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CHARACTERIZING BIOGEOCHEMICAL SHIFTS IN TWO *SALIX CAROLINIANA* MICHX.
ENCROACHED FRESHWATER SUBTROPICAL MARSHES

by

JANET HO

B.S. University of Central Florida, 2016

A thesis submitted in partial fulfillment of the requirements
for the Degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Spring Term

2018

Major Professor: Lisa G. Chambers

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ABSTRACT

Shrub encroachment is a common disturbance in wetlands, but little is known about how shrub encroachment impacts functions such as carbon (C) storage and nitrogen (N) cycling. The objective of this thesis was to identify differences in physiochemical properties (within soil, water and leaf tissue) and biogeochemical processes (soil respiration, enzyme activity, litter decomposition, and N cycling) in two subtropical freshwater marshes encroached by coastal plain willow (*Salix caroliniana* Michx.). Two study regions (Moccasin Island and Lake Apopka) were selected because of their location in the St. John's River watershed and their unique histories and hydroperiod, allowing for an investigation of how synonymous the effects of willow-encroachment are across sites. A stratified random sampling design was employed in each region, identifying three plot types: willow (>80 % willow aboveground coverage), adjacent marsh (>80% herbaceous aboveground coverage and <1 m from willows), and control marsh (>80% herbaceous aboveground coverage and >10 m from willows) plots. Triplicate soil samples were collected in each plot in the wet and dry seasons of 2017 and analyzed for physiochemical properties (bulk density, moisture, nutrient content) and used in laboratory assays to measure soil respiration, enzyme activity, and potential N mineralization and denitrification rates. Leaf tissue was collected from the dominant vegetation in each plot and analyzed for nutrient content (total C, lignin-C, and total N). Short-term litter decay rate was determined using a litter-bag field experiment. Bioavailable N and dissolved organic C (DOC) concentrations were determined from surface and porewater collected from the center of each plot. In Moccasin Island, lower decomposition rates and greater denitrification, potential N mineralization, and soil C and N content were detected in willow and adjacent marsh plots, suggesting greater C storage and N cycling in willow-encroached marshes compared to non-

encroached control marshes in Moccasin Island. Conversely, soil C and N content was lowest in willow plots in Lake Apopka. Decomposition and microbial activity (enzyme activity and respiration) were lowest in willow dominated areas and correlated to soil nutrient concentrations. In both regions, microbial compositional changes (gene copy number) were detected between plot types, mainly in bacteria (β -proteobacteria and Bacteroidetes) for Moccasin Island and archaea and fungi abundance in Lake Apopka. Ultimately, willow plots in both regions had greater lignin-C content and short-term litter C storage. Greater bioavailable N was also observed in adjacent and/or willow plots in both regions. However, soil C storage and N cycling differences were not synonymous between the two regions. Future studies of willow effects will need to look at multiple sites or risk making inaccurate generalizations. From the findings from this study, wetland processes can be altered in willow-encroached marshes and this data can help land managers decide where to allocate resources based on valued ecosystem services.

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LIST OF ABBREVIATIONS

Adjacent marsh plots – Adj.

Ammonium – NH_4^+

Carbon – C

Carbon Dioxide – CO_2

Cell wall lignin – CWL

Dinitrogen – N_2

Dissolved organic carbon – DOC

Methane – CH_4

Nitrate – NO_3^-

Nitrogen – N

Nitrous oxide – N_2O

Organic matter - OM

Phosphorous – P

Potentially mineralized nitrogen – PMN

Propidium monoazide - PMA

Quantitative polymerase chain reaction - qPCR

Soluble reactive phosphorous – SRP

Standard error - SE

CHAPTER ONE: INTRODUCTION

Wetlands provide a multitude of ecosystem services, ranging from flood and erosion control, water quality improvement, and carbon (C) sequestration (Schuyt & Brander, 2004). Unfortunately, as much as 87% of all the wetlands in the world have been lost since the 1870s, and most remaining wetlands have been compromised by urbanization and agricultural practices (USEPA, 1994; Davidson, 2014). Legislation has been implemented since the 1970s to prevent wetland loss, but the lack of focus on wetland degradation has resulted in the loss of ecosystem services, which may contribute to increased atmospheric carbon dioxide (CO₂) levels, decreased biodiversity, and eutrophication of water bodies (Barbier, 1993).

Wetland degradation is the loss of wetland functions over time due to disturbances such as nutrient enrichment, hydrological alterations, and/or shrub encroachment. Shrub encroachment in wetlands is a key process in succession that converts graminoid-herbaceous dominated wetlands into shrub dominated wetlands (Figure 1). Shrub encroachment commonly follows anthropogenic disturbances and can lead to subsequent disturbances through facilitating exotic species, altering hydrologic regimes, and changing ecosystem functions (Van Auken, 2000; Johnston, 2003). However, most shrub encroachment studies have been performed in semi-arid grassland-shrubland transition zones; it is unclear whether shrub encroachment in subtropical marsh-shrub swamp transition zones leads to degraded ecosystem functions (Saintilan & Rogers, 2014). As shrub encroachment rates rise due to increasing anthropogenic disturbances, rising atmospheric CO₂ levels, and increasing fire suppression, it is critical to

understand how marsh biogeochemical processes such as C sequestration and nitrogen (N) cycling differ in encroached marshes. (Knapp et al., 2008).

Hydrarch Model for Lakes

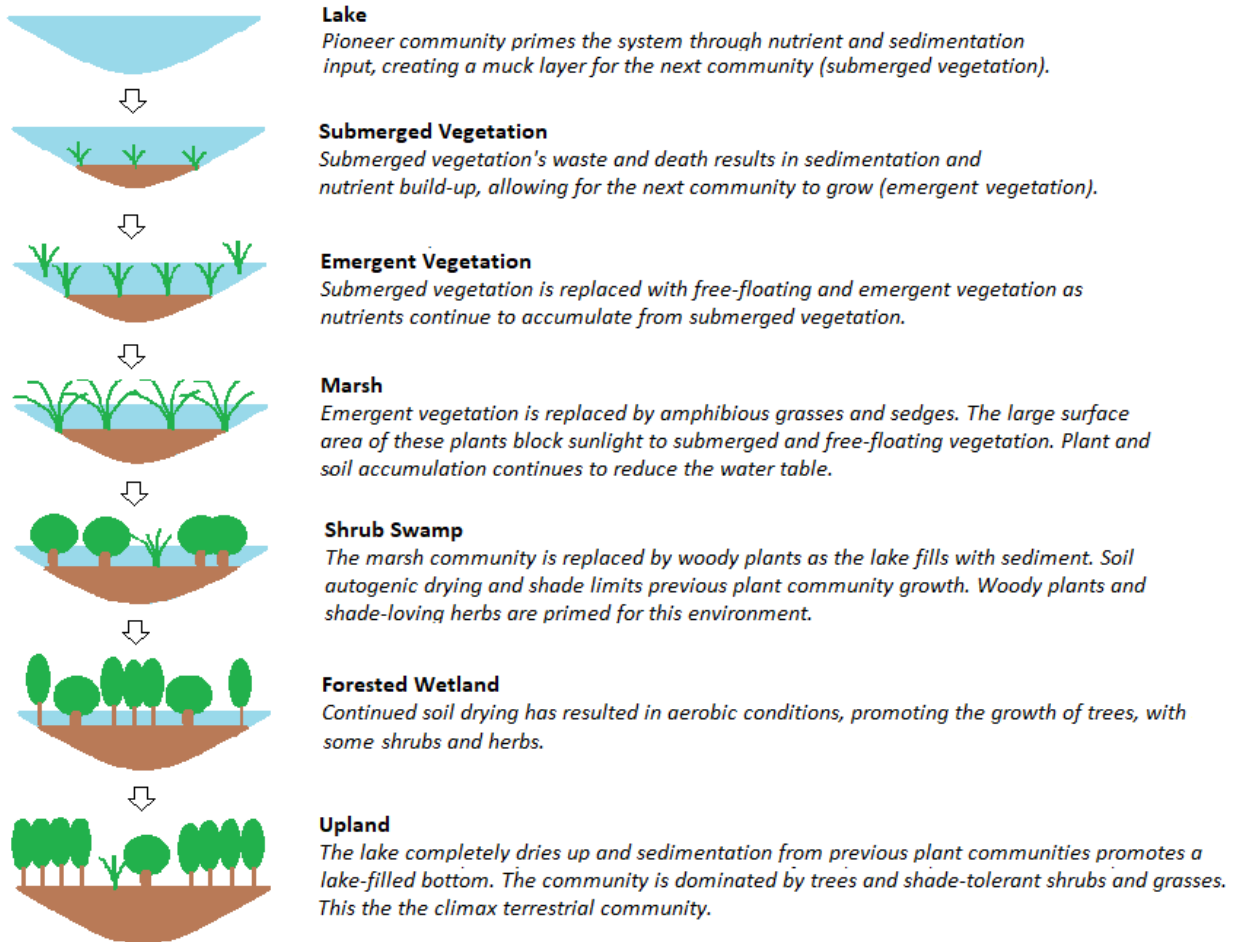


Figure 1: Hydrarch Model for Natural Marsh Succession. Following Clements' autogenic succession concept, plants prime the environment for the next plant community until reaching the upland climax community and the loss of wetland status. Figure created by author.

Marsh Biogeochemical Processes

Biogeochemical cycles include the transformation, storage, and release of nutrients (e.g., C, N, and phosphorous (P)) in the atmosphere, lithosphere, biosphere, and hydrosphere (Figure 2).

Biogeochemical processes are regulated by soil microbes (bacteria, archaea, fungi, and protists) and influenced by environmental factors. Together, environmental conditions and the soil microbial community dictate biogeochemical functions of ecosystems (Reddy & DeLaune, 2008). In general, marshes can function as C sinks and N removers due to high primary productivity and anaerobic soil conditions which promote slow decomposition rates and denitrification (Mitsch & Gosselink, 2007).

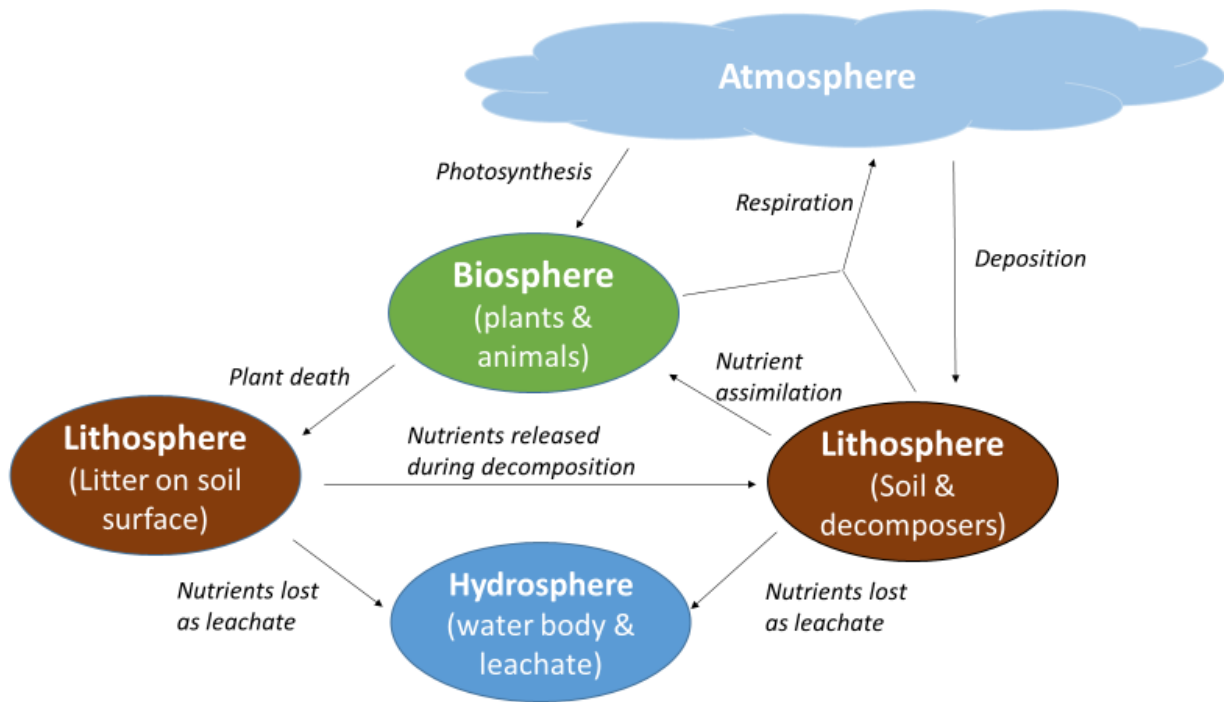


Figure 2: Biogeochemical Cycle Pathways. Nutrients are transformed throughout the atmosphere, lithosphere, biosphere, and hydrosphere. Figure created by author.

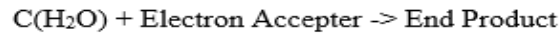
Carbon Sequestration

Decomposition/mineralization is the catabolic breakdown of organic matter into inorganic compounds by soil microbes; the process releases CO₂, dinitrogen gas (N₂), and/or methane (CH₄) into the atmosphere through cellular respiration. All microbes use cellular respiration to obtain energy for growth and reproduction. In aerobic conditions, oxygen (O₂) is used as the terminal

electron acceptor and releases CO₂ as a product. Soil microbes in anaerobic marsh soils must use less efficient terminal electron acceptors, such as nitrate (NO₃⁻), and CO₂ that is produced during mineralization (Reddy & DeLaune, 2008; Table 1). Thus, in highly productive marshes with anaerobic conditions, new plant detritus often accumulates before older detritus has been fully decomposed by soil microbes, resulting in the incorporation of older detritus into the long-term soil organic C pool (Rocha & Goulden, 2009). Despite CO₂ and CH₄ emissions from respiration, marshes typically still act as net C sinks due to their high levels of immobilization and soil C storage (Mitsch & Gosselink, 2007). Disturbances that alter litter (quantity or quality) or marsh anaerobic conditions can alter decomposition rates and C storage (Cornwell et al., 2008).

