

CARBON POOLS AND PROFILES IN WETLAND SOILS:  
THE EFFECT OF CLIMATE AND WETLAND TYPE

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## ABSTRACT

Wetlands are a large terrestrial carbon pool and play an important role in global carbon cycles as natural carbon sinks. Previous carbon studies have mainly focused on boreal peatlands; little is known about carbon pools in temperate and tropical wetlands and their soil profiles. This study analyzes the variation of soil carbon with depth in two temperate (Ohio) and three tropical (humid and dry) wetlands in Costa Rica, and compares their total soil C pool as a first step toward determining C accumulation in wetland soils. The results indicate that these temperate wetlands have significantly greater ( $P < 0.01$ ) C pools ( $17.6 \text{ kg C m}^{-2}$ ) than wetlands located in tropical climates ( $9.7 \text{ kg C m}^{-2}$ ) in the top 24 cm of soil. Carbon profiles showed a rapid decrease of concentrations with soil depth in the tropical sites, whereas in the temperate wetlands they tended to increase with depth, up to a maximum at 18-24 cm, after which they started decreasing. The two wetlands in Ohio had about ten times the mean total C concentration of adjacent upland soils (*e. g.*, in Gahanna Woods,  $161 \text{ g C kg}^{-1}$  were measured in the wetland, and  $17 \text{ g C kg}^{-1}$  in the upland site), and their soil C pools were significantly higher ( $P < 0.01$ ). Among the five wetland study sites, three main wetland types were identified – isolated forested, riverine flow-through, and slow-flow slough. In the top 24 cm of soil, isolated forested wetlands had the greatest pool ( $10.8 \text{ kg C m}^{-2}$ ), significantly higher ( $P < 0.05$ ) than the other two types ( $7.9 \text{ kg C m}^{-2}$  in the flow-through and  $8.0 \text{ kg C m}^{-2}$  in the

slough), indicating that the type of organic matter entering into the system and the type of wetland may be key factors defining its soil C pool. The flow-through wetland in Ohio (Old Woman Creek) showed a significantly higher C pool ( $P < 0.05$ ) in the permanently flooded location (18.5 kg C m<sup>-2</sup>), than in the edge location with fluctuating hydrology, where the soil is intermitently flooded (14.6 kg C m<sup>-2</sup>).

Due to the lack of a standard methodology for C analysis in wetland soils, and because of protocol inconsistencies found in the literature, this study compared five different loss on ignition procedures with the intention of finding the most accurate, precise, and replicable one for wetland soils. The ignition of 1 g (dry weight) of soil at 450°C for 4 hours provided the best results, and its comparison to a standard carbon analyzer shows that it can be used to estimate wetland soil organic matter content and obtain fairly consistent results, with higher accuracies for samples with soil organic carbon content greater than 3.5%.

## DEDICATION

Esta tesis está dedicada a todos aquellos que quiero y que, por el curso natural de la vida y el desenvolvimiento personal, no se encuentran cerca de mí. Mi padre, los amigos que me esperan en España, la buenísima gente que he conocido aquí y puedo llamar amigos, y Nicanor. Vuestro cariño enriquece mi vida y me inspira a seguir adelante.

This thesis is dedicated to all those that I love and, because of the natural process of life and personal growth, no longer live where I am. Mi father, the good friends awaiting me in Spain, the great people I met here that I can call friends, and Nicanor. Your love enriches my life and is the inspiration that keeps me going forward.

“Cáminate, no hay camino,  
se hace camino al andar”.

A. Machado

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## FIELD OF STUDY

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## CHAPTER 1

### INTRODUCTION

Wetlands are dynamic ecosystems characterized by waterlogged or standing water conditions during at least part of the year. In most wetlands, water level fluctuates seasonally instead of being stable (hydroperiod), a property that accounts for making wetlands highly productive environments (Odum et al., 1995; Mitsch and Gosselink, 2007). Accumulation of organic matter in wetland soils depends on the ratio between inputs (organic matter produced *in situ* and *ex situ*) and outputs (decomposition under waterlogged conditions (Gorham, 1998) and erosion by hydrology (Albrecht and Rasmussen, 1995) or other soil disturbance sources). Productivity among wetlands varies depending on the climate, vegetation communities, and type of wetland (Trettin and Jurgensen, 2003). Decomposition is a more complicated process as it involves aerobic and anaerobic processes (Gorham, 1998). Organic matter decomposition is often incomplete under anaerobic conditions and thus, the lack of oxygen is the main factor determining plant detritus turnover. Consequently, plant remains coming from the inflow, from the wetland biomass, or from the vegetation growing along the margins, accumulate in wetlands at different decomposition stages (Collins and Kuehl, 2001; Holden, 2005),

creating a net retention of organic matter and plant detritus (Mitsch and Gosselink, 2007). Decomposition rates in wetlands are also a function of climate (temperature and moisture enhanced microbial activity) and the quality (chemical composition) of the organic matter entering the system (Schlesinger, 1997). Hence, wetland characteristics lead to the accumulation of high amounts of organic matter in the wetland soil, serving as carbon sinks (Mitsch and Gosselink, 2007) and making them one of the most effective ecosystems accumulating soil carbon (Schlesinger, 1997; Collins and Kuehl, 2001).

### **1. 1. Goal and objectives**

The goal of this study is to provide accurate estimates and comparisons of the carbon stored in wetland soils found in different climates and hydrogeological settings. Four objectives were formulated to help reach this goal. The first two objectives are to compare the total soil carbon profiles of three types of wetlands located in one temperate (Ohio) and two tropical (Costa Rica) climates, and to identify relative differences in their soil carbon pools to better understand the factors influencing carbon sequestration in wetland soils. The third and fourth objectives of this study are to determine which loss on ignition (LOI) procedure provides more reliable results for carbon content determinations in wetland soils, and to compare these results with those obtained using a carbon analyzer.

Three hypotheses were tested as part of this study:

H1: Wetlands in the tropics have greater carbon pool in their soil than do temperate zone wetlands. The net C accumulation is expected to be greater in the tropical wetlands because tropical ecosystems are highly productive, and thus the input of organic

matter into the soil could be much greater than the amount of organic matter that is lost from the system by decomposition, seasonal wet-dry periods, or soil disturbance.

H2: Isolated forested wetland types have the highest soil carbon pool of any wetland type. Hydrological isolation of the wetland would imply that the erosion in the ecosystem is much lower, compared to flow-through wetlands, even if the wetland experiences wet-dry periods. The organic matter produced in a forested site would be more recalcitrant than any other labile plant materials, and thus harder to degrade. Hence, isolated forested wetlands must have lower losses of organic matter and carbon than the rest of the wetland types, and the net C accumulation would be greater.

H3: Loss on ignition (LOI) is a less accurate method than the carbon analyzer (TOC) to determine carbon content in wetland soils but can be a useful indicator when properly calibrated.

## **1. 2. Factors that influence carbon accumulation in wetland soils**

The chemical composition of soil organic matter consists of a labile (fresh plant materials that decompose quickly) and a resistant fraction (composed of recalcitrant humic materials that stay for long periods of time in the soil). The resistant fraction accumulates deeper in the soil profile, whereas the majority of the labile compounds that are deposited in the soil surface decompose within several months (Odum and Pigeon, 1970; Schlesinger, 1997; Wolf and Wagner, 2005). The chemical composition of organic matter depends on the type of vegetation that produced it originally: plant detritus from woody species contain more complex structures (lignin and cellulose) that are harder to

degrade by microbes and thus accumulate for long periods of time (Schlesinger, 1997). Isolated forested wetlands are considered systems of moderate productivity compared to flow-through or slow-flow wetlands (Watt and Golladay, 1999; Cronk and Fennessy, 2001). However, they are likely to store organic matter for longer periods of time as this material is more recalcitrant, even though they are only seasonally flooded and aerobic conditions might increase plant litter decomposition. Carbon accumulation in forested wetland soils is significantly greater than in upland forest soils due to the oxic/anoxic soil regime, but it is not usually differentiated from the upland forest pool in literature (Trettin and Jurgensen, 2003). Aboveground productivity of temperate wetland forests is generally greater than boreal ones (Trettin and Jurgensen, 2003), but much lower than wetland forests in the tropics (Schlesinger, 1997). Tropical wetlands are among the most productive environments, introducing large amounts of organic matter into the soil system. However, there is a strong correlation between climate and soil carbon pools where organic carbon content decreases with increasing temperatures (Kirschbaum, 1995; Albrecht and Rasmussen, 1995), because decomposition rates (microbial respiration) double with every 10°C increase in temperature (Schlesinger, 1997; Hartel, 2005). Dick and Gregorich (2004) compared relative decomposition rates of organic matter in tropical (Nigeria) and cold dry climates (Canada), and found that decomposition rates were ten times faster in the tropical site. Hence, tropical wetlands have greater carbon production than wetlands in temperate climates, but also greater decomposition. It is therefore unclear if the net carbon accumulation would be greater in temperate or in tropical wetlands.

### **1. 3. Wetlands in the global carbon cycle**

The entrance of CO<sub>2</sub> into a wetland system (mainly via photosynthesis), gives it the ability to moderate CO<sub>2</sub> concentrations in the atmosphere by sequestering this carbon and thus taking it out from the trophic exchange system (Bondavalli et al., 2000; Holden, 2005). Wetlands are also known to contribute in the release of methane (CH<sub>4</sub>) to the atmosphere, representing about the 25% of the annual methane emissions on earth (Bartlett and Harris, 1993; Bottrill, 2004; Whalen, 2005). The exact quantification of methane released by wetlands is difficult to determine due to the great variability among wetland types, environments and processes (Van der Nat and Middelburg, 2000). Most of this CH<sub>4</sub> emitted comes from the decomposition of the organic matter (Gorham, 1991), and has been estimated to be about the 3% of the net wetland production depending on the soil type and the vegetation present (Schlesinger, 1997; Jokic et al., 2003).

The balance between carbon input (organic matter production) and output (decomposition, methanogenesis, etc.), and the resulting storage of carbon in the wetland depends on several factors such as the topography and landscape position of the wetland, the hydrologic regime, the type of plants present, the temperature (and therefore climate) and moisture of the soil, the pH and salinity, and the morphology of the wetland (Collins and Kuehl, 2001). This long list of factors indicates that carbon accumulation in wetlands is a delicate process influenced by many variables. However, wetlands represent a significant sink for carbon and are a key element to consider when managing and weighing earth's carbon pedological pool. As estimated by Lal (2007), the total soil organic carbon pool is 1550 Pg (petagrams = 10<sup>15</sup> grams); wetlands are responsible for 450 Pg, one-third of this pool (Mitsch and Gosselink, 2007), despite the fact that they

only cover 6-8% of the land and freshwater surface (Roulet, 2000; Mitsch and Gosselink, 2007). Hence, wetlands represent one of the largest biological carbon pools and play a decisive role in the global carbon cycle (Chmura et al., 2003; Mitra et al., 2005). More research in this area is needed in order to quantify more accurately the extent of wetlands soil carbon pool worldwide, on the one hand, and the differences between wetland types, hydrological fluctuations, and climatic regions specifically.

#### **1. 4. Carbon measurement in wetland soils**

For more accurate determinations of wetlands carbon stock the methodology in soil sampling and carbon analysis needs to be revised, as there is no standard methods for wetlands and the standard procedures for upland soils do not necessarily apply. Loss on ignition is a widely used method to estimate organic matter content in wetland soils because of its simplicity and low costs involved. However, inconsistencies found in literature about what temperature or time protocol to use (Nelson and Sommers, 1996; Heiri et al., 2001; Cambardella et al., 2001; Collins and Kuehl, 2001; Anderson and Mitsch, 2006; Hernandez and Mitsch, 2007) indicate that this might not be the most appropriate method, particularly when comparing carbon sequestration among wetlands and with other studies. Some studies (Albrecht and Rasmussen, 1995; Heiri et al., 2001; Cambardella et al., 2001) also report overestimation of soil organic matter problems associated with loss on ignition due to carbonate minerals (inorganic constituents) oxidation at high temperatures (greater than 500°C) and loss of structural water of hydrated clays. The pre-treatment of soil samples with hydrochloric acid is suggested (Albrecht and Rasmussen, 1995; Hernandez and Mitsch, 2007) to avoid organic matter

overestimation. Pre-treatment of samples makes loss on ignition more elaborate and time-consuming, but increases the reliability of the analysis. The suitability of loss on ignition is analyzed in this study, as is its accuracy with respect to more reliable procedures (such as the use of carbon analyzers).

## CHAPTER 2

### MATERIALS AND METHODS

#### **2. 1. Study sites**

The five natural wetland sites involved in this study are located in Ohio and Costa Rica (Fig. 1) and are described in more detail below. These wetlands represent three distinct climates (temperate humid, tropical humid, and tropical dry) and three wetland hydrogeomorphic types: riverine flow-through, slow-flowing slough, and isolated forested wetlands (Table 1).

##### *2. 1. 1. Ohio*

Old Woman Creek State Nature Preserve, a protected 230-ha park on the southwestern shores of Lake Erie, has a 56-ha flow-through wetland that receives its main inflow from the 69 km<sup>2</sup> Old Woman Creek watershed. The watershed is 75% agricultural land. The wetland also receives occasional water pulses from wind-driven seiches (Herdendorf et al., 2006). The wetland accumulates substantial amounts of sediments and nutrients from the watershed, and seasonally serves as a phosphorus sink (Mitsch and Reeder, 1991). The climate of this region is temperate humid, with below zero temperatures in the winter. Water depths range from 0.4 to 1.4 m throughout the wetland (Herdendorf et al., 2006), with *Phragmites australis* in



shallower depths and *Nelumbo lutea* in deepwater areas, covering a significant portion of the marsh (site B). The soil is classified as Adrian muck (Aa) by the NRCS (2008), corresponding to outwash plains and former bogs. The sites where the soil cores A and B were extracted on July 15, 2006, were submerged under 3 and 25 cm of water, respectively.

Gahanna Woods State Nature Preserve, in the Scioto River basin of central Ohio is a 23-ha forest with vernal pools surrounded by maple (*Acer* spp.) and oak (*Quercus* spp.) swamp forests that are occasionally flooded during the year (site B). This park is an old glacial till, and highest water levels appear mainly during the winter season and after the ice thaw. The water that accumulates in these depressional isolated wetland ponds from precipitation and runoff from nearby areas (Gamble and Mitsch, 2006). The wetland basin studied here is on the southern border of the nature preserve, and standing water can be found in some sections of the wetland throughout the year (in site A, where *Typha* spp. grows). The wetland soil is classified (NRCS, 2008) as Pewamo (Pm). It was a mucky peat (hemic soil), high in organic matter where some undecomposed plant detritus could be identified, and bulk density between 0.1 and 0.2 g cm<sup>-3</sup> (Collins and Kuehl, 2001; Trettin and Jugernsen, 2003). Samples were collected on May 15, 2008, and water level at that time was 15 cm in site A and 8 cm in site B.

### 2. 1. 2. Costa Rica

EARTH University, Humedal La Reserva, is a research wetland of 116 ha in the Parismina river basin in the northeast of Costa Rica, where the climate is tropical humid with 3460±750 mm y<sup>-1</sup> of precipitation. This wetland is a slough in the middle of the rain forest reserve on the university campus where swamp palm (*Raphia*

*taedigera*) dominates (Mitsch et al., in press). The outflow end of this wetland to its north has deep water levels, and anaerobic conditions in the water column and sediments favor peat accumulation. The soil in this wetland was hydric mucky peat (partially undecomposed plant materials and bulk density of 0.1-0.2 g cm<sup>-3</sup>) and peat (undecomposed plant materials and bulk density of 0.1 g cm<sup>-3</sup>), as described by Collins and Kuehl (2001). Samples in this wetland were taken in August 17, 2006. Water level at site A, near the edge of the wetland, was 7 cm when sampled; site B was near the outflow and water depth was 90 cm during sampling.

