INVESTIGATING RE-VEGETATION PATTERNS, NUTRIENT LIMITATIONS, AND STORAGES OF NITROGEN AS DETERMINANTS OF RESTORATION SUCCESS

By

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To my daughter, Victoria, for all your patience and love!

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The role of biodiversity on the function and stability of ecosystems has long been the object of scientific debate. Past research has found that variations in ecosystem function are related to differences in the functional characteristics, especially resource capture and utilization, of the dominant plants.

The Hole-in-the-Donut (HID) region of the Everglades National Park (ENP) offers a unique opportunity to investigate the successional development of vegetation and the impact of macrophyte diversity on ecosystem functions, including productivity, nutrient-use efficiency and turnover. Historically, the HID was dominated by short hydroperiod prairies and pinelands. After 1916, farming practices were employed that altered approximately 4000 ha of natural vegetation. After 1975, farming ended and the HID was aggressively colonized by a non-native pest plant *Schinus terebinthifolius* (Brazilian pepper).

The goal of my dissertation was to determine; limitations on nitrogen and phosphorus, the nutrient-use efficiency of the vegetation, and long term nitrogen storages in each restored site as well as the surrounding native communities. Additionally, I evaluated relationships between macrophyte diversity and soil characteristics and processes.

The results of this study offers evidence that, with time, a diverse plant community similar to a native wetland community can develop after restoration by means of scraping away all soil. Additionally, this study indicated that soil processes have a greater influence on ecosystem N dynamics than does the plant community composition. We found that the soil in the native community stores significantly more N, is P-limited, and the dominate vegetation will regenerate more nutrients as compared to the restored wetland ecosystem soil and vegetation.

CHAPTER 1
INTRODUCTION

The role of biodiversity on the function and stability of ecosystems has been the object of recent scientific debate. Past research has found that variations in ecosystem function are related to differences in the functional characteristics, especially resource capture and utilization, of the dominant plants. The implication that ecosystem processes are dependent on higher levels of biodiversity and vise versa has become more evident (Loreau 2000, Chabrerie et al. 2001, Engelhardt and Ritchie 2001, Hooper et al. 2005).

Research has shown that importance should be placed on the impact of species interactions, rather than species number alone, on ecosystem function (Johnson et al. 1996). The importance of macrophyte diversity in wetland ecosystems and its link to ecosystem function (i.e., productivity, decomposition, and nutrient-use efficiency) has received little attention. A positive relationship between plant diversity and primary productivity has been demonstrated in grasslands (Naeem et al. 1994, Tilman and Downing 1994, Walker 1995, Tilman et al. 1996), but little research has been done in wetlands. Due to the high level of disturbance and destruction of natural wetlands, the loss of ecosystem function and diversity is inevitable. Through restoration and mitigation efforts, the loss of wetlands is decreasing, but the success of these efforts in terms of function and diversity is still unclear.

To understand the relationships between the vegetative community and ecosystem processes, it is important to understand what factors govern ecosystem structure. Several models have been conceptualized to explain ecosystem development and controls on soil processes and vegetative communities (Grime 1977, van der Valk 1981, Chapin et al. 1996, Lortie et al. 2004). The conceptual model proposed here combines several concepts from these existing models, but focuses more on the close linkage between the vegetation community, soil development and

ecosystem processes as well as the primary driving forces (i.e., life characteristics, competition, potential vegetation) influencing vegetative community structure (Figure 1-1). The vegetation community present in an ecosystem is closely linked to the soil properties, and the interactions between the vegetation and the soil environment influence the dominant ecosystem processes through feedback cycles.

Wetland plant communities are heavily influenced by their hydroperiod. Wetland ecosystems that are continually flooded verses seasonally flooded can have very different vegetative community structures (van der Valk 1981). The life characteristics (i.e., life span, propagule longevity, and propagule establishment requirements) of wetland vegetation will determine their success under different hydrologic conditions. In the case of restored wetland systems, the seed bank (potential vegetation) can become an important driving force controlling the community structure that develops. However, with time these early successional plant species can be driven out through competition for nutrient and other important resources by later successional plant species (Grime 1977, van der Valk 1981).

Rationale and Significance

Scientists have long acknowledged the importance of vegetation on wetland ecosystem function. Several studies have investigated the impact of nutrient enrichment (nitrogen (N) and phosphorus (P)) on vegetative communities in wetlands (Zedler 1993, Willis and Mitsch 1995, Bobbink et al. 1998, Boyer and Zedler 1998, Bedford et al. 1999, Mahaney et al. 2004, Rickey and Anderson 2004) or the nutrient removal potential of wetland plants (Reddy and DeBusk 1985, DeBusk et al. 1995, Tanner et al. 1995, Tanner 1996, Oomes et al. 1997, DeBusk et al. 2001), but few studies have been able to identify the role of vegetation diversity on wetland ecosystem processes (i.e., nutrient cycling). Changes in species composition could have long term impacts on N cycling in wetlands. From studies in grasslands, fens, and forest ecosystems,

it has been suggested that species composition may affect N cycling and storage by influencing the rate of N uptake, the use efficiency of N, the litter quality (Vitousek 1982, Johnson et al. 1996, Aerts and deCaluwe 1997, Grime 1997, Aerts et al. 1999), and therefore the rates of mineralization of soil organic N.

The amount of N available for plant uptake is determined by the balance between external inputs and outputs and by the internal cycling of nutrients (Aerts et al. 1999). As N is cycled through an ecosystem it is continuously transformed between different chemical species, mostly through biological processes. The conversion of N into various forms is important to maintain the biological requirement necessary for plant and microbial growth. Mineralization is a key process regulating the bioavailability of N for plant assimilation. It is a microbial mediated process that converts organic N to inorganic N (NH_4); therefore rates of mineralization can be significantly correlated to microbial biomass (White and Reddy 2001).

Due to the anaerobic nature of wetland soils, NH_4^+ is the most stable inorganic form of N. The oxidation of NH_4^+ to NO_3^- (nitrification) is limited to the water-soil interface where oxygen diffuses from the water column into the soil or at the root-soil interface. Through diffusion and advection flow, wetland plants can transport oxygen into the root zone creating an oxygenated rhizosphere where nitrification can occur (Reddy et al. 1989). This NO_3 is then removed by plant uptake or by microbial communities via denitrification. Denitrification is an anaerobic microbially driven process by which NO_3 is reduced to nitrous oxide (N_2O) or N_2 gas which is then released to the atmosphere.

The N availability in the soil can affect the productivity of vegetation. Plants growing in high N soils tend to have a higher tissue N concentration and higher photosynthetic rates than do plants growing in low N soils (Chapin et al. 2002). Nutrient (N or P) limitation is defined as the

increase in growth in response to the addition of the limiting nutrient (Chapin et al. 1986). However, it should be noted that plant communities adapted to nutrient poor ecosystems generally have a lower growth response to nutrient addition than to plant communities adapted to nutrient rich soils. This difference in response has been contributed to the maximum potential growth rate of the plant species adapted to the soil nutrient conditions (Grime 1977, Chapin et al. 1986). In wetland ecosystems, studies have shown that productivity has increased with N additions but at the cost of shifting the plant community from a species-rich to a species-poor community (Fojt and Harding 1995, El-Kahloun et al. 2003). This suggests that while nutrient enrichment may increase productivity it may be at the cost of decreased plant diversity.

The response of vegetation to different levels of nutrient availability is often evaluated by considering their nutrient-use efficiency (NUE). In short-lived plants (annuals), the NUE has been defined as the organic matter produced per unit of nutrient taken up or more simply the inverse of nutrient concentration in plant tissue (Chapin 1980). For long-lived perennial plants, however, it has been argued that the NUE cannot be taken simply as the inverse of plant nutrient concentration (Vitousek 1982). The NUE for perennials is thus defined as the amount (in grams) of organic matter lost from plants or permanently stored within plants per unit (in grams) of nutrient lost or permanently stored (Vitousek 1982, Birk and Vitousek 1986). In other words, the NUE is the ratio between above-ground biomass production and nutrient loss in litterfall.

Berendse and Aerts (1987), however, have suggested that the aforementioned NUE definitions are inappropriate for assessing the efficiency of N for dry matter production at the species level. They suggest that the NUE-N (NUE of nitrogen) of individual plants should include the mean residence time of the N in the plant as well as the rate of carbon fixation per unit of N in the plant (N productivity). The mean residence time of N is defined by $1/L_n$, where

L_n is the N requirement per unit of N in the plant (g N g^{-1} N yr^{-1}). The N requirement is the amount of N that is needed to maintain each unit of biomass during a given time period (g N g^{-1} dry weight yr^{-1}). The N productivity (A) is defined as dry matter production per unit of N in the plant. They suggest that N productivity is important in terms of NUE because the amount of N in the leaves of plants is one of the primary properties that determine the rate of photosynthesis. By combining the concepts of mean residence time and N productivity, the NUE at the species level is the product of the two, A/L_n (Berendse and Aerts 1987).

Yet another term often used along with NUE is that of nutrient resorption from senescing leaves. The nutrient-resorption efficiency (NRE) is defined as the ratio of the amount of nutrients resorbed from mature leaves to the maximum nutrient pool in the mature leaves expressed as a percent (Aerts et al. 1999). The NRE of N (NRE-N) from senescing leaves is typically around 40-50%, but NRE-N's as low as 0% and as high as 90% have been reported (Aerts 1996, Aerts et al. 1999, Chapin et al. 2002). It has been suggested that the large variation in NRE is in response to nutrient availability status (Aerts 1996, Killingbeck 1996, Aerts et al. 1999, Richardson et al. 1999), in other words, in nutrient limited systems the NRE of senescing leaves will be greater than in nutrient rich environments. However, reviews of the literature indicate that no such trends have been successfully supported (Chapin 1980, Aerts 1996).

At the ecosystem level, NRE could have significant implications in terms of nutrient cycling. To decrease dependence on soil nutrient availability and nutrient uptake, plants will resorb nutrients during senescence so that they are readily available for future plant growth. Past studies have suggested that efficient retranslocation or low losses of nutrients can increase the fitness of plant species in nutrient limited ecosystems (Grime 1977, Berendse 1994, Richardson et al. 1999). In addition, high NRE and NUE of vegetation can limit the

remineralization (decomposition) of nutrients in the ecosystem due to low litter nutrient content (poor litter quality) (Bridgham et al. 1995).

Decomposition of plant material is a key process for nutrient cycling within ecosystems. There are several factors that control the rates of decomposition. Mean annual temperature, precipitation, soil moisture, microbial biomass, chemical composition of the soil (i.e., available N, P, and C) and litter material (i.e., lignin, tannin, amino acids, carbohydrates, C:N:P ratios) are some of the most important factors in considering decomposition rates (Aerts and deCaluwe 1997, Gartner and Cardon 2004). Nitrogen or P concentrations can limit the rates of decomposition depending on which one is a limiting resource within the ecosystem (Aerts and deCaluwe 1997, Feller et al. 2003). Feller et al. (2002) found in sites that were N-rich and P-limited that with P fertilization the NUE-P decreased and decomposition rates increased by almost 40%. This suggests that by increasing a limiting nutrient in the soil, the litter quality can be altered and in turn increase decomposition rates and nutrient turnover.

By investigating N cycling (mineralization, nitrification, and vegetative uptake), nutrient-use efficiency, decomposition and the diversity and abundance of vegetation, inferences on mediators of vegetation communities or the influence of plant diversity on ecosystem function can be made. Controls on nutrient availability and turnover can influence to nutrient dynamics of an ecosystem and limit the amount of nutrients available for plant uptake. In addition, the ability of some plant species to capture and efficiently use these nutrients can increase their productivity and fitness allowing them to out-compete less efficient plant species.

Problem Statement

History

Invasive exotic plants are a threat to Florida's natural areas. The problems associated with foreign aquatic invasions are infringing on both disturbed and pristine ecosystems. The Exotic

Pest Plant Council (EPPC) has identified *Schinus terebinthifolius*, Raddi (Brazilian pepper or Florida holly) as one of Florida's most invasive species. *Schinus terebinthifolius* is native to Brazil, Argentina, and Paraguay and was first introduced into the United States in the 1840's as an ornamental. This evergreen dioecious tree belongs to the Anacardiaceae family and is related to poisonwood, poison oak, poison ivy, mango, and pistachio, etc. *Schinus terebinthifolius* produces dense clusters of small (1.5 mm) white flowers usually in spring. Their fruit is a cluster of small berries (6 mm diameter) which change from green to bright red as they ripen, hence the misnomer "Florida holly." These berries can have narcotic or toxic effects when eaten by birds and other wildlife (Clark 1997).

The habit of *S. terebinthifolius* is a small tree (typically to 10 feet height but can reach 40 feet in height) and it is abundant in disturbed moist to mesic sites in the southern half of the Florida peninsula. It forms dense thickets which exclude native vegetation by shading and chemical inhibition of their growth, and provide relatively poor wildlife habitat. Trees are moderately salt tolerant, withstand flooding, fire, drought, and quickly re-sprout after being cut. The root system is not considered invasive.

Schinus terebinthifolius is a pioneer of disturbed sites in Florida ranging from highways, canals, fallow fields, and drained cypress stands (Ewel et al. 1982). Furthermore it successfully colonizes many native plant communities, including pine flatwoods, tropical hardwood hammocks, and mangrove forests (Ewel et al. 1982). In addition to its threat to Florida's natural ecosystems, it poses some potential health threats. Being a relative of poison ivy, direct skin contact with its sap can cause severe skin irritation, airborne chemical emissions can result in sinus and nasal congestion and headaches. Consumption by horses and cattle has resulted in hemorrhaging, intestinal compaction, and fatal colic (Clark 1997).

In spite of *S. terebinthifolius* invasive and hazardous environmental qualities, it has several economic uses as well. As its common name (Brazilian pepper) suggests, the dried fruits are used as a spice and sold in the United States as "pink peppercorn." In areas of South America where it is native, the plant is used as a tonic and astringent. In Brazil it is considered medicinal and used in remedies to treat ulcers, respiratory problems, wounds, rheumatism, gout, tumors, diarrhea, skin ailments and arthritis. Other products include: toothpicks and a pollen source for honey bees (Clark 1997).

The invasion if *S. terebinthifolius* is recorded in the Everglades National Park (ENP) in the region called the Hole-in-the-Donut (HID) as early as 1959 (Figure 1-2). Historically, the HID was dominated by short hydroperiod prairies and pinelands. After 1916, farming practices were employed that altered approximately 4000 ha of natural vegetation. After 1975, farming ended and the HID was aggressively colonized by *S. terebinthifolius* (Dalrymple et al. 2003). The rapid spread of *S. terebinthifolius* resulted in population growth rates with increases of 20 times its density per year (Loope and Dunevitz 1981). This dense canopy in mature stands limits the ability of understory vegetation to exist or for desired indigenous vegetation to compete (Doren and Whiteaker 1990).

Attempts to control *S. terebinthifolius* through use of prescribed fire, mechanical removal in conjunction with native plantings and chemical treatment all proved unsuccessful. The dense canopy and lack of understory litter material made prescribed fire difficult and the high germination rate, high survival rate of seedlings, and rapid growth made chemical control difficult and costly (Doren and Whiteaker 1990, Dalrymple et al. 2003). These failures led to the use of mitigation funds from Miami-Dade County to employ a "scraping" method to restore wetlands in the HID. This method involves the mechanical removal of existing *S.*

terebinthifolius and underlying rock-plowed rubble and substrate leaving behind bedrock with pockets of captured substrate material. These pockets provide enough substrate for hydrophytic, herbaceous vegetation to develop on the scraped sites. A pilot project of 18 ha in 1989 proved successful in the natural recolonization of indigenous wetland vegetation and the prevention of *S. terebinthifolius* invasion (Dalrymple et al. 2003). This success led to the long-term wetland restoration project to restore the entire HID by use of the "scraping" method (Figure 1-3). Yearly restoration sites began in 1997 and will continue to completion in 2010.

The HID region of the ENP (Figure 1-4) offers a unique opportunity to investigate the successional development of vegetation and the relationship between macrophyte diversity and ecosystem functions, including productivity, nutrient-use efficiency and turnover. The desired dominant species within the HID is *Cladium jamaicense* (sawgrass). *Cladium jamaicense* is the dominant species within natural, undisturbed portions of the ENP. It is present within restored sites of the HID but it is not a dominant species. It is more abundant in the earliest restored site (1989) than it is in sites that are only a few years old. In the first few years after restoration begins, sites are dominated by weedy generalists who can tolerate harsh conditions. In the most recent survey of the restored sites, the dominate vegetation are *Typha domingensis*, *Andropogon glomeratus* and *Sagittaria lancifolia* and very little *C. jamaicense* is found (O'Hare and Dalrymple 2003). One important question in the successional development of the HID is 'what are colonization patterns for *C. jamaicense* and why is it not dominating the restored sites?'

Preliminary Work

Currently, the Everglades Research Group (ERG) conducts annual monitoring of the vegetation community, soil depth, and hydrology of each of the restored sites, as well as a reference and *S. terebinthifolius* site (Figure 1-5). For the vegetation sampling, the Braun-Blanquet method is employed to evaluate the overall vegetation community structure in

permanently establish plots every fall at peak biomass. On each restored site there are 20-10 m^2 plots (10 randomly located near natural vegetation; 10 randomly located far from natural vegetation) and 40-1 m^2 plots (20 nested in the northwest corner of each of the large plots; 20 randomly located in the intermediate strata of the site). The large plots were established to address broad characteristics of vegetation assemblages and the small plots are used to evaluate species composition with regard to soil depth, elevation, and hydrology (O'Hare and Dalrymple 2003). These data were re-evaluated for statistical relationships between the vegetation community structure, soil depth, elevation, and hydrology as well as with additional soil biogeochemical and vegetation properties collected in this study.

Project Objectives

The goal of my research was to determine the role of vegetation diversity composition on wetland ecosystem function throughout successional development after restoration and the influence of nutrient availability on macrophyte nutrient-use efficiency (NUE) of N and P. Anthropogenic impacts, such as farming practices, can drastically change the nutrient dynamics of ecosystems. Wetlands created on abandoned agricultural land are typically P-rich and N-limited. This could in turn impact the diversity and dominance of the macrophytes present. As the system develops, more N can be introduced via fixation and organic matter accretion while the P becomes tied up in the substrate shifting the dynamics of the system to a P-limited system. In response to the change in nutrient dynamics, the vegetation will also change. To assess changes in vegetation diversity and dominance the following studies were investigated in field plots of 0, 1, 2, 4, 8, and 16 years after restoration (Figure 1-6).

Study 1. Spatial and Temporal Patterns

Objective 1. Relate the diversity indices of macrophytes as well as the abundance of the dominant macrophytes and *Cladium jamaicense* to environmental characteristics by use of multivariate statistical analysis.

Objective 2. Determine productivity for above- and below-ground macrophytes and storages of carbon, nitrogen, and phosphorus of both the vegetation and the soil.

Study 2. Nitrogen Availability and Use Efficiency

Objective 1. Determine nitrogen availability and long term retention using ^{15}N stable isotope techniques.

Objective 2. Determine the nutrient-use efficiency (NUE) of nitrogen and phosphorus for community level vegetation and the dominant plant species.

Study 3. Organic Matter Turnover and Nitrogen Budget

Objective 1. Determine decomposition rates, litter quality, and nitrogen and phosphorous regeneration potential of the dominant vegetation, *Schinus terebinthifolius*, *Cladium jamaicense*.

Objective 2. Construct a nitrogen budget utilizing the information obtained from previous studies.

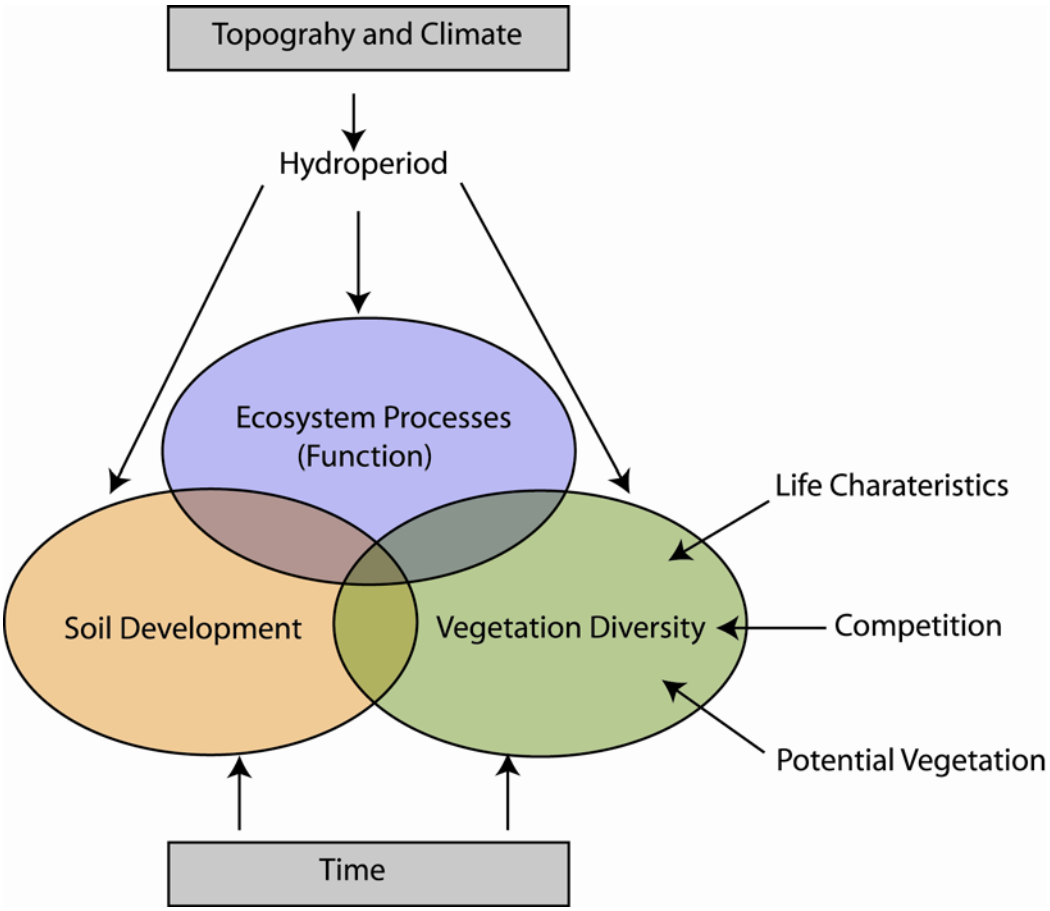

Figure 1-1. Conceptual model of the interlinking relationships between ecosystem processes, soil development, and vegetation community as well as primary driving forces that influence vegetative community structure.

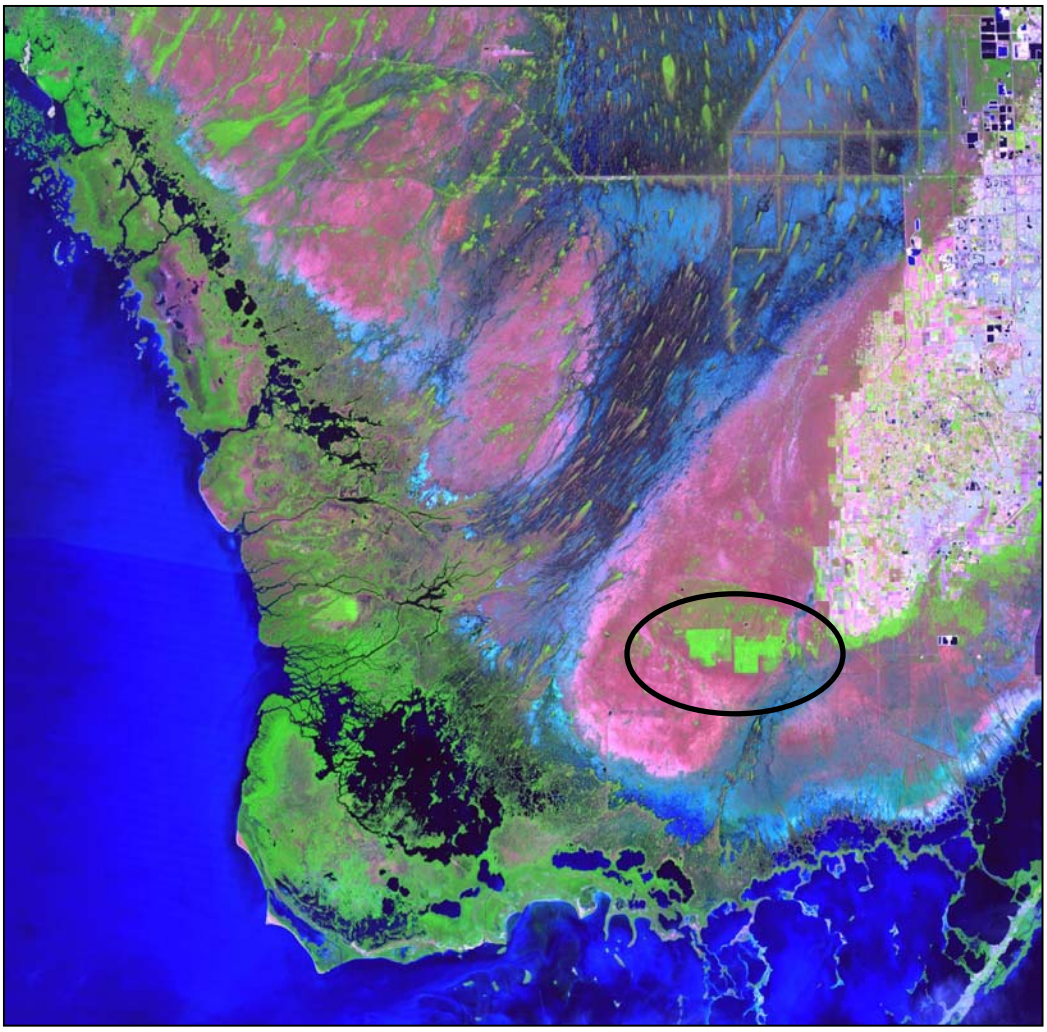

Figure 1-2. False image photo of southern Florida indicating land cover and vegetation types. The green area within the black circle is the location of the restoration project of the Hole-in-the-donut. Courtesy of South Florida Water Management District

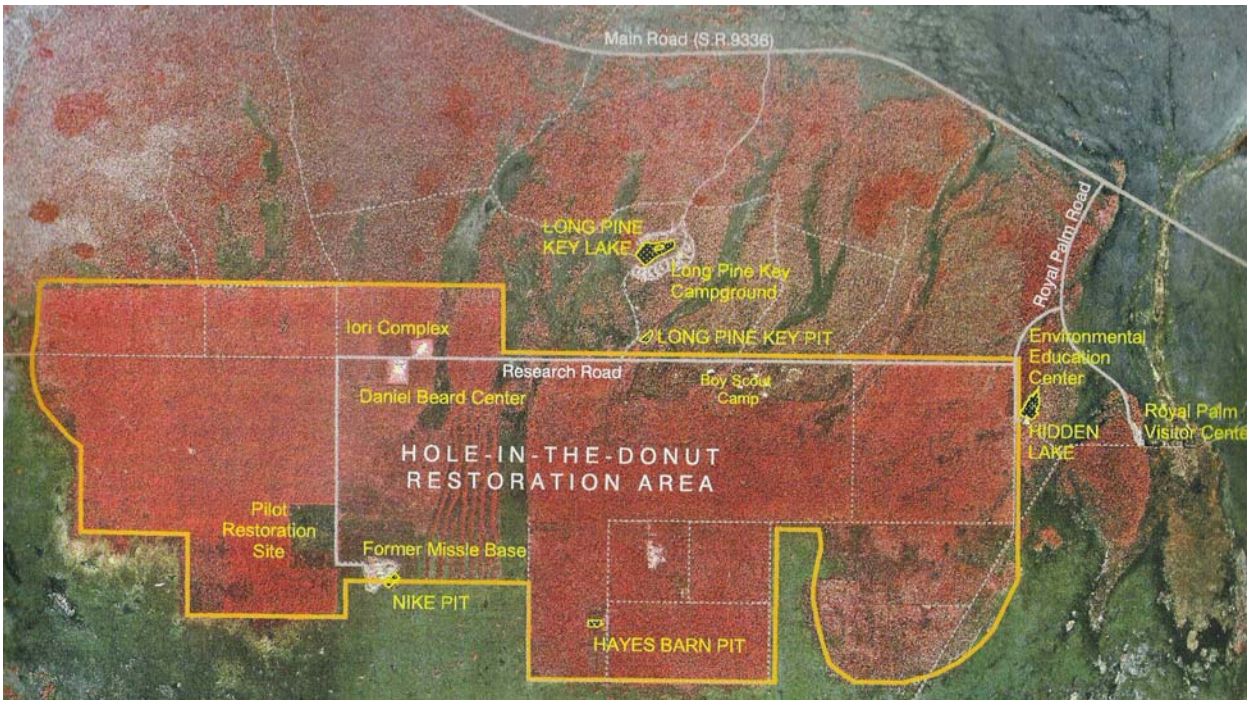

Figure 1-3. Delineation of the region of the Everglades National Park known as the Hole-in-the-Donut to be restored after the effects of farming and invasion of *Schinus terebinthifolius*. Image courtesy of the Everglades National Park.

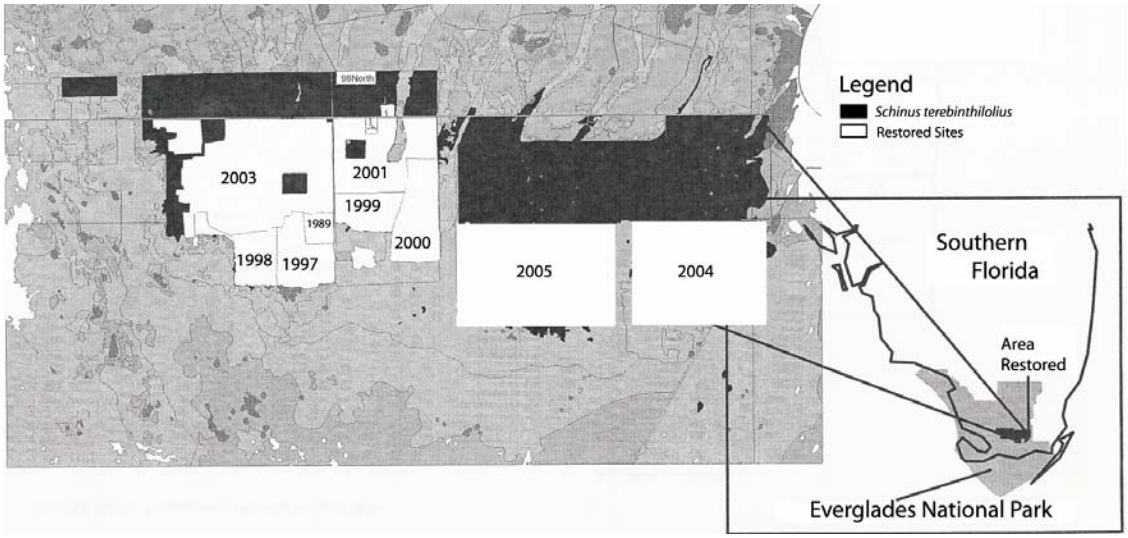

Figure 1-4. Location of the restored wetlands within the Hole-in-the-Donut in the Everglades National Park. Sites are labeled by the year in which they were scraped. Image courtesy of the Everglades National Park.

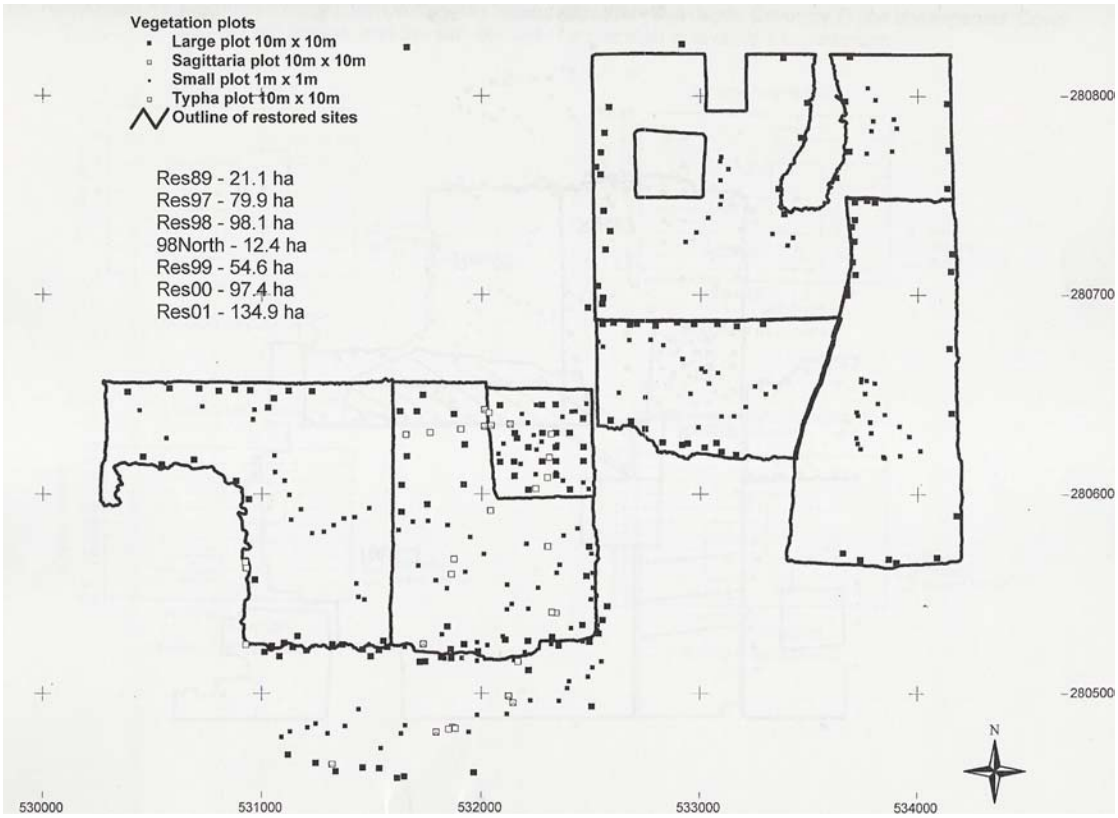

Figure 1-5. Plot locations of Everglades Research Groups reoccurring vegetation surveys in both the restored wetlands and the surrounding native communities. Image courtesy of the Everglades National Park.

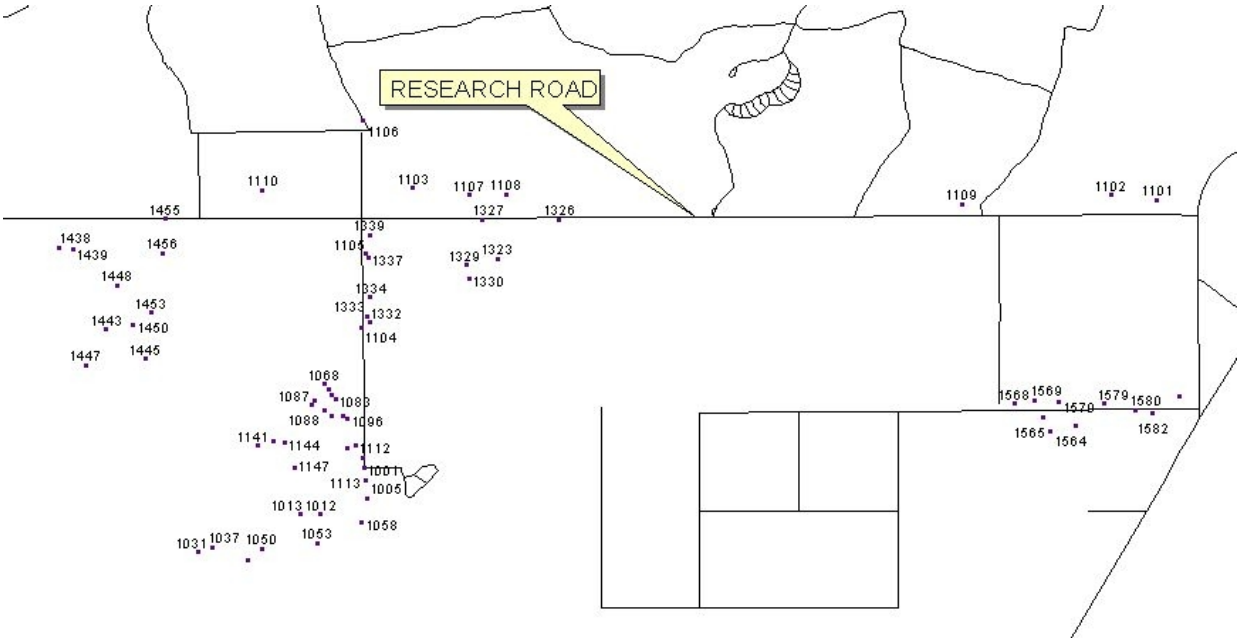

Figure 1-6. Location of each plot included in this study in the Hole-in-the-Donut region of the Everglades National Park. Image courtesy of the Everglades National Park.

CHAPTER 2
RELATIONSHIPS AMONG TIME, PLANT SPECIES RICHNESS, COMPOSITION AND ECOSYSTEM FUNCTION IN RESTORED SUBTROPICAL WETLANDS

Introduction

The loss of more the half of the original wetlands in the United States has resulted in efforts to restore or recreate previously drained wetlands (Whigham 1999). While efforts to restore and mitigate wetlands are well intended, few projects have successfully achieved natural ecosystem status in terms of vegetation structure and diversity of undisturbed wetland ecosystems; therefore, loss of wetland ecosystem function and biodiversity is inevitable. Through restoration and mitigation efforts, the loss of wetlands is decreasing, but the success of these efforts in terms of function and diversity is still unclear. The importance of macrophyte diversity in wetland ecosystems and its link to ecosystem function (i.e., productivity, decomposition, and nutrient storages) has received increasing attention (Bornette et al. 1998, Gessner et al. 2004, Gusewell et al. 2005, Whitehouse and Bayley 2005, Boers et al. 2007). Positive relationships between plant diversity and primary productivity have been demonstrated in grasslands (Naeem et al. 1994, Tilman and Downing 1994, Walker 1995, Tilman et al. 1996), but relatively little research of this kind has been done in wetlands.

