Variability in Hydrology and Ecosystem Properties and Their Role in Regulating Soil Organic Matter Stability in Wetlands of West-Central Florida

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Variability in Hydrology and Ecosystem Properties and Their Role in Regulating Soil Organic Matter Stability in Wetlands of West-Central Florida

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Abstract

Soil organic matter (SOM) provides many ecosystem services that are necessary for continued ecosystem function. The accumulation of SOM in an ecosystem is a function of its persistence time which can range from days to thousands of years. Ecosystem properties including dominant vegetation type, soil texture, and soil moisture in various habitats can regulate the persistence time of SOM.

Wetlands, because of their associated ecosystem properties, promote SOM accumulation, but little has been done to determine the ecosystem properties that regulate its persistence over time. In west-central Florida, urbanization and increased water demands have suppressed water tables in isolated wetland ecosystems via hydrological connectivity between ground and surficial waters. In this study, variability in wetland ecosystem properties, in particular dominant vegetation type and hydrological parameters, were tested as mechanisms driving SOM accumulation and stability.

Cypress wetlands had significantly more organic matter, carbon (C), and nitrogen (N) than herbaceous marshes. In addition, increased wetland inundation promoted stable SOM accumulation in forested wetlands. By increasing the percent time a forested wetland spent aerobic, decreases occurred in both labile and stable C and N pools. As large storage units of SOM, the decreases in both labile and stable C and N pools in wetland soils have large implications for global C and N cycling. Increased manipulation of wetland water levels, especially in short time scales, can mineralize both short-term and long-term storage units of C and N. Globally, the increase mineralization of large
SOC and SON stocks would exacerbate the release of air and water quality pollutants. The sensitivity of both labile and stable SOM pools draws concern when anticipating continued water demands and land use changes of the Tampa Bay region.
Introduction

Soil organic matter (SOM) exists in almost all ecosystems globally and provides many overlooked, yet critical ecosystem services. SOM is the habitat and primary energy source for soil microorganisms of detrital food webs in ecosystems (Berg and Bengtsson 2007, Palm et al. 2007). And, SOM governs soil quality by regulating macronutrient internal cycling by microorganisms. The released nutrients are essential to sustaining life and often regulate ecosystem primary production (Silver et al. 2001, Galloway 2004). Soil water quality can also be improved by SOM, as charged SOM surfaces bind chemical toxins such as aluminum through cation exchange (Craswell and Lefroy 2001, Palm et al. 2007), and as SOM facilitates nitrate (NO$_3^-$) removal by providing an energy source for heterotrophic denitrification (Schade et al. 2001, Burgin and Groffman 2012).

SOM is the primary driver of water holding capacity in soils (Craswell and Lefroy 2001), and plays a crucial role in water availability to plants by regulating water release in ecosystems (Craswell and Lefroy 2001, Palm et al. 2007, Schmidt et al. 2011). In sandy soils, large particles of SOM, i.e. particulate organic matter (POM), can increase water infiltration rate and soil aeration by increasing pore space (Rawls et al. 2003).

The ability of SOM to provide these critical functions depends on its persistence. Yet, understanding about the soil conditions that promote SOM persistence in ecosystems remains fragmented. This knowledge gap will be addressed in this thesis by identifying key ecosystem properties associated with SOM stability.
Soil organic matter can be divided into multiple pools that vary in residence and turnover time in a soil profile. Passive fractions of SOM are long-term storage pools persisting in soils hundreds to thousands of years (Parton et al. 1987). Intermediate pools, the slow SOM pools, remain in soil profiles up to a century. Both passive and slow SOM pools are relatively ecologically stable and resist biological decomposition or leaching loss from the soil profile (Schlesinger 1997). Active or labile pools, by contrast, are the fractions of SOM pools that are readily bioavailable to microorganisms for metabolic processes (Schlesinger 1997, Robertson et al. 1999b). Labile pools are readily mineralized and potentially short-lived in ecosystems (Kaye et al. 2002a).

The relative stability of the SOM pool ultimately determines the length of time it will persist and maintain ecosystem functions. Passive and slow SOM pools, i.e., stable pools of SOM, provide ecosystems with a long-term, slow release of nutrients, and aid in water storage and regulation. Globally, stable SOM provides long-term storage of large pools of important elements such as carbon (C) and nitrogen (N). Labile SOM pools, by contrast, are an easily accessible energy source for microorganisms. Microbial transformation of labile organic matter rapidly provides ecosystems with mineral nutrients available for plant or microbial uptake (Schimel et al. 1994).

The partition of SOM pools into stable and labile fractions is regulated by both biotic and abiotic ecosystem properties (Kaye et al. 2002a, 2002b, Schmidt et al. 2011). One primary ecosystem property that can control SOM stability is the vegetative community. Vegetative communities can vary in physical structure depending on the diversity and type of species present, ultimately modifying abiotic and biotic characteristics of an ecosystem. Depending on the fullness of the canopy and height of
vegetative cover, plants can cause variability in light incidence on the soil surface, creating pockets of contrasting soil temperature and moisture conditions. Vegetative species with larger canopies heavily shade the ground resulting in reduced soil temperatures, respiration rates, and net C mineralization compared to unshaded areas (Harte et al. 1995, Taggart et al. 2011). Increased shading also increases soil moisture content and decreases potential soil water evaporation (Genxu et al. 2012). Increased UV incidence from less canopy cover can also stimulate photodegradation of SOM, ultimately decreasing pool sizes by increasing the rate of litter turnover (Moorhead and Callaghan 1994).

The dominant vegetation type of an ecosystem can also differ in net primary production, litter chemical composition and structural complexity (Brinson et al. 1981, Murphy et al. 1998, Kögel-Knabner 2002). Differences in plant species composition create variability in the quality and quantity of SOM inputs (Kaye et al. 2002b). The variability in SOM inputs can develop contrasting microbial communities, each producing a distinct array of enzymes for the breakdown of specific litter chemistries (Hernandez and Hobbie 2010, Kang and Freeman 2012). Thus, the type of litter produced by plants and composition of the microbial community are important ecosystem regulators of SOM stability.

In the past, it was thought that structurally complex biomolecules such as lignin and humic acids were harder to decompose by microorganisms, and were thus commonly associated with the stable SOM pool (Melillo et al. 1982, Martin and Reddy 2010). There have been additional studies, however, illustrating that older soil C pools can also be composed of simple-structured organic molecules once assumed to be labile.
(Marshner 2008, Kleber et al. 2011). Currently, it still is uncertain how variability in litter chemistry and structural complexity combined with other ecosystem properties to contribute to the long-term stability of SOM in ecosystems (Schmidt et al. 2011, Dungait et al. 2012). One aim of this thesis is to identify ecosystem properties associated with SOM stability.

Soil texture is another important and highly-tested abiotic ecosystem property that may regulate SOM stability. Both as a textural class and mineral group, clay can preserve SOM by limiting physical accessibility of microorganisms to SOM pools. Because of small particle size, clay minerals have a large area of charged surface promoting the sorption of SOM onto clay mineral structure (Oades et al. 1988, Kleber 2007, Six et al. 2002). The formation of organominerals, or complexes of organic matter and clay particles, through sorption generates long-term SOM stability. Once SOM is associated into organominerals, adsorptive energy between SOM and clay particles is greater than the microbial energy required to access the SOM, making the SOM energetically unfavorable for microorganisms (Kleber et al. 2007, Dungait et al. 2012).

SOM, along with clay, drives formation of aggregates and other soil structures in a soil profile. Aggregation and soil structure form a physical barrier between microorganisms and SOM (Hassink 1997, Six et al. 2002, Kleber et al. 2007). In soils dominated by high sand content, SOM is the primary controller of aggregation (Hartmann and De Boote 1974). If SOM decreases, soil aggregation decreases and remaining SOM is more vulnerable to decomposition (Dungait et al. 2012). Smaller SOM particles, less than 53 μm, or mineral associated organic matter (MAOM), promote aggregation and C and N stabilization (Castellano et al. 2012). Loss of SOM also
decreases soil aggregation and soil structure leading to increased potential for soil loss from erosion (Carter 2002).

Small particle size can increase water storage capacity in soils through specific retention allowing soils to store water longer (Brady and Weil 2008). In addition, soils with smaller particle sizes (<2 mm) can significantly increase capillary rise height above the water table in comparison to larger particle sizes (>2 mm) (Brady and Weil 2008). Increased specific retention and capillary rise from smaller particle sizes can allow soils to remain saturated for longer periods of time after a flooding event, even after the water table is below the soil surface. Increased soil saturation can directly affect microbial activity and plant productivity, thus the size and persistence of SOM pools (White and Reddy 2001).

Another ecosystem property promoting stability of SOM is hydrologically-induced reducing conditions in a soil profile. Complete saturation of soils for long periods typically occurs in wetlands and riparian zones. Prolonged saturation allows substantial SOM pools to accumulate through time by at least two mechanisms (Dahm et al. 1998, DeBusk and Reddy 1998, Davidson and Janssen 2006). First, SOM pools can accumulate owing to oxygen depletion in the soil profile. Decomposition is most efficient when molecular oxygen (O$_2$) is available as an electron acceptor. In inundated soils, however, O$_2$ rapidly depletes and soils become increasingly reduced. Once soils are anoxic, facultative microbes utilize a cascade of less efficient electron acceptors for cellular respiration. Anoxia decreases the efficiency of microbial mineralization rates by nearly a third (DeBusk and Reddy 1998, White and Reddy 2001). The lower efficiency
of soil microorganisms inhibits decomposition, allowing large pools of SOM to accumulate in inundated ecosystems (David and Janssens 2006).