La Selva Biological Station, a 1600-ha National Protected Zone, is located at the confluence of two major rivers (Puerto Viejo and Sarapiquí) in the lowlands of tropical humid Costa Rica (northern Caribbean coast) where the average rainfall is 4337±520 mm yr<sup>-1</sup> (OTS, 2008). Located within a tropical and premontane wet forest, La Selva has almost two-thirds of its area under old-growth rain forest. The wetland investigated at this site is a 3-ha isolated forested swamp with alluvial soils in the middle of a mature rain forest, with some openings where grasses dominate. Samples in this wetland were taken on September 6, 2006. There was no standing water at soil core site B, but the soil was saturated. Water depth at soil core site A was 2 cm.

Palo Verde Biological Station is located in the Palo Verde National Park (2000 ha), on the Pacific slopes of southwestern Costa Rica (OTS, 2008), where the tropical climate is seasonally dry (average precipitation of 1307±271 mm yr<sup>-1</sup>). The Station is located in a seasonally dry forest between the rivers Bebedero and Tempisque. The wetland is a riverine flow-through wetland of about 1000 ha that borders the Tempisque river. Its alluvial soils are subject to flooding by the river and upland runoff during the rainy season. Samples in this wetland were taken on August 29, 2006, when water level was 2 cm in site A and 15 cm in site B.

## **2. 2. Soil sampling**

Soil cores were taken in two representative sites of each of the five wetlands. The selection of representativeness was determined by a combination of factors specific for each wetland, such as vegetation type, hydrology, soil type, human intervention, and/or wildlife disturbances. Sampling sites were deliberately chosen in different ecological communities within the wetland, and their characteristics are described in Table 1.

Three soil cores were taken in each sampling site, spaced in a triangular pattern with 40 cm between each core, to include a variation of organic matter deposition in the area (Isaksson et al., 2001; Stark et al., 2006). Individual cores were pooled together into one composite core per site (Allmaras and Kempthorne, 2002). The diameter of our sediment sampler (Fig. 2) is 7 cm, which provides a core without compaction, distortion, and disturbance (Kemp et al., 1971; Reinhardt and Cole, 2000; Grossman and Reinsch, 2002; Tan, 2005). The corer was carefully inserted into the soil and pushed down as deep as possible. Final depth obtained varied with the site. The shortest cores were obtained at Palo Verde (24 cm), where the high clay content of the soil made it difficult to reach deep soil layers. The longest cores were retrieved at La Selva and EARTH University (54-60 cm), where soft undecomposed plant materials had accumulated. The corer has a one-way check valve that creates a vacuum inside the core liner as it is pushed into the soil, and when the device is pulled out of the soil it creates a suction force that retains the sample into the tube. A similar corer device was described by Grossman and Reinsch (2002) for sampling wet soils or soils under water.

The core barrel was capped on the open end and the length of the core was measured. The soil core was removed of the tube slowly, and it was immediately

sectioned with a blade into samples 2-cm thick and packed in containers. Composite sampling was done by combining three samples corresponding from the same depth. The sample containers were sealed with parafilm and stored on ice or under 4° C to reduce volatilization losses and bacterial activity until analysis.

The water level of each sampling site was recorded. In cases where standing water was present at the sampling site and undecomposed plant materials accumulated at the sediment surface (EARTH University and La Selva), the top layer was a suspension of organic matter and sediment. The length of these layers was measured prior to soil extraction from the corer. In cases where the length of this layer was longer than 6-7 cm (EARTH University's core B, a deep water peat site; Table 1), plant materials were allowed to settle, and water was slowly drained out of the corer, carefully avoiding the loss of suspended sediments and organic matter. This transformation was needed for packing, transportation, and storage of the samples. In the rest of the cases, the watery sample (water and solid materials) was packed entirely in the containers, without discarding any of the contents of the original sample.

The corer liner is a clear polycarbonate pipe (Fig. 2), and if compaction of the soil happens during the insertion of the corer into the ground it can be easily detected by comparing the level of soil's surface inside and out the liner. If that happened, the core was discarded and the sampling was repeated. After extraction, the loss due to fraction compaction can be estimated comparing the length of the extruded core with the length of the core liner, and in that case an average correction would be applied to the entire core (Milton et al., 2001).

### **2. 3. Upland samples**

Upland soil samples in Old Womand Creek and Gahanna Woods were taken on May 10 and 15, 2008 respectively, in the forested area adjacent to the wetland. The sampled upland soil at Old Woman Creek is classified as Del Rey (DeA) silt loam, with 0 to 2 percent slopes, consisting of poorly drained lacustrine materials on lake plains (NRCS, 2008). In Gahanna Woods, the studied upland site was a Bennington (BeB) silt loam, with slopes between 2 and 6 percent, poorly drained, typical of ground and end moraines (NRCS, 2008). Two replicate cores, 35-cm long and 10-cm in diameter, divided in 5-cm increments, were extracted at each site by the core method (Grossman and Reinsch, 2002; Tan, 2005), packed and stored under 4° C until analysis.

### **2. 4. Sample analysis**

Each sample was oven-dried at 105° C for three days or until they reached constant weight, cooled down to room temperature in a desiccator, and weighed to determine bulk density (Grossman and Reinsch, 2002; Anderson et al., 2005). Dry samples were ground and sieved to pass a 2-mm particle size using a Thomas-Willey Mini Mill, which homogenizes, mixes, and reduces the heterogeneity of the sample (Tan, 2005).

Each composite sample was analyzed for carbon content using a Total Organic Carbon Analyzer (TOC-V series, SSM-50000A, Shimadzu) in April and May of 2008. Organic carbon (OC) content was determined at 900° C in 50 mg subsamples and inorganic carbon (IC) at 200° C in 50 mg subsamples pretreated with 0.3 mL of phosphoric acid 10 *M*. The sum of both values is the total carbon in the soil sample. Samples for analysis were placed in ceramic boats (acid washed and ignited at 900° C

for 30 minutes before each use) that were only touched with tweezers to avoid their contamination (Shimadzu, 2001). Standards of known carbon concentration for inorganic carbon (calcium carbonate powder, 12% carbon) and for organic carbon (dextrose anhydrous (D-Glucose) granular powder, 40% carbon) were run every time prior analysis and after every 10-15 samples for quality control. Samples were re-run whenever standards gave a deviation equal or greater than  $\pm 2\%$  from the known percentage (12% or 40%).

The same carbon analysis was performed in the 5-cm increment homogenized upland samples. Since the mass of soil per sample was much greater than in the 2-cm increment wetland samples, three replicates of each were analyzed.

The determination of soil C concentration ( $\text{g C kg}^{-1}$ ) and C pool ( $\text{kg C m}^{-2}$ ) per depth increment (equation [1] and [3], respectively) were calculated as follows:

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

$$\text{STC}_{\text{layer}} (\text{g}) = \text{dry weight}_{\text{layer}} (\text{g}) \times \text{TC}_{\text{layer}} (\%) \times 10^{-2} \quad [2]$$

$$\text{C pool}_{\text{layer}} (\text{kg C m}^{-2}) = 10^3 \times \text{STC}_{\text{layer}} (\text{g}) \times \text{A}^{-1} (\text{m}^2) \quad [3]$$

where,

$\text{TC}_{\text{layer}}$  is the percentage of Total Carbon measured with the Carbon Analyzer,

$\text{STC}_{\text{layer}}$  is the soil total carbon mass,

A is the area of the sampler barrel, *i. e.*,  $38.48 \times 10^{-4} \text{ m}^2$ .

The total C pools at 24 cm, 50 cm, and entire core depths were calculated by summing up the C pool in each soil layer to these depths (2-cm increments in wetland cores, 5-cm increments in upland cores).

#### 2. 4. 1. Loss on ignition investigation

Loss on ignition (LOI) is a widely used method for organic matter content (and organic carbon determinations) in soil samples by measuring the difference in

weight of the soil sample before and after the ignition (Cambardella et al., 2001; Tan, 2005). There is no standard LOI method for wetland soils; literature reports several temperatures and times for ignition, and even mass of dry soil ignited (Table 2). These previously reported procedures were replicated and compared in this study, by igniting ten randomly selected 2-cm increment wetland soil samples of already known carbon content (determined with the TOC) at different temperatures (400° C, 450° C, and 550° C), for different times (1 and 4 hours for 450° C and 550° C, 16 hours for 400° C), and using different masses (1 and 5 g dry weight). Heiri et al. (2001) reported that these variables lead to significant differences in the results. Ten LOI methods were compared, and the ten random samples were replicated twice for each of these ten methods. Before igniting the samples and to avoid potential interferences of carbonates, they were pretreated with hydrochloric acid 10 *M* until bubbling was ceased (Baird, 2005; Hernandez and Mitsch, 2007), oven-dried at 105° C for 24 hours, cooled to room temperature in a desiccator, weighed, and ignited as described above in a Fisher Scientific Isotemp Programmable muffle furnace (650-700 series). The mass left after ignition corresponds to the inorganic matter (minus carbonates) content. To estimate a sample's organic carbon content, 50% of the organic matter is assumed to be organic carbon (Dick and Gregorich, 2004; Schlesinger, 1997). The comparison of the ten LOI methods was carried out to determine which methods were the most accurate, precise, and replicable for organic matter determinations in wetland soils.

The most appropriate LOI method for wetland soils was then compared to the values obtained on the Carbon Analyer instrument (TOC). Twenty-nine randomly selected 2-cm increment wetland soil samples from Ohio and Costa Rica were analyzed for carbon content by means of LOI and TOC, following the steps described

above. Results were compared with the intention of determining the linear relation between organic matter determination (directly measured by LOI) and organic carbon content (measured with the TOC), on the one hand, and to further test the accuracy of LOI using TOC as a reference standard value for each of the samples analyzed.

## **2. 5. Statistical analyses**

Statistical analyses were performed with Minitab Statistical Software version 15 for Windows XP (Minitab, 2006). Student's *t*-test for two-sample independent analysis at 5% significant level was used to determine differences among wetland soil carbon pools and differences between loss on ignition methods (Fowler et al., 2003; Clarke and Cooke, 2004).

One-way analysis of variance (ANOVA) with Tukey HSD multiple comparison test and Bonferroni Post Hoc test was performed to detect differences among climates (temperate humid, tropical humid, and tropical dry), on the one hand, and among wetland types (riverine flow-through, slow-flow slough, and isolated forested), using SPSS version 15 for Windows XP (SPSS,2006).

To test accuracy and precision of loss on ignition methods, one-sample *t*-test with TOC reference value as hypothesized mean was performed. Relationship between organic matter and organic carbon was examined with Pearson product moment correlations (Clarke and Cooke, 2004; Hernandez and Mitsch, 2007).



## CHAPTER 3

### RESULTS

#### **3. 1. Carbon profile in wetland soils**

The organic carbon concentration of the studied wetland soils varied widely from 30 to 174 g C kg<sup>-1</sup> (Table 3). Assuming that 50% of organic matter is organic carbon, this means that the soil contained about 6 to 35 percent organic matter. These levels are much higher than those obtained for upland sites of the two wetlands in Ohio (Old Woman Creek and Gahanna Woods), where total C concentration was 7.1 and 16.5 g C kg<sup>-1</sup> respectively, in the top 35 cm of soil. This represented about 10% of the total C concentration of the adjacent wetlands. Inorganic carbon was low for every sample, around 0.2–1.0% of the total C, suggesting low carbonate levels in the soils and that nearly all C was organic.

The pattern in C distribution with depth was different among wetlands (Fig. 3). The C concentration of the two wetlands in Ohio tended to increase with depth in three of the four cores, reaching maximum concentrations at mid-depths (12-24 cm). One of these cores (core B, from Gahanna Wood's swamp forest) showed a continuous increase of carbon to a depth of 24-36 cm, indicating an important sustained C input into the soil. In Costa Rica, soil cores from the riverine wetland at Palo Verde showed an almost constant C concentration along its profile. The main

factors causing this uniform profile is likely to be the mixing of river sediments and disturbance of the soil by cattle and wildlife. The C content in soils from La Selva and EARTH University decreases with depth, except in core B from EARTH (dense peat site), where C levels are highest at 12-18 cm depth, below which it decreases. This core had the highest C pool of the Costa Rica sites, probably a result of the buildup of peat and the slow-flow hydrology in this slough. The profiles of the two cores from La Selva are similar, and their pattern of C distribution with depth is practically the same as EARTH's core A. This is probably due to similar hydrogeology and vegetation at each of the the three sites.

The greatest differences between cores within a wetland were found at Old Woman Creek and EARTH University (Fig. 3; Table 4). Their sites A had a pulsing hydrology (fluctuating water levels) and vegetation adapted to wet-dry periods, conditions very different to those found in sites B, where water was permanently or more frequently present and the only substantial vegetation found was floating plants adapted to deep water. This may account for differences in the C profiles at these two wetlands. The mean C pool in sites A and B was significantly different at Old Woman Creek ( $P < 0.01$ ), but not at EARTH. Differences at EARTH ( $P < 0.05$ ) were only obtained when comparing the 2-cm layers sample by sample (two sample paired t-test) instead of comparing the mean pool of the two cores (two sample t-test).

### **3. 2. Carbon pool in wetland soils**

The distribution of C per unit of volume and C concentration ( $\text{g C kg}^{-1}$ ) were similar (Fig. 3). In the 0 to 6 cm layer all the wetlands had similar amount of C per volume, even though Old Woman Creek (Ohio) and Palo Verde (Costa Rica) had lower carbon concentrations in this layer than the rest of the studied wetlands.

Gahanna Wood's (Ohio) C pool was the highest of the five wetlands in every depth increment. The C pool at Old Woman Creek was the second highest with depth, but it shows a continuous increase with each depth increment, while in the three wetlands in Costa Rica it decreased with depth.

The C pools at 24 cm for every wetland, and at 50 cm for EARTH and La Selva, are shown in Table 5. The two Ohio wetlands had the highest soil carbon pool at the 24 cm depth (Fig. 5). EARTH's and La Selva's cores were greater than 50 cm long, and the C pool to that depth was also compared. The EARTH's wetland soil (sluggish flow hydrology in a tropical rainforest) had a higher carbon pool than La Selva (in the middle of a mature tropical rainforest). Despite the longer core depth of these two tropical wetlands, the total soil carbon pool of Gahanna Woods (Ohio) was highest (Table 5), even though its cores were, on average, almost half long as those from La Selva and EARTH University.

When comparing soil C pools within wetlands, the variability was much greater at the Gahanna Woods (Ohio) isolated wetland (Fig. 6). The variation in the C pool was lowest for Palo Verde (riverine wetland; Costa Rica). EARTH University also had a low variation in its C pool, even though the C concentration of its cores (Fig. 3) was different. Core B, extracted in the peat site in this wetland, had lower soil bulk density than did site A, and that is why the C pools are similar.

Upland soil cores at Gahanna Woods and Old Woman Creek were taken to establish a reference C pool baseline for these two wetlands. The soil C concentrations in these wetlands (Table 3) were an order of magnitude higher than the carbon measured in the upland sites. The soil C pools of these wetlands were significantly different ( $P < 0.01$ ) than the pools of the uplands (Table 5). At Old Woman Creek, the wetland C pool was 14.3 kg C m<sup>-2</sup> to the 36-cm depth and the

upland had 4.5 kg C m<sup>-2</sup> in 35 cm; at Gahanna Woods, the carbon pool of the wetland was 21.0 kg C m<sup>-2</sup> at the 36-cm depth while 7.1 kg C m<sup>-2</sup> was stored to 35 cm of the nearby upland soil.

### **3. 3. The importance of climate on wetland soil's carbon pool**

In Costa Rica, two distinct climatic regions were included in this study: tropical humid climate of eastern Costa Rica and tropical dry climate of western Costa Rica. The average C pool in the top 24 cm of the temperate humid wetland soils was significantly higher than the other two tropical ones ( $P < 0.01$ , ANOVA; Fig. 7). No significant differences were found between soils of the two tropical wetlands, suggesting that the effect of temperature on carbon storage may be more important than precipitation. Our hypothesis that tropical wetlands had a greater pool than wetlands in temperate zones is rejected. There was a marked decrease in the C concentration (Fig. 3) and C pool (Fig. 4) with depth in the three tropical wetlands in Costa Rica, whereas C in the two temperate wetlands from Ohio had the tendency to increase with depth. The C pool of the upper 24 cm of wetland soil in Palo Verde, located in tropical dry Costa Rica, was the lowest of the three climatic regions (Fig. 6; Fig. 7).