In order to successfully restore ecosystem functionality, we need to understand the factors that govern the development of ecosystem function and the establishment of plant community structure. Several studies have investigated the relationships between plant community structure and ecosystem functions such as: soil organic matter accumulation (Craft and Richardson 1993, Vymazal and Richardson 1995, Callaway et al. 2003), soil nutrient pools (Ehrenfeld et al. 2001, Bengtsson et al. 2003, Zak et al. 2003, Fitter et al. 2005), biomass production (Catovsky et al. 2002, Pauli et al. 2002, Callaway et al. 2003), and decomposition (Hector et al. 2000, Catovsky et al. 2002, Gartner and Cardon 2004).

There are three common ways of measuring diversity over spatial scales: alpha, beta, and gamma diversity. Alpha diversity refers to the diversity within a particular area or ecosystem and is usually expressed as the average number of species (species richness) present in the sample units (i.e., plots) for a given ecosystem (McCune and Grace 2002). Beta diversity is a measure of the change in species diversity between two ecosystems or sample units, whereas gamma diversity is the total species pool within an ecosystem or region (McCune and Grace 2002).

Alpha diversity (or species richness) has been used extensively as a measure of species diversity (Tilman et al. 1996, Tilman et al. 1997, Bornette et al. 1998, Pollock et al. 1998, Seabloom and van der Valk 2003, Zak et al. 2003). Beta diversity (or dissimilarity in diversity) has been shown to be more important at large-scales (i.e., biomes, geographical regions with latitudinal gradients). Studies have found that beta diversity and productivity are positively related on a temperate-tropical ecosystem gradient (Francis and Currie 2003, Hawkins et al. 2003), whereas on a local or even regional scale this relationship has been weak or unimodal (Grime 1973, Tilman 1982, Grace 1999, Waide et al. 1999).

In addition to biodiversity, it is necessary to understand the importance of species composition as it relates to ecosystem function. The functional importance of species composition can be examined by determining the relative occurrences of individual plant species which provides information on species abundance (Locky and Bayley 2006). Furthermore, differences in species abundance often allude to environmental conditions controlling species composition. In subtropical Florida wetlands, for example, the dominance of *Cladium jamaicense* is associated with nutrient-poor environments, whereas, the dominance of *Typha domingensis* is found under nutrient-rich conditions (Koch and Reddy 1992, Craft et al. 1995,

Newman et al. 1996, Craft and Richardson 1997, Doren et al. 1997). Therefore, it can be useful to understand the desired habitat/environmental conditions of individual species to predict controls on species composition.

In this study, we examined patterns of species richness and composition, productivity and nutrient accumulation in undisturbed and restored subtropical wetland ecosystems that varied in time since restoration from 1 to 16 years. Our objectives were to1) determine patterns of plant species diversity and dominance in the native and restored wetland communities, 2) determine biomass production at both species and community levels, 3) quantify carbon (C), nitrogen (N), and phosphorus (P) accumulation in the biomass at the species and community level, and 4) relate species diversity indices to ecosystem function (i.e., biomass production, C, N, and P accumulation).

Methods

Site Description

This study was conducted in wetland systems restored in the Hole-in-the-Donut (HID) region of the Everglades National Park (ENP) by Miami-Dade County mitigation funds. Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season. The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Currently, the Everglades Research Group (ERG) conducts annual monitoring of the vegetation community, soil depth, and hydrology of each of the restored sites, as well as a native community. For the vegetation sampling, they employ the Braun-Blanquet method to evaluate the overall vegetation community structure in permanently establish plots every fall at peak biomass. On each restored site there are 20-10 m^2 plots and 40-1 m^2 plots (20 nested in the northwest corner of each of the large plots; 20 randomly located in the intermediate strata of the site). The large plots were establish to address the broad characteristics of vegetation assemblages and the small plots are used to evaluate species composition with regard to soil depth, elevation, and hydrology. This data will be re-evaluated for statistical relationships between the vegetation communities, soil depth, elevation, and hydrology to determine how well the current data explains the re-vegetation of the restored sites. From the intermediate strata, 10 of the pre-established small plots (1 m^2) in the restored (1, 2, 4, 8, 16 years), native sites were utilized for characterization of vegetation and soil physical and chemical properties.

Species Diversity Indices

Micro-topography variability is high in these wetland systems. In an attempt to eliminate topography and hydrology differences as variables controlling vegetation patterns, we chose 10 plots in each site at approximately 0.5 m elevation for sites restored in 2004, 2003, 2001, 1997, and 1989 and the native community. The species composition data generated was used to calculate alpha, beta and gamma diversity. Alpha diversity was determined as the average number as species found in each plot at each site. Gamma diversity was determined for the HID as a whole region (total species richness combined) and for each individual site (total species richness in each site). This allowed us to calculate beta diversity in two ways, between and within sites. Beta diversity (β) was calculated using Whittaker's measure,

$$\beta = \gamma/\alpha, \quad (2\text{-}1)$$

where γ is the gamma diversity of the HID or individual sites and α is alpha diversity for the average species richness observed in all the plots from each site.

To determine how similar or dissimilar each restored site was from the native community, we calculated Sorensen's similarity index (Cs),

$$Cs = 2*J / (\gamma_1 + \gamma_2), \quad (2\text{-}2)$$

where J is the number of shared species and γ_1 is the gamma diversity for the native community and γ_2 is the gamma diversity for the restored site being compared. This index value ranges from 0 where there are no shared species to 1 where the exact species are found in both communities.

Above-ground Biomass and Species Dominance

Above-ground biomass was determined in April 2005 (dry season; Y1), July 2005 (wet season; Y2) and July 2006 (wet season; Y3). Plant shoots within 1 m^2 plots adjacent to the pre-established plots will be clipped at the sediment surface. The plant matter was dried and weighed at 65°C to a constant mass with results expressed as g dry weight m^{-2}.

For the July 2005 and 2006 biomass sampling, each m^2 plot was divided by species contribution to biomass. The species collected in each biomass sampling were grouped based on abundance and importance to the restoration goal and ranked on frequency and percent cover. Because we were primarily interested in the contributions of dominant species to the overall biomass at each site, we grouped infrequent species (contributed less than 1% to the total above ground biomass) together in the category 'Other'. Species that were most abundant were grouped by themselves. Several Poaceae species (grasses) were observed, however, unidentifiable. Members of the Poaceae family, with the exception of Andropogon spp., found in Y3 (but not in Y2) were grouped into one category called Poaceae and counted as one species.

Relative frequency of individual species was determined as a percent of the total biomass collected for each site This was done for the HID as a whole and for each site individually to determine overall dominance and site dominance. Both species and community level vegetation samples were analyzed for C, N, and P pool sizes. Total C and total N were determined by dry combustion with a Thermo Electron Corporation Flash EA NC Soil Analyzer for the bulk above-ground plant tissue. Values for species and community level vegetation were reported as a concentration (mg g^{-1}) or pool size (mg m^{-2}).

Results

Species Composition and Diversity

The complete spatial survey of the vegetation community conducted by the Everglades Research Group found 112 different plant species (gamma diversity) present within the HID (Table 2-1and 2-2). They found 62 species in the native community and in the 1989, 1997, 2001, 2003, and 2004 restored wetlands they found 48, 51, 38, 40, and 38 species, respectively (Table 2-1 and 2-2). The alpha diversity (or species richness) was the highest in the native community at an average of 18 species per plot followed by the 1989 and 1997 at 17, 2001 at 11, 2003 at 12 and the 2004 at 9 (Table 2-2). The beta diversity for the HID as a whole system (or region) with the gamma diversity of 112, was 6 for the native site with decreasing numbers as sites increased in age since restoration (Table 2-2). Beta diversity calculated with site-specific estimates of gamma showed that the 2004 site was the most variable with a beta diversity of 7 (Table 2-2). All other sites had similar beta diversities of 3 or 4. Sorensen's similarity index ranged from 0.24 to 0.46, indicating that the oldest site (1989) was the most similar to the native community and the 2003 site was the least similar (Table 2-2).

Year 1 (Y1) biomass production resulted in the greatest biomass production in the native and 1989 sites as compared to the biomass production in year 2 (Y2) and year 3 (Y3) within the

same sites as well as across all sites ($p=0.0005$; Figure 2-1 and Table 2-3). Biomass production in Y3 was highest in the 1997 and 2003 sites as compared to Y1 and Y2. There was a significant decrease in biomass production with time in the 1989 sites ($p<0.0001$) whereas in the 2003 site, the Y3 biomass resulted in a significant increase ($p=0.0077$; Figure 2-1 and Table 2-3). No significant change occurred in the 2001 and 2004 between each sampling (0.7796 and 0.7233, respectively, Table 2-3). In Y1, the biomass production in the native and 1989 sites were significantly higher than the biomass production in the 2001, 2003, and 2004 sites, but the 1989 site was not significantly different from the 1997 site (Table 2-3 and Figure 2-1). The same was true for Y2. During Y3, the biomass production in the native community was significantly greater than all other sites, the 1989 site was significantly greater than the 1997 and 2003 , and the 1997 and 2003 were the same ($p<0.0001$; Table 2-3 and Figure 2-1).

Figure 2-2 represents the biomass contribution of each species group identified in each biomass sampling for each site (Table 2-1; species indicated by †). The amount of biomass contribution between species in the native site did not change significantly between Y2 and Y3. In the 1989 site, the amount of *C. jamaicense* did not change; the decrease in biomass production in Y3 was a result of less 'Other' production. The significant increase in Y3 for both the 1997 and 2003 sites was primarily a result of an increase in *T. domingensis*.

In addition to total above-ground biomass, the relative frequency of each species was determined for the HID as a whole and for each site individually. The most frequent species occurring in the HID was *T. domingensis* followed closely by *C. jamaicense*. *Schoenus nigricans*, and Poaceae were also frequent (Figure 2-3a). In the native community *C. jamaicense* and *S. nigricans* were co-dominant (Figure 2-3b). In the 1989 site, *C. jamaicense* and Poaceae are the two most frequently occurring species (Figure 2-3c). The 1997 and 2003 sites were

mostly dominated by *T. domingensis*; however, in the 1997 site *Andropogon* spp. were also abundant and in the 2003 site *S. sempervirens* was abundant (Figures 2-3d and e).

Nutrient Concentrations and Pools

Community level carbon and nitrogen concentrations varied from 382±88-441±68 and 6.3±1.8-10.6±2.1 mg g^{-1} across sites, respectively (Figure 2-4a and b). The community level carbon content was the lowest in the 2001 site and the highest in the native site, whereas the nitrogen content was the lowest in the 2003 site and the highest in the 2001 site. The community level phosphorus content was the lowest in the native plant communities at 0.18±0.04 mg g^{-1} and gradually increase from oldest to youngest with the 2004 site at 0.6±0.09 mg g^{-1} (Figure 2-4c).

Individual species tissue nutrient content varied both between species and among sites. *Cladium jamaicense* had the lowest C content in the 1989 site, the lowest N content in the 1997 site and the lowest P content in the native community (Table 2-4), whereas it had the highest C, N and P content in the 2004, 1989, and 2004 sites, respectively. *Schoenus nigricans* was only found in the native community. As compared to *C. jamaicense* (its co-dominate species), *S. nigricans* had higher C content and lower N and P content (Table 2-4). *Typha domingensis* was not found in the native or 1989 sites, but among restored sites, it had the lowest C, N, and P in the 2004, 2004, and 1997 sites, respectively (Table 2-4). When present, *S. lancifolia* had the highest N and P content as compared to all other species except in the 2003 (Table 2-4) where *J. megacephalus* had the highest N and *Andropogon* spp. had the highest P content.

Community level differences in C, N and P pools were a reflection of differences in species level contributions (Figure 2-5). *Schoenus nigricans* stored the most C and N of all species investigated (Figure 2-5d). Phosphorus storage was the highest in the group of 'Other' species and in *T. domingensis* and *Andropogon* spp when present (Figure 2-5f). *Sagittaria lancifolia* had the lowest C, N and P storage primarily as a result of having a low biomass

contribution (Figure 2-5d, e, and f). Community weighted C:N ratios revealed that the vegetation community present in the 2003 site was significantly higher than the ratios of the vegetation found in all other sites (Figure 2-6a). The community weighted N:P ratios of the vegetation in the 2003 and 2004 sites were significantly lower than the ratios in the vegetation in the native, 1989, 1997, and 2001 sites (Figure 2-6b). The community weighted C:P ratios of the vegetation gradually increased with age with the 2004 site having the lowest and the native vegetation with the highest; however, no significant differences were observed (Figure 2-6c).

Diversity and Biomass Relationships

Richness was weakly positively related to aboveground biomass production across the sites (r^2=0.56, d.f.=59, F=73.8, p<0.0001, n=60), with the 2004 site having the lowest above-ground biomass production and species richness and the native community having the highest (Figure 2-7). The age of each site contributed to the differences found in species richness (r^2 = 0.40, d.f=49, F=28.5, p<0.001; Figure 2-8a), whereas the age did not contribute to the trend in biomass production found across restored wetland communities (r^2 = 0.16, d.f.=49, F=8.9, p=0.0040; Figure 2-8b).

Gamma and α-diversities and biomass production were highly positively related (r^2 = 0.87, d.f.=5, F=26.4, p=0.0070 and 0.93, d.f.=5, F=53.3, p=0.0020, respectively; Figure 2-9a and b). A negative relationship was observed between the (within) beta diversity and biomass production (r2 = -0.79, d.f.=5, F=15.3, p=0.0200; Figure 2-9c).

Discussion

The Hole-in-the-Donut (HID) region of the Everglades National Park (ENP) offers a unique opportunity to study temporal patterns of relationships between species diversity and ecosystem structure and function by examining a chronosequence of restored wetland communities. Several diversity and ecosystem function experiments have been conducted under

controlled environments where the species diversity levels were planted and maintained (Tilman and Downing 1994, Tilman et al. 1996, Tilman et al. 1997, Baldwin et al. 2001, Kellogg and Bridgham 2002, Zak et al. 2003). In order to improve restoration efforts and management of large scale systems, it is important to understand how natural recruitment of plant species will develop and potentially impact ecosystem function.

All communities experience some level of disturbance which alters plant communities via removal or additions of individual species (Stiling 1999). In some cases, severe levels of disturbance (induced by either natural disasters or anthropogenic impacts) occur which result in large scale changes in ecosystem function and plant community structure which can include the invasion of non-native plant species. The loss of more the half of the original wetlands in the United States has resulted in efforts to restore and recreate previously drained wetlands (Mitsch and Gosselink 2000). While efforts to restore and mitigate wetlands are well intended, few projects have been successful in achieving natural ecosystem status in terms of vegetation composition of undisturbed wetland ecosystems. Due to the high level of disturbance and destruction of natural wetlands, the loss of wetland plant diversity is inevitable and in turn a loss of function could occur. The results of this study offer insights on the factors controlling and maintaining species composition in wetland ecosystems beginning at primary succession.

Diversity and Species Composition Patterns

Community development depends on many confounding factors ranging from soil nutrient availability, hydrology, and seed dispersal and recruitment just to name a few (van der Valk 1981). In this study, we found that the lack of soil did not inhibit plant recolonization in restored wetland ecosystems. Within six months of complete soil removal, the 2004 cleared site (age, 1 year) had a gamma diversity of 38 plant species and a α-diversity of 9 species per plot. After

this initial colonization of species, the introduction of additional species appeared to be slow. The site restored in 2001 (age, 4 years) resulted in a α-diversity increase by 2 species and the gamma diversity remained unchanged. However, by 16 years after restoration (1989 site) the α-diversity per plot almost doubled to 17 species and the gamma diversity reached 48 plant species. While this α-diversity is similar to the native communities, the gamma diversity is still significantly lower by 14 species.

While the gamma diversity may be lower in the 1989 site, the species composition is similar to the native plant community. This restored wetland had equal contributions of *C. jamaicense* to the above-ground biomass as did the native community. Additionally, *T. domingensis* was not present in any of the biomass samplings even though it has been found in the 1989 site in previous years (O'Hare and Dalrymple 2003). The most notable difference between the native plant communities and the restored wetlands is the lack of colonization by *S. nigricans* in the restored plant communities. *Schoenus nigricans* is a dominant plant species in the surrounding native communities; however it has not been recorded as present in any of the restored wetland communities since this restoration project began in 1989 (Dalrymple et al. 2003, O'Hare and Dalrymple 2003). Additional research is needed to understand the colonization patterns and environmental conditions required for this plant species to understand why it is not colonizing the restored wetland communities.

The species diversity indices may be similar during the first few years after restoration, but the species composition differs considerably. Mixed grasses contribute 50% or more to the biomass production of the 2004 and 2001 restored wetland communities, while the 2003 site is mostly dominated by *T. domingensis*. Additionally, *T. domingensis* is the dominant species found in the 1997 site (age, 8). There are some concerns on the dominance of *T. domingensis* in

these earlier restored wetland communities. While *T. domingensis* is native to the state of Florida, it is not native to this region of the ENP (Craft et al. 1995, Miao et al. 2000). It has been suggested that *T. domingensis* has allelopathic properties which inhibit the seed survival and propagation of other plant species (Prindle and Martin 1996, Domenech et al. 1997), however this has not been adequately confirmed with scientific research. More important is the invasion of *T. domingensis* into areas where the nutrient availability has been altered or enriched. Numerous studies have been conducted which have shown that *Typha* spp. will invade into nutrient enriched areas (Craft et al. 1995, Gophen 2000, Miao et al. 2000, Woo and Zedler 2002, Johnson and Rejmankova 2005). The restored wetland systems in the HID are P-rich compared to the native communities as a result of both previous farming practices and the destructive restoration technique. *Typha domingensis* has been shown to create a monoculture under such conditions (Koch and Reddy 1992, Craft et al. 1995, Doren et al. 1997, Daoust and Childers 1999, Bruland et al. 2006). While this has not occurred during the time this study was conducted, the 2004 restored wetland community has since become a *T. domingensis* monoculture (unpublished data; Everglades National Park).

Ecosystem Function

Our results indicate that the restored wetland systems with the lowest species richness also had the lowest biomass production. Additionally, we found a consistent increase in species richness with age development. Species diversity predicted 34% of the variability in total biomass production. The age of the sites had a strong influence on species richness development explaining 40% of the variability; whereas the age of each site did not explain the variability in above-ground biomass production. These results indicate that an age-driven gradient in species richness may be linked to increases in biomass production. Furthermore, this relationship between species diversity and biomass production results in increases in ecosystem function in

terms of C, N, and P accumulation. Other experimental studies that have controlled species richness levels have found similar relationships between species diversity and biomass production (Vermeer and Berendse 1983, Clement and Maltby 1996, Engelhardt and Ritchie 2001, Callaway et al. 2003, Olde Venterink et al. 2003). Our study offers further support of this relationship through natural recruitment of plant species in wetland communities.

In addition to species diversity effects on ecosystem function, the species composition of a system can have significant effects as well (Hooper and Vitousek 1997, Tilman et al. 1997, Engelhardt and Ritchie 2001, Callaway et al. 2003). Historically, the marl prairie wetlands of the ENP have been dominated by *C. jamaicense* and *S. nigricans* (Lodge 2005). Numerous studies have been conducted to conclude that *C. jamaicense* thrives under P-limited conditions and its growth and survival is inhibited when P availability increases (Davis 1991, Craft et al. 1995, Newman et al. 1996, Craft and Richardson 1997, Richardson et al. 1999, Chiang et al. 2000, Noe et al. 2001), unfortunately this same level of attention has not been given to *S. nigricans*. In another study conducted in these wetland systems it was determined that *C. jamaicense* and *S. nigricans* had high nutrient-use efficiency of P which help to maintain the P-limited environment in which they thrive by retaining more P in standing biomass (see Chapter 4). *Typha domingensis*, however, has a lower nutrient-use efficiency, potentially leading to higher litter quality and more rapid release of nutrients into the soil solution, which could reinforce high P availability under enriched conditions. All three species' use of P could have significant effects on the ecosystem function of P availability and storage.

More P is stored in the above-ground biomass of *T. domingensis* which over time would result in more P being stored in both standing biomass and litter making it unavailable for further uptake. This suggests that *T. domingensis* would facilitate the early development of the restored

wetland communities. As a result, by 16 years after restoration, the wetlands are no longer dominated by *T. domingensis*, but by the native *C. jamaicense*. Earlier vegetation surveys in these wetlands have concluded that *T. domingensis* was abundant in the 1989 site, however to what extent is unclear since these studies focused primarily on the removal of invasive species *Schinus terebinthifolius* (Dalrymple et al. 2003, O'Hare and Dalrymple 2003). Further analysis is needed on mechanistic controls of *T. domingensis* on nutrient cycling to determine the long-term effects of its P use on ecosystem function.

Applications to Wetland Restoration and Mitigation

It has been suggested that planting desired plant species and diversity levels is needed to facilitate vegetation composition development in restored wetland communities (Zedler 1993, Kellogg and Bridgham 2002, Callaway et al. 2003). In large-scale restoration applications, planting desired plant species is not always feasible. Therefore it is necessary to gain a better understanding of the natural recruitment of plant species from primary succession and the development of ecosystem function to increase the success of large-scale restoration projects. In this study, we found that natural recruitment would result in increases in species richness with time, and that the species composition would develop similarly to the native community provided enough time has past. We saw an immediate recruitment of a diverse plant community consisting of 38 individual plant species within six months of restoration. While additional increases in species diversity were slow, the diversity did increase significantly after 8 years. Additionally, it took between 8 to 16 years before the plant community developed into one representative of the native community. However, the ecosystem function was not restored in this time period, indicating that more time is needed for development of native ecosystem function.

The species composition changed significantly from site to site indicating that the plant communities are very dynamic and unstable. Understanding the seed dispersal, recruitment mechanisms, propagation requirements, and growth and survival rates of the native plant species could aid in the success of restoration efforts. If a desired native plant species has a limited seed dispersal mechanism, it could take several years before that species colonizes or dominates a restored wetland. For example, the seed dispersal mechanism of *T. domingensis* is relatively fast compared to *C. jamaicense* (van der Valk and Rosburg 1997) which could contribute to the slower colonization patterns observed for *C. jamaicense* in this study.

The results of this study offers evidence that, with time, a diverse plant community similar to a native wetland community can develop without human intervention. However, more time is clearly needed to restore ecosystem function to the level of the native system. The key here is time. Unfortunately, wetland mitigation laws require that wetlands created or restored that serve as mitigation projects are only required 5-10 years of monitoring (Clean Water Act, Section 404). This study along with many others provides ample evidence that this monitoring time period is may not be long enough to restore and maintain plant community structure or ecosystem function (Whigham 1999, Brinson and Malvarez 2002, Kellogg and Bridgham 2002, Callaway et al. 2003, Dalrymple et al. 2003, Seabloom and van der Valk 2003, Polley et al. 2005).

Conclusions

The links between diversity and function during the successional development of the wetlands in this study has implications to the management of restored ecosystems. Landscape and watershed alterations can result in severe degradation of wetland systems which result in species compositional changes and loss of biodiversity. Wetland systems are driven

predominantly by hydrology and many plant species will respond differently to fluctuations or changes in water level and flow.

To maximize species diversity and composition development similar to a native (or reference) system, it is important to understand the factors governing the native system. For this restoration project, the native vegetation community is P-limited with extremely low levels of N and P. To enhance the potential for native plant species in colonize, the nutrient rich soil was complete removed to eliminate the effects from previous farming practices. This restoration method was destructive and labor intensive, the result is the development of herbaceous wetland plant communities that, with time, has been colonized with some native species; however, overall the vegetation community structure is very different. Furthermore, more time is needed for development of ecosystem functions similar to those found in the native communites.

Table 2-1. Complete species list for all plants identified in the HID during 2005. An X indicates presence in site location. All species are listed in alphabetical order. (n=60)

Species Name	Native	1989	1997	2001	2003	2004
Agalinis fasciculata				X		
Agalinis linifolia	X					
Alestris lutea	X					
Alternanthera philoxeroides			X			
Ammania coccinea				X		
Ammania latifolia		X	X		X	X
Ampelopsis arborea		X				
Andropogon glomeratus †	X	X	X	X	X	
Andropogon virginicus †		X	X			
Aristida purpurascens	X					
Aster bracei	X			X	X	
Aster subulatus		X	X	X	X	
Axonopus furcatus			X			
Baccharis angustifolia †	X					
Baccharis glomeruliflora †	X	X	X		X	X
Baccharis halimifolia †					X	
Bacopa monnieri		X	X	X	X	X
Cassytha filiformis	X					
Centella asiatica †	X	X	X	X		X
Chara light †						X
Chara unidentified species †		X	X	X	X	X
Cirsium horridulum	X					
Cladium jamaicense †	X	X	X	X		
Coelorachis rugosa				X		
Conoclinium coelestinum	X	X	X	X		X
Cyperus elegans					X	
Cyperus haspan		X	X	X		X
Cyperus ochraceus					X	
Cyperus odoratus					X	
Cyperus polystachyos		X	X		X	X
Cyperus surinamensis			X		X	
Dichanthelium dichotomum	X	X		X		X
Diodia virginiana	X	X	X	X	X	X
Dyschoriste angusta	X					
Eclipta prostrata					X	
Eleocharis geniculata	X		X		X	X
Elytraria caroliniensis	X					
Eragrostis elliottii	X	X	X	X		

Table 2-1. Continued.

Species Name	Native	1989	1997	2001	2003	2004
Erigeron quercifolius	X					
Eupatorium capillifolium †					X	
Eupatorium leptophyllum †	X	X			X	X
Eustachys glauca	X	X	X	X		
Evolvulus sericeus	X					
Fuirena breviseta †	X	X	X	X		X
Heliotropium polyphyllum	X					
Hydrocotyle umbellata †	X	X	X		X	
Hymenocallis palmeri	X					
Hypericum hypericoides	X					X
Hyptis alata	X		X	X		X
Hyptis spicigera	X					
Ilex cassine	X					
Ipomoea sagittata	X					
Ipomoea triloba		X				
Iva microcephala	X		X			
Juncus megacephalus †		X		X	X	X
Kosteletzkya virginica				X		
Leptochloa fascicularis			X	X	X	
Lobelia glandulosa	X					
Ludwigia alata		X	X			X
Ludwigia microcarpa	X	X	X	X	X	X
Ludwigia octovalvis		X	X	X	X	X
Ludwigia peruviana †		X	X			X
Ludwigia repens †		X	X		X	
Lythrum alatum		X	X		X	X
Mecardonia acuminata			X			
Melanthera nivea	X					
Mikania scandens †	X	X	X	X	X	X
Mitreola petiolata		X	X	X	X	X
Muhlenbergia capillaris	X	X	X			
Oxypolis filiformis	X					
Panicum dichotomiflorum		X			X	
Panicum hians		X				
Panicum rigidulum			X	X	X	
Panicum tenerum	X					
Paspalum urvillei		X				
Periphyton†	X	X	X	X	X	X
Persea palustris	X					
Phyla nodiflora	X		X			X

Table 2-1. Continued.

Species Name	Native	1989	1997	2001	2003	2004
Phyllanthus caroliniensis	X	X		X		X
Pluchea odorata					X	
Pluchea rosea	X	X		X	X	X
Polygala balduinii	X					
Polygala balduinii	X					
Polygala grandiflora	X					
Polygonum hydropiperoides			X			
Polygonum punctatum			X			
Proserpinaca palustris	X	X		X		X
Rapanea punctata	X					
Rhynchospora colorata	X	X	X	X		X
Rhynchospora divergens	X					
Rhynchospora microcarpa	X	X	X	X		
Rhynchospora odorata			X	X		X
Rhynchospora tracyi	X					
Saccharum giganteum	X	X		X		
Sagittaria lancifolia †	X	X	X	X	X	
Salix caroliniana			X	X	X	X
Sarcostemma clausum †		X	X			
Schizachyrium scoparium	X					
Schoenus nigricans †	X					
Scleria verticillata	X					X
Sesbania herbacea			X		X	X
Setaria parviflora	X	X	X	X	X	X
Solidago sempervirens †	X	X			X	
Spermacoce floridana			X		X	
Spermacoce prostrata		X				
Spermacoce terminalis	X					
Stemodia durantifolia			X			
Typha domingensis †		X	X	X	X	X
Verbena scabra					X	
Vernonia blodgettii	X					X
Vigna luteola		X	X			
Vitis shuttleworthii						X
Waltheria indica	X					
Gamma Diversity Total = 112	62	48	51	38	40	38

†Represents species identified in biomass production sampling plots

Table 2-2. Diversity indices calculated from complete species list (Table 2-1) for individual sites and the HID as a whole.

Site	HID					Sites			
	alpha		beta[A]		gamma	beta[B]		gamma	Cs[C]
	Ave	SD	Ave	SD		Ave	SD		
Native	18	4.5	6	1.5	112	4	0.8	62	
1989	17	4.1	7	2.0		3	0.8	48	0.46
1997	17	4.2	7	1.9		3	0.8	51	0.36
2001	11	3.0	11	3.6		4	1.1	38	0.44
2003	12	1.9	9	1.6		3	0.5	40	0.24
2004	9	5.2	19	15.9		7	4.7	38	0.40

[A]Based on Whittaker's measure for beta-diversity for the HID as a whole region.

[B]Based on Whittaker's measure for beta-diversity with region defined as individual sites.

[C]Based on Sorensen's similarity coefficient for beta-diversity comparing the native site to each individual resored site.

Table 2-3. Summary of results from ANOVA tests with dependant variables of as either each site or year of biomass sampling. Site or year with shared lower case letters are not significantly different at $p<0.05$ based on Tukey's multiple comparisons.

Source of Variation	d.f.	prob. > F						
1 Year			Native	1989	1997	2001	2003	2004
Y1	5	<0.0001	a	ab	ab	c	bc	c
Y2	5	<0.0001	a	ab	ab	bc	bc	c
Y3	3	<0.0001	a	b	c		c	
2 Site	d.f.	prob. > F	Y1	Y2	Y3			
Native	2	0.0005	a	b	b			
1989	2	<0.0001	a	b	b			
1997	2	0.4224	n.s.	n.s.	n.s.			
2001	2	0.7796	n.s.	n.s.	n.s.			
2003	2	0.0077	ab	b	a			
2004	2	0.7233	n.s	n.s	n.s			

n.s. = not significant

Table 2-4. Summary of species carbon, nitrogen, and phosphorus content, pool sizes, and pool weighted ratios.

Species	Site	C Content (mg g^{-1}) Ave SE	N Content (mg g^{-1}) Ave SE	P Content (mg g^{-1}) Ave SE	C Pool (g m^{-2}) Ave SE	N Pool (g m^{-2}) Ave SE	P Pool (mg m^{-2}) Ave SE	Pool Wtd C:N Ave SE	Pool Wtd C:P Ave SE	Pool Wtd N:P Ave SE
C. jamaicense	Native	447 (50)	6.2 (0.7)	0.1 (0.01)	23.3 (2.3)	0.35 (0.04)	7.2 (0.9)	73 (1)	4209 (169)	57 (1.8)
	1989	405 (68)	8.9 (1.5)	0.3 (0.04)	40.6 (7.2)	0.99 (0.18)	25.7 (4.3)	62 (5)	1623 (99)	41 (7.1)
	1997	428 (58)	5.1 (2.4)	0.2 (0.05)	5.5 (2.3)	0.07 (0.33)	2.8 (0.8)	84 (3)	1955 (76)	23 (1.5)
	2001	449 (74)	6.2 (1.6)	0.1 (0.01)	31.9 (7.9)	0.44 (0.47)	9.5 (2.5)	73 (14)	3366 (169)	46 (7.1)
	2004	453 (113)	6.8 (1.7)	0.3 (0.07)	6.8 (2.5)	0.08 (0.03)	3.3 (1.1)	70 (4)	1669 (120)	24 (0.7)
S. nigricans	Native	461 (58)	5.3 (0.7)	0.1 (0.01)	112.6 (3.1)	1.31 (0.05)	19.6 (0.6)	87 (1)	5796 (80)	67 (1.5)
T. domingensis	1997	441 (74)	5.6 (0.9)	0.3 (0.05)	54.4 (8.5)	0.68 (0.11)	31.7 (6.2)	81 (3)	1908 (167)	23 (1.5)
	2001	443 (111)	8.6 (2.1)	0.4 (0.10)	14.6 (4.3)	0.22 (0.04)	9.4 (1.4)	56 (5)	1298 (182)	22 (1.1)
	2003	456 (76)	5.6 (0.9)	0.4 (0.07)	43.1 (2.4)	0.71 (0.10)	59.4 (10.4)	75 (3)	1083 (58)	14 (0.5)
	2004	422 (83)	5.4 (1.2)	0.3 (0.01)	1.2 (0.2)	0.02 (0.07)	0.9 (0.0)	78 (1)	1297 (77)	17 (0.5)
S. lancifolia	Native	450 (74)	19.3 (4.3)	1.1 (0.23)	0.9 (0.1)	0.04 (0.02)	2.1 (0.6)	23 (2)	427 (23)	18 (1.5)
	1989	458 (92)	12.1 (2.4)	0.6 (0.11)	5.0 (1.0)	0.09 (0.01)	4.0 (0.6)	47 (5)	1205 (174)	24 (1.2)
	1997	415 (104)	8.4 (2.1)	0.5 (0.13)	0.9 (0.1)	0.02 (0.004)	1.3 (0.3)	53 (4)	878 (76)	16 (0.5)
	2001	395 (52)	12.6 (2.4)	0.8 (0.12)	2.6 (0.1)	0.08 (0.01)	5.2 (0.9)	31 (1)	501 (23)	16 (0.4)
	2003	466 (84)	4.3 (0.8)	0.2 (0.03)	1.2 (0.1)	0.01 (0.003)	0.6 (0.03)	109 (2)	2097 (23)	19 (0.4)
	2004	405 (27)	11.9 (1.6)	0.7 (0.08)	1.9 (0.1)	0.06 (0.005)	3.2 (1.1)	34 (1)	601 (55)	18 (0.4)
Andropogon spp.	1989	470 (235)	5.8 (2.9)	0.3 (0.14)	8.1 (1.8)	0.10 (0.03)	5.4 (2.4)	83 (8)	2003 (555)	23 (4.6)
	1997	414 (83)	8.9 (1.8)	0.6 (0.12)	37.7 (5.9)	0.76 (0.12)	48.4 (7.9)	54 (5)	1120 (194)	18 (1.5)
	2001	425 (142)	6.8 (2.3)	0.2 (0.07)	23.6 (6.7)	0.35 (0.10)	11.0 (3.2)	74 (14)	2323 (393)	32 (0.9)
	2003	437 (2)	8.5 (1.2)	0.7 (0.06)	5.9 (2.1)	0.10 (0.02)	8.9 (2.5)	54 (8)	627 (55)	12 (0.7)
	2004	439 (69)	5.7 (0.9)	0.3 (0.06)	0.2 (0.1)	0.003 (0.001)	0.1 (0.03)	77 (3)	1318 (77)	17 (0.5)
J. megacephalus	2001	438 (146)	6.4 (2.1)	0.2 (0.08)	46.3 (20.6)	0.58 (0.25)	34.0 (17.8)	71 (5)	2241 (449)	32 (5.9)
	2003	430 (103)	9.8 (0.5)	0.4 (0.05)	6.1 (1.3)	0.14 (0.03)	5.6 (1.2)	44 (5)	1096 (77)	25 (1.0)
	2004	428 (86)	8.2 (1.6)	0.4 (0.09)	10.4 (1.1)	0.19 (0.01)	9.3 (0.9)	56 (3)	1301 (206)	22 (2.1)
Other	Native	442 (88)	6.4 (1.3)	0.3 (0.05)	9.6 (2.8)	0.17 (0.06)	4.3 (1.2)	72 (3)	1827 (129)	27 (2.4)
	1989	445 (45)	8.9 (0.9)	0.5 (0.05)	81.2 (10.4)	1.31 (0.12)	55.6 (5.3)	58 (2)	3653 (782)	51 (9.1)
	1997	443 (44)	6.3 (0.6)	0.4 (0.04)	45.5 (3.0)	0.62 (0.04)	35.8 (1.9)	72 (1)	1259 (30)	18 (0.4)
	2001	434 (43)	7.5 (0.7)	0.3 (0.03)	26.7 (2.3)	0.45 (0.04)	19.9 (1.8)	60 (1)	1697 (77)	28 (1.0)
	2003	456 (46)	5.6 (0.6)	0.7 (0.07)	29.2 (2.1)	0.38 (0.04)	40.7 (3.0)	85 (2)	744 (25)	10 (0.5)
	2004	423 (42)	8.2 (0.8)	0.5 (0.05)	23.3 (1.9)	0.43 (0.03)	24.3 (1.9)	54 (1)	982 (23)	19 (0.5)

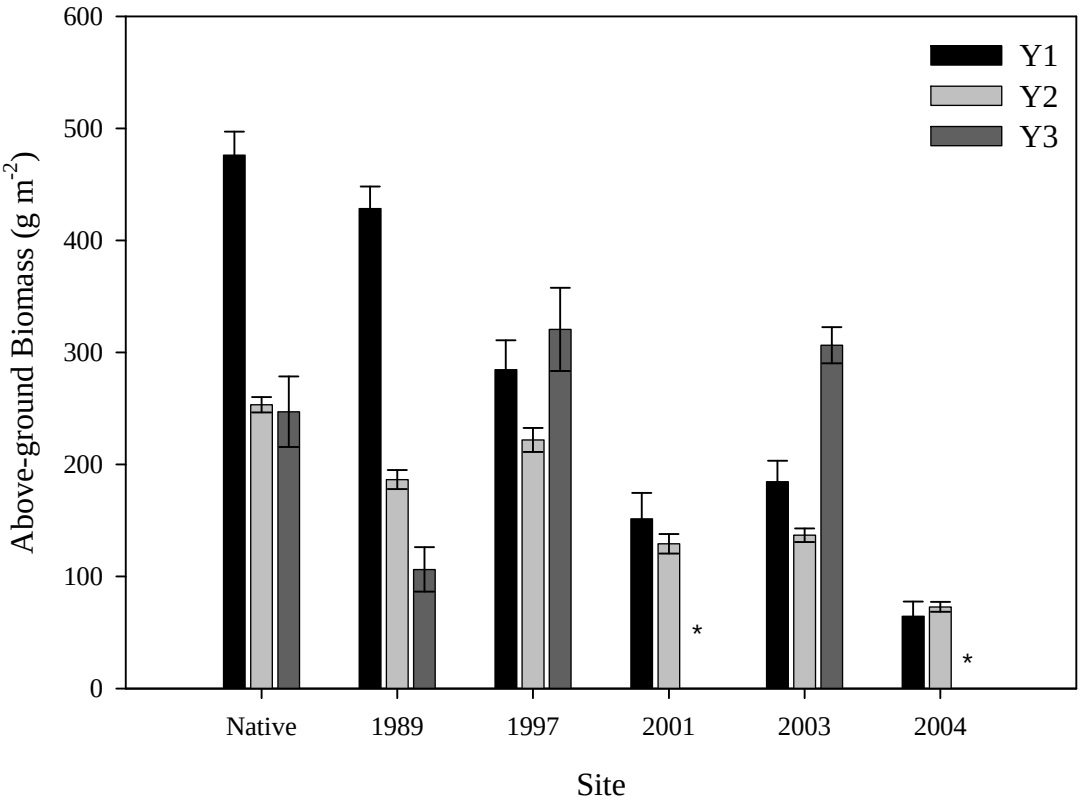

Figure 2-1. Repeated above-ground biomass collection reported as g dry wt m^{-2} for both the native communities and the restored wetlands. Y1 = dry season 2005, Y2 = wet season 2005, Y3 = wet season 2006, *not included in year 2 sampling analysis

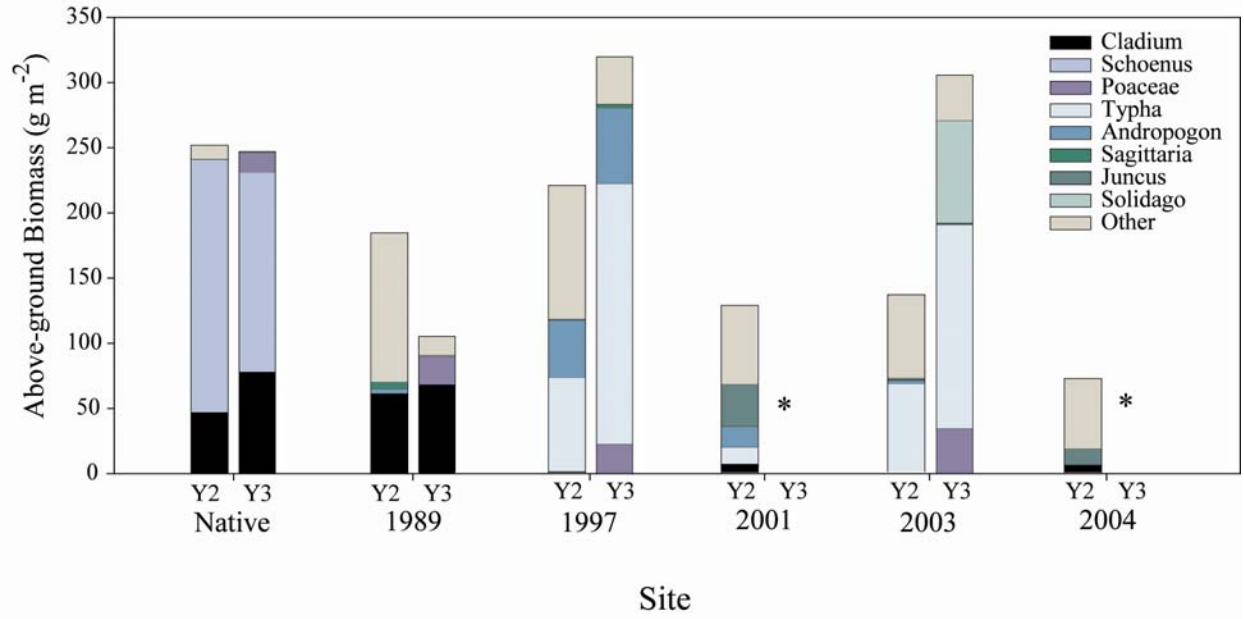

Figure 2-2. Repeated above-ground biomass collection reported as g dry wt m^{-2} and separated by species contribution for both the native communities and the restored wetlands. Y2 = wet season 2005, Y3 = wet season 2006, *not included in year 2 sampling analysis. Y1 = not separated into species contribution.