Shrub encroachment has been reported to increase primary productivity, mainly due to increased annual litterfall of shrubs compared to herbaceous vegetation (Knapp et al., 2008; Brantley & Young, 2010). Additionally, shrub litter can contain lower litter quality than herbaceous litter, altering decomposition rates (Berg & Bosatta, 1985). Two major components of litter quality are lignin-C and N content (Cornwell et al., 2008). Lignin-C is used for structural support and considered a recalcitrant-C source, meaning it is harder for microbes to degrade. Woody plants like shrubs generally contain more lignin than herbaceous species (Berg & Bosatta, 1985). Initial lignin content has been reported to be indirectly related to litter turnover rates (Sariyildiz & Anderson, 2003). Lower lignin:N ratios are considered better in terms of litter quality since N availability can limit decomposition activity (Yang et al., 2015). Studies have reported lower litter quality in shrub litter than herbaceous litter (Kaye et al., 1998). Morphological differences between shrubs and herbaceous vegetation can alter litter dynamics (litterfall and litter quality) and impact litter C storage.

Table 1: Table of Electron Acceptors and End Products in Cellular Respiration. Alternative electron acceptors such as nitrate and sulfate are less efficient than oxygen.



Electron Acceptor	O ₂	NO ₃ ⁻	Mn ⁴⁺	Fe ³⁺	SO ₄ ²⁻	CO ₂ , H ₂
End Product	H ₂ O, CO ₂	N ₂ , CO ₂	Mn ²⁺ , CO ₂	Fe ²⁺ , CO ₂	H ₂ S, CO ₂	CH ₄

← Efficiency →

Table created by author.

Nitrogen Cycling

Nitrogen cycling is predominantly controlled by the soil microbial community (Saunders & Kalff, 2000). Important processes in N cycling include N mineralization and denitrification. Bacteria in the oxic soil zone can transform organic N into inorganic N (ammonium (NH₄⁺) and NO₃⁻) which can be assimilated by plants and phytoplankton, easily exported in runoff, or diffused into the anoxic soil zone. Nitrogen mineralization can stimulate phytoplankton growth as nitrogen is often the limiting nutrient in ecosystems. These algal blooms act as a food source for microbes, promoting microbial growth and subsequently depleting available oxygen to create conditions such as hypoxia, or dissolved oxygen concentrations <2 mg L⁻¹ (Vymzal, 1995). Removal of excess bioavailable N through processes such as denitrification is critical to prevent eutrophication of major water bodies which can cause harmful algal blooms (Smith et al., 2006). Denitrifying bacteria found in the anoxic zone of wetland soils can transform NO₃⁻ into nitrous oxide (N₂O) and N₂ through sequential reduction reactions. These gases can then diffuse from the soil into the atmosphere, resulting in the removal of N from soil and runoff. (Figure 3). The presence of aerobic and anaerobic soil layers in marshes promotes specialized microbes that can transform N into bioavailable forms and remove excess N from the soil (Saunders & Kalff,

2000). Studies have reported greater evapotranspiration rates and rhizosphere oxidation in shrubs compared to herbaceous vegetation, which can result in changes to the redox status of the soil and alter N cycling (Pereira & Kozlowski, 1977; Budny & Bencotter, 2016). Shrub encroachment may result in altered physiochemical properties, raising the question of how marsh processes (C sequestration and N cycling) are altered in shrub-encroached marshes.

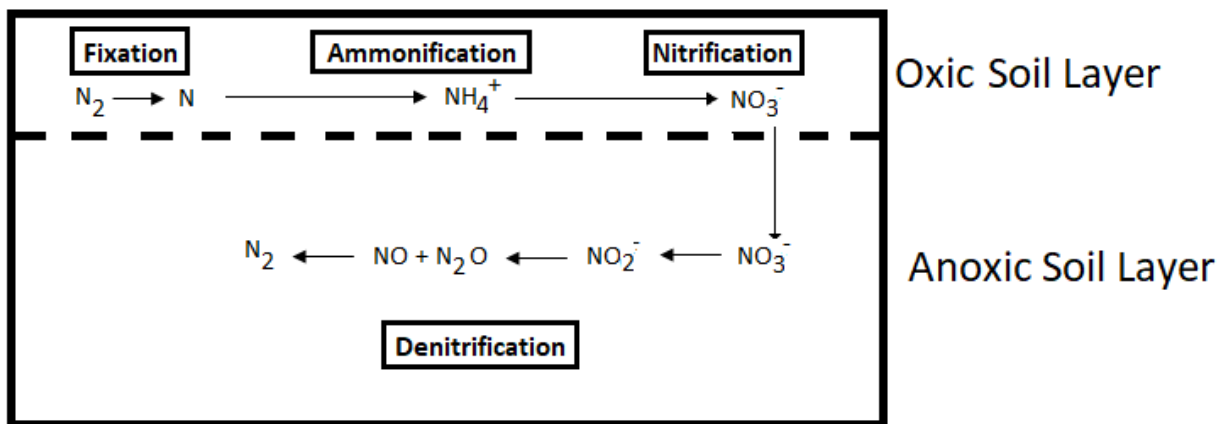


Figure 3: Simplified Nitrogen Cycle in Marshes. Figure created by author.

Site Description

In the south-east United States, willow (*Salix caroliniana* Michx.) is a common shrub encroaching freshwater marshes (Brummitt & Powell, 2002; Dahl, 2005). Willow encroachment has become a major land management issue due to willows' ability to alter plant community structure and underlying processes and its resilience to management practices (Conner et al., 2002; Hall et al., 2017). In the St. Johns River watershed (FL, USA), shrub coverage, predominantly willow, has increased anywhere from 6 to 89% in floodplain marshes in the last century, depending on location (Lowe et al., 1984; Hall et al., 2017). The expansion of willows

has been attributed to hydrological alterations and fire suppression (Quintana-Ascencio et al., 2013).

Two regions (Moccasin Island and Lake Apopka) within the St. Johns River Watershed (FL, USA) have been heavily willow-encroached since the 1970s (Hall, 1987; Murphy, 2005). Moccasin Island (28.2359° N, 80.8212° W), was hydrologically altered in the early 1900s through levee construction, producing unvegetated moist soils ideal for willow seed germination (SJRWMD, 2013). Hydrological restoration during the 1980s removed the levees impeding water flow from the St. Johns River, providing mature willows the water they needed for further expansion (Quintana-Ascencio et al. 2013). Over time, willows outcompeted the existing sawgrass (*Cladium jamaicense* Crantz.) communities due to willow's longer life span, greater resilience, and inhibitory nature towards shade intolerant grasses (Scholes & Archer, 1997; Brummitt & Powell, 2002).

Lake Apopka North Shore (28.6946° N, 81.6586° W) was used for muck-farming (corn, celery, etc.) and predominantly disturbed by nutrient enrichment and hydrological alterations in the early 1900s (Murphy, 2005). In the 1980's, the St. Johns River Water Management District began to restore the marshes within Lake Apopka's North Shore by removing levees and canals, replacing exotic species with native plant species, removing N and P from the lake through annual gizzard shad (*Dorosoma cepedianum* Lesueur) removal, and reducing nutrient discharge from agricultural farms (Schaus et al. 2010). Nutrient loading in Lake Apopka has been reduced by as much as 80% since the 1980s, and the lake has demonstrated improved water quality compared to pre-restoration nutrient concentrations (Coveney, 2016). Cattail (*Typha spp.*), which appeared following nutrient enrichment in the early 1900s, was not removed since it is a native

Florida species. Despite restoration and willow management, willow encroachment is still a prevalent issue in Lake Apopka, since willow was well established prior to nutrient reduction in the 1980s (Murphy, 2005).

Both regions, Moccasin Island and Lake Apopka, are considered warm and wet with mean annual temperature and precipitation ranges around 21 – 22 °C and 1120-1160 mm (SJRWMD, online data). Hydroperiod and soil pH differ between the two regions (Table 2). Moccasin Island’s water levels are dependent upon the St. Johns River levels, resulting in seasonal flooding during the study. Marshes in Lake Apopka are highly compacted and typically >1 m below lake level from previous agricultural practices, resulting in permanent flooding all year (Conrow et al. 2011). Despite varying hydroperiods and land histories, both regions in this watershed are willow-encroached. To date, no studies have been completed in either region on whether these willow-encroached marshes maintain similar levels of C storage and N cycling to their non-encroached counterparts.

Table 2: Study region comparison of general site conditions

	Moccasin Island	Lake Apopka
Disturbance History	Hydrological alterations	Agricultural practices
Current Vegetation	Sawgrass and willow shrub	Cattail and willow shrub
Soil Type	Hydric Haplosaprists	Hydric Haplosaprists
Soil pH	5.0 - 6.5	7.5 – 9.0
Hydroperiod	Seasonal (Aug – Dec 2017)	Permanent (all-year)

Table based on data from Hall, 1987; Murphy, 2005; USDA 2017 a&b.

Experimental Design

A stratified random sampling approach was employed to determine if differences exist in physiochemical properties (Chapter Two) and biogeochemical processes (Chapter Three) in willow-encroached marshes, relative to un-encroached marsh. Aerial photography was used to identify plots (4 x 4 m) as either shrub or herbaceous vegetation (SJRWMD, online data). Vegetation presence was verified in the field by dividing each plot into 16 quadrats (1 x 1 m) and only plots with aboveground presence of the specified vegetation (willow, sawgrass, or cattail) in > 80% quadrats were considered. After verifying vegetation coverage, herbaceous plots were further separated as either adjacent marsh (< 1m from willows) or marsh (> 10 m from willows) plots. Adjacent marsh plots were established to test if differences in willow-encroached marshes are observed beyond willow canopies, while marsh plots were used to determine how C and N biogeochemistry differs between native herbaceous marsh and the shrub-swamp that results following willow encroachment. In summary, three plot types based on vegetation type and willow proximity were designated for this study: willow plots (> 80% coastal-plain willow), adjacent marsh plots (> 80% dominant herbaceous vegetation and < 1 m from willows (~80% sawgrass and ~20% maidencane for Moccasin Island; ~80% cattail and ~20% maidencane for Lake Apopka), and marsh plots (same vegetation composition as adjacent marsh plots and >10 m from willows on all sides). Sampling occurred once in the winter (February – March) and summer (July – August) to capture seasonal variability and major deviations in hydroperiod between regions. During every sampling, five of each plot type were randomly selected from each region using a Microsoft Excel random number generator.

CHAPTER TWO: PHYSIOCHEMICAL PROPERTY DIFFERENCES IN TWO WILLOW-ENCROACHED SUBTROPICAL FRESHWATER MARSHES

Shrub encroachment, a key process in natural succession, has been increasing at unprecedented rates in the last century (Archer, 2010). Increases in anthropogenic disturbances (agriculture, urbanization, etc.), fire suppression, and climate change have all contributed to the increase in shrub encroachment worldwide (Saintilan & Rogers, 2014). Grassland research has shown major physiochemical property changes in shrub-encroached landscapes. In general, shrub encroachment results in shrub, fertility, resource, and/or hydrologic islands due to the redistribution of nutrients and water into shrub canopies from the surrounding regions (Peng et al., 2013). In response to resource competition, shrub encroachment can result in bare or unvegetated interspaces between shrub canopies (Darrouzet-Nardi et al., 2006). Greater nutrient export and eutrophication from excess bioavailable nutrients in the water column have been observed in shrub interspaces, increasing soil heterogeneity in shrub-encroached grasslands (Schade & Hobbie, 2005). These physiochemical property changes have been well identified in shrub-encroached terrestrial grassland systems, but have not been as thoroughly researched in aquatic systems (Saintilan & Rogers, 2014).

Wetland shrub encroachment is the transition from graminoid-herbaceous marshes into shrub-swamps. Morphological and physiological differences between shrubs and herbaceous plants may result in changes in important wetland functions, such as C storage and N cycling. Litterfall and litter quality changes between shrubs and their herbaceous counterparts can alter decomposition rates and net C storage. Greater CO₂ sequestration, N fixation, radial oxygen loss,

and evapotranspiration rates have been reported in shrubs compared to herbaceous vegetation in temperate and boreal wetlands (Marsh et al. 2000; Grygoruk et al. 2014). Deeper root systems, mycorrhizal associations with N-fixing bacteria and the lower water use efficiency of shrubs have been attributed to these alterations (Marsh et al., 2000; Scott et al., 2006; Brantley & Young, 2010). Differences in radial oxygen loss and evapotranspiration rates can impact anaerobic conditions in marshes, altering C and N cycling through changes in oxidation-reduction (redox) conditions. Most of these studies have been completed in temperate and boreal wetlands, resulting in a knowledge gap on shrub encroachment in subtropical wetlands, which also constitute a large portion of the world's C storage (Mitsch & Gosselink, 2007).

Willow-encroachment has become a major land management issue in subtropical marshes, such as the St. Johns River watershed, in the last century (Figure 4). Most studies completed on willow-encroachment in subtropical marshes have focused on willow removal techniques (Lee et al., 2005; Quintana-Ascencio et al., 2013; Hall et al., 2017). Limited studies within this region investigating wetland processes have reported lower decomposition and higher evapotranspiration rates in willow-encroached areas, suggesting that willow encroachment may alter physiochemical properties (Duffy, 2014; Budny & Benscoter, 2016). Changes in physiochemical properties such as nutrient availability and soil moisture are important to consider as they can impact C storage and N cycling (Barnes et al., 2012). Furthermore, as wetlands can vary greatly in hydrology and land histories, two factors linked to shrub encroachment, it is unclear whether reported changes in willow-encroached marshes are similar worldwide or even within the same watershed (Maestre et al., 2016).