### **3. 4. The effect of wetland type on carbon pool in wetland soils**

The five wetlands were grouped according to vegetation communities and hydrogeomorphology: Type 1 wetlands (Old Woman Creek and Palo Verde) were flow-through wetlands that are routinely flooded by streams or rivers; Type 2 wetlands (Gahanna Woods wetland and La Selva) were isolated with a forested canopy; and Type 3 wetland (EARTH University) was a slow-flowing slough, which

was in between the other two types (Table 1). The total C pool for the upper 24 cm of soil in each wetland type (Fig. 8) was highest in Type 2 (10.8 kg C m<sup>-2</sup>). Type 1 and 3 wetlands had similar C pools to this depth, 7.93 kg C m<sup>-2</sup> and 8.03 kg C m<sup>-2</sup>, respectively. When comparing the 2-cm layers, the mean soil carbon pool of Type 2 was significantly higher ( $P < 0.05$ , ANOVA), suggesting that the presence of trees as a source of carbon, coupled with a stagnant hydrology, are important for maximizing carbon storage in wetland soils. This finding supports the hypothesis that isolated forested wetlands have greater carbon pools than the rest of wetland types.

### **3. 5. Comparison of carbon analytical methods**

Analysis of the C concentration in wetland soils can vary considerably depending on the method used. While this study used a Carbon Analyzer (TOC) for soil analysis, many previous studies have used loss on ignition (LOI). Our study showed that the results obtained varied considerably depending on the LOI procedure followed (*i. e.*, time, temperature, and mass of soil ignited; Table 6). Among the times and temperatures tested (400°C, 16 h; 450°C, 1h; 450°C, 4 h; 550°C, 1 h; 550°C, 4 h), the ignition at 450°C for 4 hours was the method with the most accuracy, precision, and replicability compared to TOC measurements as a standard. Comparing the results obtained igniting 1 g of soil and 5 g of soil, the use of 1 g provided better results, although the differences were less obvious.

Analyzing the difference between methods at 95% confidence interval, all of them were different ( $P < 0.05$ ) with two exceptions: results after ignition at 450° C for 1 h was not significantly different to ignition at the same temperature for 4 h, and ignition at 550° C for 1 h did not differ significantly from ignition at 550° C for 4 h. This suggests that the time of ignition does not make as big of a difference as does

temperature. Comparing the use of 1 g of soil to 5 g, the results obtained are also significantly different ( $P < 0.05$ ).

The accuracy of each LOI method was tested using the % OC (percentage of organic carbon) obtained with the Carbon Analyzer as a reference value for each of the samples ignited in the muffle (Table 6). In more than half of the cases the results from LOI were significantly different ( $P < 0.05$ ) from the reference value. The method that provided fewer results that were significantly different from the reference value was 450°C for 4h, followed by 450°C for 1 h and 550°C for 1 h. The comparison between 1 and 5 g did not give sufficient information to consider one of them ideal, as both masses had similar accuracies.

To test the precision of the methods, the range and standard deviation of the % OC obtained in each case were analyzed. The method with smallest ranges was 450°C for 4h, and the one with the widest ranges was 550°C for 1 h. When comparing the mass of soil, the use of 1 g gave the smallest ranges. However, none of these ranges were significantly different. Another way to analyze variability is with the standard deviation of the mean. Standard deviations were lower for 450°C and 4h, and 1 g of soil, indicating that they give the most consistent, less variable results. Using a confidence interval of 90%, the deviations of 450°C and 4h were significantly different than all other methods. The mass, however, was not. Ignition at 450°C for 4 h provides significantly less variable results than any other method.

### **3. 6. Predicting carbon content in wetlands soils with Loss On Ignition**

Results from loss on ignition (LOI) and the carbon analyzer (TOC) gave significantly different results ( $P < 0.05$ ; Fig. 9). The greatest differences between methods occur when the organic carbon content of the soil sample was low (about

under 3.5%); the greater the % OC of the sample, the more similar the LOI results were to the TOC values, *i. e.*, the greater the accuracy of LOI. Greater error bars of LOI measurements shown in Fig. 9 in the low % OC levels indicate that LOI is less accurate in this range of carbon content.

Percent of organic carbon (OC) of the samples by LOI was estimated by assuming that 50% of the organic matter contained in the soil sample was organic carbon. A Pearson correlation test of TOC vs. LOI gave  $r = 0.526$  (Fig. 10), indicating that while LOI was not accurate in predicting carbon with this 50% assumption, it was reasonably useful in predicting organic carbon content using this relationship, particularly when the organic carbon in the soil is greater than ~ 3.5%:

$$Y = 0.433 * X - 1.734$$

where,

Y = organic carbon in the soil, and

X = the percent organic matter in the soil as measured by loss on ignition.

The regression in Fig. 10 is different from the 2:1 relation of organic matter (OM) to organic carbon (50% assumption). A slope of 0.5 and a y-intercept of (0,0) would have indicated a perfect fit of the two methods.

## CHAPTER 4

### DISCUSSION

#### **4. 1. Carbon in upland soils and wetlands**

Upland soil sampling sites in Old Woman Creek and Gahanna Woods were located in a forested area adjacent to the wetland. Forest soils have relatively high organic matter inputs from the trees and shrubs. This type of organic matter has high lignin and cellulose content, and it decomposes slowly. Despite these significant organic inputs, upland soils in both sites had low soil C concentrations ( $\text{g C kg}^{-1}$ ) compared to their adjacent wetland (about 10 times less). The climatic conditions of this region (temperate humid Ohio with low temperatures in winter) should allow soil organic matter accumulation. Since there is no reason to believe that C storage in these upland sites was being restrained by some external factors, these significant differences between uplands and wetlands indicate that wetland soils, on areal basis, can indeed function as more important C sinks than do upland soils.

Jiménez et al. (in press a, b) measured the total soil C pool to a 50-cm depth in several upland ecosystems in the dry and humid regions of Costa Rica. The gallery forests they sampled in both regions are comparable to the uplands adjacent to



all of the wetlands in this study. Their results indicate that at 30-40 cm deep the average C pool for this type of forest in Costa Rica ranged from 5.5 to 16.3 kg C m<sup>-2</sup>. The pool of our upland sites in Ohio ranged from 4.5 to 7.1 kg C m<sup>-2</sup>, at 35 cm deep. In tropical dry Costa Rica the upland pool of their gallery forest at 25 cm was 4.6 kg C m<sup>-2</sup>, whereas Palo Verde's wetland had 6.8 kg C m<sup>-2</sup> at 24 cm deep. Jiménez et al. (in press b) found that the gallery forest accumulated 21.0 kg C m<sup>-2</sup> at 50 cm. Our EARTH wetland pool at that same depth was 14.8 kg C m<sup>-2</sup>. The C pool of this wetland is much lower than their upland pool, even though the C concentration (g C kg<sup>-1</sup>) of our wetland site was high. The reason for this difference in the C pools is probably due to the presence of low density peat in the wetland. The comparison of the pools in the dry region shows that the wetland has greater pool than the upland at comparable depths, even though this wetland is likely degrading its C content due to erosion by the river, disturbance by cattle and wildlife, and marked seasonally dry seasons.

#### **4. 2. Effect of climate on wetland's soil carbon**

The results showed that wetlands located in temperate climates have significantly greater C pool ( $P < 0.01$ ) than tropical wetlands. Comparing C profiles, important differences between wetlands in Ohio and Costa Rica were observed: soil C concentration in the three tropical wetlands had the tendency to decrease with depth, whereas in the temperate region the soil C in three of the four cores extracted (both cores from Gahanna Wood's and core B from Old Woman Creek) increased with depth until 18-24 cm, after which it started decreasing. Middleton (2008) studied temperate baldcypress swamps (a forested wetland type) and reported that their soils tended to have greater organic C concentrations at mid-depths. These findings suggest

that temperate wetlands are accumulating C over time, as these deeper layers can be decades old. Bernal and Mitsch (2008) reported that the sediment at 16-18 cm depth in Old Woman Creek was ~40 years old.

The rapid decrease of C content with depth in the tropical rain forest wetland site at La Selva indicates that very little of the C that is being introduced in the soil is stored there. This is typical of a tropical rain forest where organic material and nutrients do not accumulate in the soils but are rapidly used by the biotic systems (Odum and Pigeon, 1970). La Selva is one of the five wetlands with greater C concentration in the surface layer (0-6 cm), indicating a very important introduction of organic matter into the soil system (Fig. 3). The fact that these high carbon levels are not maintained throughout the core and that about half of it is lost by 12-18 cm deep indicates that undecomposed organic materials and plant remnants in the upper wetland soil layers are readily available and labile C sources do not accumulate significantly in the soil as would older or more recalcitrant soil organic matter or humus. La Selva wetland is losing its soil C much faster than similar wetlands in temperate climates (*e. g.*, Gahanna Woods), possibly due to the higher temperatures.

The wetlands in Costa Rica represented two climates (tropical, humid and dry). The soil C pool appeared to be lower in the tropical dry region (Palo Verde), but no significant differences (ANOVA) were obtained when comparing the C pools of the two climatic regions, indicating that even though a humid climate might affect C storage, temperature may be a stronger driving force in determining carbon decomposition. The highest pool was found in the temperate humid zone, and it was significantly different from the two tropical ones because, individually, Old Woman Creek and Gahanna Woods also had the greatest carbon pools (both in the upper 24 cm and in the entire core). Total C pools of Gahanna Woods and Old Woman Creek

did not change much when measured at 24 cm and 34 cm (the entire core depth). In the three tropical wetlands, however, differences between C pools are much greater when comparing the top 24 cm to the entire cores (to a depth of 54-60 cm in EARTH and La Selva). At 24 cm, these three tropical wetlands have practically the same C pool, and that is probably why no statistical differences were found between them. This normalization at 24 cm deep was needed in order to be able to pair wetlands according to climate and compare their pools.

#### **4. 3. The effect of wetland type on carbon pools**

The significantly greater C pools of the Wetland Type 2 in both temperate and tropical regions suggest that the presence of trees and the hydrogeomorphology of the wetland are important factors to consider when studying C accumulation in wetland soils. The lower C pools of the Wetland Type 1 (riverine wetlands) were probably influenced by soil erosion, inflow of inorganic sediments from the river, other inflows such as seiches from Lake Erie into Old Woman Creek wetland, and the fluctuation of the water level. EARTH University's wetland (tropical slow flowing slough, Type 3) probably should have had a much greater pool than wetlands of Type 1 (flow-through wetlands, Old Woman Creek and Palo Verde), since it does not have significant water erosion and it is located in a developing tropical rainforest, where organic matter inputs from vegetation are high. Despite these advantages toward C build-up in its soil, EARTH's wetland had a pool similar to the C pool of Type 1 wetlands for the same depth. Unfortunately, we only had one Type 3 wetland (EARTH University) and it was located in a tropical climate where soil C decomposition is probably higher than in temperate Ohio. The effect of temperature in soil C accumulation may be more important than the hydrogeomorphology or vegetation cover in the wetland.

The high C pool of Gahanna Woods might have influenced the mean of Type 2, creating a statistical significance that might not be true otherwise. If we only consider the soil C pool of the three wetland types in Costa Rica (Table 5), the wetland soil of EARTH University's wetland slough (slow-flowing conditions) has the greatest C stock of that tropical subset.

#### **4. 4. Pulsing hydrology and soil carbon pools**

Two of the key factors that enhance carbon accumulation in wetland soils are the anaerobic conditions produced by the presence of standing water and the high productivity of wetland ecosystems (due to the standing vegetation in and around the pool of water and the net accumulation of nutrients, sediments, and organic matter coming from the vegetation cover and/or the associated body of water, *e. g.*, a river). The type of vegetation cover is determined by the presence of water (time and duration of the flood). Permanently or frequently flooded sites usually have floating plants (such as *Nelumbo lutea* in Old Woman Creek or *Eichhornia crassipes* in Palo Verde; Table 1), if any, whereas sites that are flooded only part of the year and experience wet-dry periods tend to have a dense community of macrophytes adapted to the presence of water and hydric soils (*e. g.*, *Typha* spp. in Palo Verde, Old Woman Creek, and Gahanna Woods; Table 1). The continuous presence of water in permanently flooded sites restrains organic matter decomposition due to maintenance of anaerobiosis, whereas in the sites with wet-dry periods the carbon that is retained in the soil during the flood is oxidized and lost back to the atmosphere as CO<sub>2</sub> during the dry period. Consequently, permanently flooded soils provide more suitable conditions for C accumulation, whereas intermittently flooded sites usually have greater carbon inputs.

Differences between carbon profiles within the wetland were more apparent in Old Woman Creek and EARTH University, possibly because of this permanent-pulsing effect. Carbon pools were generally greater in the permanently flooded sites than in the pulsing hydrology sites (Table 4). This finding suggests that the presence of continuous anaerobic conditions is more powerful in enhancing carbon storage in wetland soils than the presence of greater organic inputs into the soil.

Natural ecosystems are complex and it is important to consider all the factors involved in the process of carbon pool formation. Wetlands are very productive environments and even though anaerobic conditions seem to be a stronger factor determining soil carbon storage, we cannot assume that the presence of anaerobiosis by itself would enhance the carbon pool. It is rather the combination of both anaerobic conditions in the site and ecosystem productivity that makes permanently flooded wetland soils highly organic. Lake soils are also anaerobic but, compared to wetlands, are far from being as significant as carbon accumulators, unless they are highly eutrophic.

#### **4. 5. The combined effect of climate and wetland type**

The studied factors determining the soil C pool of these wetlands are climate (higher temperatures enhance decomposition of organic matter and reduction of the carbon pool), wetland type, defined by the vegetation community (significant organic matter inputs from forest canopy enhances the carbon pool), and the hydrogeomorphic settings (slowly flowing or stagnant wetlands store more carbon in the soil than do riverine wetlands). These factors made a significant difference in the wetland soils C pool. It is probably the combination of these factors that made soil carbon pool per depth unit of Gahanna Woods (a temperate, forested, isolated wetland) the highest

of all the studied wetlands, and Palo Verde (a tropical flow-through wetland) the lowest. From these results, one could think that the accumulation of organic matter and the soil C pool are determined by the decomposition rate rather than by the rate that it is produced, since decomposition must be low in sites where temperature is not high, organic matter is more recalcitrant, and hydrological erosion is low. However, no decomposition or production rates were measured in this study and we cannot be sure of the effect that climate, hydrology, or vegetation community have on both parameters; we only know the final C accumulation in the soil, and it was greater in the wetland that was in a region with cold winters, important organic inputs from woody plant species, and little or no fluvial erosion.

In Costa Rica EARTH University had the greatest C pool. It may just be consequence of the high organic matter content of its peat site (core B). Peat is a very high organic matter pool, even though its soil carbon pool is not as significant due to its low bulk density. We did not have a peat site in Ohio, so we cannot objectively estimate the effect of that type of C stock. Leaving aside this peat site in Costa Rica, La Selva (the tropical forested wetland) would have been the greatest soil C pool of the tropical wetlands studied, and Costa Rica would have soil carbon pools parallel to those found in Ohio.

#### **4. 6. Sampling method and experiment design**

The lack of a standard methodology for sampling and analysis of wetlands soils makes it harder to find the ideal procedure and sometimes more difficult to compare with other studies. Wetlands are complex ecosystems and replicability of the sites cannot guarantee that the results obtained in both will be the same. Pairing samples or wetlands into groups to obtain two or more samples or sites per group

(climate or wetland type) is a pseudo-replication. This, however, might be best approach to get a broad understanding of processes going on in the ecosystems, and was necessary because of limited time and resources to investigate more independent wetlands in this international research.

