5 mm $y^{-1}$ of soil respectively, and that this entire depressional wetland area is accumulating 23 tons $ha^{-1}$ $y^{-1}$ of sediment (Table 2.4).

### *2.4.3. Carbon sequestration rates*

On average, the depressional wetland sites accumulated almost 2.5 times more carbon per year than did the riverine sites (317 ± 93 *vs.* 140 ± 16 gC $m^{-2}$ $y^{-1}$, respectively; Table 2.4). Sequestrations are weighed averages relative to the surface area of each wetland community. The two general hydrogeomorphic types of wetlands (depressional *vs.* riverine) are significantly different ($P$ = 0.010). The riverine floating beds of water lotus were the most effective community sequestering carbon in the riverine type of wetland (160 gC $m^{-2}$ $y^{-1}$, about 45 % higher than the other two riverine sites). The forested depressional site, however, had the highest carbon sequestration rate of all the communities studied (473 gC $m^{-2}$ $y^{-1}$), more than twice the rates estimated for the other two depressional communities (202 gC $m^{-2}$ $y^{-1}$ in the shrub community and 210 gC $m^{-2}$ $y^{-1}$ in the cattail marsh). The high sequestration rate of the forested community raises the average rate of the depressional sites, but in any case all of the depressional communities sequester carbon at a faster pace than did any of the riverine communities.

## 2.5. Discussion

### *2.5.1. Carbon profiles in wetland soils*

Most of the soil carbon measured was in the organic form (85–99 %). Inorganic percentages were slightly higher in the riverine sites (particularly in the marshes), which could be due to external inputs from the river or Lake Erie. In every site, high organic carbon content could be indicating that these wetlands are receiving important organic inputs despite their different placement in the landscape and their different hydrogeomorphic classification. This is usually the case for any type of wetland – these ecosystems are so productive that they have the ability to generate large amounts of organic matter (autochtonous organic source) and store it in a semidecomposed state in the soil due to the anaerobic conditions that water saturation creates (Gorham 1998; Collins and Kuehl 2001; Mitsch and Gosselink 2007). In the cases where the wetlands receive external (allochtonous) organic inputs, their soil carbon content was even higher. For this reason, the carbon content of the wetland is much higher compared to its adjacent upland. The creek bank did not accumulate much carbon because of its slope and the constant fluvial processes of the creek and the occasional seiches, but it was higher than the concentration in the adjacent upland for two possible reasons: (1) being under water for most of the time could be retarding the decomposition of the organic matter contained in its soil, and (2) the water coming back and forth to the creek bank can be a source of stress (erosive agent) but also a constant subsidy of nutrients and organic matter (Odum 1988; Odum et al. 1995; Mitsch and Gosselink 2007). All these organic rich sediments that are being eroded from the creek bed are entering the wetland, where they are deposited and temporarily stored in its soil, in a way similar to runoff from the uplands surrounding the wetlands bringing nutrient-rich sediments and organic materials into the wetland.

Soil carbon concentration increased with depth in most of our sites. Chin et al. (1998) measured total dissolved carbon in the pore water of the riverine wetland at Old Woman Creek and also found that the concentration increased with depth, up to a point where it was significantly greater than the concentration in the standing water. This increase is particularly evident in the floating beds of *N. lutea*, a community that has increased in area considerably in Old Woman Creek in the last two decades (Francko and Whyte 1999; Whyte et al. 2003; Herdendorf et al. 2006; Cornell and Klarer 2008), augmenting the net primary productivity of the entire wetland area and thus the organic matter inputs into the wetland soil. The increase of carbon content with depth is frequent in sites where there is significant organic matter accumulation and the decomposition rate (carbon output) is slower than the organic matter deposition (carbon input), as described by Becker-Heidmann and Scharpenseel (1992), Gorham (1998), Schlesinger (1997), and Wolf and Wagner (2005). In the wetlands of this study, that could be due to the constant or semiconstant presence of water slowing down microbial activity (in the cattail marsh, the water lotus marsh, the reed – bulrush marsh, and the mudflat), and because of the recalcitrant nature of the organic matter entering the system (particularly in the depressional wetland sites).

The depressional isolated wetlands of Gahanna Woods are surrounded by a forested wetland community dominated by *Q. palustris*, which is able to survive because of seasonal flooding (Mitsch et al. 2009). When the site is not flooded the soil is exposed and organic matter accumulated can oxidize. But overall, this site seems to be accumulating enough organic matter to compensate carbon for the loses under aerobic conditions, which could be consequence of the recalcitrant character of the organic matter coming into the soil from the trees. Trees litter reaches the wetland directly or through runoff or wind. Plant litter from woody species is rich in lignin and complex polysaccharides, difficult to degrade by microorganisms

(Schlesinger 1997; Wolf and Wagner 2005; Berg and McClaugherty 2008) and thus, remaining longer in the soil and accumulating deeper in the profile than rapidly degradable labile compounds (Schlesinger 1997; Trumbore 1997; Wolf and Wagner 2005). Also, water from precipitation falling through the tree canopy onto the wetland is likely to contain more organic compounds than water from direct precipitation onto the wetland. Dalva and Moore (1991) studied this phenomenon and found that water richer in dissolved organic carbon after passing through the forest canopy. The profiles of these depressional isolated wetland sites are more similar among themselves than are the ones in at the riverine sites, probably because the depressional communities were closer to each other and likely received similar organic inputs from the surrounding forest. The more recalcitrant woody plant input could be a reason why the carbon soil content in the depressional communities is about one order of magnitude higher than in the riverine sites.

Despite the similar or higher carbon accretion rates on the depressional sites compared to the riverine ones, total accumulation of sediment is lower because its soil has lower density. The high annual sediment accretion in the forested community can be interpreted as evidence of the large amount of organic matter accumulating in this site from the trees litter, probably due to its high productivity and recalcitrant character.

### *2.5.2. Carbon sequestration in freshwater temperate wetlands*

The studied forested isolated wetland sites had greater carbon sequestration rates (317 ± 93 gC $m^{-2}$ $y^{-1}$) than did the riverine sites (140 ± 16 gC $m^{-2}$ $y^{-1}$), but also

greater variability. The high productivity of this forested site raises the average rate of all of our depressional sites. The carbon sequestration rates of the marsh and shrub depressional communities studied, however, are also higher compared to any of the riverine ones. Such a significant difference between our two wetland types is likely due to the high productivity of the forest in which they are located, cooler temperatures provided by the shade that retards litter decomposition, and the recalcitrance of the organic matter being introduced
in the wetland (Fierer et al. 2005). Within the riverine sites, the sites with highest carbon sequestration rate were the floating beds of water lotus (160 gC $m^{-2}$ $y^{-1}$), probably because of the combined effect of the permanent anaerobic conditions in this deepwater area and the reported high productivity of this community. It is therefore important to take into account these differences when comparing the efficiency of wetlands as carbon sequestering systems. The flooding duration and the type of vegetation growing in the wetland are factors that control soil carbon accumulation, and therefore can be managed to enhance the natural ability of a wetland to accumulate carbon, while maintaining other valuable wetland functions and ecosystem services.

To put the carbon sequestration rates obtained in this study in perspective, we compare them with other carbon sequestration rates estimated for freshwater temperate wetlands reported in literature (Table 2.5). Most of these wetland studies used radiometric dating with $^{137}$Cs and/or $^{210}$Pb. The range of carbon sequestration in temperate zone inland wetlands range over an order of magnitude from 56 to 504 gC $m^{-2}$ $y^{-1}$ (Table 2.5). The mean of all these temperate zones estimates is 174 gC $m^{-2}$

$y^{-1}$ and the median is 131 gC $m^{-2}$ $y^{-1}$. Eliminating outliers, most of the numbers range from 100 to 280 gC $m^{-2}$ $y^{-1}$. Our carbon sequestration estimates (317 ± 93 and 140 ± 16 gC $m^{-2}$ $y^{-1}$; Table 2.4) fall within the range of previously published rates. The average values for isolated, depressional, and/or forested wetlands reported in literature (188 ± 33 gC $m^{-2}$ $y^{-1}$), however, are more similar to those of riverine, flow-through wetlands (164 ± 37 gC $m^{-2}$ $y^{-1}$) than to our forested wetland measurements. All these temperate zone mineral soil wetland estimates are, in general, considerably higher than the average rates of carbon sequestration estimated for boreal peatlands (10–61 gC $m^{-2}$ $y^{-1}$; Mitsch and Gosselink 2007), a much less productive type of wetlands where most of the wetland carbon studies are focused. Many wetland carbon budgets are based on peatland carbon pools and sequestration rates, which may lead to a general underestimation of the role of wetland ecosystems in global carbon budgets and to the misconception that every wetland has a similar low accretion rate.

### *2.5.3. Implications of this study*

Wetlands are important sinks of carbon, as evidenced in the significant differences between wetland and non-wetland sites in this study. However, we have shown that not all wetlands are equal in their ability to sequester carbon. In our study the six temperate wetland communities differed in their hydrogeomorphic type and placement in the landscape, thus allowing different vegetation communities to dominate. These differences resulted in significantly different carbon content in the soil (greater in all the depressional wetland sites than in the riverine ones, the greatest in the forested community, and greater in the riverine deeper areas than in the shallower ones). The hydrogeomorphic types of these wetland communities also had significantly different carbon sequestration rates (on average, 2.5 times higher in the depressional wetland areas than in the riverine sites). Not every wetland is equally effective in sequestering carbon; it is important to address differences in wetland types and vegetation communities when assessing the role of wetlands as carbon sinks in global carbon budgets.

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| **Wetland community** | **Dominant vegetation** | **Hydrogeomorphic category** | **Hydrologic features** | **Soil type *** | **Location** |
|---|---|---|---|---|---|
| Shrub | *Cephalanthus occidentalis* | Depressional, isolated | Intermittently flooded, shallow edge | Pw | Central Ohio |
| Forested | *Quercus palustris* | Depressional, isolated | Intermittently flooded, shallow | Pw/Cc | Central Ohio |
| Marsh | *Typha* spp. | Depressional, isolated | Permanently flooded, deep water | Cc | Central Ohio |
| Marsh | *Phragmites australis*, *Scirpus fluviatilis* | Riverine, flow-through | Intermittently flooded, shallow edge | FnA | Northern Ohio |
| Mudflat | *Leersia oryzoides* | Riverine, flow-through | Intermittently flooded, shallow | FnA/Aa | Northern Ohio |
| Floating bed | *Nelumbo lutea* | Riverine, flow-through | Permanently flooded, deep water | FnA | Northern Ohio |

* Pw (Pewamo), Cc (Carlisle muck), FnA (Fluvaquents), Aa (Adrian muck).

Table 2.1. Description of the six wetland communities included in this study.

| Wetland community | Hydrogeomorphic category | Total Carbon (gC kg$^{-1}$ soil) | Inorganic Carbon (gC kg$^{-1}$ soil) | Organic Carbon (gC kg$^{-1}$ soil) |
|---|---|---|---|---|
| Shrub | Depressional, isolated | 134.4 ± 5.2 (6) | 1.7 ± 0.1 (6) | 132.7 ± 5.1 (6) |
| Forested | Depressional, isolated | 143.6 ± 6.4 (7) | 2.7 ± 0.6 (7) | 141.0 ± 6.9 (7) |
| Marsh | Depressional, isolated | 159.4 ± 6.8 (7) | 2.7 ± 0.8 (7) | 156.7 ± 7.5 (7) |
| All depressional wetland sites | | 146.4 ± 4.2 (20) | 2.4 ± 0.4 (20) | 144.0 ± 4.3 (20) |
| Upland of depressional wetland | | 16.5 ± 6.3 (7) | 1.0 ± 0.1 (7) | 15.5 ± 6.3 (7) |
| Floating bed | Riverine, flow-through | 86.6 ± 8.4 (7) | 5.4 ± 1.6 (7) | 81.3 ± 9.7 (7) |
| Marsh | Riverine, flow-through | 26.6 ± 1.8 (7) | 4.3 ± 0.9 (7) | 22.3 ± 1.1 (7) |
| Mudflat | Riverine, flow-through | 35.1 ± 3.2 (6) | 0.7 ± 0.3 (6) | 34.9 ± 3.4 (6) |
| All riverine wetland sites | | 50.1 ± 6.9 (20) | 3.6 ± 0.8 (20) | 46.6 ± 6.9 (20) |
| Creek bank | | 9.1 ± 1.1 (7) | 0.3 ± 0.0 (7) | 8.9 ± 1.1 (7) |
| Upland of riverine wetland | | 7.1 ± 2.9 (7) | 1.1 ± 0.0 (7) | 5.9 ± 2.9 (7) |

Table 2.2. Mean concentrations of total, organic and inorganic soil carbon (gC kg$^{-1}$) expressed as average ± standard error (*n*), and ratio of organic carbon over total carbon in the six wetland sites and the three non-wetland sites included in this study. All site cores are 35 cm long, except those from the shrub and the mudflat communities that are 30 cm long.

| Wetland community | Hydrogeomorphic category | Carbon pool (kgC m$^{-2}$) | Bulk density (Mg m$^{-3}$) |
|---|---|---|---|
| Shrub | Depressional, isolated | 3.72 ± 0.27 (6) | 0.56 ± 0.05 (6) |
| Forested | Depressional, isolated | 3.93 ± 0.34 (7) | 0.54 ± 0.03 (7) |
| Marsh | Depressional, isolated | 4.62 ± 0.57 (7) | 0.57 ± 0.05 (7) |
| All depressional wetland sites | | 4.18 ± 0.25 (20) | 0.57 ± 0.01 (20) |
| Upland of depressional wetland | | 1.01 ± 0.32 (7) | 1.60 ± 0.01 (7) |
| Marsh | Riverine, flow-through | 1.10 ± 0.08 (7) | 0.82 ± 0.02 (7) |
| Mudflat | Riverine, flow-through | 1.31 ± 0.10 (6) | 0.75 ± 0.02 (6) |
| Floating bed | Riverine, flow-through | 2.73 ± 0.24 (7) | 0.63 ± 0.02 (7) |
| All riverine wetland sites | | 1.50 ± 0.19 (20) | 0.77 ± 0.06 (20) |
| Creek bank | | 0.38 ± 0.05 (7) | 0.84 ± 0.01 (7) |
| Upland of riverine wetland | | 0.64 ± 0.22 (7) | 1.68 ± 0. 03 (7) |

Table 2.3. Average ± standard error (number of samples) of soil carbon pool (kgC m$^{-2}$) and bulk density (Mg m$^{-3}$) in the six wetland communities (upper 35 cm in all communities except the shrub and the mudflat communities, which were 30 cm), three non-wetland communities (upper 35 cm), and overall hydrogeomorphic wetland types.

| Wetland community | Hydrogeomorphic category | Annual Sediment Accretion (mm $yr^{-1}$) | Annual Sediment Accumulation (tons $ha^{-1}$ $yr^{-1}$) | Carbon Sequestration Rate (gC $m^{-2}$ $yr^{-1}$) |
|---|---|---|---|---|
| Shrub | Depressional, isolated | 3.0 | 14 | 202 |
| Forested | Depressional, isolated | 6.2 | 34 | 473 |
| Marsh | Depressional, isolated | 3.4 | 15 | 210 |
| All depressional wetland sites | | 4.5 | 23 | 317 |
| Floating bed | Riverine, flow-through | 3.8 | 25 | 160 |
| Marsh | Riverine, flow-through | 4.3 | 35 | 105 |
| Mudflat | Riverine, flow-through | 4.3 | 31 | 112 |
| All riverine wetland sites | | 4.1 | 28 | 140 |

Table 2.4. Average sedimentation and carbon accumulation in the six wetland sites and weighted average of the hydrogeomorphic wetland types.

| Wetland Site | Carbon sequestration rate (g $m^{-2}$ $yr^{-1}$) | Reference |
|---|---|---|
| ***Isolated, depressional, and/or forested wetlands*** | | |
| Dismal swamp, Virginia | 105 | Craft et al. 2008 |
| Cypress swamp, Florida | 122 | Craft et al. 2008 |
| Pocosin, North Carolina | 127 | Bridgham and Richardson 1993 |
| Okefernokee peat swamp, Georgia | 25 - 82 | Schlesinger 1978 *; Craft et al. 2008 |
| Peat meadow, Netherlands | 280 | Hendricks et al. 2007 * |
| Danube floodplain, Austria | 180 | Zehetner et al. 2009 |
| Cypress-gum floodplain, Georgia | 107 | Craft and Cassey 2000 |
| Upper St. Johns floodplain, Florida | 117 - 244 | Brenner et al. 2001 |
| Pin oak swamp, Ohio | 473 | This study |
| Buttonbush swamp, Ohio | 202 | This study |
| Cattail marsh, Ohio | 210 | This study |

Continued.

Table 2.5. Comparison of carbon sequestration rates of freshwater wetlands located in temperate regions reported in literature. Carbon sequestration rates estimated in this study are included in each appropriate wetland type. All studies except those with an asterisk used $^{137}$Cs and/or $^{210}$Pb to determine carbon sequestration rates.

Table 2.5 continued.

| **Wetland Site** | **Carbon sequestration rate (g $m^{-2}$ $yr^{-1}$)** | **Reference** |
|---|---|---|
| ***Riverine and/or flow-through wetlands*** | | |
| Cattail marsh, nutrient enriched, Florida | 264 | Reddy et al. 1993 |
| Cattail-sawgrass marsh, Florida | 104 - 167 | Reddy et al. 1993; Craft and Richardson 1993 |
| Sawgrass marsh, Florida | 124 | Reddy et al. 1993 |
| Grass-sedge marsh, Georgia | 56 | Craft and Cassey 2000 |
| Reed-bulrush marsh, Oregon | 116 | Graham et al. 2005 |
| Reed marsh, Denmark | 504 | Brix et al. 2001 * |
| Arum arrow marsh, Virginia | 97 | Whiting and Chanton 2001 * |
| Anderson tule marsh, California | 106 - 155 | Kim 2003 |
| Everglades, Florida | 99 - 190 | Craft and Richardson 1993 |
| Reed-bulrush marsh, Ohio | 105 | This study |
| Cutgrass mudflat, Ohio | 112 | This study |
| Water lotus marsh, Ohio | 160 | This study |

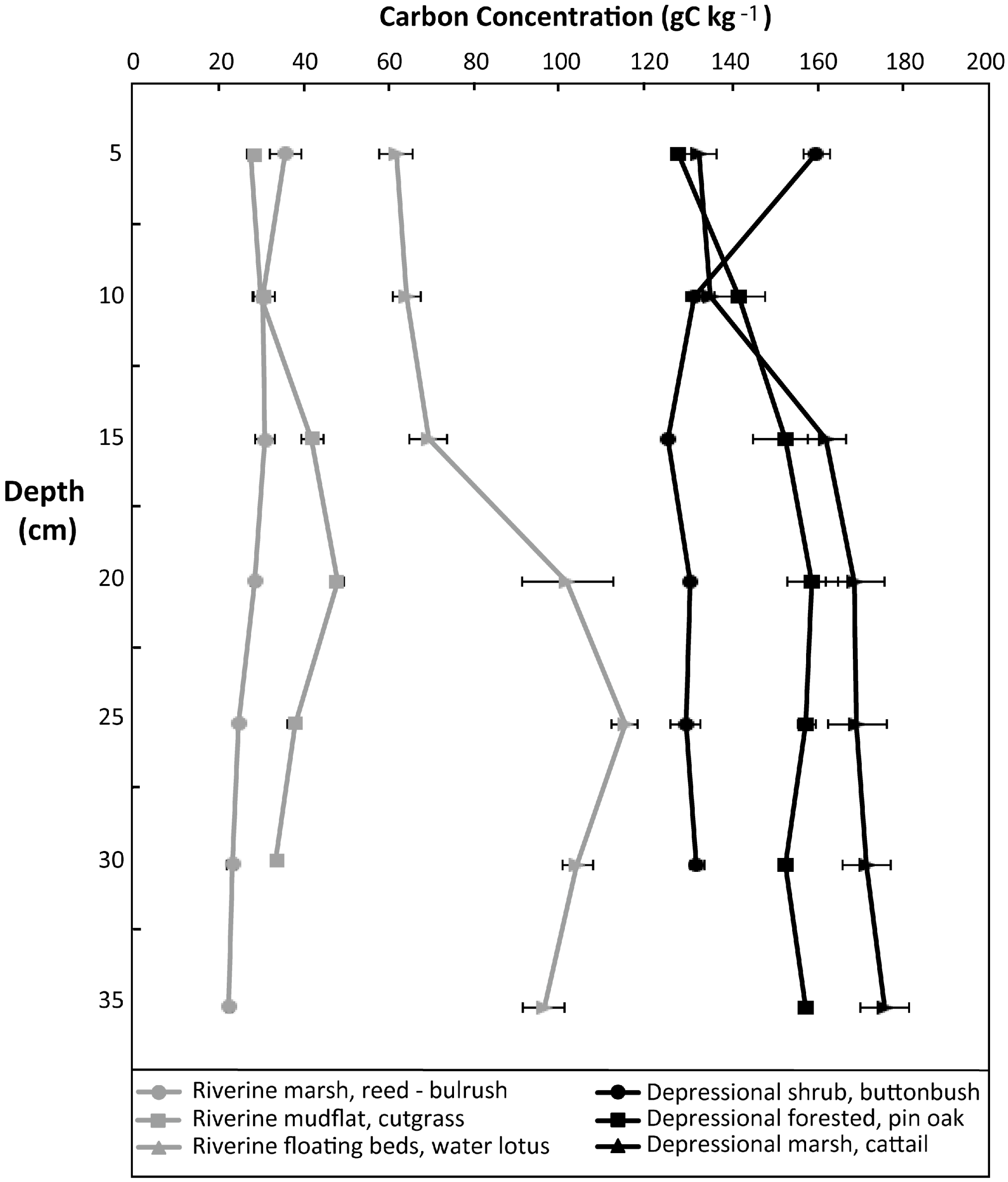

Figure 2.1. Profile of carbon concentration in the soil (gC kg$^{-1}$ soil) of the six wetland communities at the isolated depressional wetlands (in black) and the riverine flow-through site (in gray). Concentration for each depth increment is the average of that depth (*n* = 3), and the error bars represent standard error of the average.