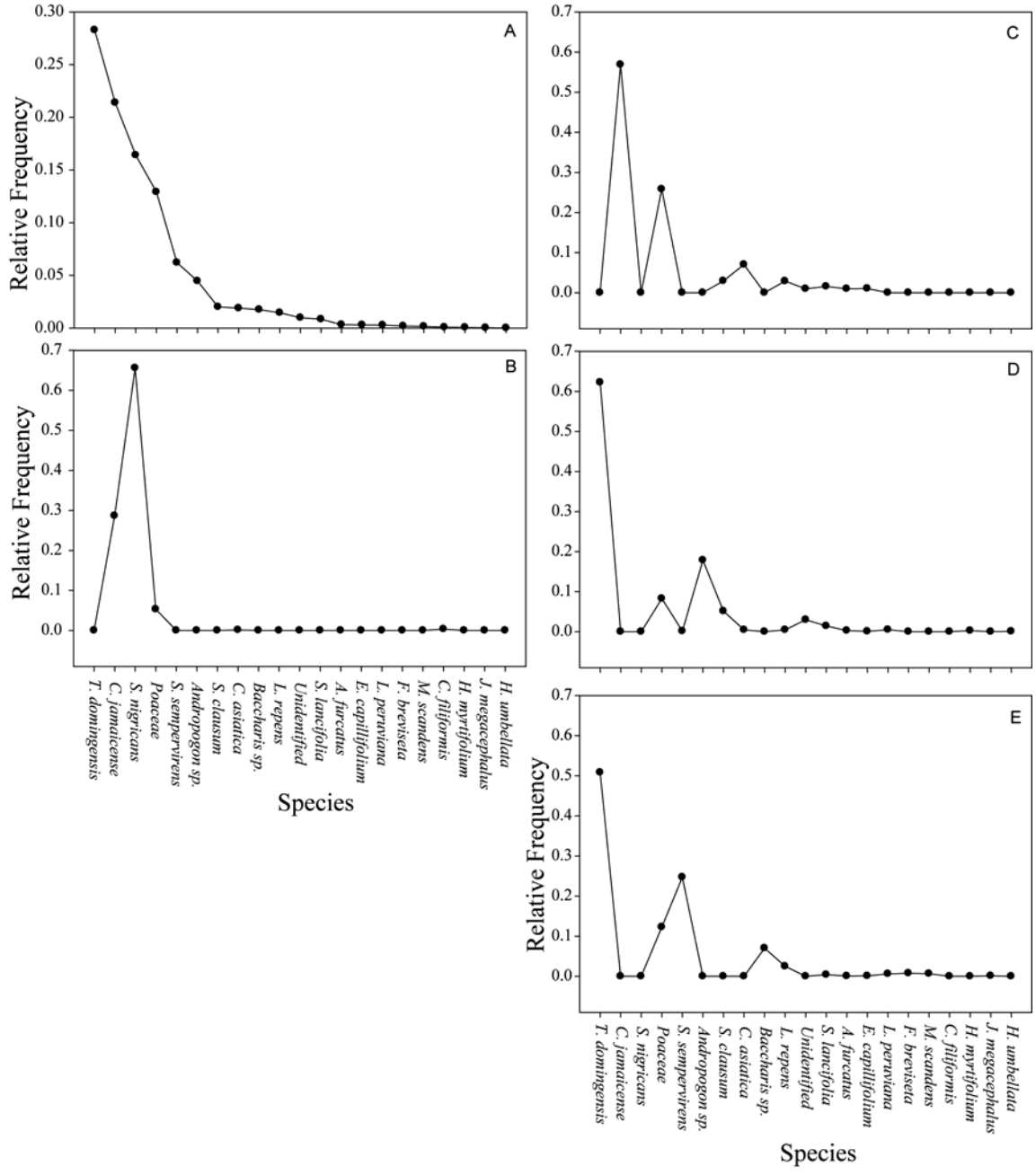

Figure 2-3. Relative frequency of vegetation species collected in the biomass sampling within the restored and native communities of the HID. Species are ordered from left to right as most frequent to least frequent. A) All sites combined in HID. B) Native. C) 1989. D) 1997. E) 2003.

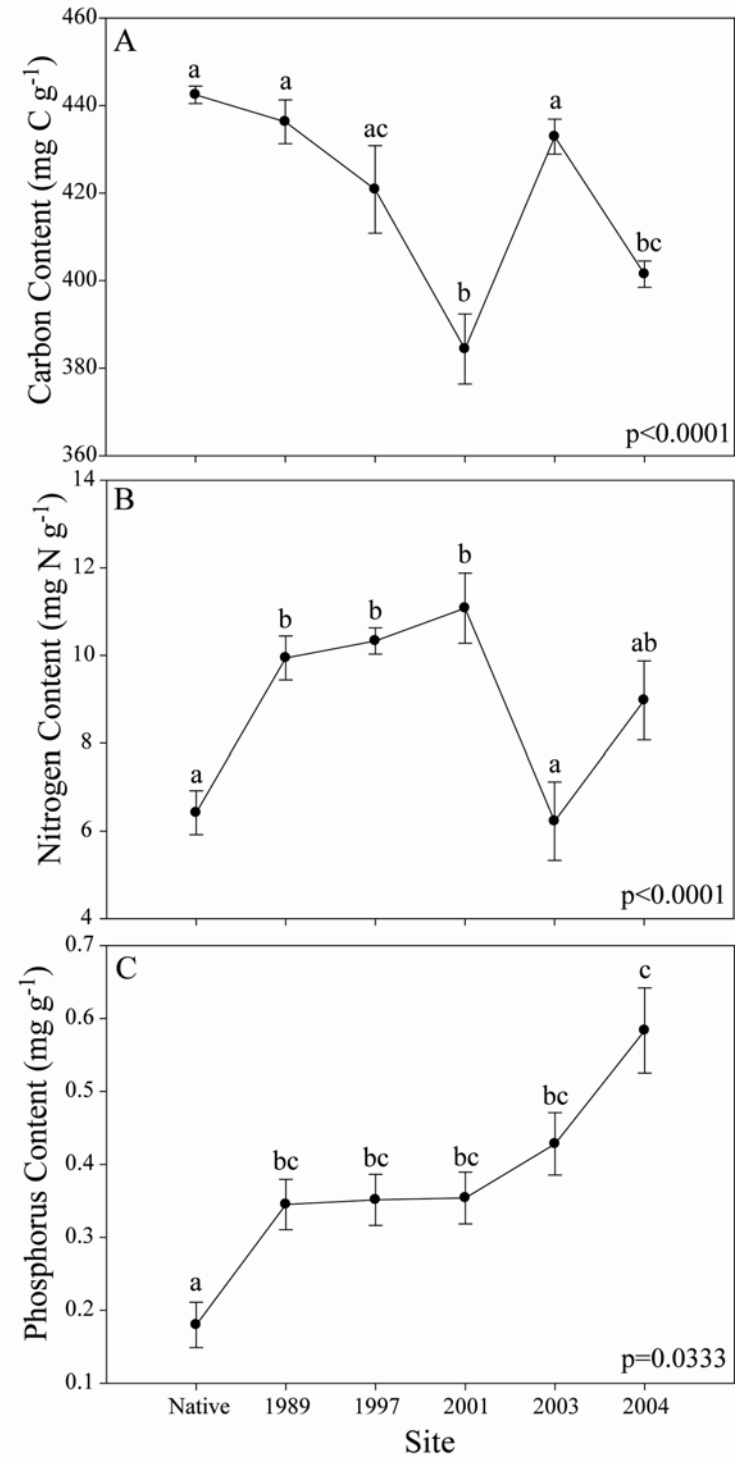

Figure 2-4. Mean tissue content for the community level vegetation for each site in the HID during wet season, Y2. A) Carbon; d.f.=5, F=9.7. B) Nitrogen; d.f.=5, F=17.2. C) Phosphorus, d.f.=5, F=2.7. n=60, 10 for each site average.

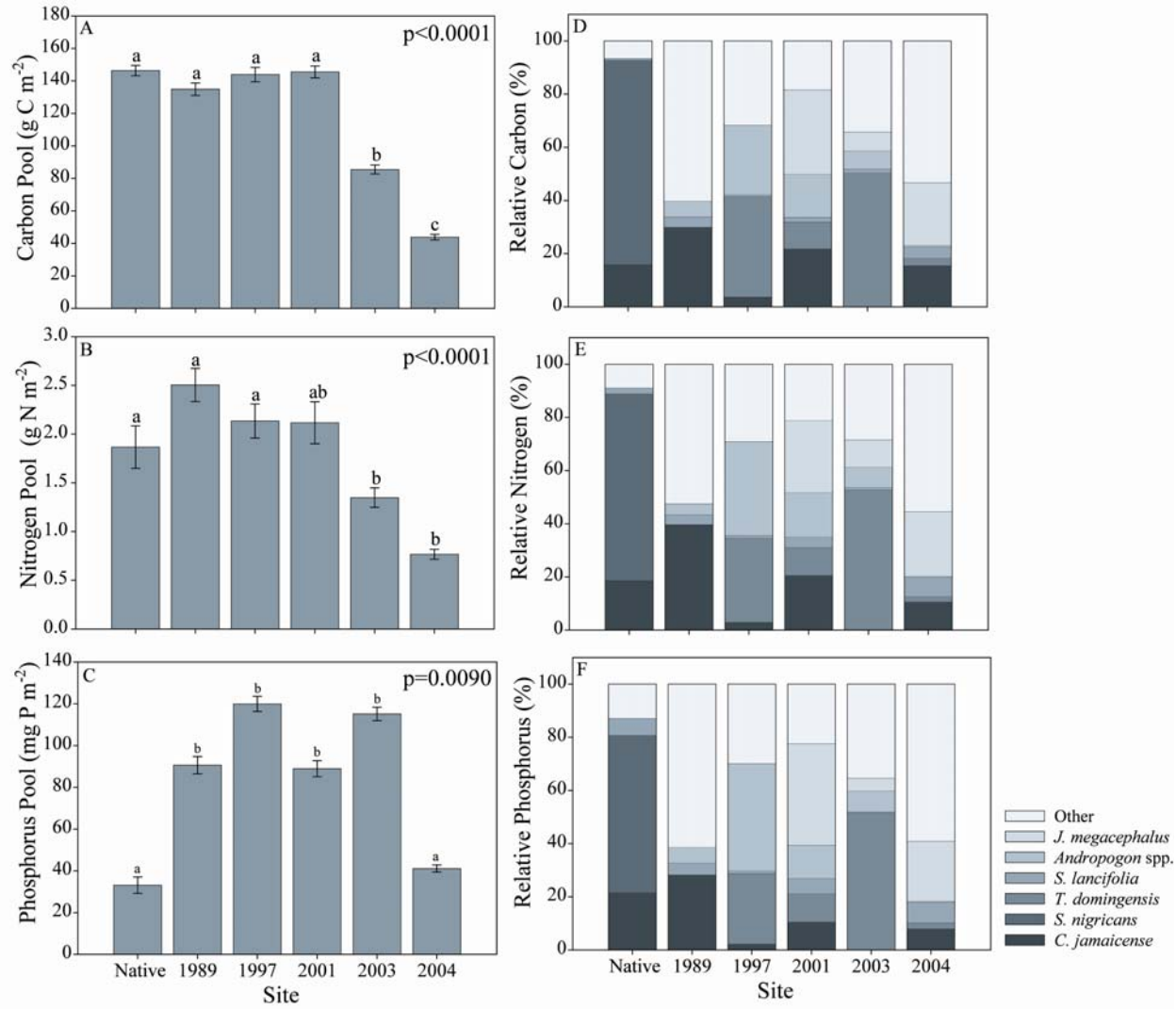

Figure 2-5. Mean carbon, nitrogen, and phosphorus accumulation in above-ground biomass and relative contribution of plant species found in the HID. A) Carbon pool; d.f.=5, F=6.8. B) Nitrogen pool; d.f.=5, F=7.7. C) Phosphorus pool; d.f.=5, F=3.2. D) Relative % carbon. E) Relative % nitrogen. F) Relative % phosphorus.

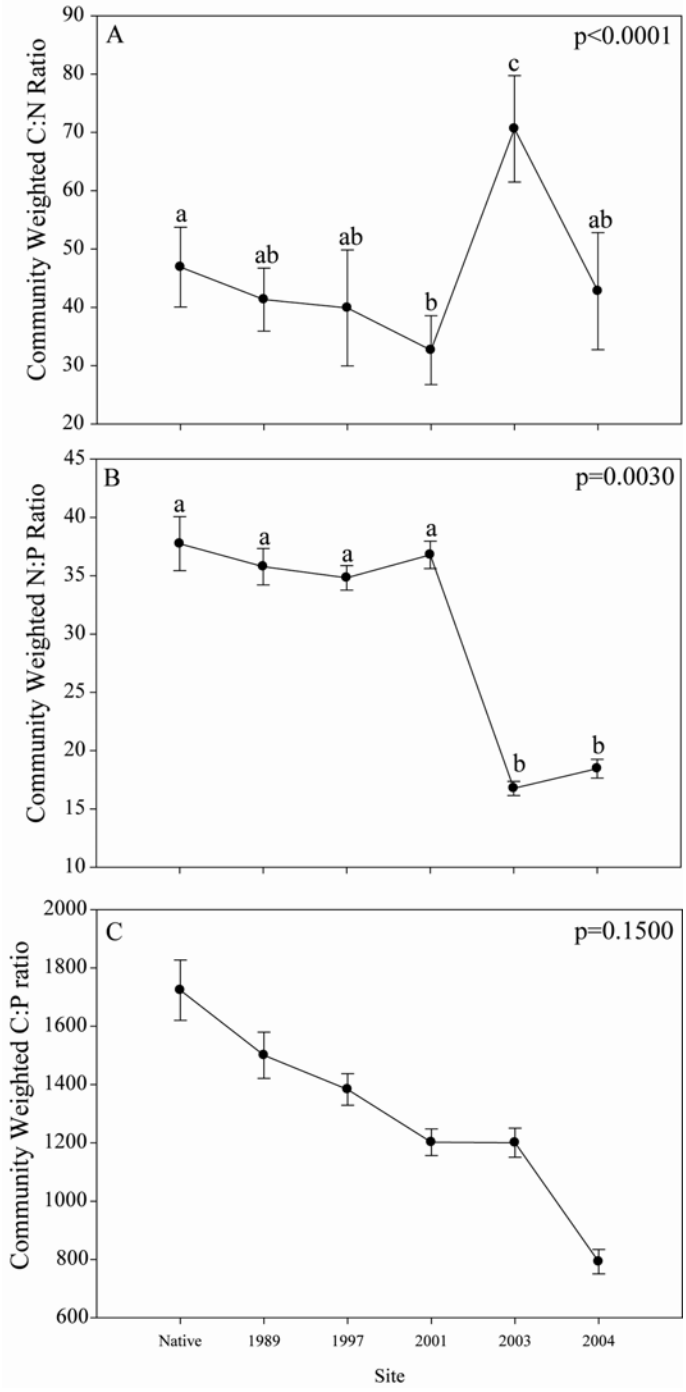

Figure 2-6. Trends in community weighted carbon, nitrogen, and phosphorus ratios between native and restored wetland communities. A) Community weighted C:N ratio; d.f.=5, F=27.1, p<0.0001. B) Community weighted N:P ratios; d.f.=5, F=4.2, p=0.0030. C) Community weighted C:P ratios; d.f.=5, F=1.7, p=0.1500.

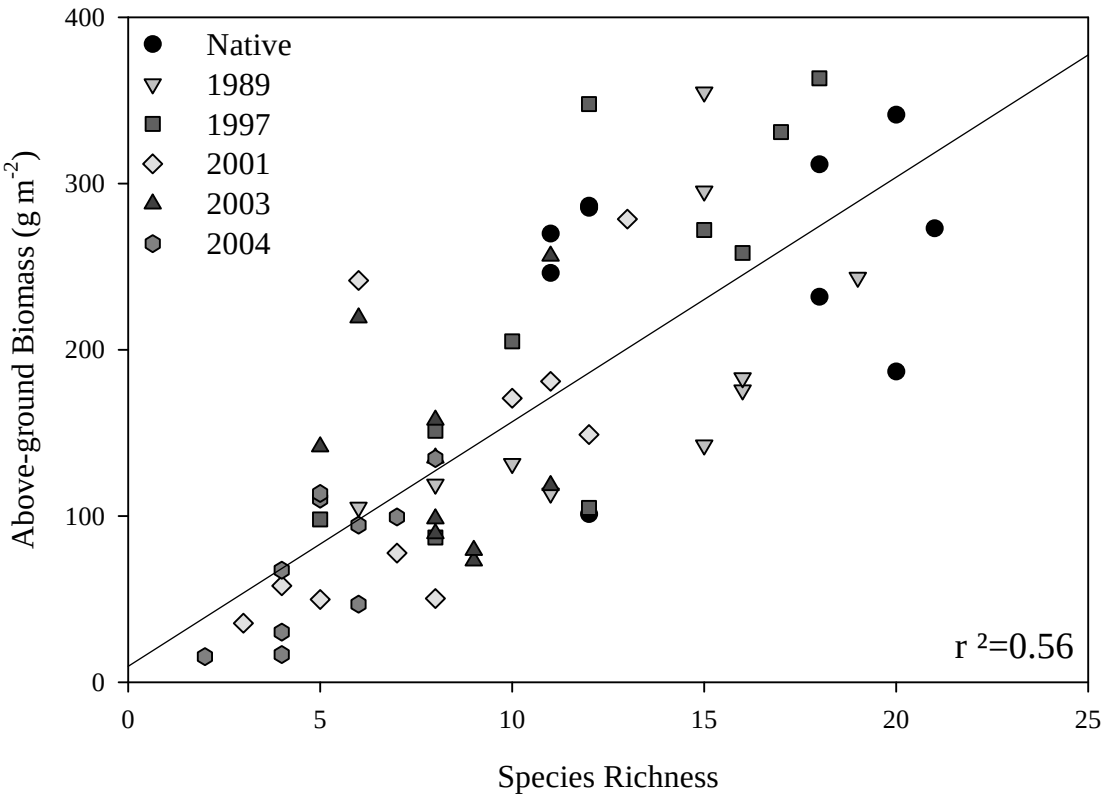

Figure 2-7. Relationship between above-ground biomass production and species richness for the HID during the wet season, Y2. Additionally, each site has been coded to indicate site differences and relationships. (d.f.=59, F=73.8, p<0.001, N=60)

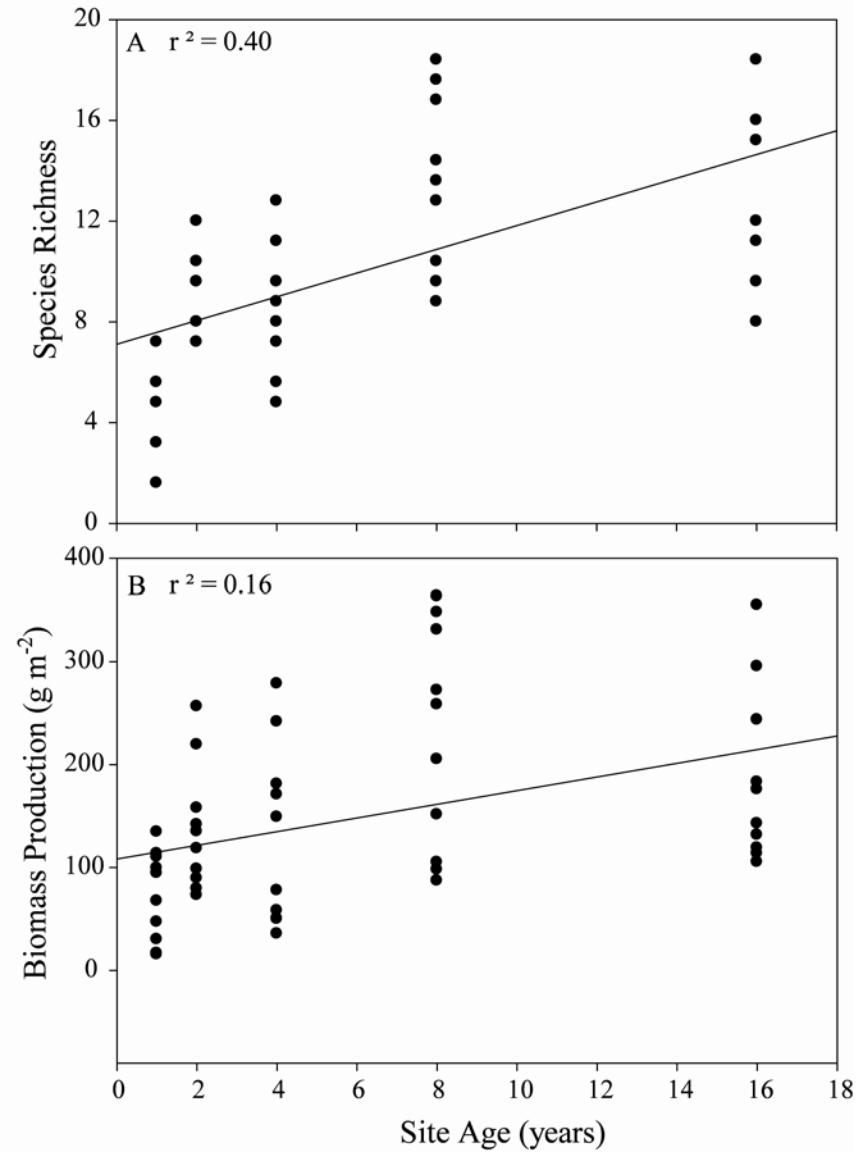

Figure 2-8. Relationships with the age of each restored site for the restored wetland communities in the HID. A) Species richness, d.f.=49, 28.5, p<0.0001. B) Above-ground biomass production, d.f.=49, F=8.9, p=0.0040. n=50, 10 for each site.

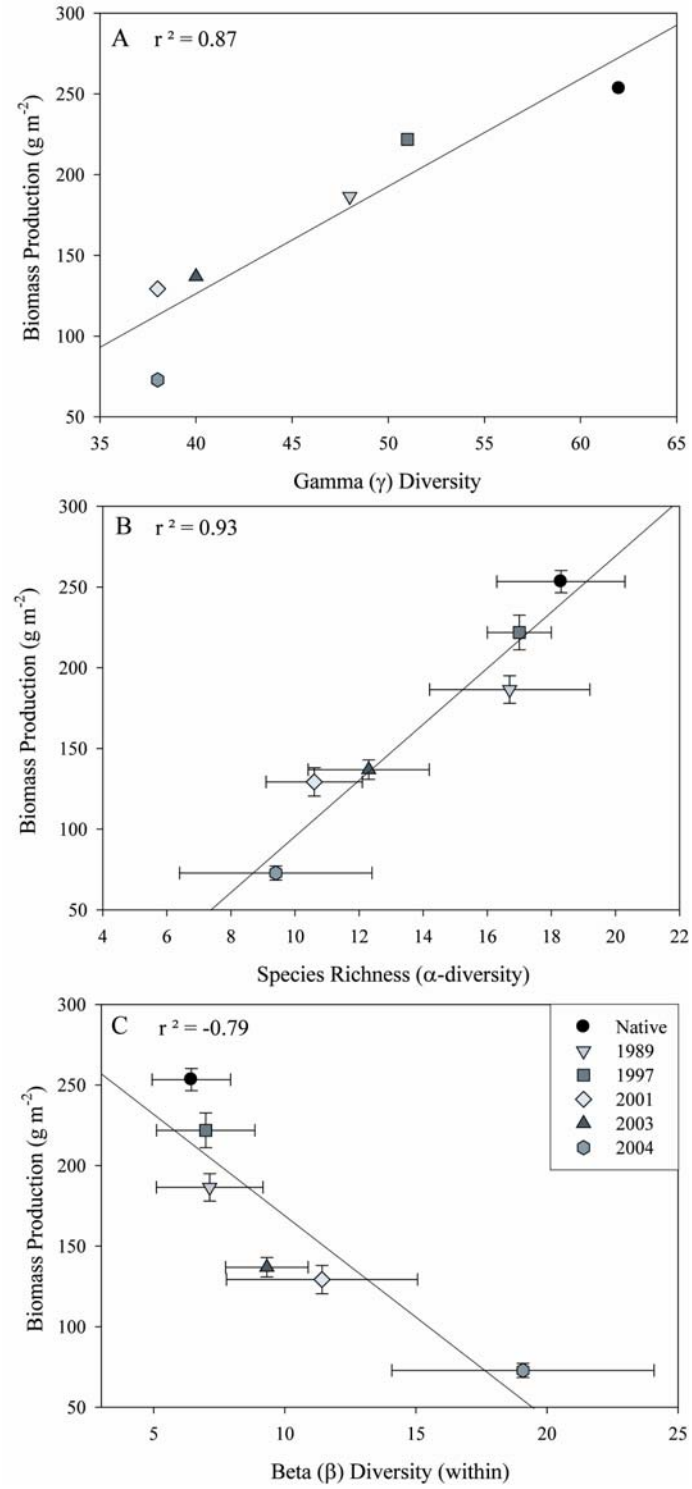

Figure 2-9. Relationships with above-ground biomass production for the sites in the HID. A) Gamma diversity; d.f.=5, F=26.4, p=0.007. B) Species richness (a-diversity), d.f=5, F=53.3, p=0.002. C) Beta diversity (within), d.f.=5, F=15.3, p=0.02. n=60, 10 for each site.

CHAPTER 3
MULTIVARIATE ANALYSIS OF PLANT COMMUNITY STRUCTURE AND RELATIONSHIPS TO ECOSYSTEM CHARACTERISTICS IN SUBTROPICAL RESTORED WETLANDS

Introduction

The loss of more the half of the original wetlands in the United States has resulted in efforts to restore and recreate previously drained wetlands (Whigham 1999). While efforts to restore and mitigate wetlands are well intended, few projects have been successful in achieving natural ecosystem status in terms of vegetation composition of undisturbed reference wetland ecosystems. Hydrology, nutrient availability, and invasion of exotic plant species are the dominant factors impacting the spatial variation of wetland plant communities. Several studies have investigated the impact of these factors on vegetative community structure in wetlands and the nutrient removal potential of wetland plants (Bornette et al. 1998, Kellogg and Bridgham 2002, Olde Venterink et al. 2002, Murphy et al. 2003, Seabloom and van der Valk 2003, Ehrenfeld 2004). Due to the high level of disturbance and destruction of natural wetlands, the loss of wetland plant diversity is inevitable and in turn a loss of function could occur. Therefore, it is important to understand the factors controlling and maintaining species composition in wetland ecosystems.

Hydrology is a dominant factor controlling the development of spatial variation in wetland plant community structure and can be the primary control over propagation and recruitment of wetland vegetation. Experimental studies have shown that variability in hydrological regime can result in different patterns of species development from the seed bank (van der Valk 1981, Pollock et al. 1998, Baldwin et al. 2001). In a series of field and greenhouse controlled seed bank experiments, Baldwin et al. (2001) found that continuously non-flooded soils had almost twice the species richness and 55% greater total stem length than did the

continuously flooded soils. Whereas in studies of riparian wetlands, the wetlands which experienced greater number of flood events or days of inundation per year resulted in a greater species richness (Bornette et al. 1998, Pollock et al. 1998, Murphy et al. 2003).

Hydrology can drive the nutrient availability which can also alter the plant species composition of wetlands. River water is often nutrient rich and an increase in flood events can increase the amount of nutrients made available to the vegetation community in riparian wetlands. In a study of riparian wetlands with high and low connectivity to the river, wetlands with the highest connectivity (increased hydroperiod) resulted in greater species richness and an increase in nutrient availability (Bornette et al. 1998). A wetland with a hydroperiod of 3 d y^{-1} resulted in species richness of 10 and nitrate concentrations of 1.15 mg L^{-1}, whereas a wetland with a hydroperiod of 37 d y^{-1} resulted in species richness of 38 and nitrate concentrations of 10 mg L^{-1} (Bornette et al. 1998). In this case, the increase in flooding had an indirect positive effect on species richness due to the import of more nutrients. This suggests that nutrient availability could also govern plant species composition and structure.

Despite the increase in wetland protection, they continue to be threatened by nutrient enrichment due to anthropogenic impacts. This nutrient enrichment can be in the form of nitrogen (N), phosphorus (P) or both. Agricultural nutrient loading can result in the decrease of plant diversity in wetland ecosystems. The mechanism responsible for this decrease is generally accepted as increased competition or a heightened productivity in a nutrient loving plant (i.e., cattail) that shades and crowds out other smaller or native plants (Craft et al. 1995, Rivero et al. 2007).

An increase in one nutrient can result in a limitation of another. Some wetland ecosystems can naturally be P limited and increasing the N availability in this system can augment this

problem and vise versa. This change in nutrient availability can result in changes in species composition and decreases in species diversity. In a review of fens and bogs, the wetlands that were relatively undisturbed showed that high species diversity was frequently associated with low nutrient status and that species rich wetlands typically have moderate productivity and standing crop (Bedford et al. 1999). However, this relationship has not always been observed. Changes in species composition are not always associated with increases in productivity. Several studies have shown that with increased nutrients, species diversity decreases but productivity increases (Willis and Mitsch 1995, Boyer and Zedler 1998, Mahaney et al. 2004, Rickey and Anderson 2004). Some wetland plants, like *Typha* spp. or *Phragmites* spp., will produce tall dense monospecific stands (high above-ground biomass) under nutrient rich environments and in response they out-compete smaller plants for light which decreases species diversity.

In this study, we combined vegetation composition analysis with environmental characteristics of the soil and plants in restored and native wetland communities to discern relationships among the two. By gaining a clear understanding of what factors govern vegetation community structure we can then evaluate the level of restoration success. The objectives were to 1) determine differences and/or similarities within the development of plant community structure in each restored site; and 2) relate the vegetation community structure of these sites to soil and plant properties.

Methods

Site Description

This study was conducted in wetland systems restored in the Hole-in-the-Donut (HID) region of the Everglades National Park (ENP). Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian

pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season. The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Currently, the Everglades Research Group (ERG) conducts annual monitoring of the vegetation community, soil depth, and hydrology of each of the restored sites, as well as a native community. For the vegetation sampling, the Braun-Blanquet method was employed to evaluate the overall vegetation community structure in permanently established plots every fall at peak biomass. On each restored site there are 20-10 m^2 plots and 40-1 m^2 plots (20 nested in the northwest corner of each of the large plots; 20 randomly located in the intermediate strata of the site). The large plots were establish to address the broad characteristics of vegetation assemblages and the small plots were used to evaluate species composition with regard to soil depth, elevation, and hydrology. This data will also be used in conjunction with additional data collected in this study.

From the intermediate strata, 10 of the pre-established small plots (1 m^2) in the restored (1, 2, 4, 8, 16 years), native sites were utilized for characterization of vegetation and soil physical and chemical properties.

Soil Physical and Chemical Analysis

Soil samples were collected during July 2005 (wet season) from each plot via a 7.6 cm diameter PVC soil core collector to the depth of bedrock and stored at 4°C until laboratory analysis was performed. Before analyses were performed, all rocks, roots and litter material

were removed from the soil. Bulk density was calculated by determining the moisture content of each core by drying a subsample at 60°C until a constant weight was achieved. Soil depth was measured from the top of the soil to the bedrock. Soil exposure was defined as the percent of bare soil without vegetation cover. Organic matter content was determined as loss on ignition (LOI) by combusting 0.5 g of dry soil at 550°C for 4 hours. LOI was calculated as the percent of organic matter lost after combustion.

Ammonium and nitrate were determined by K_2SO_4 extraction (Bundy and Meisinger 1994) and analyzed by flow injection with a Bran Luebbe Auto Analyzer 3 Digital Colorimeter for NH_4 (EPA Method 350.1) and a Alpkem Rapid Flow Analyzer 300 Series for NO_3 (EPA Method 353.2). A subsample of the K_2SO_4 extract was digested for total kjeldahl nitrogen (TKN) via kjeldahl block digestion and analyzed by flow injection with a Bran Luebbe Auto Analyzer 2 Colorimeter (EPA Method 351.1). Total organic carbon (TOC) was analyzed from the extract with a Shimadzu TOC-5050A Total Organic Carbon Analyzer equipped with a ASI-5000A auto sampler. Total phosphorus was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Total carbon (TC) and total nitrogen (TN) were determined by dry combustion with a Thermo Electron Corporation Flash EA NC Soil Analyzer. Carbon, N and P ratios were calculated on a mass basis as C:N, C:P, and N:P.

Vegetation Nutrient Analysis

Total C and TN were determined by dry combustion with a Thermo Electron Corporation Flash EA NC Soil Analyzer for the bulk above-ground plant tissue. Total phosphorus was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer

(EPA Method 119-A rev3) (Anderson 1976). Carbon, N and P ratios were calculated on a mass basis as C:N, C:P, and N:P.

Statistical Analysis

A non-metric multidimensional scaling (NMS) ordination was used with PC-ORD (autopilot, thorough mode) (McCune and Mefford 1999) to determine differences and/or similarities in the plant community structure within the 1997, 2001, 2003, and 2004 restored wetlands at six months after they were restored. Data for the 1989 site was not made available for six months after restoration; therefore it was not included in this analysis. To evaluate the 1989 site, a NMS ordination was applied to investigate the 1989 and 1997 site at eight years after restoration to determine differences and/or similarities in long term developmental patterns in plant community composition.