Grossman and Reinsch (2002) reported that the most suitable soil sampler for wet soils or soils under water is the one we used, a piston that creates a vacuum to retain the soil sample in the tube. They and others (Kemp et al., 1971; Reinhardt and Cole, 2000) suggest that in order to obtain undisturbed sediment cores, the diameter of the sampler should be from 5 to 10 cm, to avoid compaction and distortion of the core. Our sediment sampler has a 7-cm diameter, and its clear polycarbonate core barrel made compaction of the core very easy to identify by comparing the surface level of the soil inside and outside the barrel. All these indicate that our sediment sampler is appropriate for soil sampling in wetlands.

The sampling method determines the accuracy of the volume of soil extracted, and therefore bulk density calculations which, in the end, translates into the accuracy and reliability of the soil C pool estimation of the wetland. Wetlands soils are, for the most part, rich in organic matter and water-saturated, two factors that reduce considerably the bulk density of the soil (compared to upland soils). Mineral upland soils typically range from 1.0 to 1.8 g cm<sup>-3</sup> (Hartel, 2005), but wetland soil bulk densities are often much lower. Collins and Kuehl (2001) stated that on a dry weight basis, bulk density of muck and peat in wetlands ranges from 0.1 to 0.2 g cm<sup>-3</sup>; Anderson and Mitsch (2006) obtained bulk densities of ~ 0.5 g cm<sup>-3</sup> in surface soils of created temperate flow-through wetlands in central Ohio; Reinhardt and Cole (2000) found bulk density ranging from 0.45 to 1.05 g cm<sup>-3</sup> in freshwater wetland soils; Trettin and Jurgensen (2003) studied wetland forests soils and obtained values of 0.1

to  $1.05 \text{ g cm}^{-3}$  for their bulk density. Our results fall in the range of these reported values. For that reason, and given the suitability of our sediment sampler, we have no reason to believe that our sampling methodology is biased nor underestimating bulk density of the extracted soil cores, and we can consider our results reliable.

All samples were dried at  $105^\circ \text{ C}$  prior to analysis to determine dry weight and bulk density. The rationale behind this procedure was to use a consistent method to be able to compare between all the samples taken. However, the combustion at  $105^\circ \text{ C}$  may be too high for the peat samples. Peat is mostly plant material, and to determine dry weight of plants,  $65^\circ \text{ C}$  is conventionally used (Collins and Kuehl, 2001; Grossman and Reinsch, 2002). By drying peat samples at  $105^\circ \text{ C}$  we may have caused an error that in the end translates to lower carbon content and dry weight estimates per unit volume. More research is needed to assess whether combustion temperature would have made a difference in the total C pool of the highly organic sites studied.

The 2:1 conversion factor between organic carbon and organic matter (*i. e.*, 50% of the organic matter is organic carbon) might also be misleading. The ratio of organic carbon to organic matter is known to vary with many factors, such as organic matter type or soil type (Collins and Kuehl, 2001; Cambardella et al., 2001). Some other conversion factors have been also reported in literature, such as the Van Bemmelen factor (Collins and Kuehl, 2001), which assumes that 58% of the organic matter is organic carbon or the ratio of Glucose to organic matter, which assumes 40% of organic matter is carbon. The ratio 2:1 in this study was used as an approximation, and it was applied to all the samples studied for loss on ignition (LOI). Hence, the selection of the most suitable LOI method for wetland soils carried out in this study is not compromised; only the regression equation between LOI and TOC (Carbon Analyzer instrument) should be applied carefully remembering that it was



based on a 50% factor. Results can, however, be corrected in future comparisons if another factor (such as the Van Bemmelen factor) is used.

#### **4. 7. Loss on ignition**

The selection of the most appropriate loss on ignition (LOI) method is a complicated process because the three variables analyzed (time, temperature, and mass) are closely interconnected. That is probably why no obvious results were obtained for the mass of dry soil to be used. Previous studies report the importance of mass in this type of carbon analysis (Cambardella et al., 2001), but in our study it seemed like its importance was masked by the effect of other more powerful variables, such as temperature. For that reason, bias and mistakes that temperature causes in the result will be translated to the other two variables. Since LOI's accuracy was lower at low organic carbon percentage ranges in the soil, the key factor determining the accuracy of the result was the carbon content of the original soil sample introduced in the furnace in every single method. Perhaps it would have been better to perform more tests with the high organic matter content samples, as they seemed to provide more accurate results, so the selection of the best LOI method would have been dependent only on the effect of the three variables, *i. e.*, temperature, time, and mass.

The estimation of organic carbon in LOI was based on a 2:1 ratio of organic matter (OM) to organic carbon (OC). The results suggest that this estimation was not completely accurate (Fig. 10). The linear equation from the regression was calculated in order to get a better conversion between OM and OC, applicable only when combusting 1 g (dry weight) of soil at 450° C for 4 hours.

Methodologically, even though pre-treatment of the samples was needed to account for possible carbonates in the soil, it might have biased the results. The residue that the dry acid might have left on the dried sample after the pretreatment is likely to be insignificant in large samples (5 or 10 g), but could potentially have a greater effect when using low amounts of soil (1 g or less). Despite this possibility, the use of 1 g was still the best option.

The comparison between TOC and LOI gave results that were strong enough to draw some conclusions, but there were differences between the methods. To improve the significance of the results, it would be good to analyze more samples to get more data points in the comparison. Given the variability of the LOI results, these methods should always be used with a number of sample replicates large enough to reduce errors and/or bias. Perhaps a confidence interval of 95% is too high for this analysis; 90% or 85% would probably bring out greater differences, even though the statistical power would be reduced. This selected method seems to be satisfactory enough for carbon determination in wetland soils, but it is clearly not the best option (compared to the TOC, a method more complicated and expensive but more accurate).

## CHAPTER 5

### CONCLUSIONS

1. Wetlands accumulate significant amounts of carbon in their soils compared to adjacent upland sites. This accumulation is consequence of many factors, one of the most important being the anaerobic conditions produced by the presence of water.
2. Soil carbon pools in the wetlands located in temperate climates were significantly greater than similar wetlands located in tropical regions, probably due to greater temperatures in tropics. No significant differences were found when comparing wetland carbon pools in tropical humid and tropical dry climates.
3. The wetland type, defined in this study by vegetation community and hydrogeomorphology, is key in developing a soil carbon pool. Isolated forested wetlands had greater soil carbon content than non-forested or flow-through wetlands. The combination of these two factors with the climatic variable indicated that, in this study, forested isolated wetlands in temperate climates had the optimum conditions for carbon accumulation in wetland soils.
4. Significant differences in carbon pools at different sites within the riverine wetland in northern Ohio (Old Woman Creek) indicate that frequency of flooding

is an important factor to take into account in wetland carbon budgets. Further research on this topic is needed.

5. Estimates of carbon and organic content in wetland soils varied widely, depending on the loss on ignition procedure followed. Compared to a Carbon Analyzer instrument, loss on ignition was inaccurate at low carbon content ranges (below 3.5%) in wetland soils.
6. With the appropriate calibration to convert organic matter content to organic carbon, the ignition of wetland soil samples (1 g, dry weight) at 450° C for 4 hours provides results comparable to those obtained using a Carbon Analyzer.

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APPENDIX A

TABLES

Wetland	Location	Climatic Region	Wetland Type	Soil	Core	Hydrology	Dominant vegetation
<b>GW*</b>	Ohio	Temperate	Isolated,	Pm**	A	Deep water	<i>Typha angustifolia</i> (cattail)
		Humid	forested		B	Shallow water	Forested swamp
<b>OWC</b>	Ohio	Temperate	Riverine	Aa; Mr	A	Wetland Edge	<i>Typha angustifolia</i> (cattail)
		Humid	flow-through		B	Deep water	<i>Nelumbo lutea</i> (water lotus)
<b>LS</b>	Costa Rica	Tropical	Isolated,	Alluvial;	A	Wetland Edge	<i>Spathiphyllum friedrichsthalian</i> (lily)
		Humid	forested	hydric	B	Shallow	Forested swamp, grasses
<b>EA</b>	Costa Rica	Tropical	Slow-flowing	Mucky	A	Wetland Edge	<i>Raphia taedigera</i> (swamp palm)
		Humid	slough	peat	B	Deep Water	<i>Spathiphyllum</i> spp., understory
<b>PV</b>	Costa Rica	Tropical	Riverine	Alluvial,	A	Seasonally Flooded	<i>Typha domingensis</i> (cattail)
		Dry	flow-through	hydric	B	Continuously Flooded	<i>Eichhornia crassipes</i> (water hyacinth)

\*GW, Gahanna Woods; OWC, Old Woman Creek; LS, La Selva; EA, EARTH University; PV, Palo Verde.

\*\*Pm, Pewamo (silty clay loam); Aa, Adrian muck (outwash plains, former bogs); Mr, Miner (silty clay loam).

Table 1. Description of the five wetlands in Ohio and Costa Rica used in this study.

<b>temperature (°C)</b>	<b>time (h)</b>	<b>mass (g)</b>	<b>reference</b>
400	16		Nelson and Sommers, 1996; Collins and Kuehl, 2001
450	4	10	Cambardela et al., 2001
550	4		Heiri et al., 2001
550	1		Anderson and Mitsch, 2006; Hernandez and Mitsch, 2007
550	3	5	Nahlík and Mitsch, 2008

Table 2. Loss on ignition procedures reported in literature.

Climatic Region	Wetland	Core	Total Carbon (g kg <sup>-1</sup> )	Inorganic Carbon (g kg <sup>-1</sup> )	Organic Carbon (g kg <sup>-1</sup> )	OC/TC ratio
TmH*	GW**	A	176.7 ± 6.0 (18)	2.2 ± 0.4 (18)	174.6 ± 6.5 (18)	0.99
		B	144.2 ± 10.4 (17)	2.5 ± 0.8 (17)	141.8 ± 10.4 (17)	0.99
		mean	<b>160.9 ± 6.4 (35)</b>	<b>2.3 ± 0.4 (35)</b>	<b>158.6 ± 6.6 (35)</b>	<b>0.99</b>
OWC	UP***	UP	16.5 ± 6.3 (7)	1.0 ± 0.1 (7)	15.5 ± 6.3 (7)	0.94
		A	91.1 ± 4.7 (19)	7.1 ± 1.4 (19)	84.0 ± 5.7 (19)	0.92
		B	28.8 ± 2.2 (17)	7.9 ± 1.1 (17)	20.9 ± 1.5 (17)	0.73
	mean	mean	<b>61.7 ± 5.9 (36)</b>	<b>7.5 ± 0.9 (36)</b>	<b>54.2 ± 6.1 (36)</b>	<b>0.82</b>
		UP	7.1 ± 2.9 (7)	1.1 ± 0.0 (7)	5.9 ± 2.9 (7)	0.84
		A	36.4 ± 7.3 (28)	5.4 ± 0.9 (28)	30.9 ± 6.8 (28)	0.85
TrH	LS	B	40.9 ± 6.7 (30)	9.9 ± 0.3 (30)	31.0 ± 6.7 (30)	0.76
		mean	<b>38.7 ± 4.9 (58)</b>	<b>7.7 ± 0.5 (58)</b>	<b>31.0 ± 4.7 (58)</b>	<b>0.80</b>
		A	69.3 ± 9.2 (27)	9.9 ± 0.4 (27)	59.4 ± 9.1 (27)	0.86
TrD	PV	B	113.3 ± 13.3 (28)	10.6 ± 0.2 (28)	102.8 ± 13.1 (28)	0.91
		mean	<b>91.7 ± 8.6 (55)</b>	<b>10.2 ± 0.2 (55)</b>	<b>81.5 ± 8.5 (55)</b>	<b>0.88</b>
		A	31.2 ± 3.5 (12)	2.2 ± 0.2 (12)	29.1 ± 3.7 (12)	0.93
mean	B	38.5 ± 2.1 (13)	2.3 ± 0.2 (13)	36.2 ± 2.2 (13)	0.94	
	mean	<b>35.0 ± 2.1 (25)</b>	<b>2.2 ± 0.2 (25)</b>	<b>32.8 ± 2.2 (25)</b>	<b>0.94</b>	

\*TmH, Temperate Humid; TrH, Tropical Humid; TrD, Tropical Dry.

\*\*GW, Gahanna Woods; OWC, Old Woman Creek; LS, La Selva; EA, EARTH University; PV, Palo Verde.

\*\*\*UP, Upland.

Table 3. Mean concentrations of soil carbon (total, inorganic, and organic), ave. ± std. err. (n), and ratio of organic carbon (OC) over total carbon (TC) in twelve cores collected in five wetlands (2-cm layers) and two upland sites (5-cm layers) in three climatic regions.

Wetland	Mean Carbon Pool (kgC m <sup>-2</sup> ) <sup>†</sup>		Total Carbon Pool (kgC m <sup>-2</sup> )	
	Pulsing (Core A)	Permanent / Frequent (Core B)	Pulsing (Core A)	Permanent / Frequent (Core B)
<b>Old Woman Creek</b>	0.47 ± 0.04 (17) <sup>a</sup>	1.02 ± 0.06 (19) <sup>b</sup>	14.57	18.48
<b>EARTH University</b>	0.53 ± 0.03 (27)*	0.59 ± 0.03 (28)*	14.20	16.35

<sup>†</sup> Means in the same row followed by different letter are significantly different ( $P < 0.01$ ).

\* Not significantly different in a two sample t-test ( $P > 0.05$ ); significantly different in a two sample paired t-test ( $P < 0.05$ ).

Table 4. Mean carbon pools (ave. ± std. err. (n)) and total carbon pools of cores (2-cm layers) extracted in two wetlands in sites with distinct flooding frequency.

depth range (cm)	Gahanna Woods	Old Woman Creek	La Selva	EARTH University	Palo Verde
0--6	2.05	2.21	2.47	2.21	2.07
6--12	4.05	2.23	1.94	2.06	2.17
12--18	4.30	2.09	1.26	1.82	1.22
18--24	4.33	2.51	1.23	1.94	1.36
24--30	3.24	2.47	0.67	1.96	
30--36	3.03	2.77	0.43	1.67	
36--42			0.37	1.46	
42--48			0.37	1.09	
48--54			0.37	1.06	
54--60			0.79		
<b>24-cm C pool</b>	<b>14.73</b>	<b>9.03</b>	<b>6.89</b>	<b>8.03</b>	<b>6.82</b>
<b>50-cm C pool</b>	-	-	<b>8.91</b>	<b>14.75</b>	-
<b>Core Total C pool</b>	<b>21.01</b>	<b>14.26</b>	<b>9.89</b>	<b>15.28</b>	<b>6.82</b>
25-cm Upland C pool*	6.41	3.87	-	-	-
35-cm Upland C pool**	7.05	4.49	-	-	-

\*Pool at 25 cm depth in the upland is comparable to the pool at 24 cm depth in the wetland.

\*\*Pool at 35 cm depth in the upland (entire core) is comparable to the pool at 36 cm depth in the wetland (entire core).

Table 5. Soil carbon ( $\text{kg C m}^{-2}$ ) with depth in the five studied wetlands, and total soil carbon pool ( $\text{kg C m}^{-2}$ ) for 24 cm, 50 cm, and entire depth of extracted wetland cores. Upland soil carbon pools for 25 cm and for the entire soil cores (35 cm) extracted in Ohio are also indicated.

TOC % OC	LOI mass (g)		LOI calculated % OC				
	400°16h	450°1h	450°1h	450°4h	550°1h	550°4h	
0.7	1	5.6	4.6	3.8	9.1	7.4	
	5	4.8	5.2	6.9	6.0	8.1	
9.1	1	10.1	10.4	9.8	11.5	13.1	
	5	13.0	10.3	8.1	10.8	11.5	
0.8	1	9.6	5.0	5.1	10.8	9.5	
	5	6.3	5.4	6.1	8.7	9.4	
2.1	1	6.8	6.4	4.4	7.5	6.7	
	5	4.4	3.2	5.4	5.9	7.6	
3.8	1	4.5	3.5	4.1	4.7	5.4	
	5	4.2	2.8	3.8	5.0	4.9	
10.8	1	10.0	9.9	10.2	11.6	11.7	
	5	9.5	10.2	9.8	11.5	10.8	
3.2	1	5.9	5.3	5.9	6.6	7.2	
	5	5.2	5.3	5.4	6.2	7.1	
3.9	1	7.3	6.0	5.1	9.8	8.7	
	5	8.1	9.2	5.8	7.4	8.1	
3.7	1	6.3	5.9	6.2	9.4	11.1	
	5	5.2	3.7	6.0	9.7	10.0	

Table 6. Comparison of percentage OC (organic carbon) obtained with ten different loss on ignition (LOI) procedures, and reference Carbon Analyzer (TOC) value for nine random wetland soil samples from Ohio and Costa Rica.