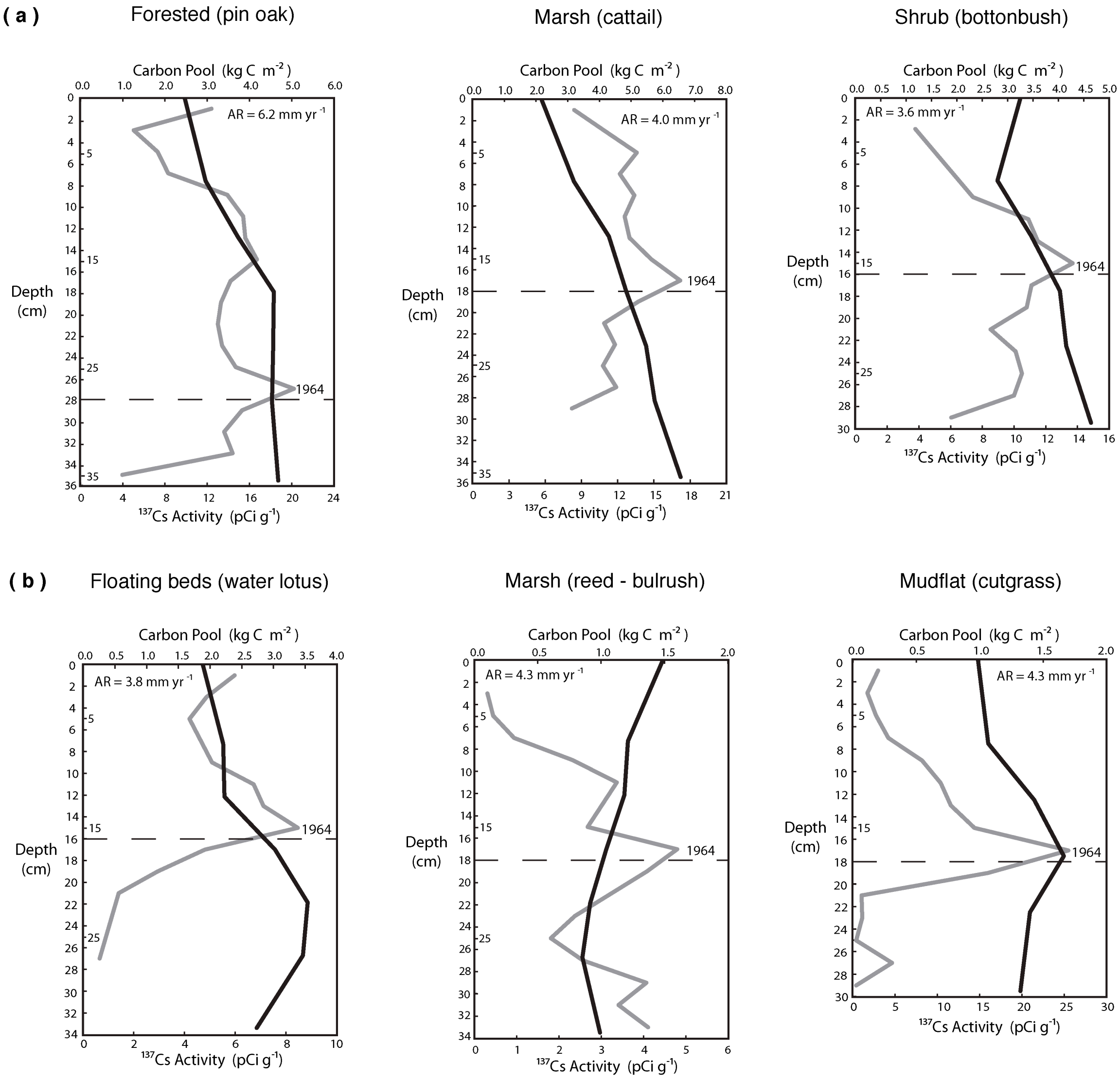

Figure 2.2. $^{137}$Cs activity per unit mass of soil (pCi g$^{-1}$) in soil cores (in gray) and carbon pool profile (in black) in six wetland communities at the (a) isolated depressional wetlands and the (b) riverine flow-through wetlands. The peak of Cs activity corresponds to the year 1964; the dotted line at that depth represents the amount of soil accumulated since that year. Sediment accretion rates (*AR*) are shown for each community.

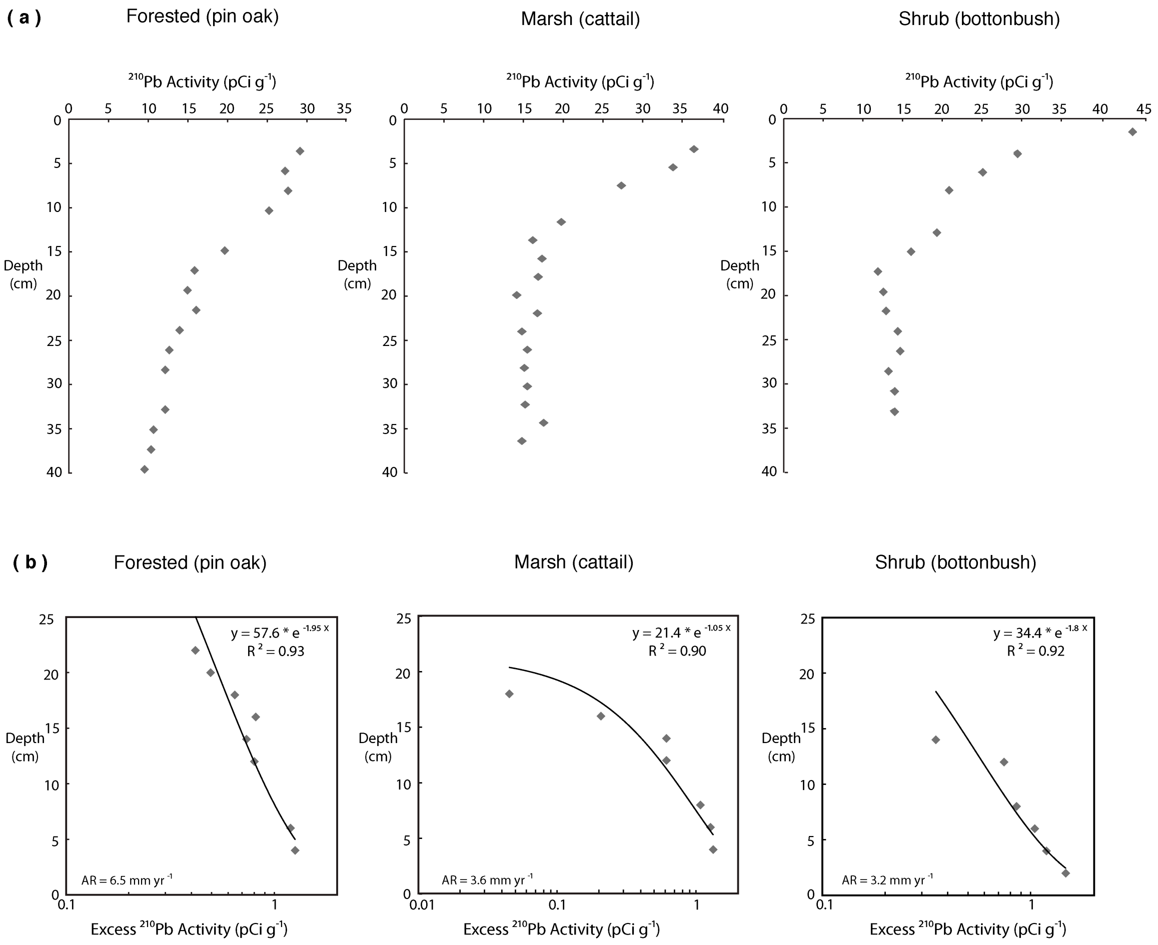

Figure 2.3. (a) Total $^{210}$Pb activity per unit mass of soil (pCi g$^{-1}$) in soil cores from three wetland communities at the isolated depressional wetlands; and (b) excess $^{210}$Pb activity profiles with estimated sediment accretion rate (*AR*) of each.

# CHAPTER 3

# CARBON SEQUESTRATION IN TROPICAL FRESHWATER WETLANDS

## 3.1. Abstract

Tropical wetlands are typically productive ecosystems that can introduce large amounts of carbon into the soil. However, high temperatures and little or seasonal water availability can hinder the ability of wetland soils to sequester carbon efficiently. We determined the carbon sequestration rate of twelve wetland communities in four different tropical wetlands — a riverine flow-through wetland with a marked wet and dry season, a seasonal floodplain of an inland delta, an isolated depressional wetland in a rainforest, and a slow-flowing rainforest swamp — with the intention of finding conditions that favor soil carbon accumulation in tropical wetlands. Triplicate soil cores were extracted in these communities and analyzed for total carbon content to determine the wetland soil carbon pool. We found that the sites in the humid tropics had greater carbon content ($P \leq 0.05$) than the tropical dry ones (96.5 and 34.8 gC $kg^{-1}$, respectively). While the tropical dry wetlands had similar sequestration rates (63 ± 10 $gCm^{-2}$ $y^{-1}$ on average), the tropical

humid ones differed significantly ($P < 0.001$), with high rates in a slow-flowing slough (306 ± 77 gC $m^{-2}$ $y^{-1}$) and low rates in a tropical rain forest depressional wetland (84 ± 23 gC $m^{-2}$ $y^{-1}$). The carbon accumulating in all of these wetlands was mostly organic (92–100 %). These results suggest the importance of differentiating between types of wetland communities and their hydrology when estimating overall rates at which tropical wetlands sequester carbon, and the need to include tropical wetland carbon sequestration in global carbon budgets.

## 3.2. Introduction

Wetland protection has been achieved around the world by the recognition of wetland services and by programs such as the designation many international wetlands as "Ramsar Wetlands of International Importance" (*www.ramsar.org*). Many tropical wetlands, however, are not protected by international recognition and remain vulnerable to destructive alterations – tropical wetlands are generally considered free resources of land, water, and biomass, and the important hydrological and ecological functions they perform in the landscape are frequently disregarded (de la Cruz 1986; Roggeri 1995; Junk 2002; Mitsch and Gosselink 2007). This lack of protection is sometimes due to the difficulty in estimating with accuracy the land coverage of tropical wetlands, given their inaccessibility when located in tropical wet rainforests, and the differences in some tropical climates between the permanent area of a wetland (the area that remains flooded during the dry season) and the seasonal area (area flooded during the wet season), which can often be several orders of magnitude greater than the permanent wetland area (de la Cruz 1986; Junk 1997,2002).

The loss of tropical wetlands entails the loss of their functions. Most of the studies on tropical wetland functions address water availability, community structure, and species diversity; less is known about the biogeochemistry of these wetlands. However, recent recognition of the ecosystem services of wetlands on global carbon cycles and net greenhouse gas exchange with the atmosphere has led to estimations of carbon budgets in tropical wetlands, mostly by means of remote sensing (e.g. Melack et al. 2004) and eddy covariance (e.g. Saunders et al. 2007), with fewer studies measuring *in situ* soil carbon accumulation (e.g. Franzluebbers et al. 2001; Chimner and Ewel 2005). The general goal of these estimations is to assess the role of tropical wetlands in global carbon budgets and their potential effect on climate change as sinks or sources of carbon-based greenhouse gases to the atmosphere. This assessment is of critical importance because (1) current biogeochemical cycles in tropical wetlands can provide an approximation of what temperate wetlands could come to be in a changing climate where temperatures are expected to rise (IPCC 2007), and (2) the current carbon stock of tropical wetlands could be released quickly to the atmosphere if these wetlands change due to increased evapotranspiration or to changes in the regional hydrology.

Despite the importance of defining carbon balances in tropical wetlands it is difficult to make generalizations, given the specific characteristics of the different types of tropical wetlands (Junk 2002). Most of the freshwater tropical wetlands belong to the floodplain type (such as those of the Amazon, Magdalena, or Orinoco in South America; the Okavango, Nile, or Zaire in Africa; Ganges, Mekong, or Tigris and Euphrates in Asia), and are subject to different extent to seasonal changes in water supply, i.e., hydrologic pulses (de la Cruz 1986; Junk 2002). Hydrologic pulses generally increase wetland productivity (Odum et al. 1995), but the lack of water in a tropical wetland during the dry season can allow the fast decomposition of the organic matter accumulating in the soil (Kirschbaum 1995; Franzluebbers et al. 2001; Saunders et al. 2007). Some studies on tropical floodplains suggest that floodplain wetlands might be a source of carbon to the atmosphere (Melack et al. 2004; Sousa Moura et al. 2008), but uncertainties on their carbon budgets are high (Melack et al. 2004; Chimner and Ewel 2005). On the contrary, tropical peatlands usually have permanent high water table, which slows down organic matter decomposition and allows the accumulation of deep layers of peat (Franzluebbers et al. 2001; Chimner and Ewel 2005; Saunders et al. 2007). This allows tropical peatlands to account for up to 30 % of the globe's total peat carbon pool, despite of covering just 10 % of the total peatland area in the world (Chimner and Ewel 2005; Jauhiainen et al. 2005). While boreal peatlands accumulate on average about 29 gC $m^{-2}$ $y^{-1}$ (Gorham 1991), tropical peatlands have reported sequestration rates as high as 300 gC $m^{-2}$ $y^{-1}$ (Chimner and Ewel 2005), 480 gC $m^{-2}$ $y^{-1}$ (Saunders et al. 2007), and 527 gC $m^{-2}$ $y^{-1}$ (Suzuki et al. 1999).

We measured *in situ* the soil carbon sequestration rates at four different tropical freshwater wetland sites (a depressional isolated wetland, slow-flow swamp, a riverine wetland, and an inland delta) located in two different climates (tropical humid, i.e. warm and wet year-round; and a tropical dry, i.e. with two marked seasons, warm and wet, and hot and dry) with the intention of finding the settings or wetland features that favor carbon accumulation in the soil of tropical wetlands. We also estimated the sequestration rates of their different wetland communities within these 4 sites to assess the variability within each wetland site to better understand the environmental and ecological conditions that affect carbon sequestration in tropical wetlands.

## 3.3. Materials and methods

### *3.3.1.Study sites*

Our freshwater wetlands in the humid tropics are located in the heart of the rainforest of northeastern Costa Rica, one on the campus of EARTH University (10° 13' N, 83° 34' W) and the other in La Selva Biological Station (10° 25' N, 84° 0' W).

The wetland at EARTH University is a 112-ha slow-flow slough that receives water from precipitation, runoff, and the overflow of nearby creeks that feed the Rivers Parismina and Dos Novillos. Abundant precipitation (3460 ± 750 mm $y^{-1}$) and poorly drained alluvial soils (classified as Aquepts by Vásquez Morera 1983) maintain the wetland with standing water year-round, up to 1 m in the deepest sites (Bernal and Mitsch 2008). The swamp area is dominated by *Raphia taedigera* (swamp palm) and *Pentaclethra macroloba* (oil tree) with numerous and diverse woody and herbaceous understory, while the flooded area is dominated by water-tolerant species that conform three distinct wetland communities: open water areas where water level is highest and peat accumulates; a shallower but permanently flooded area densely vegetated by *Chamaedorea tepejilote* (pacaya palm), *Spathiphyllum friedrichsthalii* (peace lily), *Pentaclethra macroloba* (oil tree), and *Calathea crotalifera* (rattlesnake plant); and an edge community dominated by *Spathiphyllum friedrichsthalii* (peace lily) where muck accumulates. More detailed information about this wetland can be found in Mitsch et al. (2008, 2010) and Nahlik and Mitsch (2011).

The 3-ha wetland at La Selva is located in a primary, evergreen rainforest (McDade and Hartshorn 1994), developed over alluvial soils classified as Typic and Histic Tropaquepts (Sollins et al. 1994) between the Rivers Puerto Viejo and Sarapiquí. This rainforest hosts numerous small depressional ponds, mainly fed by precipitation and runoff. Despite of receiving abundant rainfall (4337 ± 520 mm $y^{-1}$), standing floods do not remain for more than 3-5 days (Hartshorn and Hammel 1994), leaving the mucky substrate of the wetland with permanent saturated conditions but intermittent standing water. The forest vegetation in the area is a dense and diverse canopy, subcanopy, and understory mixture of woody species, dominated by *Pentaclethra macroloba*, *Carapa nicaraguensis* (andiroba or crabwood), and *Pachira aquatica* (money tree), among others (Hartshorn and Hammel 1994; Nahlik and Mitsch 2011). The flooded or saturated areas present different wetland communities dominated by *Spathiphyllum friedrichsthalii* in the edge of the wetland, a combination of shrubs and grasses such as *Acalypha diversifolia* (palito feo) and *Gynerium sagittatum* (wild cane) in the permanent ponds, and open water areas that remain as shallow mudflats.

The freshwater wetlands of the dry tropic included in this study are located in Palo Verde Biological Station, in Western Costa Rica (10° 20' N, 85° 20' W), and in the Okavango Delta, in Northern Botswana (19° 32' S, 23° 09' E). Both wetlands experience marked warm wet and hot dry seasons every year, having high water levels in the peak of the rainy season and/or the following seasonal river discharge, and dry soils with a few permanent ponds that in the dry months survive the strong evapotranspiration.

The wetland of Palo Verde (1200 ha) is on a coastal plain that receives water from precipitation (1240 ± 252 mm $y^{-1}$) and the occasional overflow of the adjacent Tempisque River, when its water level is high enough to overpass the sediment barrier along the wetland margin (McCoy and Rodríguez 1994). The alluvial Vertisols (Bernal and Mitsch 2008) retain the water in the wetland long enough to support floating aquatic and emergent plants, that are relegated to the scarce permanent ponds and saturated soils during the dry season (November – April). The wetland has been managed for the last three decades for cattle grazing and the control of *Typha domingensis* (Southern cattail), as described by McCoy and Rodríguez (1994) and Trama et al. (2009). Three wetland communities can be identified in this marsh. The permanently flooded sites are dominated by *Eichhornia crassipes* (water hyacinth) and *Thalia geniculata* (aligator flag), while *Typha domingensis* dominates the flooded or saturated areas. The sections of the wetland that dry down during driest season remain as mudflats with grasses and sedges (Trama et al. 2009), such as *Eleocharis* sp. (spikerush), *Paspalidium* sp. (panicgrass), and *Oxycaryum cubense* (cuban bulrush). The wetland is bordered by a secondary dry forest (Hernández et al. 2002) dominated by *Guazuma ulmifolia* (West Indian elm).

The inland delta of the Okavango River is a major wetland complex in Africa (de la Cruz 1986). This vast floodplain in the semi-arid basin of the Kalahari River covers almost 1.5 x $10^6$ ha, 80 % of which are seasonal wetlands (de la Cruz 1986; Ramberg et al. 2006a). The rainy season in this region (700 – 200 mm $y^{-1}$, Wolski et al. 2006; Mitsch et al. 2010) occurs in the months of November to March, with peak river flows between March and May (Mitsch et al. 2010). The highest water levels in the floodplain, however, occur during the months of July to September (Ramberg et al. 2006a,b). The area presents stream channels, and permanently and seasonally flooded floodplains. Pristine oligotrophic waters and sandy soils (classified as Gleyic Arenosols by Simmonds 1998) lead to a wetland where the dominant plant species are grasses and sedges, such as *Cyperus articulatus* (piri piri), *Oryza longistaminata* (longstamen rice), and *Schoenoplectus corymbosus* (isolepis corymbosa), all of them present in the seasonal and the permanent marshes. More information about the hydrology and vegetation cover of this inland delta can be found in Ramberg et al. (2006a,b), Wolski et al. (2006), and Mitsch el at (2010).

### *3.3.2.Sample collection and analysis*

Sediment cores were collected in each of the three identifiable communities of the four wetlands included in this study, described above and listed in Table 1. Two sets of cores were collected in each community of the wetlands in Costa Rica, one for analysis of carbon content and one for radiometric dating, as well as an extra upland set for carbon content determination (Bernal and Mitsch 2012). Cores for carbon analysis consisted on triplicate cores divided on 5 cm increments, analyzed individually; cores for radiometric analyses ($^{137}$Cs and $^{210}$Pb) consisted on composite cores divided on 2 cm increments (three cores per composite sample, whose corresponding layers were pooled together into one sample per depth increment in each community; Isaksson et al. 2001; Stark et al. 2006; Bernal and Mitsch 2012). Due to permitting and regulation restrictions only one set of cores was collected in each of the three wetland communities in the Okavango Delta. This single set of cores was used both for carbon analysis and radiometric dating, and it consisted on composite cores collected and prepared the same way as the composite cores for radiometric analysis from Costa Rica.

The set of samples from Costa Rica for carbon analysis were dried at 65 °C, ground to a < 2 mm particle size, and homogenized. Total, organic and inorganic soil carbon content was measured in each sample using a Total Carbon Analyzer (TOC-V series, SSM-5000A, Shimadzu), and reported as carbon concentration (gC kg$^{-1}$) and carbon pool (kgC m$^{-2}$), as described by Bernal and Mitsch (2012). Total soil nitrogen was measured in a Nitrogen Combustion Analyzer (varioMax CN, Elementar Analysensysteme) to estimate soil C:N ratios. The set of samples from Costa Rica for radiometric analysis, as well as the single set of samples from Botswana, were dried at 105 °C, ground to a < 2 mm particle size, and homogenized. Some studies report significant differences on soil carbon content when very organic soils are dried at temperatures higher than 65 °C (Grossman and Reinsch 2002). To ensure that the different drying temperature used here (65 and 105 °C) did not affect the carbon content determination of the sandy wetland soils of the Okavango, we dried a subset of 60 samples from Costa Rica (from Palo Verde's wetland communities) at 65 and 105 °C, and measured their carbon content using the procedure described above. The carbon content of these 60 samples ranged from 1.6 % to 7.0 %, and no significant differences were found on their soil carbon under different drying temperatures ($P = 0.4$). Consequently, we analyzed the carbon content of Okavango Delta's soils following the method similar to the analysis performed for the Costa Rican samples.

Accretion rates of each of the twelve tropical sites site were estimated non-destructively by measuring $^{137}$Cs and $^{210}$Pb activity in the soil, using a high efficiency Germanium Detector (GL 2820R, Canberra). $^{137}$Cs was used to estimate sediment accretion rates in the cores where a peak on $^{137}$Cs activity, corresponding to the year 1964 (year of maximum deposition rate on the globe), was identified in the core profile (Craft and Casey 2000; Smith et al. 2000; Graham et al. 2005; Ilus and Saxén 2005). The measurement of $^{210}$Pb activity and calculation of excess $^{210}$Pb allowed us to estimate accretion rates in every site by using the constant activity model, described extensively in literature (Appleby and Oldfield 1978; Oldfield and Appleby 1984; Faure and Mensing 2005) and used for many wetland studies (Briker-Urso et al. 1989; Craft and Casey 2000; Graham et al. 2005; Bernal and Mitsch 2012).

Knowing the sediment accretion rate of each wetland site (cm $y^{-1}$) we calculated the carbon sequestration rate (gC $m^{-2}$ $y^{-1}$) multiplying the accretion rate by the soil bulk density and by the carbon content of each site. More detailed information on soil sample collection, preparation, analyses, and calculations can be found in Bernal and Mitsch (2012).

### *3.3.3. Statistical analysis*

In every dataset (wetland communities, wetlands, and climates) we did an exploratory data analysis (EDA), normality checks with *Q-Q* plots and Kolmogorov-Smirnov tests, and homogeneity of variances checks with Levene statistic. Statistical analysis of variance (ANOVA) with Tukey HSD was used to determine differences between climates and between wetlands on carbon content, carbon pools, accretion rates, and carbon sequestration rates. Independent *t*-tests were performed to compare carbon content between each wetland and their adjacent upland site. *P* values ≤ 0.05 and ≤ 0.10 indicate significant differences at 95% and 90% confidence level, respectively (Fowler et al. 2003). Statistical analyses were performed with IBM SPSS Statistics version 19.0 for Macintosh (SPSS Inc.).