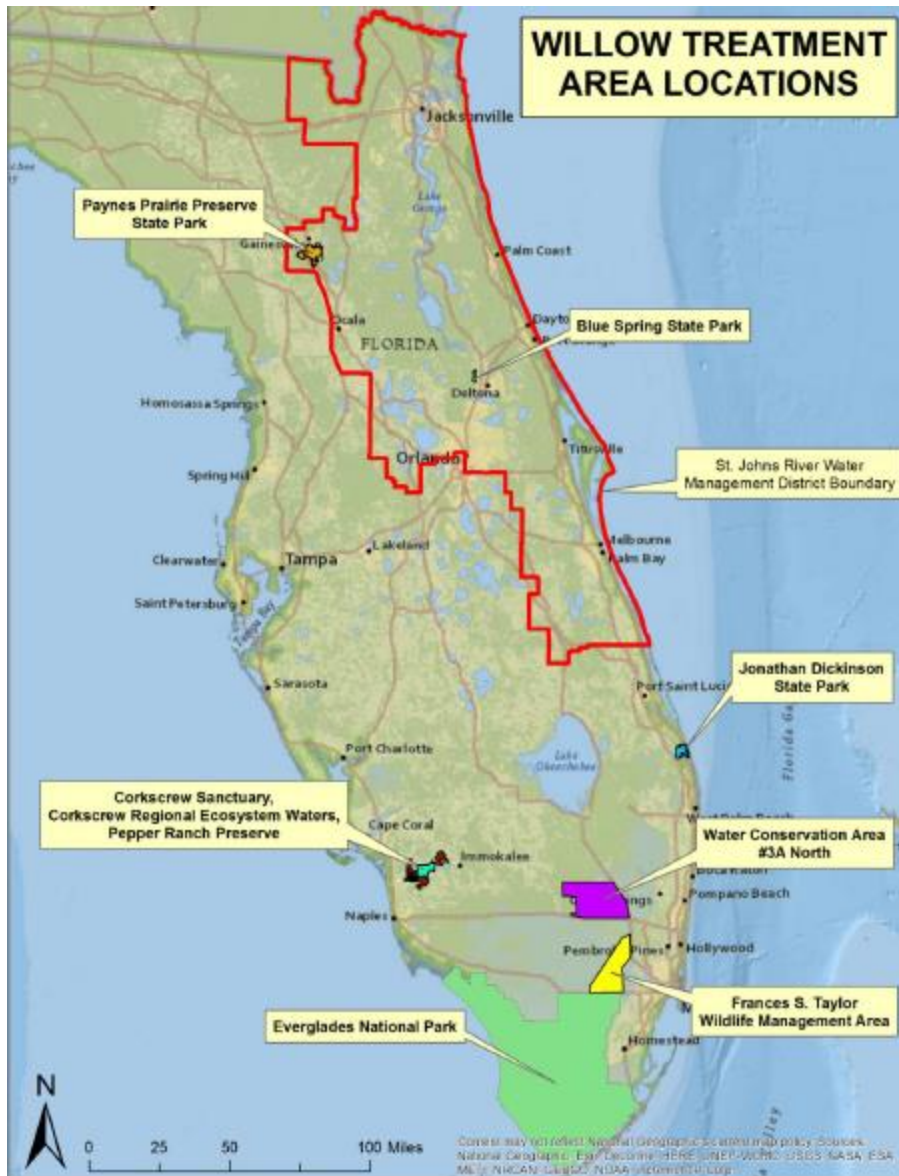


Figure 4: Map of various willow removal treatment areas in Florida. The red area represents the St. Johns River Watershed. Figure from: Hall et al., 2017

The purpose of this study was to characterize physiochemical property differences in two willow-encroached subtropical marshes (Moccasin Island and Lake Apopka) within the St. Johns River watershed that are willow-encroached and have different histories and hydroperiods. In this study, I quantified physiochemical differences (bulk density, soil moisture, nutrient (C, N, and phosphorous (P) content) in the soil, surface and porewater, and leaf tissue of willows and

herbaceous vegetation (sawgrass and cattail). This study did not attempt to understand if willow encroachment was the cause of physiochemical property differences, but provides data on how ecosystem services may differ between willow-encroached and non-encroached marshes. A secondary objective was to see if differences detected in this study were synonymous within the same watershed or similar to findings from shrub encroachment in terrestrial systems.

Materials & Methods

Soil sampling and analysis

In both regions, five randomly selected plots from each plot type (willow, adjacent marsh, and marsh) were sampled. Three 0-15 cm soil cores were taken in every plot to account for heterogeneity using the push core technique and extruded in the field. Samples were immediately placed on ice and transported back to the Aquatic Biogeochemistry Lab at UCF where they were homogenized by hand and stored at 4°C until analysis. Soil surface redox potential and temperature was measured at every sampling point. A subset of field moist sample was dried in a ThermoFisher Heratherm oven (ThermoFisher Scientific, Waltham, MA) at 70°C for 72 hours and weighed to determine gravimetric moisture content and soil bulk density. Porosity was calculated as the % pore space = $1 - (\text{bulk density} / \text{particle density})$, with 2.65 g cc^{-1} used as particle density (Boyd, 1995). Afterwards, the oven dried samples were ground with a SPEX Sample Prep Mixer Mill 8000M (Metuchen, NJ) to a fine powder for analysis of soil organic matter (OM) content and total nutrient (C, N, and P) analysis. A subset of the dried, ground sample (~0.005 g) was analyzed on the Elementar Vario Micro Cube (Elementar Americas Inc., Mount Laurel, NJ) for total C and N. Percent OM was determined on dried,

ground subsamples by ashing for four hours at 550°C in a ThermoFisher Isotemp muffle furnace (ThermoFisher Scientific, Waltham, MA) via the loss-on-ignition method. The ashed subsamples were then digested with 50 mL of 1 M HCl at 150°C for 30 minutes after Andersen (1976) and analyzed for total P on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, WI) using EPA method 134-A Rev. 5. Additionally, a subset of the wet sample (~ 5 g) was extracted with 2 M KCl for extractable nutrients (nitrate (NO_3^-), ammonium (NH_4^+), and soluble reactive phosphorous on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, WI; AGR-231, AGR-210, and EPA-118 respectively). Extractable N was calculated as the sum of extractable NO_3^- and NH_4^+ .

Water sampling

While this study did not look at the cause of willow encroachment, I acknowledge that hydrological differences between plots could play a role in observed physiochemical property differences. Continuous automated surface water level loggers were placed in representative willow and marsh plots for each region throughout the entire study, as well as manual water level measurements recorded to the nearest cm in each plot during each sampling visit. No significant water level differences between plot types were detected in either region throughout the study.

Surface water (~500 mL) was collected in acid-washed Nalgene bottles from the center of all flooded plots with corresponding pH, turbidity, conductivity, temperature, and dissolved oxygen measurements determined on a ProDSS YSI (Yellow Springs Instruments, Yellow Springs, OH). All surface water samples were filtered through a 0.45 μm membrane filter and preserved with double distilled concentrated sulfuric acid to a pH <2 within 24 hours and

analyzed for bioavailable surface water nutrients: NO_3^- , NH_4^+ , orthophosphate, and dissolved organic carbon (DOC; EPA-127, EPA-103, and EPA-118 respectively).

Porewater was sampled in the summer using porewater equilibrators ('peepers'), rectangular acrylic boards with 35 wells at 1 cm depth intervals. Each well was filled with N_2 -purged DI water, the entire profile lined with $0.45\mu\text{m}$ membrane filter paper, and maintained under anaerobic conditions until deployment in the field. One peeper was installed vertically in the soil profile (0-35 cm) in the center of every plot and left to stabilize for seven days. After seven days, porewater was extracted and pooled into the following segments (0-5 cm, 5-10 cm, 10-15 cm, and 15-30 cm). Samples were preserved in the field with double distilled concentrated sulfuric acid to a pH <2 and analyzed for bioavailable porewater nutrients: NO_3^- , NH_4^+ , orthophosphate, and DOC using the methods described above. Bioavailable N was calculated as the sum of bioavailable NO_3^- and NH_4^+ .

Leaf tissue sampling

Plots were evenly split into 9 quadrats and ~10 live mature leaves from the dominant plant species in the plot (willow, cattail, or sawgrass) were randomly collected at eye level in each quadrat. Willow leaves were collected in willow plots and sawgrass or cattail leaves were collected in adjacent marsh and marsh plots. All leaves were brought back to the lab and rinsed with deionized water to remove sediment, larvae, and pollen. Samples were dried and ground with a SPEX Sample Prep Mixer Mill 8000M (Metuchen, NJ) to a fine powder for analysis of total nutrient (C, N, and P) analysis as described above. Cell wall lignin (CWL) content was

determined by the acetyl bromide method and analyzed at 280 nm on a HTX Synergy MicroPlate Reader (Moreira-Vilar et al. 2014; Biotek Instruments, Winooski, VT).

Statistical analyses

The study regions were individually analyzed for statistical analysis due to the different histories and hydrology between the regions. For each region, seasonal (winter and summer) data were combined for analyses because paired t-tests indicated there were no significant differences in each parameter between season, except for surface water in Moccasin Island, which was only flooded seasonally (stats package). Therefore, all data presented (except for surface water) had an $n = 10$ for each plot type, and for each site. All statistical analyses were performed in R Statistics (R Foundation for Statistical Computing, Vienna, Austria). All data sets were tested for normality (Shapiro-Wilk) and homogeneity (Levene's test). For data that did not meet the assumptions (bulk density data from Moccasin Island and soil phosphorous data from Lake Apopka), data was log transformed. Differences between plot types and physiochemical properties were analyzed using a one-way ANOVA (stats package). If significant differences were detected between plot types, Tukey's test was used to determine which plots differed from each other (lsmeans package). Statistical significant was determined at $\alpha=0.05$ for all tests. All results reported are the untransformed means \pm standard error (SE) and p-values shown are from ANOVA models.

Results

Soil physiochemical properties

At Moccasin Island, willow and adjacent marsh plots had greater porosity ($p < 0.001$; $82 \pm 2\%$; $81 \pm 1\%$) than marsh plots ($67 \pm 4\%$). Resultantly, lower soil compaction and greater soil moisture content ($p < 0.01$; $p < 0.001$) were observed in willow ($0.40 \pm 0.03 \text{ g cc}^{-1}$; $86 \pm 1\%$) and adjacent marsh plots ($0.50 \pm 0.02 \text{ g cc}^{-1}$; $79 \pm 2\%$) than in marsh plots ($0.98 \pm 0.21 \text{ g cc}^{-1}$; $52 \pm 9\%$). Redox potential ($p < 0.01$) was lowest in willow and adjacent marsh plots ($23 \pm 21 \text{ mV}$; $-6 \pm 13 \text{ mV}$) compared to marsh plots ($82 \pm 13 \text{ mV}$). Soil temperatures were greater in adjacent marsh plots ($29.8 \pm 0.41^\circ\text{C}$) than in willow ($28.7 \pm 0.42^\circ\text{C}$) and marsh plots ($28.5 \pm 0.42^\circ\text{C}$). Greater soil nutrient (OM, C, N, and P) concentrations and higher C:N were observed in willow and adjacent marsh soils compared to marsh soils ($p < 0.01$; Table 3). Bulk extractable SRP and N (NO_3^- and NH_4^+) did not differ significantly between plot types.

At Lake Apopka, porosity and bulk density was similar in all plot types, but less soil moisture ($p = 0.03$) was observed in willow ($47 \pm 5\%$) soils compared to adjacent marsh soils ($57 \pm 4\%$) and marsh soils ($63 \pm 3\%$). While not significant, soil temperatures were generally greater in adjacent marsh plots ($31 \pm 1.2^\circ\text{C}$) in comparison to willow ($29.3 \pm 1.3^\circ\text{C}$) and marsh plots ($30.9 \pm 1.2^\circ\text{C}$). Less total C content ($p = 0.03$) was detected in willow plots ($162 \pm 30 \text{ g kg}^{-1}$) compared to adjacent marsh ($224 \pm 26 \text{ g kg}^{-1}$) and marsh plots ($282 \pm 33 \text{ g kg}^{-1}$). Total N, P, C:N, extractable SRP, and extractable N (NO_3^- and NH_4^+) were similar in all plot types.

Table 3: Mean \pm SE for soil nutrient concentrations in Moccasin Island and Lake Apopka by plot type.

Study Region	Plot Type	SOM [g kg⁻¹ soil]	Total C [g kg⁻¹ soil]	Total N [g kg⁻¹ soil]	Total P* [g kg⁻¹ soil]	C:N
<i>Moccasin Island</i>						
	Marsh	518 \pm 89 ^a	245 \pm 28 ^a	13 \pm 0.5 ^b	5.6 \pm 0.6 ^a	11.6 \pm 0.1 ^a
	Adj.	792 \pm 24 ^b	413 \pm 8.3 ^b	7.7 \pm 0.5 ^a	9.0 \pm 1.0 ^b	12.2 \pm 0.1 ^b
	Willow	831 \pm 29 ^b	399 \pm 11 ^b	8.9 \pm 0.3 ^a	9.4 \pm 0.9 ^b	12.1 \pm 0.5 ^b
<i>Lake Apopka</i>						
	Marsh	468 \pm 76 ^a	281 \pm 33 ^b	12.5 \pm 0.8 ^a	5.5 \pm 0.5 ^a	15.3 \pm 1.0 ^a
	Adj.	380 \pm 63 ^a	224 \pm 34 ^{ab}	11.5 \pm 0.8 ^a	5.8 \pm 0.8 ^a	14.9 \pm 0.7 ^a
	Willow	295 \pm 57 ^a	162 \pm 34 ^a	11.3 \pm 0.8 ^a	5.4 \pm 1.1 ^a	15.9 \pm 0.8 ^a

* All statistical analyses for total P at Lake Apopka were ran using log transformed data, but untransformed total P results are shown here for interpretation.