APPENDIX B  
FIGURES

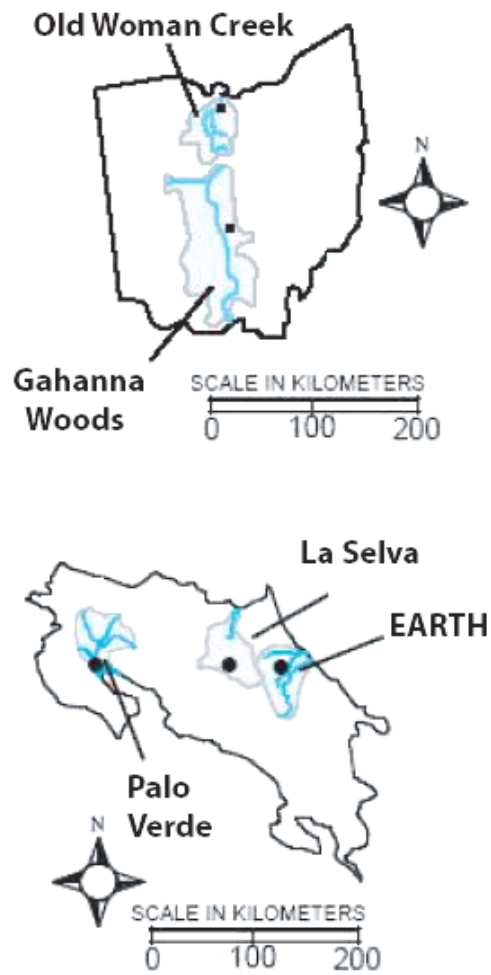


Figure 1. Location of the studied wetlands in Ohio (upper map) and Costa Rica (lower map).

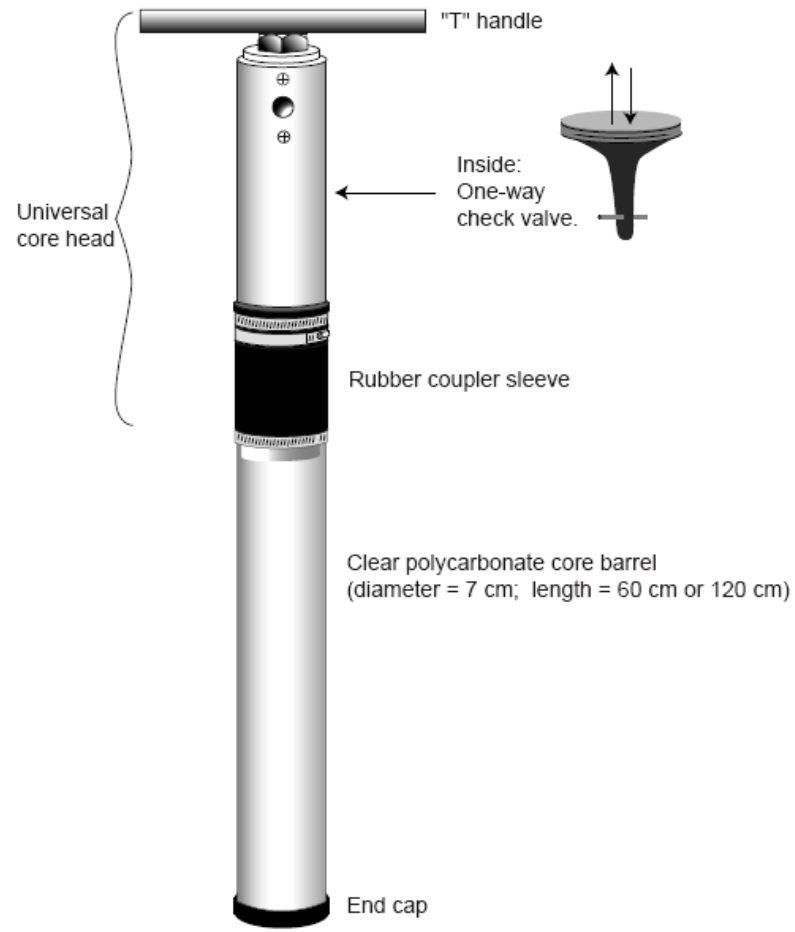


Figure 2. Sediment sampler for wetland soils used in this study. Drawing by Anne Mischo.

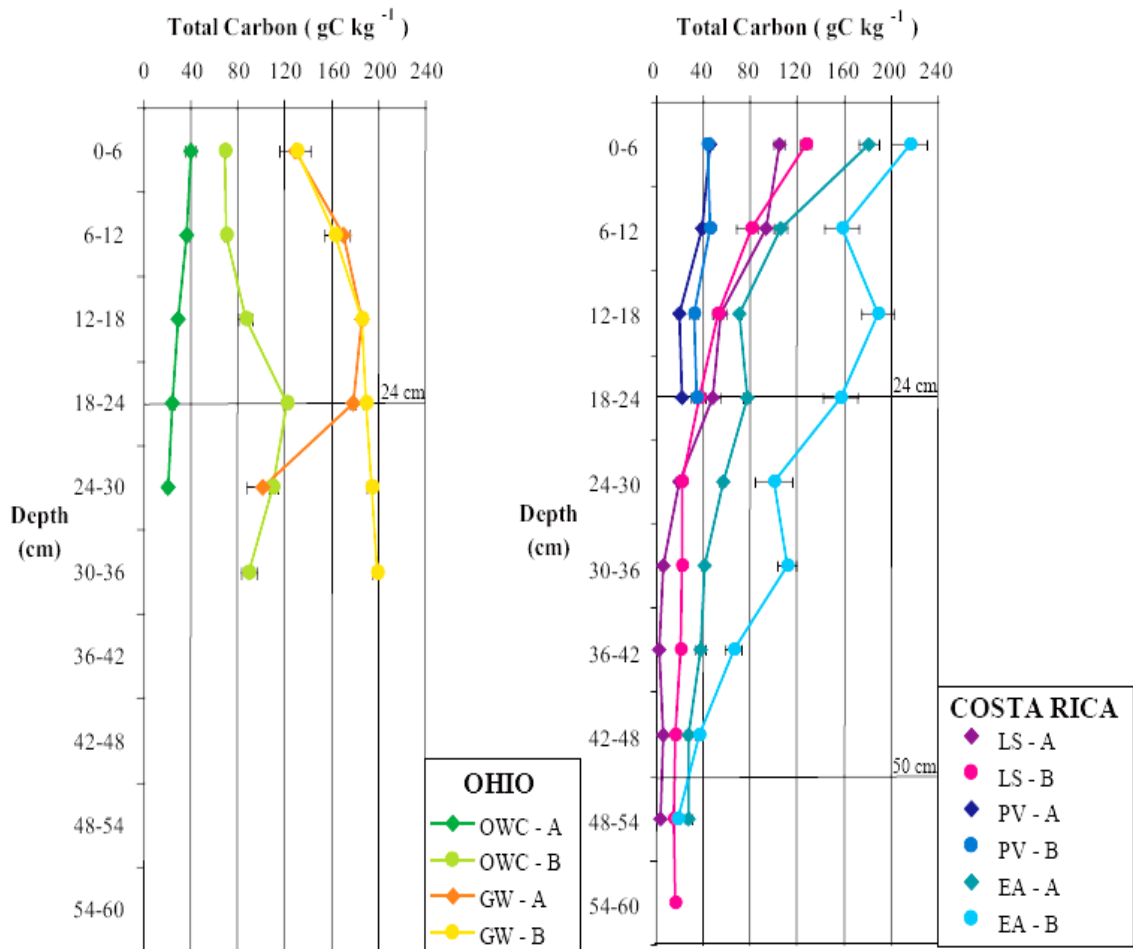


Figure 3. Distribution of total soil carbon with depth in five wetlands, two cores per wetland (A and B). Each point in the graph is the mean of that depth range ( $n = 3$ ); bars represent standard errors. Left: profile for the two wetlands in Ohio – OWC (Old Woman Creek) and GW (Gahanna Woods). Right: profile for the three wetlands on Costa Rica – LS (La Selva), PV (Palo Verde), and EA (EARTH University).

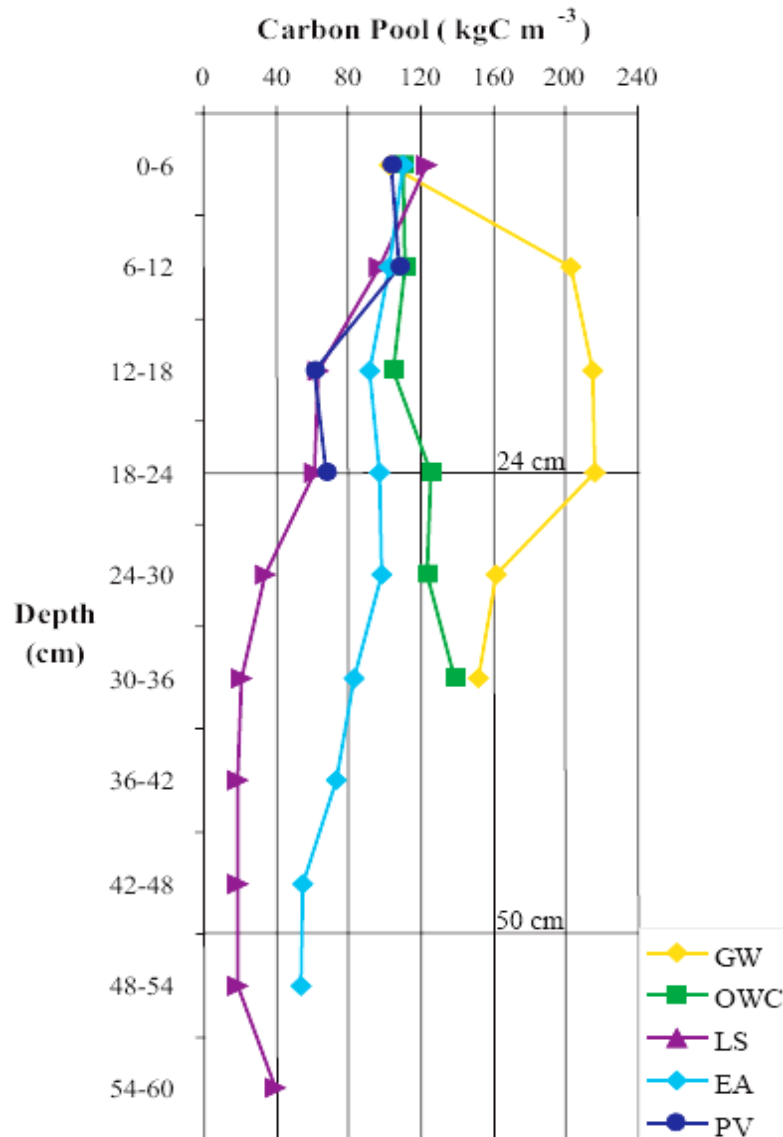


Figure 4. Distribution of total carbon pool with depth in the soil of five wetlands, OWC (Old Woman Creek), GW (Gahanna Woods), LS (La Selva), PV (Palo Verde), and EA (EARTH University). Each point in the graph is the mean of the core(s) at that depth range ( $n = 6$  in every point except the last one for OWC, GW, and LS, where  $n = 3$ ).

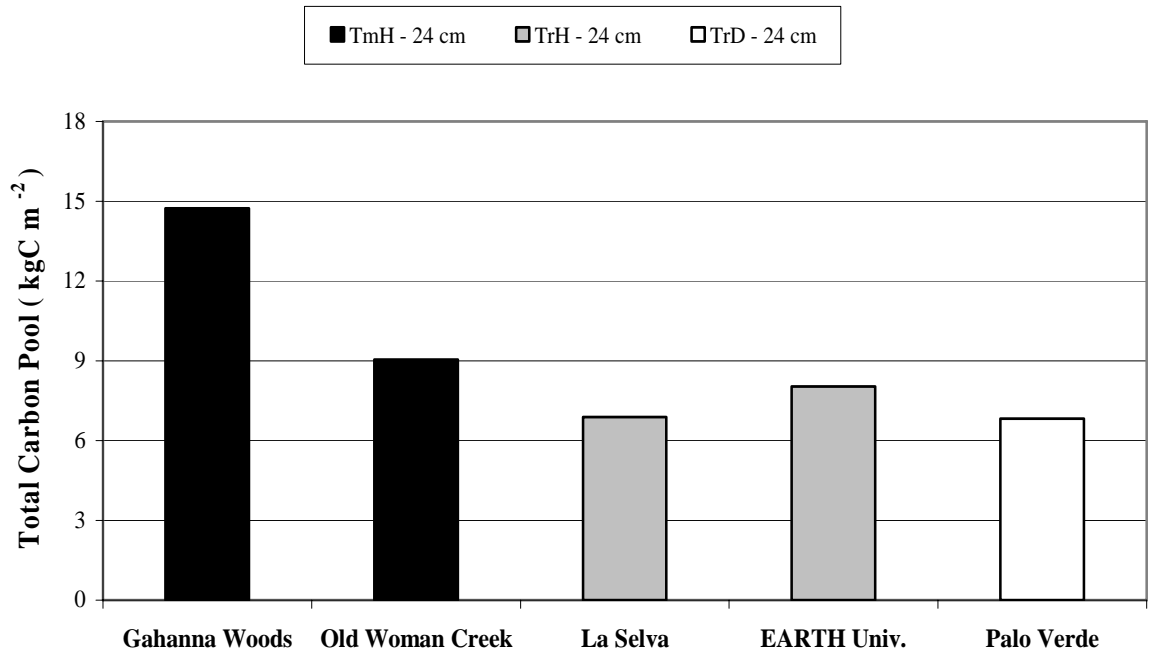


Figure 5. Total soil carbon pool for the top 24 cm of soil in five wetlands located in the temperate humid (TmH), tropical humid (TrH), and Tropical dry (TrD) regions.

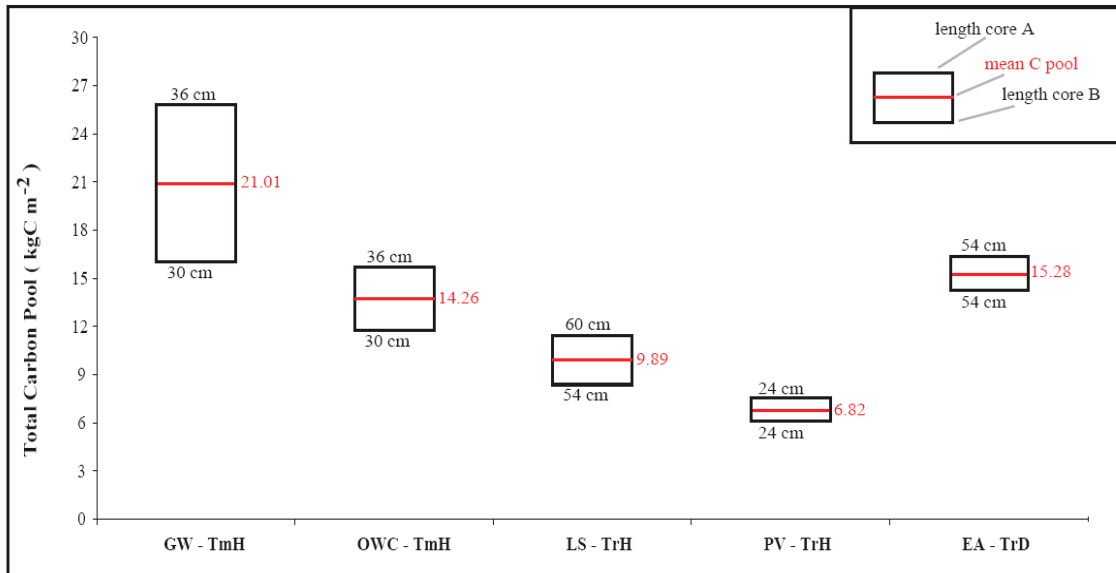


Figure 6. Total soil carbon pool of five wetlands located in the temperate humid (TmH), tropical humid (TrH), and tropical dry (TrD) climatic regions. Each box represents the total pool of each of the cores extracted in the wetland (top line for the longest core, bottom line for the shortest one). Red line in the middle of each box is the mean pool of the wetland. OWC, Old Woman Creek; GW, Gahanna Woods; LS, La Selva; PV, Palo Verde; and EA, EARTH University.