## 3.4. Results

### *3.4.1. Soil profiles of tropical wetlands*

The carbon content in the three wetland communities of the isolated rainforest wetland at La Selva were very similar, with averages ranging from 31.6 to 61.3 gC kg$^{-1}$ ($P$ = 0.18); Table 3.2. The carbon content was highest in the top 20 – 25 cm of soil (up to 78.6 gC kg$^{-1}$) of every wetland site, decreasing sharply from that depth below, down to 21.7 gC kg$^{-1}$. Similar bulk densities (0.5 Mg m$^{-3}$, on average) and carbon contents yielded similar soil carbon pools in these sites ($P$ > 0.7), even though in the permanent ponds the average carbon pool was 40 % higher that in the other two communities (1.64 kgC $m^{-2}$ *vs* 1.17 kgC $m^{-2}$; Table 3.2). The average soil carbon pool for the entire wetland was 1.33 kgC $m^{-2}$ to a depth of 55 cm. Similarly to the soil carbon content, the carbon pools decreased from the top to the deeper layers (Figure 3.1), ranging from 31.8 kgC $m^{-3}$ to 7.1 kgC $m^{-3}$. The carbon stored in this wetland is mostly organic (95–99 %), having slightly higher inorganic content in the edge community. Carbon and nitrogen content in the soil of these communities were significantly correlated ($P < 0.001$, $r = 0.99$, linear $R^2 = 0.98$), and their C:N ratio was, on average, 9.6 (Table 3.2).

The analysis with $^{210}$Pb (Figure 3.2) indicated that the soil accretion rates of these three wetland communities ranged from 4.0 mm $y^{-1}$ in the non-vegetated site to 4.8 mm $y^{-1}$ in the permanent pond. The sedimentation rate is correlated with bulk density and thus, the permanent pond had the lowest sedimentation rate (2.17 ± 0.09 kg $m^{-2}$ $y^{-1}$) despite of having the highest accretion (Table 3.2).

The slough swamp in the rainforest at EARTH University had very rich organic soils, even in the non-peat sites (98–100 % of the total carbon was organic). Carbon content in the peat community was significantly higher ($P < 0.001$) than in the rest of wetland (325 ± 43 gC $kg^{-1}$ in the peat site, *vs.* 71 ± 12 gC $kg^{-1}$ in the permanently flooded vegetated community and 57 ± 11 gC $kg^{-1}$ in the vegetated mucky edge; Table 3.2). While carbon content decreased with depth in all the non-peat sites (ranging from 97 gC $kg^{-1}$ on the surface layers to 34 gC $kg^{-1}$ at 45 cm deep), it increased with depth in the peat community, being 236 gC $kg^{-1}$ in the surface and

454 gC $kg^{-1}$ at 45 cm deep. The high organic matter content of the peat site reduced the soil bulk density, being almost half of the bulk density measured in the rest of the wetland sites (Table 3.2). Bulk density of the peat site decreased with depth. However, the carbon pool of this area increased with depth (Figure 3.1) because of carbon the content here was increasing as one goes deeper into the soil. The carbon pool remained somewhat constant with depth in the other two wetland communities, being on average 1.2 kgC $m^{-2}$, significantly less ($P < 0.001$) than the average pool in the peat site (3.4 kgC $m^{-2}$; Table 3.2). Soil carbon and nitrogen were significantly correlated in these wetland sites ($P < 0.001$, $r = 0.99$, and linear $R^2 = 0.99$ in the peat community; $P < 0.001$, $r = 0.98$, and linear $R^2 = 0.96$ in the other two communities), and the C:N ratio was almost twice as high in the peat site than in the other two wetland communities (Table 3.2).

Accretion and sedimentation rates were highest in the vegetated areas at the edge (10.0 mm $y^{-1}$ and 4.5 ± 0.5 kg $m^{-2}$ $y^{-1}$) and the permanently flooded area (9.7 mm $y^{-1}$ and 3.4 ± 0.4 kg $m^{-2}$ $y^{-1}$). The accretion rate in the peat site was much than in the rest of the wetland (7.8 mm $y^{-1}$; Figure 3.2), and its sedimentation rate was one-third of the sedimentation in the edge community (1.4 ± 0.1 kg $m^{-2}$ $y^{-1}$; Table 3.2).

Palo Verde's three riverine wetland communities had similar soil carbon content ($P > 0.4$), ranging from 39 gC $kg^{-1}$ in the permanent ponds to 45 gC $kg^{-1}$ in the vegetated edge (Table 3.2). This carbon was mostly organic, with 8 to 6 % inorganic content. Having similar carbon content and similar bulk densities (0.66 Mg $m^{-3}$, on average) the soil carbon pools of these three wetland communities was very similar as well ($P > 0.4$), ranging on the top 30 cm from 1.35 kgC $m^{-2}$ in the permanent ponds to 1.35 kgC $m^{-2}$ in the mudflats (Table 3.2) and being relatively constant along the soil profiles (Figure 3.1). The carbon content, however, showed a marked decrease with soil depth, being as high as 65.1 gC $kg^{-1}$ in the top 10 cm of the wetland edge and as low as 25.4 gC $kg^{-1}$ in the mud flats at 30 cm deep. The C:N ratio was highest in the vegetated edge (11.0 ± 0.3) and lowest in the mudflats (9.9 ± 0.1), but both carbon and nitrogen contents were significantly correlated in these sites ($P < 0.001$, $r = 0.99$, linear $R^2 = 0.98$).

Accretion rates in these wetland communities, estimated with $^{210}Pb$ (Figure 3.2), were relatively constant (3.0 mm $y^{-1}$, 3.2 mm $y^{-1}$, and 3.3 mm $y^{-1}$ in the edge, permanent pond, and mudflat, respectively). Their sedimentation rates were very similar as well, ranging from 1.96 kg $m^{-2}$ $y^{-1}$ to 2.24 kg $m^{-2}$ $y^{-1}$ (Table 3.2).

The sandy wetland soils of the Okavango Delta had high bulk density (0.96 – 1.02 Mg $m^{-3}$) and low carbon content. The lowest carbon content was in the deep riverine marsh (16.7 ± 3.4 gC $kg^{-1}$) and highest in the shallow and seasonally flooded marsh (33.3 ± 7.3 gC $kg^{-1}$; Table 3.2), although differences were not significant ($P > 0.2$). The carbon pools of these two communities in the top 30 cm of soil, however, were significantly different ($P = 0.02$), with the shallow seasonal marsh carbon pool almost twice of the pool in the riverine marsh (1.5 kgC $m^{-2}$ and 0.75 kgC $m^{-2}$, respectively; Table 3.2), while the deep seasonal marsh had a carbon pool midway between those two marshes (1.25 kgC $m^{-2}$). None of this carbon was inorganic. The carbon profiles of both seasonal marshes was very similar, being higher in the top

10–15 cm of soil (152.7 – 37.4 gC $kg^{-1}$) and decreasing below to a somewhat constant level around 13.8 gC $kg^{-1}$. The carbon pools of the seasonal marshes decreased sharply with depth (Figure 3.1). Being somewhat constant below 10–15 cm deep in the deep seasonal marsh. In the riverine marsh the carbon content varied less with soil depth than in the other communities, from 39.7 gC $kg^{-1}$ at the surface layers to 4.1 gC $kg^{-1}$ at a depth of 30 cm, and the carbon pool was showing a similar trend (Figure 3.1).

$^{210}Pb$ activity (Figure 3.2) indicated that the deep and shallow seasonal marshes had about the same accretion rates (1.8 mm $y^{-1}$ and 1.9 mm $y^{-1}$, respectively) and sedimentation rates (1.8 kg $m^{-2}$ $y^{-1}$ and 1.9 kg $m^{-2}$ $y^{-1}$, respectively).

### *3.4.2. Carbon sequestration in tropical wetlands*

Differences on carbon content and sedimentation rates were accentuated in the carbon sequestration rates of each wetland and each community. The isolated wetland in the rainforest at La Selva sequestered, on average, 84 ± 23 gC $m^{-2}$ $y^{-1}$. The sequestration rates of its open and vegetated edge communities were very similar (60.6 and 60.7 gC $m^{-2}$ $y^{-1}$; Table 3.2), but the rate in the permanent pond (130.9 gC $m^{-2}$ $y^{-1}$) was 2.2 higher than the other two communities ($P < 0.01$). The swamp at EARTH University (the other rainforest wetland) had a carbon sequestration rate of 306 ± 77 gC $m^{-2}y^{-1}$, a rate significantly higher than the sequestration estimated for the wetland at La Selva ($P < 0.001$). There were also significant differences on carbon sequestration between EARTH's peat community and the vegetated ones ($P < 0.001$) since the peat community had a sequestration rate of 465 gC $m^{-2}$ $y^{-1}$, twice the average rate of the other two communities in this wetland (Table 3.2). In the riverine seasonal wetland of Palo Verde, the wetland soils are sequestering 84 ± 3 gC $m^{-2}$ $y^{-1}$. The individual rates of the three communities are very similar ($P > 0.4$), being highest in the mudflat (84.2 gC $m^{-2}$ $y^{-1}$) and lowest in the vegetated edge (79.9 gC $m^{-2}$ $y^{-1}$; Table 3.2). Our second seasonal wetland, the floodplain of the Okavango inland delta, had the lowest carbon sequestration rates of this study, being on average 42 ± 6 $m^{-2}$ $y^{-1}$ for the entire wetland. Its riverine permanent marsh had significantly lower carbon sequestration (33 gC $m^{-2}$ $y^{-1}$) than the shallow seasonal one (53 gC $m^{-2}$ $y^{-1}$), $P = 0.1$ (Table 3.2). Sequestration rates of the Okavango were significantly different than the rates estimated for Palo Verde ($P = 0.05$). When grouping these twelve wetland communities by climate, however, differences are significant with $P = 0.08$ but communities from La Selva are not statistically different to those from Palo Verde or Okavango.

## 3.5. Discussion

### *3.5.1. Carbon in tropical wetland soils*

Tropical wetlands have been classified by Junk (1997,2002) as saline swamps and saltpans, periodically flooded forests, coastal wetlands, river floodplains, and periodically flooded savannas. Except for saline swamps, saltpans, and coastal wetlands, our study covered the remaining freshwater wetland types well. Thus, these four wetlands are collectively a good representation of freshwater tropical wetlands in the world.

The wetland communities within each of the wetland sites included in this study had very similar carbon content; the only one markedly different to the other communities of the site was the peat-accumulating humid slough at EARTH University which, given its characteristics, could be considered a completely different wetland. Wetland communities are an indication of the different hydrogeomorphic features occurring in a wetland (Boutin and Keddy 1993; Mitsch and Gosselink 2007; Bernal and Mitsch 2012). These features (hydrology, vegetation, and placement in the landscape, among others) usually influence wetland functions such as the ability to sequester carbon, because they define the biogeochemistry of the site. In our wetlands, the fact that there were no big differences between wetland communities on carbon content within each wetland suggests that the environmental conditions are playing a role as important as the community characteristics in the biogeochemistry of these wetlands.

In our three communities of the isolated rainforest wetland at La Selva Biological Station, abundant rains and warm temperatures kept biomass productivity in the ecosystem high, but the lack of standing water for prolonged periods of time due to evapotranspiration and high temperatures likely led to organic matter oxidizing quickly (Kirschbaum 1995; Franzluebbers et al. 2001; Saunders et al. 2007), keeping it from accumulating deeper in the soil. Consequently, carbon content of these soils decreased sharply with depth, and the sequestration rate of each community were very similar. The community at this isolated wetland whose soils are more frequently under water than the rest, however, had significantly higher carbon sequestration rate than the others (Table 3.2). The effect of standing water on soil carbon is even more evident in the wetland sites of EARTH University, where the constant presence of water (supplied by adjacent creeks) hindered organic matter decomposition and enhanced considerably its soil carbon content and sequestration rate, either by maintaining anaerobic conditions in the soil, by reducing soil temperature respect to ambient temperature, or both (Chimner and Ewel 2005; Saunders et al. 2007). These carbon-favoring conditions are especially fostered in the peat site, where deep water levels (one meter or more, depending on the season; Bernal and Mitsch 2008; Nahlik and Mitsch 2011) and consistent input of recalcitrant organic matter from the dense canopy of trees (woody plant debris) and their rhizomes led to the accretion of partially undecomposed plant material (i.e. peat) deep down into the wetland soil (Trettin and Jurgensen 2003; Jauhiainen et al. 2005; Chimner and Ewel 2005). These characteristics created a profile where soil carbon content increases its concentration as we go deeper in the soil profile instead of decreasing, which is an indication of the recalcitrant character of the organic matter that is accumulating here for the long term, as long as the site remains undisturbed (Saunders et al. 2007). Bridgham et al. (1998), Chimner and Ewel (2005), and Jauhiainen et al. (2005) found similar profiles in other tropical peatlands and pointed out the highly recalcitrant character of the organic matter contained in this type of wetlands, much more recalcitrant than in temperate and boreal peatlands since labile plant debris (e.g. leaves) decompose very quickly in warm and humid climates, where biologically active carbon (i.e. microbial communities) are much more active than in colder climates (Franzluebbers et al. 2001), leaving behind only the organic matter that is hard to decompose. The high C:N ratio of our peat site (about twice as much than any other wetland community included in this study; Table 3.2) is also an indication of the recalcitrant nature of the organic matter accumulating in this community (Stevenson and Cole 1999; Chimner and Ewel 2005; Sousa Moura et al. 2008). The presence of water and the constant input of organic matter increased the sediment accretion rate as well in these wetland sites, while the communities at La Selva's isolated wetland had accretion rates less than half of the rates at EARTH University's swamp (Table 3.2), possibly due to the erosion and compaction that the organic soils at this isolated wetland might be experimenting when the lack of standing water exposes them to the atmosphere (Grossman and Reinsch 2002). Other studies report similarly high accretion rates for tropical peatlands (Maas 1996; Chimner and Ewel 2005), ranging from 4 to 10 mm $y^{-1}$, considerably higher than the average 1 mm $y^{-1}$ reported for boreal peatlands (Gorham 1991).

Soil carbon content and sequestration rates were even more similar between wetland communities at the riverine wetland at Palo Verde in western Costa Rica. In this wetland the permanent or semipermanent presence of water in some shallow pools did not cause significantly greater soil carbon than in the other wetland communities. These similarities on soil carbon between wetland communities are probably because of a combination of several environmental factors affecting the biogeochemistry of the wetland. First, these wetland soils are Vertisols, i.e. they shrink as they dry and swell as they become moist, creating deep, wide cracks in the soil that let warm air deep down into the soil profile, potentially enhancing organic matter oxidation and microbial activity (Franzluebbers et al. 2001). Second, these wetland soils are frequently disturbed by the presence of cattle grazing in the wetland, compacting and/or resuspending sediments, and making it difficult for carbon to accumulate in the soil. Third, the frequent exposure of the wetland soil due to the seasonal water fluctuation produces strong respiration pulses with the drying and re-wetting of the soil (Miller et al. 2005; Saunders et al. 2007). Fourth, the seasonality of the wetland due to the lack of precipitation and high temperatures during the dry season is likely reducing organic matter inputs to the soil as wetland vegetation dies off under prolonged periods of draught. The net effect of these factors is probably the responsible of a homogeneous carbon profile throughout this wetland. Likewise, the soils of the deep and shallow seasonal marshes in the Okavango Delta have similar low carbon content and sequestration rates that might be due to a high organic matter oxidation, consequence of a marked dry season that lasts several months (Ramberg et al. 2006a,b; Mitsch et al. 2010) and a constant trafficking of wildlife that resuspends the sediment – some of the wild animals like the hippopotamus (*Hippopotamus amphibious*), very abundant in this savanna, are well known for creating deep ruts that form new creeks and change the flow of the water in this floodplain (Ramberg et al. 2006b). The soils in this wetland are sandy soils and thus have very low reactivity, which reduces the ability of the soil to bind organic matter particles to the soil fraction (Stevenson and Cole 1999), making them very unlikely to accumulate significant amounts of carbon. Additionally, the little soil organic matter that builds up in this savanna is periodically burned in the recurring fires of the Delta, very frequent in the Okavango's floodplain during the dry season before the flood (Heinl et al. 2006; Mitsch et al. 2010). These fires not only oxidize quickly the carbon contained in the soil, they also reduce the amount of plant residue that is potentially entering the soil of the floodplain, and keep the landscape under the domination of grasses and sedges (van Langevelde et al. 2003), whose carbon is much more labile than woody plant residues (Wolf and Wagner 2005). The few woody species present in the floodplain are lost or reduced in size by the combined effect of fire and herbivores such as giraffes, *Giraffa camelopardalis*, and elephants, *Loxodonta africana*, two of the very abundant herbivores in the Delta (van Langevelde et al. 2003; Ramberg et al. 2006b). The permanent marsh adjacent to the river had significantly lower carbon content than the rest of the communities in this floodplain despite of being under water year-round, free from fires and under constant anaerobiosis. However, being on the edge of the river, these soils are likely suffering constant fluvial erosion and sedimentation, impeding organic matter accumulation in the area (Lal 2005; Bernal and Mitsch 2012).

### *3.5.2. Climate and carbon sequestration in tropical wetlands*

There were significant differences on carbon sequestration between wetlands within each climatic region ($P < 0.001$ for the tropical humid sites, $P = 0.05$ for the tropical dry ones). If we compare the sequestration in the tropical humid region with the sequestration in the tropical dry one, the carbon sequestration rates are significantly higher in the humid tropic ($P = 0.03$). However, when comparing the four wetlands together, carbon sequestration in the tropical dry wetlands of Palo Verde and Okavango Delta were not significantly different to those from the La Selva wetland in the humid tropics. The high carbon content of the soils in the humid tropical wetland at EARTH University are responsible of this site being statistically higher than the other sites. We compared the carbon content of the wetlands in the two climatic regions with and without the peat site as a separate group (Figure 3.3). We did this because, even though tropical peatlands are typical from the tropical humid regions, we did not want it to mask the potential differences between the other tropical humid wetland communities and the tropical dry ones. In either case the soil carbon contents at significantly different at the 90% confidence interval ($P$ = 0.08 for the comparison of the tropical humid peatland, the tropical humid wetlands, and the tropical dry wetlands; $P$ = 0.05 for the comparison of tropical humid *vs.* tropical dry ones).

Following the lead of Brown and Lugo (1982), we explored the relationship between soil carbon sequestration in our twelve tropical wetland communities and climate using the ratio of mean annual precipitation, $P$ (mm $y^{-1}$), over mean annual air temperature, $T$ (°C). This ratio serves as a climatic indicator of potential water availability in the regions where our wetlands are located. The result was a bell-shaped curve (Figure 3.4) that suggests that a middle point in precipitation and temperature for tropical wetlands are ideal to enhance soil carbon sequestration ($R^2$ = 0.72; $R^2$ = 0.90 if we exclude the peat site). Even though the ideal conditions for carbon sequestration seem to be converging in the type of frequently flooded rainforest wetland of EARTH University, given its climate (represented by the $P/T$ ratio) and densely vegetated forest coverage, one also has to take into account the fact that this is the only wetland that is flooded the entire year, and that is probably why the sequestration rate of every one of its wetland communities were significantly higher than the rates of the communities in the isolated wetland of La Selva, where climate and rainforest productivity can be considered the same but standing water is not permanent. Hydrology is a fundamental forcing factor in a wetland (Junk 2002; Mitsch and Gosselink 2007), and it is especially relevant for carbon accumulation in the tropical wetlands since the presence of water retards organic matter decomposition, preserving the carbon in the soil for much longer than it would, given the high decomposition rates in tropical ecosystems (Schlesinger 1997; Stevenson and Cole 1999; Franzluebbers et al. 2001; Chimner and Ewel 2005; Saunders et al. 2007). Previous studies on the matter suggest that decomposition is actually the main factor determining organic matter accumulation in tropical wetlands, rather than productivity (Franzluebbers et al. 2001; Chimner and Ewel 2005; Bernal and Mitsch 2008). Our results support that hypothesis, since in our wetland sites productivity is likely to be higher in the two wetlands located in the rainforest (at La Selva and at EARTH University), but decomposition is likely to be lowest at the one in EARTH University. Given these differences, we compared the carbon sequestration rates of our wetlands based on wetland type (Figure 3.5), which is defined by the hydrogeomorphic setting of the wetland. Thus, we have three types of wetlands, as described in Table 3.1 – a slough swamp in a rainforest (EARTH University), a depressional isolated pond in a rainforest (La Selva), and two riverine wetlands in a seasonally flooded river floodplain (Palo Verde and Okavango Delta). Carbon sequestration in the slough was significantly higher ($P < 0.01$) than in the isolated and the riverine wetlands, whose sequestration rates were not different. Given that the slough and the isolated wetland have similar climate and ecosystem productivity, we can assume that the significant difference on carbon sequestration between these two wetlands is because of their different hydroperiod, i.e. their differences on water levels or "hydrologic signature" (Mitsch and Gosselink 2007).

### *3.5.3. Significance of this study*

Tropical wetlands represent a significant carbon sink, especially tropical peatlands. Soil carbon sequestration has been rarely measured in tropical wetlands directly; most of the studies that report carbon budgets and storages on tropical wetlands are based on discounting respiration (aerobic and anaerobic, $CO_2$ and $CH_4$ respectively) from ecosystem productivity, leaving a wide range of uncertainty (Melack et al. 2004; Chimner and Ewel 2005). This has led to the misconception that tropical wetlands, like the vastly studied Amazon floodplain (Melack et al. 2004; Sousa Moura et al. 2008), are a source of carbon rather than a sink, even though (1) only 1–3 % of wetland's plant productivity is emitted as methane (Whiting and Chanton 1993; Schlesinger 1997; Melack et al. 2004); (2) the carbon stored in wetland plants is greatly exceeded by the wetland soil carbon pool (Saunders et al. 2007); and (3) upland forests in the Amazon are also a source of methane even though they are not under anaerobic conditions (Carmo et al. 2006; Kirschbaum et al. 2006; Sousa Moura et al. 2008), increasing the uncertainty on tropical wetland ecosystem carbon budgets. We have shown that there are significant differences on carbon sequestration between tropical wetland types as well as between some tropical wetland communities, and the same is true for methane emissions (Nahlik and Mitsch 2011). It is therefore inaccurate to report the role of tropical wetlands in global carbon budgets as if they were only represented by river floodplains (low soil carbon sequestration and high $CH_4$ emissions) or only by tropical peatlands (high soil carbon sequestration and low $CH_4$ emissions).

Tropical wetlands may be more vulnerable than temperate and boreal wetlands because of the overexploitation of their water and biomass for the sake of economic development (de la Cruz 1986; Junk 2002; Chimner and Ewel 2005). Under a changing climate with temperature increases, freshwater tropical wetlands are at high risk because a change in temperature and precipitation patterns will alter the evapotranspiration rates, seasonality of the wetland, and fire frequency (Brown and Lugo 1982; Roggeri 1995; Heinl et al. 2006), potentially modifying the wetland soil carbon accumulation rate (Franzluebbers et al. 2001; Chimner and Ewel 2005) and changing the wetland hydroperiod, which in turn could "switch" the wetland from being a sink of carbon to a source (Jones and Humphries 2002; Jauhiainen et al. 2005; Saunders et al. 2007).