Superscript letters represent significantly different means by Tukey's HSD test.

Table created by author.

Surface and Porewater

Moccasin Island was only seasonally flooded from August – December. Water level did not differ by plot type and ranged from 72 – 92 cm during sampling. Of the bioavailable surface water nutrients measured, only DOC differed by plot type ($p = 0.01$). Adjacent marsh plots had greater DOC concentrations ($16.5 \pm 0.8 \text{ mg L}^{-1}$) than willow ($14.1 \pm 1.0 \text{ mg L}^{-1}$) and marsh ($12.6 \pm 2.1 \text{ mg L}^{-1}$) plots. In contrast, porewater DOC concentrations from 0-30 cm in the soil profile did not differ at any depth by plot type. By depth, both willow and adjacent marsh plots contained more porewater orthophosphate than marsh plots in the upper 15 cm ($p < 0.05$; Figure 5a). Porewater bioavailable N (NH_4^+ and NO_3^- , predominantly in the form of NH_4^+) was similar

in all plots except for the upper 5 cm, where adjacent marsh plots contained more N than all other plot types ($p = 0.03$; Figure 5b).

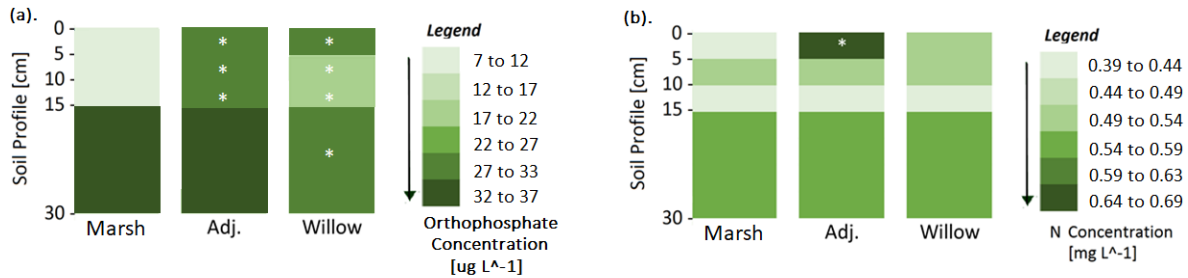


Figure 5: Mean value for porewater (a) orthophosphate and (b) N (NH_4^+ and NO_3^-) composition in soil profile segments in Moccasin Island by plot type. * represents a significantly different mean value compared to marsh plots using Tukey's HSD test. Figure created by author.

Lake Apopka was permanently flooded during the study. Water level did not differ by plot type and ranged from 38 – 81 cm during sampling times. The only difference in trends between seasons in the entire study was in surface water DOC concentrations. During the winter sampling, willow plots contained more DOC ($95 \pm 8 \text{ mg L}^{-1}$) than adjacent marsh plots ($81 \pm 4 \text{ mg L}^{-1}$). However, a general equilibrium of DOC concentrations between willow and adjacent marsh plots was observed in the summer sampling, suggesting possible transport of DOC throughout the system (Table 4). This was supported by personal observations of willow litter in both willow and adjacent marsh plots during the summer. In both seasons, surface water bioavailable NH_4^+ was greatest in willow plots ($p < 0.01$; Table 4). Surface water orthophosphate concentrations were similar for all plots. In contrast to the surface water results, porewater DOC did not differ by plot type or depth. Willow and adjacent marsh plots had twice as much porewater orthophosphate, particularly in the 15-30 cm segment ($p = 0.02$; Figure 6a). Adjacent marsh plots contained twice as much total porewater bioavailable N (NH_4^+ and NO_3^- , predominantly NH_4^+) as marsh and willow plots ($p = 0.01$; Figure 6b). By soil depth, the

majority of N was in the 0-10 cm segment for willow plots and observed in the lower 10-30 cm for adjacent marsh plots. In summary, greater surface water (NH_4^+ and DOC) and porewater (ortho-P and $\text{NH}_4^+ + \text{NO}_3^-$), bioavailable nutrients were detected in willow and/or adjacent marsh plots.

Table 4: Mean value \pm SE for surface water nutrient concentrations in Lake Apopka during each sampling by plot type. During both samplings, willow plots had the highest bioavailable NH_4^+ concentrations. Dissolved organic C concentrations was highest in willow plots during the winter sampling and a general equilibrium of DOC concentration was observed in the summer sampling.

Sampling	Plot Type	DOC [mg L ⁻¹]	NH_4^+ [mg L ⁻¹]	NO_3^- [mg L ⁻¹]	SRP [mg L ⁻¹]
<i>Winter</i>					
	Marsh	88.4 \pm 7.3 ^{ab}	0.10 \pm 0.01 ^a	0.04 \pm 0.01 ^b	0.01 \pm 0.00 ^a
	Adj.	81.1 \pm 4.0 ^a	0.08 \pm 0.03 ^a	0.02 \pm 0.01 ^a	0.01 \pm 0.00 ^a
	Willow	94.6 \pm 8.6 ^b	0.20 \pm 0.01 ^b	0.03 \pm 0.01 ^{ab}	0.02 \pm 0.01 ^a
<i>Summer</i>					
	Marsh	80.7 \pm 6.3 ^a	0.10 \pm 0.01 ^a	0.03 \pm 0.01 ^b	0.02 \pm 0.01 ^a
	Adj.	85.7 \pm 5.5 ^a	0.08 \pm 0.03 ^a	0.01 \pm 0.01 ^a	0.01 \pm 0.00 ^a
	Willow	83.1 \pm 3.6 ^a	0.20 \pm 0.01 ^b	0.02 \pm 0.01 ^{ab}	0.02 \pm 0.01 ^a

Superscript letters denote significantly different means by Tukey's HSD test.

Table created by author.

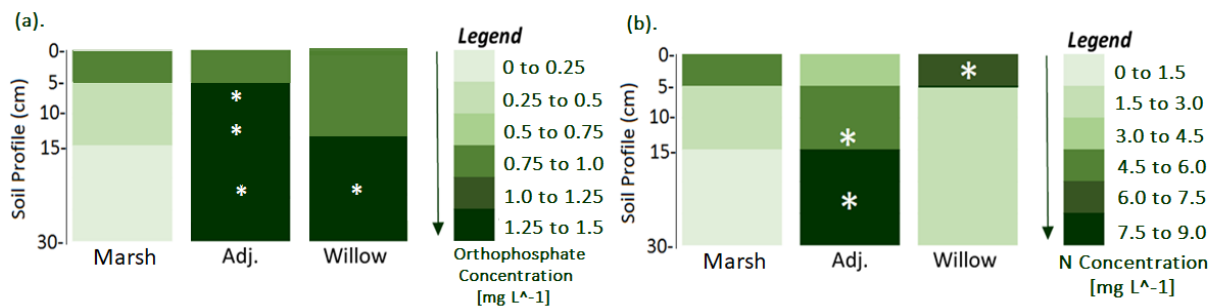


Figure 6: Mean value for porewater (a) orthophosphate and (b) N ($\text{NH}_4^+ + \text{NO}_3^-$) composition in soil profile segments in Lake Apopka by plot type. * represents a significantly different mean compared to marsh plots using Tukey's HSD test. Figure created by author.

Leaf Tissue Chemical Composition

At Moccasin Island, willow leaf tissue had greater total C content ($p < 0.001$; 479 ± 10 g kg^{-1} leaf tissue) than sawgrass leaves in adjacent marsh (443 ± 3 g kg^{-1} leaf tissue) and marsh (441 ± 3 g kg^{-1} leaf tissue) plots. Marsh and adjacent marsh plots contained the same vegetation composition but greater N content was observed in sawgrass leaves in adjacent marsh plots ($p < 0.001$; Table 5). As a result, sawgrass leaves in adjacent marsh plots contained the highest quality (lowest C:N) and willow leaves contained the lowest litter quality ($p < 0.001$). Similarly, willow leaves had the greatest cell-wall lignin content ($p = 0.05$). Total P content did not differ by plot type.

Table 5: Mean value \pm SE for leaf tissue for each region by plot type. In both regions, greater C and CWL content was detected in willow leaf tissue. N content was lower in willow leaves than sawgrass leaves.

Study Region	Plot Type	Total C [g kg^{-1} leaf]	CWL [g kg^{-1} leaf]	Total N [g kg^{-1} leaf]	Total P [g kg^{-1} leaf]	C:N
<i>Moccasin Island</i>						
	Marsh	441 ± 3^a	30.7 ± 9.4^a	26.3 ± 2.0^b	1.7 ± 0.2^a	17.0 ± 1.4^b
	Adj.	443 ± 3^a	33.7 ± 2.0^a	35.5 ± 0.8^c	1.6 ± 0.1^a	12.5 ± 0.3^a
	Willow	479 ± 10^b	55.1 ± 8.0^b	20.4 ± 2.0^a	1.7 ± 0.2^a	24.1 ± 1.3^c
<i>Lake Apopka</i>						
	Marsh	467 ± 5^a	19.8 ± 2.1^b	21.9 ± 2.1^a	1.3 ± 0.1^a	22.3 ± 2.6^a
	Adj.	470 ± 4^a	12.3 ± 2.6^a	23.0 ± 5.0^a	1.5 ± 0.3^a	21.6 ± 3.7^a
	Willow	488 ± 4^b	43.9 ± 14^c	20.0 ± 2.5^a	1.2 ± 0.2^a	25.0 ± 4.0^a

Superscript letters represent significantly different means by Tukey's HSD test.

Table created by author.

Likewise, at Lake Apopka, total C and CWL content differed by plot type ($p < 0.01$ and $p = 0.03$ respectively). Greater total C and CWL content was observed in willow leaves (488 ± 4 g

kg⁻¹ leaf tissue; 44 ± 14 g kg⁻¹ leaf tissue) compared to cattail leaves in adjacent marsh (470 ± 4 g kg⁻¹ leaf tissue; 12 ± 3 g kg⁻¹ leaf tissue) and marsh (467 ± 5 g kg⁻¹ leaf tissue; 20 ± 2 g kg⁻¹ leaf tissue) plots. No significant differences in total N, total P, or C:N were detected (Table 5).

Discussion

Physiochemical properties differed significantly by plot type in the two willow-encroached marshes. While vegetation and water properties differences were directionally similar in both regions, soil physiochemical property differences were highly dissimilar by region (Figure 7). Soil C and N concentrations were 2x greater in willow and adjacent marsh plots at Moccasin Island while willow plots at Lake Apopka had lower C concentrations and soil moisture than marsh and adjacent marsh plots. The directional difference of physiochemical properties between these two willow-encroached marshes within the same watershed suggests that studies focused on singular study regions risk making inaccurate generalizations of wetland shrub encroachment effects.

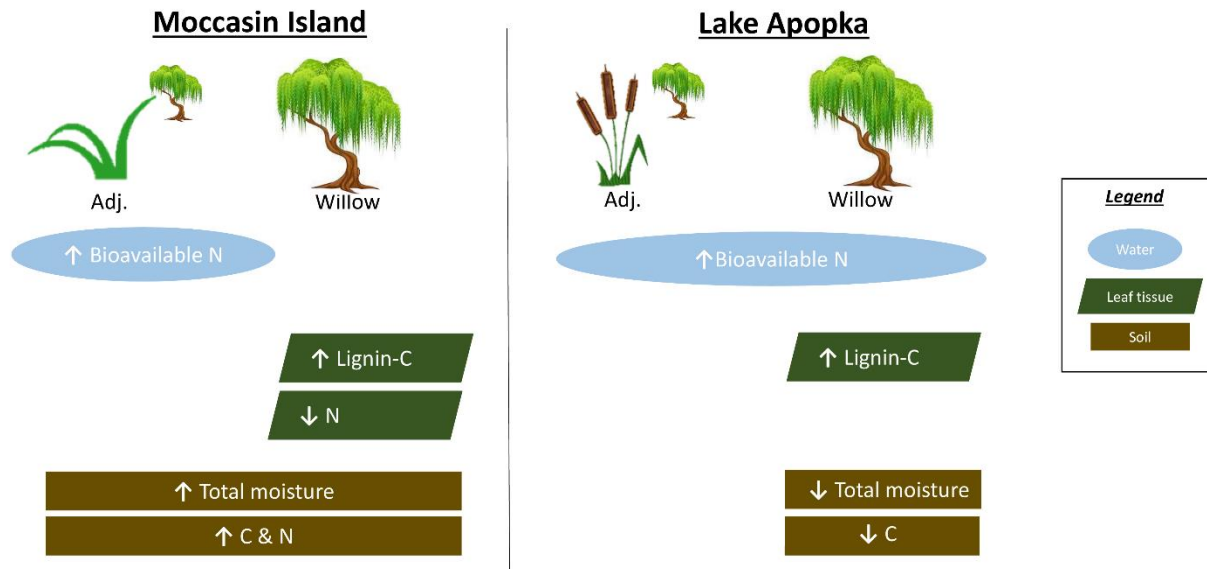


Figure 7: Key physiochemical property differences in willow and adjacent plots compared to marsh plots by region. Boxes overlaying both adj. and willow plots signify differences in both plot types ($p < 0.05$). Figure created by author.