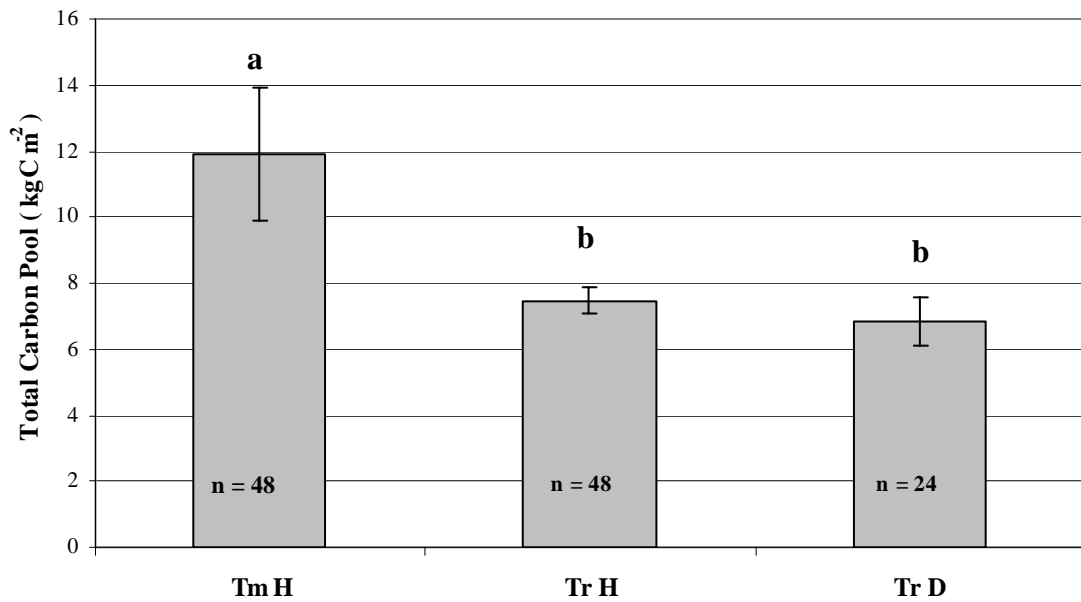
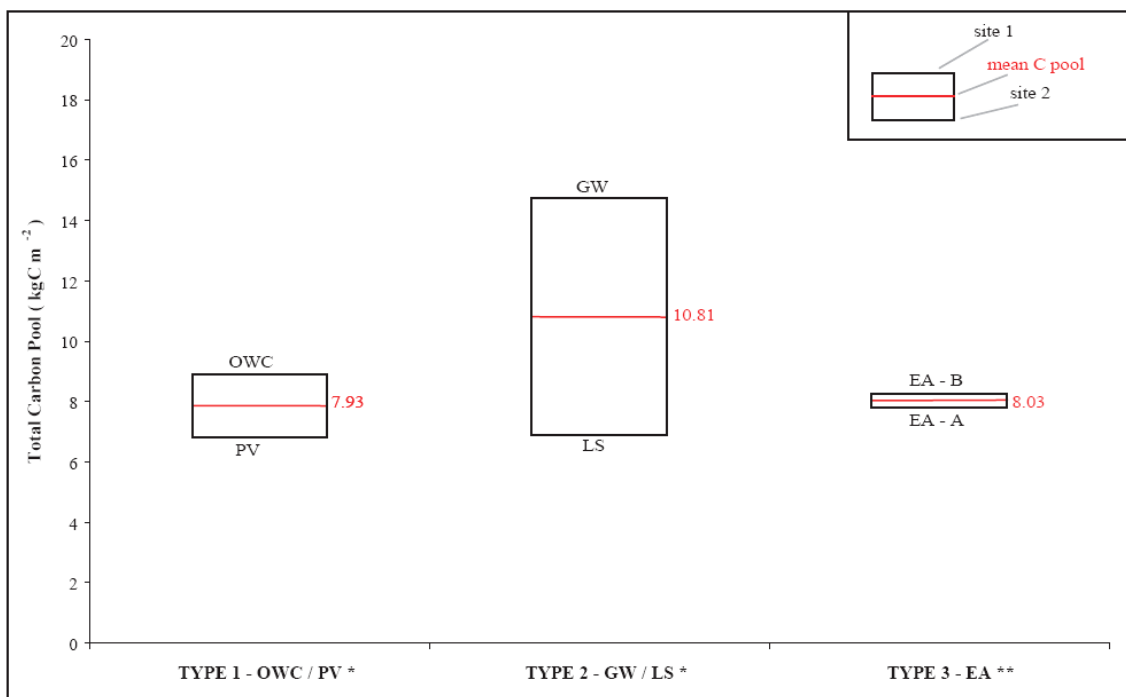


Figure 7. Comparison of the total carbon pool of the top 24 cm of soil in wetlands located in temperated humid (TmH), tropical humid (TrH), and Tropical dry (TrD) regions. Number of 2-cm samples from each region (n) is indicated. Bars represent standard errors. Different letters over error bars indicate significant differences between pools at  $P < 0.01$  significance.





\* mean of two wetlands, four cores divided in 2-cm increments (n = 48). OWC, Old Woman Creek; PV, Palo Verde; GW, Gahanna Woods; LS, La Selva.

\*\* one wetland, mean of two cores A and B divided in 2-cm increments (n = 24). EA, EARTH University.

Figure 8. Total soil carbon pool for the top 24 cm of soil of three types of wetlands. Boxes of wetland type 1 and 2 represent the mean total pool of each wetland (average of the total pool of the two cores extracted in the wetland); top line for the wetland with higher C pool, bottom line for the one with lower pool. Box of wetland type 3 represents the total pool of the two cores extracted in the wetland (top line for the core with higher C pool, bottom line for the one with lower pool). Red line in the middle of each box is the mean pools of the wetland type.

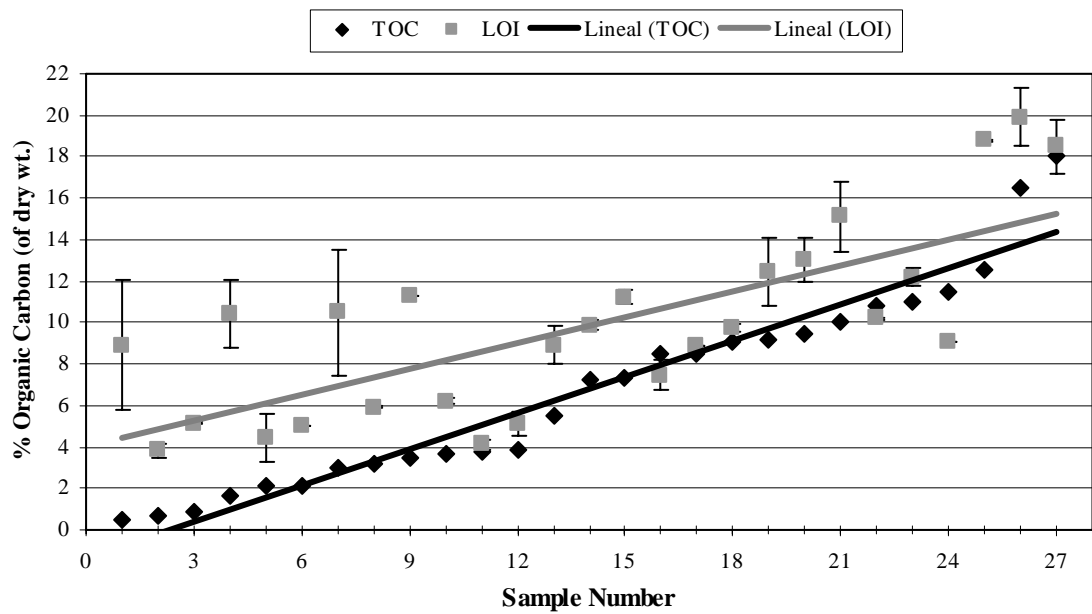


Figure 9. Percentage of organic carbon in dry weight measured the same 27 samples with the carbon analyzer (TOC) and by loss on ignition (LOI). Bars in LOI represent standard error. Trend lines for both methods are provided. Sample number refers to sequence of TOC samples from lowest to highest percentage of organic carbon.

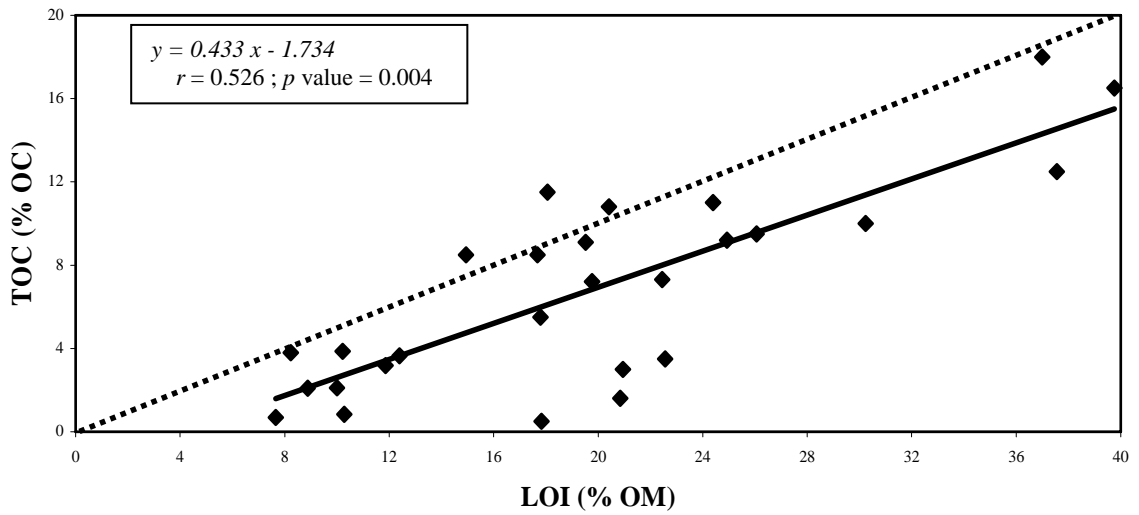


Figure 10. Regression graph of organic mater (OM) percentages obtained by loss on ignition (LOI) versus organic carbon (OC) percentages obtained with the carbon analyzer (TOC). Solid line represents regression line, dotted line represents 2:1 relationship. Equation of the linear regression,  $r$  correlation index (Pearson correlation test), and  $p$  value of the correlation are provided.

APPENDIX C  
ORIGINAL DATA

Old Woman Creek, Core A.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
OWC	2	0--6	5.103	1.303	3.800	65.690	0.853	3.352	871.130
OWC	4	0--6	3.692	1.459	2.233	67.334	0.875	2.486	646.033
OWC	6	0--6	3.171	1.026	2.145	65.288	0.848	2.070	538.007
OWC	8	6--12	3.276	0.779	2.497	62.058	0.806	2.033	528.323
OWC	10	6--12	3.863	1.091	2.772	63.724	0.828	2.462	639.714
OWC	12	6--12	3.881	1.107	2.774	61.528	0.799	2.388	620.546
OWC	14	12--18	3.082	1.170	1.912	63.232	0.822	1.949	506.440
OWC	16	12--18	2.927	0.938	1.989	60.008	0.780	1.756	456.446
OWC	18	12--18	2.808	1.282	1.526	56.471	0.734	1.586	412.078
OWC	20	18--24	2.943	1.199	1.744	60.504	0.786	1.781	462.735
OWC	22	18--24	2.121	0.315	1.806	63.831	0.829	1.354	351.828
OWC	24	18--24	2.138	0.251	1.887	58.958	0.766	1.261	327.573
OWC	26	24--30	2.506	0.240	2.266	58.146	0.755	1.457	378.668
OWC	28	24--30	1.758	0.391	1.367	62.504	0.812	1.099	285.552
OWC	30	24--30	1.803	0.372	1.431	59.461	0.773	1.072	278.603

Old Woman Creek, Core B.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
OWC	2	0--6	7.049	1.534	5.515	43.293	0.562	2.747	713.748
OWC	4	0--6	6.888	1.445	5.443	48.872	0.635	3.030	787.323
OWC	6	0--6	6.824	1.588	5.236	53.712	0.698	3.299	857.256
OWC	8	6--12	7.198	0.925	6.273	51.408	0.668	3.330	865.462
OWC	10	6--12	7.043	1.491	5.552	56.122	0.729	3.557	924.467
OWC	12	6--12	6.665	1.276	5.389	55.826	0.725	3.349	870.243
OWC	14	12--18	7.461	0.886	6.575	49.648	0.645	3.334	866.372
OWC	16	12--18	8.529	1.017	7.512	45.448	0.590	3.489	906.605
OWC	18	12--18	10.040	0.946	9.094	44.067	0.573	3.982	1034.788
OWC	20	18--24	12.870	1.256	11.614	48.659	0.632	5.636	1464.698
OWC	22	18--24	12.040	0.093	11.947	42.828	0.556	4.641	1206.034
OWC	24	18--24	11.640	0.124	11.516	44.989	0.585	4.713	1224.801
OWC	26	24--30	11.610	0.130	11.480	54.645	0.710	5.710	1483.835
OWC	28	24--30	10.950	0.149	10.801	56.386	0.733	5.557	1444.074
OWC	30	24--30	10.380	0.077	10.303	43.600	0.566	4.073	1058.501
OWC	32	30--36	10.530	0.099	10.431	45.750	0.594	4.336	1126.748
OWC	34	30--36	8.289	0.107	8.182	39.370	0.512	2.937	763.264
OWC	36	30--36	8.025	0.143	7.882	46.808	0.608	3.381	878.558

Gahanna Woods, Core A.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
GW	2	0--6	10.760	1.245	9.515	23.222	0.302	2.249	584.418
GW	4	0--6	11.930	0.353	11.577	27.889	0.362	2.994	778.178
GW	6	0--6	16.150	0.156	15.994	39.667	0.515	5.766	1498.324
GW	8	6--12	15.790	0.174	15.616	49.723	0.646	7.066	1836.285
GW	10	6--12	17.490	0.126	17.364	49.501	0.643	7.792	2024.895
GW	12	6--12	17.850	0.149	17.701	53.723	0.698	8.630	2242.847
GW	14	12--18	18.690	0.105	18.585	48.778	0.634	8.205	2132.253
GW	16	12--18	18.560	0.110	18.450	41.112	0.534	6.867	1784.615
GW	18	12--18	18.310	0.940	17.370	44.889	0.583	7.800	2027.043
GW	20	18--24	17.960	0.145	17.815	47.334	0.615	7.256	1885.613
GW	22	18--24	18.350	0.102	18.248	49.612	0.645	8.193	2129.229
GW	24	18--24	17.370	0.096	17.274	46.500	0.604	7.269	1889.123
GW	26	24--30	13.150	0.107	13.043	52.445	0.681	6.207	1612.994
GW	28	24--30	9.791	0.095	9.696	48.778	0.634	4.298	1117.009
GW	30	24--30	7.528	0.121	7.407	48.834	0.634	3.309	859.812

Gahanna Woods, Core B.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
GW	2	0--6	12.720	0.722	11.998	23.889	0.310	1.434	372.741
GW	4	0--6	12.860	0.497	12.363	29.945	0.389	1.526	396.561
GW	6	0--6	13.510	0.205	13.305	36.889	0.479	1.770	459.930
GW	8	6--12	14.100	0.515	13.585	35.111	0.456	1.843	478.916
GW	10	6--12	17.320	0.419	16.901	37.500	0.487	2.855	741.861
GW	12	6--12	17.450	0.091	17.359	38.334	0.498	3.013	783.073
GW	14	12--18	18.580	0.117	18.463	39.389	0.512	3.409	885.833
GW	16	12--18	18.230	0.091	18.139	40.945	0.532	3.290	855.029
GW	18	12--18	18.970	0.124	18.846	50.501	0.656	3.552	922.963
GW	20	18--24	18.660	0.130	18.530	52.501	0.682	3.433	892.266
GW	22	18--24	18.700	0.138	18.562	45.556	0.592	3.445	895.345
GW	24	18--24	19.370	0.102	19.268	42.667	0.554	3.712	964.775
GW	26	24--30	19.520	0.142	19.378	44.278	0.575	3.755	975.797
GW	28	24--30	18.750	0.104	18.646	46.667	0.606	3.477	903.489
GW	30	24--30	19.830	0.115	19.715	39.556	0.514	3.887	1010.052
GW	32	30--36	19.540	0.118	19.422	36.834	0.479	3.772	980.250
GW	34	30--36	19.420	0.122	19.298	43.278	0.562	3.724	967.770
GW	36	30--36	20.600	0.156	20.444	54.167	0.704	4.179	1086.104



EARTH University, Core A.