The NMS ordination technique was used to determine relationships between environmental characters and vegetation community structure for restored sites compared to native sites performed with PC-ORD (autopilot, thorough mode) (McCune and Mefford 1999). The environmental characters were divided into three groups, soil chemical, soil physical, and vegetation chemical parameters with three separate NMS ordinations performed. The parameters from each analysis that indicated the strongest correlation with respect to differences in native communities verses restored sites were them subjected to further analysis. Regressions were performed to determine if any relationships existed between these parameters among the three divided groups (SAS, JMP) (SAS 2005).

Results

Species Composition

A total of 22 vegetation species were identified within the 60 1-m^{-2} plots to determine biomass production in the restored and native communities included in this study (Table 3-1).

These species were grouped based on abundance and importance to the restoration goal and ranked based by frequency and percent cover. Species that were that most abundant were grouped by themselves. Several species observed in the sites were rare and contributed less than 1% to the total above ground biomass. In an effort to eliminate clutter and confusion these rare species were grouped together and called 'Other' for ordination analysis. Several Poaceae species (grasses) were observed, however, unidentifiable to the species level. All Poaceae species were grouped into one group called Poaceae and counted as one species and weighted based on its relative frequency in each site.

Environmental Parameters

The results of the soil chemical analysis are summarized as averages with standard errors and listed for each site in Table 3-2. Nitrate had non detectable limits for all sites which was not surprising since this analysis was performed during the wet season. The 2004 site had considerably less extractable NH_4, TKN, and TOC concentration as compared to all the other sites. For TC and TN there is a split in the sites with the Native, 1989, and 1997 sites having similar values higher than what was observed in the 2001, 2003, and 2004 sites. This resulted in the same trend with the C:N ratios. The TP values for the sites showed no trend with restoration age. However, the native communities had the lowest TP values of 0.33 g kg^{-1} and the 2003 site had the highest at 0.87 g kg^{-1}. Interestingly, the native site had the highest TN values and the 2003 site had the lowest, 11.0 and 6.2 g kg^{-1}, respectively. This resulted in N:P ratios following the same trend with 48.3 in the native and 7.6 in the 2003 sites.

The soil physical properties did not result in the same variability that was seen with the soil chemical parameters (Table 3-3). There was not a significant difference in the elevation across the sites. However, the 2004 site had a lower elevation than all the others due to its lower geographical location relative to the other sites. The native sites had an average soil depth of

14.6 cm, whereas the restored sites had a range of soil depths from 0.93 to 2.8 cm. The percent moisture was similar across sites except for the 2004 site which had the lowest moisture content of 46.4%. The native and 2004 sites had similar bulk densities of 0.41 and 0.39 g cm^{-3} and the 1989, 1997, 2001, and 2003 sites had similar densities of 0.28, 0.27, 0.27, and 0.30 g cm^{-3}. The low bulk densities are a result of high levels of organic matter present in the soil. This is supported by the high levels of %LOI which is a measure of organic matter content.

The vegetation nutrient analysis results are found in Table 3-4. The lowest TN levels were found in the vegetation in the 2003 at 6.2 g kg^{-1}, which is the same as what was found in the soil in the 2003 site. The vegetation in the native sites had the lowest amount of TP at 0.31 g kg^{-1} and the vegetation in the 2004 site had the highest at 0.57 g kg^{-1}. The N:P ratios were similar for the native, 1989, 1997, and 2001 sites ranging from 34.8 to 37.7 with the native being the highest. The 2003 and 2004 sites had relatively low N:P ratios comparably at 16.8 and 18.1.

Soil and Plant Relationships

The NMS ordinations comparing the development of the 1997, 2001, 2003, and 2004 sites at six months of development concluded that a 2-dimensional solution (two axis) was best for each ordination performed with a Monte Carlo test p-value = 0.092. The final stress for the 2-dimensional solutions was 12.4 after 90 iterations. The NMS ordination concluded that there were some difference in initial plant community development within the 1997, 2001, 2003, and 2004 restored wetlands (Figure 3-1). The initial plant colonization was similar in the 2001 and 2003 restored wetlands at six months after complete soil removal. These two sites were grouped with overlapping plots in ordination space. The 1997 site plant colonization at six months was clearly different from the 2001 and 2003 sites. The site restored in 2004 had the greatest variability in species composition across plots. This site had similarities in species composition with the 1997, 2001 and 2003 restored wetland communities (Figure 3-1).

The NMS ordinations comparing the 1989 and 1997 sites at eight years of development concluded that a 2-dimensional solution (two axis) was best for each ordination performed with a Monte Carlo test p-value = 0.073. The final stress for the 2-dimensional solutions was 12.9 after 90 iterations. The NMS ordination investigating differences the relationships between plant community development of the 1989 and 1997 sites at eight years after restoration indicates that theses two sites are similar in species composition at this age of development (Figure 3-2). The ordination suggests variability between plots in both sites. However due to the mixed single group of plots, it is concluded that these two sites are similar in species composition.

The NMS ordinations of species composition comparison to environmental characteristics concluded that a 2-dimensional solution (two axis) was best for each ordination performed with a Monte Carlo test p-value = 0.0196. The final stress for the 2-dimensional solutions was 10.36 after 91 iterations. With vegetation community structure, the ordination resulted in the native communities clearly being different in vegetation composition as compared to the restored sites (Figures 3-3, 3-4, and 3-5). There is grouping with the restored sites, but they all overlap at some point in ordination space indicating areas of similarity. *Schoenus nigricans* was the dominant species present in the native communities followed by *C. jamaicense*. *Schoenus nigricans* was not found in any of the restored sites, but *C. jamaicense* was found in small numbers in all sites except the 2003 site. The absence of *S. nigricans* in any of the restored sites is largely responsible for the distinct grouping of the native communities as indicated by the placement of *S. nigricans* in ordination space (Figures 3-3, 3-4, and 3-5). Additional species found in the native sites include *S. lancifolia,* Poaceae, and Other. Poaceae occurred in similar frequencies in all sites. *Sagittaria lancifolia* were found in all sites, but in small numbers with the fewest in the native communities.

Typha domingensis, *Andropogon glomeratus*, *Andropogon virginicus* (*Andropogon* spp.), and *Juncus megacephalus* were all absent from the native communities. *Typha domingensis* was present in all restored sites but the most abundant in the 1997 and 2003 which is supported by the placement of *T. domingensis* between the groupings of the 1997 and 2003 sites in the ordination (Figures 3-3, 3-4, and 3-5). *Andropogon* spp. was also present in all restored sites but the most abundant in the 1997 site. *Juncus megacephalus* was limited to the 2001, 2003, and 2004 sites and was most abundant in the 2001 restored site.

The overlain joint plot for soil chemical parameters on the NMS ordination indicates that difference in vegetation community composition was driven by C:P, N:P, and TP variables (Figure 3-3). The length of each vector is proportional to correlations with the vegetation composition, the longer the length the stronger the relationship. These vectors also indicate that TP is inversely related to the C:P and N:P ratios (r^2 = -0.773 and -0.738, respectively). In addition, due to the weak correlation of TN with N:P ratios (r^2 = 0.308), this suggests that the N:P ratios are driven by the amount of TP in the system.

Of the soil physical properties that were investigated, only soil depth correlated with the vegetation composition (Figure 3-4). The direction and length of this vector indicates that the soil depth maintained in the native communities is an important parameter controlling overall vegetation composition.

The overlain joint plot for the vegetation nutrient parameters did not indicate any strong correlations with the vegetation composition (Figure 3-5). The lengths of all the vectors are short relative to what was found with the other ordinations. The direction of the vectors for the nutrient ratio, however, is similar to the direction of the same ratios for the soil chemical analysis.

To evaluate relationships between soil chemical, physical and vegetation nutrient parameters, regressions were performed between the parameters that were identified from each of the NMS ordinations. The C:P and N:P ratios of both the soil and vegetation and the soil depth was chosen for regression analysis. A strong correlation was found to exist between the soil N:P and C:P ratios and the soil depth with an $r^2 = 0.76$ ($F=49.2$, $p<0.0001$, d.f.=59) and 0.59 ($F=36.0$, $p<0.0001$, d.f.=59), respectively (Figure 3-6a, and b). Due to the large differences in soil depth between the native and restored sites, there was a cluster of point at the low soil depths with the linear regression extended by the deeper depths of the native communities. To investigate if this was a false relationship, the regressions were ran without the native sites to see if this relationship still existed within the cluster of restored sites. The soil N:P ratios of the restored sites resulted in an $r^2 = 0.39$ ($F=5.7$, $p=0.02$, d.f.=49; Figure 3-6c). While this correlation is not as strong as with the inclusion of the native site data, the result is the same positive relationship. The same was found to be true for the C:P ratio and soil depth with the exclusion of the native sites ($r^2 = 0.36$, figure not shown).

A relationship between vegetation N:P and C:P ratios with the soil depth was not seen. Both ratios resulted in a positive relationship, but the correlation was not significant ($r^2 = 0.18$; $F=6.2$. $p=0.05$, d.f.=59 and 0.26; $F=9.8$, $p=0.003$, d.f.=59 for N:P and C:P, respectively; Figure 3-7a, and b).

Discussion

The desired species composition of the restored wetlands in the HID is the community found in the native sites investigated, a *Schoenus nigricans* and *Cladium jamaicense* dominated system. In the first few years after restoration, sites are comprised of species associated with disturbance; i.e. *Typha domingensis* and *Baccharis* spp (O'Hare and Dalrymple 2003). However, as the sites develop with time, the native plant species *C. jamaicense* is becoming more

abundant. *Cladium jamaicense* is present in small numbers within restored sites but *S. nigricans* is not present at all. The increased abundance of *C. jamaicense* in the 1989 site relative to the more recently restored sites, gives encouragement that *C. jamaicense* may become more abundant in all sites with time.

Research has shown that planting desired vegetation communities in restored wetlands could result in faster community development similar to a reference wetland ecosystem (Kellogg and Bridgham 2002). Restored wetlands that relied on natural re-vegetation had much lower species richness and an increase in exotics after a five to seven year period as compared to reference wetlands (Seabloom and van der Valk 2003). This suggests that planting is necessary to insure the desired composition in wetland vegetative community structure of restored wetlands. An analysis of species diversity development in restored wetlands of the HID indicated that planting was not necessary to restore high levels of species diversity and composition (See Chapter 2). However, the NMS ordination performed in this study did not indicate similarities between the 1989 and the native plant communities. There is a clear differentiation between these two sites in ordination space. The most distinct contributing factor to this difference in ordination analysis is due to the presence of *S. nigricans* in the native community. The dominance of this plant species in the native community and the lack of its presence in any restored wetland system outweigh any similarities found between the native and 1989 site when analyzed statistically.

Research on *S. nigricans* within the ENP has been limited and no information on its recruitment and germination requirements in the ENP are known. Studies have been performed in the Netherlands and Mediterranean regions on its behavior in salt marsh dunes (Ernst and Vanderham 1988, Ernst and Piccoli 1995, Ernst et al. 1995, Bakker et al. 2007). *Schoenus*

nigricans has been shown to be dependent on waterlogged, silicon rich conditions in order to produce fruit (Ernst et al. 1995). Additionally, it has been shown to tolerate dry conditions, however optimal growth occurs under wet conditions (Ernst and Piccoli 1995). In opposition, a more recent study found that *S. nigricans* had optimal germination and recruitment at moist to dry conditions, suggesting that a retreating groundwater table would provide optimal conditions for new recruitment (Bakker et al. 2007). While the fluctuating water table of the HID may be an ideal environment for *S. nigricans*, the silicon concentration could be limiting seed production. Silicon is typically high during early stages of primary succession in areas where new soil is derive from volcanic material and have been shown decrease with weathering processes (Hedin et al. 2003). In the HID, all soil has been removed and soil development is dependent upon biological growth and processes which could result in soil limited in silicon.

By comparing the native and 2003 communities (the two extremes), we found that the native communities are P-limited and that the 2003 restored site may be N-limited immediately following restoration. The soil and vegetation in the native community indicates a P-limited system (N:P ratios of 48 and 38, respectively), whereas the soil and vegetation in the 2003 site indicates a possible N-limited system (N:P of 8 and 17). It has been suggested that a N:P < 14 results in an N-limitation and a N:P > 16 results in a P-limitation (Koerselman and Meuleman 1996). However, more importantly is the significant difference between the N:P ratios from the native and 2003 sites.

The farming practices that previously occurred in the HID resulted in elevated P and decreased N concentrations relative to the native regions of the ENP. The differences in ratios are a result of the native site having the highest N and the lowest P values and the 2003 site having the lowest N and the highest P values. This imbalance in the N and P concentrations is a

contributing factor that led to the initial invasion by *S. terebinthifolius*. After restoration, this imbalance in nutrient concentrations still exists but it is not as extreme. In northern parts of the ENP, *Typha* spp. has been found to invade areas that have been disturbed via enrichments of P concentrations. A good example is Water Conservation Area 2A (WCA-2A), levels of P have increased resulting in a shift from a *C. jamaicense* dominated system to one dominated by *Typha* spp. (either *T. domingensis* or *T. latifolia*) (Reddy et al. 1999, Rivero et al. 2007).

Conclusions

One important question in the successional development of the HID is 'what are colonization patterns for *C. jamaicense* and *S. nigricans* and why are they not dominating the restored sites?' *Schoenus nigricans* has not been found to colonize any of the restored wetland communities. This brings up some interesting life history question on the required conditions in order for *S. nigricans* to successfully recruit, propagate and survive. More research is needed on this species in the ENP to determine germination, recruitment and seed production requirements.

Cladium jamaicense, however, is dominant in the 1989 site and is present in more recently restored sites, indicating that with time it will inhabit these wetland areas even if the environmental conditions are not restored to native conditions. This study provides evidence that a nutrient limitation could be responsible for plant community composition in subtropical restored wetland communities. Therefore, restoration projects need to take into account the environmental nutrient condition needed to develop and maintain native plant community structure.

Table 3-1. List of all species identified during vegetation biomass collection in the restored sites and the native communities. (n=60 plots)

Species Name	[C]Species Group	Species Name
Macrophyte Community		[A]Algae Mats
[B]*Andropogon glomeratus*	Andropogon	Chara light
[B]*Andropogon virginicus*	Andropogon	Chara unidentified species
Baccharis angustifolia	Other	Periphyton unidentified species
Baccharis glomeruliflora	Other	
Baccharis halimifolia	Other	
Centella asiatica	Other	
Cladium jamaicense	Cladium	
Eupatorium capillifolium	Other	
Eupatorium leptophyllum	Other	
Fuirena breviseta	Other	
Hydrocotyle umbellata	Other	
Juncus megacephalus	Juncus	
Ludwigia peruviana	Other	
Ludwigia repens	Other	
Mikania scandens	Other	
[B]Poaceae	Poaceae	
Sagittaria lancifolia	Sagittaria	
Sarcostemma clausum	Other	
Schoenus nigricans	Schoenus	
Solidago sempervirens	Other	
Typha domingensis	Typha	

[A]Not included in NMS ordindation, but present at all sites

[B]All Poaceae species were grouped together with the exception of the two Andropogon species which were group together as one group named Andropogon

[C]All species in species group Other contributed less than 1% of the total above ground biomass at the time of sampling and therefore were weighted with less importance over the most abundant plant species observed

Table 3-2. Average values for soil chemical parameters for each site used in non-metric multidimensional ordination with vegetation community data (n=10 for each site). NDL = non detectable limits.

	Native		1989		1997		2001		2003		2004	
	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE
NH_4-N (ug g^{-1})	33	(1.3)	53	(0.9)	72	(3)	70	(4)	58	(3)	16	(0.4)
NO_3-N (ug g^{-1})	NDL		NDL		NDL		NDL		NDL		NDL	
TKN (ug g^{-1})	62	(2.0)	107	(4)	93	(4)	129	(10)	100	(6)	21	(2)
TOC (ug g^{-1})	1073	(23.2)	1236	(30)	1467	(32)	1217	(77)	1132	(28)	806	(57)
TC (g kg^{-1})	188	(1.7)	178	(2)	186	(2)	157	(2)	153	(0.9)	158	(3)
TN (g kg^{-1})	11	(0.2)	10	(0.1)	10	(0.1)	7	(0.1)	6	(0.1)	7	(0.2)
TP (g kg^{-1})	0.3	(0.03)	0.7	(0.03)	0.7	(0.03)	0.4	(0.02)	0.9	(0.02)	0.5	(0.02)
C:N	17	(0.2)	18	(0.2)	19	(0.1)	25	(0.3)	25	(0.4)	22	(0.3)
C:P	865	(44.3)	344	(19)	383	(46)	597	(45)	191	(7)	393	(14)
N:P	48	(2.1)	18	(0.8)	20	(2)	25	(2)	8	(0.3)	19	(0.8)

Table 3-3. Average values for soil physical properties for each site used in non-metric multidimensional ordination with vegetation community data. (n=10 for each site)

	Native		1989		1997		2001		2003		2004	
	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE
Elevation (m)	0.5	(0.004)	0.5	(0.005)	0.5	(0.04)	0.5	(0.05)	0.5	(0.004)	0.4	(0.01)
Soil depth (cm)	15	(0.7)	3	(0.1)	1	(0.1)	3	(0.2)	1	(0.1)	1	(0.00)
Moisture (%)	61	(5)	64	(4)	69	(7)	56	(1)	59	(8)	46	(5)
Bulk Density (g cm^{-3}	0.41	(0.02)	0.28	(0.01)	0.27	(0.04)	0.27	(0.02)	0.30	(0.01)	0.39	(0.01)
LOI (%)	22	(0.6)	23	(3.1)	24	(4.2)	15	(0.4)	14	(4.0)	20	(1.1)

Table 3-4. Average values for vegetation nutrient parameters for each site used in non-metric multidimensional ordination with vegetation community data. (n=10 for each site)

	Native		1989		1997		2001		2003		2004	
	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE
TC (g kg^{-1})	436	(21)	432	(12)	418	(18)	376	(33)	433	(23)	395	(26)
TN (g kg^{-1})	9	(1)	11	(0.1)	11	(2)	12	(1)	6	(1)	10	(1)
TP (g kg^{-1})	0.31	(0.01)	0.34	(0.01)	0.35	(0.02)	0.35	(0.01)	0.43	(0.02)	0.57	(0.2)
C:N	47	(7)	41	(5)	40	(1)	33	(6)	71	(9)	42	(1)
C:P	1723	(104)	1500	(79)	1383	(54)	1202	(46)	1200	(50)	769	(32)
N:P	38	(2)	36	(2)	35	(1)	37	(1)	17	(1)	18	(1)

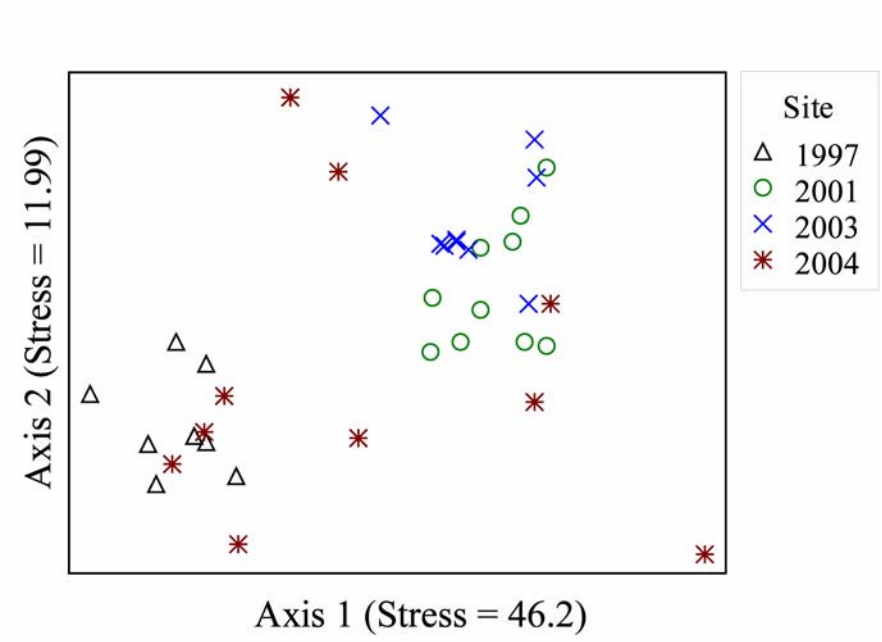

Figure 3-1. Non-metric multidimensional scaling (NMS) ordination of vegetation community for each restored site at six months after restoration. The symbols represent each site with the year being the year the site was cleared. Data for the 1989 site was not available for six months after restoration.

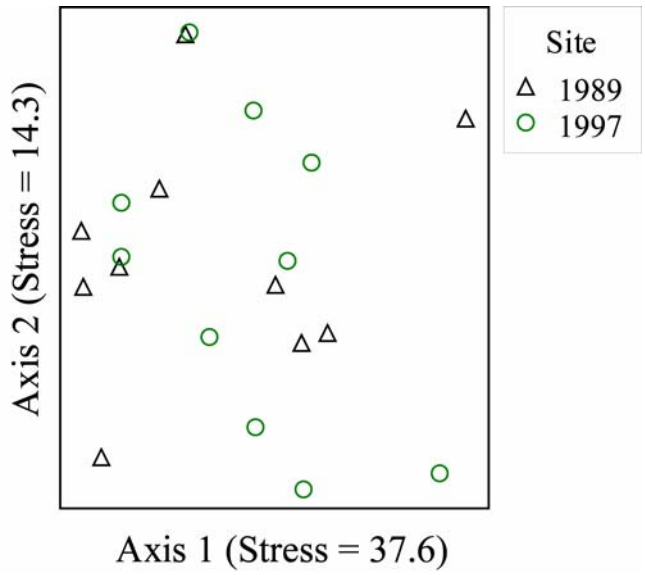

Figure 3-2. Non-metric multidimensional scaling (NMS) ordination of vegetation community data for the 1997 and 1998 restored sites at 8 years after restoration. The symbols represent each site with the year being the year the site was cleared.

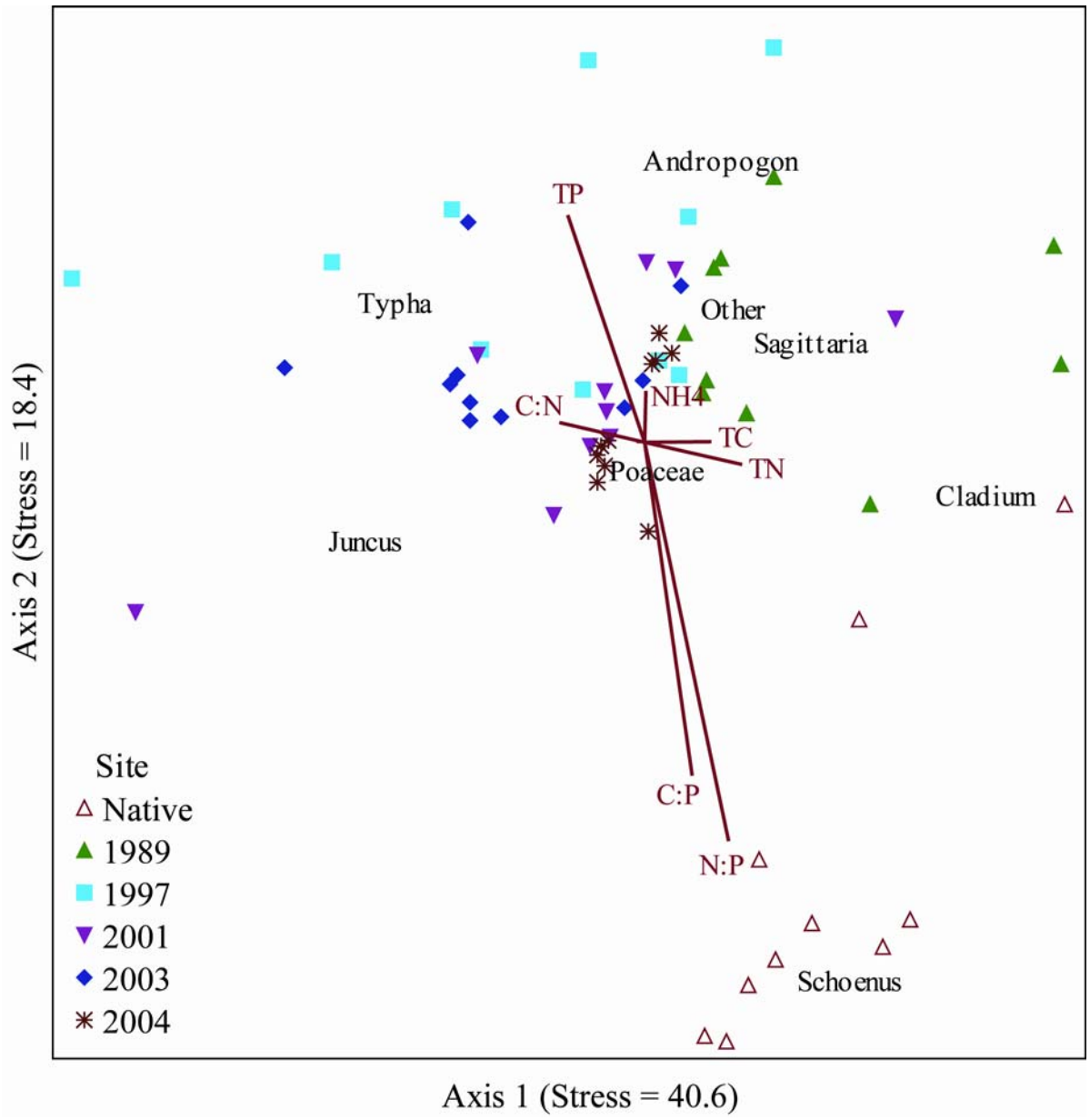

Figure 3-3. Non-metric multidimensional scaling (NMS) ordination of vegetation community data for restored and native sites. The symbols represent each site with the year being the year the site was cleared. The overlain line vectors represent significant correlations between vegetation composition and each soil chemical parameter.

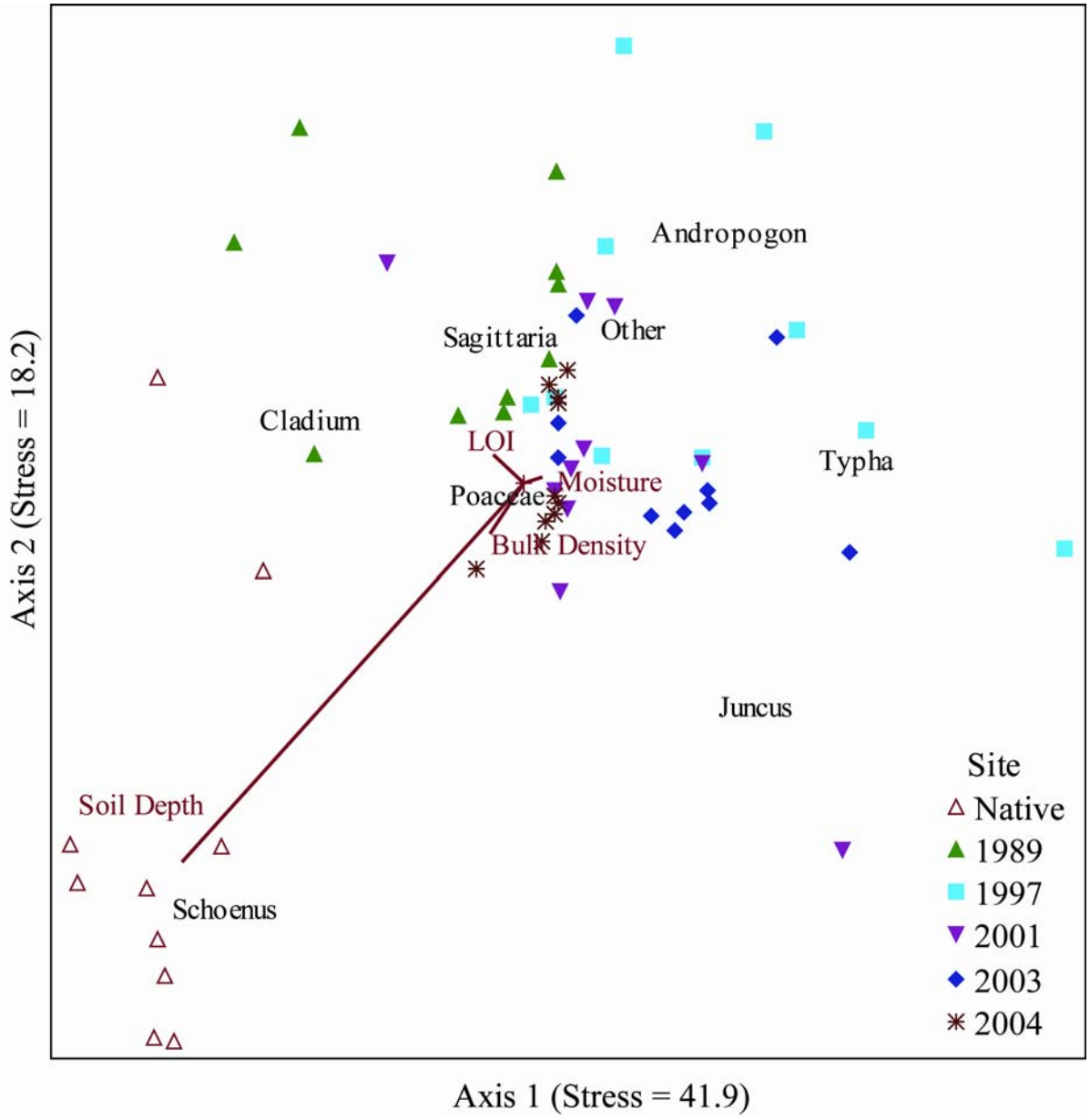

Figure 3-4. Non-metric multidimensional scaling (NMS) ordination of vegetation community data for restored and native sites. The symbols represent each site with the year being the year the site was cleared. The overlain line vectors represent significant correlations between vegetation composition and each soil physical properties.

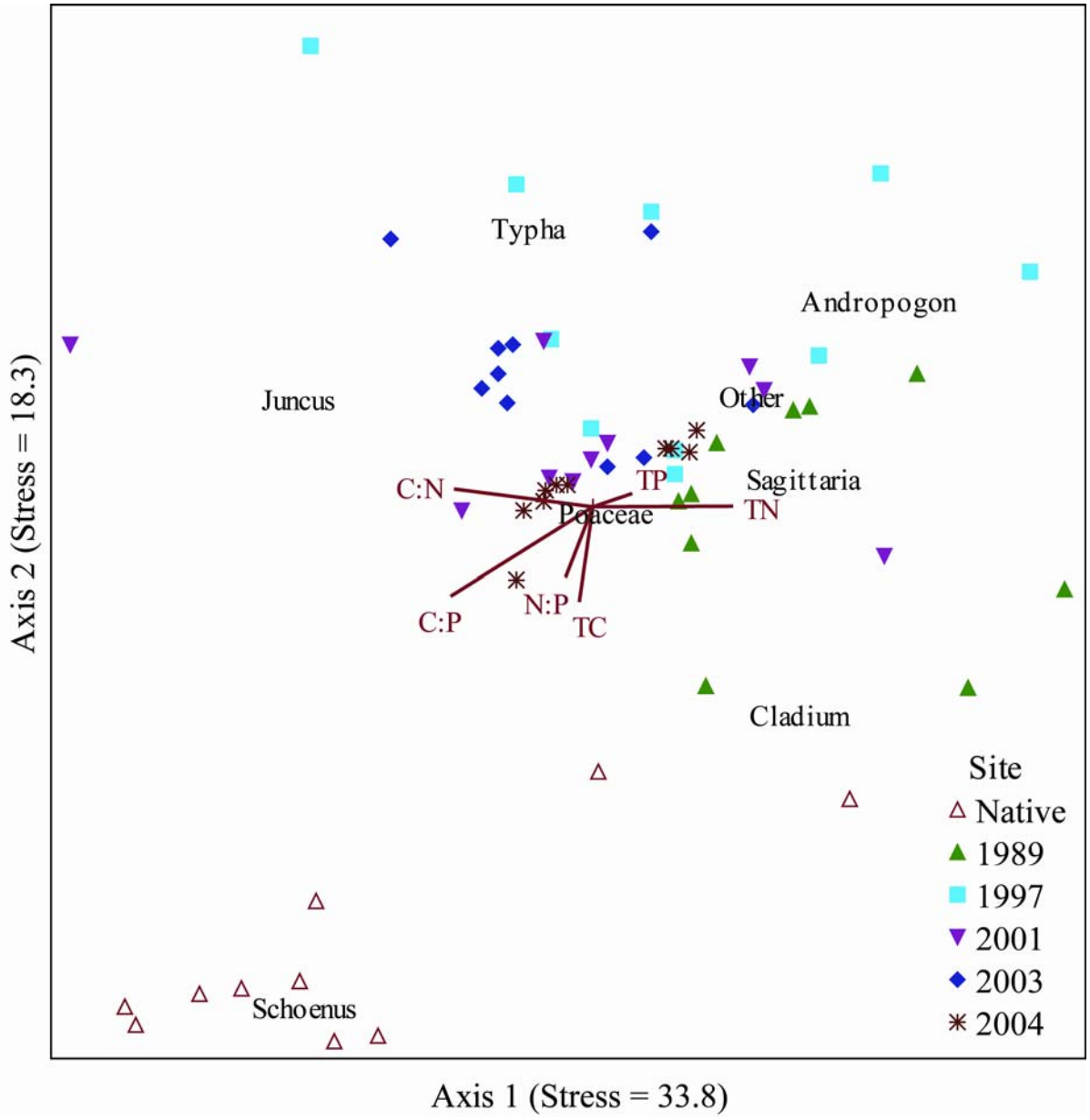

Figure 3-5. Non-metric multidimensional scaling ordination (NMS) of vegetation community data for restored and native sites. The symbols represent each site with the year being the year the site was cleared. The overlain line vectors represent significant correlations between vegetation composition and each vegetation nutrient parameter.

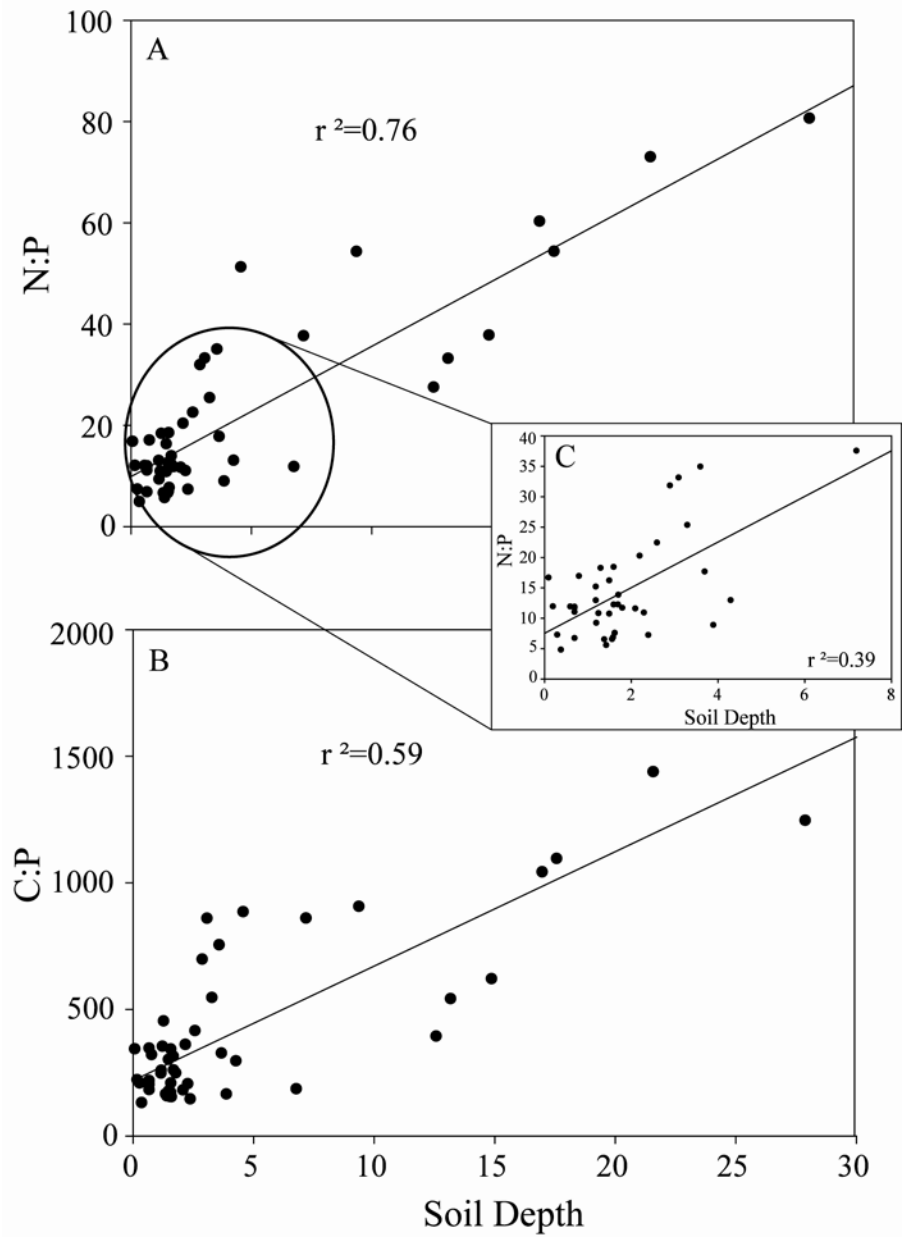

Figure 3-6. Relationship between soil depth and soil N:P and C:P ratios for the native and restored wetlands within the HID. A) Soil N:P ratio; d.f.=59, F=49.2, p<0.0001. B) Soil C:P ratio; d.f=59, F=36.0, p<0.0001. C) Soil N:P ratio without native site; d.f.=49, F=5.7, p=0.0214.