Second, hydrologically-driven anoxia also facilitates build-up of phenolic compounds. Phenol oxidase initiates decomposition by breaking down inhibitory phenolic compounds, but its activity is hindered when oxygen is not present (Freeman et al. 2004, Fenner and Freeman 2011). In soils that are inundated for long periods of time, phenolic compounds accumulate until water levels recede and oxygen is re-introduced into the soil profile (Ahn 2009, Fenner and Freeman 2011). Once O$_2$ is re-introduced and phenol oxidase is produced, a cascade of other enzymes is activated, and decomposition more readily proceeds (Freeman et al. 2004).

Ecosystems with properties promoting accumulation of stable SOM are increasingly important since SOM contains the largest pools of carbon (C) and nitrogen (N) in the terrestrial biosphere (Schlesinger 1977, Bowden 1987, Davidson and Janssens 2006, Marschner 2008). Wetlands occupy only 6% of the global landscape but accumulate a third of global SOM and the most C relative to other terrestrial ecosystems (Schlesinger 1997, Batzer and Sharitz 2006, Davidson and Janssens 2006). Wetland SOM pools are thought to be stable (Raich and Schlesinger 1992), but it is uncertain if SOM stability derives from its chemical composition or from other ecosystems properties that protect soil organic C (SOC) and soil organic N (SON) from decomposition. For instance, what happens to the stability of SOM when ecosystem properties, such as water table levels, are manipulated over decadal time scales? Although substantial progress has been made in documenting the chemical complexity of different detrital sources (Kögel-Knaber 2002), and in identifying the mechanisms by which soil aggregation and anoxia
reduce decomposition (Six et al. 2002, Fenner and Freeman 2011), much uncertainty remains in how these factors interact over long time scales to regulate in situ development of labile and stable SOM pools.

Land use changes from urban development and agriculture have caused substantial losses of wetland ecosystems globally. In the conterminous U.S., 53% of wetlands have been lost, with some Midwestern states converting over 80% of wetlands to agricultural land (Dahl 1990). Of all anthropogenic changes, suppression in wetland water levels and basin drainage for agriculture have had detrimental effects on wetland function, such as the storage of SOC and SON on the landscape. Lowered water levels increase oxygen availability, creating aerobic conditions in wetland soils and stimulating microbial decomposition of accumulated SOM pools (Haag and Lee 2010, Ye et al. 2009).

As a substantial storage pool of terrestrial C, soils have a potential for contributing global C feedbacks to already changing atmosphere chemistry (Schlesinger 1997). Due to large C stocks in soil, carbon dioxide (CO$_2$) emission from soil respiration is ten times the amount of anthropogenic contributions from fossil fuel combustion [50-75 Pg C/year compared to 5 Pg C/year] (Raich and Schlesinger 1992), so small increases in this large flux could dramatically add to atmospheric CO$_2$ loading. Of additional sources of CO$_2$, increasing soil disturbance from land-use change is the second greatest anthropogenic source to the atmosphere only outmatched by fossil fuel combustion (Le Quéré 2009, Erisman 2011). Wetland drainage, among other soil disturbances, decreases C storage in terrestrial landscapes and also decreases the future potential to sequester new
C and mediate effects of global climate change (Raich and Schlesinger 1992, Le Quéré 2009).

Enhancing microbial breakdown of SOM also alters wetland cycling and storage of SON. Aerobic soil conditions stimulate mineralization of SON into ammonium ($\text{NH}_4^+$). The $\text{NH}_4^+$ then undergoes nitrification and is transformed to nitrite ($\text{NO}_2^-$) and further to $\text{NO}_3^-$ (Schlesinger 1997). Increased $\text{NO}_3^-$ concentration in soils can pollute water sources and cause eutrophication of downstream rivers and estuaries (Vitousek et al. 1997, Erisman 2011). Aside from being a water quality pollutant, $\text{NO}_3^-$ can also undergo denitrification, producing nitrous oxide ($\text{N}_2\text{O}$) and $\text{NO}_x$ (nitric oxide, NO, and nitrogen dioxide, NO$_2$) (Hall et al. 1996, Vitousek et al. 1997, Hall and Matson 1999). As almost 300 times more potent than CO$_2$, N$_2$O is a long-lived greenhouse gas that has profound global warming potential even in small volumes (Erisman et al. 2011). Once produced, $\text{NO}_x$ can combine with ozone and contribute to photochemical smog, can be further oxidized to produce nitric acid (HNO$_3$) to increase acid rain deposition, and cause plant mortality (Schlesinger 1997, Vitousek et al. 1997, Gregg et al. 2003). N-fixation has already tripled from application and production of synthetic fertilizers, agricultural planting of N-fixing plants, and fossil fuel combustion (Galloway et al. 2004, 2008). The release of mineralized N from accumulated SOM pools, such as present in wetlands ecosystems, could continue to accelerate the global cycling of N. Increased mineralization of SON pools shifts the function of wetlands from a global sink of N into a source. The adverse consequences of accelerated C and N mineralization, coupled with the very large global pools of these elements in wetland soils, lend urgency to the
objective of the current thesis: identifying ecosystem properties that facilitate SOC and SON stability in wetland ecosystems.

Context

In Florida, wetlands occupy the most land area of any other U.S. state, covering over 11.4 million acres or 29% of the landscape (Haag and Lee 2010). Freshwater wetlands make up 90% of total wetland area in Florida (Dahl 2000, Dahl 2005, Haag and Lee 2010). Wetlands in Florida store more SOM and C per hectare than other terrestrial ecosystems (Craft and Chaing 2002, Ahn et al. 2009). With increased population growth, urban development, and continuing agricultural practices, Florida has lost the most wetland area of any state in the coterminous U.S. (Dahl 1990). It is important to understand the stability of the C and N currently stored in wetland soils because land use changes will continue to eliminate wetlands from the landscape or replace them with mitigated wetlands whose compensatory function is questionable (Mossman et al. 2012).

West-central Florida has a landscape characterized by low-relief and a shallow water table with strong hydrological connectivity between surficial water bodies and aquifers below. The hydrogeology of the area consists of three aquifers including, from deepest to shallowest, the Floridan aquifer, the intermediate aquifer, and the surficial aquifer (Haag and Lee 2010). The Floridan aquifer is a thick limestone sequence overlain by either the intermediate aquifer or a variably-confining clay layer. The intermediate aquifer is composed of variable sedimentary deposits and is primarily involved in the exchange of water between the other two aquifers (Arthur et al. 2008). The surficial aquifer resides in unconfined sandy deposits at the land surface. The
surficial aquifer is permeable to aquifers below in most locations and perched by lower semi-confining sediments in others (Haag and Lee 2010).

In Florida, closed-basins form on the landscape when karstic limestone bedrock below the surface collapses and causes overlying sand and clay sedimentary deposits to fill in void space within a newly-formed cavern. With little relief on the landscape, the subsequent slump in overlying sedimentary deposits fills in the void space creating basins close to the water table. These closed basins can be seasonally saturated or inundated with water table rise during the wet season creating wetlands (Haag et al. 2005). The water table in closed basins is largely controlled by the effects of rainfall and evapotranspiration in instances where the water table connected to the surficial aquifer. The effects of groundwater pumping on the potentiometric surface of the underlying Floridan aquifer are also important, however, especially where the intermediate aquifer is absent (Lee et al. 2009, Nilsson et al. In Press). In closed-basin wetlands in Florida, the water table level is the primary driver of soil moisture and soil redox conditions and is highly responsive to the fluctuating water table (Sun et al. 1995, Thomas et al. 2009).

Variability in wetland basin hydrology and wildfire frequency has created a landscape sprinkled with both forested and non-forested wetlands often in close proximity to one another. Forested wetlands, such as cypress domes, occur in basins characterized by longer hydroperiods and less frequent fire when compared to non-forested, herbaceous marsh wetlands (Duever et al. 1986). Forested and non-forested wetlands in also vary in organic matter chemistry and primary production (Brinson et al. 1981, Mitsch and Gosselink 2007, Bernal and Mitsch 2012). Cypress tree (*Taxodium ascendens*) litter has larger C:N ratios and a chemical composition higher in lignin
content when compared to herbaceous vegetation (Martin and Reddy 2010, Mahaney 2010, Bernal and Mitsch 2012). Cypress wetlands also have higher net primary productivity than freshwater marshes (Brinson et al. 1981). Though large differences exist in substrate quality in wetland vegetation, little work has been done comparing vegetative differences in SOM stability, especially in wetlands.

Similar to other coastal areas, west-central Florida has experienced population increases and rapid urbanization. Regional population growth has placed an increased demand on water supply from local aquifers, primarily the Upper Floridan aquifer (Haag and Lee 2010). The increased extraction of groundwater and prominent hydroconnectivity between aquifers has caused observable suppression of surface water levels in local wetlands (SWFWMD 1996). Because of the variability in hydrogeological connectivity between the surficial and Florida aquifer, individual wetlands have varied in water level response to increased ground water extraction. Some wetlands have experienced little notable changes to water levels and continue to have regular seasonal periods of inundation and hydric soils. By contrast, other wetlands have experienced decades of lowered water table level with increased soil oxidation and subsidence (Fig. 1) (Haag et al. 2005). In addition, decreased water levels in these wetlands have caused decreases in soil nitrogen and carbon storage (Powell 2008).

In the Tampa Bay region, spatial variability in wetland hydrology on the landscape, coupled with additional stresses from groundwater extraction, has created an experimental gradient of whole-ecosystem, long-term hydrology manipulations. Coupled with hydrology, contrasts in dominant wetland vegetation communities and soil mineralogy of isolated wetlands across the region have set up an opportunity to make
direct connections between ecosystem properties and SOM dynamics. Local land managers have recorded 25 or more years of water levels in individual forested and non-forested wetlands providing an ideal opportunity to quantitatively test vegetative, edaphic, and hydrological controls on SOM storage and stability.