One possible explanation for the dissimilar directional shift of soil physiochemical properties is hydroperiod differences. Permanent flooding, like at Lake Apopka, may reduce the amount of litterfall reaching the soil surface in willow plots, decreasing soil C content. Moccasin Island was only seasonally flooded, permitting direct litterfall deposition onto the soil surface when it was not flooded. The differences in soil nutrient results between sites aligns with terrestrial shrub encroachment studies where drier sites captured more organic C while wetter sites lost organic C in shrub encroached landscapes (Schlesinger et al., 1996; Jackson et al., 2002; Bhark & Small, 2003; Schade & Hobbie, 2005), further supporting the hypothesis that the hydroperiod of a wetland may play a key role in determining the unique properties of shrub-encroached areas relative to marsh areas. Future studies on litter-water dynamics using more marshes with different hydroperiods will need to be done to corroborate this. As wetlands can be highly variable in hydrology, which is tightly linked to shrub encroachment, it is important to

understand the role hydrology may play on soil physiochemical properties in shrub-encroached wetlands.

Soil physiochemical property differences, particularly soil nutrient availability, redox potential, and moisture, are important to consider as these properties are all associated with biogeochemical processes such as C storage and N cycling (Hartman et al., 2008). Greater soil nutrient availability detected in willow plots at Moccasin Island could increase soil microbial community size, altering C storage and N cycling by accelerating decomposition rates (Hartman et al., 2008; Cederlund et al., 2014). Less saturated soils can promote aerobic microbes such as fungi who are well known for breaking down recalcitrant-C sources, altering decomposition rates (Ewing et al., 2007; Hartman et al., 2008). Denitrification, an important process in regulating water quality, occurs in reduced soil conditions (Faulkner & Richardson, 1988). Willow and adjacent marsh plots in Moccasin Island had lower redox potentials which can promote denitrification and alter N cycling. The detection of soil property differences in this study supports the need for future studies on how biogeochemical processes (decomposition and denitrification) differ between willow-encroached marshes and non-encroached marshes.

Other physiochemical property differences in this study included nutrient differences in water and leaf tissue. In both regions, I observed greater bioavailable nutrients (NH_4^+ and orthophosphate) in water (surface and/or porewater) in willow and adjacent marsh plots. Depending on the wetlands' capacity to remove excess bioavailable N, greater bioavailable N concentrations in willow and adjacent marsh plots could pose a eutrophication risk, especially in areas like Lake Apopka which are already at high risk from possible persistent nutrient pollution (Coveney et al., 2016). Bioavailable N in water can also be linked to leaf N allocation, which

may explain why sawgrass in adjacent marsh plots contained more leaf N than sawgrass in marsh plots (Davis, 1990). As a limiting nutrient, greater leaf N content in sawgrass can improve litter quality and accelerate decomposition rates in adjacent marsh plots (Yang et al., 2015). However, the lower litter quality (lignin content, C:N) of willow leaves could impede decomposition and result in greater litter C storage in willow-encroached marshes (Duffy, 2014). In summary, differences in leaf chemical composition and water nutrients in these regions may alter litter C storage and N cycling in willow-encroached marshes.

Major physiochemical property differences were detected in willow plots and adjacent plots relative to non-encroached marsh plots in both study regions. As physiochemical properties are linked to biogeochemical processes, the next step will be to determine if and how wetland processes such as C storage and N cycling are altered in willow-encroached marshes. Dissimilarities in physiochemical property differences were seen between regions, therefore it will be important to disentangle why these differences were observed and use appropriate sampling designs in future studies to ensure inaccurate generalizations are not made. Information like this is valuable to improve our understanding of the impacts shrub encroachment has on wetland function and aiding land managers in making informed decisions on willow management depending on their marsh system and desired ecosystem functions.

CHAPTER THREE: DIFFERENCES IN BIOGEOCHEMICAL PROCESSES IN TWO WILLOW-ENCROACHED SUBTROPICAL FRESHWATER MARSHES

Freshwater wetlands play a critical role in C storage and N cycling (Schuyt & Brander, 2004). Freshwater wetlands can store twice as much soil C stored as terrestrial systems and are commonly used to treat stormwater due to their ability to remove excess bioavailable N (38 - 83 g N m⁻² yr⁻¹) via denitrification (Coveney et al., 2002; Mitsch et al., 2012; Adame et al., 2015). Marshes provide these services in large part due to the soil microbial community which acts as fundamental transformers of nutrients (Mitsch & Gosselink, 2007). However, these microbes are especially vulnerable to environmental stress as marshes are commonly disturbed by hydrological alterations, nutrient enrichment, shrub encroachment, and more (Hartman *et al.*, 2008).

Environmental stress can alter the controlling factors of microbial communities, which include soil chemistry, nutrient input, and hydrology. For example, increased nutrient deposition and litterfall from shrub encroachment can increase microbial community size and modify C storage (Dhillon, 1997; Duffy, 2014). Compositional shifts between anaerobes and aerobes or oligotrophs and copiotrophs from hydrological alterations and nutrient enrichment respectively, can alter microbial C and N use efficiency (Ajwa & Tabatabai, 1994). Furthermore, studies have shown that even if restored, wetlands may show decreased bacterial diversity compared to undisturbed wetlands (Hartman et al., 2008). Changes in microbial functions such as enzyme activity and CO₂ production can also act as indicators of biogeochemical changes in disturbed landscapes (Langenheder et al., 2006). Accordingly, it is important to identify size, compositional,

and functional changes in the soil microbial community to understand if C storage and N cycling is altered in marshes under environmental stress.

Shrub encroachment, an increasingly common phenomenon in wetlands, can result in environmental stress through vegetation transitions. Studies have demonstrated that decomposition and evapotranspiration can be altered in willow-encroached marshes (Duffy 2014; Budny & Benscoter, 2016). However, few studies have focused on soil microbial differences in willow-encroached marshes, despite the key role microbes play in biogeochemical processes. Based on the detection of major physiochemical property differences in Chapter Two, it is possible that microbial-mediated biogeochemical processes (C storage and N cycling) can also be altered. Therefore, this study focused on identifying differences in the soil microbial community and biogeochemical processes in two willow-encroached marshes (Moccasin Island and Lake Apopka) within the St. Johns River watershed.

From physiochemical differences in the soil, water and leaf tissue at Moccasin Island and Lake Apopka (see Chapter Two), I predicted that increasing recalcitrant lignin-C found in willow litter would result in lower decomposition rates and greater litter C storage in willow plots for both regions (Ewing et al., 2007; Xu et al., 2008). At Moccasin Island, greater nutrient availability, recalcitrant litter, and saturated soil conditions combined with anaerobic and aerobic periods due to seasonal flooding will promote N mineralization and denitrification rates in willow and/or adjacent marsh plots. At Lake Apopka, lower nutrient availability would depress enzyme activity, denitrification, and decomposition rates in willow plots. Ultimately, I predicted that lower litter quality in willow litter would depress decomposition rates and increase litter C storage while soil nutrient availability and soil moisture would influence N cycling in each region.

Materials & Methods

Soil sampling

Sampling for all assays below were completed from soil samples collected in the summer (July – August) except for potential mineralized N (PMN) rates, which was an additional study and sampled in December 2017. In both regions, five of each plot type were randomly selected for sampling. For each analysis described below, three sampling points were randomly selected in each plot. Soil temperature was measured at each sampling point prior to collecting the sample. Two 0-10 cm soil cores were collected around each sampling point using the push core technique, extruded in the field and composited into one sample to account for soil heterogeneity. All soil cores were collected in sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) and transported on dry ice to the University of Central Florida for analyses.

Microbial community size and composition

Microbial biomass carbon

A subsample (~5 g) of wet soil of each sample was extracted with 25 mL of 0.5 M K_2SO_4 after incubating at 25°C and 150 rpm for one hour and designated as the non-fumigate sample. A duplicate subsample was chloroform fumigated for 24 hours and subsequently extracted with 0.5 M K_2SO_4 for an hour (Vance et al., 1987). Both the non-fumigate and fumigate samples were vacuumed filtered using Supor 0.45 μ M filters, preserved using DD H_2SO_4 , and stored at 4°C until analysis. Organic flocculants formed after filtration were separated from the supernatant and dissolved in 1 M H_2SO_4 . Both the supernatant and dissolved

flocculants were analyzed for DOC on a Shimadzu TOC-L analyzer (Shimadzu Instruments, Kyoto, Japan). Soil microbial biomass-C was calculated as the difference in DOC between the fumigate and non-fumigate for every sample.

Viability qPCR

For propidium monoazide (PMA) optimization, a non-antibiotic resistant human *Staphylococcus aureus* (*S. aureus*) nasal isolate of sequence type 8 (provided by the University of Central Florida's Cole Lab) was used. Cultures were designated as viable or non-viable for PMA optimization. The *S. aureus* batch designated as non-viable was immersed in a 90°C water bath for 15 minutes and then cultured on luria broth agar overnight at 37°C to verify that the culture was not viable. Quantification was measured on a BioTek Synergy HTX multi-mode reader at 600 nm (BioTek Instruments, Winooski, VT). To determine optimal PMA conditions, sets (consisting of sterilized soil spiked with either viable or non-viable *S. aureus*) were designated with different PMA concentrations (20 – 70 uM) and photoactivation times (15 - 50 min) using a 600 W LED lamp placed 20 cm above the samples. After photoactivation, all samples were extracted using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany). DNA quality was verified by ensuring A260/A230 and A260:A280 were > 1.5. Samples were quantified through quantitative polymerase chain reaction (qPCR) on a CFX96 Touch Real-Time PCR detection system (BioRad, Hercules, CA). Each 20 uL consisted of 10 uL of PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA), 1.25 ul of universal bacteria primers Eub338 and Eub518 (10 uM; Thermo Fisher Scientific, Waltham, MA), 5 ul of template DNA (0.5 ng ul), and 3.75 ul of qPCR grade water. Samples were analyzed in triplicate in polypropylene 96-well plates with an absolute standard curve (serial dilutions of *S. aureus*).

All plates were ran for 15 min at 95°C, followed by 40 cycles of 95°C for 1 min, 30 s at 53°C, 72°C for 1 min, and a melt curve at 95°C for 10 seconds. Sets with high PMA concentrations and/or long photoactivation times resulted in low recovery rates of live *S. aureus* detected due to prolonged or intense intercalation of viable DNA with PMA. Out of the two sets with > 90% recovery rates of viable *S. aureus*, the set with minimal experiment manipulations (20 uM PMA dye and 15 min of photoactivation) was selected as optimal PMA conditions (Nocker et al., 2007; Desneux et al., 2015).

Soil microbial composition identification followed the protocol described below. Soil samples were wet sieved (2 mm mesh) and injected with 20 uM PMA dye and incubated for 15 minutes prior to DNA extraction. Extracted DNA samples were run in triplicate on a CFX96 qPCR System with the same reaction mix from PMA optimization. Following a modified taxon-specific qPCR method, samples were run for 10 min at 95°C, 40 cycles of 95°C for 60 seconds, annealed at the temperatures listed in Table 6 for 30 seconds, and heated at 72°C for 60 seconds (Fierer et al., 2007; Table 6). Microbial DNA specific to each microbial taxon were extracted and quantified to create standard curves for absolute quantification during qPCR (Carolina Biological Supply, Burlington, NC; Table 6). Melt disassociation curves were run after the reaction at 95°C for 10 sec. Post-amplification products were run on a 2% agarose gel to verify amplicon length.

All bacteria, fungi and archaea abundance was calculated as the total amount of gene copies in each soil sample from each assay using domain-specific universal primers. Six of the most common bacteria taxa observed in soils were quantified using taxon-specific primers (Fierer et al. 2005; Table 6). Bacteria composition of each taxa was calculated as the gene copy number for the specific bacteria taxa over total number of all bacteria gene copies. Bacteria

classified as “other bacteria” was calculated as the remaining amount of all bacteria gene copies not identified as one of the six bacteria taxa quantified.

Table 6: qPCR Cycle Properties. Table modified from Fierer et al., 2005.

Target group	DNA Standard	Forward primer*	Reverse primer*	Amplicon length (bp)	Annealing temp (°C)
All Bacteria	<i>Staphylococcus aureus</i>	Eub338	Eub518	200	53
<i>α</i>-Proteobacteria	<i>Rhodospirillum rubrum</i>	Eub338	Alf685	365	60
Acidobacteria	<i>Acidobacterium capsulatum</i>	Acid31	Eub518	500	50
<i>β</i>-Proteobacteria	<i>Spirillum volutans</i>	Eub338	Bet680	360	60
Actinobacteria	<i>Micrococcus luteus</i>	Actino235	Eub518	300	60
Firmicutes	<i>Staphylococcus aureus</i>	Lgc353	Eub518	180	60
Bacteroidetes	<i>Novosphingobium capsulatum</i>	Cfb319	Eub518	220	65
All Fungi	<i>Eurotium chevalieri</i>	5.8s	ITS1f	300	53
All Archaea	<i>Halobacterium salinarum</i>	Arch967F	Arch-1060R	140	60

* Nucleotide sequence of primers provided in Appendix A.

Table created by author.

Microbial functions

Enzyme activity

Alkaline phosphatase, β -1-4-glucosidase, β -N-acetylglucosaminidase, β -xylosidase and cellobiose were measured with a methylumbiferone (MUF) substrate assay within 24 hours of field collection (Freeman et al., 1995). A soil slurry (~0.5 g wet soil and 39 mL of autoclaved distilled water) was incubated at 25°C for 1 hour at 140 rpm. 150 μ L of the slurry and 100 μ L of substrate (MUF-x) were incubated at 25°C for 24 hours and fluorescence activity was measured at excitation/emission wavelengths 360/460 nm on a BioTek Synergy HTX multi-mode reader at the 0 and 24-hour mark (BioTek Instruments, Winooski, VT). Enzyme activity rate was determined as the difference between the final and initial fluorescence over 24 hours.