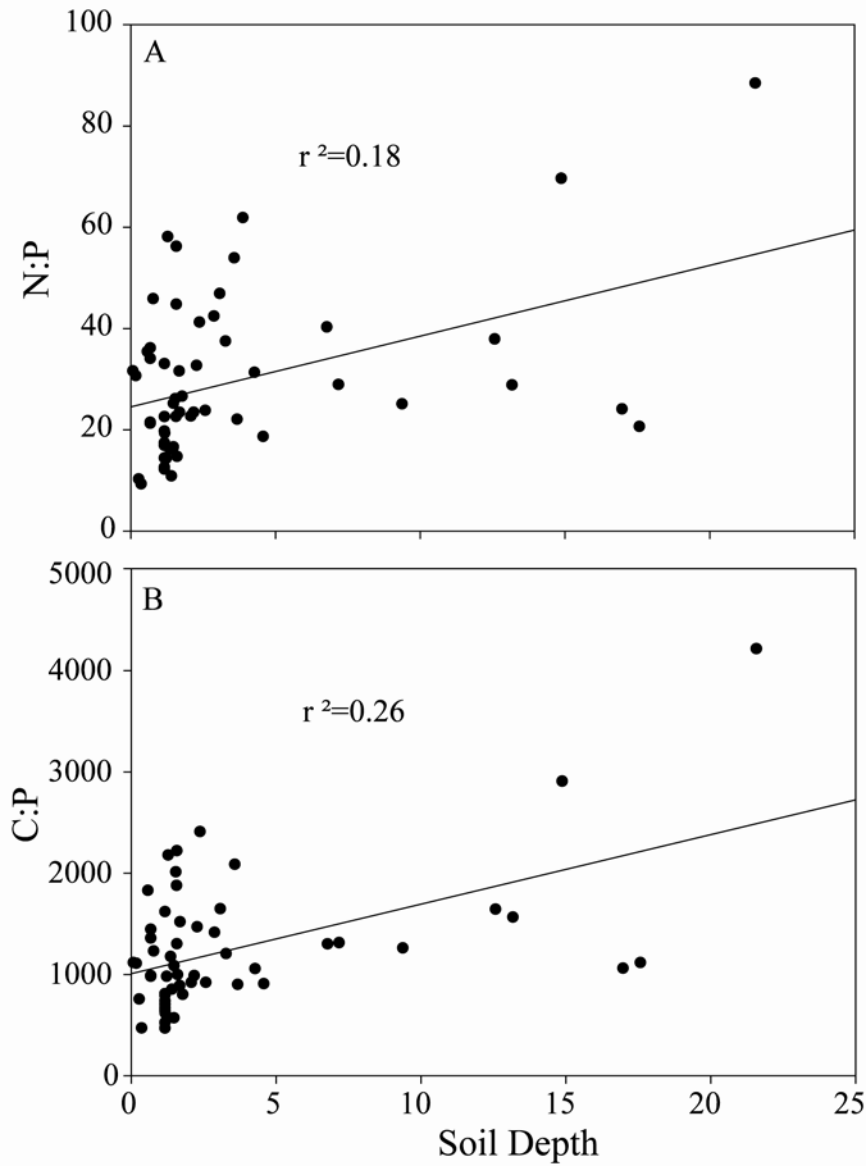

Figure 3-7. Relationship between soil depth and vegetation N:P and C:P ratios for the native and restored wetlands within the HID. A) Vegetation N:P ratio; d.f.=59, F=6.2, p=0.0156. B) Vegetation C:P ratio, d.f.=59, F=9.8, p=0.0027.

CHAPTER 4
NUTRIENT-USE EFFICIENCY AND POTENTIAL NUTRIENT LIMITATIONS IN SUBTROPICAL RESTORED WETLANDS

Introduction

A common indicator used to assess nutrient limitations to vegetation communities is nitrogen to phosphorus (N:P) ratios. A high N:P ratio at the vegetation level suggests a phosphorus (P)-limited ecosystem and a low N:P ratio suggests a nitrogen (N)-limited ecosystem. A review of fertilization studies in wetland vegetation communities found that N:P ratios < 14 resulted in a N-limited system whereas a N:P ratio > 16 would be P-limited (Koerselman and Meuleman 1996). This finding was further supported by a more extensive review of wetland communities, however, indicating that a critical ratio lower than 14 might be more suggestive of a N-limited system (Güsewell and Koerselman 2002). A more resent review of fertilization studies which included a wider range of terrestrial plant communities suggests that N:P ratio < 10 and > 20 are more suggestive of N and P limitations, respectively (Güsewell 2004). The ranges of N:P ratios between either 14 to 16 or 10 to 20 is suggested to indicate a possible co-limitation in N and P.

When evaluating N:P ratios as a tool to assess nutrient limitations there are several factors to keep in mind. These factors include spatial and temporal variations, elevational differences, and potential potassium limitations, all of which can result in variations in tissue N and P concentrations (Shaver and Chapin 1995, Güsewell and Koerselman 2002, Olde Venterink et al. 2002, Olde Venterink et al. 2003). Spatial, temporal and differences in elevation can have localized affects on water levels which can in turn control nutrient availability. Studies have shown that under dry conditions, vegetation N concentrations will increase resulting in higher N:P ratios and under wet conditions N concentrations will decease resulting in a lower N:P ratio (Güsewell et al. 2000, Olde Venterink et al. 2002).

The response of vegetation to different levels of nutrient availability is often evaluated by considering their nutrient-use efficiency (NUE) and nutrient-resorption efficiency (NRE). In annuals, the NUE has been defined as the organic matter produced per unit of nutrient taken up or more simply the inverse of nutrient concentration in plant tissue (Chapin 1980). For perennial plants, however, it has been argued that the NUE cannot be taken simply as the inverse of plant nutrient concentration (Vitousek 1982). The NUE for perennials is thus defined as the amount of organic matter lost from plants or permanently stored within plants per unit of nutrient lost or permanently stored (Vitousek 1982, Birk and Vitousek 1986). In other words, the NUE is the ratio between above-ground biomass production and nutrient loss in litterfall.

Berendse and Aerts (1987), however, have suggested that the aforementioned NUE definitions are inappropriate for assessing the efficiency of N for dry matter production at the species level. They suggest the NUE of individual plants should include the mean residence time of the N in the plant as well as the rate of carbon (C) fixation per unit of N in the plant (N productivity). The mean residence time of N is defined by $1/L_n$, where L_n is the N requirement per unit of N in the plant (g N g^{-1} N yr^{-1}). The N requirement is the amount of N that is needed to maintain each unit of biomass during a given time period (g N g^{-1} dry weight yr^{-1}). The N productivity (A) is defined as dry matter production per unit of N in the plant. They suggest that N productivity is important in terms of NUE because the amount of N in the leaves of plants is one of the primary properties that determine the rate of photosynthesis. By combining the concepts of mean residence time and N productivity, the NUE at the species level is the product of the two, A/L_n (Berendse and Aerts 1987).

The nutrient-resorption efficiency (NRE) is defined as the ratio of the amount of nutrients resorbed from mature leaves to the maximum nutrient pool in the mature leaves expressed as a

percent (Aerts et al. 1999). The NRE of N from senescing leaves is typically around 40-50%, but NRE's as low as 0% and as high as 90% have been reported (Aerts 1996, Aerts et al. 1999, Chapin et al. 2002). It has been suggested that the large variation in NRE is in response to nutrient availability status (Aerts 1996, Killingbeck 1996, Aerts et al. 1999, Richardson et al. 1999), in other words, in nutrient limited systems the NRE of senescing leaves will be greater than in nutrient rich environments. While earlier reviews of the literature indicated that no such trends have been successfully supported (Chapin 1980, Aerts 1996), a recent study on wetland graminoids found that the NRE of both N and P were on average higher in P-limited systems (Güsewell 2005).

At the ecosystem level, NRE could have significant implications in terms of nutrient cycling. To decrease dependence on soil nutrient availability and nutrient uptake, plants will resorb nutrients during senescence so that they are readily available for future plant growth. Past studies have suggested that efficient retranslocation or low losses of nutrients can increase the fitness of plant species in nutrient limited ecosystems (Grime 1977, Berendse 1994, Richardson et al. 1999). In addition, high NRE and NUE of vegetation can limit the regeneration (decomposition) of nutrients in the ecosystem due to low litter nutrient content (poor litter quality) (Bridgham et al. 1995).

The goal of this study was to determine if a nutrient limitation gradient existed in the Hole-in-the-Donut (HID) region of the Everglades National Park (ENP). This region contains a chronosequence of restored wetland communities that involved complete soil removal to bedrock. The HID was heavily farmed in the early to mid 1900's resulting in a P-rich area in the middle of a naturally P-limited ENP. Anthropogenic impacts, such as farming practices, can drastically change the nutrient dynamics of ecosystems. Wetlands created on abandoned

agricultural land are typically P-rich and N-limited. As the system develops, more N can be introduced via fixation and organic matter accretion while the P can become tied up in the substrate shifting the dynamics of the system to a P-limited system. We hypothesized that immediately following soil removal that the restored sites would be N-limited and that with time they would shift to a P-limited system. The objectives of the study were to 1) determine if a N:P ratio gradient existed in the chronosequence of restored wetlands; 2) determine if a vegetation community level N- or P-limitation exists; and 3) determine if a species level N- or P-limitation exists.

Methods

Site Description

This study was conducted in wetland systems restored within the Hole-in-the-donut region of the Everglades National Park. Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season. The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Soil Analysis

In April 2005 (dry season) and July 2005 (wet season), soil samples were collected with a 7.6 cm diameter PVC core from 10 plots randomly distributed throughout sites restored in 1989 (16), 1997 (8), 2001 (4), 2003 (2), and 2004 (1) as well as the surrounding native communities (see Chapter 1 for a more detailed site description). The number in parentheses indicates the

number of years since restoration was completed at the time of this study. Elevation was kept constant at 0.5 m to eliminate hydrology differences as a driving factor of nutrient availability. The soil cores were transported to the laboratory and stored at 4°C until analysis. Before analyses were performed, all rocks, roots, and litter material was removed from the soil. Within 24 to 48 hours of sample collection, each soil sample was extracted for ammonium (NH_4) with K_2SO_4 (Bundy and Meisinger 1994) and set up for incubation for potentially mineralizable nitrogen (PMN) (or biologically available nitrogen) (Keeney 1982, Bundy and Meisinger 1994, White and Reddy 2000). Soil extracts were analyzed via flow injection analysis with a Bran Luebbe Auto Analyzer 3 Digital Colorimeter (EPA Method 350.1). A subsample of each soil was dried at 60°C for 3 days then ground with a ball grinder to a fine powder for total N and P analysis. Dry soil samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

Vegetation Analysis

Nutrients

Nitrogen and P were determined for both the composite biomass (community level) and selected individual plant species (species level) within 10, 1 m^2 plots in each site. To determine nutrients in composite biomass, all the vegetation in a 1 m^2 plot was cut at the soil surface, separated by live and senescent plant tissue, bulked and dried at 70°C until all moisture was removed. Once dry, all vegetation from each plot was passed through a Wiley Mill tissue grinder equipped with a 2-mm mesh screen to achieve homogeneity. A subsample was ball ground to a fine powder for N and P analysis. Individual plant species of *Cladium jamaicense*, *Schoenus nigricans*, *Typha domingensis*, and *Sagittaria lancifolia* were collected from each site

near each plot when available. Each species was not always present at each site. Plant fractions of live, senescence, and litter were collected for each species. The samples were dried at 70°C until moisture was removed and ground with a Wiley Mill tissue grinder equipped with a 2-mm mesh screen. A subsample was ball ground to a fine powder. All plant samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

Nutrient-use efficiency and nutrient-resorption efficiency

The NUE and NRE were determined following methods outlined by Berendse and Aerts (1987), Aerts et al. (1999), and Feller et al. (2002). The live and senescent fraction of each biomass collection and individual species were used in the following calculations. The NRE was calculated as the percentage of N (or P) recovered from senescing leaves before stem fall:

$$NRE = (N_{live\ stems} - N_{senescent\ stems})/(N_{live\ stems}) * 100\ (\%) \quad (4\text{-}1)$$

The NUE of the individual plants was calculated as:

$$NUE = A/L_n, (g\ biomass\ mg^{-1}\ N) \quad (4\text{-}2)$$

where A is the N productivity, dry matter production per unit of N in the plant and is calculated as:

$$A = biomass\ production\ (g\ dry\ wt\ m^{-2}\ yr^{-1})/biomass\ N\ (mg\ N\ m^{-2}\ y^{-1}), (g\ dry\ wt\ mg^{-1}\ N) \quad (4\text{-}3)$$

and L_n is the N requirement per unit of nitrogen in the plant and is calculated as:

$$L_n = N_{live\ plant}\ (mg\ m^{-2}) / N_{senescent\ plant}\ (mg\ m^{-2}), (unitless) \quad (4\text{-}4)$$

The N requirement is the fraction of N that is remaining in the biomass during a given time period. The NUE at the ecosystem level was taken as biomass production per unit of N in senescent leaves (g dry wt biomass mg^{-1} N).

Statistical Analysis

All data collected were analyzed statistically using Fit Model in JMP Version 5.1 (SAS 2005). Analysis of variance (ANOVA) was performed to investigate site and seasonal differences in soil, composite biomass, and individual plant species for nutrients and each nutrient index. Regressions were performed to determine if any strong relationships existed between variables. Multiple comparisons were made using the Least Square Means test and to determine Pearson's correlation coefficients between all variables.

Results

N and P Concentrations

Nitrogen and P concentrations in the soil ranged from 5.8-11 and 0.29-0.93 g kg^{-1}, with the native site having the highest N and lowest P concentrations (Table 4-1). Significant differences were observed for soil N, P, and N:P ratios across sites, but not between seasons (Table 4-2). The native, 1989, and 1997 sites were not significantly different from each other in N concentration but were significantly different from the 2001, 2003, and 2004 site ($p<0.0001$). For P, the native, 2001, and 2004 sites were not significantly different from each other, but were significantly different from the 1989, 1997, and 2003 sites ($p<0.0001$). The soil N:P ratios in the native site were significantly higher than all the restored sites with a $p<0.0001$. Within the restored sites the soil N:P was significantly different between the 2001 and 2003 sites but neither were significantly different for the 1989, 1997, or 2004 sites ($p<0.001$).

While soil N and P concentrations were unaffected by seasonality (wet verses dry season), the composite vegetation N concentrations were affected by dry and wet seasonal changes (Table 4-3). The biomass N concentration resulted in a significant difference between site and season (Table 4-2). With the exception of the 2003 restored site, the N concentration in the vegetation was significantly less in the wet season as compared to the dry season ($p<0.0001$). A similar

trend was observed for the vegetation N:P ratios ($p=0.0473$). Phosphorus concentrations in the vegetation did not result in a significant difference between season ($p=0.6563$), but were significantly different between sites ($p<0.0001$). The native vegetation P was significantly less than all restored sites except for 2001. In addition, during the wet season the native community N:P ratio was significantly higher than the restored communities ($p<0.0001$).

At the species level, the two native species (*C. jamaicense* and *S. nigricans*) had significantly higher N:P ratios than the two restored site species included in this study ($p=0.0003$; Table 4-2 and 4-4). *Sagittaria lancifolia* contained almost twice the concentration of N and two to four times as much P over the other three species with an average between all sites at 18.9 and 1.4 g kg^{-1}, respectively ($p<0.0001$; Table 4-4). None of the species included were significantly different across sites for N, P or N:P (Table 4-2).

Nutrient Limitations

To determine if a nutrient limitation gradient existed between native and restored sites, we evaluated relationships between N and P concentrations of the vegetation and soil, drivers of N:P ratios, nutrient-resorption efficiencies (NRE), and nutrient-use efficiencies (NUE) of the vegetation at both the community level and species level.

Relationships between N and P concentrations for vegetation samples collected are represented in Figure 4-1. We included lines to represent the cut-off for N:P of 14 and 16 to assess where N- and P-limitations may occur. The critical N:P ratios we used are N:P<14 (N-limited) and N:P>16 (P-limited). These critical ratios have been proven valid for several wetland vegetation communities (Koerselman and Meuleman 1996, Güsewell and Koerselman 2002). Any points which fall between the N:P 14 and 16 lines represent possible co-limitations. During the dry season, few points fell between the lines or above the N:P = 14 line indicating little N-limitation exists. The majority of the points fell below the N:P = 16 line, which strongly

suggests a P-limitation in most sites (Figure 4-1a). For the wet season, the data suggests that the 2003 site and portions of the 1997 and 2004 site have an N-limitation or co-limitation (Figure 4-1b). During both seasons, the native vegetation communities have N:P ratios greater than 16 and therefore are P-limited.

To gain more insight on the type of limitations present within the HID, we plotted N and P concentrations against the N:P ratios for both the vegetation biomass and soil (Figure 4-2). The N:P ratios in both the soil and the vegetation biomass are controlled by P alone. Weak relationships exists between N concentration and the N:P ratios, no definite trends were observed (Figures 4-2b and d). Interestingly, the same exponential change in P concentration to N:P ratio exists in both the vegetation biomass and the soil (Figures 4-2a and c). This relationship suggests that there is a critical P concentration that could result in a shift from an N-limitation towards a P-limitation.

In an attempt to determine the point at which a critical P concentration was reach, we performed linear regressions on each half of the curve (Figures 4-2a and c). We statistical determined the mid-way point with step-wise regressions until maximum r^2 values were achieved for each slope. The regression equations were solved to determine at which point the two lines would cross. This is the critical P concentration and N:P ratio. For the vegetation biomass, the critical P concentrations and N:P ratio is 0.44 g kg^{-1} and 18.3, respectively (Figure 4-2a), and the soil is 0.55 g kg^{-1} and 13.7, respectively (Figure 4-2c). The vegetation is then P-limited at N:P ratios great than 18.3. Due to the lack of a relationship between N concentration and N:P ratios, it is hard to say with certainty that at N:P ratios less than 18.3 would result in an N-limitation.

The community level nutrient-resorption efficiency of N (NRE-N) was low for the native sites (appox. 20%) and as high as 60% for the 1989 restored site (Figure 4-3a). Significant

difference were found between sites for NRE-N, but not between dry and wet season ($p=0.0034$ and 0.1874, respectively; Table 4-5). The NRE-N in the vegetation biomass in 1989 site was found to be significantly higher than that of the native vegetation (Figure 4-3a). The native vegetation was not significantly different from any of the other restored sites.

Significant differences were found between sites and wet and dry seasons for the nutrient-resorption efficiency of P (NRE-P) of the vegetation biomass (Table 4-5). There were no significant differences found during the dry season across sites for NRE-P, but during the wet season the 2004 sites had significantly lower NRE-P as compared to the 1989 and 1997 sites, but were not significantly different from the native, 2001 or 2003 sites (Figure 4-3b). In addition the NRE-P was considerably higher than the NRE-N (Figures 4-3a and b).

The community level nutrient-use efficiency of N (NUE-N) was significantly lower in the 2004 site as compared to the vegetation found in the 1989, 1997, and 2003 (Figure 4-3c and Table 4-5). This suggests that the vegetation found in the 2004 site is more efficient with nitrogen because it is less available. The NUE-N of the native site vegetation was not significantly different from any of the vegetation in the restored sites. Significant seasonal differences were observed between the 1989, 2001, and 2003 sites ($p=0.0172$; Table 4-5 and Figure 4-3c). For all three of these sites, the NUE-N was significantly less during the dry season as compared to the wet season.

The nutrient-use efficiency of P (NUE-P) was significantly greater in the native vegetation over all the vegetation found in the restored sites ($p<0.0001$; Table 4-5 and Figure 4-3d). This increased efficiency with P in the native communities could be a result of a greater P-limitation. There was no significant differences found between dry and wet season for NUE-P for any of the vegetation communities ($p=0.7591$; Table 4-5).

We regressed the N:P ratios of the vegetation biomass with the NUE-N and -P to determine if any relationships existed that could further indicate a N- or P-limitation. We found that a positive relationship exists between N:P ratio and NUE-P (r^2=0.48; F=29.6, p<0.0001, d.f.=33; Figure 4-4b) and no relationship exists between N:P ratio and NUE-N (r^2=0.016; F=0.53, p=0.47, d.f.=33; Figure 4-4a). With increasing N:P ratios of the vegetation in the HID, P use becomes more efficient, suggesting that when P is limited the vegetation use it more wisely.

To investigate species level response to potential nutrient limitations, we determined the NRE-N and -P and the NUE-N and -P for the dominant plant species found in each site. The dominant species found in the native communities was *C. jamaicense* and *S. nigricans* and the two dominant restored species found were *T. domingensis*, and *S. lancifolia*. *C. jamaicense* was also found in the 1989 restored site and *S. nigricans* was only present in the native communities.

No significant differences were found between sites or species for NRE-N or -P (Table 4-5). The NRE-N and -P was at the species level (Figure 4-5a) was similar to what was found at the community level (Figure 4-3a). The NRE-N ranges from 17.6±16.2 to 44.1±2.6% and the NRE-P ranged from 66.3±13.2 to 82.6±3.3%.

The NUE-N in *S. lancifolia* is significantly less than what was found for *C. jamaicense*, *S. nigricans*, or *T. domingensis* (p<0.0001; Figure 4-5c and Table 4-5). No significant difference was found for NUE-N between sites at the species level (p=0.2005).

Significant differences were observed for NUE-P at the species level both between sites and individual species. The native community species had significantly higher NUE-P over all the species in each restored site (p=0.0199). Sagittaria had significantly less NUE-P than Cladium or Schoenus but not Typha. The NUE-P of Typha was significantly less than Cladium

and Schoenus that was present in the native communities but not the Cladium found in the 1989 site (Figure 4-5d).

Multivariate analyses were performed to obtain Pearson's correlation coefficients for each vegetation parameter against soil and vegetation properties. We conducted this multivariate test twice, at the community level (Table 4-6) and species level (Table 4-7). We considered any correlation coefficient that was ±0.5 significant. At the community level, the NRE-N was strongly correlated to both plant N and P concentration. NRE-P was not correlated to any of the variables included. NUE-N was positively correlated to available NH_4, but not to PMN. NUE-P was positively correlated to soil and plant N:P and was negatively correlated to plant P concentration. The plant N:P ratio was positively correlated to the soil N:P ratio and negatively correlated to soil and plant P concentration. In addition to being correlated to NRE-N, plant N was also positively correlated to plant P.

At the species level (Table 4-7), NRE-N was negatively correlated to plant N:P ratios. The NUE-N was positively correlated to NRE-P and NUE-P and negatively correlated to plant N and P concentration. The NUE-P was positively correlated to plant N:P ratios and negatively correlated to plant N and P concentrations. In addition, the NUE-P was positively correlated to soil N:P ratios but negatively correlated to soil P concentration. Plant N:P ratios negatively correlated to plant P, soil P, and soil NH_4. However, plant N:P ratios positively correlated to soil N:P ratios. Plant N concentration positively correlated to plant P concentrations. Additionally, plant P positively correlated to soil P and negatively correlated to soil N:P ratios.

Discussion

We investigated the three objective of this study by looking at relationships between soil and plant N and P concentration and N:P ratios as well the NRE and NUE of the vegetation at

the community and species level. We utilized these tools to evaluate the success of restoration in terms of nutrient limitations as a control over vegetation community structure.

Community Level Limitations

Large variations occur in N content of the vegetation, but the variation was small for P content. It has been suggested that these variations are a result of differences in the availability of P and N in the soil (Koerselman and Meuleman 1996). This implies that plants will respond accordingly to increases in nutrient availability. In other words, if the availability of N or P increases, it will result in an increase in plant N or P content. In this study, no relationship was found between soil N availability indices and plant N or N:P ratios. However, we did find that with increases in soil P there was an increase in plant P and a decrease in plant N:P ratios. While the soil N and P concentration are not direct measures of nutrient availability, they provide an indication that the HID is driven by P.

From N:P ratios, we find that a community level P-limitation persists in all sites except the restored 2003 site. Based solely on N:P ratios, the 2003 site appears to be N-limited (Figure 4-1). Past research has shown that N:P ratios of community level vegetation clearly differentiate between N- and P-limitations (Koerselman and Meuleman 1996, Güsewell and Koerselman 2002, Tessier and Raynal 2003, Güsewell 2004). The evidence to support the application of the N:P ratio as an indicator of nutrient limitation comes from a series of fertilization studies. These studies have allowed scientists to develop critical N:P ratios that could indicate shifts from N- to P-limitations.

No relationship was found between the N:P ratios and the N concentrations, therefore we were unable to define a critical N concentration level (Figures 4-2b and d). The lack of this relationship limits us from concluding with certainty that a N-limitation exists. However, since P is clearly limiting and driving the system, we were able to determine a critical N:P ratio. As long

as either N or P is limiting, a critical N:P ratio can be determined (Aerts and Chapin 2000). The community level critical N:P ratio is 18 for the HID (Figure 4-2a). This is slightly higher than what has been shown in the literature, most likely as a result of seasonal variation in hydrology. Güsewell (2004) found that critical N:P ratios as high as 20 were more appropriate when dealing with upland ecosystems. These wetlands are dry for approximately half of the year and as a result the critical N:P ratio could be higher.

It could be argued that due to the nature of the restoration method (complete soil removal) that the strong relationship found between the soil P and plant P (and soil N:P and plant N:P) is because some of the soil P is derived from the vegetation community. However, by taking a closer look at the ratios themselves, the soil and plant N:P ratios are very different with the exception of the 2003 site (Table 4-1 and 4-3). In addition, yearly surveys of the vegetation community structure indicate plant communities have changed with time (O'Hare and Dalrymple 2003), therefore the vegetation contribution to the soil nutrient pools could also change with time.

While significant differences were found between the sites for both community level NRE-N and NUE-N, these differences did not relate to the nutrient limitations suggested by the N:P ratios. In addition, we found that as N availability increased (based on extractable NH_4; Güsewell and Koerselman 2002) so did the NUE-N, supplying further evidence that N is not limiting the community level vegetation. As a result, N is not limiting in the native or restored plant communities and the differences observed in NRE-N and NUE-N are likely driven by individual species traits (i.e., partitioning, productivity, reproduction and translocation), not a nutrient limitation. These results are the opposite of what has been observed in experimental

fertilization studies in wetland systems where the NUE-N of the vegetation was found to increase when N is limiting (Meuleman et al. 2002, Feller et al. 2003).

Examining the NRE-P and NUE-P at the community level provides additional support that P controls the plant community nutrient availability. Not only did the vegetation have high rates of NRE-P, the native communities have a much greater NUE-P over all the restored sites. We found that the native communities had the highest N:P ratios and therefore are the most P-limited. Since the ENP is naturally P-limited, it was not surprising that the vegetation community of the native sites had the highest NUE-P. Furthermore, there was a strong relationship between soil and plant N:P ratios and the NUE-P, concluding that when P is limiting the vegetation community will use P more efficiently (Figure 4-4). This relationship also reveals that the 2003 plant community has the lowest N:P ratios and NUE-P while the native community has the highest N:P and NUE-P. We see no evidence within the other restored communities to suggest that a gradient in nutrient limitation exists based on site age.

Species Level Limitations

It has been found that individual species can differ from community level N:P ratios, therefore, making it difficult to determine species level nutrient limitations (Aerts and Chapin 2000). Koerselman and Meuleman (1996) attempted to address the question of whether or not species level critical N:P ratios were identical to that of community level. They argued that many of the studies they included in their review were near monocultures and therefore interspecific differences in critical N:P ratios were likely to be insignificant. In a later study, Güsewell and Koerselman (2002) found that the N:P ratios of individual plants species varied considerably and that interspecific variation of species could make it difficult to determine species level nutrient limitations.

We found that the N:P ratios of the species included in this study were similar to the community level N:P ratios in the 1997 and 2003 sites, but not in the native and 1989 sites (Tables 4-3 and 4-4). The community present in the 1997 and 2003 site are dominated by *T. domingensis* (~50-60% of the above-ground biomass; see Chapter 2) and therefore the community level N:P ratios are driven by *T. domingensis*. The native site is dominated by *S. nigricans* (~60% of the above-ground biomass) and *C. jamaicense* (~30 of the above-ground biomass), whereas the 1989 site is dominated by *C. jamaicense* (~40-60%; see Chapter 2). At this dominance level, the community level N:P ratios were higher than the species level N:P ratios. Even though all three of these species have similar dominance levels in their perspective sites, their N:P ratios do not necessarily predict community level N:P ratios. Therefore, we conclude that species level critical N:P ratio may be different than community level critical ratios.

The NRE-N and NUE-N indicate differences between species but not across sites (Figure 4-5a and c). This suggests that they are driven by species differences, not site nutrient limitations. At the species level, we found that with increases in both plant N and P concentrations the NUE-N would decrease (Table 4-7), however, this was not observed at the community level. This indicates, that at the species level, the NUE-N is driven by species N and P requirements not nutrient availability. In addition, this suggests that there was not a N-limitation at the species level within the HID ecosystem.

The NUE-P offers a different conclusion about P-limitations at the species level. We observed that the NUE-P varies between species as well as across sites (Table 4-5 and Figure 4-5). *Cladium jamaicense* has a lower NUE-P in the 1989 site than in the native communities. Since the 1989 site is not as P-limited as the native site based on N:P ratios, *C. jamaicense* was

not as efficient with P. A similar relationship was observed between species level plant P and community level plant P with the soil P and soil N:P ratios (Table 4-6 and 4-7). From this, we concluded at both the community level and species level, as soil P becomes more limited, the plants became more efficient with their use of P.

It should be noted, without conducting a N and P fertilization study directly, it is difficult to say with certainty that a N- or P-limitation exists (Vitousek 2004). Over the past decade, several review papers of fertilization studies in terrestrial systems have been conducted to develop critical N:P ratios which allow us to make inferences about nutrient limitations without conducting a fertilization study (Koerselman and Meuleman 1996, Güsewell and Koerselman 2002, Güsewell 2004). These studies focused primarily on community level limitations that did not consider multi-species interactions. Therefore, it is difficult to make conclusions about species level nutrient limitations solely by considering their N:P ratios. The use of additional tools, such as the nutrient-resorption efficiency (NRE) and nutrient-use efficiency (NUE), could be used in conjunction with the N:P ratios of individual species to potentially determine species level nutrient limitations (Vitousek 1982, Vitousek 1984, Berendse and Aerts 1987, Aerts and Decaluwe 1994, Feller et al. 2003, Güsewell 2005).

Conclusions

The vegetation communities which have developed in the restored wetlands of the HID are very different than the surrounding desired native plant communities. Several factors can control re-vegetation patterns after disturbances. In this study we evaluated potential nutrient limitations as a control over vegetation community structure and restoration success.

We found that soil N and P content varied considerably within the restored sites but the N:P ratios were less variable. The N:P ratios of the native plant community were two to three times greater than the ratios found in the restored communities. Additionally, soil P and soil N:P

ratios exhibited controls on plant N:P at both community and species levels. No such conclusion can be made in terms of soil N.

At the vegetation community level, the native plant community has N:P ratios and NUE-P that are two to four times greater than that of restored plant communities. The species level N:P ratios and NUE-P of the native communities were also two to four times greater than the species found in the restored communities. This suggests at the community and species levels, native sites are more P-limited than the restored sites.

Our study shows that a P-limitation is prevalent in the native communities as well as most of the restored communities. Little evidence was found to support a N-limitation in any of the sites. The N:P ratios of the site restored in 2003 imply that it may be N-limited not P-limited, however, no other data collected at the community or species level indicates a N-limitation is prevalent. At the community level, we found no differences in the NUE-N between sites, additionally; the differences observed in species level NUE-N could be due to plant traits not a N-limitation.

To conclude, due to differences in the level of P-limitations in the restored sites verses the native communities, the increased levels of soil P could influence the re-vegetation patterns of the restored wetlands. At these higher levels of P, the desired vegetation composition (*Cladium jamaicense* and *Schoenus nigricans* co-dominance) is not achieved. A greater understanding of the lasting impacts of the residual P in the restored wetlands is necessary to determine if the desired native vegetation species will inhabit these wetlands with time.

Table 4-1. Summary of the average nitrogen, phosphorus, and N:P ratios of the soil for each of the sites during the dry and wet season. (n=10)

		N (g kg^{-1})		P (g kg^{-1})		N:P	
Site	Season	Ave	SD	Ave	SD	Ave	SD
Native	Dry	11.0	1.6	0.29	0.15	45	19.1
	Wet	11.0	2.2	0.33	0.31	48	21.0
1989	Dry	10.5	1.6	0.81	0.40	15	5.0
	Wet	9.7	1.2	0.66	0.32	18	8.1
1997	Dry	9.4	1.3	0.93	0.35	12	6.2
	Wet	10.1	1.3	0.74	0.30	20	23.5
2001	Dry	7.0	1.2	0.46	0.22	18	9.5
	Wet	6.5	1.4	0.37	0.19	25	20.4
2003	Dry	5.8	1.0	0.90	0.19	7	2.0
	Wet	6.2	1.4	0.87	0.24	8	2.7
2004	Dry	7.6	5.0	0.44	0.11	18	10.1
	Wet	7.5	2.5	0.45	0.17	19	8.3

Table 4-2. Summary of results from a two-way ANOVA test with dependant variables of nitrogen (N) concentration, phosphorus (P) concentration, and N:P ratios for the soil, community level vegetation (composite biomass), and species level

	N ($g\ kg^{-1}$)			P ($g\ kg^{-1}$)			N:P		
Source of Variation	d.f.	F stat	prob. > F	d.f.	F stat	prob. > F	d.f.	F stat	prob. > F
Soil									
Site	5	21.7	<0.0001	5	16.1	<0.0001	5	18.9	<0.0001
Season	1	0.01	0.9284	1	1.9	0.1706	1	2.1	0.1467
Site*Season	5	0.4	0.8595	5	0.6	0.7168	5	0.3	0.9414
Vegetation Community									
Site	5	10.5	<0.0001	5	7.5	<0.0001	5	22.2	<0.0001
Season	1	63.8	<0.0001	1	0.2	0.6563	1	4.0	0.0473
Site*Season	5	4.3	0.0010	5	3.0	0.0134	5	6.5	<0.0001
Species									
Site	3	1.1	0.3741	3	0.7	0.5642	3	0.4	0.7870
Species	4	52.6	<0.0001	4	16.5	<0.0001	4	9.6	0.0003

Table 4-3. Summary of the average nitrogen, phosphorus, and N:P ratios of the vegetation community (composite biomass) for each of the sites during the dry and wet season. (n=10 for dry season, n=20 for wet season)

		N (g kg^{-1})		P (g kg^{-1})		N:P	
Site	Season	Ave	SD	Ave	SD	Ave	SD
Native	Dry	9.4	1.1	0.31	0.12	38	23.2
	Wet	5.9	1.0	0.14	0.08	54	20.4
1989	Dry	10.6	1.2	0.34	0.13	36	15.7
	Wet	8.2	3.3	0.42	0.31	23	8.6
1997	Dry	11.0	2.2	0.35	0.16	35	10.5
	Wet	6.9	2.4	0.41	0.23	19	6.1
2001	Dry	11.7	1.3	0.35	0.14	37	11.8
	Wet	7.6	2.0	0.31	0.17	29	10.6
2003	Dry	6.2	0.9	0.43	0.21	17	6.1
	Wet	6.3	2.0	0.58	0.25	12	5.6
2004	Dry	9.6	1.8	0.58	0.22	18	8.0
	Wet	7.8	1.9	0.42	0.14	20	6.1

Table 4-4. Comparison of nutrient characteristics at the species level for nitrogen (N) concentration, phosphorus (P) concentration, and N:P ratio. Biomass numbers are an average for all plots at each site. (n=3)

			N (g kg^{-1})		P (g kg^{-1})		N:P		Biomass	% Total
Species	Site	Type	Ave	SD	Ave	SD	Ave	SD	(g m^{-2})	Biomass
C. jamaicense	Native	Live	8.4	0.98	0.22	0.03	39	0.3	78.2	24.0
		Std dead	5.8	0.79	0.05	0.02	129	25.7		
		Litter	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.		
	1989	Live	9.6	1.07	0.26	0.07	39	12.4	69.4	28.9
		Std dead	8.0	1.99	0.08	0.01	96	12.3		
		Litter	8.5	1.78	0.13	0.01	69	19.7		
S. nigricans	Native	Live	8.0	1.15	0.23	0.02	35	5.1	153.1	47.1
		Std dead	5.7	0.60	0.05	0.00	113	1.5		
		Litter	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.		
S. lancifolia	1989	Live	19.1	0.32	1.30	0.60	16	7.3	0.9	0.4
		Std dead	16.7	8.58	0.84	1.01	48	46.5		
		Litter	13.7	-	0.34	-	40	-		
	1997	Live	18.5	1.85	1.33	0.13	14	0.1	3.0	1.1
		Std dead	15.1	5.64	0.47	0.26	34	7.3		
		Litter	11.6	-	0.12	-	94	-		
	2003	Live	19.9	-	1.53	-	13	-	1.1	0.5
		Std dead	14.7	-	0.39	-	38	-		
		Litter	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.		
T. domingensis	1989	Live	10.7	1.38	0.65	0.06	16	0.8	93.8	39.0
		Std dead	6.6	1.18	0.17	0.09	43	16.2		
		Litter	7.3	1.15	0.14	0.10	71	36.6		
	1997	Live	9.7	2.12	0.68	0.23	15	1.7	199.8	72.5
		Std dead	6.0	0.40	0.08	0.01	78	8.4		
		Litter	7.1	0.58	0.10	0.03	78	21.2		
	2003	Live	10.3	0.29	0.79	0.02	13	0.5	156.3	74.7
		Std dead	6.8	0.47	0.16	0.03	44	6.3		
		Litter	7.2	0.44	0.18	0.04	42	8.3		

Table 4-5. Summary of results from a two-way ANOVA test with dependant variables of nutrient-resorption efficiency of nitrogen (NRE-N) and phosphorus (NRE-P), and nutrient-use efficiencies of nitrogen (NUE-N) and phosphorus (NUE-P) for the community level vegetation (composite biomass), and species level.