**Figure 1**: Variability in wetland hydrological conditions between two cypress dome wetlands. Starkey Cypress “R” continues to have frequent periods of inundation while Starkey Cypress “D” has experienced shorter and less frequent periods of inundation. The baseline of each hydrograph represents the elevation at which each wetland is dry. Shifts in baselines are caused by relocating the staff gage or land managers performing hang augers to estimate subsurface water levels. Data courtesy of Southwest Florida Water Management District (SWFWMD).
Proposed Questions and Predictions

By exploiting landscape variability in isolated wetland ecosystem properties, I asked two questions regarding SOM dynamics. First, how do edaphic properties, such as mineral texture and bulk density, along with dominant vegetation type, influence SOC and SON stability in Florida wetlands? Soils with smaller mineral and organic particle sizes should encourage the stability of SOC and SON pools through soil aggregation and binding of SOM into mineral complexes. Smaller particle sizes should also increase soil water holding capacity keeping soils saturated for longer periods of time than with larger particle sizes. The size and stability of soil C and N pools should differ between wetlands with herbaceous vegetation and those dominated by woody-tissue plants, as many of the vegetative controls on SOM dynamics discussed above (i.e., litter chemistry, microclimate, etc.) likely differ between these contrasting plant forms.

The second question I ask in two components. First, does wetland hydrology regulate the size and stability of SOM pools in wetland soils? Secondly, if hydrology indeed has regulatory role, at what time scale does historical wetland hydrology influence SOM persistence? I predict that in wetlands with histories of minimal inundation, labile C and N pools will be small. A reduced hydroperiod (% of time a wetland is inundated) will have facilitated a history of aerobic conditions that support high heterotrophic respiration, which should disproportionately decompose the more easily mineralized labile pools (Fig. 2). By contrast, I predict that stable C and N pools will be unrelated to hydroperiod, as stable pools are inherently resistant to mineralization even under oxidizing conditions. In addition, if hydrology does have a regulatory role, it is important to indicate at what temporal extent historical hydrology drives the decrease
in C and N pools. I thus investigate whether pool size versus hydroperiod relationships are evident at each of several temporal extents (e.g. 6 months, 1 year, 5 years, etc. up to 25 years) over which wetland hydroperiod (% time aerobic) is calculated.

**Figure 2:** Hypothesized relationship between SOM stability and the percentage of time a soil exhibits aerobic conditions. As wetlands experience more time aerobic, only labile C and N pools will decrease owing to increased mineralization. Stable C and N pools, by definition, should not change with hydrological manipulation.
Methods

Study Area and Site Selection

To investigate these questions, closed-basin herbaceous marsh and forested cypress dome wetlands were chosen as study sites in Pasco and Hillsborough counties in west-central Florida (Fig 3). Wetlands were chosen in the Cypress Creek, Morris Bridge, and J.B. Starkey wellfields located in the northern Tampa Bay region. The wellfields are 3 of the 11 blocks of state-owned land set aside around Tampa Bay for the installation and maintenance of multiple wells that extract millions of gallons of water from local aquifers for municipal use. Besides minimal infrastructure to support groundwater extraction, this land is undeveloped, so all study wetlands are surrounded by a matrix of native pine flatwoods upland habitat. Five or six cypress dome and four or five marsh wetlands were selected in each wellfield, for a total of 16 cypress domes and 13 marshes (Appendix Tables A-1a through A-1c).

Each selected wetland had 25 years of surface water device observations recorded monthly from 1985 through the soil sampling date. Additionally, wetlands of each vegetation type for each wellfield varied in water table fluctuations over the 25 year time period. Given the intentional gradient in hydrology, sites differed in soil type. Most wetlands over the three wellfields contained three soil series classified as poorly-drained Inceptisols or Entisols, three wetlands were classified as Mollisols, and two as Spodosols (Appendix Tables A-1a through A-1c).
Figure 3: Isolated wetland locations in J.B. Starkey, Cypress Creek, and Morris Bridge wellfields. J.B. Starkey and Cypress Creek wellfields are located in Pasco County and the Morris Bridge wellfield in Hillsborough County.

Field Sampling

Prior to sampling, five equidistant linear transects were established radiating from the wetland edge inward to the deepest point of each basin (Fig. 4). Individual transects were initiated at the elevation of the basin edge, or normal pool, determined using the roots of saw palmetto (*Serenoa repens*). The lower limit of *Serenoa repens* was chosen as the indicator of normal pool because it was present at the wetland-upland edge of both cypress dome and freshwater marsh wetlands. The species is used as a wetland-upland boundary in pine flatwood habitats of Florida because of its very slow growth rate in newly established seedlings (Abrahamson and Abrahamson 2009). Seedlings take
decades to reach reproductive age minimizing the establishment of new individuals into the interior of wetlands. Saw palmetto is also resistant to landscape changes and has been well correlated with other historic normal pool indicators (Carr et al. 2006, Abrahamson and Abrahamson 2009).

![Wetland Elevation Zones](image)

**Figure 4:** Aerial view of established transects and soil core locations for an individual wetland. Elevation zonation of a wetland was pre-determined regulating agencies.

Each of the 5 transects intersected zones of elevation that correspond with pre-defined ecological zonation, and have thus been established for purposes of long-term hydrological and vegetation monitoring by environmental and water utility managers. Each wetland is split by elevation offsets from normal pool into transition, outer deep, and deep zones (Figs. 4 & 5) (SWFWMD and Tampa Bay Water 2005). The boundary separating the transition zone and outer deep zone is approximately a 15 cm elevational offset from the elevation of normal pool. An additional elevational offset of approximately 15 cm partitions the wetland basin into the outer deep and deep zones.
Figure 5: Vertical zonation of a wetland basin with soil core locations. Each of the 5 transects began at the base of *Serenoa repens* and ended near the deepest portion of the basin in close proximity to the wetland staff gage.

Soil core locations along each transect were determined using a manometer to measure normal pool elevation offsets of 10 cm and 40 cm (Fig. 5). Two 30-cm deep soil cores were taken on each of the five transects (one at the 10-cm offset and one at the 40-cm offset) using a sliding hammer corer or a constructed PVC corer for inundated soils. Both soil corers had a pointed tip and holes near the top of the core body to allow water and air from soil pore space to escape minimizing soil compaction when sampling. In each wetland, the five soil cores from the 10 cm offset were combined to form a composite transition zone sample, and the five soil cores from 40-cm offset were similarly combined to form a deep zone composite. Soil sampling for all wetlands was completed at the end of the wet season in the fall of 2010.

**Physical Soil Properties**

A 20 g soil subsample was dried at 60°C until a constant mass was reached to measure soil moisture and calculate bulk density. Once dry, this sample was later
combusted in a muffle furnace for 2 hours at 550°C for mass loss on ignition loss
calculation of soil organic matter (SOM) content (Cambardella 2001). The following
equations were used in the calculation of percent soil moisture (%$S_m$), bulk density ($D_b$)
and percent soil organic matter (% SOM), which were modified from Brady and Weil
(2008) and Robertson et al. (1999a):

Percent Soil Moisture:

$$% S_m = \left( \frac{[\text{wet field soil mass (g)} - \text{oven dry soil mass (g)}]}{\text{wet field soil mass (g)}} \right) \times 100$$

Bulk Density:

$$D_b = \frac{\text{oven dry soil mass (g)}}{\text{soil core volume (cm}^3\text{)}}$$

Percent Soil Organic Matter:

$$% \text{SOM} = \left( \frac{[\text{oven dry soil mass (g)} - \text{furnace combusted soil mass (g)}]}{\text{oven dry soil mass (g)}} \right) \times 100$$

Soil Texture

After combustion, mineral soil texture was determined using a modified version
of the rapid method (Kettler et al. 2001). A 15 gram subsample of post-combusted soil
was shaken in a plastic bottle with 45 mL of 3% sodium hexametaphosphate (HMP)
solution. After shaking was complete, the soil and HMP slurry were then wet-sieved
with the 3% HMP solution through a 53 μm sieve over a 600 mL beaker. Mineral material on the sieve was rinsed with water into a pre-weighed beaker and dried at 60°C and reweighed 48 hours later for the sand mass. Clay and silt particles that had passed through the sieve into the beaker were re-suspended in the solution. Settling time of silt was calculated similarly to the hydrometer method using Stokes’ Law (Gee 1986). After all silt had settled, the clay and HMP solution was decanted from the silt and the remaining silt portion was dried and weighed. The mass of silt was small relative to the sand mass so it was corrected for residual mass of HMP after drying. Clay mass was calculated as total initial mineral mass minus the mass of sand and silt fractions.

Particulate Organic Matter

Another dried subsample was used to categorize the physical size of SOM for each composited sample. This analysis divided the total SOM mass into particulate organic matter (POM) particles (>53 μm), and mineral associated organic matter (MAOM) (Camberdella et al. 2001). A dried 10 gram subsample was shaken in a plastic bottle with 30 mL of 3% HMP solution, then sieved through a 53 μm sieve. Material that did not pass through the sieve was collected, oven dried, and combusted with POM calculated as mass loss on ignition. MAOM was then calculated as total SOM minus POM.