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| | Location | Mean annual precipitation ($mm\ y^{-1}$) * | Wetland type | Wetland communities | Hydrologic features |
|---|---|---|---|---|---|
| ***Tropical humid*** | | | | | |
| *La Selva Biological Station* | NE Costa Rica, Central America. 10° 25' N, 84° 0' W | 4337 ± 520 | Depressional isolated rainforest wetland | open mudflat | frequently flooded, saturated soil |
| | | | | vegetated edge | frequently flooded, saturated soil |
| | | | | shallow marsh | permanently/frequently flooded |
| *EARTH University* | E Costa Rica, Central America. 10° 13' N, 83° 34' W | 3460 ± 750 | Slow flow rainforest swamp | swamp | permanently flooded |
| | | | | vegetated edge | frequently flooded, saturated soil |
| | | | | peatland | permanently flooded, deep water |
| ***Tropical dry*** | | | | | |
| *Palo Verde Biological Station* | W Costa Rica, Central America. 10° 20' N, 85° 20' W | 1240 ± 252 | Seasonal riverine wetland | permanent pond | permanently/frequently flooded |
| | | | | vegetated mudflat | seasonally flooded, saturated soil |
| | | | | vegetated edge | seasonally flooded, saturated soil |
| *Okavango Delta* | N Botswana, Africa. 19° 32' S, 23° 09' E | 700 – 200 | Seasonal riverine floodplain | shallow marsh | seasonally flooded, shallow water |
| | | | | deep marsh | seasonally flooded |
| | | | | riverine deep marsh | permanently flooded, deep water |

* from Mitsch et al. (2010) and Nahlik and Mitsch (2011).

Table 3.1. Description of the twelve tropical wetland communities at the four wetland sites included in this study.

| | **Bulk Density (Mg m$^{-3}$)** | **Carbon Content (gC kg$^{-1}$)** | **OC:TC ratio** | **C:N ratio** | **Carbon Pool (kgC m$^{-2}$)** | **Sed. Rate (kg m$^{-2}$ y$^{-1}$)** | **Acc. Rate (mm y$^{-1}$)** | **Carbon Sequestration Rate (gC m$^{-2}$ y$^{-1}$)** |
|---|---|---|---|---|---|---|---|---|
| ***Tropical humid*** | | | | | | | | |
| Depressional, open mudflat | 0.56 ± 0.04 | 32.5 ± 14.3 | 0.99 ± 0.00 | 9.50 ± 0.38 | 1.2 ± 0.1 | 2.2 ± 0.3 | 4.0 | 61 ± 15 |
| Depressional, vegetated edge | 0.53 ± 0.04 | 31.6 ± 12.8 | 0.95 ± 0.02 | 9.79 ± 0.20 | 1.1 ± 0.1 | 2.5 ± 0.4 | 4.6 | 61 ± 12 |
| Depressional, shallow marsh | 0.45 ± 0.06 | 61.3 ± 7.6 | 0.99 ± 0.00 | 9.52 ± 0.21 | 1.6 ± 0.1 | 2.2 ± 0.1 | 4.8 | 131 ± 12 |
| Swamp, perm. flooded | 0.36 ± 0.04 | 71.4 ± 11.8 | 0.98 ± 0.00 | 11.53 ± 0.28 | 1.2 ± 0.1 | 3.4 ± 0.4 | 9.7 | 222 ± 13 |
| Swamp, vegetated edge | 0.45 ± 0.08 | 56.9 ± 10.5 | 0.98 ± 0.00 | 11.14 ± 0.31 | 1.2 ± 0.1 | 4.5 ± 0.5 | 10.0 | 232 ± 14 |
| Swamp, deep peat | 0.19 ± 0.03 | 325.0 ± 42.9 | 1.00 ± 0.00 | 19.28 ± 2.94 | 3.4 ± 0.4 | 1.4 ± 0.1 | 7.8 | 465 ± 34 |

Continued.

Table 3.2. Average ± standard error (*n*) of physiochemical conditions, pools and rates of the twelve tropical wetland communities included in this study. The depressional communities are located at La Selva Biological Station, the swamp communities at EARTH University, the riverine communities at Palo Verde Biological Station, and the floodplain communities at the Okavango Delta. Soil carbon pools are calculated for the top 45 cm in the tropical humid sites and for the top 30 cm in the tropical dry ones.

Table 3.2 continued.

| | Bulk Density (Mg m$^{-3}$) | Carbon Content (gC kg$^{-1}$) | OC:TC ratio | C:N ratio | Carbon Pool (kgC m$^{-2}$) | Sed. Rate (kg m$^{-2}$ y$^{-1}$) | Acc. Rate (mm y$^{-1}$) | Carbon Sequestration Rate (gC m$^{-2}$ y$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| ***Tropical dry*** | | | | | | | | |
| Riverine, perm. flooded | 0.70 ± 0.28 | 39.1 ± 2.3 | 0.92 ± 0.03 | 10.81 ± 0.50 | 1.4 ± 0.1 | 2.2 ± 0.3 | 3.2 | 84 ± 3 |
| Riverine, mudflat | 0.65 ± 0.07 | 44.1 ± 4.2 | 0.94 ± 0.02 | 9.92 ± 0.11 | 1.4 ± 0.1 | 2.1 ± 0.3 | 3.3 | 89 ± 3 |
| Riverine, vegetated edge | 0.65 ± 0.03 | 44.9 ± 1.8 | 0.92 ± 0.01 | 11.02 ± 0.34 | 1.4 ± 0.1 | 2.0 ± 0.4 | 3.0 | 80 ± 4 |
| Floodplain, shallow marsh | 0.99 ± 0.05 | 33.3 ± 7.3 | 1.00 ± 0.00 | - | 1.5 ± 0.1 | 1.9 ± 0.1 | 1.9 | 53 ± 7 |
| Floodplain, deep marsh | 1.02 ± 0.07 | 30.7 ± 10.5 | 1.00 ± 0.00 | - | 1.3 ± 0.1 | 1.8 ± 0.1 | 1.8 | 41 ± 9 |
| Floodplain, riverine marsh | 0.96 ± 0.07 | 16.7 ± 3.4 | 1.00 ± 0.00 | - | 0.8 ± 0.1 | 2.5 ± 0.2 | 2.6 | 33 ± 3 |

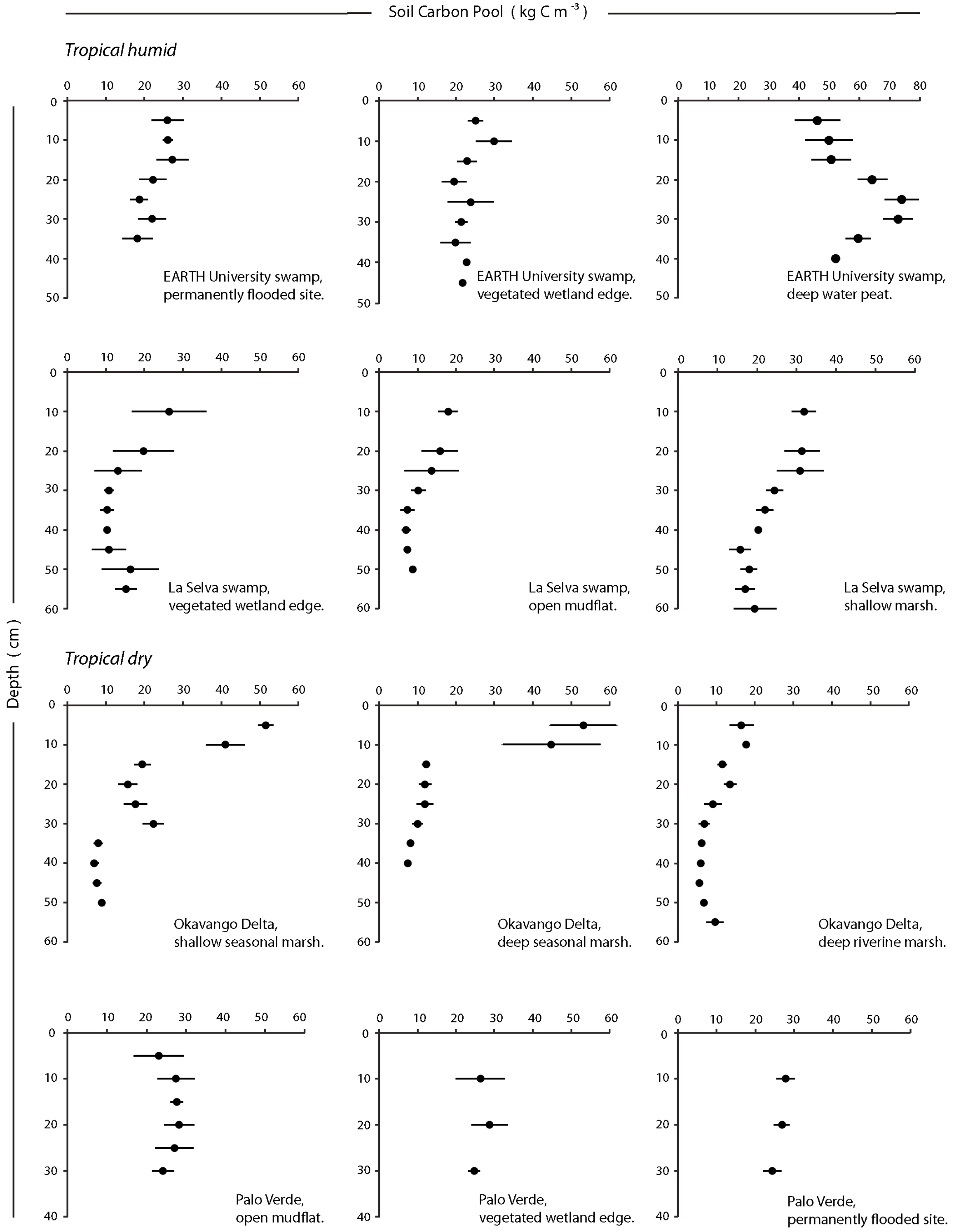

Figure 3.1. Profile of soil carbon pools (kgC $m^{-3}$) over depth (cm) of the twelve wetland communities at the four tropical humid and tropical dry wetland sites. The pool for each depth increment is the average of that depth of the three replicate cores per community (*n* = 3), and the error bars represent standard error of the average.

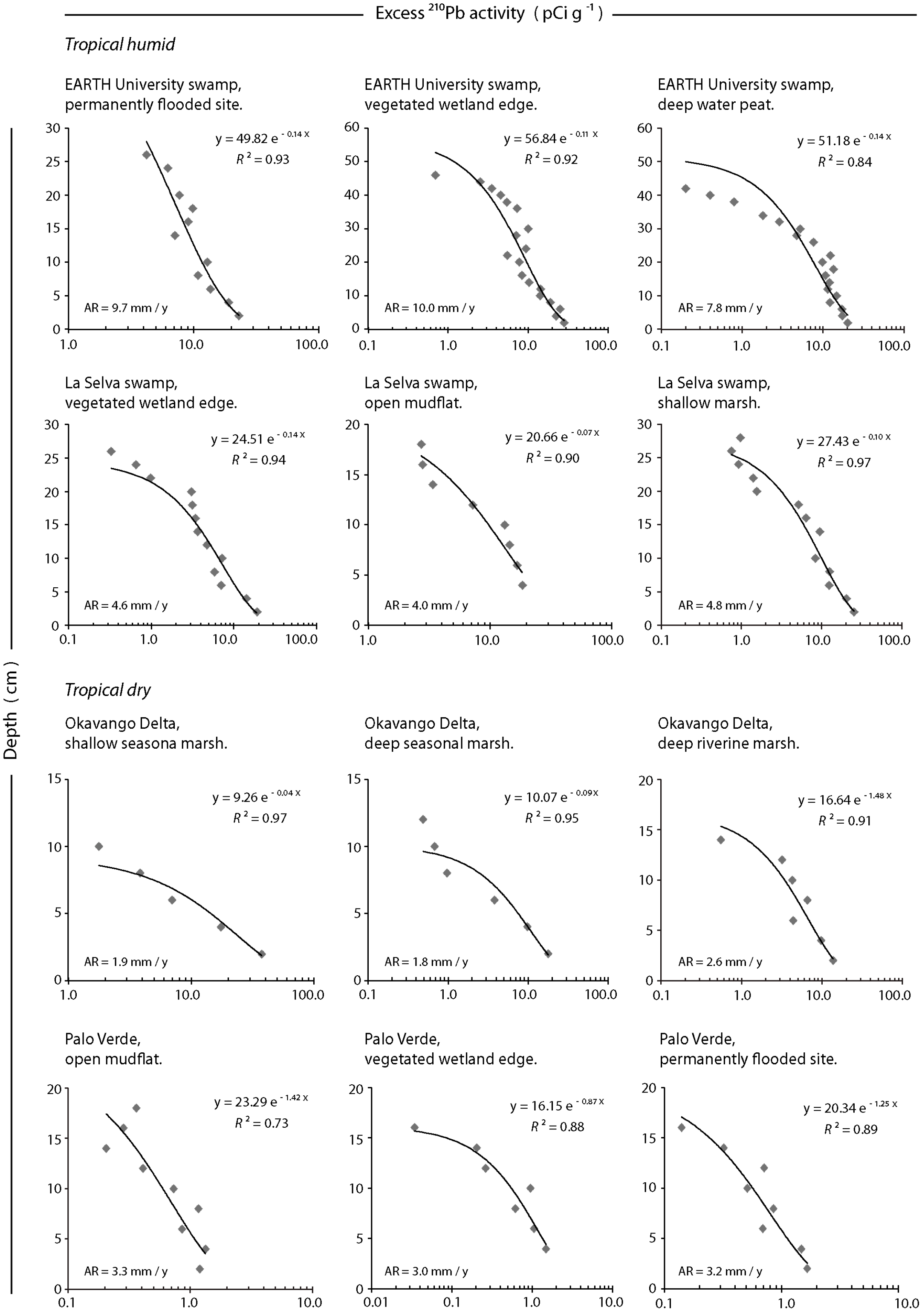

Figure 3.2. Excess $^{210}$Pb activity per unit mass of soil (pCi g$^{-1}$) profiles in soil cores of the twelve wetland communities at the tropical humid and tropical dry wetland sites, with estimated sediment accretion rate (*AR*) of each.

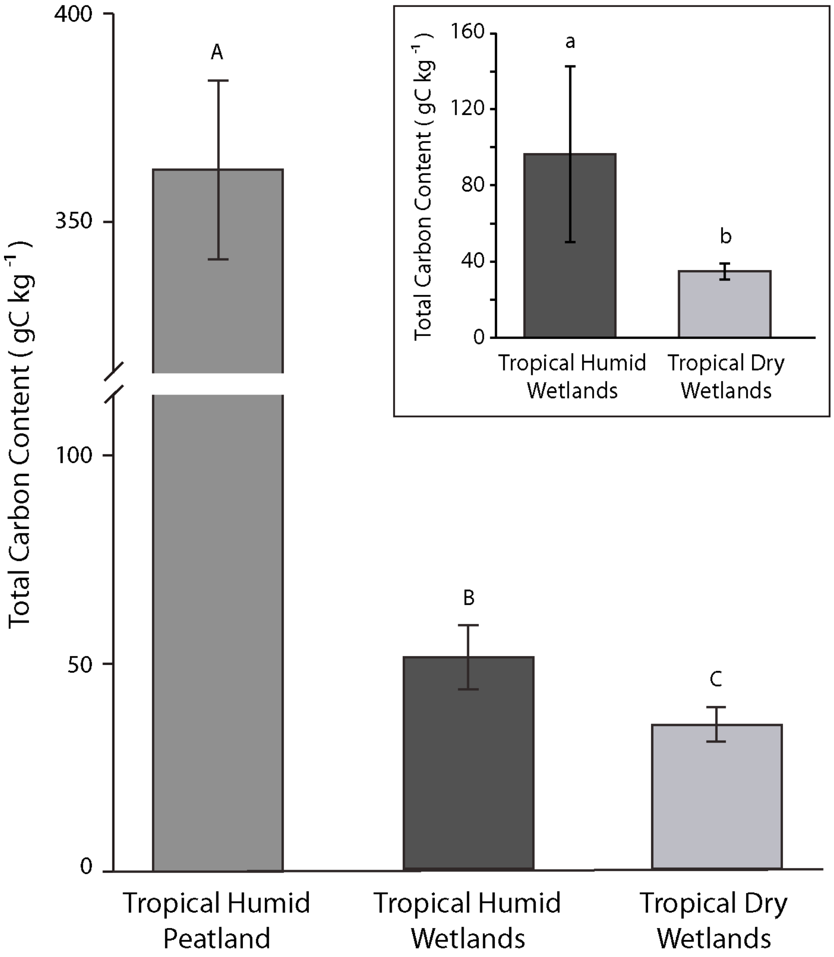

Figure 3.3. Average total carbon content (gC kg$^{-1}$ soil) of the tropical humid peatland, tropical humid wetland communities, and tropical dry wetland communities; and comparison of all the tropical humid wetland communities *vs.* all the tropical dry ones. Error bars represent standard error of the average. Different letters on top of the bars indicate significant differences at the 90 % significance level, lower case for the comparison of tropical humid *vs.* tropical dry ($P$ = 0.05) and upper case for the comparison of tropical humid peatland *vs.* tropical humid wetlands *vs.* tropical dry wetlands ($P$ = 0.08).

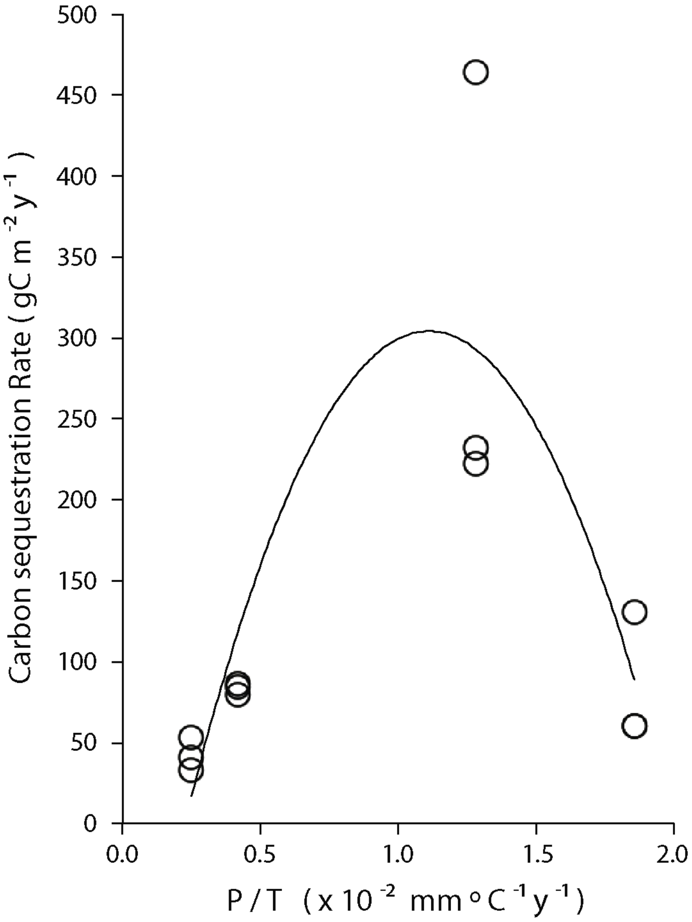

Figure 3.4. Average carbon sequestration rate (gC $m^{-2}$ $y^{-1}$) of the twelve tropical wetland communities included in this study, classified according to the climatic index *P/T* (precipitation over temperature, in mm $y^{-1}$ and °C, respectively). The bell shape curve ($R^2$ = 0.72) indicates the trend of the regression.

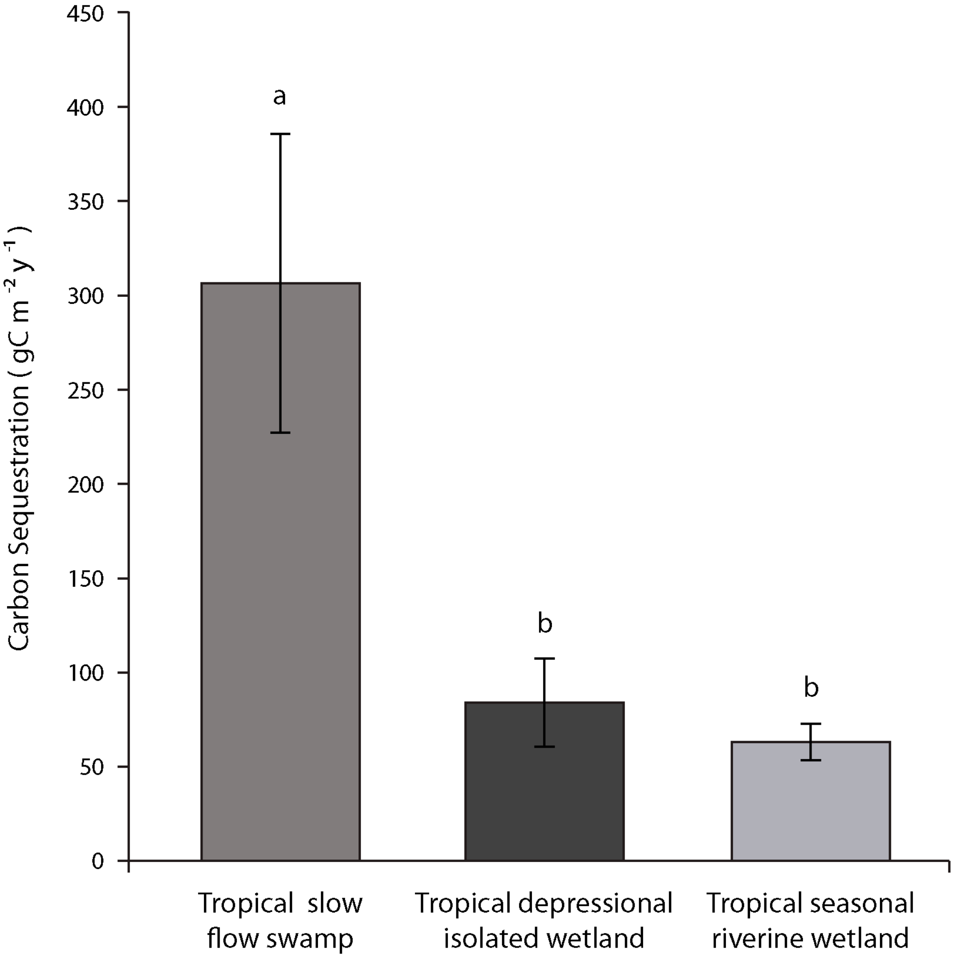

Figure 3.5. Average carbon sequestration rate (gC $m^{-2}$ $y^{-1}$) of the wetland communities included in this study, grouped according to their major wetland type, i.e. tropical slow-flow swamp (*n* = 3), tropical depressional isolated wetland (*n* = 3), and tropical seasonal riverine wetland (*n* = 6). Error bars represent standard error of the average. Different letters on top of the bars indicate significant differences between averages ($P < 0.01$).