	NRE-N			NRE-P			NUE-N			NUE-P		
Source of Variation	d.f.	F stat	prob. > F	d.f.	F stat	prob. > F	d.f.	F stat	prob. > F	d.f.	F stat	prob. > F
Vegetation Community												
Site	5	5.0	0.0034	5	3.3	0.0229	5	3.3	0.0228	5	11.3	<0.0001
Season	1	1.9	0.1874	1	9.4	0.0056	1	6.6	0.0172	1	0.1	0.7591
Site*Season	5	0.6	0.7015	5	1	0.4393	5	1.4	0.2708	5	0.2	0.9726
Species												
Site	3	1.2	0.3542	3	1.1	0.3938	3	1.7	0.2005	3	4.3	0.0199
Species	4	2.8	0.0598	4	1.3	0.3025	4	13.0	<0.0001	4	3.2	0.0379

Table 4-6. Pearson's correlation coefficients for the seven plant community level (composite biomass) characteristics addressed in this study. Table abbreviations are nutrient-resorption efficiency of nitrogen (NRE-N) and phosphorus (NRE-P), and nutrient-use efficiencies of nitrogen (NUE-N) and phosphorus (NUE-P), NH^4 is an extractable ammonium, potential mineralizable nitrogen (PMN).

	NRE-N	NRE-P	NUE-N	NUE-P	Plant N:P	Plant N	Plant P
Soil Variables							
NH_4	0.1604	0.0305	0.6074	0.0066	-0.0468	-0.1210	-0.0080
PMN	0.4727	0.4779	0.0598	-0.0212	-0.0776	0.3437	0.2796
Soil N	0.2508	0.3849	0.1371	0.3519	0.3201	0.1623	-0.0560
Soil P	0.4850	0.2928	0.1598	-0.4159	-0.5839	0.2965	0.6798
Soil N:P	-0.3620	-0.0451	0.0111	0.5758	0.7282	-0.2998	-0.6675
Plant Variables							
NRE-N		0.4810	0.2799	-0.1825	-0.2631	0.7832	0.6290
NRE-P			0.3716	0.4671	-0.0267	0.1888	0.1947
NUE-N				0.2135	-0.1273	-0.3205	-0.0518
NUE-P					0.6930	-0.3019	-0.6307
Plant N:P						-0.1613	-0.7637
Plant N							0.6146
Plant P							

Table 4-7. Pearson's correlation coefficients for the seven species level characteristics addressed in this study. Table abbreviations are nutrient-resorption efficiency of nitrogen (NRE-N) and phosphorus (NRE-P), and nutrient-use efficiencies of nitrogen (NUE-N) and phosphorus (NUE-P), NH^4 is an extractable ammonium, potential mineralizable nitrogen (PMN).

	NRE-N	NRE-P	NUE-N	NUE-P	Plant N:P	Plant N	Plant P
Soil Variables							
NH_4	0.1826	0.0310	-0.1915	-0.4383	-0.5637	0.2733	0.4540
PMN	-0.2020	-0.1249	-0.3172	-0.2919	-0.0354	0.2116	0.0591
Soil N	0.0523	0.0130	0.2372	0.2950	0.1390	-0.2380	-0.2833
Soil P	0.4312	0.0245	-0.0966	-0.5488	-0.7357	0.3002	0.5682
Soil N:P	-0.2312	0.0874	0.3365	0.7680	0.7166	-0.4282	-0.6095
Plant Variables							
NRE-N		0.1850	0.1696	-0.1481	-0.5885	0.3555	0.4733
NRE-P			0.6975	0.4313	-0.0403	-0.4052	-0.3749
NUE-N				0.7005	0.1761	-0.7975	-0.6513
NUE-P					0.6694	-0.6782	-0.7861
Plant N:P						-0.4411	-0.7668
Plant N							0.8667
Plant P							

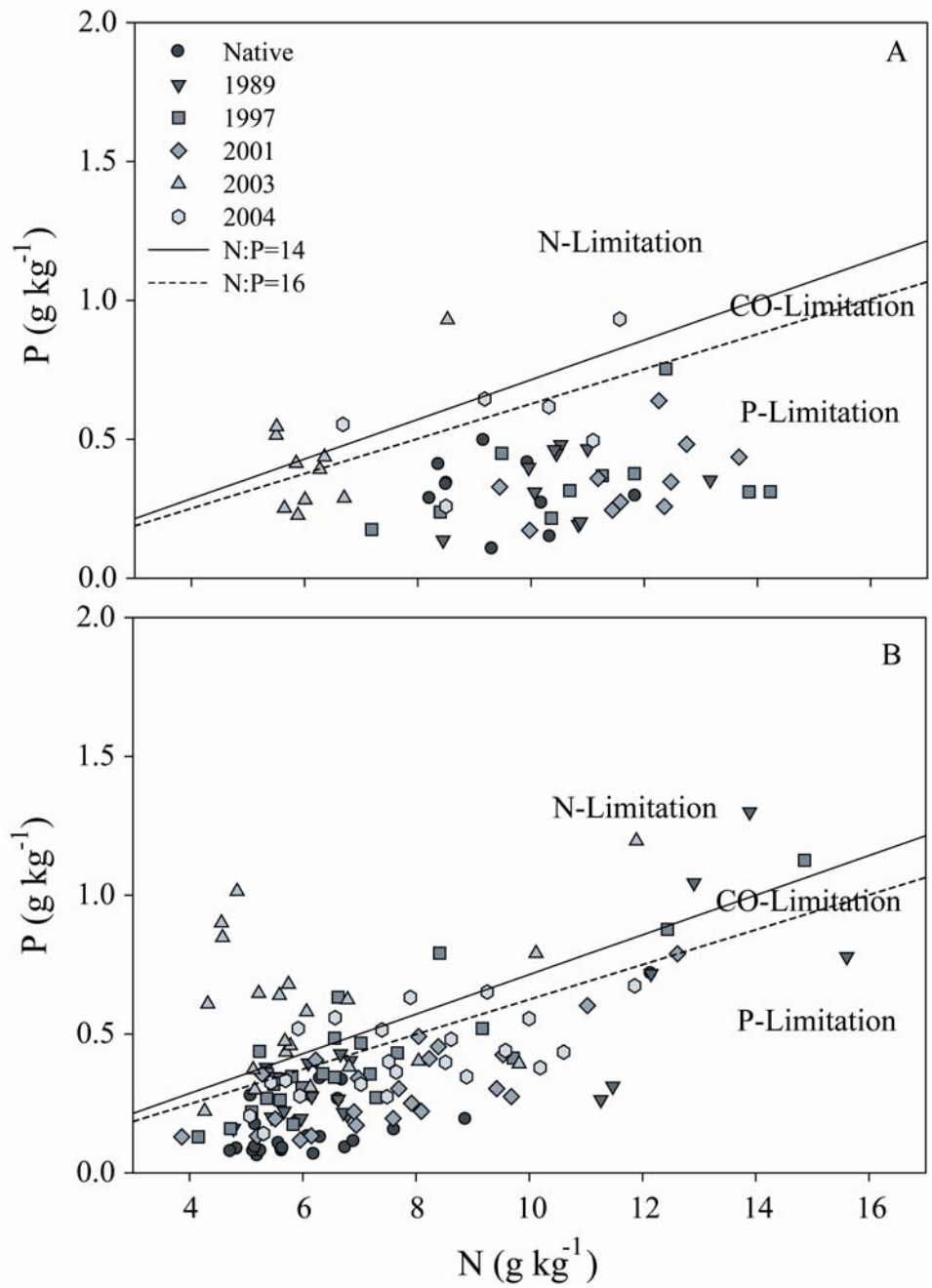

Figure 4-1. Relationship between community level vegetation N and P concentration for each site in the HID. A) Dry season. B) Wet season. The solid line on each graph depicts N:P ratio of 14 and the dashed line depicts N:P ratio of 16 (mass basis).

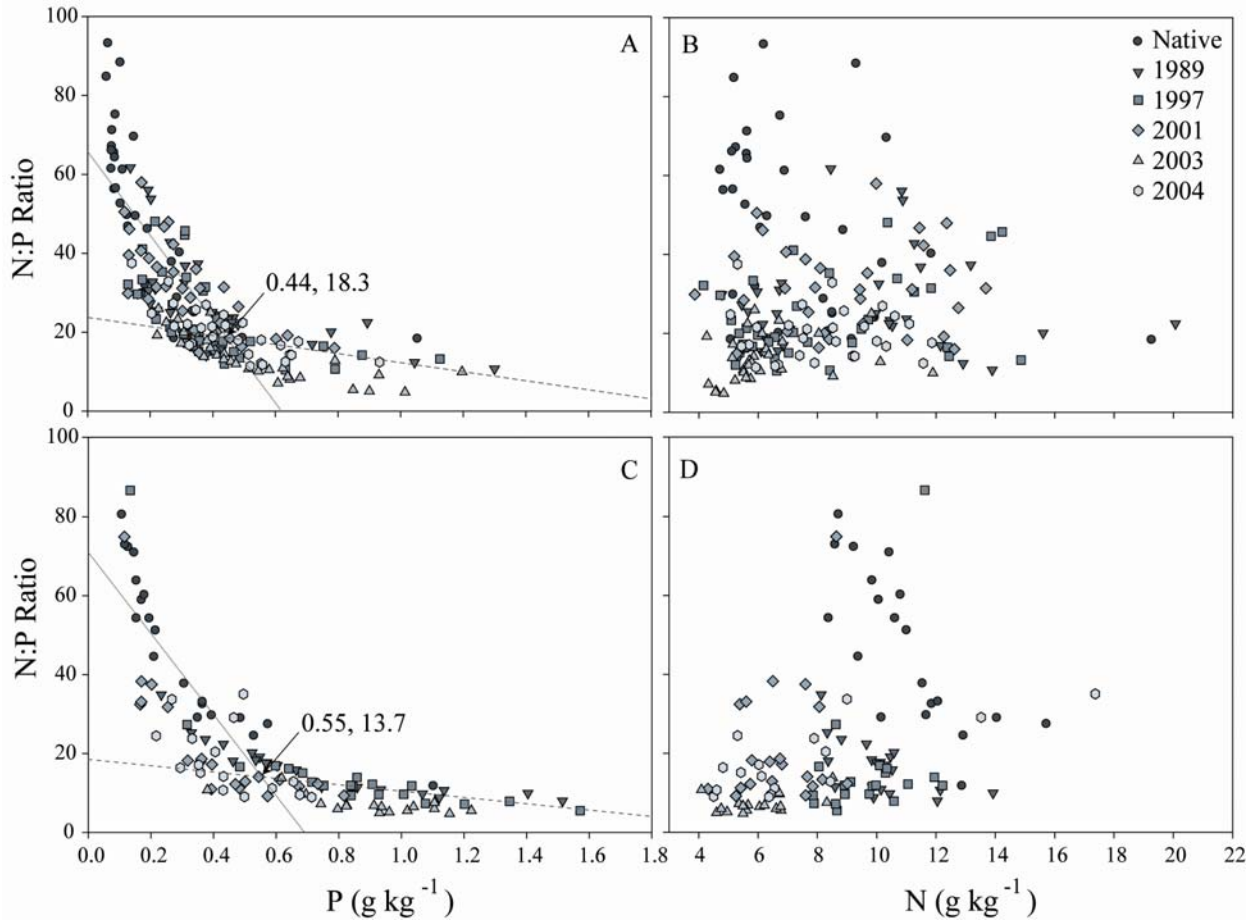

Figure 4-2. Relationships between nitrogen (N) and phosphorus (P) concentration and N:P rations. A) Vegetation P. B) Vegetation N. C) Soil P. D) Soil N. Data points are coded by sites. The lines on A and C depict linear regressions for the two slopes found by splitting the data at the midway point. The point in which the two lines cross as labeled as the critical P concentration and N:P ratio.

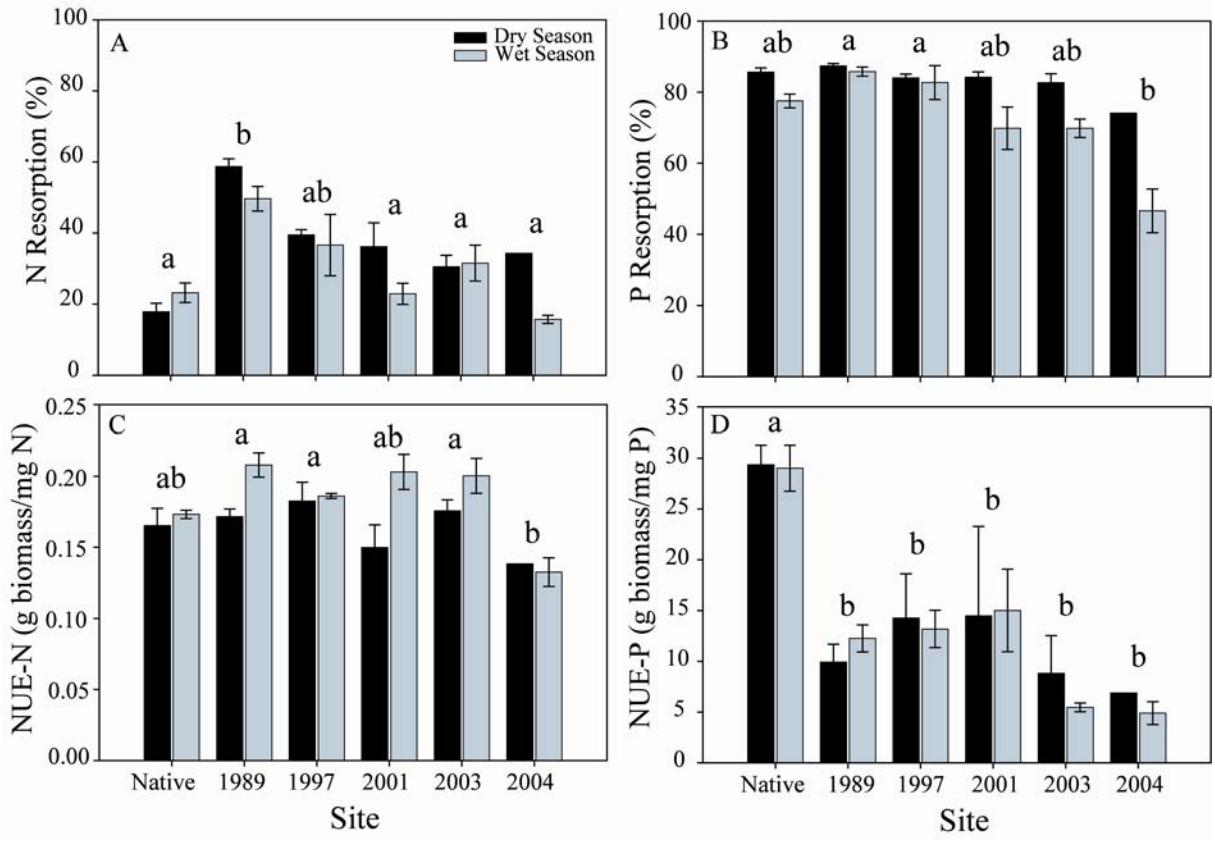

Figure 4-3. Nutrient-use efficiency and nutrient-resorption efficiency of nitrogen and phosphorus of the vegetation community (composite biomass). A) Nitrogen-resorption efficiency (NRE-N). B) phosphorus-resorption efficiency (NRE-P). C) Nitrogen-use efficiency (NUE-N). D) Phosphorus-use efficiency (NUE-P). Bars within each site with the same lowercase letters that are not significantly different at $p<0.05$. (n=6)

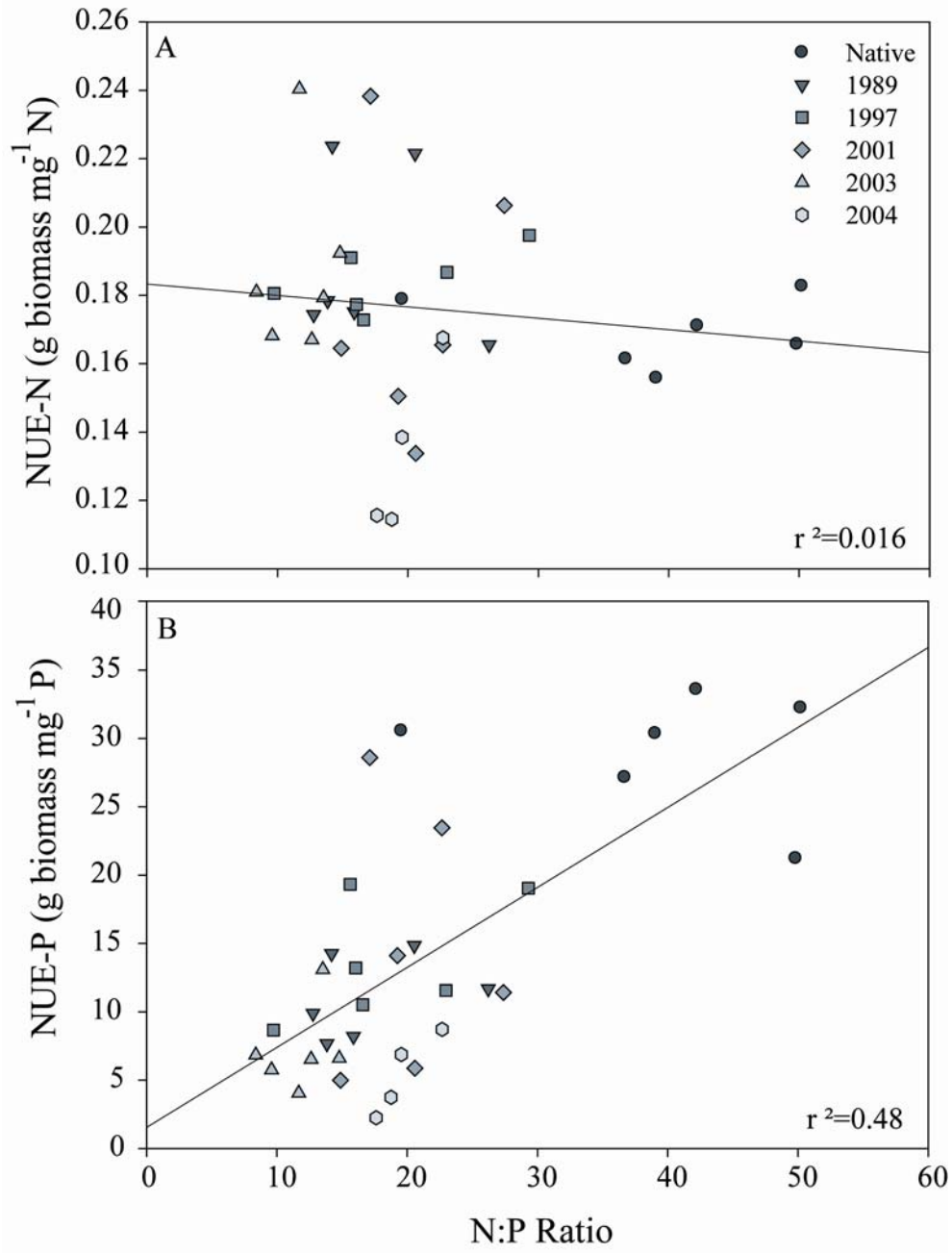

Figure 4-4. Linear regression between N:P ratio of the vegetation community (composite biomass). A) nutrient-use efficiency of nitrogen (NUE-N); F=0.53, p=0.47, d.f.=33. B) Nutrient-use efficiency of phosphorus (NUE-P); F=29.6, p<0.0001, d.f.=33.

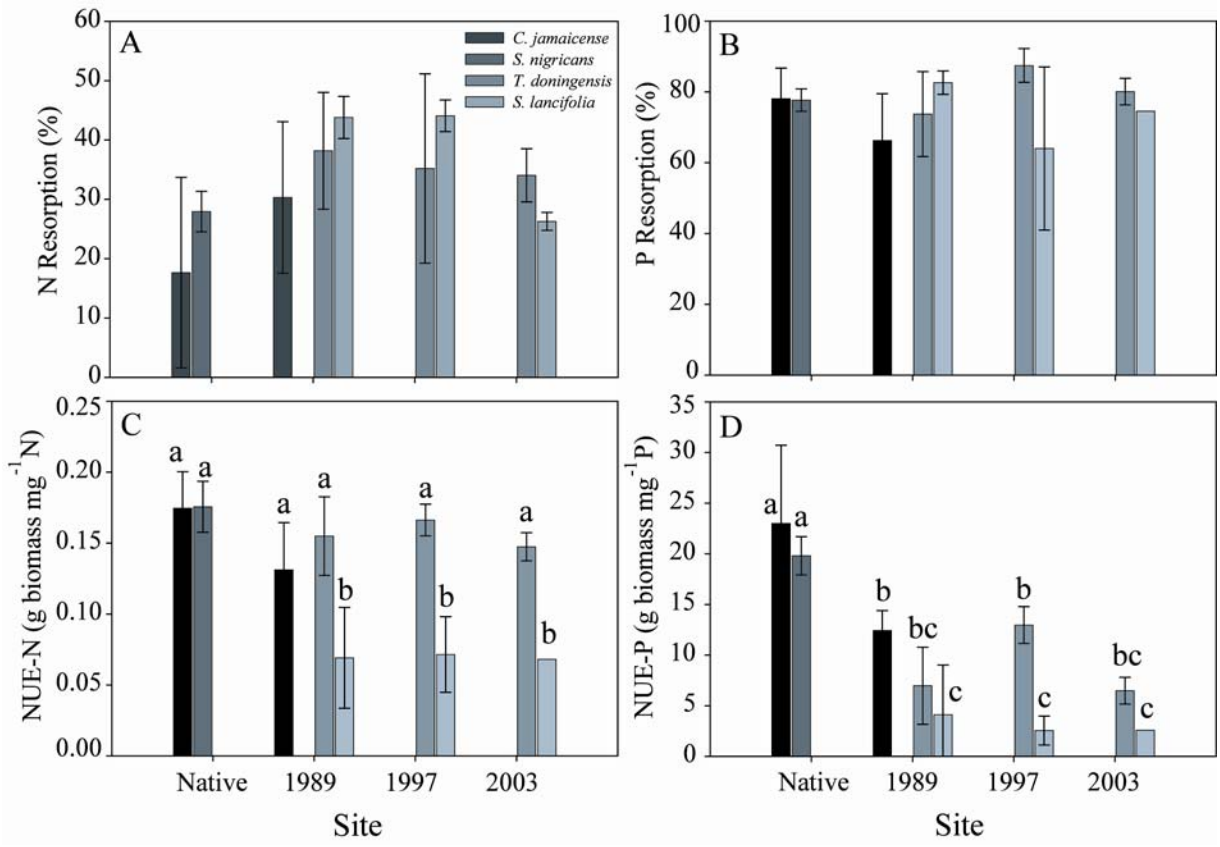

Figure 4-5. Nutrient-use efficiency and nutrient-resorption efficiency of nitrogen and phosphorus for the individual species included in this study. A) Nitrogen-resorption efficiency (NRE-N). B) Phosphorus-resorption efficiency (NRE-P). C) Nitrogen-use efficiency (NUE-N). D) Phosphorus-use efficiency (NUE-P). Bars for each species with the same lowercase letters that are not significantly different at $p<0.05$. (n=3)

CHAPTER 5
CONTROLS ON REGENERATION OF NUTRIENTS FROM THE DOMINANT VEGETATION IN NATIVE AND RESTORED WELTANDS

Introduction

Decomposition of plant litter material is a key process for nutrient cycling within ecosystems. Wetland ecosystems are often described as detritus-based systems where decomposition of macrophyte litter is considered an important source of energy (Mann 1988). Emergent macrophytes often constitute a major portion of the primary production and in turn contribute largely to the organic matter accumulation in wetlands (Wetzel 1990). Decay of senescent plant material begins in the standing dead plants before falling to the ground (Kuehn and Suberkropp 1998). Plant matter decomposition of fallen dead material can be divided into three distinct phases: an initial rapid loss due to leaching, microbial colonization and degradation, and physical and biological fragmentation (Valiela et al. 1985, Megonigal et al. 1996).

There are several factors that control the rates of decomposition. Mean annual temperature, precipitation, soil moisture, microbial activity (i.e., enzymes), chemical composition of the soil and litter material (i.e., available nitrogen (N), phosphorus (P), carbon (C), and C:N:P ratios), and litter cellular quality (i.e., lignin, tannin, amino acids, carbohydrates,) are some of the most important factors that can control decomposition rates (Aerts and deCaluwe 1997, Gartner and Cardon 2004).

Extra-cellular enzymes have long been recognized as key contributors that control rates of decomposition (Schimel and Weintraub 2003). These enzymes are the primary means by which complex organic compounds are degraded by microbial organisms. Enzyme production is both energy and N intensive (Koch 1985). When nutrients are limiting, microbes will produce extra-cellular enzymes to mobilize nutrients from detrital material. In contrast, high nutrient

availability often negatively correlates with enzyme activities that release nutrients (Cróst 1991, Sinsabaugh and Moorhead 1994). Plus, the quality of litter material can also be an important determinant in both microbial production of enzymes and rates of decay (Corstanje et al. 2006).

The microbial communities responsible for decomposition will both mineralize and immobilize nutrients from the litter material. Furthermore, decomposition rates have been found to increase with the additions of N and P (Jordan et al. 1989). Rates of decomposition may be limited if either N or P concentrations are a limiting resource within the ecosystem (Aerts and deCaluwe 1997, Feller et al. 2003). By increasing a limiting nutrient in the soil, the litter quality can be altered and in turn increase decomposition rates and nutrient turnover. Nutrient limitations in plant communities have been assessed by calculating N:P ratios of the plant material (Koerselman and Meuleman 1996, Tessier and Raynal 2003, Güsewell 2004). Additionally, N:P ratios can be utilized to determine which nutrient (N or P) may limit decomposition (Güsewell and Freeman 2005, Güsewell and Verhoeven 2006).

In addition to N and P ratios being good predictors of potential decay, the lignin concentrations, lignin:N and lignin:P ratios of plant litter material can also be useful indices to predict decay rates (Meentemeyer 1978, Melillo et al. 1982, Aerts and deCaluwe 1997). A large portion of the C stored in emergent macrophytes is found in recalcitrant material (i.e, lignin) that is not easily degraded (Mann 1988). In response, the mass loss of litter during the decomposition process is often correlated with secondary compounds like cellulose and lignin or lignin:N and :P ratios (Vitousek et al. 1994, Aerts and deCaluwe 1997, Rejmankova and Houdkova 2006).

Isolated, closed wetlands systems (i.e., depressional wetlands) primarily receive water and nutrients from precipitation (Bedford et al. 1999, Battle and Golladay 2007). Frequently these wetland systems are nutrient-limited and tend to have minimal abiotic influences (i.e., waves,

currents) that limited physical fragmentation of litter material. It comes to reason that if these wetland systems are nutrient-limited, the vegetation community in these wetlands would also be nutrient limited. As a result, the litter quality of the vegetation would be poor due to influences of the nutrient-limited environment. Additionally, these nutrient-limited environments depend on the decomposition of litter material for availability of nutrients (Aerts and deCaluwe 1997).

In this study, we aimed to compare the decomposability of litter material of *Cladium jamaicense* and *Typha domingensis* across a chronosequence of restored wetland communities in the Everglades National Park (ENP). *Typha domingensis* is the dominant plant species found in restored wetlands and *C. jamaicense* is the dominant native plant species. The primary objectives were to: 1) determine initial litter quality differences between these two species, 2) determine if site conditions had controls on rates of decomposition (restored verses native wetlands), 3) evaluate potential litter quality effects on decomposition rates between species and sites, 4) determine the regeneration of vital nutrients (N and P) from the litter material and 5) assess potential microbial limitations on decomposition between species and sites.

We tested the these objectives with the investigation of the following hypothesis: H1) Due to the natural oligotrophic (P-limited) conditions of the ENP, *C. jamaicense* would have poorer litter quality than *T. domingensis* and, therefore, would have slower rates of decomposition than *T. domingensis*, H2) The restored wetland communities would have greater rates of decay due to elevated nutrient concentrations relative to the native conditions, and H3) the microbial activity associated with the litter material will be greater with *C. jamaicense* and the native site due to greater nutrient limitations.

Methods

Site Description

This study was conducted in wetland systems restored within the Hole-in-the-Donut region of the Everglades National Park. Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season (Figure 5-1). The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Decomposition Experiment

We used an in situ litterbag decomposition experiment to estimate mass loss and nutrient regeneration potentials of *C. jamaicense* and *T. domingensis*. Sites included in the study were the surrounding native marl prairie grassland communities of the ENP and the sites restored in 1989, 1997, and 2003.

In December 2005, we collected live above-ground, live roots, senescent, and litter material for *C. jamaicense* and *T. domingensis* to determine partitioning of N and P in each plant component. For the litter bags, recently senescent material that was still attached to the plant and standing was collected for *C. jamaicense* and *T. domingensis*. *C. jamaicense* material was only collected from the native communities were it dominated. *T. domingensis* material was collected throughout the 1997 and 2003 restored communities where it dominated. The litter material was dried at 30°C for 48 hours and cut into 6 cm segments for placement in bags. Each bag was 10 x

10 cm in size and made with 1-mm mesh fiberglass screen. Each bag contained 5 g dry weight of either *C. jamaicense* or *T. domingensis*.

In each of the fours sites, three 4 m^2 plots were established for a total of 12 plots. The litter bags were placed on top of the soil substrate within each 4 m^2 plots to be collected in triplicate at times 6, 12, 24, and 52 weeks equaling 72 litter bags per sample period. Upon sampling the bags were stored at 4°C until analysis. In the laboratory, any soil and root material was separated from the remaining litter material. The wet weight plus mesh bag was recorded before any analysis was performed. Within 24 hours of collection, 1.0 g wet weight was separated for extra-cellular enzyme activities (EEA) and 1.0 g wet weight was separated for microbial biomass N and C analysis (MBN and MBC). The remaining litter was then reweighed, washed, frozen and freeze dried to remove all moisture.

After drying, the litter material collected from each bag as well as the initial litter collected was weighed to determine remaining litter mass dry weight. Once dry, the litter was passed through a Wiley Mill tissue grinder equipped with a 1-mm mesh screen to achieve homogeneity. A subsample was ball ground to a fine powder for total C, N and P analysis. All litter samples were analyzed for total C and N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

Litter Fractionation

Initial litter samples of each species as well as all litterbags collected at time 168 and 365 days were analyzed for litter quality. A sequential extraction method was used to determine soluble cellular content (sugars, carbohydrates, lipids, etc.), hemi-cellulose, α-cellulose, and

lignin (Rowland and Roberts 1994). The coarsely ground litter material (Wiley Mill, 1-mm mesh screen) was weighed at 1.0 g each and sealed into filter bags that were extracted with neutral detergents to remove soluble cellular contents followed by an acid detergent extraction for removal of hemi-cellulose using a Ankom A200 Fiber Analyzer. To remove α-cellulose a 72% H_2SO_4 regent was used leaving residual litter containing lignin and ash. The residual litter was combusted at 550°C for 4 hours to determine ash content. Each plant fraction was reported as mg g^{-1} litter.

Assessment of Litter Quality

We classified *C. jamaicense* and *T. domingensis* as high or low quality based on percent lignin, lignin:N, lignin:P ratios, C:N and C:P ratios and decomposition rate constants. Rates of N mineralization or immobilization are highly influenced by lignin content and C:N ratios (Figure 5-2) (Brady and Weil 1999). Litter with high lignin content and high C:N ratios are considered poor in quality and would have slow rates of decomposition. Lignin contents of 20-25% and C:N ratios greater than 30 would be considered high (poor quality) (Brady and Weil 1999). Differing amounts of lignin in combination with varying C:N ratios can effect rates of N regeneration from litter material. Figure 4-2 illustrates the combined effects of lignin and C:N content and whether or not we can predict net immobilization or net mineralization of N.

To determine a decomposition constant, k, for *C. jamaicense* and *T. domingensis* we assumed an exponential rate of mass loss for both species. The following equation was utilized to calculate k;

$$M_f = M_i e^{-kt} \quad (5\text{-}1)$$

where M_i is the initial mass of the litter, M_f is the final mass of the litter and t is the time at M_f. The decomposition constant, k, was calculated for both litter materials at each site. The mean

residence time, or time required for the litter to decompose under steady state, was calculated as 1/k (Chapin et al. 2002).

Microbial Biomass and Extra-cellular Enzyme Activities

Microbial biomass C and N (MBC and MBN) were extracted from each litter sample during all sample times and microbial biomass P (MBP) was extracted at 365 days only with a chloroform fumigation-extraction incubation method (Brookes et al. 1985). Both the chloroform fumigated and non-fumigated samples were extracted with 0.5M K_2SO_4 solution for MBC and N and a $NaHCO_3$ solution for MBP. To determine MBC, each sample was analyzed for TOC with a Schimadzu TOC-5050A Total Organic Carbon Analyzer equipped with an ASI-5000A auto-sampler. To determine MBN, each sample extract was digested for TKN via kjeldahl digestion (Brookes et al. 1985) and MBP extracts were digested via H_2SO_4 via block digestion. Both digests were analyzed for N and P content with a Seal AQ2+ Automated Discrete Analyzer (TKN: EPA Method 111-A rev1, and TP: EPA 119-Arev3). The MBC, MBN, and MBP were computed as the difference between the fumigated and non-fumigated samples. No efficiency correction factors were used in the calculation of this data.

Enzyme activities were performed via a common fluorometric method (Hoppe 1993). Fluorescent artificial substrates methyl-umbelliferone (MUF)-β-D-glucoside, MUF-phosphate, and L-leucine-7-amino-4-methylcoumarin were used to determine the EEA of β-1,4-glucosidase (βGA; EC 3.2.1.21), alkaline phosphatase (APA; EC 3.1.3.1), and L-leucine-aminopeptidase (L-LAA; EC 3.4.11.1), respectively.

Sample suspensions were made by homogenizing 1.0 g of litter material in 50 ml of ultra pure water with a tissue shredder. Each sample suspension was dispensed in triplicate in white, 96-well microplates by pipetting 100 µl of sample and 100 µl of the appropriate substrate in each

well. Each plate was allowed to incubate in the dark at room temperature for 2 hours. This time was determined by conducting an enzyme kinetic test on each litter type with each substrate. After 2 hours, the plates were read with a Bio-Tek FL600 fluorometric plate reader with excitation wavelengths of 360/40 and emission wavelengths of 460/40. Enzymes activities were expressed as μg MUF g^{-1} litter hr^{-1} for βGA and APA and μg AMC g^{-1} litter hr^{-1} for L-LAA. Each enzyme activity was normalized for MBC.

Statistical Analysis

All data were analyzed statistically using Fit Model in JMP Version 5.1 (SAS 2005). Analysis of variance (ANOVA) was performed to investigate site and species differences related to decomposition rates, litter quality and microbial activity. Regressions were performed to determine relationships between variables.

Results

Decomposition Rates

Cladium jamaicense and *T. domingensis* had similar rates of decomposability (Table 5-1). Differences in decomposition constants were seen between sites, but were minimal between species. Both species had the highest k in the 1997 site and the lowest in the 1989 site, 1.5±0.05 and 0.76±0.21 yr^{-1}, respectively. The residence time of each litter was also similar between species but varied across sites. The 1997 site had the lowest and the 1989 site had the highest residence time at 0.65 and 1.31 years, respectively.

The seasonal changes in hydrology had an impact on the rate of mass loss across all sites (Figure 5-3). The sites were dry for the first two sampling periods of this study. We observed little change in the mass of either species during this time period. Within two weeks following day 84 sample time, the wet season began and all sites were inundated with water.

Litter Quality

We assessed initial litter quality based on percent lignin, C:N and C:P ratios (Table 5-1). We found no significant differences between *C. jamaicense* and *T. domingensis* for %Lignin and C:N, but there was significantly higher C:P ratio for *T. domingensis* compare to *C. jamaicense* ($p<0.0001$). Both *C. jamaicense* and *T. domingensis* had low lignin content at 6.7 and 4.1% and high C:N ratios of 75.5 and 84.6, respectively. Based on these results, the litter for *C. jamaicense* and *T. domingensis* has a mid level quality rating and indicates that N would be immobilized initially to decrease the C:N ratio of the litter. Once the C:N ratio has decreased to a ratio more suitable for microbial decomposition, net mineralization will occur.

The initial C:N, C:P, and N:P ratios varied considerable for both *C. jamaicense* and *T. domingensis* for each plant component analyzed (Figure 5-4). No significant differences were found for C:N ratios between *C. jamaicense* or *T. domingensis* for any of the plant components analyzed. *Cladium jamaicense* had significantly higher C:P and N:P ratios in all plant parts compared to *T. domingensis* except for senescent material ($p<0.0001$).

There was no significant difference observed in the initial litter fractionation of soluble cellular content, hemi-cellulose, α-cellulose and lignin between *C. jamaicense* and *T. domingensis* (Figure 5-4a). After 168 and 365 days, a significant difference was found in the litter fractionation between species and sites (Figure 5-5b and 5-5c). Additionally, we found soluble cellular content for *T. domingensis* was on average higher than the content found in *C. jamaicense* litter ($p<0.0001$ for both times) (Table 5-2). However, the lignin content of *T. domingensis* was found to be significantly less than *C. jamaicense* ($p<0.0001$ for both times) (Table 5-2). No significant differences were found for hemi-cellulose or α-cellulose content between species after 168 or 365 days of decomposition. For comparisons of each species

between sites, a significant difference was found between sites for the hemi-cellulose fraction of *C. jamaicense* at 168 days (p=0.0166), but no significant differences were found for any fractions of *C. jamaicense* at 365 days. For *T. domingensis,* a significant difference was observed for all fractions of litter quality between sites at both 168 and 365 days of decomposition (Table 5-2).