Chemical Soil Properties

Total Soil C and N

Total C and N content were measured on a separate dried subsample that was milled using a Spex SamplePrep 8000M Mixer/Mill. Small masses of the milled samples
were rolled into 5x9 mm Costech tin capsules and combusted on a NC 2100 Elemental Analyzer. Atropine (70.56% Carbon, 4.84% Nitrogen) was used as an elemental standard and replicated for each sample plate.

**SOC and SON Incubation Biological Assays**

Labile C and N were separated from the respective stable pool using a year-long biological assay (Nadelhoffer 1990, Robertson and Paul 2000, Kaye et al. 2002a). Labile C and N pool sizes are defined as the amount of C and N mineralized over a one year period under conditions that intentionally maximized mineralization of extant C and N available at the time of soil collection. Soils were thus incubated for a year in warm, moist, aerobic conditions. Throughout the year soils were repeatedly leached with a C- and N-free leaching solution to deprive microorganisms of mineral nutrients, and no additional C or N was added to the soil subsample forcing microbial decomposition of SOC and SON initially present in the soil. In summary, this assay identifies biogeochemical pools, namely, the fractions of soil C and N that are potentially mineralizable at the time of sample collection; the assay is not necessarily indicative of actual field mineralization rates.

The incubations were initiated with a 50 g subsample of the field composite sample and were incubated at 25°C for 365 consecutive days. Soil samples were sprayed daily with deionized water to prevent water limitation and structural changes caused by drying. During the incubation, soils were nested in a series of filters in a 93mm diameter perforated Buchner cups with attachable funnels. A thick glass fiber 9 cm filter (Whatman GF/F), followed by a thinner 9 cm glass fiber filter (Whatman GF/D) and
finally layer glass wool were all placed beneath the soil respectively. An 11 cm glass fiber filter (Whatman GF/D) was placed over the top to prevent soil dispersion (Fig. 6).

![Diagram](image)

**Figure 6:** Sample leaching apparatus for the year-long carbon and nitrogen bioavailability assay.

Labile C was estimated as the amount of SOC mineralized to CO$_2$ throughout the year-long incubation. To quantify mineralized C, daily CO$_2$ flux was measured daily on 30 days during the year: 1, 3, 7, 24, 32, 39, 51, 61, 72, 81, 93, 107, 126, 143, 157, 178, 184, 199, 212, 227, 240, 256, 268, 284, 298, 318, 332, 347, and 365. After all 30 measurements had been made, daily CO$_2$ flux rates were integrated over the year to estimate the labile C pool.

To measure a daily CO$_2$ flux, Buchner cups were sealed individually in airtight 1 L plastic jars fixed with septa on the lids. Headspace CO$_2$ concentration was measured at the time of initial seal and repeated through the septa after 24 hours. Headspace samples
were collected using a syringe with a removable needle and inserted into an airtight flowpath in a static benchtop PP Systems EGM-4 infrared gas analyzer. The difference between CO₂ concentration between the initial seal and 24 hours later was converted to µg of C per g of soil per day (Appendix Figs. A-1a and A-1b).

Similarly to labile C, labile N was estimated as the amount of N mineralized during the year. To determine mineralized N, and to minimize re-immobilization of mineral N, soils were leached 15 times using a low-ion, C- and N-free leaching solution following a measurement of CO₂ flux. The leaching solution was composed of micro-nutrients as follows: 4.0 mM CaCl₂, 2.0 mM KH₂PO₄, 1.0 mM K₂SO₄, 1.0 mM MgSO₄, 25µM H₃BO₃, 2.0 uM MnSO₄, 2.0 uM ZnSO₄, 0.5uM CuSO₄, and 0.5 uM Na₂MoO₄ (Nadelhoffer 1990). During each leaching event, soils were submerged in 200 mL of leaching solution and allowed to equilibrate with the solution for an hour. To collect the leachate, funnels were attached to the bottom of each Buchner cup and placed over a sidearm flask using a rubber stopper. The sample apparatus was attached to a vacuum pump through a hose to the sidearm flask until the added leaching solution had been collected. Leaching events took place on incubation days 1, 3, 7, 16, 24, 39, 61, 81, 107, 143, 184, 227, 268, 318 and 365.

The collected leachate was analyzed for NO₃⁻ and NH₄⁺ using microplate analysis on a Biotek Epoch microplate spectrophotometer (Doane and Horwath 2003, Hood-Nowotny 2010). Standards for NO₃⁻ were prepared from KNO₃ powder dissolved in the leaching solution to known concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1, and 2 mg N/L. Triplicate 150 µL aliquots of each sample and prepared standards were pipetted into a well in a flat bottom 96-well plate along with 150 µL of a reducing reagent composed of
vanadium chloride (III), sulfanilamide, and N-(1-naphthyl)ethylenediamine dihydrochloride. The vanadium chloride reduced NO$_3^-$ to NO$_2$ which when interacting with sulfanilamide causes a color change reaction. Well plates were covered with parafilm and placed into a dark drawer overnight. The following morning the well plate was analyzed for NO$_3^-$ as absorbance at a wavelength of 540 nm.

To prepare NH$_4^+$ standards, NH$_4$Cl powder was dissolved into a leaching solution matrix in the same known concentrations ranging from 0 to 2 mg N/L. Triplicate 175 µL volumes of each sample and standard was pipetted into individual wells of the 96-well plate. Three prepared reagents were subsequently added to each well in the following order and volume: 25 µL of citrate, 50 µL of salicylate-nitroprusside, 25 µL of hypochlorite. The well plate was placed on a reciprocating well plate shaker at 400 rpm for 50 minutes. After shaking was complete, the well plate was immediately read on the Biotek Epoch microplate spectrophotometer at 650 nm. Gen5 software was used to collect, modify, and export raw data for both NO$_3^-$ and NH$_4^+$ analysis (Appendix Figs A-2a and A-2b).

**Microbial Biomass**

Microbial biomass N was measured at the end of the incubation assay as an index of actively cycling N still present and the completeness of bioavailable N removal. Microbial biomass N was determined with chloroform (CHCl$_3$) fumigation using a direct extraction as described in Brookes et al. (1985) and modified by Perakis and Hedin (2001). Following the year-long incubation, two 10 g subsamples of the post-incubation
soil from each Buchner cup was processed for microbial-N biomass. One 10 g soil subsample was placed into a 125 mL Nalgene bottle with 50 mL of 0.5M K₂SO₄ and shaken for an hour. The soil slurry was then gravity filtered through a Fisherbrand G6 filter into a flask. The collected filtrate was analyzed for NO₃⁻ and NH₄⁺ using the same method described in the year-long biological assay with a 0.5M K₂SO₄ standards matrix.

Filtrate was analyzed for total dissolved N (TDN) using a potassium persulfate oxidation (K₂S₂O₈) reagent (D’Elia et al. 1977, Cabrera and Beare 1993). Ammonium oxidation standards as well as alanine (C₃H₇NO₂) and nicotinic acid (C₆H₅NO₂) organic N digestions standards were prepared in known concentrations of 2.0 and 5.0 mg N/L in 0.5M K₂SO₄. Then 10 mL of each sample and standard with 10 mL of K₂S₂O₈ were combined in tightly sealed glass vials. All samples and standards were then autoclaved at 121°C for 40 minutes. Once cooled, digested samples and standards were analyzed on the microplate reader for NO₃⁻ concentration. If sample dilutions were necessary, the digested blank (0 ppm) standard was used to dilute high concentration samples.

The second 10 g soil sample was placed into a 250 mL amber bottle with 0.6 mL of CHCl₃ and sealed immediately under a fume hood for 5 days (Brooks et al. 1985). After 5 days the sample bottles were opened and allowed to aerate for an additional 3 days. Following the three day aeration period, samples were placed with the bottle open into a vacuum desiccator. Using a vacuum pump the atmosphere in the desiccator was exhausted, removing remaining CHCl₃ from the soil. Each sample was exhausted 3 times. After exhaustion of the sample, chloroform fumigated soils were processed similarly to the direction extraction (unfumigated) subsample, above. Post-fumigated samples were extracted with 0.5M K₂SO₄ and the extracts were run for TDN.
To calculate N associated microbial biomass, differences in TDN were calculated between the fumigated and unfumigated extracts (shown below). The values were not corrected for a fumigation efficiency factor, \( k \), since estimates of this value for wetland soils in Florida are unknown (Robertson et al. 1999).

Microbial Biomass N (mg N / g dry soil) = \( N_F - N_c \)

Where

\[ N_F = \text{N concentration in fumigated K}_2\text{SO}_4 \text{ extract (mg N/g dry soil)} \]

\[ N_C = \text{N concentration in unfumigated K}_2\text{SO}_4 \text{ extract (mg N/g dry soil)} \]

Mineral N Extractions

A 10 gram subsample of the field composite was extracted in 50 mL of 2M KCl to measure extractable inorganic N prior to the biological assays (Robertson et al. 1999). Soil was placed in a 125 mL nalgene bottle with 50 mL of 2M KCl and shaken for one hour. The soil slurry was then gravity filtered through Fisherbrand G6 (nominal pore size 1.6 µm) filters over a flask. Collected filtrate was analyzed for NO\(_3^-\) and NH\(_4^+\) using a similar method to the leachate samples from the year-long biological assay. The standards prepared with the KNO\(_3\) and NH\(_4\)Cl powders were dissolved in a 2M KCl matrix of known concentrations ranging from 0 to 2 mg-N/L in the same increments as the leachate analysis. Because of the potentially large concentrations of NH\(_4^+\) in the extraction samples, a smaller volume of 60 µL of sample was used along with 115 µL of 2M KCl matrix to dilute the NH\(_4^+\) in the sample.
Hydrological Data Analysis

Hydrological data for the selected wetlands were provided by the Southwest Florida Water Management District (SWFWMD) and Tampa Bay Water (TBW), a water resource regulator and wholesale water provider, respectively, charged with monitoring wetlands for ecological effects of groundwater withdrawal. Data from wetland wells and the wetland staff gage were provided in raw form. Staff gage data were used as the primary data source and were manually composited with supplemental wetland well data when water was below the surface elevation of the wetland (i.e. below the lowest reading on the staff gage). In instances of a recorded dry staff gage before a wetland well was installed, the water level was necessarily below the elevation of both soil cores. If data were noted marked missing or compromised from device error by the field observer, they were removed from the period of record.