# CHAPTER 4

# CARBON SEQUESTRATION IN CREATED RIVERINE WETLANDS

## 4.1. Abstract

The increase of greenhouse gases in the atmosphere has raised awareness on climate change, creating the need to find effective ways to accumulate and store carbon to keep it from being released back to the atmosphere. Wetlands are significant carbon sinks; high productivity and waterlogged conditions give them the ability to accumulate large amounts of soil organic matter. We found that two similar 15-year-old created wetlands in central Ohio are, on average, accumulating 242 gC $m^{-2}$ $y^{-1}$, a rate 70 % higher than a similar natural wetland in the region and 26 % higher than the rate estimated for these same wetlands 10 years after their creation, suggesting that they become more efficient sequestering carbon during their "teenage" years. When these wetlands were created, one was planted with wetland macrophytes while the other was left unplanted to be naturally colonized. After 15 years, the naturally colonized wetland has higher carbon sequestration rate (267 ± 17 gC $m^{-2}$ $y^{-1}$) than the planted wetland (219 ± 15 gC $m^{-2}$ $y^{-1}$; $P < 0.05$). These

rates are positively correlated with aboveground net primary productivity, although in both wetlands the open water community had the greatest rate (287 ± 27 and 240 ± 31 gC $m^{-2}$ $y^{-1}$, respectively). Soil carbon was 81–97 % organic, and the soil C:N ratios are 11.5–14.4, uncorrelated to C:N in the water. This is one of the few studies on carbon sequestration in created wetlands in the world. If created wetlands are proven to be efficient carbon sinks, they could function as natural cost-effective mitigation tools to sequester carbon and abate the net greenhouse effect of carbon emissions.

## 4.2. Introduction

The increased wetland loss rates in the last century and the recognition of wetland values has lead to a "no net loss" policy in the United States (Dahl 2000; NRC 2001; Mitsch and Gosselink 2007) where wetlands are often created to replace lost or damaged natural ones. There are concerns about created wetlands being able to function as natural ones because of the time that it can take the wetland to develop its structure (e.g., hydric soil and vegetation cover) and functions (e.g., water quality improvement and wildlife haven). Wetland creation and restoration have been thoroughly evaluated over the years to assess the success of the new wetlands in their ability to replace functional natural ones, and the accomplishment of the no net wetland loss policy (Mitsch and Wilson 1996; Zedler and Calloway 1999; Kentula 2000; Campbell et al. 2002; Gutrich et al., 2009). Through the failure of some of these mitigation projects (Erwin 1991; Spieles 2005; Matthews and Endress 2008), we have learned that the typical 5-year jurisdictional monitoring period might not be long enough for the achievement of wetland structure and function. This is particularly true for some wetland functions such as soil organic matter accumulation, which depends greatly in the successful establishment of vegetation and hydrology (Campbell et al. 2002; Craft et al. 2003; Bruland and Richardson 2005; Anderson and Mitsch 2006; Fennessy et al. 2008; Hossler and Bouchard 2010). As hydric soils are developed, the waterlogged conditions and high productivity give created wetlands the potential to be carbon sequestering systems as effective as natural wetlands are.

The use of natural systems to accumulate carbon is one of the most cost-effective tools to reduce the net effect of greenhouse gas emissions and abate climate change (Hanley and Spash 2003; IPCC 2005; Stern 2007; Lal 2008). Wetlands are known to be significant carbon sinks – their high productivity introduces large amounts of organic matter into the soil while the semi-permanent presence of water retards its decomposition (Collins and Kuehl 2001; Mitsch and Gosselink 2007). Even though anaerobic decomposition under the presence of water produces methane (making wetlands accountable for about 25 % of the yearly emissions; Bartlett and Harris 1993; Whalen 2005; IPCC 2007), wetland's soil carbon stock represents one-third to one-half of the organic terrestrial carbon pool (Mitra et al. 2005; Bridgham et al. 2006; Mitsch and Gosselink 2007; Lal 2008). These characteristics give wetlands the natural ability to be successful carbon sequestering systems, and thus policy makers have considered creating wetlands for carbon capture and sequestration to counteract the increasing levels of greenhouse gases in the atmosphere (IPCC 2007; Schrag 2007; Stern 2007). This would entail that wetlands could be created to serve a specific purpose (in this case sequester carbon) rather than solely compensate for the loss of a similar ecosystem. To get to this point much research is still needed for a fully understanding of the conditions that enhance carbon sequestration in wetlands, while keeping methane emissions low.

In order to efficiently sequester carbon, we need a healthy, fully functionally wetland, i.e. a wetland with the proper hydrology and supporting wetland vegetation. In created wetlands this point is reached at different ages depending on the initial wetland design. Once it is achieved, the two ways to enhance its soil carbon pool are by (1) increasing carbon inputs and by (2) decreasing the carbon outputs. Enhancing wetland productivity can potentially increase the amount of organic matter introduced in the soil and thus, enhance the capacity to sequester carbon. Flow-through and pulsing wetlands are typically more productive because they receive additional nutrients and organic matter from the connected water body (Mitsch 1988; Odum et al. 1995; Mitsch and Reeder 1991; Cronk and Fenessy 2001), retaining and transforming them as they flow through the wetland, and thus removing them from the water column and improving water quality. Numerous studies have investigated the use of wetlands as effective water treatment systems, especially for nitrate ($NO_3^-$) removal (Reddy and Patrick 1984; Davidsson and Ståhl 2000; Mitsch et al. 2001; Hamersley et al. 2003) by means of denitrification (transformation of nitrate into $N_2$ gas) and immobilization (accumulation in the soil as organic matter or bound to the clay fraction as $NH_4^+$; Schlesinger 1997; Stevenson and Cole 1999; Vepraskas and Faulkner 2001).

Carbon and nitrogen cycles are closely related. The C:N ratio varies with soil types, ranging usually between 10:1 and 14:1 depending on the decomposition stage of the soil organic matter (Schlesinger 1997; Stevenson and Cole 1999). Nitrogen is a limiting nutrient, and its presence in the soil increases plant productivity, which in turn introduces more organic matter into the system and increases carbon inputs (Alvarez 2005). It also helps in the formation of more stable soil carbon compounds through the humification process and stabilization of carbon, increasing the resilience of carbon in the soil and improving its sequestration (Cambardella and Elliott 1994; Stevenson and Cole 1999; Alvarez 2005). On the other hand, nitrogen is also a limiting nutrient for microbes, and the presence of available nitrogen can enhance microbial activity and the concomitant decomposition of organic matter if the environmental conditions are adequate (Mack et al. 2004; Bragazza et al. 2006), potentially increasing the carbon output of the system. In consequence, nitrogen influences inputs and outputs of carbon in the soil, but information on the net effect of nitrogen on carbon pools reported in literature is inconsistent and often contradictory, making it difficult to predict a straightforward relationship between both pools (Neff et al. 2002). Most of these studies have been done in agricultural soils because of its relevance for crop production and, more recently, because of the increasing interest on enhancing soil's capacity to sequester carbon. Currently there are no studies found in the literature analyzing this relationship between carbon and nitrogen in wetlands.

Wetland hydrogeochemistry is also a key factor affecting carbon accumulation in the soil. There is usually a gradient of inundation frequency, from the deepest and typically permanently flooded area of the wetland to the shallower and semi-permanently flooded edges (Mitsch and Gosselink 2007). This gradient determines the vegetation communities being established – open water, algae mats, and floating plants typically dominate the deepest areas of the wetland, while emergent macrophytes develop in the intermediate and shallower ones (Boutin and Keddy 1993; Cronk and Fenessy 2001; Mitsch and Gosselink 2007). Edge vegetation communities tend to be more productive and densely vegetated since they are more affected by hydrological pulses (Odum 1969; Odum et al. 1995) and less limited by the permanent presence of water. Thus, these areas are potentially introducing larger amounts of organic matter into the soil than open water and floating communities. On the other hand, deeper sites have lower organic matter decomposition rates due to the permanent anaerobic conditions of the soil, while edge areas experience strong respiration pulses when the soil is dried and re-wetted (Stevenson and Cole 1999; Jassal et al. 2005; Miller et al. 2005), potentially decreasing the carbon stock in the soil. It is therefore unclear which communities would have greater soil carbon accumulation; Bernal and Mitsch (2012) found in a temperate riverine wetland higher carbon sequestration rates in the floating communities of the deep water sites, but typically carbon accumulation studies in wetlands do not differentiate between wetland communities as differences are usually not significant (Bruland and Richardson 2005; Gutrich et al. 2009; Hossler and Bouchard 2010), and because traditionally the concern has been the effect of nutrient gradient on soil properties rater than vegetation gradients (Reddy et al. 1993; Craft and Richardson 1993; Neff et al. 2002; Mack et al. 2004; Stern et al. 2007).

In this study we explore the soil carbon and nitrogen content of two 15-year created riverine wetlands in central Ohio with the intention of finding evidence, by means of difference in the C:N ratios, of a positive correlation of both pools in the soil (nitrogen enhances carbon content, i.e., carbon is greater in sites where nitrogen is greater) or negative one (nitrogen reduces carbon content, i.e., carbon is lower in sites where nitrogen is greater and higher where nitrogen is lower), and the potential effect of nitrogen on the stable carbon fraction of the soil (positively or negatively correlated as well). We expect to find higher total soil carbon content and higher stable carbon in the soils where soil nitrogen is higher. We also expect to find higher soil nitrogen in the inflow area of the wetlands, where water has higher nitrogen concentrations. In addition, we investigate the potential effect on soil carbon accumulation of the different vegetation communities present in the wetlands, with the intention of finding a pattern that correlates positively or negatively vegetation cover with soil carbon pool and sequestration rate. Lastly, we estimate carbon pool and sequestration rate of these wetlands 15 years after they were created and compare them to their carbon pool and sequestration rate when they were 10 years old. We expect to find that, after 15 years, these wetlands are increasing their soil carbon pool at a greater rate than the one reported by Anderson et al. (2005) and Anderson and Mitsch (2006) after these wetlands were

10 years old, because of an increase in the biomass cover in these wetlands over the last 5 years (compared to the previous 10) that is potentially introducing greater amounts of carbon into the soil, and because a greater thickness of the hydric soil layer.

## 4.3. Materials and methods

### *4.3.1. Site description*

This research was conducted at the Olentangy River Wetland Research Park (40° 01' N, 82° 01' W), where two symmetrical 1-ha wetlands were created in 1994 adjacent to the third order Olentangy River. Water from the river has been pumped through these wetlands since their creation in 1994 following the water level of the river, to mimic the hydrologic pulses that a natural riverine wetland in that location would have (i.e., often higher water levels in December-June and lower in July-November; Mitsch et al. 1998,2005a,2005b,2012). Both wetlands receive the same amount of water, experience the same pulsing hydrology, and have similar water retention times. Their hydric wetland soil developed over the non-hydric parent material (Ross silt loams; NRCS 2010) shortly after flooding started, and sediment accretion in the two wetland basins has been occurring since (Anderson et al. 2005). Both wetlands are diversely vegetated with wetland plants (*OBL* and *FACW*, among others; Reed 1988). One of these wetlands (the western basin) was planted in 1994 with 13 native species macrophytes, while the eastern basin was left unplanted to be colonized naturally (Mitsch et al. 1998, 2012). This is the only design difference between both wetlands, which over time resulted in differences on aboveground biomass and net primary productivity (cumulatively greater in the unplanted wetland than in the planted one after 15 years, because it was rapidly colonized by dominant wetland species such as *Typha* spp.), and in diversity of vegetation communities (which increased over the years in both wetlands but has been historically higher in the planted one). Despite this original difference, both wetlands have converged in number of species (101 in the planted wetland and 97 in the naturally colonized one, 15 years after creation), with a total of 22 identifiable vegetation communities (including open water and algal mats) over that time (Mitsch et al. 2012). Water quality has also been measured in these wetlands for 15 years in the inflow and outflow of the basins (Mitsch et al. 1998,2005,2012). These analyses reveal that both wetlands function as sinks of nutrients (nitrogen and phosphorous), i.e., inflow concentrations are greater than outflow levels, while carbon in the water remain somewhat constant throughout the wetlands. More information about how these wetlands developed and changed 3, 6, 10 and 15 years after creation can be found in Mitsch et al. (1998), Mitsch et al. (2005a), Mitsch et al. (2005b), and Mitsch et al. (2012), respectively.

### *4.3.2.Soil sampling and samples preparation*

A total of 44 soil cores (22 per wetland) were extracted in May 2009, 15 years after the wetlands were created, following a 10-m grid spatial pattern that covers both wetlands entirely (Figure 4.1). This same grid was used as a reference in 1993 before the wetlands were flooded, one year after flooding (1995), and 10 years after flooding, in 2004 (Anderson et al. 2005; Anderson and Mitsch 2006). The cores were 7-cm in diameter, and their length varied depending on the depth of the sediment accumulated over the underlying non-hydric soil (between 10 and 35 cm). Extracted cores were immediately divided in the field into 5-cm increments, and packed in sealed containers that were stored under 4 °C until analysis. The cores were extracted in the same grid points where Anderson et al. (2005) and Anderson and Mitsch (2006) collected their 10-year soil samples, in order to be able to compare our 15-year rates with their 10-year ones. Sampling points in both wetlands were evenly distributed in the inflow, middle, and outflow sections (seven, eight, and seven cores, respectively). In every section they present shallow areas that are not flooded when the water level drops, and deeper areas that are permanently flooded. Permanently flooded areas have zones with and without vegetation (emergent wetland plants adapted to the presence of water). As a result, there are three distinct generic communities in each wetland section: open water (deeper area of the wetland, where water level is higher, soils are permanently flooded, and no emergent macrophytes grow), emergent community (area where the water level is high, the soil is permanently flooded, and wetland plants grow), and edge community (where wetland macrophytes grow but water level is shallow and the soil is not flooded when water level drops). Soil sampling points were also evenly distributed in these three communities throughout both wetlands. As reference non-wetland site, two extra cores (15-cm deep and 10-cm in diameter) were collected in the forested upland area between both wetland basins by the core method (Grossman and Reinsch 2002; Tan 2005). All the soil samples were oven-dried until constant weight was reached (60 °C for the wetland soils, 105 °C for the upland ones, according to the standard methods of soil analysis; Grossman and Reinsch 2002; Bernal and Mitsch 2012), weighed to determine the bulk density (Mg $m^{-3}$), ground to a 2-mm particle size, and homogenized.

*4.3.3. Determination of soil carbon and nitrogen content*

Carbon content of the soil samples was determined by combusting triplicates of 150 mg of soil in a Total Carbon Analyzer for Soil Samples (TOC-V series, SSM-5000A; Shimadzu) at 900 °C for total carbon content and at 200 °C with a 10 M $H_3PO_4$ pre-treatment for inorganic carbon content. Organic carbon was determined as the difference between both. The soil carbon concentration (gC $kg^{-1}$) and pool (kgC $m^{-2}$) of each core were calculated following the equations described in Bernal and Mitsch (2008,2012):

$$\text{C concentration (gC kg}^{-1}) = 10 \times TC\ (\%) \quad [1]$$

$$TC\ (\text{g}) = W\ (\text{g}) \times TC\ (\%) \times 10^{-2} \quad [2]$$

$$\text{C pool (kgC m}^{-2}) = 10^{-3} \times TC\ (\text{g}) \text{ x } A^{-1}\ (\text{m}^2) \quad [3]$$

$TC$ is total carbon, $W$ is the dry weight of soil, and $A$ is the area of the sediment sampler.

Similarly, total nitrogen in the soil was measured in a Nitrogen Combustion Analyzer (vario Max CN; Elementar Analysensysteme), and the soil nitrogen concentration (gN $kg^{-1}$) and pool (kgN $m^{-2}$) were determined by the same calculations described above for carbon. Inorganic soil nitrogen content (μg$NO_3$-N $g^{-1}$ and μg$NH_4$-N $g^{-1}$) was measured by doing a 2 $M$ KCl extraction and a flow injection analysis (Mulvaney 1996). The soil C:N ratios of each wetland, section, and community were determined by dividing their total carbon by their total nitrogen.

Chemically stable soil carbon pool was determined with chemical oxidation in a triplicated subset of half of the samples from each community in the naturally colonized wetland, by dispersing 0.5 g of soil in 250 mL of distilled water mixed with 20 g of $Na_2S_2O_4$ and buffered with 22 g of $NaHCO_3$. The mixture soil solution was incubated at 80 °C for 48 hours and washed with 40 mL of distilled water until neutral pH (Helfrich et al. 2007). Carbon content after chemical oxidation was determined by the same procedure and calculations used to determine of soil carbon pool.

The carbon, nitrogen, and sediment accumulation since the wetlands were created in 1994 was calculated by estimating the total soil carbon, nitrogen, and sediment pool of the hydric layer, i.e., from the soil surface to the underlying non-wetland soil over which the wetland soil was developed, in each point of the grid. The rates were determined by dividing the pools in each point of the grid by the age of the wetland at the time of sampling (15 years). Given the age of these wetlands, other common methods to estimate sequestration rates in natural wetlands such as $^{137}Cs$ (Craft and Richardson 1993; Bernal and Mitsch 2012) could not be used here. Individual rates were averaged by community, section, and wetland; total wetland averages were weighed based on the surface area of each community – between 2004 and 2009 (10 to 15 years after creation), the planted wetland averaged 35 % open water and 65 % vegetated area (emergent and edge communities) while the colonized one had 30 % open water and 70 % vegetated area. To estimate the carbon sequestration rate in the upland area, we compared the upland soil carbon pool before the wetlands were created (in 1993, from Anderson et al. 2005) with the upland 15 years after wetland creation (2009).

### *4.3.4. Aboveground Net Primary Productivity and Water Quality*

Details on procedures for water quality measurements and aboveground net primary productivity (ANPP) determinations in these two wetlands since they were created can be found in Mitsch et al. (2005a,2012). Fifteen-year data on ANPP and a decade of dissolved carbon and nitrogen (TKN and $NO_3$-N) were used for comparisons with our soil physicochemical analysis.

### *4.3.5. Statistical analysis*

Statistical analyses were performed with IBM SPSS Statistics version 19.0 for Macintosh (SPSS Inc.). In every data set we did an EDA (exploratory data analysis), normality checks (with *Q-Q* plot, Kolmogorov-Smirnov test, and Shapiro-Wilk test) to ensure that they fit the normal distribution, and tested the homogeneity of their variances with Levene Statistic. A statistical analysis of variance (ANOVA) with Tukey HSD indicated the effect of these independent variables (section and community) in the carbon and the nitrogen pools as well as in the carbon sequestration rates (dependent variables), and determined the significance of differences in carbon sequestration between the two wetlands. Carbon and nitrogen content in the soil and in the water were examined with Pearson product moment correlations to find a potential relationship between carbon and nitrogen soil pools, and between soil content and water concentrations. Soil carbon sequestration and net primary productivity were examined as well with Pearson correlations to explore the relationship between aboveground biomass and soil carbon sequestration (Fowler et al. 2003).

## 4.4. Results

### *4.4.1. Wetland soil carbon and nitrogen content*

Average total soil carbon content was significantly higher by 30 % ($P < 0.01$) in the naturally colonized wetland (Table 4.1). Total soil carbon was not significantly different when comparing the three wetland communities or the vegetated and non-vegetated areas, but differences between open water and vegetated areas were greater in the naturally colonized wetland (42.7 ± 1.1 and 48.1 ± 4.6 gC kg$^{-1}$, for open water and vegetated areas respectively) than in the planted one (35.2 ± 2.5 and 36.7 ± 2.1 gC kg$^{-1}$). The highest soil carbon content was found in the edge community of the colonized wetland (46.5 ± 5.6 gC kg$^{-1}$), while emergent and open water communities had about 85 % of that carbon content (Table 4.1).

There were no significant differences on carbon content in the inflow, middle, and outflow of the wetlands. However, in the planted wetland the content increased from inflow to outflow while in the naturally colonized one it decreased from inflow to outflow. Most of the carbon accumulated in the soil was organic (90 % organic, on average, in the originally planted wetland and 93 % in the colonized one), while the highest inorganic carbon content was found in the open water areas (19 % in both wetlands), and the lowest in the vegetated area of the naturally colonized wetland basin (less than 1 %). The analysis of stable carbon revealed that 19.8 ± 6.7 % of the carbon contained in the open water sites was resistant to chemical oxidation, while in the vegetated areas the chemically resistant fraction was 10.5 ± 2.7 %.

Total soil nitrogen content was significantly different in both wetlands ($P$ = 0.04), even though their weighed average is similar (3.1 ± 0.1 gN $kg^{-1}$ in the planted one, 3.2 ± 0.1 gN $kg^{-1}$ in the colonized one, Table 4.1). It increased significantly ($P$ < 0.1) from inflow to outflow in both cases, but no differences were found when comparing between communities. The total nitrogen in the soil was mostly organic. $NO_3$-N was significantly higher ($P$ = 0.01) in naturally colonized wetland, while there was no difference in their soil $NH_4$-N. When looking at the wetland communities, no differences were found on $NO_3$-N content (Table 4.1) but $NH_4$-N was significantly higher in the open water ($P$ < 0.001). In every community, $NO_3$-N was consistently higher in the naturally colonized wetland, while $NH_4$-N was always higher in the planted one.

The C:N ratios are somewhat constant in both wetlands (12.4 ± 0.5, on average), although it was consistently higher in the naturally colonized basin (Table 4.1). The edge communities had the highest ratio (14.4 ± 1.3), while the open water sites had the lowest (11.5 ± 0.4; Table 4.1). Total soil carbon and total soil nitrogen are significantly correlated in these two wetlands ($P$ < 0.001), and fit a linear regression line with $R^2$ = 0.60 (Figure 4.2a). When looking at the relationship of each individual wetland, the correlation is still significant ($P$ < 0.001 in the planted wetland; $P$ < 0.01 in the colonized one) but the linear regression is stronger in the planted wetland ($R^2$ = 0.75) than in the naturally colonized one ($R^2$ = 0.38).

The naturally colonized wetland had a greater carbon pool than the planted one (4.0 ± 0.3 kgC $m^{-2}$ and 3.2 ± 0.2 kgC $m^{-2}$, respectively; $P = 0.02$). The lowest carbon pool was found in the emergent communities (3.2 ± 0.3 kgC $m^{-2}$), and the highest in the open water sites (4.0 ± 0.3 kgC $m^{-2}$); $P \leq 0.05$. The nitrogen pool was similar in both wetlands, but it followed the same trend as the carbon pool, i.e., highest in the open water (0.31 ± 0.02 kgN $m^{-2}$) and lowest in the emergent areas (0.25 ± 0.02 kgN $m^{-2}$); Table 4.1.

### *4.4.2. Sedimentation rates*

With similar bulk densities and hydric layer depth, both wetlands yielded almost the same estimated sedimentation rates (6.0 ± 0.4 kg $m^{-2}$ $y^{-1}$ in planted wetland, 5.9 ± 0.4 kg $m^{-2}$ $y^{-1}$ in colonized one; Table 4.1). The open water sites had greater sedimentation rate than the vegetated areas (6.6 ± 0.5 kg $m^{-2}$ $y^{-1}$), but their soil depth was also the thickest (17.9 ± 1.7 cm) and the least dense (0.59 ± 0.04 Mg $m^{-3}$). On the contrary, the edge communities had the highest bulk density (0.75 ± 0.06 Mg $m^{-3}$) and the least accretion depth (Table 4.1).