In addition to differences between species and sites, significant differences between the two time periods were investigated. For *C. jamaicense,* a significant change in all litter fractions between the two time periods was observed (Table 5-2). There was a significant increase in both soluble cellular content and lignin and significantly less hemi-cellulose and α-cellulose at 365 days for *C. jamaicense* (Figure 5-5b and 5-5d). For *T. domingensis,* a significant change in all litter fractions except hemi-cellulose between the two time periods was found (Table 5-2). There was a significant increase in both soluble cellular content and lignin and significantly less α-cellulose at 365 days for *T. domingensis* (Figure 5-5c and 5-5e).

Relationships between lignin, N, and P as an indicator of litter quality were evaluated. In addition to the lignin content, the Lignin:N ratio can be a good predictor of the recalcitrance of the litter material. Both *C. jamaicense* and *T. domingensis* followed the same trends with respect to Lignin:N ratios. An inverse relationship between the N content and the Lignin:N ratio was observed (r^2=0.37) (Figure 5-6a) and a positive relationship between lignin content and the Lignin:N ratio was found (r^2=0.80) (Figure 5-6b). We also calculated Lignin:P ratios and investigated relationships between this ratio and lignin and P content for each species. With respect to Lignin:P ratios, *C. jamaicense* and *T. domingensis* behaved differently. No relationship was observed between the Lignin:P ratio and the lignin content for each species (Figure 5-7b). For *C. jamaicense* there was an inverse relationship between P content and the

Lignin:P ratio (r^2=0.62) (Figure 5-7a). An inverse relationship was observed for *T. domingensis* as well, but the relationship was weaker (r^2=0.39) (Figure 5-7a).

The C:N, C:P and N:P ratios were significantly less for *T. domingensis* than *C. jamaicense* in most sites after 365 days (Figure 5-8 and Table 5-3). The N:P ratio was the only ratio that resulted in a significant difference between sites as well as between species (Table 5-3). The C:N ratios of *C. jamaicense* after 365 days was similar to the initial C:N ratio determined, whereas the C:N ratio of *T. domingensis* was half the initial ratio (Figures 5-4a, 5-8a and 5-8b). The final C:P ratio of *C. jamaicense* was three times less than the initial and the C:P ratio of *T. domingensis* was half the initial ratio (Figures 5-4b, 5-8c and 5-8d). The final N:P ratio for *C. jamaicense* was as much as five times less than the initial ratio (Figures 5-4c and 5-8e). The final N:P ratio of *T. domingensis* was the same as the initial in the native site and half the initial ratio in the 2003 site (Figures 5-4c and 5-8f).

Cladium jamaicense mineralized both N and P for all times sampled during this decomposition study (Figure 5-9a and 5-9b). *Typha domingensis* immobilized N for the first 168 days of decomposition, but by 365 days N was mineralized from this litter material (Figure 5-9a). Phosphorus was immobilized in *T. domingensis* litter throughout the year long study (Figure 5-9b).

To investigate potential controls on nutrient mineralization or immobilization, regressions between the change in N or P with their respective Lignin:N or :P ratio were performed. A positive relationship was observed between the change in N and the Lignin:N ratio (p=0.71; Figure 5-10). This relationship was the same for both *C. jamaicense* and *T. domingensis*; however, *T. domingensis* had a lower range of data than *C. jamaicense*. The relationship between change in P and Lignin:P ratio was different for both species with *T. domingensis*

having a lower range than *C. jamaicense* (Figure 5-11). Both species had a weak positive relationship, *T. domingensis* with an r^2=0.35 and *C. jamaicense* with an r^2=0.42.

Microbial Activity

The microbial biomass (MBC, N, and P) associated with the litter material varied considerably between species and sites during the decomposition study (Table 5-4). In addition, there was a considerable increase in MBC and N with time. For example, the MBC associated with *C. jamaicense* was 2815 mg kg^{-1} at 42 days and 18,739 mg kg^{-1} at 365 days in the native community (Table 5-4). This trend was found for *C. jamaicense* in all sites for both MBC and MBN. *Typha domingensis* litter material had a similar trend for MBC and MBN in the native site (Table 5-4); however, in the restored sites, the MBC and MBN associated with *T. domingensis* litter increased considerably by 168 days with an additional increase by 365 days. The MBP associated with *C. jamaicense* was similar for the native and 1989 sites (54 and 56 mg kg^{-1}, respectively), whereas, it was almost double in the 1997 and 2003 sites (91 and 111 mg kg^{-1}, respectively) (Table 5-4).

Significant differences were observed for MBC and MBN between species during all time periods sampled (Table 5-5). No significant differences were observed between sites or site*species interactions for MBC and MBN except during the wet season (time period 168 days) (Table 5-5). However, no significant differences were observed at 168 days for MBC:N ratios between species, sites or site*species interactions. The MBP was significantly different between sites and species but had no site*species interaction effects (Table 5-5).

For the enzyme βGA no significant differences were found between sites for *C. jamaicense* or *T. domingensis* for the first two sample periods of 48 and 84 days; however, there was a significant difference between sites for the two species after 168 and 365 days of decomposition

(Table 5-6 and Figure 5-12). Significant differences in βGA were observed between both species at each sample period (Table 5-6, Figure 5-12a and b). Site and species interactions were not observed for βGA.

We found significant difference between sites for L-LAA at both 84 and 168 days, but not at 48 and 365 days (Table 5-6). Significantly less L-LAA was found in association with *T. domingensis* litter material as compared to *C. jamaicense* litter at 42 and 84 days, but no significance was observed between species at 168 and 365 days (Table 5-6 and Figure 5-12c and d). Site and species interactions were only found to be significant at day 84 ($p=0.0005$) (Table 5-6). Enzyme APA was only determined at 365 days of decomposition. We found no significant difference between sites or species ($p=0.3021$ and 0.9232, respectively) (Table 5-6 and Figure 5-13). Additionally no site and species interactions were found ($p=0.4548$) (Table 5-6).

Discussion

The decomposition rates of *C. jamaicense* and *T. domingensis* did not differ from each other even though there were significant differences in litter quality indices of each species. This suggests that decomposition of these two species was not controlled by differences in species litter quality indices investigated. While differences were not found between species, we observed variation in decay coefficients between sites (Table 5-1) suggesting that there may be site characteristics (i.e., nutrient availability, moisture, and microbial community) that alter rates of decay equally between litter types. Both species had rapids rates of decay in the 1997 and 2003 sites, however, slower rates were observed in the native communities and the 1989 site. In a study comparing *C. jamaicense* and *T. latifolia*, they found that these two litter types had significantly different decay coefficients even under the same soil characteristics (Corstanje et al. 2006). One notable difference between this study and ours was the initial N:P ratios of each

litter type. Corstanje et al. (2006) found initial litter N:P ratios to be 12 and 26 for *T. latifolia* and *C. jamaicense*, respectively. These ratios are considerably lower than the ratios of 90 and 290 for *C. jamaicense* and *T. domingensis*, respectively, found in the litter material of our study suggesting that the P-limitation in the litter from our study had significant controls on nutrient regeneration but not decay coefficients.

Based on similarities in initial litter quality of soluble cellular content, hemi-cellulose, α-cellulose, lignin (Figure 5-5a) and initial C:N ratios (Figure 5-4a), we expected to see similar patterns in N regeneration between *C. jamaicense* and *T. domingensis*. However, the decomposition patterns of N were different in these two species. The microbial community associated with *C. jamaicense* mineralized N steadily during the decomposition period suggesting that N did not limit the decay of *C. jamaicense* (Figure 5-9a). However, *T. domingensis* litter immobilized N during the first 168 days of the decomposition study and mineralization of organic N did not occur until day 365 (Figure 5-9a). Furthermore, the amount of N mineralized from *T. domingensis* at day 365 was approximately 3.5 times less than what was mineralized from *C. jamaicense*.

The significant differences observed between the initial C:P and N:P ratios suggested that *T. domingensis* would immobilized P at much faster rates than *C. jamaicense* (Figure 5-4b and 5-4c). The initial C:P ratio for *C. jamaicense* was approximately 5 times less than the C:P ratio of *T. domingensis* and the initial N:P ratio was 3 times less (Figure 5-1). Surprisingly, P was found to be mineralized from *C. jamaicense* at every time period during the decomposition study, whereas P was found to be immobilized by *T. domingensis* through out this study (Figure 5-9b). No differences were found for either species in terms of N or P regeneration between sites.

These findings on N and P indicate that secondary compound content of *C. jamaicense* and *T. domingensis* have some controls over N or P regeneration regardless of nutrient content. An increase in N or P content is usually attributed to immobilization by the microbes associated with the litter material because the material that is being decomposed is limited in either N or P content (Güsewell and Freeman 2005). Secondary compounds such as lignin have been shown to limit decomposition rates (Webster and Benfield 1986) and in turn could limit nutrient regeneration. Lignin is more recalcitrant that other plant compounds and is often found to increase over time (Webster and Benfield 1986).

The differences found in Lignin:N and :P ratios of *C. jamaicense* and *T. domingensis* did not affect the decomposition rate of each litter type. While differences were found in nutrient regeneration of both N and P for each litter type, an opposite relationship between N and P regeneration and the Lignin:N and :P ratios were observed. *Cladium jamaicense* had the highest Lignin:N and :P ratios (more recalcitrant material) which suggests that N and P would more likely be immobilized compared to *T. domingensis*. Conversely, we found the opposite to be true. We expected that the Lignin:N ratios would be negatively related to the change in N, but a positive relationship was observed (Figure 5-10). As a result, more N was mineralized (regenerated) from *C. jamaicense* than *T. domingensis* (Figure 5-9a). The lack of organic N mineralization from litter material that appeared to be N-limited due to litter quality indices has been observed in other studies (Harris et al. 1995, Scheffer and Aerts 2000, Corstanje et al. 2006, Güsewell and Verhoeven 2006). This implies that litter quality indices are a poor predictor of N mineralization.

With increases in P content the litter quality of each species increases (Figure 4-7a). Again, *C. jamaicense* and *T. domingensis* formed two distinct groups; however, each species

relationship followed difference curves. In addition, no relationships were observed between the Lignin:P ratios and the lignin content (Figure 5-7b). These results give further support that a P-limitation in the litter could have significant controls on nutrient regeneration. *Typha domingensis* had significantly lower Lignin:P ratios as compared to *C. jamaicense*. As was the case with the change in N, we anticipated that the Lignin:P ratio would be negatively correlated with the change in P, although the opposite was observed. We had hypothesized that *T. domingensis* would mineralize more P than *C. jamaicense* due to higher initial quality, but the opposite was found (Figure 5-9b). Not only was the initial nutrient quality of *C. jamaicense* greater, P was consistently mineralized from this litter material throughout the study.

It has been shown the P rarely limits decomposition in field experiments even with extremely limiting N:P ratios (Aerts et al. 2001, Aerts et al. 2003, Güsewell and Verhoeven 2006). Additionally, it has been shown that P does not limit decay or result in immobilization until P concentrations of 0.3 mg g^{-1} have been reach (Xu and Hirata 2005, Güsewell and Verhoeven 2006). In this study, the P concentrations of both these litter types were well below this limit. The initial P content of *C. jamaicense* and *T. domingensis* was 0.10 and 0.02 mg g^{-1}, respectively. While the apparent P-limitations of the litter material of *C. jamaicense* and *T. domingensis* did not limit rates of decay, they did affect the ability of both N and P to be mineralized from *T. domingensis*.

Final nutrient ratios of *C. jamaicense* and *T. domingensis* do not offer any conclusions on the similarity in decay rates for these two litter types. Considerable variability was found between each litter type and between sites. In general, *C. jamaicense* had higher final C:N and C:P ratios as compared to *T. domingensis* (Figure 5-8). *Cladium jamaicense* had much higher final N:P ratios in the native site but had similar ratios to *T. domingensis* in all other sites. This

difference in final nutrient ratios implies that these two litter types would have different decay coefficients not only across sites but between species. We had hypothesized that due to the natural oligotrophic (P-limited) conditions of the ENP, *C. jamaicense* would have poorer litter quality than *T. domingensis* and, therefore, would have slower rates of decomposition than *T. domingensis.* This hypothesis was not confirmed by the initial nutrient or cellular content analysis. However, the final litter material of *C. jamaicense* is indeed of poorer nutrient quality. In a comparable study, litter decomposition was found to be limited by P only at N:P ratios greater than 22, but when litter had lower nutrient ratios, decomposition could be either N or P limited (Güsewell and Freeman 2005). In addition, they found that establishing critical N:P ratios could be difficult due to threshold differences among species and that species-specific critical N:P ratios could be highly influenced by growth conditions. This suggests that the site differences in which *C. jamaicense* and *T. domingensis* are grown in could have considerable controls on rates of decomposition rather than nutrient ratios alone. Furthermore, since both initial litter types are extremely P-limited, the effects of this limitation could have equal weight on the rates of decomposition.

Enzyme production is often the limiting step in litter decomposition in aquatic systems (Cróst 1991). In this study, the initial nutrient analysis of both litter types suggested that *T. domingensis* would result in N mineralization as compared to *C. jamaicense*, however, the opposite was found. By investigating the β-1,4-glucosidase (βGA) and L-leucine-aminopeptidase (L-LLA) activities, we found significantly greater activities of βGA associated with the litter of *C. jamaicense* as compared to the litter of *T. domingensis* during the first six months of decomposition and a greater association of L-LLA through the first 84 days. After six months the L-LLA production with each litter type was the same. While differences in L-LLA

production was not found between species at six months, the litter material placed in the native community has significantly higher L-LLA production compared to the other sites. External environmental factors have a greater impact on L-LLA production as compared to internal litter quality. Other studies have also demonstrated external environmental controlled on L-LLA production (Burns and Ryder 2001, Rejmankova and Sirova 2007).

Unfortunately, the enzyme activity of alkaline phosphatase (APA) was not included until the final sampling at 365 days; therefore, we can only speculate that this activity would also have had a greater association with *C. jamaicense* over *T. domingensis*. With greater enzyme activity more N and P would be released from the litter material. The microbial communities associated with *C. jamaicense* are activity acquiring more nutrients from this litter material and as a result more N and P is mineralized.

Conclusions

Even though *C. jamaicense* and *T. domingensis* had different initial nutrient contents and differences in litter quality throughout the decomposition study, their decay rates and coefficients were the same. Regardless of the path each litter type followed, the end result in terms of organic matter input into each system was equivalent.

With regards to nutrient regeneration, *C. jamaicense* indicated a much greater potential to release N and P for further utilization as compared to *T. domingensis* regardless of site location and litter quality indices. This indicates that in the restored communities, where *T. domingensis* is dominating, fewer nutrients would be regenerated and available for future plant or microbial uptake. This has severe implications in terms of native plant community establishment in restored wetlands. It is clear that *T. domingensis* has an impact on both N and P cycling which could prohibit native plant communities from colonizing these areas. Competition studies for

uptake of N and P is needed to determine how *C. jamaicense* and *T. domingensis* interact for nutrients to determine what effect *T. domingensis* litter decay may have.

Under native conditions, both the microbial communities and *C. jamaicense* are thriving under nutrient limited conditions. In response, the microbes produce extra-cellular enzymes to acquire needed nutrients from litter associated with *C. jamaicense*. A greater understanding of this plant-microbe interaction is needed to gain more insight to why the microbial communities across all sites are putting more energy into nutrient acquisition from a litter source of inferior quality.

Table 5-1. Summary of initial litter quality of *Cladium jamaicense* and *Typha domingensis* for microbial decomposition. The decomposition constant, k, was determined from mass loss curves from field decomposition study. The residence time of the litter material at each site was determined as $^{1}/_{k}$.

Species	Lignin %	C:N	C:P	Site	k (yr^{-1})	$^{1}/_{k}$ (yr)
C. jamaicense	6.7	75.5 (11.3)	3874 (581)	Native	1.05 (0.05)	0.96
				1989	0.91 (0.07)	1.10
				1997	1.54 (0.08)	0.65
				2003	1.38 (0.17)	0.72
T. domingensis	4.1	84.6 (12.7)	18729 (2809)	Native	1.04 (0.01)	0.96
				1989	0.76 (0.21)	1.31
				1997	1.53 (0.10)	0.65
				2003	1.22 (0.03)	0.82

Table 5-2. Summary of results from a two-way ANOVA test with variables as species, site, and time for the litter fractionation of *Cladium jamaicense* and *Typha domingensis*.

		168 days			365 days		
	Source of Variation	d.f.	F-stat	prob. > F	d.f.	F-stat	prob. > F
Soluble Cellular Content							
	Species	1	29.2	<0.0001	1	19.1	<0.0001
	Site						
	C. jamaicense	3	2.9	0.0680	3	0.8	0.5240
	T. domingensis	3	23.1	<0.0001	3	3.4	0.0483
	Time*						
	C. jamaicense	1	43.9	<0.0001			
	T. domingensis	1	10.1	0.0032			
Hemi-cellulose							
	Species	1	1.6	0.2147	1	2.7	0.1091
	Site						
	C. jamaicense	3	4.8	0.0166	3	2.1	0.1433
	T. domingensis	3	21.4	<0.0001	3	4.8	0.0172
	Time*						
	C. jamaicense	1	24.2	<0.0001			
	T. domingensis	1	4.0	0.0532			
α-Cellulose							
	Species	1	0.1	0.7087	1	0.7	0.4094
	Site						
	C. jamaicense	3	2.7	0.0853	3	0.3	0.8287
	T. domingensis	3	11.9	0.0004	3	4.1	0.0281
	Time*						
	C. jamaicense	1	64.4	<0.0001			
	T. domingensis	1	29.5	<0.0001			
Lignin							
	Species	1	51.8	<0.0001	1	23.9	<0.0001
	Site						
	C. jamaicense	3	0.3	0.8428	3	0.9	0.4253
	T. domingensis	3	6.6	0.0054	3	4.1	0.0272
	Time*						
	C. jamaicense	1	108.9	<0.0001			
	T. domingensis	1	108.7	<0.0001			

*Time is a comparison between analysis of litter at sample periods 168 and 365 days

Table 5-3. Summary of results from a two-way ANOVA test with dependant variables of C:N, C:P, and N:P ratios for the litter material of *Cladium jamaicense* and *Typha domingensis*.

	C:N ratio			C:P Ratio			N:P Ratio		
Source of Variation	d.f.	F-stat	prob. > F	d.f.	F-stat	prob. > F	d.f.	F-stat	prob. > F
Site	3	2.3	0.0906	3	1.9	0.1432	3	4.2	0.0137
Species	1	74.8	<0.0001	1	21.6	<0.0001	1	15.0	0.0006
Site*Species	3	1.1	0.3390	3	0.9	0.4658	3	1.3	0.3029

Table 5-4. Summary of microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) as well as the ratios of MBC:N, MBC:P, and MBN:P.

Species	Days	Site	MBC (mg kg^{-1})	MBN (mg kg^{-1})	MBP (mg kg^{-1})	MBC:N	MBN:P	MBC:P
C. jamaicense	42	Native	2815 (91)	370 (4)		8 (0.3)		
	84	Native	3713 (4)	207 (20)		19 (1.9)		
	168	Native	3631 (84)	217 (11)		19 (0.6)		
	365	Native	18739 (1347)	708 (41)	54.1 (2.2)	26 (0.9)	13 (0.4)	346
	42	1989	1786 (218)	238 (21)		7 (0.4)		
	84	1989	3834 (162)	215 (18)		18 (0.8)		
	168	1989	6559 (926)	433 (79)		16 (1.6)		
	365	1989	19092 (715)	850 (27)	55.5 (1.6)	22 (0.3)	15 (0.2)	344
	42	1997	3107 (278)	308 (32)		11 (1.3)		
	84	1997	3635 (208)	281 (20)		13 (0.2)		
	168	1997	9730 (194)	982 (27)		10 (0.4)		
	365	1997	26677 (1471)	1018 (114)	90.8 (5.6)	28 (2.0)	11 (1.3)	294
	42	2003	2235 (78)	240 (15)		12 (0.9)		
	84	2003	3709 (106)	267 (15)		14 (0.4)		
	168	2003	8636 (119)	681 (32)		13 (0.7)		
	365	2003	26744 (2704)	1123 (131)	111.3 (4.5)	24 (0.6)	10 (0.8)	240
T. domingensis	42	Native	5391 (132)	438 (16)		13 (0.3)		
	84	Native	7917 (499)	433 (5)		18 (1.2)		
	168	Native	5590 (952)	295 (65)		20 (1.2)		
	365	Native	26418 (238)	1041 (56)	24.5 (2.1)	26 (1.5)	43 (2.1)	1076
	42	1989	5155 (196)	327 (19)		16 (1.5)		
	84	1989	7455 (129)	347 (8)		22 (0.3)		
	168	1989	23486 (1327)	2050 (212)		12 (1.0)		
	365	1989	29607 (4334)	1325 (155)	74.4 (4.7)	22 (1.3)	18 (1.4)	398
	42	1997	5597 (167)	359 (36)		17 (1.8)		
	84	1997	7509 (444)	366 (5)		20 (1.0)		
	168	1997	19894 (530)	1322 (43)		15 (0.2)		
	365	1997	33068 (1642)	1065 (66)	147.8 (0.4)	31 (1.3)	7 (0.4)	224
	42	2003	5793 (253)	429 (55)		15 (2.5)		
	84	2003	6518 (240)	346 (14)		19 (1.4)		
	168	2003	19124 (1166)	1278 (106)		16 (1.7)		
	365	2003	32171 (612)	1484 (40)	150.6 (6.9)	22 (0.4)	11 (0.4)	214

Table 5-5. Summary of results from a two-way ANOVA test with dependant variables of microbial biomass (C, N, and P), and microbial biomass ratios (C:N, N:P, and C:P) for the litter material of *Cladium jamaicense* and *Typha domingensis*.

	42 days			84 days			168 days			365 days	
Source of Variation	df	F-stat	prob> F	df	F-stat	prob> F	df	F-stat	prob> F	df	F-stat	prob> F
MBC												
Site	3	1.2	0.3349	3	0.6	0.6437	5	21.5	<0.0001	3	2.2	0.1048
Species	1	90.8	<0.0001	1	88.6	<0.0001	1	71.5	<0.0001	1	9.7	0.0041
Site*Species	3	0.8	0.5076	3	0.5	0.6561	5	7.2	0.0010	3	0.3	0.8564
MBN												
Site	3	1.9	0.1399	3	1.6	0.2150	3	19	<0.0001	3	2.5	0.0840
Species	1	5	0.0331	1	9.5	0.0054	1	35.2	<0.0001	1	6.9	0.0134
Site*Species	3	0.4	0.7228	3	0.3	0.7898	3	8.4	0.0004	3	0.6	0.6250
MBP												
Site										3	6.9	0.0013
Species										1	4.5	0.0430
Site*Species										3	0.5	0.6889
MBC:N												
Site	3	3.1	0.0441	3	4.4	0.0144	5	1.1	0.3624	3	6.6	0.0017
Species	1	2.3	0.1443	1	12.7	0.0018	1	0.01	0.9054	1	0.03	0.8630
Site*Species	3	0.9	0.4783	3	1.7	0.2011	5	0.4	0.7867	3	0.8	0.5137
MBN:P												
Site										3	5.7	0.0036
Species										1	3.6	0.0665
Site*Species										3	3.7	0.0236
MBC:P												
Site										3	4.8	0.0081
Species										1	2.7	0.1108
Site*Species										3	2.8	0.0580

Table 5-6. Summary of results from a two-way ANOVA test with dependant variables of βGA, L-LAA, and APA for the litter material of *Cladium jamaicense* and *Typha domingensis*.

	42 days			84 days			168 days			365 days		
Source of Variation	df	F-stat	prob> F	df	F-stat	prob> F	df	F-stat	prob> F	df	F-stat	prob> F
βGA												
Site	3	1.9	0.1458	3	2.5	0.0834	5	25.5	<0.0001	3	2.8	0.0033
Species	1	14.9	0.0006	1	39.0	<0.0001	1	2.3	0.0290	1	6.3	0.0184
Site*Species	3	2.4	0.0868	3	1.9	0.1626	5	0.6	0.6191	3	1.2	0.3174
L-LAA												
Site	3	1.2	0.3204	3	3.4	0.0361	3	9.4	0.0002	3	1.0	0.4174
Species	1	28.5	<0.0001	1	40.3	<0.0001	1	4.5	0.4350	1	1.7	0.2045
Site*Species	3	1.2	0.3413	3	8.8	0.0005	3	2.4	0.0887	3	1.6	0.2028
APA												
Site										3	1.3	0.3021
Species										1	0.009	0.9232
Site*Species										3	0.9	0.4548

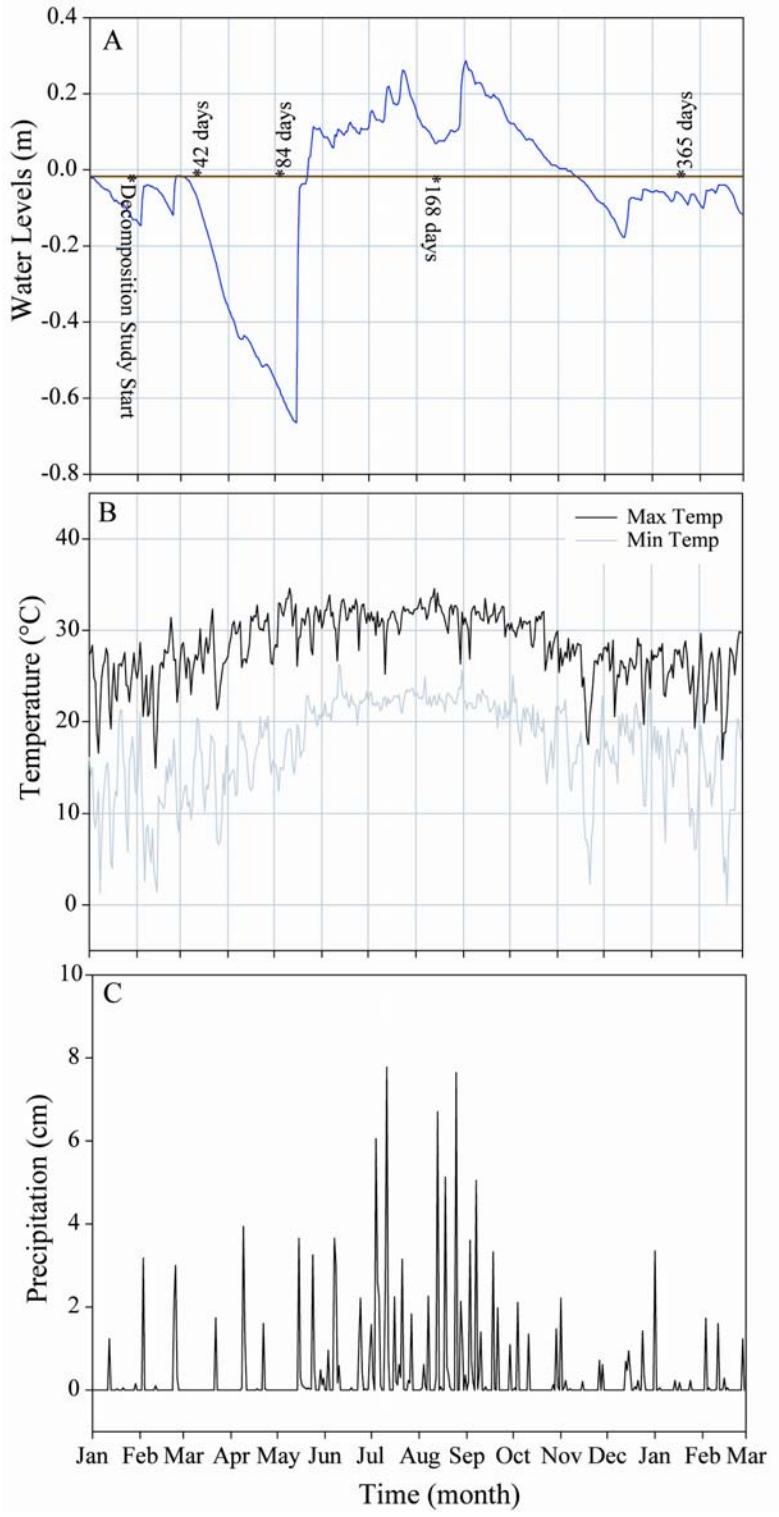

Figure 5-1. Climate data from 2006 obtained from Florida Automated Weather Network. A) Hydroperiod reported as groundwater level above NAVD 1988 (obtained from USGS National Water Information System). B) Daily temperature. C) Daily precipitation.

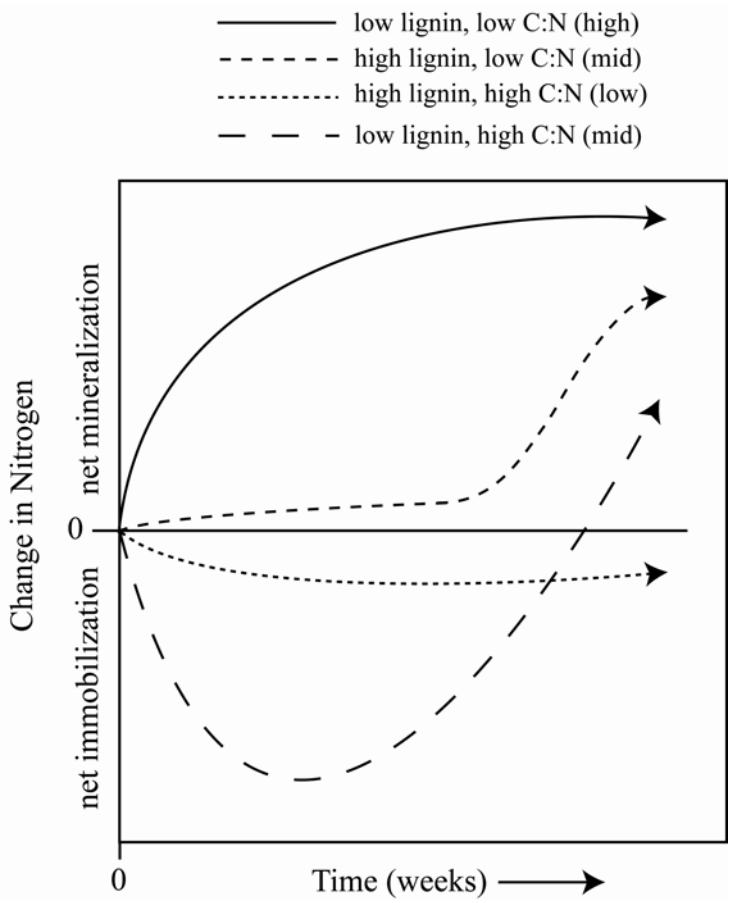

Figure 5-2. Conceptual diagram of temporal patterns of nitrogen release from litter material of differing quality available for microbial decomposition. Lignin content greater than 20% and C:N ratios greater than 30 would be considered high in content and be considered poor quality and therefore would be limited in microbial mineralization of nitrogen. High, mid, and low indicated litter quality rating (modified from Brady and Weil, 1999)

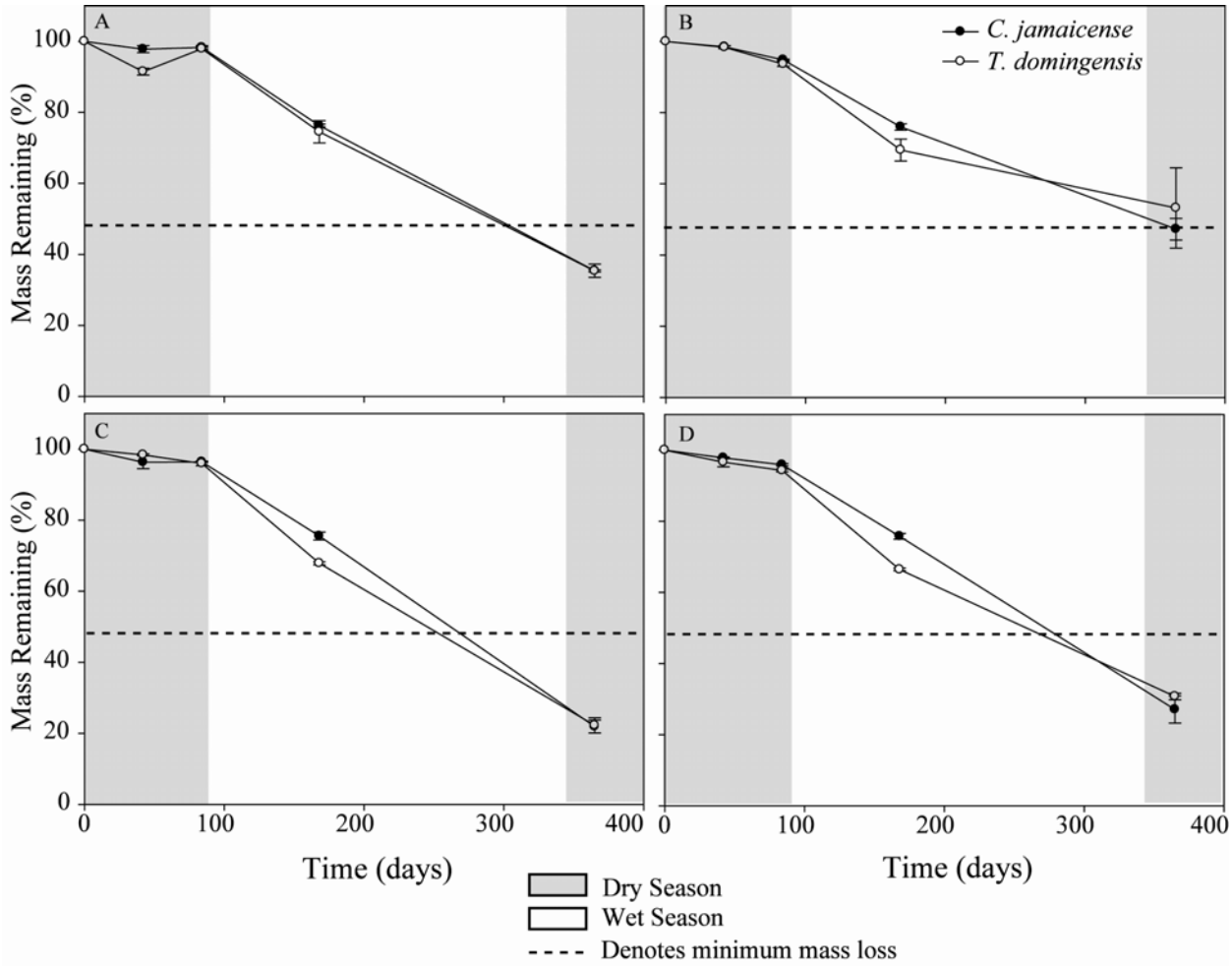

Figure 5-3. Percent mass remaining for *Cladium jamaicense* and *Typha domingensis* from time zero to 365 days for each site. A) Native. B) 1989. C) 1997. D) 2003. The grey areas on each graph illustrates when the sites were dry and the white areas illustrates when the sites were inundated with water.

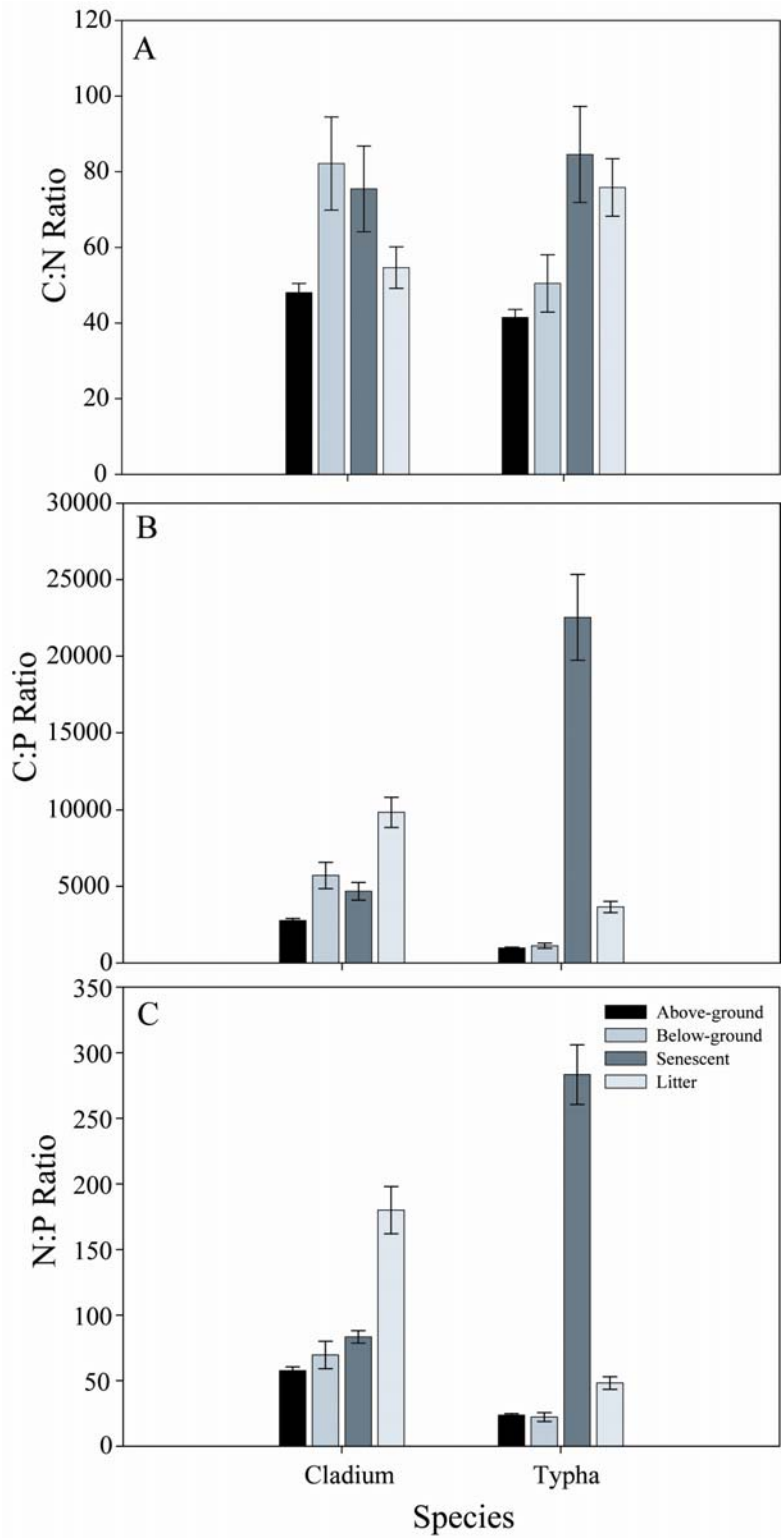

Figure 5-4. Initial nutrient ratios for *Cladium jamaicense* and *Typha domingensis* plant compartments. A) C:N. B) C:P. C) N:P.