Once a manual composite had been organized for all recorded water level observations, the period of record was truncated to the 25 years preceding the exact date of the soil sampling event. The composited hydrological record of each wetland was graphed with the known elevation of individual soil cores from each zone in a sampled wetland. During the hydrological analysis, a soil core was considered inundated if water level elevation was greater than the elevation of the top of the soil core. The percent time each soil core spent aerobic (defined as not inundated) or inundated, frequency of inundation (the number of inundating events over a time period), average duration of inundating events, and longest duration of an inundating event were calculated for each wetland. These parameters were calculated using 6 month, 1 year, 5 year, 10 year, 15 year, 20 year, and 25 year windows preceding sample collection (Fig. 7).
Figure 7: Method of analysis for calculating parameters of hydrology by blocks of time prior to the date of soil sampling. This method was used to calculate percent time aerobic, duration of inundating events, frequency of events, and longest duration of an inundating event for both soil core elevations.

Statistical Analysis

Statistical analyses were performed using the student edition of SPSS Statistics version 20. Split plot analysis of variance (ANOVA) was used to investigate the response of soil traits to the interactive influence of wetland vegetation type and zone
with wetland site number (unique to each wetland) treated as a random factor. When testing for significance of vegetation type on a soil trait, the denominator of the $F$-ratio was the mean square of the wetland number nested within vegetation type. To test the effects of zone and the vegetation type x zone interaction, the denominator of the $F$-ratio was the mean square of the interaction between zone and the wetland number nested within the vegetation type: zone x wetland number (vegetation type). Significant effects of vegetation type were taken to indicate support for the first hypothesis, that vegetation type influences stable and labile C and N pools. A transition zone vs. deep zone comparison, because of basin characteristics, is one test of the hydrology hypothesis. The transition and deep zones of a wetland control for vegetation type while contrasting inundation time. Thus, a significant zonal effect is support for the hydrology hypothesis, particularly if deep zone C and N pools, in compared with transition zone pools, have a larger proportion of labile material.

In addition to the statistical tests by wetland zone described above, the hypothesis regarding hydrological controls on SOM pool size and stability was also tested among wetlands by evaluating whether labile and stable C and N pools correlated with hydroperiod using linear regression. This analysis only used the cypress deep zone samples. In the remaining three treatments (vegetation type x elevation zone combinations), hydrological variance was small, with limited variability in time soil cores spent under aerobic conditions. Marsh deep zones ranged in time spent aerobic from 67.4% to 100% while cypress deep zones ranged from 20.4% to 99.8% over the 25 year hydrological record. Thus cypress deep zones provided a more informative contrast in hydrological extremes, while controlling for a common vegetation type and elevation
zone. SOM pools were also the largest and most variable in this wetland type and zone (see Results). These continuous variables were analyzed using linear regression. All statistical tests were significant with $\alpha \leq 0.05$. 
Results

**Ecosystem Properties of Marsh and Cypress Wetlands**

Several ecosystem properties differed significantly between cypress dome and herbaceous marsh wetlands (Tables 1 and 2). In cypress wetlands, deep zone bulk density was significantly lower than in the transition zone (Table 1). Of all vegetation type x zone combinations, cypress deep zones had the lowest bulk density values and the most variability. In marsh wetlands, bulk density did not significantly vary between transition and deep zones.

Mineral texture almost entirely comprised sand for both cypress dome and marsh wetlands (Table 1). Cypress deep zones had significantly lower sand content with a larger proportion of silt and clay than other vegetation types and zones (Table 2). Though mineral texture varied among treatments, all soil samples were classified as sand using the NRCS textural triangle classification (Brady and Weil 2008). POM was not significantly different between wetland types or zones, however, freshwater marsh deep zones had the smallest fraction of POM by percent mass.

Soil moisture varied significantly between wetland vegetation type, zone, and their interaction (Table 2). Cypress deep zones had the highest average soil moisture content with the most variability within a treatment group (Table 1). In cypress deep zones, soil moisture ranged from 12.5% to 61.6%. Marsh transition zones had the lowest average, minimum and maximum soil moisture content by mass in comparison to all
other zones. Soil moisture in the marsh deep zones was greater than in marsh transition zones but was only about half that of cypress wetland deep zones.

Table 1: Summarized average values for measured ecosystem properties.

<table>
<thead>
<tr>
<th>Ecosystem Property</th>
<th>Cypress</th>
<th>Marsh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deep</td>
<td>Transition</td>
</tr>
<tr>
<td>Bulk Density (g/cm$^3$)</td>
<td>0.9 (0.1)</td>
<td>1.3 (0.0)</td>
</tr>
<tr>
<td>% Soil Moisture</td>
<td>32.8 (3.5)</td>
<td>18.6 (1.6)</td>
</tr>
<tr>
<td>% Sand</td>
<td>91.9 (0.9)</td>
<td>96.1 (0.2)</td>
</tr>
<tr>
<td>% Silt</td>
<td>6.1 (0.7)</td>
<td>2.9 (0.2)</td>
</tr>
<tr>
<td>% Clay</td>
<td>2.1 (0.3)</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>% POM</td>
<td>48.1 (3.2)</td>
<td>49.7 (3.8)</td>
</tr>
<tr>
<td>% SOM</td>
<td>8.4 (1.1)</td>
<td>3.5 (0.3)</td>
</tr>
</tbody>
</table>

Note: Summarized mean (one standard error) values for each measured ecosystem property. See full ANOVA table results in the Appendix Table A-2a.

Table 2: $P$-values for physical attributes from split plot ANOVAs.

<table>
<thead>
<tr>
<th>Ecosystem Property</th>
<th>Vegetation Type</th>
<th>Elevation Zone</th>
<th>Interaction $(Vegetation Type \times Elevation Zone)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Density</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% Soil Moisture</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% Sand</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>% Silt</td>
<td>0.001</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>% Clay</td>
<td>0.003</td>
<td>0.005</td>
<td>0.011</td>
</tr>
<tr>
<td>% POM</td>
<td>0.664</td>
<td>0.105</td>
<td>0.227</td>
</tr>
<tr>
<td>% SOM</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Summarized $P$-values for split-plot ANOVA statistical testing of response of ecosystem properties to vegetation type, elevation zone, and the interaction. See full ANOVA table details in Appendix Table A-2a.
Percent SOM by mass contrasted significantly among wetland vegetation type and elevation zones (Table 2). Overall, the pool of SOM was significantly larger in cypress wetlands than in marsh wetlands (Fig. 8). Deep zones in cypress wetlands possessed the most organic matter, with over double the SOM than in deep zones in marsh wetlands. On average, SOM was similar in marsh transition and deep zones. Percent SOM had the greatest variability in cypress deep zone, with values ranging from 3.1% to 20.6% (standard deviation 4.6%).

![Figure 8](image)

**Figure 8**: Average percent SOM by soil mass for each wetland vegetation type and zone. Error bars are one SEM.

Cypress domes had significantly larger SOC and SON pools than did marsh wetlands (Fig. 9, Split-plot ANOVA, vegetation type $P<0.001$). When considering only the zonal treatment, on average, SOC and SON pools were also significantly larger in wetland deep zones than in wetland transition zones. These two main effects (larger pools in cypress than in marsh wetlands and in deep zones than in transition zones)
appear to be driven by very large pools in cypress deep zones, relative to smaller and similar pools among the other three vegetation type x elevation zone treatments. Among all four vegetation type x zone combinations, cypress deep zones had the largest SOC and SON pool as well as the most variability (40.25 ± 4.26 g C/kg\textsuperscript{-1} soil, 2.00 ± 0.26 g N/kg\textsuperscript{-1} soil).

**Ecosystem Properties as Regulators of SOM Stability**

Like total C and N pools, labile and stable C and N pools varied significantly among the four treatment groups (Fig. 9, Split-plot ANOVA, vegetation type x zone, labile C \(P=0.005\), labile N \(P=0.001\), stable C \(P=0.001\), stable N \(P=0.001\)). Labile C and N pools were two to three times greater in cypress deep zones than in the other three vegetation type x elevation zone treatment combinations. Labile C and N pools were smaller in marsh than in cypress wetlands, with labile C lowest in the marsh deep zones and labile N lowest in the marsh transition zones. Labile C and N pool sizes were also influenced by total SOC and SON pool sizes (Linear regression, labile C vs. total SOC, \(R^2 = 0.758\), \(P<0.001\) and labile N vs. total SON \(R^2 = 0.655\), \(P<0.001\)). The microbial N pool measured in incubated soil at the end of the incubations was relatively small, containing < 1.0% of the total SON pool, indicating that the incubation was effective in removing actively cycling N from the soil and thus in quantifying labile material. Though a small pool, this post-incubation microbial N was greatest in cypress deep zones (8.4 ± 1.04 mg-N/kg\textsuperscript{-1} soil) and lowest in marsh transition zones (3.2 ± 0.37 mg-N/kg\textsuperscript{-1} soil).
Figure 9: Labile and stable SOC and SON pools by wetland vegetation type and zone. Note the difference in vertical scales among panels. Error bars are one SEM. Full details for each ANOVA are in the appendix (Table A-2b).