### *4.4.3. Carbon sequestration*

These differences in carbon pools yielded a similar trend in carbon sequestration rates, being significantly higher in the wetland that was naturally colonized than in the planted one (267 ± 17 gC $m^{-2}$ $y^{-1}$ and 219 ± 15 gC $m^{-2}$ $y^{-1}$, respectively; $P = 0.02$; Table 4.1). No differences were found between the inflow, middle, and outflow sections of either of the wetlands. However, like the soil total carbon content, carbon sequestration increased from inflow to outflow in the planted wetland and from outflow to inflow in the colonized one, being lowest in the inflow of the one planted (201 ± 29 gC $m^{-2}$ $y^{-1}$) and highest in the inflow of the one colonized (291 ± 26 gC $m^{-2}$ $y^{-1}$, 45 % more than in the inflow of the planted wetland). When exploring the wetland communities, carbon sequestration in open water was consistently higher in both wetlands (240 ± 31 and 201 ± 18 gC $m^{-2}$ $y^{-1}$ respectively in open water and vegetated areas of the planted wetland, *vs.* 287 ± 27 and 255 ± 21 gC $m^{-2}$ $y^{-1}$ in open water and vegetated areas of the naturally colonized one), and this difference was significant at the 85 % confidence level ($P$ = 0.13). Carbon sequestration is lowest in emergent communities (212 ± 20 gC $m^{-2}$ $y^{-1}$). Significant differences at the 85 % confidence level were also found between open water (267 ± 21 gC $m^{-2}$ $y^{-1}$) and emergent communities ($P$ = 0.12), but none between the edge ones (255 ± 19 gC $m^{-2}$ $y^{-1}$) and the rest (Table 4.1).

### *4.4.4. Wetland vs. upland*

No hydric conditions developed on the upland soil adjacent to the wetlands and its bulk density remained high (1.22 ± 0.08 Mg $m^{-3}$), almost twice of the average density in both wetlands (0.67 ± 0.02 Mg $m^{-3}$). The carbon content is lower than in the wetlands (24.7 ± 4.1 gC $kg^{-1}$, 40 % less that the content of the average of both wetland soils), but it is also highly organic (84 %, slightly more than in the wetland open water communities). In 15 years the upland sites have increased their total soil carbon pool by 1.5 kgC $m^{-2}$, yielding a carbon sequestration rate of 99 gC $m^{-2}$ $y^{-1}$, i.e. a pool and a rate 2.5 times lower than the pool and rate of these created wetlands in the same 15 years. The total nitrogen content of the upland soil (2.4 ± 0.1 gN $kg^{-1}$) was lower than the one measured in these wetlands, which was on average about 31 % higher. From this nitrogen content, $NO_3$-N contributed to 3.2 ± 0.3 µg $NO_3$-N $g^{-1}$ while $NH_4$-N was 9.04 ± 1.3 µg $NH_4$-N $g^{-1}$, much less than the $NH_4$-N measured in the wetlands (41.1 µg $NH_4$-N $g^{-1}$, on average; Table 4.1). These carbon and nitrogen contents result in a C:N ratio for the upland area of 10.6 ± 0.3.

## 4.5. Discussion

### *4.5.1. Carbon in the soil and vegetation cover*

The carbon that accumulates in the soil is consequence of the environmental conditions. We considered these two wetlands a replica of the same ecosystem since they were created identical and have undergone the same forcing functions (same hydrology, same nutrient load, same weather), with the only difference that one (western basin) was originally planted and the other (eastern basin) was not. In these 15 years, this has led to a major difference on vegetation cover between these two wetlands – the unplanted wetland was dominated by resilient and rapid growing plant species (such as cattail) that produce high aboveground biomass, while the planted one has consistently been much less dominated by these plants and thus had lower plant biomass despite higher plant community diversity (Mitsch et al. 2012). These cattail patches are not evenly distributed; they are covering densely the inflow of the originally unplanted wetland, while its outflow remains mostly open water. This open water patch in the outflow is probably due to extensive herbivory of the *Typha* spp. in the years 2000-2003 (Mitsch et al. 2012) by muskrats (*Ondatra zibethicus*) and Canada Geese (*Branta canadensis*), given its historically lower diversity in plant species than the planted basin. In the planted wetland cattail has been historically more of an edge community until the last few years, where it has been dominating part of the outflow of the basin. As a result, the aboveground net primary productivity (ANPP) of the naturally colonized wetland was historically higher than in the planted one (394 ± 29 gC $m^{-2}$ $y^{-1}$ and 342 ± 22 gC $m^{-2}$ $y^{-1}$, respectively; Figure 4.3), being a significant increase of ANPP from inflow to outflow in the planted wetland basin ($P = 0.11$, linear $R^2 = 0.89$) and a significant decrease from inflow to outflow in the colonized one ($P = 0.01$ and linear $R^2 = 0.99$). Carbon sequestration follows the same trends, with $P = 0.07$ and linear $R^2 = 0.95$ in the planted wetland, and with $P = 0.09$ and linear $R^2 = 0.92$ in the wetland that was naturally colonized (Figure 4.3). This correlations and the fact that carbon sequestration was higher in the sections where ANPP was higher, indicate that the carbon that accumulates in the soil is not necessarily favored by the nutrients in the water (otherwise it would be higher in the inflow of both wetlands, where nutrient concentrations are higher).

The carbon dissolved and suspended in the water at the inflow and the outflow of both wetlands remains somewhat constant. Therefore, assuming that the main source of carbon into the soil is the vegetation, we find that the originally planted wetland is storing into the hydric soil 64 % of what is coming into the system as aboveground biomass (ANPP), while the soil of the naturally colonized wetland sequesters 68 % of the carbon that enters the system as ANPP (Figure 4.4). The remaining is likely to leave the wetland, mostly in the form of a carbon gas (primarily methane and some carbon dioxide), but part of it also suspended or dissolved in the water and transferred laterally by animals or winds (Chapin III et al. 2006). In ecosystems such as these two wetlands where organic matter and biomass accumulates significantly, ecosystem respiration is lower than its productivity, i.e., $P/R < 1$, a quality attributed to "young mature" ecosystems in early stages of ecological succession (Odum 1969; Odum and Barrett 2004).

### *4.5.2. Wetland communities and carbon sequestration*

We explored the effect that the type of wetland community may have on soil carbon sequestration by comparing the sequestration rates of each community in each wetland. We found that rates were higher in the open water community than in the vegetated ones, a difference that is due to the emergent communities having the lowest carbon sequestration rates. These differences, however, are significant at the 85 % confidence interval, a significance level commonly not considered very impressive in ecosystem sciences, but very frequently used in other fields because of its appropriateness in experiments with little replications (Christensen 1998). Our intention pointing out this difference is to remark the effect that permanent anaerobic conditions have on carbon accumulation in these wetlands. Open water communities are not expected to be as productive as those with vegetation (emergent and edge communities in this study), although floating plants and algae mats can introduce important amounts of carbon into the soil (Wu and Mitsch 1998; Bernal and Mitsch 2012). In these two created wetlands the open water communities are likely receiving great organic inputs from the plant debris produced by the other communities, given the small size of the wetland and the closeness of the communities. If the open water sites are accumulating more carbon than the emergent communities it is probably because of the retarded decomposition of the soil organic matter under permanent anaerobiosis in the open water sites, while the vegetation cover in the emergent communities introduces oxygen into the soil through the roots creating oxic conditions throughout the soil profile (Schlesinger 1997; Stevenson and Cole 1999; Mitsch and Gosselink 2007). In the edge community the carbon sequestration rates were very similar to those in the open water areas, despite of the intermittent anaerobic conditions. In these edge sites, however, the inputs of carbon into the soil are likely to be highest, compared to the other sites, given the dense vegetation and the closeness to the upland (potentially and extra source of carbon by means of woody plant debris and runoff).

We studied the possible interaction between the wetlands (planted and colonized) and the communities (open water, emergent, and edge) on carbon sequestration to see if there was a difference in carbon accumulation in the communities depending on whether the wetland was planted or colonized. Sequestration rates of each community type were not significantly different in both wetlands, and there was no interaction effect between community and wetland type ($P$ = 0.94; $F$ = 0.66). Therefore, the rates of each community do not depend on the wetland they are located, and *vice versa*.

### *4.5.3. Carbon and Nitrogen*

The nitrogen contained in the soil increased towards the outflow in both wetlands, opposite to the nitrogen contained in the water. These two wetlands effectively remove nitrogen in the water (Mitsch et al. 2005a,2005b,2012), although denitrification is not the main pathway (Hernandez and Mitsch 2007; Song et al. 2012) – soil accumulation is estimated to be much more important (Mitsch et al. 2012). Neither denitrification study at these created wetlands (Hernandez and Mitsch 2007; and Song et al. 2012) shows a clear denitrification difference or pattern between inflow and outflow, suggesting that the decrease of nitrogen content in the water might be related to the increase of nitrogen in the soil rather than the denitrification process itself. Being mostly organic, part of this nitrogen is likely to be coming from the organic material that is entering the ecosystem in the water from the adjacent eutrophic river and, for the most part, produced within the wetland (as plant biomass). To further explore this point we compared the C:N ratios in both wetlands' soil (Figure 4.2a) and water (Figure 4.2b). The water carbon and nitrogen contents show no relationship ($P$ = 0.79, linear $R^2$ = 0.002). In the soil, however, the C:N ratios remain somewhat constant in both wetlands (Table 4.1) and are strongly correlated (Figure 4.2a). The correlation is not as strong in the naturally colonized wetland compared to the planted one, probably because the range of total soil carbon in the colonized wetland was narrower and thus the values are more clustered than in the one that was planted. This positive correlation between carbon and nitrogen suggests that total nitrogen is derived from the organic matter (Cunqui et al. 2007), and thus carbon and nitrogen contents are changing together under the different conditions that these wetlands present.

Carbon and nitrogen can be related in the soil organic matter in the form of labile organic compounds (C:N of 20–30 or higher; Stevenson and Cole 1999) that remain undecomposed due to the anaerobic conditions, or as stable or humified resilient organic material (C:N of 10–12; Stevenson and Cole 1999). Carbon content is greater in the vegetated communities (especially edge ones), and in consequence so are the C:N ratios. The soil nitrogen content, however, is the same. Therefore, we cannot assume that the C and N are mainly bound through labile organic compounds, which would be higher where fresh organic material is higher. On the other hand, the stable carbon fraction is higher in the open water sites (20 %) than in the vegetated areas (10 %), making it difficult to correlate it with the constant soil nitrogen levels. Carbon could be more stable in the open water sites because in the vegetated areas labile carbon increases due to the root exudates and plant detritus (Altor and Mitsch 2008), or because of the potential stabilizing effect of nitrogen in the form of $NH_4^+$ (significantly higher in the open water community, Table 4.1) that binds strongly to clay particles. $NH_4^+$ levels are higher in the open water sites of these wetlands because of the permanent anaerobic conditions that prevent aerobic oxidation of $NH_4^+$ to $NO_3^-$ (Reddy and Patrick 1984; Stevenson and Cole 1999; Davidsson and Ståhl 2000), which was lower in these open water sites but not significantly.

In light of these results, we cannot say with confidence that nitrogen enhances carbon accumulation in the soil, given that even though C:N ratios are relatively constant throughout the wetlands we do not necessarily see higher carbon content where nitrogen is higher; the driver is likely to be the organic matter produced in the different wetland communities, source of carbon and nitrogen. However, we can say that nitrogen is not decreasing the carbon pool and thus, the idea that nitrogen (and especially $NH_4$-N; Stevenson and Cole 1999) in the soil reduces soil carbon content by means of enhanced microbial decomposition of organic matter does not appear to apply in our wetlands. We can also affirm that the nutrient rich waters coming into the wetlands are not directly responsible of enhancing nor decreasing carbon content in the soil, given the lack of correlation between both variables, but it is likely to be affecting it indirectly by increasing aboveground biomass (i.e., "fertilizing" the wetland), especially cattail, a plant species that spreads rapidly in nutrient rich waters.

### *4.5.4. Carbon sequestration over time*

Anderson and Mitsch (2006) measured the carbon content, pool and sequestration rate of these two wetlands 10 years after they were created. From year 10 to 15 (Table 4.2), the thickness of the hydric layer has increased, on average, from 9 to 14 cm. This increase, along with an increase in bulk density of 0.2 Mg $m^{-3}$, increased the soil carbon pool to 76 % more of what Anderson and Mitsch (2006) reported, even though the actual total carbon content has not increased as dramatically (from 38.5 gC $kg^{-1}$ after 10 years to 41.3 gC $kg^{-1}$ after 15). The average nitrogen content, however, decreased slightly (9 %) from year 10 to 15. Over time, these two wetlands have experienced an exponential increase in their soil carbon pool. The soil carbon content and pool, however, is also increasing over time (Figure 4.5) but its average rate is not as fast in the recent years as it was in the first decade. If we look at the carbon content of the two wetlands individually, we see that shortly after creation and at the age of 10 years both wetlands had about the same carbon content. However, in 2009 (15 years after they were created), the two wetlands appear to be diverging on their soil carbon content – even though aboveground biomass has increased over time in both, the naturally colonized wetland is increasing its soil carbon pool at a faster rate than the planted wetland.

An increase in the carbon pool results in an increase in the carbon sequestration rate of 26 %, from 190 gC $m^{-2}$ $y^{-1}$ when these wetlands were 10 years old to 242 gC $m^{-2}$ $y^{-1}$ when they were 15 years old. This means that, to obtain an average sequestration rate of 242 gC $m^{-2}$ $y^{-1}$ in 15 years, in the last five years (from 2004 to 2009) the actual carbon sequestration rate would have been 346 gC $m^{-2}$ $y^{-1}$. In the long term and as long as these wetlands are not disturbed, they will probably reach "maturity" and their soil carbon pool will likely increase at a lower rate (Odum 1969; Mitsch et al. 2012), approaching the rate of similar natural riverine wetlands of the temperate region (Bernal and Mitsch 2012). Therefore, it is important to address the age of a created wetland when assessing its carbon sequestration capacity. We compared our two created wetlands with a similar flow-through wetland in Ohio (our reference natural wetland described in Table 4.2 and in detail by Bernal and Mitsch 2012). This reference natural wetland had similar hydrology, nutrient loading, vegetation, and climate to our two created wetlands, and it sequestered 140 gC $m^{-2}$ $y^{-1}$ on average, 43 % less of what our two created wetlands are currently sequestering.

### *4.5.5. Creating wetlands to sequester carbon*

From these results it seems feasible to create wetlands to efficiently sequester carbon. Macrophyte productivity appears to be one of the main factors enhancing carbon accumulation in the wetland soil. In these created wetlands, aboveground net primary productivity was high, and interestingly highest in the unplanted, naturally colonized wetland, while the planted wetland consistently had higher plant community diversity (Mitsch et al. 2012). Nutrient rich waters favor vegetation growth and thus, enhancing the soil carbon sequestration. Our results suggest that the nitrogen in the soil is more likely to come through plant productivity than directly from the water, but we cannot conclusively state the role of this nitrogen on the soil carbon pool. What seems clear from our analysis is that soil nitrogen does not affect negatively the soil carbon. Hydrologically, open water sites have high carbon sequestration rate, similar to the vegetated edge communities; while the former is likely to have slow organic matter decomposition rates, the later is likely to be more productive.

For a full assessment of the net effect of a created wetland as sink or source of carbon to the atmosphere, one would have to take into account the soil carbon sequestration rate and the two main fluxes of carbon (typically) of a wetland ecosystem, i.e. the $CO_2$ taken from the atmosphere (mainly through biomass productivity), and the $CO_2$ and $CH_4$ emitted through aerobic and anaerobic respiration. Soil carbon sequestration rates serve as an indication of how efficient an ecosystem is in functioning as a carbon sink. However, the current debate on climate change has raised much interest on greenhouse gas emissions from a system (mostly $CH_4$ in wetlands, and $N_2O$ to a lesser extent) and their relation to the sequestration rate. Methane is a powerful greenhouse gas, but the amount emitted from a wetland is estimated to be 1–3 % of the wetland biomass productivity (Whiting and Chanton 1993; Schlesinger 1997; Melack et al. 2004), which in turn is greatly exceeded by the rate of carbon accumulation in the soil, as indicated in this study and others (Mitsch and Gosselink 2007; Saunders et al. 2007). Thus, if wetlands were going to be created to sequester carbon, in order to be able to single out the ideal conditions to develop in the system for carbon accumulation it would be necessary to assess which of these conditions present least methane emissions as well. Previous studies in these two created wetlands from Altor and Mitsch (2008), Nahlik and Mitsch (2010), and Sha et al. (2011) indicate that while methane emissions are difficult to predict, the open water sites had consistently higher emission rates than the other sections of the wetlands, and over all they are higher under steady flow rather than under a pulsing hydrology (Altor and Mitsch 2008). Additionally, the greater the wetland productivity the more carbon is available in the soil and thus the greater the methane emissions (Nahlik and Mitsch 2010). Consequently, if one has to choose the ideal conditions for carbon sequestration, seems reasonable to create a wetland with pulsing hydrology and shallow water levels that maintain carbon sequestration rates high (like our edge community) while methane emissions are minimized, even though the presence of the three communities (open water, emergent, and edge) are probably necessary in a fully functional and structured wetland.

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| | **PLANTED** [1] **WETLAND** | **COLONIZED** [1] **WETLAND** | **WETLAND COMMUNITY** | | |
|---|---|---|---|---|---|
| | | | **open water** | **emergent** | **edge** |
| ***Physiochemistry*** | | | | | |
| **Bulk density (Mg m$^{-3}$)** | 0.63 ± 0.02 (17) | 0.71 ± 0.04 (19) | 0.59 ± 0.04 (12) | 0.67 ± 0.03 (15) | 0.75 ± 0.06 (9) |
| **Hydric soil depth (cm)** | 14.6 ± 1.3 (17) | 13.2 ± 1.2 (19) | 17.9 ± 1.7 (12) | 12.0 ± 0.8 (15) | 11.7 ± 0.8 (9) |
| **Total C content (gC kg$^{-1}$)** | 36.1 ± 1.6 (17) $^{A}$ | 46.5 ± 2.8 (19) $^{B}$ | 39.6 ± 1.6 (12) | 39.9 ± 2.8 (15) | 46.5 ± 5.6 (9) |
| **Organic C : Total C ratio** | 0.90 ± 0.03 (17) | 0.93 ± 0.01 (19) | 0.81 ± 0.04 (12) | 0.97 ± 0.02 (15) | 0.97 ± 0.01 (9) |
| **Total N content (gN kg$^{-1}$)** | 3.1 ± 0.1 (17) $^{A}$ | 3.2 ± 0.1 (19) $^{B}$ | 3.1 ± 0.2 (12) | 3.2 ± 0.3 (15) | 3.2 ± 0.3 (9) |
| **$NH_4$-N content (µg g$^{-1}$)** | 45.1 ± 3.9 (17) | 37.0 ± 5.1 (19) | 60.2 ± 7.0 (12) $^{A}$ | 31.2 ± 4.3 (15) $^{B}$ | 33.9 ± 5.1 (9) $^{B}$ |
| **$NO_3$-N content (µg g$^{-1}$)** | 2.1 ± 0.2 (17) $^{A}$ | 3.0 ± 0.2 (17) $^{B}$ | 2.4 ± 0.3 (12) | 2.6 ± 0.3 (14) | 2.7 ± 0.5 (8) |
| **C : N ratio** | 11.6 ± 0.3 (17) | 13.1 ± 0.8 (19) | 11.5 ± 0.4 (12) | 11.9 ± 0.6 (15) | 14.4 ± 1.3 (9) |
| ***Rates and pools*** | | | | | |
| **Soil C pool (kgC m$^{-2}$) *** | 3.2 ± 0.2 (17) $^{A}$ | 4.0 ± 0.3 (19) $^{B}$ | 4.0 ± 0.3 (12) $^{A}$ | 3.2 ± 0.3 (15) $^{B}$ | 3.8 ± 0.3 (9) $^{A,B}$ |
| **Soil N pool (kgN m$^{-2}$) *** | 0.29 ± 0.01 (17) | 0.30 ± 0.01 (19) | 0.31 ± 0.02 (12) | 0.25 ± 0.02 (15) | 0.28 ± 0.02 (9) |
| **Sedimentation rate (kg m$^{-2}$ y$^{-1}$)** | 6.0 ± 0.4 (17) | 5.9 ± 0.4 (19) | 6.8 ± 0.5 (12) | 5.3 ± 0.3 (15) | 5.9 ± 0.6 (9) |
| **C accumulation rate (g m$^{-2}$ y$^{-1}$)** | 219 ± 15 (17) $^{A}$ | 267 ± 17 (19) $^{B}$ | 267 ± 21 (12) $^{a}$ | 212 ± 20 (15) $^{b}$ | 255 ± 19 (9) $^{a,b}$ |

[1] Weighted averages based on corresponding surface area of open water and vegetated communities (i.e. emergent and edge).

* Soil C and N pools calculated for the average hydric soil depth in each wetland.

Table 4.1. Average ± standard error (*n*) of physiochemical conditions, pools and rates of the planted wetland and the naturally colonized at the Olentangy River wetland Research Park 15 years after they were created, and of their three wetland communities (open water, emergent, and edge). Statistical significance of the differences between the two wetlands and their communities is indicated (capital letters for significance at $\alpha \leq 0.05$ and lower case letters for significance at $\alpha < 0.15$).

| | Bulk Density (Mg $m^{-3}$) | Soil Accretion (cm) | Total Carbon content (gC $kg^{-1}$) | Carbon Pool * (kgC $m^{-2}$) | Carbon accumulation rate (gC $m^{-2}$ $y^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| **Created wetlands** | | | | | | |
| **10 y since creation (1994 - 2004)** | 0.5 | 9 | 38.5 | 2.3 | 190 | Anderson and Mitsch 2006 |
| **15 y since creation (1994 - 2009)** | 0.7 | 14 | 41.3 | 3.7 | 242 | this study |
| **change 2004 – 2009 (%)** | + 37 % | + 55 % | + 7 % | + 76 % | + 26 % | this study |
| **Reference natural wetland** | | | | | | |
| | 0.8 | - | 50.1 | 1.5 | 140 | Bernal and Mitsch 2012 |

* Carbon pool to average hydric soil depth in created wetlands, and to 35 cm in reference wetland.

Table 4.2. **.** Comparison of physiochemical conditions 10 and 15 years after the wetlands were created (average of both wetlands), including the percentage change in the conditions between both periods, and the values for the reference natural wetland.