Soil carbon production rates

To determine potential soil production rates for CO₂ and CH₄, a microcosm was created in a 120 mL serum bottom using ~ 5 g of fresh wet soil and 10 mL of N₂ purged site water. All serum bottles were closed with a rubber septa and aluminum crimp and evacuated with N₂ gas. Samples were placed in the orbital shaker at 150 rpm and 25°C for 96 hours. At every sampling time (12, 24, 48, 72, and 96-hours), headspace gas samples and pressure readings were taken to determine total gas production at a constant temperature using Henry's Law. The headspace gas was analyzed for CO₂ and CH₄ using a Shimadzu Gas Chromatograph (Shimadzu, Kyoto, Japan). Standard curves were made with known concentrations of pure mixtures of gases and a linear regression was fitted to measure the production rate of every soil sample.

Litter decay

Each plot was split into 9 quadrats and ~10 live mature leaves from the dominant plant species in the plot (willow, cattail, or sawgrass) were randomly collected at eye level in each quadrat in March 2017. All leaf tissue was rinsed with nanopore water before being oven dried at 60°C for 72 hours. A subset of fresh leaf tissue from each plot was ground and total C and N content were determined. Lignin-C content was also analyzed using the acetyl bromide method (Moreira-Vilar et al. 2014). Twelve litter bags (dimensions: 31 x 31 cm; 2 mm mesh) were filled with ~1 g of dried leaf tissue representative of the plot's vegetation composition and anchored in a circular pattern to the ground using wooden stakes in March 2017. Four random bags from each plot were collected in June 2017 and brought back to the University of Central Florida for analysis. Litter was rinsed with deionized water and dried at 60°C for 72 hours before being weighed. Mass decay rates were determined as the mass loss over time (Wieder & Lang, 1982). Nutrient (total C, lignin-C, and total N) content was determined on the litter mass remaining. In September 2017, the study regions were affected by Hurricane Irma, preventing the collection of litter bags beyond the four-month collection.

Nitrogen cycling

Potential mineralized nitrogen rates

Potential mineralized nitrogen rates were determined through laboratory incubations (White & Reddy, 2000). A subset of fresh wet soil (~5 g) was extracted with 2M KCl and allowed to incubate at 150 rpm and 25°C for 1 hour before being analyzed for NO_3^- and NH_4^+ on a Seal AQ2 Discrete Analyzer for initial extractable N content using EPA methods EPA-127 and

EPA-103 respectively. A duplicate subset was placed in a 120 ml glass serum bottle and crimp sealed with a rubber septa and aluminum cap. Samples were purged with N₂ for 10 minutes and injected with 10 ml of N₂ purged distilled H₂O to create anaerobic conditions. After shaking at 150 rpm and 25°C for ten days at room temperature, samples were extracted with 2M KCl at 150 rpm and 25°C for 1 hour and analyzed for NH₄⁺ on an AQ2 Discrete Analyzer for final extractable N concentrations. PMN rates were calculated as the difference between the initial and final extractable N concentrations over time.

Potential denitrification rates

To determine denitrification potential, a microcosm was created in a 120 mL serum bottom using ~ 5 g of fresh wet soil, 17 mL of C₂H₂ and 10 mL of 50 ppm nitrate glucose solution. Acetylene was used to stop denitrification at N₂O, which was measured using a Shimadzu Gas Chromatograph (Yoshinari & Knowles, 1976). Samples were incubated at 150 rpm and 25°C for 96 hours. The headspace gas and pressure measurements were collected at 12, 24, 48, 72, and 96-hours for analysis on the gas chromatograph. Standard curves were made with known concentrations of pure mixtures of gases and a linear regression was fitted to measure the gas concentration of every soil sample. The gas pressure, constant temperature, and headspace gas concentration were used to calculate total gas volume and thus, the denitrification potential of every soil sample.

Statistical Analyses

Each study region was statistically analyzed independently in R statistics. For every measured property, the three samples in each plot were averaged for a mean plot value to be used

in statistical analyses. Therefore, all data presented had an $n = 5$ for each plot type, and for each site. All data sets were tested for normality (Shapiro-Wilk) and homogeneity (Levene's test). For data that did not meet the assumptions (all extracellular enzyme activity rates for both regions), data was log transformed to meet the assumptions. Differences between measured properties and plot types were analyzed using a one-way ANOVA (stats package). If significant differences were detected between plot types, Tukey's test was used to determine which plots differed from each other (lsmeans package). Relationships between previously reported soil physiochemical properties (see Chapter Two) and the measured responses in Chapter Three were analyzed using linear regressions ($df = 13$) at a significance of $p < 0.05$. All results are reported as the untransformed mean \pm SE and p-values shown are from ANOVA models.

Results

Soil microbial community size and composition

Microbial composition

Microbial biomass C and viability qPCR showed no changes in microbial community size or general composition (total bacteria:archaea:fungi) between plot types at Moccasin Island. Half of the bacteria in this region were designated as "other bacteria" (DNA identified as bacteria, but not identified as any of the six dominant bacteria taxa studied). Out of the six bacteria taxa quantified, major compositional shifts within bacteria was detected. Bacteroidetes and Betaproteobacteria abundance was lowest in adjacent marsh plots ($p = 0.02$; $p < 0.001$). Betaproteobacteria and Bacteroidetes abundance was inversely related to soil temperature ($R^2 = 0.65$ and 0.98 respectively).

Table 7: Mean \pm SE % bacteria gene compositions for major bacteria taxa in Moccasin Island by plot type.

Taxon [% bacteria gene copies]	Marsh	Adj.	Willow
<i>Alphaproteobacteria</i>	1.75 \pm 0.71 ^a	1.24 \pm 0.89 ^a	0.85 \pm 0.29 ^a
<i>Acidobacteria</i>	39.8 \pm 26.0 ^a	39.7 \pm 23.7 ^a	30.9 \pm 24.6 ^a
<i>Actinobacteria</i>	3.76 \pm 0.89 ^a	2.37 \pm 1.21 ^a	3.42 \pm 0.47 ^a
<i>Bacteriodetes</i>	7.68 \pm 0.93 ^b	3.84 \pm 0.80 ^a	5.43 \pm 0.71 ^a
<i>Betaproteobacteria</i>	1.44 \pm 0.07 ^b	0.56 \pm 0.04 ^a	2.82 \pm 0.15 ^c
<i>Other bacteria</i>	45.6 \pm 6.50 ^a	52.3 \pm 8.34 ^{ab}	56.6 \pm 1.50 ^b

Superscript letters represent significantly different means by Tukey's HSD test.

Table created by author.

At Lake Apopka, willow plots had the greatest microbial community size, due to the increase in archaea abundance ($p < 0.001$; Figure 8). Fungi were found in small quantities (< 5000 gene copies g^{-1} soil) and only in willow and adjacent marsh plots. Despite no significant differences in total bacteria community size, shifts in major bacteria taxa abundance were observed by plot type. Only Actinobacteria and other bacteria abundance differed by plot type ($p < 0.001$; $p = 0.01$; Figure 9). Willow and adjacent marsh plots had more "other bacteria" (gene copies that identified as bacteria but were not within the six common bacteria taxa studied) than marsh plots ($p < 0.001$). Actinobacteria gene copy abundance was lower in adjacent marsh and willow plots (21 ± 0.81 % bacteria gene copies; 18 ± 0.4 % bacteria gene copies) compared to marsh plots (39 ± 0.4 % bacteria gene copies) and directly related to soil DOC concentrations ($R^2 = 0.55$, $p = 0.04$).

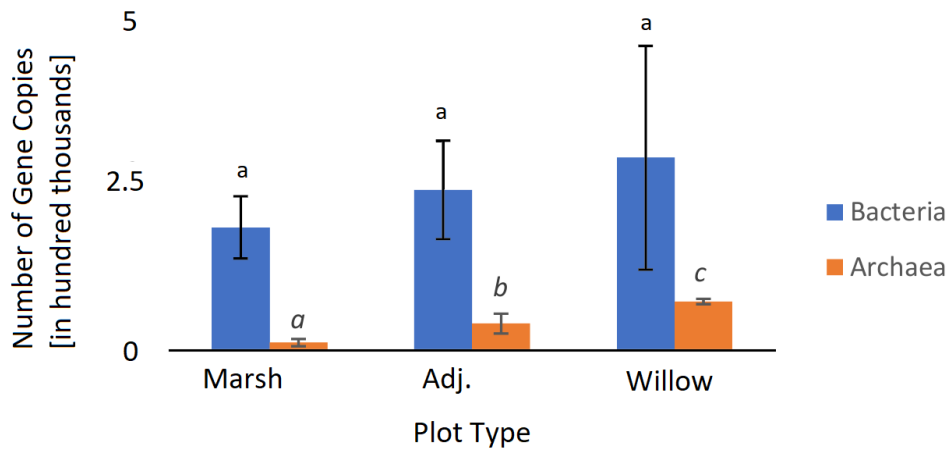


Figure 8: Mean gene copy number \pm SE for total bacteria and archaea based on universal primers in Lake Apopka by plot type. Letters signify significantly different mean rates as determined by Tukey's HSD test. Figure created by author.

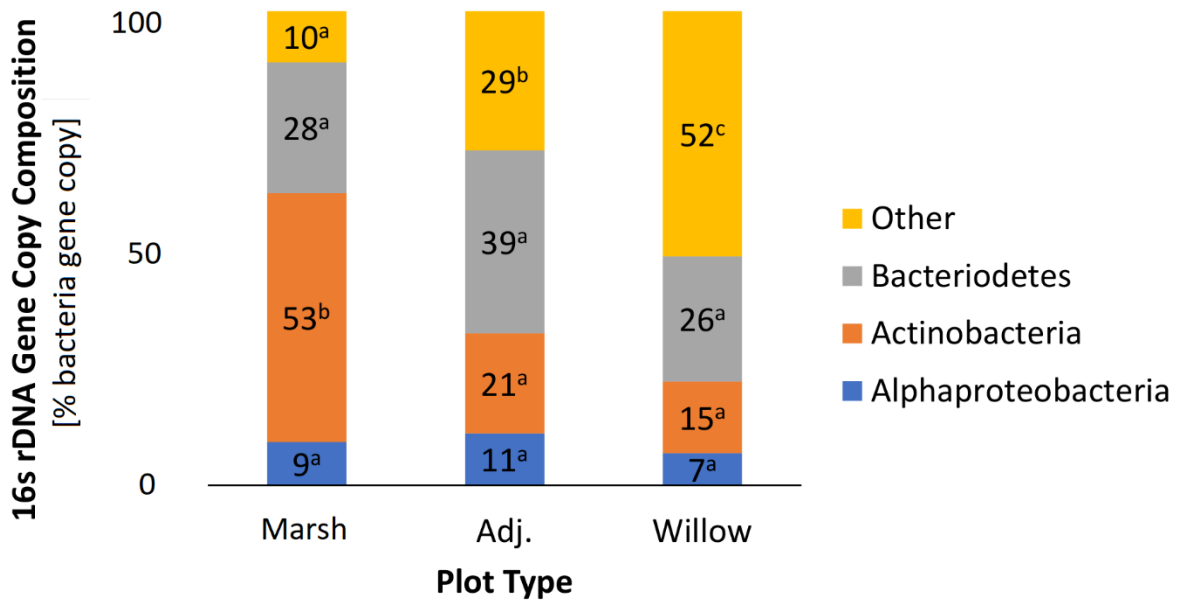


Figure 9: Mean gene copy % for bacteria taxa in Lake Apopka by plot type. Value represents mean gene copy % total bacteria and letters signify significantly different means as determined by Tukey's HSD test. Figure created by author.

Microbial functions

All statistical analyses were run on log-transformed extracellular enzyme activity rates.

For interpretation, all results and figures are shown as untransformed means \pm SE. In Moccasin

Island, both β -1-4-glucosidase and β -N-acetylglucosaminidase activity followed an increasing activity gradient from marsh to willow plots ($p = 0.05$; Figure 10a & b). Willow plots had greater alkaline phosphatase activity than all other plot types ($p < 0.01$; Figure 10c). Extracellular enzyme activity was not highly correlated to any soil physiochemical property but in general, β -1-4-glucosidase activity increased with soil DOC concentrations while β -N-acetylglucosaminidase and alkaline phosphatase activity were directly related to soil moisture content. Like enzyme activity rates, willow and adjacent marsh plots had the highest CO_2 production rates ($p < 0.01$; $1.9 \pm 0.2 \mu\text{g CO}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$; $2.1 \pm 0.2 \mu\text{g CO}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$) while marsh plots had the lowest CO_2 production rates ($1.1 \pm 0.1 \mu\text{g CO}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$). CO_2 production was directly related to soil C content ($R^2 = 0.60$, $p = 0.03$). Methanogenesis was minimal in all plots.

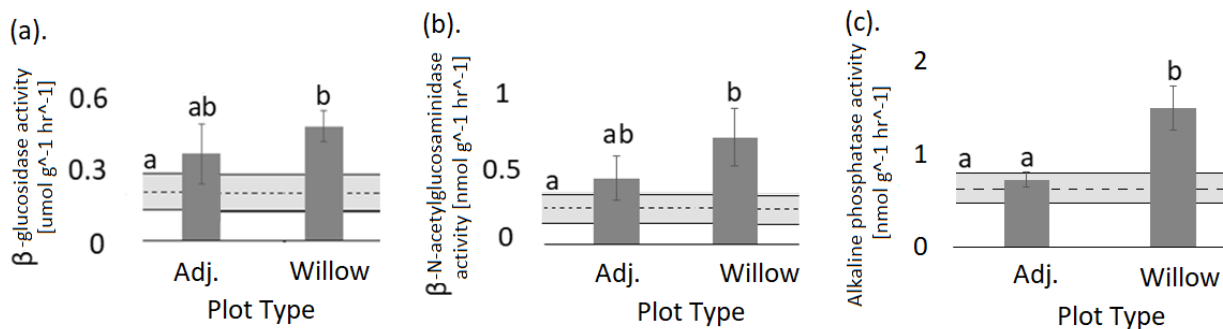


Figure 10: Untransformed mean value \pm SE extracellular enzyme activity rates in Moccasin Island by plot type. Horizontal bar represents mean value \pm SE for marsh plots. Letters signify significantly different activity rates as determined by Tukey's HSD test. Figure created by author.