Labile C and N composed only a small portion of the total SOC and SON pool (Figure 10). In cypress deep zones, labile C only averaged 5.47% of the total SOC pool,
and was a smaller proportion of total SOC than in other vegetation type x zone combinations. Although cypress dome deep zones had the largest labile C pools, their total SOC pools were most strongly dominated by stable C (contrary to the hypothesis, as explored in the Discussion). Marsh deep zones had the highest variability in the proportion SOC that was labile, with values ranging from 2.7% to 13.0%. Labile N pools were only a small fraction of the total SON pool over all treatments, ranging from 2.8% to 13.2%. Labile C and labile N as proportions of total SOC and SON were significantly lower in deep zones than in transition zones (Split–plot ANOVA, proportion labile C vs. zone $P=0.010$, proportion labile N vs. zone $P<0.001$), but did not respond to vegetation type or the vegetation type x zone interaction.

**Figure 10:** Labile SOC and SON as a percent of total soil C and N pool sizes. Error bars are one SEM.
Aside from wetland vegetation type and elevation zone, other ecosystem properties were significant indicators of SOM stability. Percent soil organic matter was the best predictor of both stable C and stable N pool sizes of all measured ecosystem properties (Table 3). Stable C and N pools had similar statistical values, suggesting that both pools are highly responsive to soil ecosystem properties. Stable C and N pools responded similarly to measured ecosystem properties both in significance (P-values) and variability (R values) (Table 3). Both stable C and N pools did not respond significantly to particulate organic matter. Stable N significantly responded to soil C:N, however, it had a low R² value illustrating a highly variable relationship.

Table 3: Ecosystem properties linear regression with stable SOM pools.

<table>
<thead>
<tr>
<th>Ecosystem Property</th>
<th>Stable C (mg C g⁻¹ soil)</th>
<th>Stable N (mg N g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Bulk Density (g/cm³)</td>
<td>-0.858</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% Soil Moisture</td>
<td>+0.860</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% Sand</td>
<td>-0.666</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% POM</td>
<td>+0.032</td>
<td>0.989</td>
</tr>
<tr>
<td>% SOM</td>
<td>+0.946</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Microbial N (mg N g⁻¹ soil)</td>
<td>+0.767</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C:N Ratio</td>
<td>-0.214</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Note: The table above illustrates linear regression relationships between stable SOM pools and measured ecosystem properties. A separate linear regression analysis was performed between each ecosystem property and stable C and N pool sizes. For each linear regression N=58 (16 cypress wetlands and 13 marsh wetlands x 2 zones) total replicates.

**Hydrological Controls on SOM Stability in Cypress Wetlands**

In both zones of marsh wetlands, soils were aerobic (not inundated) 86-100% of the time, whether observed for 6 months or 25 years prior to soil sampling. There was
thus minimal hydrological variability among marsh wetlands, regardless of the temporal extent of observation. Owing to the small amount of variability in percent time aerobic observed in marsh in cypress dome transition zones, the relationship between biogeochemical pool sizes and percent time aerobic was explored for only the cypress dome deep zones. Percent time aerobic was used as the primary calculated hydrological metric since it had the strongest statistical relationship with C and N pool sizes than the other calculated hydrological attributes (frequency of inundation, average event duration, and longest duration of an inundating event). It is important to note, however, that cypress deep zone soils on average had longer flooding events which occurred more frequently than in marsh deep zones (Appendix Fig. A-3).

Figure 11: Labile C pool size vs. percent time a wetland was aerobic. Labile C was compared against 1 year (left) and 25 years (right) prior to sample collection. Note the shift in the x-axis scale and the curvilinear relationship between labile C as a function of hydrology for both hydrological time spans. An exponential decline model was not fit to the data because the range of the x-axis was truncated at 100% aerobic thus estimating an asymptote was not possible or valid. There was more variability around the relationship between labile C and hydrology for the longer 25-yr period of record.
Figure 12: Stable C pool size vs. percent time a wetland was aerobic. Again, stable C was plotted during 1 year (left) and over 25 years (right) prior to sample collection. A linear regression was performed for stable C pool size vs. percent time aerobic for all cypress deep zone wetlands (n=16). Again note the increase variability in the relationship with longer hydrological records.

In cypress deep zones, wetlands with greater time in aerobic conditions contained significantly smaller pools of total SOC and SON (linear regression, SOC vs. % time aerobic over 25 years, \( P<0.001, R^2 = 0.678 \) and SON vs. % time aerobic over 25 years \( P<0.001, R^2 = 0.454 \)). Labile C and N pool sizes decreased sharply with increasing percent time that wetlands were aerobic, regardless of the observation window (6 months - 25 years) over which percent time aerobic was calculated (Fig. 11). Labile C and N had a decreasing, curvilinear relationship with percent time aerobic and became increasingly variable as the hydrological analysis included longer periods of record. Labile C and N pools declined sharply as wetlands approached 50% aerobic conditions, and declined gradually toward a lower non-zero limit as wetlands approached 100% aerobic conditions. Labile C and N as a percent of total C and N did not correlate with any calculated hydrological parameters.
Figure 13: Strength of C and N pool versus hydroperiod relationships at multiple temporal scales of hydroperiod analysis. The relationship between stable element pool size and % time a wetland is aerobic ($R^2$) plotted against the temporal extent over which wetland hydrograph data were evaluated to calculate % time aerobic. Stable C and N versus the changing period of record length in cypress deep zones. Stable C and N pool sizes were in mg per g-1soil. The zero time point is % soil moisture of the soil on the date of soil sampling. The linear relationships had a negative slope value with the exception of percent soil moisture which was positive. The critical Pearson’s correlation coefficient for a two-tailed test was $R=0.426$ for n=16 (d.f.=14) and is squared in the figure above to illustrate all regression $R^2$ values were highly significant ($P \leq 0.002$).
Discussion

The purposes of this study were to document variability in soil properties and determine the primary controls on SOM stability in closed-basin freshwater marsh and cypress dome wetlands in west-central Florida. Wetland vegetation type, soil texture, soil moisture, and organic matter content were tested as controls on C and N pool size and stability. Variability in inundation time both (i.) between elevation zones within wetlands and (ii.) among cypress dome wetlands provided opportunistic experiments to test the hypothesis that SOM stability is regulated by wetland hydroperiod at temporal scales ranging from days to decades.

SOM by Vegetation Type and Zone

Addressing the first proposed question, most ecosystem soil properties varied between forested and non-forested wetlands and had highly significant effects on both SOM pool size and stability. Bulk density, soil moisture, texture, and soil organic matter content all significantly varied between marsh and cypress wetlands and both wetland zones (Tables 1 and 2). On average, marsh wetlands had higher bulk densities and lower percent soil moisture, which may be explained by the lower organic matter content and coarser textured sediments (Table 1, Fig. 8). The high sand content increases soil porosity, rapidly decreasing water holding capacity and soil moisture. In addition, the results of this study are consistent with other studies that have determined that low
amount of SOM also decreases a soil’s capacity to retain water, especially in sandy soils (Hudson 1994, Rawls 2003).

Cypress dome wetlands had significantly higher amounts soil organic matter, total carbon, and total nitrogen than isolated freshwater marsh wetlands (Figs. 8-9). Larger SOM pools in cypress wetlands may reflect basin development. Cypress wetlands form in basins characteristic of longer duration of inundation keeping soils anaerobic longer (Duever et al. 1986). The Cypress basins in this sample set, as represented by the deep zone soil core, were also characterized by longer periods of inundation and a greater number of inundating events than marsh basins (Appendix figure A-3). Through increased inundation and anaerobic soil conditions, conditions protecting SOM from microbial decomposition would allow larger C and N stocks to accumulate in cypress domes than in marsh wetlands. Cypress wetlands also had finer sediments than marshes which could mediate leaching loss of small SOM particles through binding to cation exchange sites. In addition, the smaller particle sizes would also increase water holding capacity and keep soils saturated longer.

Aside from the abiotic characteristics of the wetland basin, wetland vegetation creates variability in ecosystem properties. Cypress dome wetlands promote higher SOM accumulation because cypress trees have greater litter inputs from higher net primary productivity than in herbaceous marshes (Brinson et al. 1981). As the dominant component of ecosystem structure, cypress tree canopies also mediate light incidence more than marsh grasses by decreasing solar radiation from 2096 kcal/m$^2$ x day outside the canopy to 596 kcal/m$^2$ x day inside of the vegetation cover (Mitsch 1986). In cypress domes, the decrease in solar radiation from canopy cover could possibly create isolated
pockets of decreased soil temperature which could be inhibiting to decomposition. Lignin is also a primary component of cypress tree litter similar to upland pine species (Martin and Reddy 2010). This provides microorganisms a more complex substrate for respiration and could allow the accumulation of litter, specifically stable litter, in this wetland type (Figure 9 and 10).

Since SOM is the primary source of SOC and SON, it is not surprising that cypress wetlands with higher amounts of SOM also have larger C and N pools than freshwater marsh wetlands (Fig 9). On average, C pool sizes by soil mass in this study are similar to previously measured C pools in Florida wetlands, which are >3X the pool sizes observed in other terrestrial habitats and land uses in Florida (Silveria et al. 2008, Ahn et al. 2009). In cypress domes, soil C and N pools were similar to those found in cypress domes in nearby Orange County, Florida (Coultas and Duever 1986).