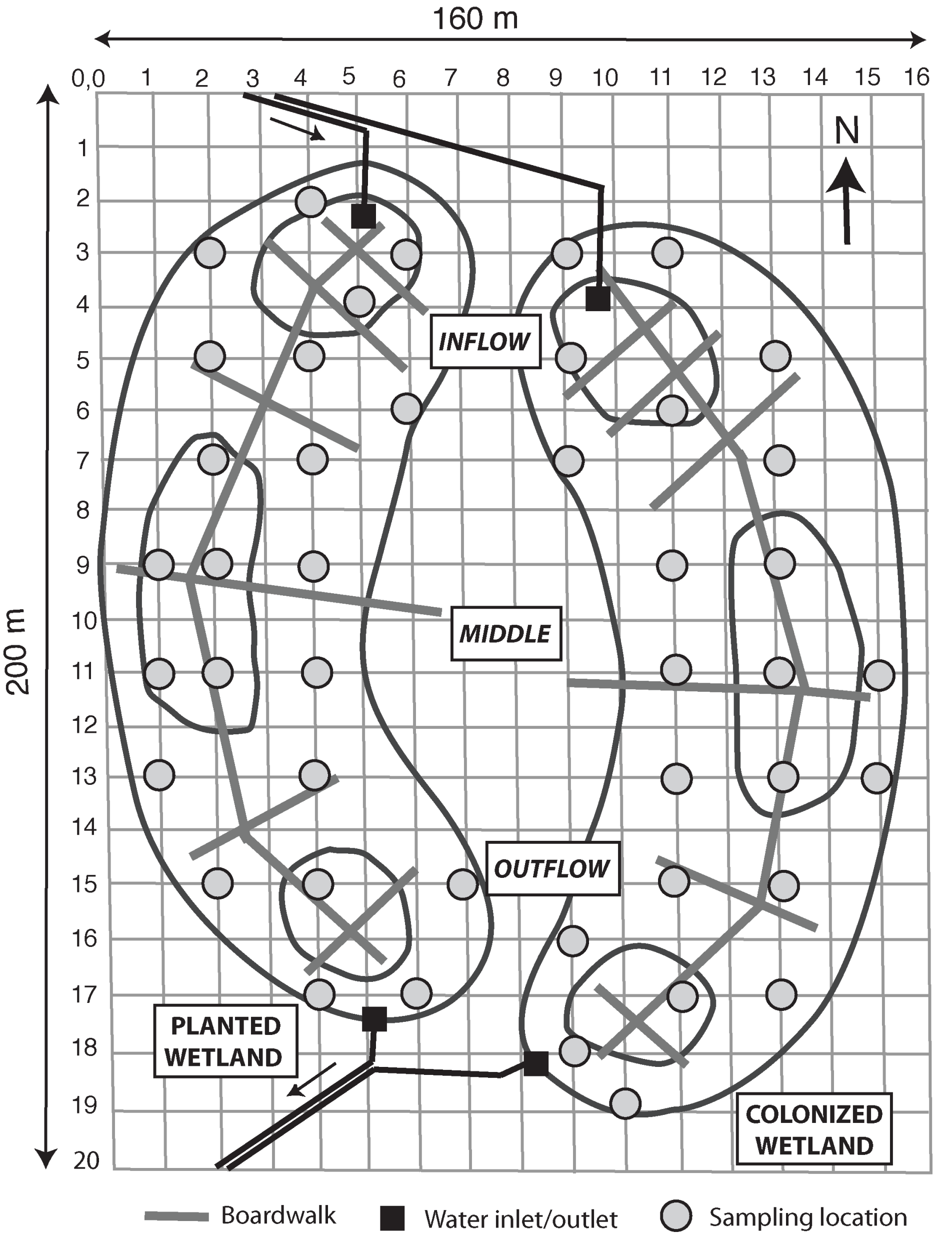

Figure 4.1. Schematic of the two created wetlands at the Olentangy River Wetland Research Park, within the 10-m grid, including the representation of the soil sampling locations, water inflows and outflows, direction of the flow, and boardwalks. The wetland sections (inflow, middle, and outflow) and the three major open water zones (enclosed areas within each wetland) are also indicated.

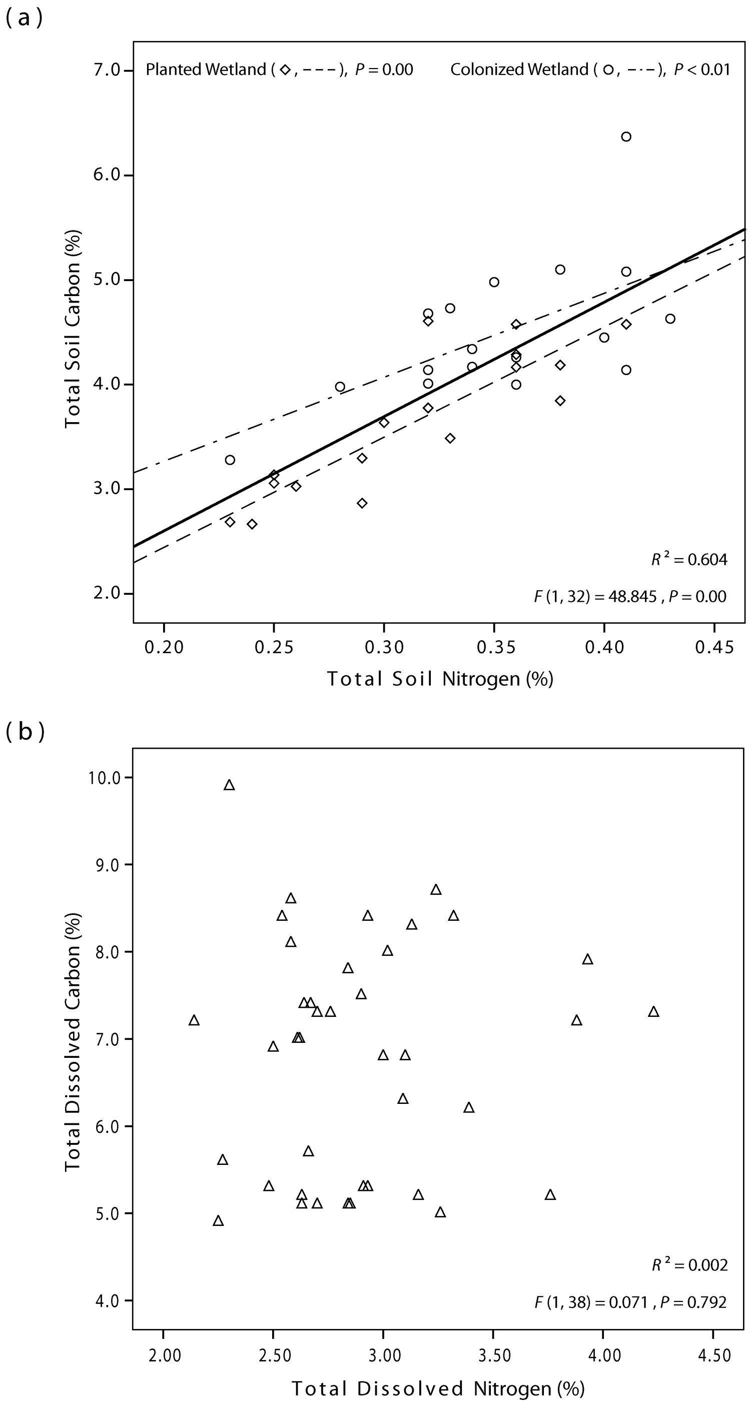

Figure 4.2. Total carbon content (%) *vs.* total nitrogen content (%) in (a) the soil and (b) the water of the two created wetlands at the Olentangy River Wetland Research Park, with significance of the Pearson correlation (*F* (*df*) and *P* values) and linear $R^2$. In the soil graph (a), solid line indicates the average trend of both wetlands (corresponding to the reported *F*, *P*, and $R^2$ values); the individual planted and naturally colonized wetland percentages, trend, and significance of the correlation are also indicated.

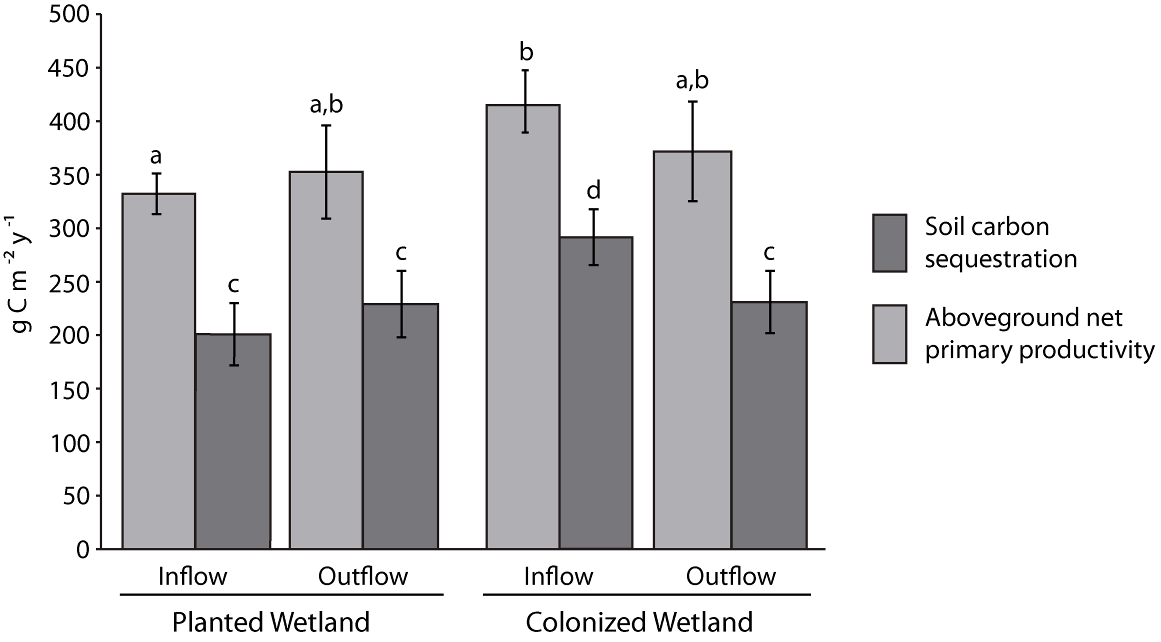

Figure 4.3. Comparison of soil carbon sequestration (dark grey) and aboveground net primary productivity (light grey) rates (gC $m^{-2}$ $y^{-1}$) in the inflow and outflow sections of both created wetlands at the Olentangy River Wetland Research Park. Bars represent standard error, and different letters indicate significant differences at $\alpha < 0.15$.

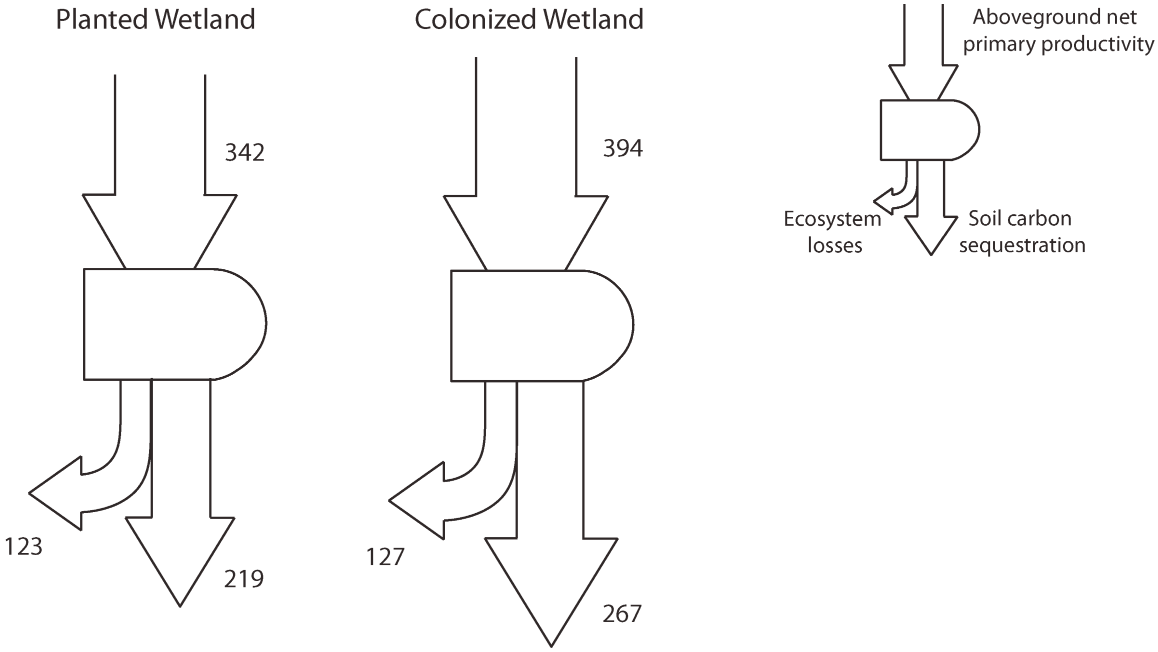

Figure 4.4. Diagram summarizing wetland carbon inputs (aboveground net primary productivity) and outputs (soil carbon sequestration and ecosystem carbon losses). Rates for each flow are indicated next to the arrows (in gC $m^{-2}$ $y^{-1}$), whose width is proportional to their rate. *Illustration after Twilley et al. 1986 for mangrove swamps litter dynamics*.

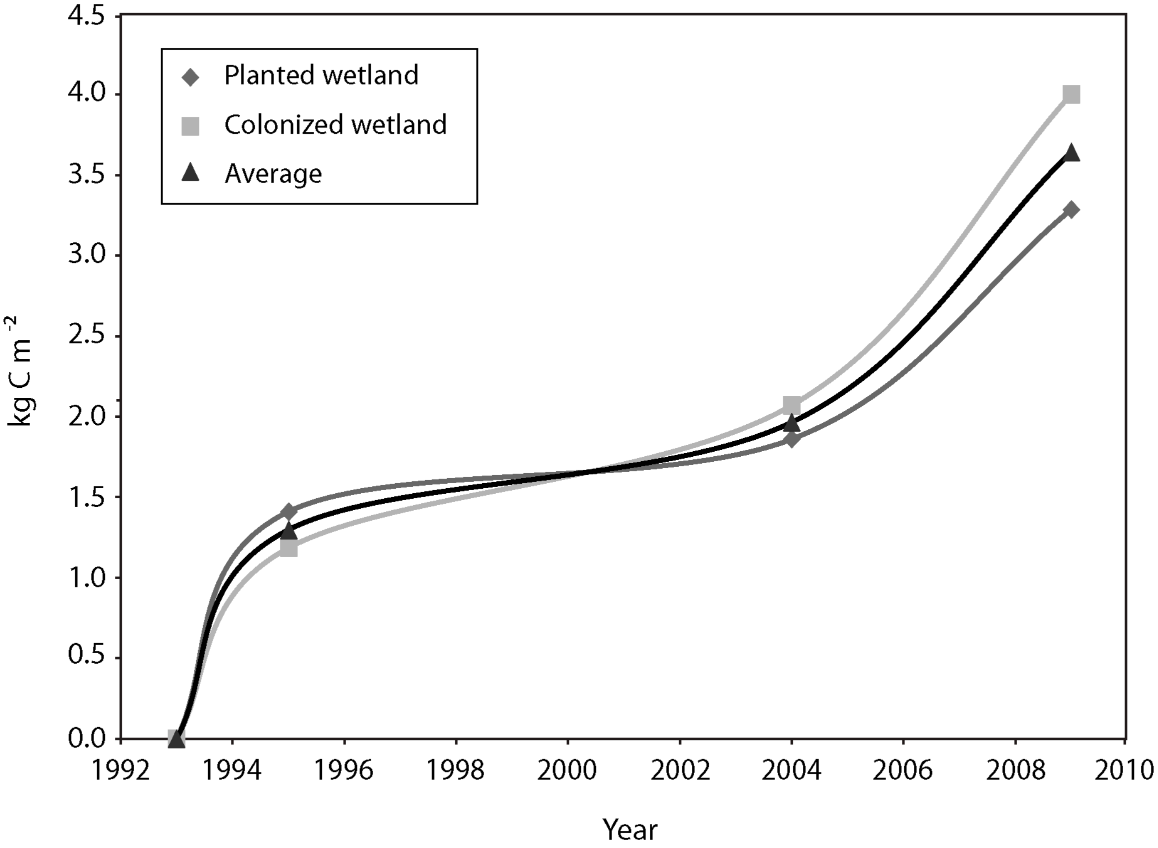

Figure 4.5. Trend of total soil carbon (kgC $m^{-2}$) accumulating in the created wetlands at the Olentangy River Wetland Research over 15 years (since they were created in 1994 to 2009). Carbon data of years 1995 and 2004 are from Anderson et al. (2005) and Anderson and Mitsch (2006). Individual carbon pool of planted and naturally colonized wetlands are represented in dark and light grey, respectively; back line represents the average of both wetlands.

# CHAPTER 5

# CONCLUSIONS

This dissertation focused on soil carbon sequestration in natural and created freshwater wetlands located in temperate and tropical regions. The three studies have generated conclusions that contribute to the fields of wetland biogeochemistry, wetland management, and carbon capture and sequestration (CCS). These conclusions are grouped below by the individual study that generated them (described in detail in Chapters 2, 3, and 4 of this dissertation), followed by a set of integrated conclusions and suggestions for future research based on the findings reported here.

## 5.1. Carbon sequestration rates in natural temperate freshwater wetland communities

(1) Soil carbon sequestration differed significantly in the wetland communities of the two hydrogeomorphic wetland types studied – isolated

depressional wetland sites connected to the groundwater table, and riverine flow-through wetland sites that receive water from an agricultural watershed.

(2) These wetland sites are significantly more effective in accumulating carbon in the soil than are adjacent non-wetland sites.

(3) Results from this study suggest that (a) carbon sequestration in wetland soils is favored by the permanent presence of water (in the riverine wetland, sequestration rate was highest in the permanently flooded site), and that (b) recalcitrant plant debris is more effective enhancing soil carbon sequestration capacity than plant material from emergent wetland vegetation (all the depressional sites received woody plant material and had greater sequestration rates than any of the riverine sites).

(4) Hydrogeomorphic conditions are important to predict carbon sequestration in wetlands, as they determine both the vegetation communities that dominate the ecosystem and thus the carbon inputs (carbon source, i.e. organic matter type) and the outputs (aerobic and anaerobic decomposition).

## 5.2. Carbon sequestration in natural tropical freshwater wetlands

(1) Tropical wetlands can be significant carbon sinks when the hydrologic conditions favor peat accumulation.

(2) Wetlands in the tropical humid climate had significantly higher carbon sequestration rate than did those in tropical dry regions.

(3) The slow-flow rainforest swamp sequestered significantly more carbon than our other tropical wetland sites. This swamp is the only one with standing

water year-round, suggesting that even though it may have similar biomass productivity (i.e., carbon inputs) similar to the other rainforest wetland in this study, the permanent presence of water likely retarded organic matter decomposition and thus playing a more important role favoring carbon sequestration.

## 5.3. Carbon sequestration in created riverine wetlands

(1) Created wetlands can be efficient carbon sequestering systems, compared to uplands and even natural wetlands in the same climate.

(2) Created wetlands appear to increase their efficiency in sequestering carbon during their “teenage” years (carbon sequestration rates were higher after 15 years of creation than after 10), but eventually this accumulation rate may slow to rates similar to natural wetlands.

(3) Carbon sequestration (and consequently soil carbon pool) was correlated with aboveground biomass productivity in these created wetlands.

(4) Soil carbon sequestration had no correlation with the nitrogen concentrations in the water, suggesting that nutrient rich waters do not necessarily favor carbon accumulation in the soil directly, even though they enhance biomass productivity and thus soil carbon inputs.

(5) Soil C:N ratios were higher where biomass productivity was higher, but nitrogen content was constant throughout these wetlands, suggesting that soil nitrogen does not decrease carbon content in the soil.

(6) Stable carbon fractions were higher in the open water sites in the created wetlands, possibly due to their lower labile carbon inputs compared to vegetated

communities, to higher $NH_4^+$ levels in the soil in the open water areas, or both. Total soil nitrogen is not correlated with changes in stable carbon fraction.

(7) Carbon sequestration was highest in the open water communities, suggesting the importance of permanent anaerobic conditions to sequester carbon in the soil and of the vegetation from surrounding communities that are likely introducing organic matter in the open water sites.

## 5.4. Integrative conclusions

(1) Overall, our temperate wetland sites were sequestering carbon at a faster rate (233 gC $m^{-2}$ $y^{-1}$, on average) than the tropical ones (151 gC $m^{-2}$ $y^{-1}$, on average), suggesting that higher temperatures in the tropics may hinder carbon sequestration.

(2) In the tropics, where temperature might to be a limiting factor for carbon sequestration in wetland soils, the presence of water seems to be of critical importance to enhance carbon sequestration rate (by maintaining a soil temperature lower than the ambient soil temperature and by maintaining anaerobic conditions).

(3) In temperate regions, where temperature is unlikely to limit carbon sequestration in wetland soils, permanent anaerobic conditions seem to be the most favorable condition for carbon accumulation. However, when organic matter inputs are high (i.e., high biomass productivity) or recalcitrant (e.g., woody plant debris), their role in favoring soil carbon sequestration is as important or more than the permanent presence of water.

(4) The creation of wetlands for carbon capture and sequestration (CCS) is something that can be achieved successfully, as long as the carbon inputs and outputs to the system are acknowledged. Understanding the conditions that favor soil carbon accumulation in wetlands can help in managing the sink capacity of these ecosystems.

## 5.5. Suggestions for future research

Wetlands are proven to be significant carbon sinks, and can potentially serve as mitigation tools to abate the net greenhouse effect of carbon gases to the atmosphere. But to efficiently perform this task, there are some questions that rise from this study and need to be explored more in depth for a successful management of wetlands' carbon stock. Some of them are listed below.

(1) The effect of organic matter inputs on soil carbon sequestration in wetland ecosystems – is carbon accumulation more favored by the biomass productivity (i.e. amount of organic matter entering the soil) or by the recalcitrance of the plant detritus?

(2) The role of nitrogen on soil carbon – can it be estimated in a system where the carbon accumulation is not primarily affected by biomass productivity?

(3) What is the role of belowground biomass productivity on carbon sequestration in wetland soils?

(4) Methane emissions and net primary productivity – they are the two main flows of carbon out and into a wetland, and they should be measured along with carbon sequestration rates to provide an accurate carbon budget of the wetland.

Methane is of importance since it is a powerful greenhouse gas. Methane emissions from the wetlands included in this study can be found in Nahlik and Mitsch 2010 (for these temperate natural and created wetlands) and in Nahlik and Mitsch 2011 (for these tropical wetlands). In the wetlands where methane production is unknown it should be measured along with the sequestration rate.

(5) Management practices – if wetlands can be created to sequester carbon wetland creation can be encouraged by assigning an economic value to the carbon sequestered, which would require an estimation of the difference in carbon pool in a site before and after creating a wetland.

(6) To ensure that the results obtained in these studies are representative of the wetland types in temperate and tropical zones, a similar study of additional wetlands in the same climates should be done, which is a typical pitfall in whole ecosystem studies because of the challenge in having true experimental replications.

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# Appendix A: C and BD Data (Natural Wetland Sites)

A.1. Gahanna Woods Nature Preserve

| **WETLAND SITE** | **Bulk Density (g/cm$^3$)** | **Depth (cm)** | **n Cores** | **Mean TC (%)** | **OC/TC ratio** | **C pool (kgC/m$^2$)** |
|---|---|---|---|---|---|---|
| GW - Sh | 0.41 | 5 | 3 | 15.99 | 0.99 | 3.24 |
| GW - Sh | 0.43 | 10 | 3 | 13.09 | 0.99 | 2.80 |
| GW - Sh | 0.55 | 15 | 3 | 12.48 | 0.99 | 3.46 |
| GW - Sh | 0.62 | 20 | 3 | 12.99 | 0.99 | 4.03 |
| GW - Sh | 0.64 | 25 | 3 | 12.90 | 0.99 | 4.15 |
| GW - Sh | 0.70 | 30 | 3 | 13.16 | 0.99 | 4.62 |
| GW - Tr | 0.39 | 5 | 3 | 12.73 | 0.95 | 2.49 |
| GW - Tr | 0.42 | 10 | 3 | 14.18 | 0.99 | 2.96 |
| GW - Tr | 0.49 | 15 | 3 | 15.28 | 0.99 | 3.72 |
| GW - Tr | 0.58 | 20 | 3 | 15.89 | 0.99 | 4.57 |
| GW - Tr | 0.58 | 25 | 3 | 15.75 | 0.99 | 4.56 |
| GW - Tr | 0.59 | 30 | 3 | 15.26 | 0.99 | 4.53 |
| GW - Tr | 0.59 | 35 | 3 | 15.75 | 0.98 | 4.65 |
| GW - Ms | 0.34 | 5 | 3 | 13.22 | 0.94 | 2.23 |
| GW - Ms | 0.47 | 10 | 3 | 13.51 | 0.98 | 3.20 |
| GW - Ms | 0.53 | 15 | 3 | 16.24 | 0.99 | 4.30 |
| GW - Ms | 0.57 | 20 | 3 | 16.90 | 0.99 | 4.85 |
| GW - Ms | 0.65 | 25 | 3 | 16.95 | 0.99 | 5.48 |
| GW - Ms | 0.67 | 30 | 3 | 17.17 | 0.99 | 5.75 |
| GW - Ms | 0.75 | 35 | 2 | 17.59 | 0.99 | 6.55 |

Sh – Shrub; Tr – Trees; Ms – Marsh.