Wetland	Depth (cm)	Depth Range (cm)	%IC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
EA	2	0--6	19.980	0.943	19.037	19.335	0.251	3.477	903.550
EA	4	0--6	17.970	1.327	16.643	19.489	0.253	3.152	819.111
EA	6	0--6	16.460	1.186	15.274	19.861	0.258	2.942	764.611
EA	8	6--12	11.550	0.805	10.745	29.450	0.383	3.061	795.563
EA	10	6--12	10.900	1.290	9.610	30.982	0.403	3.039	789.834
EA	12	6--12	9.248	1.273	7.975	29.211	0.380	2.431	631.835
EA	14	12--18	7.202	1.105	6.097	27.820	0.361	1.803	468.617
EA	16	12--18	6.682	0.879	5.803	31.015	0.403	1.865	484.711
EA	18	12--18	7.399	0.988	6.411	30.691	0.399	2.044	531.115
EA	20	18--24	7.300	0.979	6.321	31.899	0.414	2.096	544.628
EA	22	18--24	8.361	0.784	7.577	29.052	0.377	2.186	568.120
EA	24	18--24	7.401	0.769	6.632	27.874	0.362	1.857	482.502
EA	26	24--30	6.243	0.971	5.272	36.400	0.473	2.045	531.499
EA	28	24--30	5.244	0.875	4.369	41.056	0.533	1.938	503.549
EA	30	24--30	5.426	0.957	4.469	42.360	0.550	2.069	537.570
EA	32	30--36	4.434	0.783	3.651	49.125	0.638	1.960	509.446
EA	34	30--36	4.074	1.248	2.826	49.314	0.641	1.808	469.883
EA	36	30--36	3.748	0.726	3.022	47.413	0.616	1.599	415.628
EA	38	36--42	3.456	0.751	2.705	48.995	0.637	1.524	396.030
EA	40	36--42	4.779	0.789	3.990	45.967	0.597	1.977	513.792
EA	42	36--42	2.897	0.830	2.067	54.221	0.704	1.414	367.382
EA	44	42--48	3.061	0.832	2.229	50.723	0.659	1.397	363.136
EA	46	42--48	2.722	1.029	1.693	51.325	0.667	1.257	326.751
EA	48	42--48	2.347	1.224	1.123	50.588	0.657	1.069	277.694
EA	50	48--54	3.240	1.152	2.088	53.540	0.696	1.561	405.720
EA	52	48--54	2.158	1.125	1.033	60.517	0.786	1.175	305.446
EA	54	48--54	2.910	1.194	1.716	72.184	0.938	1.890	491.289

EARTH University, Core B.

Wetland	Depth (cm)	Depth Range (cm)	%IC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
EA	2	0--6	18.770	1.176	17.594	13.858	0.180	2.341	608.352
EA	4	0--6	20.690	1.290	19.400	13.893	0.180	2.587	672.280
EA	6	0--6	25.240	1.186	24.054	11.161	0.145	2.535	658.877
EA	8	6--12	18.910	1.136	17.774	14.930	0.194	2.541	660.312
EA	10	6--12	15.940	0.899	15.041	17.298	0.225	2.482	644.903
EA	12	6--12	12.560	1.239	11.321	20.363	0.265	2.302	598.190
EA	14	12--18	15.760	1.174	14.586	18.622	0.242	2.641	686.428
EA	16	12--18	19.450	1.049	18.401	15.976	0.208	2.797	726.762
EA	18	12--18	21.470	1.225	20.245	14.923	0.194	2.884	749.380
EA	20	18--24	19.290	1.186	18.104	15.180	0.197	2.635	684.860
EA	22	18--24	14.430	0.986	13.444	24.627	0.320	3.198	831.150
EA	24	18--24	13.420	1.079	12.341	24.249	0.315	2.929	761.118
EA	26	24--30	13.760	1.065	12.695	28.531	0.371	3.533	918.215
EA	28	24--30	8.938	1.224	7.714	39.234	0.510	3.156	820.168
EA	30	24--30	7.363	1.109	6.254	35.609	0.463	2.360	613.226
EA	32	30--36	13.090	0.816	12.274	27.456	0.357	3.235	712.146
EA	34	30--36	10.220	1.065	9.155	25.587	0.332	2.353	611.606
EA	36	30--36	10.140	1.002	9.138	26.078	0.339	2.380	618.466
EA	38	36--42	8.318	1.194	7.124	33.585	0.436	2.514	653.378
EA	40	36--42	6.056	0.864	5.192	36.331	0.472	1.980	514.603
EA	42	36--42	5.453	0.959	4.494	36.785	0.478	1.805	469.145
EA	44	42--48	4.440	0.977	3.463	40.543	0.527	1.620	421.015
EA	46	42--48	3.637	0.972	2.665	52.292	0.679	1.712	444.814
EA	48	42--48	2.912	0.913	1.999	51.998	0.676	1.363	354.143
EA	50	48--54	2.307	0.977	1.330	68.909	0.895	1.431	371.815
EA	52	48--54	1.719	0.855	0.864	72.737	0.945	1.125	292.440
EA	54	48--54	1.443	0.936	0.507	75.862	0.986	0.985	256.031

Palo Verde, Core A.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
PV	2	0--6	4.471	0.213	4.258	65.805	0.855	2.648	688.125
PV	4	0--6	3.997	0.174	3.823	57.768	0.751	2.078	540.042
PV	6	0--6	5.132	0.144	4.988	59.237	0.770	2.736	711.025
PV	8	6--12	4.495	0.164	4.331	73.610	0.956	2.978	773.869
PV	10	6--12	3.450	0.276	3.174	83.495	1.085	2.593	673.727
PV	12	6--12	3.728	0.110	3.618	79.174	1.029	2.656	690.339
PV	14	12--18	2.290	0.204	2.086	76.459	0.993	1.576	409.510
PV	16	12--18	1.769	0.316	1.453	66.634	0.866	1.061	275.694
PV	18	12--18	1.599	0.166	1.433	70.865	0.921	1.020	265.023
PV	20	18--24	2.086	0.159	1.927	67.196	0.873	1.262	327.840
PV	22	18--24	2.122	0.394	1.728	68.307	0.887	1.305	339.013
PV	24	18--24	2.348	0.281	2.067	71.213	0.925	1.505	391.075

Palo Verde, Core B.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
PV	2	0--6	4.132	0.275	3.857	78.154	1.015	2.906	755.293
PV	4	0--6	4.548	0.248	4.300	68.203	0.886	2.792	725.482
PV	6	0--6	4.194	0.234	3.960	73.831	0.959	2.787	724.217
PV	8	6--12	4.736	0.151	4.585	63.252	0.822	2.696	700.628
PV	10	6--12	4.823	0.130	4.693	72.787	0.946	3.159	821.063
PV	12	6--12	4.167	0.122	4.045	69.145	0.898	2.593	673.889
PV	14	12--18	3.942	0.134	3.808	63.717	0.828	2.261	587.458
PV	16	12--18	3.048	0.182	2.866	70.864	0.921	1.944	505.177
PV	18	12--18	2.571	0.252	2.319	65.915	0.856	1.525	396.360
PV	20	18--24	2.478	0.333	2.145	62.040	0.806	1.384	359.561
PV	22	18--24	4.235	0.215	4.020	63.510	0.825	2.421	629.065
PV	24	18--24	3.541	0.348	3.193	80.712	1.049	2.572	668.447

La Selva, Core A.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
LS	2	0--6	11.240	1.376	9.864	21.567	0.280	2.182	566.966
LS	4	0--6	9.474	1.164	8.310	25.413	0.330	2.167	563.114
LS	6	0--6	10.763	0.776	9.987	28.598	0.372	2.770	719.911
LS	8	6--12	10.737	1.189	9.548	33.423	0.434	3.230	839.316
LS	10	6--12	9.585	0.765	8.820	31.532	0.410	2.720	706.877
LS	12	6--12	7.793	0.941	6.852	32.665	0.424	2.291	595.380
LS	14	12--18	6.621	0.787	5.834	29.115	0.378	1.735	450.863
LS	16	12--18	5.529	0.136	5.393	32.065	0.417	1.596	414.651
LS	18	12--18	4.317	0.126	4.191	34.874	0.453	1.355	352.114
LS	20	18--24	6.008	1.134	4.874	37.189	0.483	2.011	522.577
LS	22	18--24	5.165	0.808	4.357	46.193	0.600	2.147	558.014
LS	24	18--24	3.295	1.005	2.290	52.476	0.682	1.556	404.411
LS	26	24--30	1.925	0.837	1.088	50.788	0.660	0.880	228.660
LS	28	24--30	2.442	1.129	1.313	51.580	0.670	1.134	294.599
LS	30	24--30	1.416	0.811	0.605	45.360	0.589	0.578	150.224
LS	32	30--36	0.950	0.780	0.170	47.203	0.613	0.404	104.880
LS	34	30--36	0.393	0.117	0.276	47.356	0.615	0.167	43.528
LS	36	30--36	0.318	0.108	0.210	44.479	0.578	0.127	33.082
LS	38	36--42	0.227	0.152	0.075	42.503	0.552	0.087	22.566
LS	40	36--42	0.323	0.153	0.170	45.250	0.588	0.132	34.184
LS	42	36--42	0.248	0.078	0.170	46.025	0.598	0.103	26.696
LS	44	42--48	0.493	0.142	0.351	46.508	0.604	0.206	53.626
LS	46	42--48	0.581	0.101	0.480	47.664	0.619	0.249	64.769
LS	48	42--48	0.746	0.124	0.622	49.903	0.648	0.335	87.070
LS	50	48--54	0.413	0.137	0.276	47.695	0.620	0.177	46.071
LS	52	48--54	0.353	0.134	0.219	50.517	0.656	0.160	41.708
LS	54	48--54	0.240	0.105	0.135	48.623	0.632	0.105	27.293

La Selva, Core B.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
LS	2	0--6	13.865	1.285	1.580	34.519	0.448	4.307	1119.385
LS	4	0--6	12.554	0.734	1.820	32.477	0.422	3.669	953.575
LS	6	0--6	11.897	1.127	10.770	36.149	0.470	3.870	1005.845
LS	8	6--12	10.990	0.900	10.090	29.519	0.384	2.920	758.752
LS	10	6--12	7.097	1.026	6.071	30.852	0.401	1.971	512.110
LS	12	6--12	6.094	0.680	5.414	32.000	0.416	1.755	456.100
LS	14	12--18	6.026	1.022	5.004	31.889	0.414	1.729	449.444
LS	16	12--18	5.634	1.194	4.440	34.445	0.448	1.747	453.883
LS	18	12--18	4.177	1.036	3.141	41.037	0.533	1.543	400.911
LS	20	18--24	4.572	1.061	3.511	34.593	0.449	1.423	369.911
LS	22	18--24	3.313	0.954	2.359	40.741	0.529	1.215	315.688
LS	24	18--24	2.913	0.952	1.961	42.519	0.552	1.115	289.685
LS	26	24--30	2.256	1.026	1.230	40.519	0.526	0.823	213.796
LS	28	24--30	2.582	1.174	1.408	43.112	0.560	1.002	260.347
LS	30	24--30	1.815	0.783	1.032	44.186	0.574	0.722	187.569
LS	32	30--36	1.960	0.818	1.142	44.704	0.581	0.789	204.931
LS	34	30--36	2.659	0.966	1.693	41.435	0.538	0.992	257.683
LS	36	30--36	1.928	0.951	0.977	45.593	0.592	0.791	205.593
LS	38	36--42	2.130	1.033	1.097	43.445	0.564	0.833	216.432
LS	40	36--42	2.272	1.157	1.115	44.000	0.572	0.900	233.813
LS	42	36--42	1.836	0.993	0.843	48.630	0.632	0.804	208.824
LS	44	42--48	2.202	1.199	1.003	46.704	0.607	0.926	240.534
LS	46	42--48	1.453	0.918	0.535	46.056	0.598	0.602	156.515
LS	48	42--48	1.181	0.702	0.479	51.556	0.670	0.548	142.407
LS	50	48--54	1.507	1.202	0.305	57.501	0.747	0.780	202.670
LS	52	48--54	1.615	1.267	0.348	55.239	0.718	0.803	208.650

La Selva, Core B, continued.

Wetland	Depth (cm)	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
LS	54	48--54	1.420	0.746	0.674	65.890	0.856	0.842	218.831
LS	56	54--60	1.352	0.665	0.687	66.445	0.863	0.808	210.108
LS	58	54--60	1.569	0.949	0.620	66.167	0.860	0.934	242.812
LS	60	54--60	1.870	1.176	0.694	76.779	0.998	1.292	335.803

Upland sites, Ohio.

Upland	Depth Range (cm)	%TC	%IC	%OC	Dry Weight (g)	Bulk Density (g cm <sup>-3</sup> )	STC (g)	C pool (g C m <sup>-2</sup> )
OWC	0--5	1.893	0.087	1.806	695.833	1.767	13.170	1676.907
OWC	5--10	1.343	0.129	1.213	708.342	1.799	9.511	1211.033
OWC	10--15	0.528	0.115	0.413	711.308	1.806	3.756	478.190
OWC	15--20	0.286	0.081	0.205	726.796	1.846	2.076	264.346
OWC	20--25	0.243	0.097	0.146	759.327	1.928	1.842	234.489
OWC	25--30	0.240	0.090	0.150	777.962	1.976	1.865	237.473
OWC	30--35	0.390	0.098	0.292	785.595	1.995	3.064	390.097
GW	0--5	3.988	0.094	3.894	488.984	1.242	19.502	2483.056
GW	5--10	2.893	0.063	2.830	481.648	1.223	13.933	1774.007
GW	10--15	1.853	0.050	1.803	506.643	1.287	9.388	1195.327
GW	15--20	0.888	0.062	0.826	509.901	1.295	4.526	576.247
GW	20--25	0.592	0.202	0.390	503.306	1.278	2.978	379.156
GW	25--30	0.608	0.179	0.428	501.539	1.274	3.049	388.220
GW	30--35	0.398	0.098	0.300	505.361	1.283	2.011	256.067



APPENDIX D  
STATISTICAL ANALYSES

**Climate comparison.**

ANOVA					
Carbon Pool					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4351903.3	2	2175951.664	15.158	.000
Within Groups	16795628	117	143552.372		
Total	21147531	119			

Multiple Comparisons							
Dependent Variable: Carbon Pool							
	(I) Climate	(J) Climate	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	368.71821*	77.33918	.000	185.1218	552.3146
		3.00	422.51921*	94.72076	.000	197.6605	647.3779
	2.00	1.00	-368.71821*	77.33918	.000	-552.3146	-185.1218
		3.00	53.80100	94.72076	.837	-171.0577	278.6597
	3.00	1.00	-422.51921*	94.72076	.000	-647.3779	-197.6605
		2.00	-53.80100	94.72076	.837	-278.6597	171.0577
Bonferroni	1.00	2.00	368.71821*	77.33918	.000	180.8700	556.5665
		3.00	422.51921*	94.72076	.000	192.4530	652.5854
	2.00	1.00	-368.71821*	77.33918	.000	-556.5665	-180.8700
		3.00	53.80100	94.72076	1.000	-176.2652	283.8672
	3.00	1.00	-422.51921*	94.72076	.000	-652.5854	-192.4530
		2.00	-53.80100	94.72076	1.000	-283.8672	176.2652

\*. The mean difference is significant at the .05 level.