Similar to other SOM fractionation studies of wetlands both in Florida and in northern habitats, stable C and N pools dominated the total C and N pools in both wetland vegetation types (Bridgham et al. 1998, McLatchey and Reddy 1998, Kaye et al. 2002b, Ahn et al. 2009). Though not a large proportion of total C or N, labile C and N pool sizes were heavily influenced by the total amount of C and N in a given treatment group, and were thus highest in cypress deep zones (Figs. 9 and 10).

In comparison to marsh wetlands, cypress deep zones may have larger labile C and N pool sizes based on larger total C and N pools. Even with differences in labile C and N pool sizes, marsh and cypress wetlands had similar percentages of labile C and N to total C and N pool size (Fig. 10). The similarity between the two vegetation types was
also supported by the non-significance between the proportion of labile C and N as a function of total C and N. Thus it is unclear if vegetation litter chemistry as an inherent quality of an ecosystem dictates labile pool sizes or proportions of labile C and N to the total pool (Kleber and Johnson 2010, Dungait et al. 2012). It does, however, suggest that additional ecosystem properties other than litter chemistry complexity and vegetative structure must be influencing SOM stability in these ecosystems.

Stable SOM pool sizes were more responsive to the total amount of organic matter present in the soil and the percent soil moisture than to other ecosystem properties (Table 3). Since labile C and N pools were only a portion of the total C and N pools, it is not surprising that stable SOM pool sizes are heavily dependent on total SOM in a wetland. The most interesting observation is the large significant dependence of stable SOM pool sizes on soil moisture in a wetland soil. The high significance suggests that soil moisture, regulated by wetland hydrology, plays a dominant role as an ecosystem property regulating SOM persistence.

Hydrological Influence on SOM Stability

The hydrology hypothesis between labile C and N pool size and wetland zonation treatments had significant, yet unsupportive results. While stable C and N pool sizes (mass element per gram soil) responded to several factors, labile SOC and SON as a proportion of total C and N only responded to wetland zonation (Fig. 10). The proportion of labile SOM relative to total SOM pool sizes was larger in transition zones regardless of wetland vegetation type. Therefore, the wetland zone that had experienced
more time inundated (the deep zone) had more SOM, but did not contain the largest proportion of labile SOM relative to total SOM. The observation that deep zones had lower proportions of labile SOM does not support the hypothesis that inundation protects large labile SOM pools from decomposition. Rather, it suggests that deep zones, which are inundated more frequently due to basin morphology, accumulate stable SOM at a quicker rate than they accumulate labile SOM. The observed relationship between the proportions of labile SOM with zone suggests once again the increasing importance of hydrology as a primary ecosystem control on SOM stability.

Two differing mechanisms could explain increased SOM stability in zones with increased soil inundation. First, inundation may lead to accumulation of stable SOM. This will be discussed in greater detail in the succeeding section. Second, water limitation may occur in the transition zones of wetland perimeters causing stocks of labile SOM to accumulate until water is reintroduced. In Florida, over 60% of precipitation happens during summer, with November through February being the driest with drought-like conditions (Stankey 1981). After water tables in wetlands drop in late summer, water could limit microbial activity especially in areas with little vegetative cover (Mitsch 1986). Water limitation of microbial activity in the transition zone could be exacerbated by poor moisture retention in the sandy, organic matter-depleted soils (cf. Sponseller 2007).

The second way the hydrology hypothesis was addressed in this study is best discussed as two components: first, the effect of hydrology on SOM stability, and second, the timescale at which this relationship is evident. It is important to recall that during this study, labile SOC and SON pools were fractionated from the total SOM pool through an
incubation assay that was intentionally designed to maximize mineralization rates. Microorganisms were incubated in aerobic, moist, and warm conditions, and persistently deprived of mineral N by repeated leaching.

**Modified Relationship:**

![Modified Relationship Diagram](image)

**Figure 14:** Modified relationship between SOM stability and increased time a wetland underwent aerobic conditions. As time spent aerobic increases, labile and stable C and N pools decrease.

Based on the assumption that stable pools do not readily turnover, it was expected that only the labile pool would decompose under aerobic conditions, and that total SOC and SON pool sizes would vary according to hydrological effects on labile pools (Fig. 2). However, the results of this study indicate a need to modify the original hypothesized relationship between percent time aerobic and C and N pool dynamics (Figs 11 and 12). The modified relationship illustrates that as wetlands experienced increasingly aerobic conditions, both labile and stable SOM pools decreased but at different rates of decay (Fig. 14). Labile pools exhibited an exponential decay type relationship with hydroperiod, declining rapidly with even small amounts of time spent in aerobic
conditions (Fig. 11). Stable pools, on the other hand, decreased steadily with decreasing hydroperiod (Fig. 12).

The decrease in labile C and N pools happens more rapidly with decreasing hydroperiod than predicted in the conceptual model (Figs. 2 and 11). The observation that labile C and N pools decrease non-linearly has a direct impact on the persistence time of even potentially short-term pools. Even with small hydrological changes, i.e. increasing time spent aerobic by 20% in a year, can cause a rapid decline in labile C and N pools. The mineralization of the labile C and N pools can have direct consequences to the release of potential air and water quality pollutants. Thus it is important to preserve accumulated labile C and N pools by keeping wetlands inundated, especially in those wetlands which have limited experience under aerobic conditions.

Stable pools, on the other hand, continually decreased with increased exposure to aerobic soil moisture conditions at a lower rate (Fig 12). Decreasing stable pools is contrary to the expectation that stable pool size would not change with increased exposure to aerobic soil conditions. Several mechanisms could explain the decrease in stable pools with suppression of wetland hydrology. One explanation can be the release phenol oxidase, an enzyme that triggers decomposition. As in situ wetland soils become aerobic, the phenolic oxidase enzyme becomes available, and microbial activity breaks down aromatic phenolic compounds from the SOM pool into smaller degradates. Once phenols are broken into smaller degradates, a variety of additional enzymes are triggered, and the decomposition cascade can take place on the smaller organic molecules (Freeman et al. 2004, Fenner and Freeman 2011). Large stable SOM pools in mostly inundated wetlands may, therefore, result from the accumulation of phenolic compounds in the
absence of phenol oxidase under anaerobic conditions. It is true that, if phenolic compounds require anoxia, these compounds should have decomposed and been counted as “labile” by my aerobic incubations. However, at the outset of the year-long incubation, a phenol oxidase “jump start” needed to occur before the decomposition cascade could begin. Thus mineralization rates of incubated soils from inundated wetlands would have been smaller relative to those from aerobic wetlands.

A larger stable pool (calculated as total minus labile) under anaerobic conditions could also be explained through underestimates of the labile pool due to soil aggregation processes. As wetlands become more aerobic, the total organic matter pool decreased. As the primary driver of aggregate formation in sandy soils, loss in SOM promotes loss of soil structure, in turn decreasing aggregate formation (Chaney and Swift 1984, Carter 2002). Therefore, wetlands that are increasingly anaerobic, through SOM accumulation, could have more potential labile C and N protected in soil aggregates. Aggregate formation and stability increases with soils that undergo several wet and dry cycles (Denef et al. 2001). Thus wetlands that only spend a short time aerobic (few wet-dry switches) have not experienced conditions that promote soil aggregate formation. With few dry/wet cycles, thus aggregates, C and N pool will be more susceptible to decomposition, thus accounted for in the labile C and N pool sizes.

An additional interpretation of the modified relationship of pool size percentages with hydroperiod is that as soils become more aerobic, stable SOM pools decrease. When comparing the percentage of labile SOM to total pool size between zones, the deep zone had a smaller percentage of labile SOM than transition zones of the same wetland type (Fig. 10). The relationship between zone and C and N pool size illustrates that as
wetlands become more aerobic, labile SOM pools increase faster than stable SOM pools or that SOM pools in general decrease. However, based on the documented relationship comparing among wetlands, declines occur in stable SOM with increasingly aerobic conditions (Fig. 12). Thus, whether comparing between zones or within the same zone, it is evident that stable SOM, indeed, is declining as time spent aerobic increases.

One mechanism that could cause declines in stable SOM pools is a shift in microbial community composition. In the scope of this study, stable C and N pools were defined as not decomposable in a year in the lab which depends on the taxonomic composition of the microbes present. The microbial community could vary from wetland to wetland as well as the classes of organic molecules defined as stable SOM. Likewise, as wetlands increase in time spent aerobic, microbial communities could shift in composition redefining the types of organic molecules defined as stable. The shift in microbial community would change biomolecules once classified as stable in the presence one taxa of microorganisms, to be redefined as bioavailable, or labile, to the succeeding microbes. If wetlands continue to remain aerobic for extended periods of time, upland plant species encroach, altering the inputs of litter (Rochow 1985, Haag and Lee 2010). Changes in plant litter chemistry could stimulate microbial community composition shifts by providing biomolecules which would be stable to the preceding microbial community but labile to the resulting community. The community shift, as described above, could redefine stable SOM as bioavailable to the new onset of microorganisms depleting stable C and N pools.

Increasingly aerobic conditions in cypress wetlands also cause treefall which can open the canopy increasing light incidence on the soil surface and promoting SOM
decomposition (Rochow 1985, Moorhead and Callaghan 1994). Increasing light incidence could promote photooxidation of molecules that biologically would be considered stable. Also, changes in canopy cover could directly increase soil temperature or decrease soil moisture could also stimulate decomposition of previously defined stable SOM.

The results of this study indicate that suppression of wetland water tables does in fact regulate the pools size and modifies the relative stability of SOM through time. However, at what temporal extent does this modified relationship best explain the stability of SOM? Addressing the second component of the hydrology hypothesis, both stable C and stable N pool sizes significantly responded to percent time aerobic for all temporal extents over which percent time aerobic was calculated (Fig. 13). Thus there is support that hydrology regulates stable SOM pool sizes.