All extracellular enzyme activity rates were log-transformed to meet assumptions for statistical analyses. For interpretation, all results and figures are shown as untransformed means \pm SE. At Lake Apopka, adjacent marsh and/or willow plots generally had lower β -1-4-glucosidase, β -N-acetylglucosaminidase and β -xylosidase activity rates than marsh plots ($p = 0.2$; Figure 11a-c). Only alkaline phosphatase activity significantly differed by plot type ($p <$

0.001). Adjacent marsh plots had the greatest alkaline phosphatase activity ($5.1 \pm 0.9 \mu\text{mol g}^{-1}$ soil hr^{-1}) than all other plot types (Figure 10d). In general, adjacent marsh plots ($1.8 \pm 0.2 \mu\text{g CO}_2 \text{g}^{-1}$ soil hr^{-1}) produced less CO_2 than marsh ($3.2 \pm 0.8 \mu\text{g CO}_2 \text{g}^{-1}$ soil hr^{-1}) and willow plots ($2.5 \pm 0.2 \mu\text{g CO}_2 \text{g}^{-1}$ soil hr^{-1} ; $p = 0.1$). Methanogenesis was minimal in all plots. Soil physiochemical properties explained little of the variance in enzyme activity or respiration rates except for alkaline phosphatase activity, which was directly related to soil moisture content ($R^2 = 0.57$, $p = 0.02$).

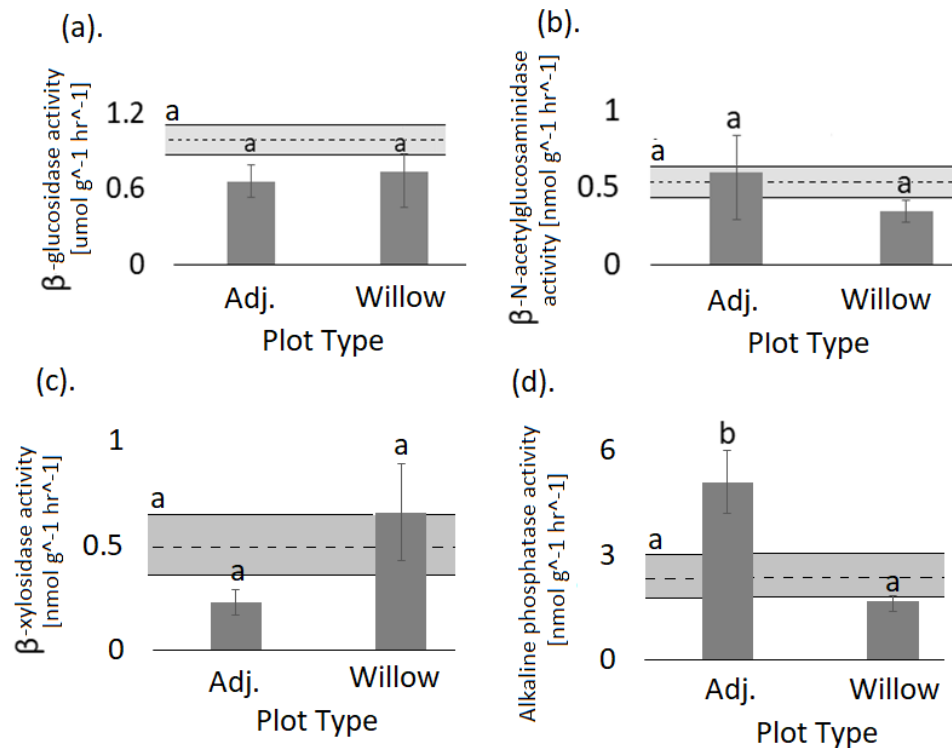


Figure 11: Untransformed mean value \pm SE extracellular enzyme activity rates in Lake Apopka by plot type. Horizontal bar represents mean value \pm SE for marsh plots. Letters signify significantly different activity rates as determined by Tukey's HSD test. Only alkaline phosphatase activity significantly differed by plot type. Figure created by author.

Litter decay

At Moccasin Island, short-term (4 month) mass decay was lowest in willow litter ($p < 0.01$; 56 ± 1 % remaining) compared to sawgrass litter in adjacent marsh (35 ± 4 % remaining) and marsh plots (39 ± 5 % remaining). Mass decay rates were directly related to leaf lignin-C content ($R^2 = 0.78$, $p = 0.02$). Initially, greater C and lignin-C content was observed in willow leaf tissue compared to sawgrass leaf tissue, resulting in lower litter quality ($p = 0.01$). After only four months of decomposition, major chemical changes were detected in the litter. Lignin-C content drastically decreased by 75% in willow litter ($p = 0.02$; Figure 12a). Willow litter had the greatest litter N concentrations ($p < 0.001$) and as a result, willow plots contained the lowest total C:N and lignin:N ratios following four months of in-situ decomposition ($p = 0.05$; $p = 0.04$; Figs 12b-d).

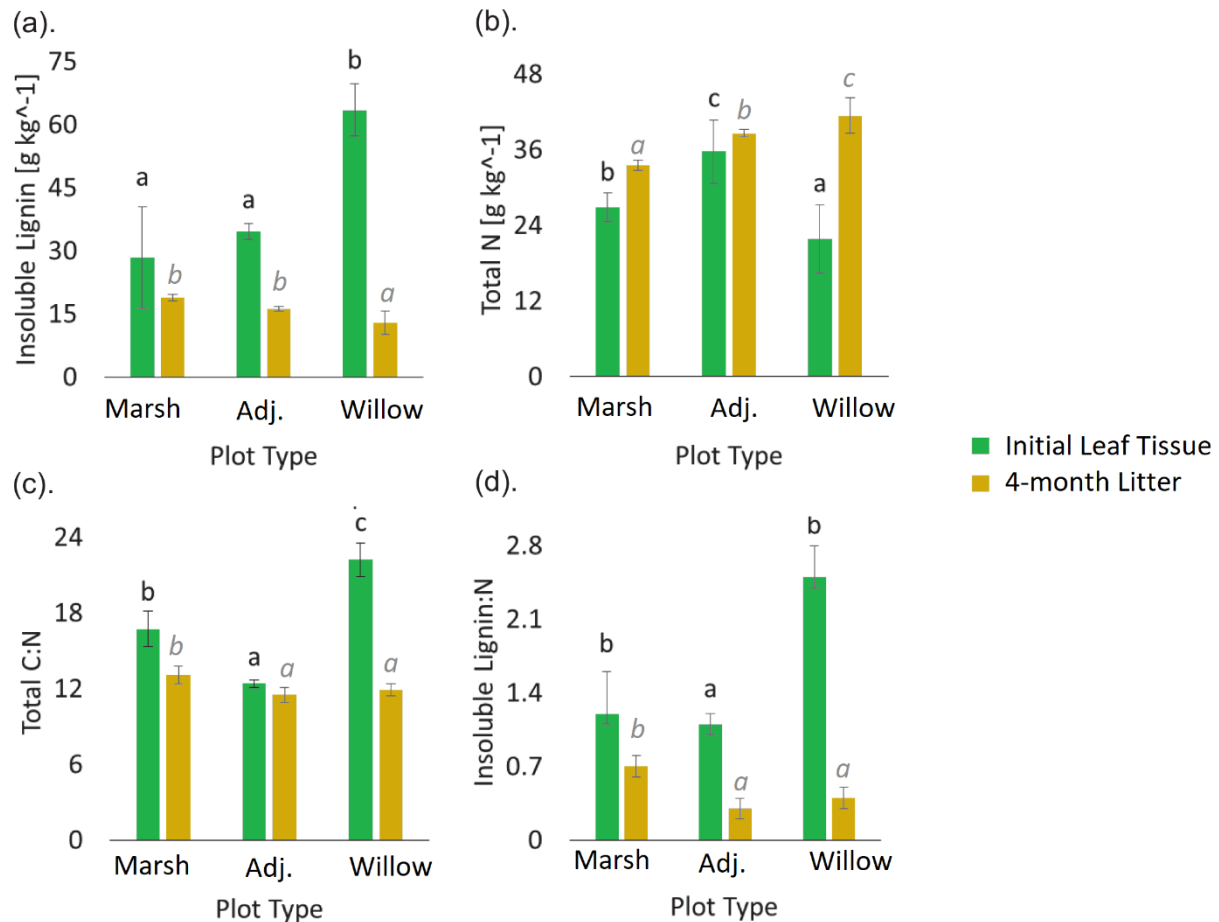


Figure 12: Mean value \pm SE for initial leaf tissue and 4-month litter in Moccasin Island by plot type. Letters represent significantly different means determined by Tukey's HSD test for initial leaf tissue. Italicized letters represent significantly different means determined by Tukey's HSD test for 4-month litter. Figure created by author.

Similarly, at Lake Apopka, mass decay was lower in willow (43 ± 5 % remaining) plots compared to marsh plots (20 ± 2 % remaining; $p < 0.001$). Greater lignin and total N content was detected in willow litter compared to herbaceous litter after four months of decomposition ($p < 0.01$; Figure 13a & b). Resultantly, remaining willow litter had greater or similar litter quality (C:N and lignin:N) as cattail litter in marsh plots (Figure 13 c & d).

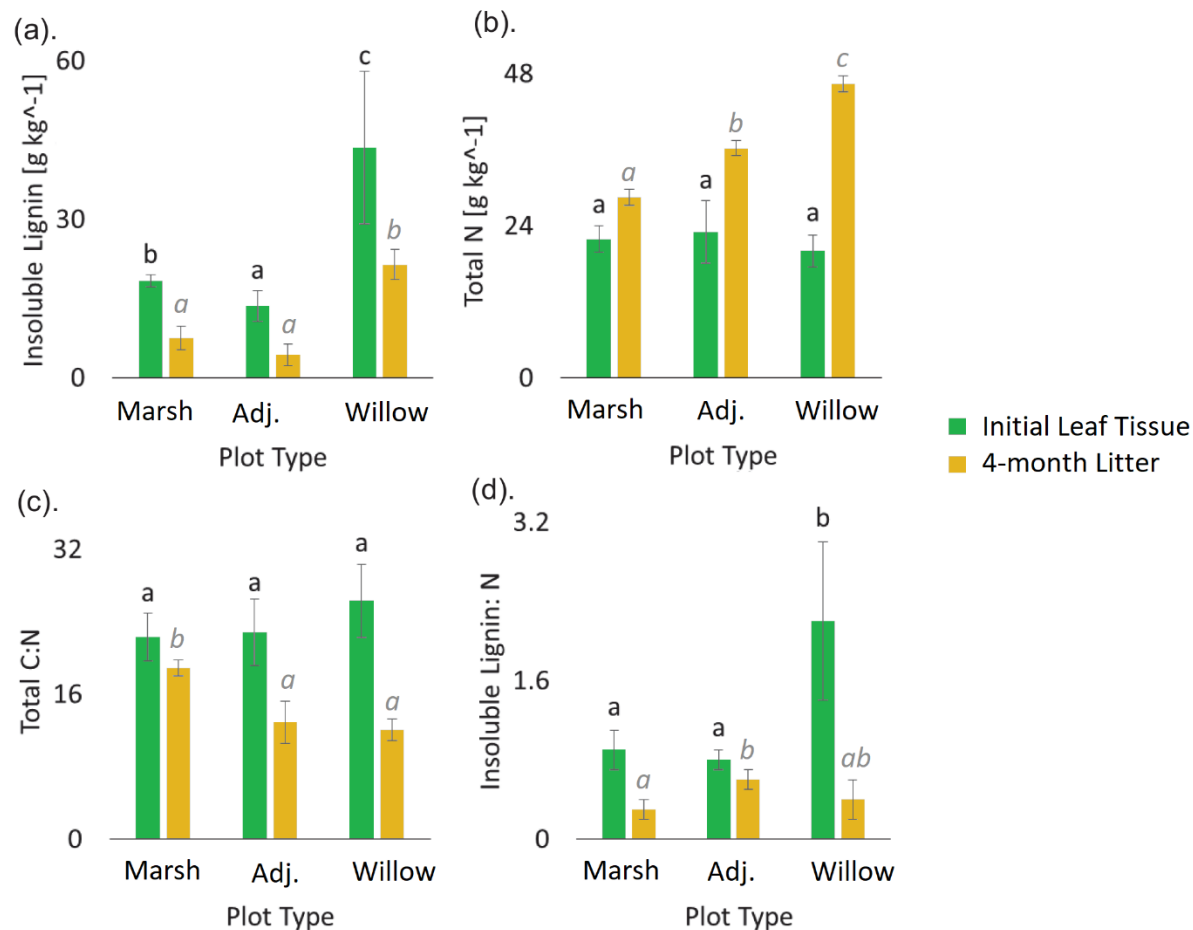


Figure 13: Mean value \pm SE for initial leaf tissue and 4-month litter in Lake Apopka by plot type. Letters represent significantly different means determined by Tukey's HSD test for initial leaf tissue. Italicized letters represent significantly different means determined by Tukey's HSD test for 4-month litter. Figure created by author.