A.2. Old Woman Creek Nature Preserve

| WETLAND SITE | Bulk Density ($g/cm^3$) | Depth (cm) | n Cores | Mean TC (%) | OC/TC ratio | C pool ($kgC/m^2$) |
|---|---|---|---|---|---|---|
| OWC - Fb | 0.63 | 5 | 3 | 6.05 | 0.83 | 1.91 |
| OWC - Fb | 0.70 | 10 | 3 | 6.31 | 0.84 | 2.20 |
| OWC - Fb | 0.65 | 15 | 3 | 6.81 | 0.89 | 2.22 |
| OWC - Fb | 0.60 | 20 | 3 | 10.10 | 0.93 | 3.02 |
| OWC - Fb | 0.62 | 25 | 3 | 11.46 | 0.99 | 3.54 |
| OWC - Fb | 0.67 | 30 | 3 | 10.34 | 0.99 | 3.46 |
| OWC - Fb | 0.57 | 35 | 3 | 9.55 | 0.99 | 2.73 |
| OWC - Ms | 0.86 | 5 | 3 | 3.44 | 0.80 | 1.48 |
| OWC - Ms | 0.83 | 10 | 3 | 2.92 | 0.82 | 1.21 |
| OWC - Ms | 0.80 | 15 | 3 | 2.95 | 0.80 | 1.18 |
| OWC - Ms | 0.77 | 20 | 3 | 2.71 | 0.77 | 1.04 |
| OWC - Ms | 0.78 | 25 | 3 | 2.32 | 0.92 | 0.91 |
| OWC - Ms | 0.78 | 30 | 3 | 2.18 | 0.89 | 0.85 |
| OWC - Ms | 0.93 | 35 | 2 | 2.10 | 0.92 | 0.98 |
| OWC - Md | 0.76 | 5 | 3 | 2.62 | 0.92 | 0.99 |
| OWC - Md | 0.75 | 10 | 3 | 2.85 | 0.96 | 1.07 |
| OWC - Md | 0.70 | 15 | 3 | 4.06 | 0.99 | 1.43 |
| OWC - Md | 0.71 | 20 | 3 | 4.67 | 0.99 | 1.66 |
| OWC - Md | 0.77 | 25 | 3 | 3.65 | 0.99 | 1.40 |
| OWC - Md | 0.83 | 30 | 3 | 3.19 | 0.99 | 1.33 |

Fb – Floating bed; Ms – Marsh; Md – Mudflat.

A.3. EARTH University

| WETLAND SITE | Bulk Density ($g/cm^3$) | Depth (cm) | n Cores | Mean TC (%) | OC/TC ratio | C pool ($kgC/m^2$) |
|---|---|---|---|---|---|---|
| EA - PF | 0.27 | 5 | 3 | 9.66 | 0.99 | 1.30 |
| EA - PF | 0.28 | 10 | 3 | 9.44 | 0.99 | 1.31 |
| EA - PF | 0.25 | 15 | 3 | 11.00 | 0.99 | 1.36 |
| EA - PF | 0.34 | 20 | 3 | 6.54 | 0.99 | 1.11 |
| EA - PF | 0.37 | 25 | 3 | 4.99 | 0.98 | 0.93 |
| EA - PF | 0.45 | 30 | 3 | 4.89 | 0.98 | 1.10 |
| EA - PF | 0.53 | 35 | 3 | 3.43 | 0.97 | 0.91 |
| EA - Ed | 0.26 | 5 | 3 | 9.63 | 0.99 | 1.25 |
| EA - Ed | 0.35 | 10 | 3 | 8.60 | 0.99 | 1.49 |
| EA - Ed | 0.41 | 15 | 3 | 5.54 | 0.98 | 1.14 |
| EA - Ed | 0.52 | 20 | 3 | 3.73 | 0.97 | 0.98 |
| EA - Ed | 0.46 | 25 | 3 | 5.15 | 0.98 | 1.19 |
| EA - Ed | 0.53 | 30 | 3 | 4.04 | 0.98 | 1.07 |
| EA - Ed | 0.64 | 35 | 3 | 3.12 | 0.96 | 0.99 |
| EA - Pt | 0.20 | 5 | 3 | 23.56 | 1.00 | 2.31 |
| EA - Pt | 0.21 | 10 | 3 | 24.11 | 1.00 | 2.49 |
| EA - Pt | 0.19 | 15 | 3 | 27.15 | 1.00 | 5.57 |
| EA - Pt | 0.19 | 20 | 3 | 33.83 | 0.99 | 3.21 |
| EA - Pt | 0.19 | 25 | 3 | 38.06 | 1.00 | 3.70 |
| EA - Pt | 0.18 | 30 | 3 | 41.12 | 1.00 | 3.63 |
| EA - Pt | 0.13 | 35 | 3 | 46.34 | 1.00 | 2.98 |

PF – Permanently flooded; Ed – Edge; Pt – Peat.

A.4. La Selva Biological Station

| WETLAND SITE | Bulk Density (g/cm$^3$) | Depth (cm) | n Cores | Mean TC (%) | OC/TC ratio | C pool (kgC/m$^2$) |
|---|---|---|---|---|---|---|
| LS - Ed | 0.27 | 20 | 3 | 6.69 | 0.99 | 3.59 |
| LS - Ed | 0.52 | 25 | 3 | 2.65 | 0.97 | 0.68 |
| LS - Ed | 0.68 | 30 | 3 | 1.52 | 0.93 | 0.51 |
| LS - Ed | 0.66 | 35 | 3 | 1.11 | 0.92 | 0.37 |
| LS - Ed | 0.67 | 40 | 3 | 1.06 | 0.91 | 0.35 |
| LS - Md | 0.35 | 20 | 3 | 7.53 | 1.00 | 5.28 |
| LS - Md | 0.61 | 25 | 3 | 2.16 | 0.99 | 0.66 |
| LS - Md | 0.62 | 30 | 3 | 1.74 | 0.99 | 0.54 |
| LS - Md | 0.65 | 35 | 3 | 1.58 | 0.99 | 0.51 |
| LS - Md | 0.67 | 40 | 3 | 1.55 | 0.99 | 0.52 |
| LS - Md | 0.61 | 45 | 3 | 1.75 | 0.98 | 0.54 |
| LS - Md | 0.58 | 50 | 3 | 2.80 | 0.99 | 0.82 |
| LS - Md | 0.62 | 55 | 3 | 2.44 | 0.99 | 0.76 |
| LS - Ms | 0.40 | 20 | 3 | 7.86 | 0.99 | 6.36 |
| LS - Ms | 0.44 | 25 | 3 | 7.07 | 0.99 | 1.55 |
| LS - Ms | 0.49 | 30 | 3 | 4.94 | 0.99 | 1.22 |
| LS - Ms | 0.47 | 35 | 3 | 4.66 | 0.99 | 1.10 |
| LS - Ms | 0.54 | 40 | 3 | 3.77 | 0.99 | 1.02 |
| LS - Ms | 0.55 | 45 | 3 | 2.86 | 0.98 | 0.78 |
| LS - Ms | 0.68 | 50 | 3 | 2.65 | 0.99 | 0.90 |
| LS - Ms | 0.59 | 55 | 3 | 2.87 | 0.99 | 0.85 |
| LS - Ms | 0.67 | 60 | 3 | 2.92 | 0.99 | 0.97 |

Ed – Edge; Md – Mudflat; Ms – Marsh.

## A.5. Palo Verde Biological Station

| WETLAND SITE | Bulk Density (g/cm³) | Depth (cm) | n Cores | Mean TC (%) | OC/TC ratio | C pool (kgC/m²) |
|---|---|---|---|---|---|---|
| PV - Ed | 0.41 | 10 | 3 | 6.51 | 0.91 | 2.64 |
| PV - Ed | 0.73 | 20 | 3 | 3.96 | 0.91 | 2.88 |
| PV - Ed | 0.83 | 30 | 3 | 2.99 | 0.95 | 2.48 |
| PV - PF | 0.54 | 10 | 3 | 5.11 | 0.98 | 2.78 |
| PV - PF | 0.72 | 20 | 3 | 3.74 | 0.93 | 2.68 |
| PV - PF | 0.84 | 30 | 3 | 2.89 | 0.86 | 2.44 |
| PV - Md | 0.44 | 10 | 3 | 58.09 | 0.96 | 2.53 |
| PV - Md | 0.55 | 20 | 3 | 51.20 | 0.97 | 2.79 |
| PV - Md | 0.63 | 30 | 3 | 44.11 | 0.96 | 2.80 |

Ed – Edge; PF – Permanently flooded; Md – Mudflat.

## A.6. Okavango Delta

| WETLAND SITE | Bulk Density (g/cm$^3$) | Depth (cm) | Mean TC (%) | OC/TC ratio | C pool (kgC/m$^2$) |
|---|---|---|---|---|---|
| BW - Sw | 0.47 | 2 | 10.23 | 0.99 | 0.97 |
| BW - Sw | 0.62 | 4 | 8.93 | 0.99 | 1.10 |
| BW - Sw | 0.90 | 6 | 5.68 | 0.99 | 1.02 |
| BW - Sw | 0.88 | 8 | 4.00 | 0.99 | 0.70 |
| BW - Sw | 0.99 | 10 | 3.74 | 1.00 | 0.74 |
| BW - Sw | 1.02 | 12 | 2.11 | 1.00 | 0.43 |
| BW - Sw | 1.03 | 14 | 2.12 | 1.00 | 0.44 |
| BW - Sw | 1.11 | 16 | 1.37 | 1.00 | 0.30 |
| BW - Sw | 1.09 | 18 | 1.08 | 1.00 | 0.23 |
| BW - Sw | 1.11 | 20 | 1.82 | 1.00 | 0.40 |
| BW - Sw | 1.12 | 22 | 1.03 | 1.00 | 0.23 |
| BW - Sw | 1.14 | 24 | 1.87 | 1.00 | 0.43 |
| BW - Sw | 1.19 | 26 | 1.69 | 1.00 | 0.40 |
| BW - Sw | 1.06 | 28 | 2.61 | 1.00 | 0.55 |
| BW - Sw | 1.15 | 30 | 1.67 | 1.00 | 0.38 |
| BW - Dp | 0.26 | 2 | 15.27 | 0.99 | 0.78 |
| BW - Dp | 0.64 | 4 | 8.31 | 0.99 | 1.06 |
| BW - Dp | 1.08 | 6 | 6.47 | 0.99 | 1.40 |
| BW - Dp | 1.11 | 8 | 3.36 | 0.99 | 0.75 |
| BW - Dp | 1.13 | 10 | 2.39 | 0.99 | 0.54 |
| BW - Dp | 1.03 | 12 | 1.18 | 0.99 | 0.24 |
| BW - Dp | 1.11 | 14 | 0.94 | 1.00 | 0.21 |
| BW - Dp | 1.22 | 16 | 1.17 | 1.00 | 0.29 |
| BW - Dp | 1.30 | 18 | 1.01 | 1.00 | 0.26 |
| BW - Dp | 0.98 | 20 | 0.88 | 1.00 | 0.17 |
| BW - Dp | 1.11 | 22 | 1.44 | 1.00 | 0.32 |
| BW - Dp | 1.03 | 24 | 0.85 | 1.00 | 0.17 |
| BW - Dp | 1.17 | 26 | 0.94 | 1.00 | 0.22 |
| BW - Dp | 1.03 | 28 | 1.15 | 1.00 | 0.24 |
| BW - Dp | 1.08 | 30 | 0.67 | 1.00 | 0.14 |

Sw – Shallow marsh; Dp – Deep marsh.

A.6. Okavango Delta – Continued.

| **WETLAND SITE** | **Bulk Density ($g/cm^3$)** | **Depth (cm)** | **Mean TC (%)** | **OC/TC ratio** | **C pool ($kgC/m^2$)** |
|---|---|---|---|---|---|
| BW - Ms | 0.27 | 2 | 3.97 | 0.99 | 0.21 |
| BW - Ms | 0.41 | 4 | 4.99 | 0.99 | 0.41 |
| BW - Ms | 0.74 | 6 | 2.46 | 0.99 | 0.37 |
| BW - Ms | 0.81 | 8 | 2.10 | 0.99 | 0.34 |
| BW - Ms | 0.93 | 10 | 1.92 | 0.99 | 0.36 |
| BW - Ms | 0.96 | 12 | 1.45 | 1.00 | 0.28 |
| BW - Ms | 1.03 | 14 | 0.91 | 1.00 | 0.19 |
| BW - Ms | 1.13 | 16 | 1.00 | 1.00 | 0.22 |
| BW - Ms | 1.06 | 18 | 1.58 | 1.00 | 0.33 |
| BW - Ms | 1.09 | 20 | 1.16 | 1.00 | 0.25 |
| BW - Ms | 1.15 | 22 | 1.18 | 1.00 | 0.27 |
| BW - Ms | 1.13 | 24 | 0.66 | 1.00 | 0.15 |
| BW - Ms | 1.25 | 26 | 0.48 | 1.00 | 0.12 |
| BW - Ms | 1.18 | 28 | 0.41 | 1.00 | 0.10 |
| BW - Ms | 1.21 | 30 | 0.78 | 1.00 | 0.19 |

Ms – Riverine marsh.

## Appendix B: C and BD Data (Created Wetland Sites)

B.1. ORW Wetland 1

| SECTION | COMMUNITY | Core length (cm) | Bulk Density (g/cm$^3$) | Mean TC (%) | OC/TC ratio | C pool (kg C/m$^2$) |
|---|---|---|---|---|---|---|
| IN | EM | 10 | 0.62 | 3.12 | 0.99 | 1.95 |
| | ED | 10 | 0.64 | 4.56 | 0.97 | 2.95 |
| | OW | 20 | 0.66 | 3.76 | 0.71 | 5.00 |
| | EM | 15 | 0.48 | 4.17 | 0.99 | 3.03 |
| | EM | 10 | 0.84 | 2.67 | 0.86 | 2.25 |
| | ED | 15 | 0.63 | 3.04 | 0.99 | 2.89 |
| MD | EM | 15 | 0.58 | 4.56 | 0.99 | 4.00 |
| | OW | 20 | 0.69 | 2.85 | 0.89 | 3.92 |
| | EM | 10 | 0.57 | 3.28 | 0.99 | 1.89 |
| | OW | 10 | 0.72 | 3.01 | 0.99 | 2.17 |
| | OW | 20 | 0.41 | 4.15 | 0.56 | 3.46 |
| | EM | 15 | 0.62 | 3.62 | 0.99 | 3.40 |
| OU | EM | 10 | 0.68 | 2.65 | 0.99 | 1.80 |
| | ED | 15 | 0.72 | 4.27 | 0.87 | 4.63 |
| | OW | 15 | 0.59 | 3.83 | 0.88 | 3.43 |
| | ED | 10 | 0.72 | 4.59 | 0.99 | 3.33 |
| | EM | 20 | 0.57 | 3.47 | 0.80 | 4.01 |

IN – Inflow; MD – Middle; OU – Outflow; OW – Open water; EM – Emergent; ED – Edge.

B.2. ORW Wetland 2

| SECTION | COMMUNITY | Core length (cm) | Bulk Density (g/cm³) | Mean TC (%) | OC/TC ratio | C pool (kg C/m²) |
|---|---|---|---|---|---|---|
| IN | ED | 15 | 0.88 | 3.28 | 0.99 | 4.34 |
| | OW | 10 | 0.83 | 4.73 | 0.95 | 3.96 |
| | EM | 15 | 0.81 | 4.68 | 0.99 | 5.70 |
| | OW | 15 | 0.47 | 4.01 | 0.68 | 2.84 |
| | ED | 10 | 1.18 | 3.98 | 0.99 | 4.72 |
| | EM | 10 | 0.72 | 6.37 | 0.99 | 4.63 |
| MD | EM | 10 | 0.73 | 5.10 | 0.99 | 3.75 |
| | OW | 25 | 0.52 | 4.00 | 0.73 | 5.20 |
| | EM | 10 | 0.77 | 4.17 | 0.99 | 3.21 |
| | OW | 25 | 0.47 | 4.26 | 0.68 | 5.04 |
| | EM | 10 | 0.82 | 2.42 | 0.99 | 2.00 |
| | OW | 25 | 0.47 | 4.14 | 0.82 | 4.92 |
| | ED | 10 | 0.56 | 8.85 | 0.99 | 4.98 |
| OU | EM | 10 | 0.74 | 5.08 | 0.99 | 3.81 |
| | EM | 10 | 0.50 | 4.45 | 0.99 | 2.24 |
| | ED | 10 | 0.60 | 4.98 | 0.99 | 2.98 |
| | OW | 20 | 0.64 | 4.14 | 0.86 | 5.30 |
| | OW | 10 | 0.62 | 4.63 | 0.96 | 2.87 |
| | ED | 10 | 0.83 | 4.34 | 0.99 | 3.61 |

IN – Inflow; MD – Middle; OU – Outflow; OW – Open water; EM – Emergent; ED – Edge.

# Appendix C: N Data

## C.1. ORW Created Wetland 1 – Soil

| SECTION | COMMUNITY | Mean TN (%) | NH4-N (ug/g) | NO3-N (ug/g) |
|---|---|---|---|---|
| IN | EM | 0.23 | 23.70 | 2.67 |
| | ED | 0.41 | 39.39 | 1.89 |
| | OW | 0.32 | 55.34 | 2.75 |
| | EM | 0.25 | 49.23 | 1.93 |
| | EM | 0.38 | 43.20 | 1.88 |
| | ED | 0.25 | 31.58 | 2.24 |
| MD | EM | 0.30 | 26.54 | 1.55 |
| | OW | 0.36 | 67.89 | 3.42 |
| | EM | 0.36 | 20.6 | 2.78 |
| | OW | 0.26 | 79.42 | 1.40 |
| | OW | 0.29 | 46.45 | 14.2 |
| | EM | 0.29 | 23.15 | 1.79 |
| OU | EM | 0.24 | 35.70 | 1.54 |
| | ED | 0.32 | 55.27 | 2.37 |
| | OW | 0.38 | 52.28 | 2.70 |
| | ED | 0.36 | 45.45 | 1.23 |
| | EM | 0.33 | 48.55 | 2.54 |

IN – Inflow; MD – Middle; OU – Outflow; OW – Open water; EM – Emergent; ED – Edge.

C.2. ORW Created Wetland 2 – Soil

| **SECTION** | **COMMUNITY** | **Mean TN (%)** | **NH4-N (µg/g)** | **NO3-N (µg/g)** |
|---|---|---|---|---|
| IN | ED | 0.23 | 27.04 | 2.56 |
| | OW | 0.32 | 41.95 | 2.17 |
| | EM | 0.32 | 30.39 | 6.98 |
| | OW | 0.34 | 72.25 | 1.96 |
| | ED | 0.28 | 18.79 | 4.59 |
| | EM | 0.41 | 13.95 | 2.74 |
| MD | EM | 0.39 | 36.46 | 2.97 |
| | OW | 0.36 | 63.28 | 2.95 |
| | EM | 0.18 | 12.79 | 4.47 |
| | OW | 0.32 | 55 | 2.25 |
| | EM | 0.34 | 31.95 | 2.8 |
| | OW | 0.36 | 70.42 | 2.12 |
| | ED | 0.36 | 12.15 | 0.56 |
| OU | EM | 0.4 | 30.18 | 2.05 |
| | EM | 0.41 | 44.84 | 3.75 |
| | ED | 0.34 | 22.76 | 4.39 |
| | OW | 0.43 | 26.46 | 2.42 |
| | OW | 0.41 | 92.11 | 3.46 |
| | ED | 0.35 | 43.34 | 2.02 |

IN – Inflow; MD – Middle; OU – Outflow; OW – Open water; EM – Emergent; ED – Edge.

## C.3. ORW Created Wetlands – Water

| **WETLAND 1** | | | **WETLAND 2** | | |
|---|---|---|---|---|---|
| **SECTION** | **TOC (mg L$^{-1}$)** | **TN (mg L$^{-1}$)** | **SECTION** | **TOC (mg L$^{-1}$)** | **TN (mg L$^{-1}$)** |
| IN | 5.2 | 3.76 | IN | 5.2 | 3.76 |
| | 5.0 | 3.26 | | 5.0 | 3.26 |
| | 6.8 | 3.00 | | 6.8 | 3.00 |
| | 7.5 | 2.90 | | 7.5 | 2.90 |
| | 5.7 | 2.66 | | 5.7 | 2.66 |
| | 6.8 | 3.10 | | 6.8 | 3.10 |
| | 7.9 | 3.93 | | 7.9 | 3.93 |
| | 8.4 | 2.93 | | 8.4 | 2.93 |
| MD | 5.2 | 3.16 | MD | 6.2 | 3.39 |
| | 5.1 | 2.84 | | 5.1 | 2.85 |
| | 7.0 | 2.62 | | 7.0 | 2.61 |
| | 7.3 | 2.70 | | 7.8 | 2.84 |
| | 5.3 | 2.48 | | 5.1 | 2.63 |
| | 7.4 | 2.67 | | 8.3 | 3.13 |
| | 7.2 | 3.88 | | 7.3 | 4.23 |
| | 6.3 | 3.09 | | 8.0 | 3.02 |
| OU | 5.3 | 2.91 | OU | 5.3 | 2.93 |
| | 5.1 | 2.70 | | 5.2 | 2.63 |
| | 6.9 | 2.50 | | 7.2 | 2.14 |
| | 7.4 | 2.64 | | 7.3 | 2.76 |
| | 4.9 | 2.25 | | 5.6 | 2.27 |
| | 8.6 | 2.58 | | 8.1 | 2.58 |
| | 8.7 | 3.24 | | 8.4 | 3.32 |
| | 9.9 | 2.30 | | 8.4 | 2.54 |

IN – Inflow; MD – Middle; OU – Outflow.

C.4. Natural Wetlands – Soil

| **WETLAND** | **COMMUNITY** | **Mean TN (%)** |
|---|---|---|
| OWC | Fb | 0.43 |
| OWC | Ms | 0.18 |
| OWC | Md | 0.24 |
| EA | PF | 0.55 |
| EA | Ed | 0.44 |
| EA | Pt | 1.53 |
| LS | Ed | 0.19 |
| LS | Md | 0.20 |
| LS | Ms | 0.32 |
| PV | Ed | 0.41 |
| PV | PF | 0.36 |
| PV | Md | 0.47 |

Fb – Floating bed; Ms – Marsh; Md – Mudflat; PF – Permanently flooded; Ed – Edge; Pt – Peat.