As the period of hydrological record increases, it is not surprising that the relationship between stable C and hydroperiod weakens. At longer temporal extents, ecosystem properties other than hydrology may increasingly play a role in regulating stable C and N pools. Since the strength of the stable pool-hydrology relationship is neither consistent nor random with increasing temporal extent in the hydrology record, an argument can be made that gradual changes in ecosystem properties alter the fraction of SOM defined as stable. As discussed in-depth previously, one gradual change coinciding with increasingly aerobic conditions is shifts in dominant vegetative cover. The gradual shift in dominant vegetation cover would change net primary production, canopy cover, and structural complexity of new SOM inputs. The establishment of a dominant species, however, is a gradual process that would slowly diminish the relationship between
percent time aerobic and stable SOM. As litter inputs or microbial community composition changes, the bioavailable pool of C and N may gradually change as well.

Local observations of soil subsidence supports the idea that the definition of stability may shift with time spent aerobic. If stable SOM pools were resistant to decomposition, then soil subsidence would only deplete the labile portions. The labile portions, however, were small in proportion to total SOM pools and would not represent the substantial SOM loss in aerobic wetland soils. Decreasing of both labile and stable SOM pools is supportive of observations and more reasonable for the notable soil subsidence in local wetlands. Whether it is vegetation or microbial community shifts, or other mechanisms, stable C and N pools decrease with increased aerobic conditions.

Even more surprising than decreasing stable pools is that the least amount of variability of a defined stable, i.e. a pool with a longer persistence time of 1 year, has the best relationships with time records of less than one year. Specifically, percent soil moisture by mass, indicative of hydrological conditions on the soil sampling date, had the least amount of variation and strongest relationship with the amount of stable SOC and SON. The observed relationship was also true with labile SOC and SON pools. This suggests that even short-term changes in hydrology can influence SOC and SON accumulation in wetlands.

Stable SOM was methodologically defined as the fraction of C and N not mineralized during full year under conditions that intentionally maximize mineralization. This technique is a very effective at stripping soil of labile material (Nadelhoffer 1990, Kaye et al. 2002a,b, my post-incubation microbial N results). Given this effectiveness, it is interesting that the correlation between stable pools and hydrology would change with
relatively small increases in the hydrology period of record, or even change at all. Given that stable pools were determined with a one-year incubation, it is noteworthy that the stable pool-hydrology relationship began to weaken even before the temporal extent for hydrology calculations had been expanded to one year. One explanation for a weakening pattern is that SOM turnover may be even more rapid in wetland ecosystems than was determined in laboratory incubations, even for the lab-defined “stable” fraction. Interactions with SOM in the ecosystem differ than in the incubation since in situ soils have the opportunity to interact with plant roots, additional soil fauna, and the water table. Depending on the dominant source of water for the wetland basin, interactions with groundwater can bring in nutrients at much higher concentrations than the leaching solution used throughout the laboratory incubation (Whigham and Jordan 2003).

Regardless of the proposed mechanisms, the results of this study suggest that the stable pool may not be a single pool of SOM with a uniform turnover time, i.e. non-labile, but composed of several SOM pools with varying persistence times under certain ecosystem conditions. In this study, labile and stable pools were distinguished after one year of incubation time as defined by the method. Toward the end of the year incubation, C and N was still being mineralized but at slower rates. Stable C pools in marsh and cypress wetlands had a similar calculated mean estimated persistence time of approximately 25 years in the deep zones. If the same mineralization rate of C (mean cypress deep zones 3.63 µg C (g⁻¹ soil x day⁻¹), mean marsh deep zones 1.73 µg C (g⁻¹ soil x day⁻¹)) continued until the remaining pool had been respired (mean cypress deep zone 38.12 mg C g⁻¹ soil, mean marsh deep zone 15.76 mg C g⁻¹ soil), it would take 28.72 years for the cypress and 24.93 years for the marsh to completely deplete. Thus it
is hard to infer the size fraction of the labile and stable pools other than what was defined by the method. Decreasing stable pools with increasing time spent aerobic does, however, suggests that wetland SOM pools are only stable under increased anaerobic conditions inherent of wetlands as an ecosystem property.

Regardless of methodological definition and ecosystem controls, it is important to reiterate the importance of reduced hydroperiod resulting in the loss of stable C and N in addition to labile C and N from wetland ecosystems. Even if a small proportion of a wetland stable SOM pool, along with the labile SOM pool, is mineralized under increasingly aerobic conditions it results in a huge additional, unpredicted flux of active C and N into global cycles. The increased pool size of mineralizeable SOM could lead to larger fluxes of CO₂ and nitrogen enrichment of both air and water. Stable C and N pools are presumed to be long-term storage units of C and N and are a large proportion of total ecosystem SOM in current global change models and may be underrepresented in flux potential. Even though wetlands store most of the world’s SOM, stable SOM also dominates other terrestrial habitats including boreal and temperate forests (Bridgham et al. 1998, Kaye et al. 2002a,b).
Conclusion

Forested wetlands had larger SOM, SOC, and SON pools. In the United States, forested wetland area is decreasing at a faster rate than other wetland types (Dahl 2000). In the place of forested wetlands, mitigation often replaces the lost forested acreage with marsh or unvegetated ponds. This study, however, illustrates that mitigated wetlands, in comparison to forested wetlands, may not be replacing the SOC and SON production or storage function on the landscape.

Wetlands store significant SOM that is assumed to be almost entirely stable. However, as shown in this study, when wetlands experienced a decreased hydroperiod, both labile and stable C and N pools significantly decreased. Previous studies, though few, have documented a static snapshot, or single average value, between labile and stable pools between ecosystems (Kaye et al. 2002b, Ahn 2009). Even fewer, however, have addressed the possibility of stable pools changing, i.e. a dynamic component to SOM stability, in response to changing properties within the same ecosystem type. In this study, it is evident that within the same wetland type, altering a regulating ecosystem property, i.e. hydroperiod, alters the stability of SOM. The possibility that stable SOM pools change on short time scales when ecosystem properties are manipulated, especially in wetlands with large C and N pools, has potential implications at a global scale. If reduced hydroperiods in wetlands decrease the accumulated labile and stable SOM pools, then the quantity of C and N vulnerable to mineralization and export from water-stressed wetlands have been grossly underestimated.
As wetlands contain one-third of global SOM, the dynamic nature of wetland C and N bioavailability suggests that changes in ecosystem properties that regulate microbial activity could result in large C and N fluxes (Schlesinger 1997). Because wetlands tightly link surface and groundwater, increased release of mineralized SON as NO$_3^-$ from wetland soils can further eutrophy receiving water bodies including local bays and estuaries. Increased soil respiration from dense wetland SOC stocks will continue to contribute to increasing CO$_2$ fluxes to the atmosphere. The steady release of stable SOC and SON pools would also deplete large C and N storage units on the landscape which may have taken decades to accumulate. As shown in this study, even short term changes in hydrology, i.e. less than a year, can significantly change the dynamic relationship between labile and stable pools.

The results of this study stimulate a growing need to understand the regulation of SOM stability by ecosystems, especially those with accumulated C and N. Globally, wetlands will continue to experience stress in regulating ecosystem properties. Water will increasingly become limited with population growth and degraded in quality by pollutants. Wetlands with reduced hydroperiods not only lose accumulated C and N stocks, but also lose their capacity to store new inputs of nutrients and pollutants because SOM is removed from the system. In addition, wetlands store accessible freshwater and often rely on stressed groundwater systems to create surface inundation conditions. As a result, as society damages wetland soils to overcome water quantity problems, we also create potential water quality problems by damaging nature’s filter. Thus, as shown in this study, decreased hydroperiods, even short-term, may have larger impacts on the stability of SOM pools and global C and N cycles then once predicted.
Literature Cited


Appendix
Table A-1a: Study sites located in the Cypress Creek Wellfield. SWFWMD name is the monitoring name given to the wetland for monitoring purposes by the water management district. The Tampa Bay Water name is also named only for monitoring purposes.

<table>
<thead>
<tr>
<th>SWFWMD Wetland ID</th>
<th>Wetland Type</th>
<th>SWFWMD Wetland Name</th>
<th>Tampa Bay Water Wetland Name</th>
<th>Soil Series Map Unit</th>
<th>Soil Order</th>
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<td>190</td>
<td>Marsh</td>
<td>Cypress Creek Site &quot;E&quot; (CCWF &quot;E&quot;)</td>
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<td>196</td>
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<td>Inceptisol</td>
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### Table A-1b: Study sites located in the Morris Bridge Wellfield

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<td>MBWF Well Marsh</td>
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<td>Basinger Entisol</td>
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<tr>
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Table A-1c: Study sites located in the J.B. Starkey Wellfield.

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<td>Sellers</td>
<td>Inceptisol</td>
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Figure A-1a: Average carbon dioxide flux from cypress wetland soil samples over the year-long incubation. Error bars are one SEM.

Figure A-1b: Average carbon dioxide flux from marsh wetland soil samples over the year-long incubation. Error bars are one SEM.
Figure A-2a: Average nitrate leached from cypress and marsh wetland soil samples over the year-long incubation. Error bars are one SEM.

Figure A-2b: Average ammonium leached from cypress and marsh wetland soil samples over the year-long incubation. Error bars are one SEM.
Table A-2a: Detailed ANOVA table for measured ecosystem properties.

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Table A-2b: Detailed ANOVA table for carbon and nitrogen pools.

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Figure A-3: Average duration and frequency of inundating events for sample wetlands. The average duration and frequency below is represented by the deep zone soil core elevation over a 10 year hydroperiod calculation.