Nitrogen cycling

At Moccasin Island, PMN and denitrification rates differed by plot type ($p = 0.02$; $p = 0.001$). Specifically, adjacent marsh ($16.1 \pm 1.3 \text{ mg g}^{-1} \text{ soil day}^{-1}$) and willow plots ($16.2 \pm 2.1 \text{ mg g}^{-1} \text{ soil day}^{-1}$) had greater PMN rates than marsh plots ($12.8 \pm 2.0 \text{ mg g}^{-1} \text{ soil day}^{-1}$). Adjacent marsh soils had greater potential denitrification rates ($0.31 \pm 0.05 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$) compared to willow ($0.22 \pm 0.04 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$) and marsh soils ($0.16 \pm 0.05 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$).

Denitrification potential was directly related to soil moisture content ($R^2 = 0.66$, $p = 0.01$).

At Lake Apopka, PMN rates were similar in all plots and only differences in denitrification rates were observed ($p < 0.001$). Willow and adjacent marsh plots had lower denitrification rates ($0.22 \pm 0.05 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$; $0.24 \pm 0.04 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$) than marsh plots ($0.34 \pm 0.02 \mu\text{g N g}^{-1} \text{ soil hr}^{-1}$). Denitrification potential was directly related to soil total N content ($R^2 = 0.55$, $p = 0.03$).

Discussion

This study emphasizes the significance of shifting vegetation communities within wetlands, as major differences in microbial activity and biogeochemical processes were detected within willow and/or adjacent marsh plots and between study regions (Figure 14). I found that microbial compositional changes were highly related to labile-C pools and temperature, while nutrient availability and soil moisture greatly impacted microbial functions. Resultantly, C storage and N cycling differences in willow-encroached marshes were detected and not synonymous between study regions within the same watershed.

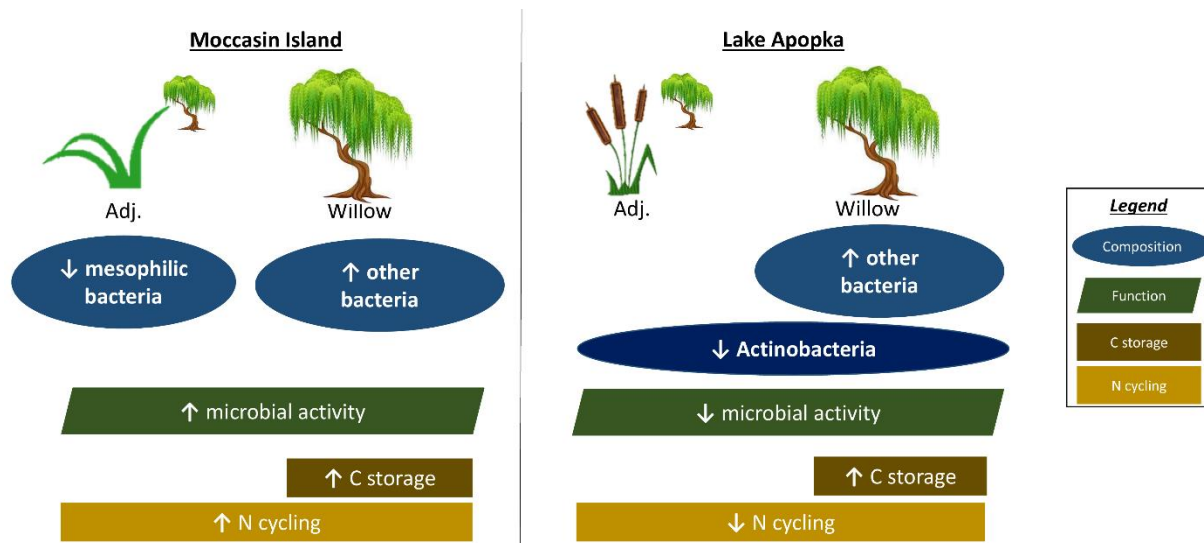


Figure 14: Key differences in biogeochemical processes in willow and adjacent marsh plots compared to marsh plots by region. Boxes overlaying both adj. and willow plots signify differences in both plot types ($p < 0.05$). Figure created by author.

Labile-carbon pools and temperature are related to microbial compositional changes

Correlations between soil microbial composition and physiochemical properties in the study regions demonstrated that changes in litter quality and temperature were related to bacteria community composition differences. Soil DOC, often considered the labile-C pool microbes can easily access for metabolism, was observed as a correlate of Actinobacteria abundance (Gonet & Debaska, 2006). Some studies have shown no relationship between oligotrophs like Actinobacteria to labile-C (Fierer et al., 2007) sources while Actinobacteria increased in response to labile-C amendments in other studies (Goldfarb et al., 2011). In this study, Actinobacteria abundance was directly related to soil DOC concentrations with lower Actinobacteria abundance in adjacent marsh and willow plots at Lake Apopka. At Moccasin Island, soil temperatures played a larger role in determining microbial community shifts. Soil temperature was highly correlated to β -Proteobacteria and Bacteroidetes abundance, where less

gene copies from these taxa were observed in adjacent marsh plots which also had the highest temperatures. Previous studies have shown both taxa to be considered mesophilic bacteria (Green et al., 2006). Temperature played a larger role at Moccasin Island than at Lake Apopka due to Moccasin Island's seasonal flooding status. This may have permitted greater direct radiance onto the soil surface compared to Lake Apopka, where surface water levels were always > 30 cm and surface temperatures did not differ between plots. In sum, C quality or soil temperatures were major indicators of bacteria abundance shifts in willow-encroached marshes.

Nutrient availability and soil moisture linked to microbial functions

In this study, enzyme activity and respiration rates differed between regions and was associated with nutrient availability and soil moisture. At Moccasin Island, greater microbial activity was observed in willow and/or adjacent marsh plots where greater nutrient availability and soil moisture were detected. At Lake Apopka, willow plots had lower microbial activity, nutrient availability, and moisture. Relationships between soil physiochemical properties and biogeochemical processes were consistent with the current literature. CO₂ production was directly related to soil C availability, while denitrification potential was related to soil N availability and soil moisture. Greater nutrient (C and N) availability can allow for greater microbial biomass size and respiration (Hartman et al., 2008). Greater soil moisture content can inhibit O₂ diffusion, promoting reduced soils conditions (-50 to 50 mV) and denitrification (Wierer et al., 1993). Overall, greater microbial activity occurred where larger soil nutrient pools were detected and denitrification activity was linked to soil moisture.

Litter carbon storage

Despite a slight increase in CO₂ production in Moccasin Island's willow plots, mass decay was halved in the same plots, suggesting greater litter C storage potential. At Lake Apopka, both CO₂ production and mass decay rates were depressed in willow plots. Mass decay rates were highly correlated to recalcitrant lignin-C content in the plots' respective litter. Other studies have also found similar results; litter quality decreases with shrub encroachment and depresses decomposition rates (Duffy, 2014). However, it is important to note that the rates reported in this study and many others only demonstrate short-term decomposition rates. It has been assumed that long-term decomposition rates may be magnitudes slower than short-term decomposition rates, as labile-C rapidly breaks down in fresh litter, but older litter has slower turnover times as mainly recalcitrant-C sources remain (Bazter & Sharitz, 2014). Conversely, this study reported litter chemistry changes that suggest willow litter quality may improve in as little as four months of decomposition, potentially speeding up the rate of decomposition in the long-term. The greater fungi abundance in willow and adjacent marsh plots may also aid in quicker turnover of recalcitrant litter in willow plots. In this study, willow-encroached marshes increased as potential short-term C sinks due to willow's lower initial litter quality. While short-term decomposition rates show depressed mass loss of willow litter, litter chemistry changes and microbial composition differences suggest that long-term decomposition may not be linear and willow-encroached marshes could store less litter C in the long-term. Future studies on long-term decomposition can help elucidate on long-term litter C storage in willow-encroached marshes.

Nitrogen cycling

Freshwater marshes are commonly at risk of eutrophication from agriculture and urbanization and it is important to consider how shrub encroachment can alter N cycling. Greater N mineralization and lower denitrification in willow plots may result in eutrophication and decreased water quality over time as less net bioavailable N is removed from the water. At Moccasin Island, greater N cycling (mineralization and denitrification) was detected in willow and adjacent marsh plots, while Lake Apopka's willow plots had lower denitrification potential than marsh plots. These trends are consistent with previous studies on N cycling and nutrient amendments; N mineralization and denitrification rates were directly related to nutrient availability and moisture content respectively (Peng et al., 2007; Barnes et al., 2012). N cycling was highly dependent upon the region and associated with soil physiochemical properties.

Structural differences between sawgrass and cattail may also contribute to N cycling differences between regions. The presence of hypertrophied lenticels and secondary aerenchyma in cattail and willows (*S. nigra*, *S. viminalis*) can promote oxidation hotspots via radial oxygen loss, allowing for coupled nitrification/denitrification in the rhizosphere (Pereira & Kozlowski, 1977; Li et al., 2010; Randersen et al., 2011). At Lake Apopka, no differences in N mineralization was observed between cattail and willow plots. However, at Moccasin Island, both willow and adjacent marsh plots (potential willow root penetration) had greater N mineralization rates than sawgrass control plots. Future studies on radial oxygen loss between these three species will need to be done to corroborate this.

Major differences in soil microbial composition and function were observed in willow and adjacent marsh plots, resulting in slower decomposition and changes in N cycling. Differences in the microbial community and biogeochemical processes in willow-encroached marshes were highly dependent upon physiochemical properties such as litter quality, nutrient availability, and soil temperature. The notable differences in C storage and N cycling in willow-encroached marshes in this study emphasizes the importance of understanding how shrub encroachment can impact wetland functions. Furthermore, it is important to acknowledge that marshes, even within the same watershed, may have different responses to willow-encroachment. This is fundamentally important for land managers who deal with shrub encroachment in a multitude of marshes that are inevitably different in hydroperiod, vegetation, etc. Based on these results and findings from future studies, land managers can make better informed decisions on whether to continue willow removal or to maintain willow populations based on desired wetland functions and marsh conditions.

CHAPTER FOUR: CONCLUSION

Physiochemical properties and biogeochemical processes differed in willow and/or adjacent marsh plots compared to non-encroached marsh plots. Physiochemical properties (nutrient availability, soil moisture, and soil temperature) were highly associated with observed changes in microbial composition and function. Together, the differences in physiochemical properties and biogeochemical processes altered these marshes' ability to sequester C and cycle N. Greater short-term litter C storage was observed in both regions, but N cycling changes differed by region. As land managers are currently investing resources to remove willows from a variety of wetlands, it is critical for land managers to have data that can help them make informed decisions based on desired ecosystem functions. For example, greater C storage was detected in willow plots in both regions, but previous studies have shown greater evapotranspiration rates in subtropical willow-encroached marshes (Budny & Benscoter, 2016). Depending on if C storage or water storage is a desired function, it can impact whether land managers decide to keep or remove willow. Long-term decomposition and nutrient runoff studies are needed to gain a clearer understanding of C and N cycling in willow-encroached marshes and to aid land managers in the decision-making process. These findings provide the foundation for future functional studies as it demonstrated that C and N cycling were altered in willow-encroached marshes.

The differences observed in willow-encroached marshes were dissimilar to terrestrial systems, as previously assumed. A common effect of shrub encroachment in terrestrial systems is the creation of fertility islands, where greater soil nutrients are observed within shrub canopies

(Schlesinger & Pilmanis, 1998). However, less soil C was observed in willow plots at Lake Apopka, potentially due to litter suspension in the water column and the dispersion of litter and nutrients from willow plots via water. Only at Moccasin Island, where litter and nutrients were deposited directly onto the soil surface during seasonal dry-down, were fertility islands detected. Nevertheless, even at Moccasin Island where fertility islands were present, greater nutrient concentrations were also detected in adjacent marsh plots. This is contrary to terrestrial studies where there is generally a decrease in nutrients in the adjacent regions (unvegetated bare soil). In highly productive wetlands such as the marshes in this study, adjacent plots are rarely unvegetated. The presence of vegetation in adjacent plots promoted nutrient deposition whereas unvegetated soils in terrestrial systems promote the loss of soil nutrients over time as they are dispersed by wind or water. A common theme in both studies was the dissimilarities between freshwater marshes within the same watershed, highlighting the need for studies to use appropriate sampling regions to reduce the risk of making inaccurate generalizations. Ultimately, this study emphasizes the need for more research in aquatic systems to truly understand how wetland functions will be impacted by shrub encroachment.

As one of few characterizations of physiochemical properties and biogeochemical processes in willow-encroached marshes, it has provided the foundation for future research, such as isolating the less common bacteria taxa detected but unidentified in willow soils and investigating how they might impact biogeochemical processes. Also, the greater litter quality of willow litter compared to herbaceous litter after only four months of decomposition raises the question of how long-term decomposition will be altered in willow-encroached marshes. In summary, this thesis begins to inform a large knowledge gap in wetland disturbance ecology and

demonstrates the importance of studying the impact of shrub encroachment on valuable ecosystem functions like C storage and N cycling.

APPENDIX A: PRIMER SEQUENCES FOR QPCR

Table 8: Primer Sequences for qPCR

Primer	Primer sequence (5'-3')
Eub338	ACT CCT ACG GGA GGC AGC AG
Eub518	ATT ACC GCG GCT GCT GG
Alf685	TCT ACG RAT TTC ACC YCT AC
Bet680	TCA CTG CTA CAC GYG
Actino235	CGC GGC CTA TCA GCT TGT TG
Lgc353	GCA GTA GGG AAT CTT CCG
Cfb319	GTA CTG AGA CAC GGA CCA
Acid31	GAT CCT GGC TCA GAA TC
ITS1f	TCC GTA GGT GAA CCT GCG G
5.8sr	CGC TGC GTT CTT CAT CG
Arch967F	AATTGGCGGGGGAGCAC
Arch-1060R	GGCCATGCACCWCTCTC

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