**Wetland type comparison.**

ANOVA					
Carbon Pool					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1622114.8	2	811057.384	4.860	.009
Within Groups	19525416	117	166883.898		
Total	21147531	119			

Multiple Comparisons							
Dependent Variable: Carbon Pool							
	(I) wetland type	(J) wetland type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-240.14640*	83.38762	.013	-438.1012	-42.1916
		3.00	-8.73013	102.12856	.996	-251.1743	233.7140
	2.00	1.00	240.14640*	83.38762	.013	42.1916	438.1012
		3.00	231.41627	102.12856	.065	-11.0279	473.8604
	3.00	1.00	8.73013	102.12856	.996	-233.7140	251.1743
		2.00	-231.41627	102.12856	.065	-473.8604	11.0279
Bonferroni	1.00	2.00	-240.14640*	83.38762	.014	-442.6856	-37.6072
		3.00	-8.73013	102.12856	1.000	-256.7890	239.3288
	2.00	1.00	240.14640*	83.38762	.014	37.6072	442.6856
		3.00	231.41627	102.12856	.076	-16.6426	479.4752
	3.00	1.00	8.73013	102.12856	1.000	-239.3288	256.7890
		2.00	-231.41627	102.12856	.076	-479.4752	16.6426

\*. The mean difference is significant at the .05 level.

### Hydrology, OWC and EA.

Two-Sample T-Test and CI: EA-IF; EA-PF

	N	Mean	StDev	SEMean
<b>EA-IF</b>	27	526	164	32
<b>EA-PF</b>	28	594	174	33

Difference = mu (EA-IF) - mu (EA-PF)

Estimate for difference: -68.4

95% CI for difference: (-159.8; 23.0)

T-Test of difference = 0 (vs not =): T-Value = -1.50 **P-Value = 0.139** **DF = 52**

Paired T-Test and CI: EA-IF; EA-PF

	N	Mean	StDev	SE Mean
<b>EA-IF</b>	27	525.9	163.9	31.5
<b>EA-PF</b>	27	605.7	166.2	32.0
<b>Difference</b>	27	-79.8	177.9	34.2

95% CI for mean difference: (-150.2; -9.4)

T-Test of mean difference = 0 (vs not = 0): T-Value = -2.33 **P-Value = 0.028**

Two-Sample T-Test and CI: OWC-IF-; OWC-PF

	N	Mean	StDev	SE Mean
<b>OWC-IF</b>	17	1.819	0.602	0.15
<b>OWC-PF</b>	19	3.926	0.928	0.21

Difference = mu (OWC-IF) - mu (OWC-PF)

Estimate for difference: -2.107

95% CI for difference: (-2.633; -1.580)

T-Test of difference = 0 (vs not =): T-Value = -8.16 **P-Value = 0.000** **DF = 31**

**Wetland and upland, OWC and GW.**

Two-Sample T-Test and CI: OWC; OWC-UP

	N	Mean	StDev	SE Mean
<b>OWC</b>	6	2.377	0.250	0.10
<b>OWC-UP</b>	7	0.642	0.571	0.22

Difference = mu (OWC) - mu (OWC-UP)

Estimate for difference: 1.735

95% CI for difference: (1.185; 2.285)

T-Test of difference = 0 (vs not =): T-Value = 7.27 **P-Value = 0.000** **DF = 8**

Two-Sample T-Test and CI: GW; GW-UP

	N	Mean	StDev	SE Mean
<b>GW</b>	6	3.501	0.899	0.37
<b>GW-UP</b>	7	1.007	0.849	0.32

Difference = mu (GW) - mu (GW-UP)

Estimate for difference: 2.493

95% CI for difference: (1.407; 3.580)

T-Test of difference = 0 (vs not =): T-Value = 5.11 **P-Value = 0.000** **DF = 10**

### Loss On Ignition

Variable	N	Mean	StDev	SE Mean	95% CI
400°16h	40	6.987	2.632	0.416	(6.145; 7.829)
450°1h	40	6.283	2.769	0.438	(5.397; 7.169)
450°4h	40	6.174	2.066	0.327	(5.514; 6.835)
550°1h	40	8.460	2.604	0.412	(7.627; 9.292)
550°4h	40	8.792	2.474	0.391	(8.001; 9.583)

Paired T for 400°16h - 450°1h

	N	Mean	StDev	SE Mean
400°16h	40	6.987	2.632	0.416
450°1h	40	6.283	2.769	0.438
Difference	40	0.704	2.115	0.334

95% CI for mean difference: (0.028; 1.380)

T-Test of mean difference = 0 (vs not = 0): T-Value = 2.11 **P-Value = 0.042**

Paired T for 400°16h - 450°4h

	N	Mean	StDev	SE Mean
400°16h	40	6.987	2.632	0.416
450°4h	40	6.174	2.066	0.327
Difference	40	0.813	1.965	0.311

95% CI for mean difference: (0.184; 1.441)

T-Test of mean difference = 0 (vs not = 0): T-Value = 2.62 **P-Value = 0.013**

Paired T for 400°16h - 550°1h

	N	Mean	StDev	SE Mean
400°16h	40	6.987	2.632	0.416
550°1h	40	8.460	2.604	0.412
Difference	40	-1.473	1.969	0.311

95% CI for mean difference: (-2.102; -0.843)

T-Test of mean difference = 0 (vs not = 0): T-Value = -4.73 **P-Value = 0.000**

Paired T for 400°16h - 550°4h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
400°16h	40	6.987	2.632	0.416
550°4h	40	8.792	2.474	0.391
Difference	40	-1.805	2.095	0.331

95% CI for mean difference: (-2.475; -1.135)

T-Test of mean difference = 0 (vs not = 0): T-Value = -5.45 **P-Value = 0.000**

Paired T for 450°1h - 450°4h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
450°1h	40	6.283	2.769	0.438
450°4h	40	6.174	2.066	0.327
Difference	40	0.109	1.950	0.308

95% CI for mean difference: (-0.515; 0.733)

T-Test of mean difference = 0 (vs not = 0): T-Value = 0.35 **P-Value = 0.726**

Paired T for 450°1h - 550°1h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
450°1h	40	6.283	2.769	0.438
550°1h	40	8.460	2.604	0.412
Difference	40	-2.177	2.333	0.369

95% CI for mean difference: (-2.923; -1.430)

T-Test of mean difference = 0 (vs not = 0): T-Value = -5.90 **P-Value = 0.000**

Paired T for 450°1h - 550°4h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
450°1h	40	6.283	2.769	0.438
550°4h	40	8.792	2.474	0.391
Difference	40	-2.509	2.146	0.339

95% CI for mean difference: (-3.195; -1.823)

T-Test of mean difference = 0 (vs not = 0): T-Value = -7.40 **P-Value = 0.000**

Paired T for 450°4h - 550°1h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
450°4h	40	6.174	2.066	0.327
550°1h	40	8.460	2.604	0.412
Difference	40	-2.285	2.052	0.324

95% CI for mean difference: (-2.942; -1.629)

T-Test of mean difference = 0 (vs not = 0): T-Value = -7.04 **P-Value = 0.000**

Paired T for 450°4h - 550°4h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
450°4h	40	6.174	2.066	0.327
550°4h	40	8.792	2.474	0.391
Difference	40	-2.618	1.582	0.250

95% CI for mean difference: (-3.124; -2.112)

T-Test of mean difference = 0 (vs not = 0): T-Value = -10.47 **P-Value = 0.000**

Paired T for 550°4h - 550°1h

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
550°4h	40	8.792	2.474	0.391
550°1h	40	8.460	2.604	0.412
Difference	40	0.332	1.653	0.261

95% CI for mean difference: (-0.196; 0.861)

T-Test of mean difference = 0 (vs not = 0): T-Value = 1.27 **P-Value = 0.211**

Paired T for 1g - 5g

	<b>N</b>	<b>Mean</b>	<b>StDev</b>	<b>SE Mean</b>
1g	100	7.695	2.600	0.260
5g	100	6.984	2.812	0.281
Difference	100	0.711	2.241	0.224

95% CI for mean difference: (0.266; 1.155)

T-Test of mean difference = 0 (vs not = 0): T-Value = 3.17 **P-Value = 0.002**



LS-D56 ; Test of mu = 0.687 vs not = 0.687

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	5.178	0.529	0.265	(4.335; 6.020)	16.96	0.000
450°1h	4	4.898	0.551	0.276	(4.021; 5.775)	15.28	0.001
450°4h	4	5.350	2.020	1.010	(2.140; 8.570)	4.620	0.019
550°1h	4	7.530	2.190	1.100	(4.050; 11.02)	6.250	0.008
550°4h	4	7.759	1.401	0.700	(5.530; 9.987)	10.10	0.002
1g	10	6.106	2.149	0.679	(4.569; 7.643)	7.980	0.000
5g	10	6.183	1.609	0.509	(5.032; 7.334)	10.80	0.000

OWC-A28; Test of mu = 9.094 vs not = 9.094

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	11.550	3.030	1.520	(6.730; 16.370)	1.62	0.203
450°1h	4	10.324	0.503	0.251	(9.524; 11.124)	4.90	0.016
450°4h	4	8.916	0.998	0.499	(7.328; 10.503)	-0.36	0.744
550°1h	4	11.156	1.066	0.533	(9.459; 12.852)	3.87	0.031
550°4h	4	12.275	1.098	0.549	(10.527; 14.022)	5.79	0.010
1g	10	10.955	1.381	0.437	(9.967; 11.943)	4.26	0.002
5g	10	10.734	2.296	0.726	(9.092; 12.377)	2.26	0.050

LS-D42; Test of mu = 0.843 vs not = 0.843

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	7.935	1.930	0.965	(4.864; 11.006)	7.35	0.005
450°1h	4	5.227	0.543	0.272	(4.362; 6.092)	16.14	0.001
450°4h	4	5.603	0.533	0.266	(4.755; 6.451)	17.87	0.000
550°1h	4	9.753	1.615	0.808	(7.183; 12.323)	11.03	0.002
550°4h	4	9.449	0.498	0.249	(8.656; 10.242)	34.53	0.000
1g	20	7.186	2.261	0.505	(6.128; 8.244)	12.55	0.000
5g	20	6.242	2.028	0.453	(5.293; 7.191)	11.91	0.000

PV-D14; Test of mu = 2.086 vs not = 2.086

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	5.610	2.150	1.080	(2.190; 9.030)	3.27	0.047
450°1h	4	4.789	1.962	0.981	(1.667; 7.912)	2.76	0.070
450°4h	4	4.946	1.120	0.560	(3.164; 6.727)	5.11	0.015
550°1h	4	6.697	1.074	0.537	(4.988; 8.405)	8.59	0.003
550°4h	4	7.133	1.867	0.933	(4.163; 10.103)	5.41	0.012
1g	10	6.357	1.599	0.506	(5.213; 7.501)	8.45	0.000
5g	10	5.312	1.888	0.597	(3.961; 6.662)	5.40	0.000

OWC-B2; Test of  $\mu = 3.8$  vs not = 3.8

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	4.330	0.337	0.168	(3.794; 4.865)	3.15	0.051
450°1h	4	3.171	0.679	0.340	(2.090; 4.252)	-1.85	0.161
450°4h	4	3.947	0.453	0.227	(3.225; 4.668)	0.65	0.564
550°1h	4	4.807	0.805	0.403	(3.526; 6.088)	2.50	0.088
550°4h	4	5.146	0.310	0.155	(4.652; 5.640)	8.67	0.003
1g	10	4.430	0.755	0.239	(3.890; 4.970)	2.64	0.027
5g	10	4.130	0.970	0.307	(3.436; 4.824)	1.07	0.310

OWC-A18; Test of  $\mu = 10.801$  vs not = 10.801

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	9.792	0.363	0.182	(9.214; 10.370)	-5.55	0.012
450°1h	4	10.037	0.643	0.322	(9.013; 11.060)	-2.38	0.098
450°4h	4	9.979	0.349	0.174	(9.424; 10.534)	-4.71	0.018
550°1h	4	11.569	1.059	0.530	(9.884; 13.255)	1.45	0.243
550°4h	4	11.250	0.806	0.403	(9.968; 12.532)	1.11	0.346
1g	10	10.680	0.957	0.303	(9.996; 11.365)	-0.40	0.699
5g	10	10.371	1.019	0.322	(9.641; 11.100)	-1.34	0.215

PV-D10; Test of  $\mu = 3.174$  vs not = 3.174

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	5.549	0.864	0.432	(4.174; 6.923)	5.50	0.012
450°1h	4	5.290	0.545	0.272	(4.424; 6.157)	7.77	0.004
450°4h	4	5.664	0.510	0.255	(4.853; 6.475)	9.77	0.002
550°1h	4	6.427	0.755	0.377	(5.226; 7.628)	8.62	0.003
550°4h	4	7.111	0.224	0.112	(6.755; 7.468)	35.14	0.000
1g	10	6.186	0.839	0.265	(5.587; 6.786)	11.36	0.000
5g	10	5.830	0.928	0.294	(5.166; 6.494)	9.05	0.000

PV-C2; Test of  $\mu = 3.857$  vs not = 3.857

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	7.705	1.078	0.539	(5.990; 9.421)	7.14	0.006
450°1h	4	7.63	3.59	1.79	(1.920; 13.340)	2.10	0.126
450°4h	4	5.467	0.635	0.318	(4.456; 6.478)	5.07	0.015
550°1h	4	8.60	2.70	1.35	(4.300; 12.900)	3.51	0.039
550°4h	4	8.389	0.633	0.316	(7.383; 9.396)	14.33	0.001
1g	10	7.386	2.340	0.740	(5.712; 9.060)	4.77	0.001
5g	10	7.731	2.142	0.677	(6.199; 9.263)	5.72	0.000

EA-D32; Test of mu = 3.651 vs not = 3.651

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
400°16h	4	5,782	0,924	0,462	(4,311; 7,253)	4,61	0,019
450°1h	4	4,78	2,24	1,12	(1,21; 8,34)	1,00	0,390
450°4h	4	6,101	0,458	0,229	(5,372; 6,830)	10,70	0,002
550°1h	4	9,527	0,257	0,129	(9,118; 9,936)	45,69	0,000
550°4h	4	10,547	0,733	0,366	(9,381; 11,713)	18,82	0,000
1g	10	7,775	2,298	0,727	(6,131; 9,419)	5,68	0,000
5g	10	6,918	2,815	0,890	(4,904; 8,931)	3,67	0,005

### Loss On Ignition versus Carbon Analyzer

Two-sample T for TOC vs LOI				
	N	Mean	StDev	SE Mean
<b>TOC</b>	28	6.17	4.23	0.80
<b>LOI</b>	28	9.87	4.54	0.86
Difference = mu (TOC) - mu (LOI)				
Estimate for difference: -3.70				
95% CI for difference: (-6.06; -1.35)				
T-Test of difference = 0 (vs not =): T-Value = -3.16 <b>P-Value = 0.003</b> <b>DF = 53</b>				

<b>Correlations: TOC; LOI</b>
<b>Pearson correlation of TOC and LOI = 0.526</b>
<b>P-Value = 0.004</b>