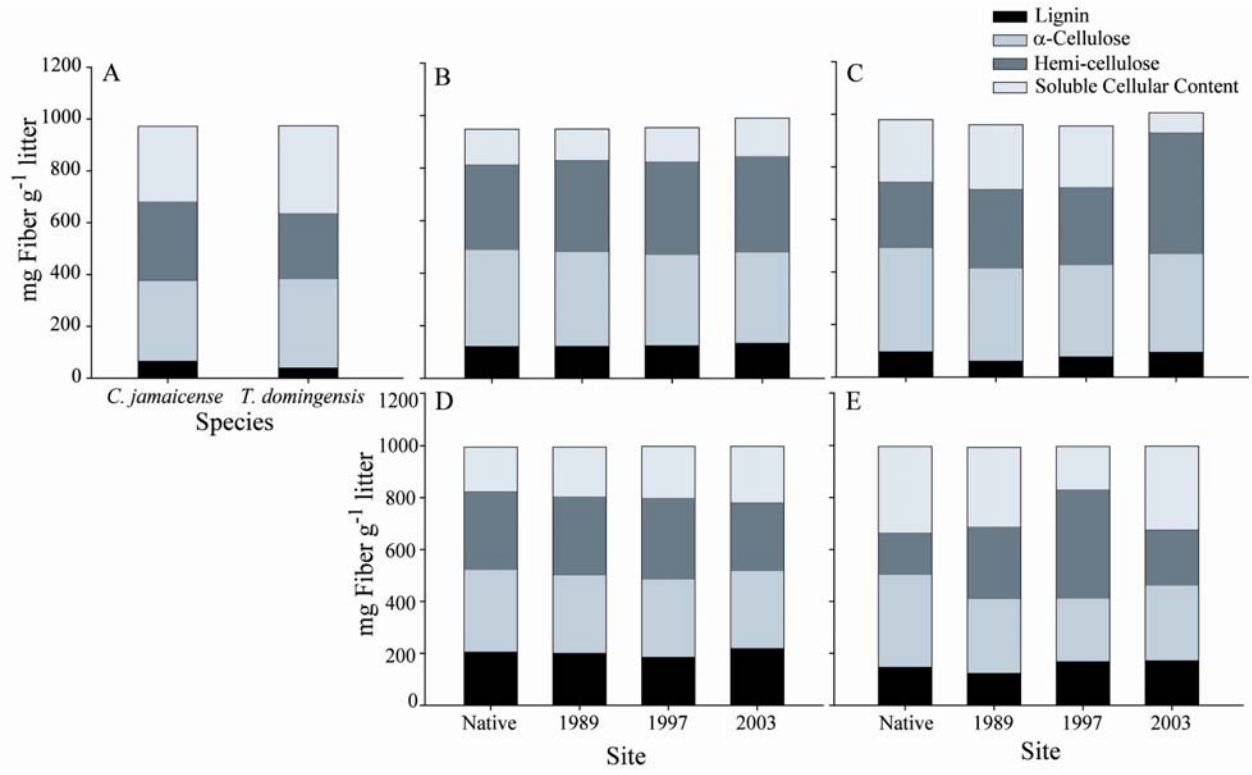

Figure 5-5. Litter fractionation for initial *Cladium jamaicense* and *Typha domingensis* senescent litter and at 168 and 365 days. A) Initial content of *Cladium jamaicense* and *Typha domingensis*. B) *Cladium jamaicense* for each site at 168 days. C) *Typha domingensis* for each site at 168 days. D) *Cladium jamaicense* for each site at 365 days. E) *Typha domingensis* for each site at 365 days.

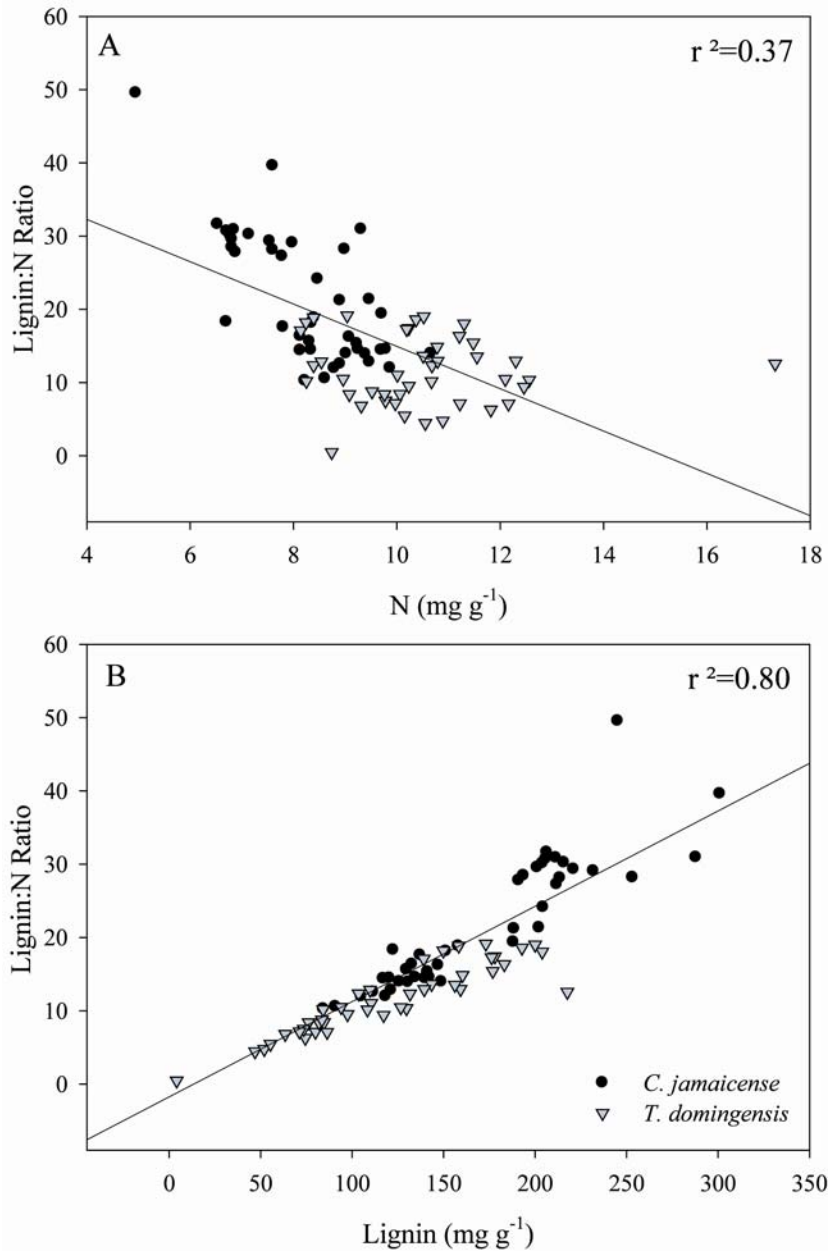

Figure 5-6. Relationships between litter quality indices of *Cladium jamaicense* and *Typha domingensis*. A) N content, d.f.=71, F=52.5, p<0.0001. B) Lignin content to the Lignin:N ratio; d.f.=71, F=232.9, p<0.0001.

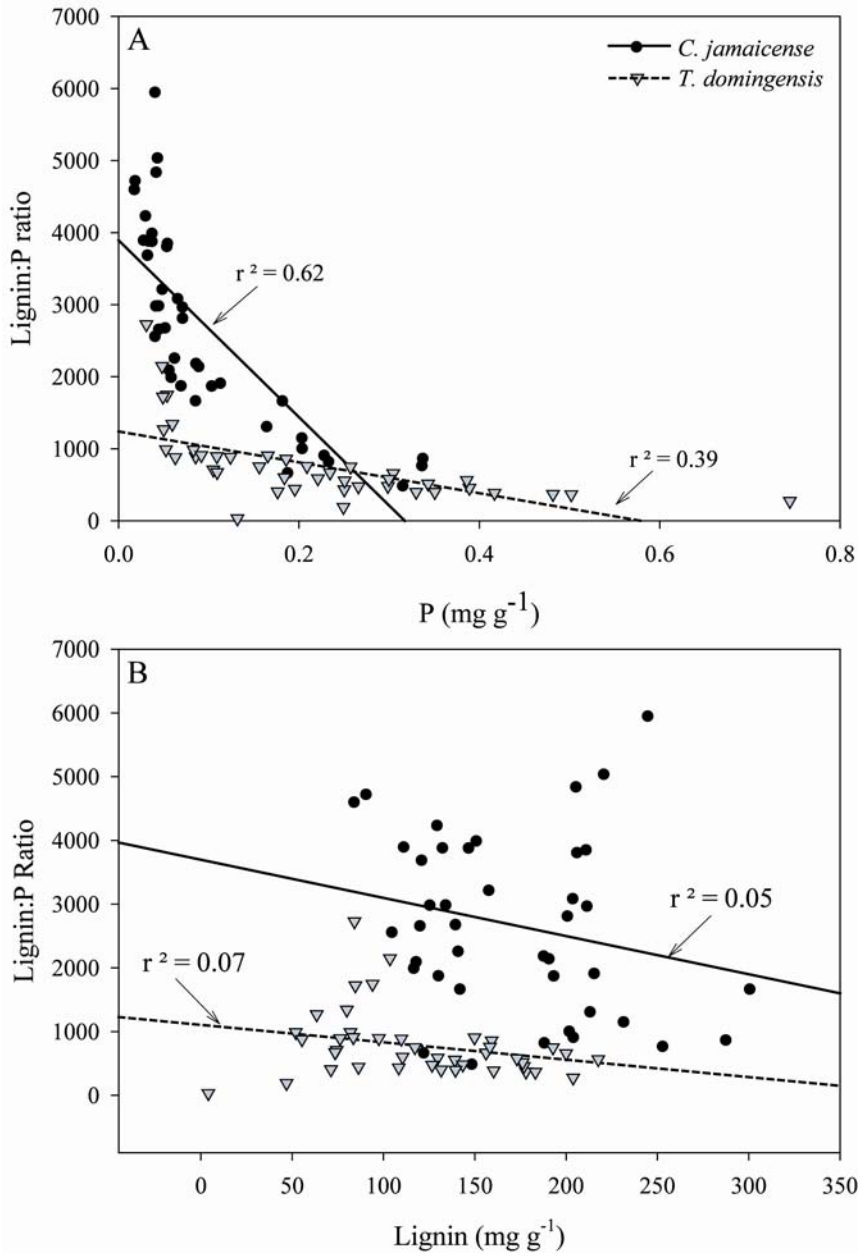

Figure 5-7. Relationships between litter quality indices of *Cladium jamaicense* and *Typha domingensis*. A) P content to lignin:P ratio; *C. jamaicense* – d.f.=35, F=42.9, p<0.0001, *T. domingensis* – d.f.=35, F=32.5, p<0.0001. B) Lignin content to lignin:P ratio; *C. jamaicense* – d.f.=35, F=0.4, p=0.5273, *T. domingensis* – d.f.=35, F=7.0, p=0.0122.

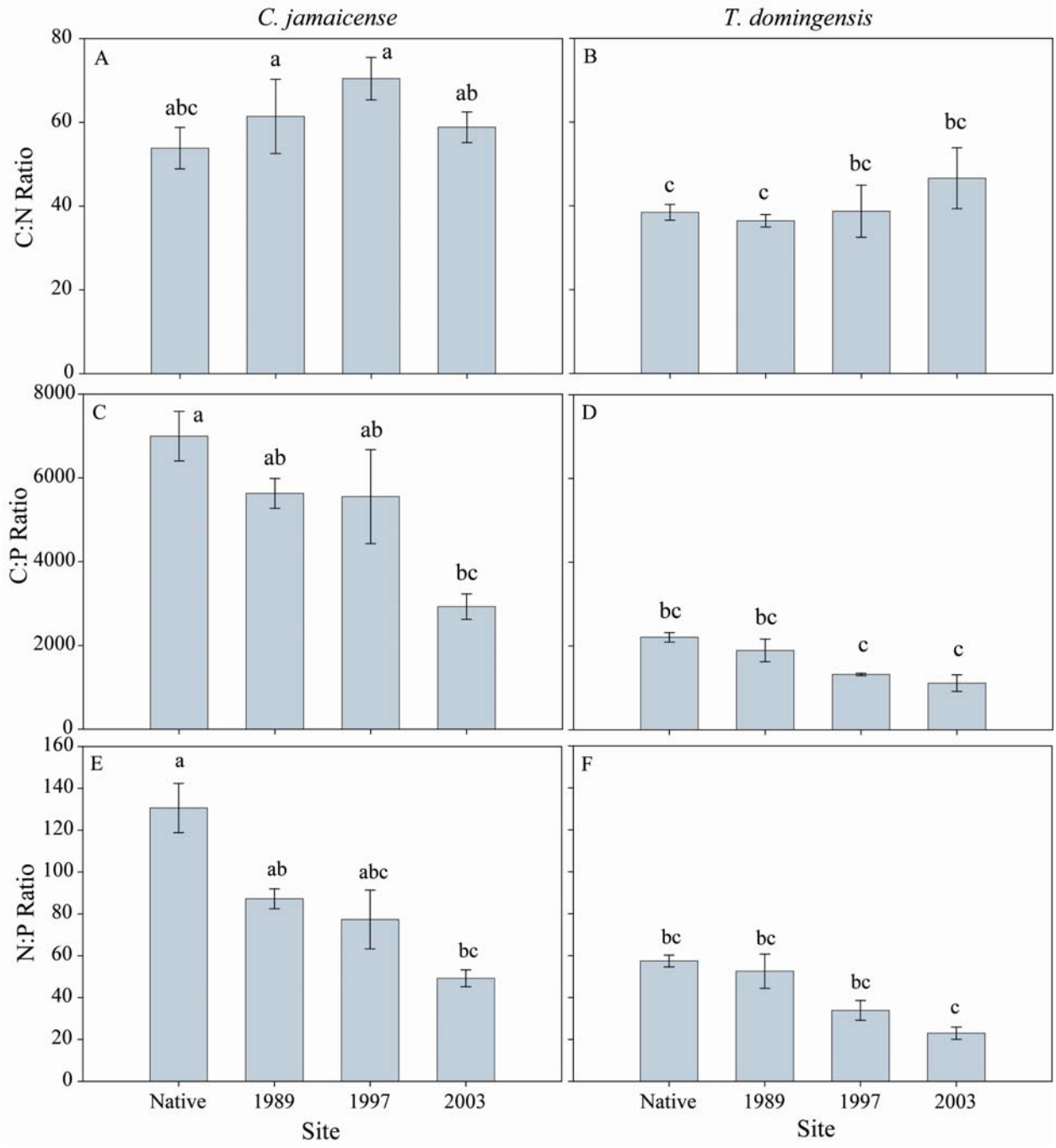

Figure 5-8. Final ratios after 365 days of decomposition for *Cladium jamaicense* and *Typha domingensis*. A) C:N of *Cladium jamaicense*. B) C:N of *Typha domingensis*. C) C:P of *Cladium jamaicense*. D) C:P of *Typha domingensis*. E) N:P of *Cladium jamaicense*. F) N:P of *Typha domingensis*.

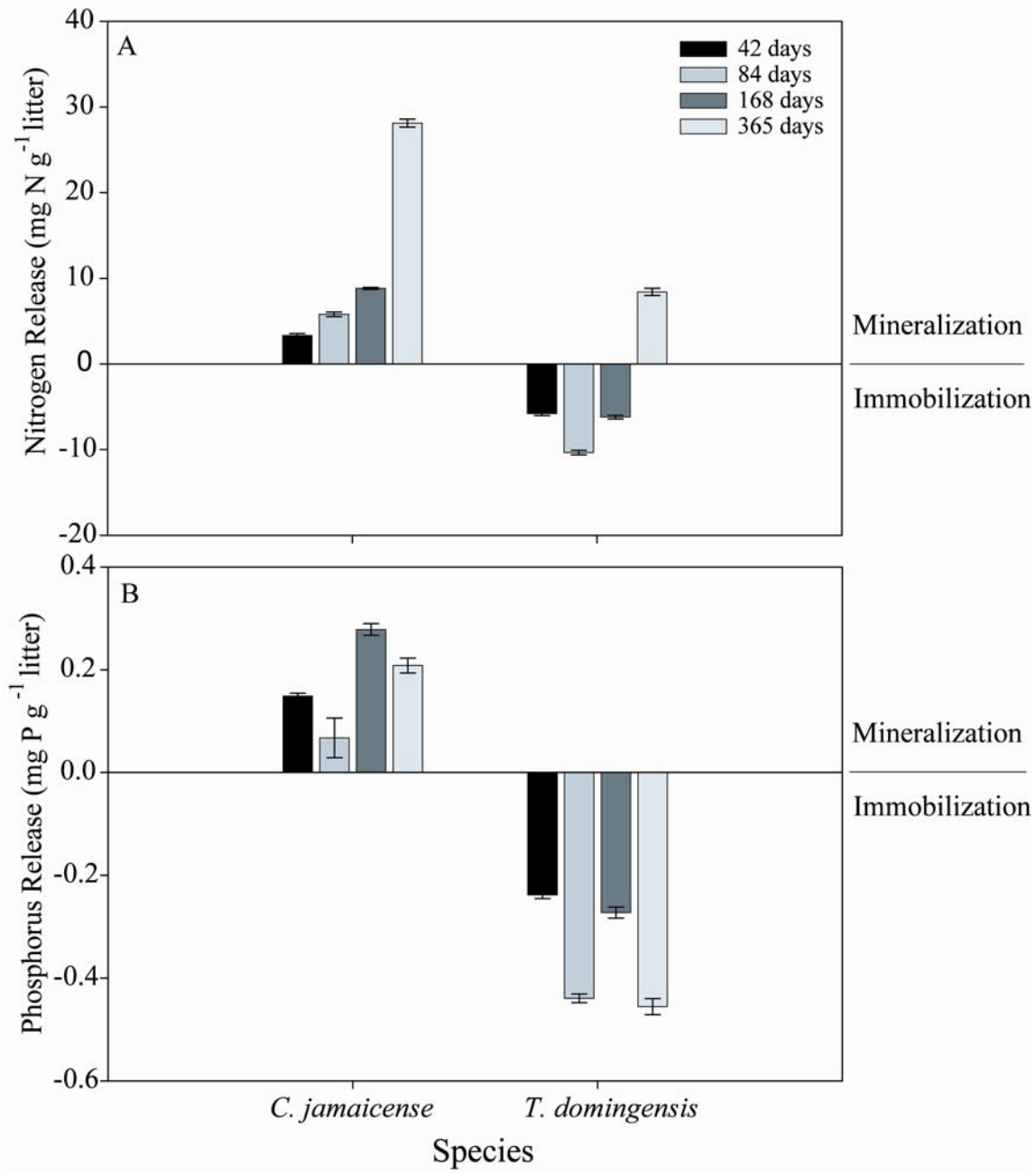

Figure 5-9. Change in nutrients for *Cladium jamaicense* and *Typha domingensis* for each time period analyzed. A) Nitrogen. B) Phosphorus. Positive numbers above the line represent net mineralization of each nutrient and negative numbers below the line represent net immobilization of each nutrient.

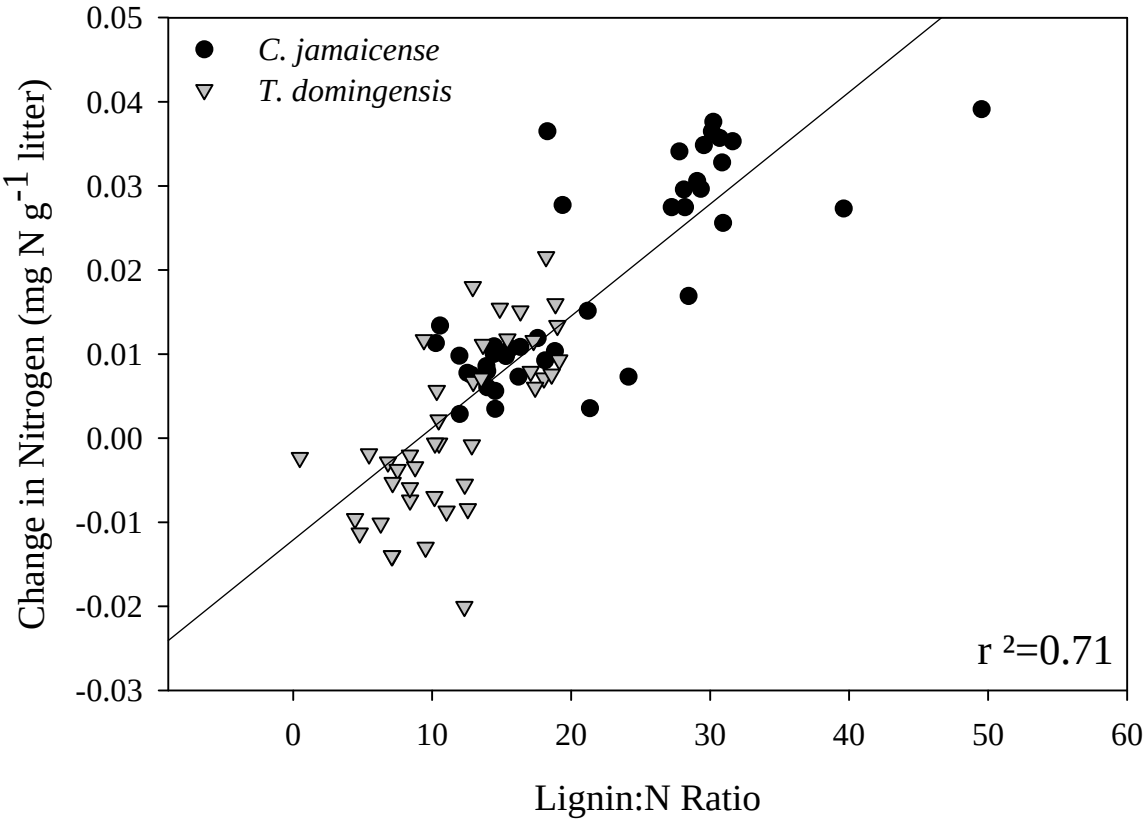

Figure 5-10. Relationship between Lignin:N ratio and the change in nitrogen content in the litter material. Points associated with *Cladium jamaicense* and *Typha domingensis* are designated; d.f.=83. F=198.6, p<0.0001.

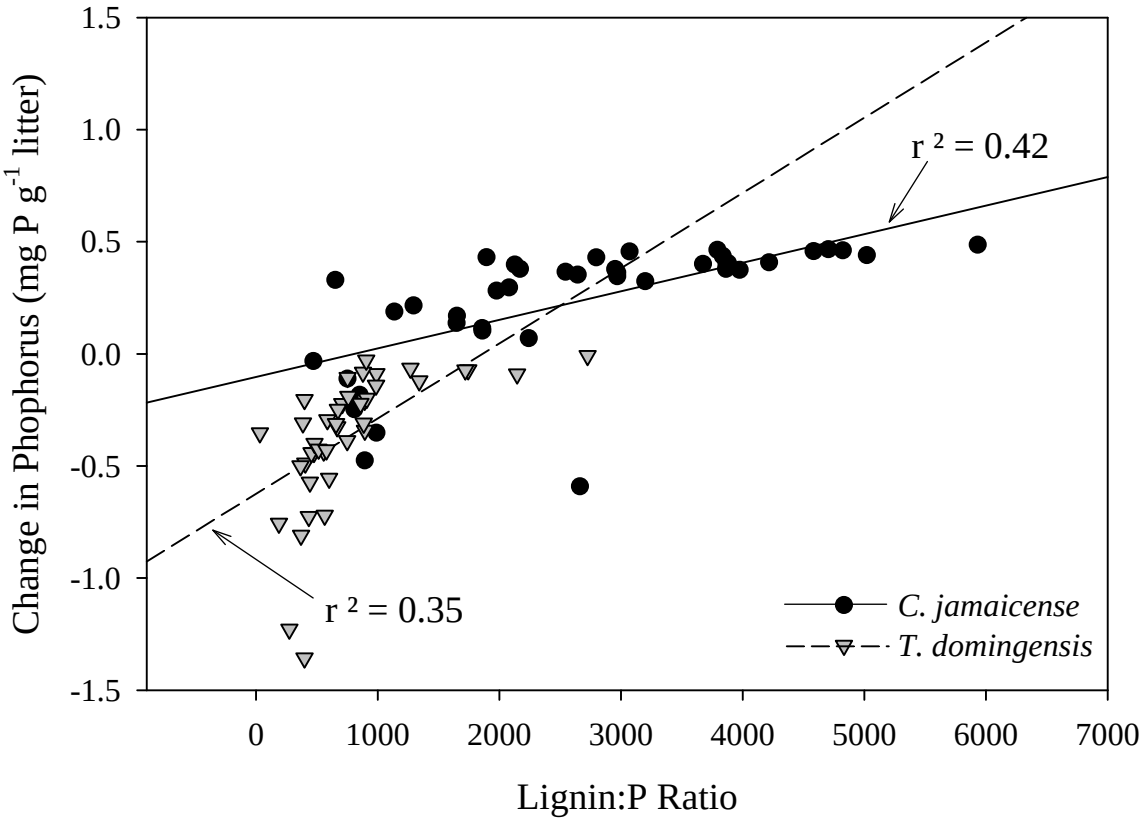

Figure 5-11. Relationship between Lignin:P ratio and the change in phophorus content in the litter material. Points associated with *Cladium jamaicense* and *Typha domingensis* are designated; *C. jamaicense* – d.f.=41, F=7.51, p=0.0098, *T. domingensis* – d.f.=41, F=21.6, p<0.0001.

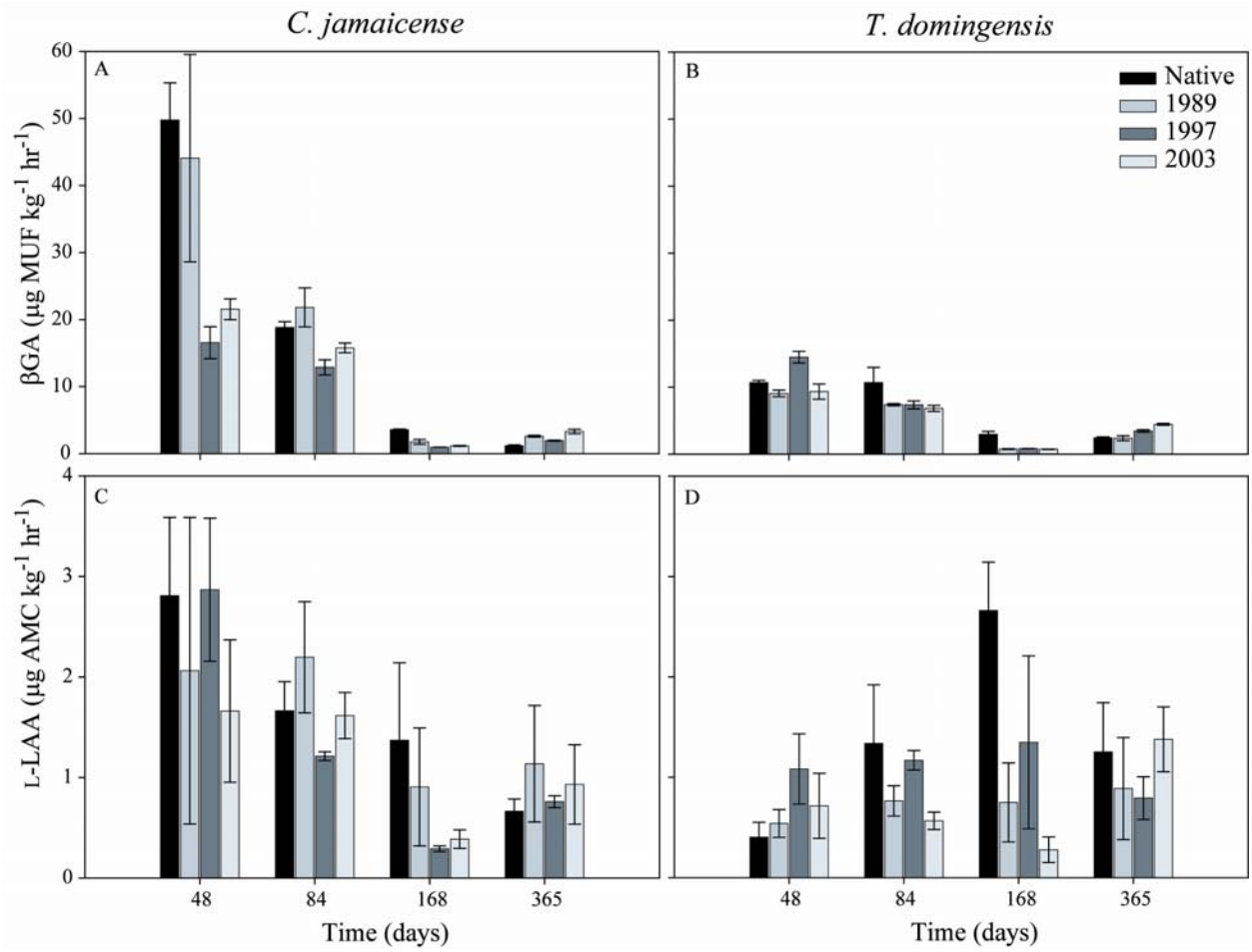

Figure 5-12. Enzyme activities associated with *Cladium jamaicense* and *Typha domingensis* during decomposition. A) β-glucosidase (βGA) of *Cladium jamaicense*. B) β-glucosidase (βGA) of *Typha domingensis*. C) L-leucine-aminopeptidase (L-LLA) of *Cladium jamaicense*. D) L-leucine-aminopeptidase (L-LLA) of *Typha domingensis*.

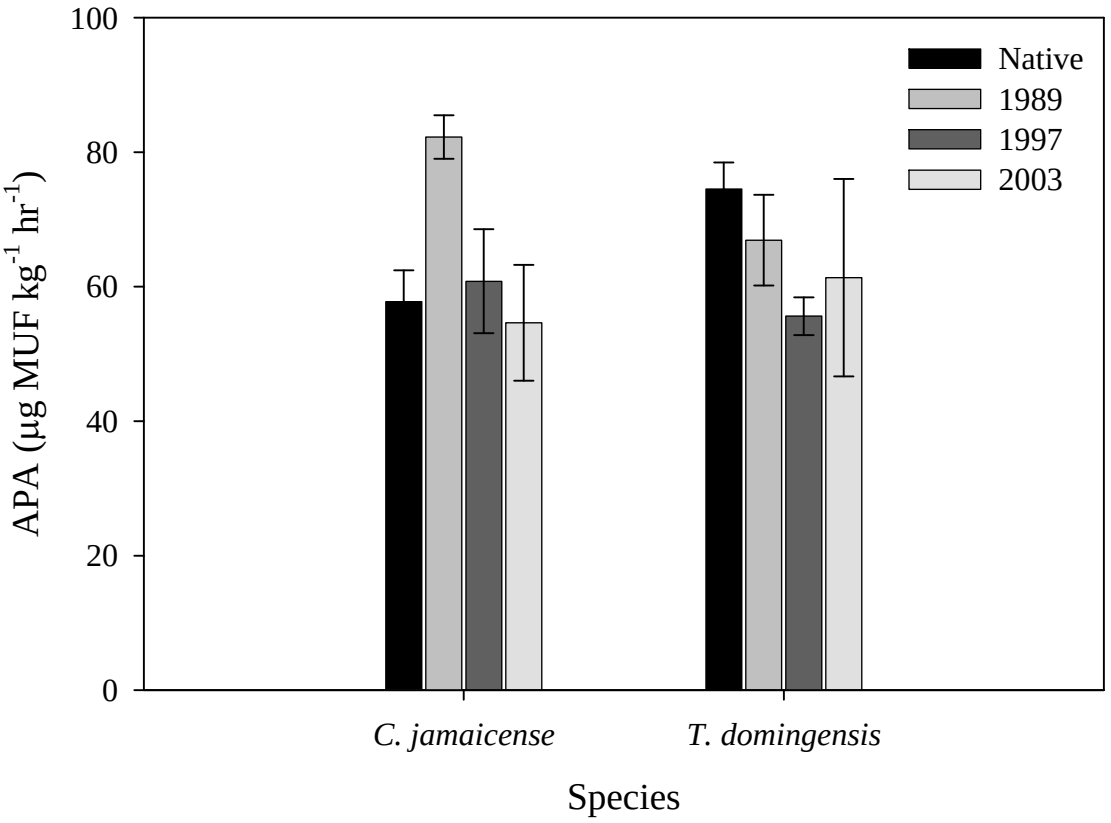

Figure 5-13. Alkaline phosphatase (APA) enzyme activities associated with *Cladium jamaicense* and *Typha domingensis* during decomposition.

CHAPTER 6
RETENTION OF ^{15}N IN SOIL AND VEGETATION: SEASONAL VARIATION AND LONG TERM NITROGEN STORAGE IN RESTORED WETLANDS

Introduction

Hydrologically isolated wetland systems are often nitrogen (N) poor and rely on N inputs via precipitation, deposition, and internal nutrient cycling (i.e., mineralization) (Fennessy and Cronk 2001). Nitrogen plays a major role in the functioning of wetland soils and plant communities (Verhoeven et al. 1996, Olde Venterink et al. 2002, Picking and Veneman 2004). Therefore it is important to understand seasonal variability and long term storages of N to adequately assess restoration success of these types of wetland systems.

Internal rates of N transformations are important determinants of nutrient availability in wetland plant communities (Mitsch and Gosselink 2000). The continuous transformations and numerous chemical species of N can complicate the study of N budgets (White and Howes 1994, Martin and Reddy 1997). Most of these transformations involve microbial activities, which may either facilitate access to or compete with plants for available N. The latter may be driven by incorporation of N into microbial biomass (Kuehn et al. 2000, Catovsky et al. 2002, Inubushi and Acquaye 2004) or removal of N from the system via gaseous transformations (Firestone et al. 1980, Reddy et al. 1989, Bodelier et al. 1996, Chiu et al. 2004). Additionally, rates of these microbially driven processes can alter long term N storage in various ecosystem pools (i.e., NH_4^+ and NO_3^-).

Several studies have determined rates of N transformations and quantified N pools for wetland ecosystems (Hemond 1983, Martin and Reddy 1997, Oomes et al. 1997, Senzia et al. 2002). The amalgamation of this information into whole system budgets to summarize N cycling and storage has been utilized extensively to create ecosystem mass balances which can assist in determining net N retention and loss (Martin and Reddy 1997, Oomes et al. 1997).

However, this approach has many shortcomings in that the determination of key N processes that control N transformation carry a significant amount of error and variability (White and Howes 1994, Gribsholt et al. 2005). Principally, the determination of N flux rates is predominantly restricted to laboratory assays, which only estimate rates of transformations. There are several different methods which have been utilized to assess denitrification, mineralization, and nitrification rates (Keeney 1982, Bundy and Meisinger 1994, Hart et al. 1994, Mosier and Klemedtsson 1994, Weaver and Danso 1994) and different methodologies can result in contradictory rates. While these rates may provide representative estimates for N processes in these ecosystems, when utilized together these estimates can have confounding effects on errors in calculating total ecosystem net N retention and loss.

In addition to microbially driven soil N processes, plant communities and individual plant species can have considerable direct and indirect effects on ecosystem N cycling (Aerts et al. 1999, Engelhardt and Ritchie 2001, Epstein et al. 2001, Ehrenfeld 2003). Community and species level N uptake and use can directly control rates of nutrient flux through competition with microbes for different N species and the turnover time of N in plant biomass. Plant species may vary in their capacity to uptake NH_4^+, NO_3^- or even amino acids as a source of N (Jackson et al. 1989, Schimel and Chapin 1996, Streeter et al. 2000, Henry and Jefferies 2003a, Schimel and Bennett 2004) and plant species have been shown to vary substantially in the use and residence time of N in plant biomass. Plants can also indirectly affect nutrient mineralization via the effects of their litter quality on microbial decomposition (Mason and Bryant 1975, Hector et al. 2000, Gartner and Cardon 2004).

The use of in situ stable isotope tracers to follow N throughout an ecosystem has provided a greater understanding of N processes and long term storages across a multitude of ecosystems

(Hobbie and Chapin 1998, Mulholland et al. 2000, Fry and Smith 2002, Gerzabek et al. 2004, Fry 2006). Through additions of NH_4^+ or NO_3^- highly enriched in ^{15}N relative to natural abundance levels can assist in determining rates of plant uptake (Merbach et al. 2000, Dinkelmeyer et al. 2003, Ruckauf et al. 2004, Templer and Dawson 2004), transformations (Mulholland et al. 2000, Tank et al. 2000, Templer et al. 2003, Gribsholt et al. 2005), and storage (White and Howes 1994, Epstein et al. 2001).

The primary objective of this study was to investigate the differences in ecosystem N retention and partitioning in restored wetland systems. Many times in the restoration or mitigation of wetland systems the focus is solely on the vegetation community; ecosystem function is often overlooked. The restored wetlands investigated in this study have very different plant community composition and diversity compared to the native or desired plant community structure. The native communities are co-dominated by *Schoenus nigricans* and *Cladium jamaicense*, whereas the restored wetlands are dominated by *Typha domingensis*. This study aimed to 1) determine which soil N pools had the greatest influence on ecosystem N retention, 2) determine potential influences of both community and species level contributions to ecosystem N retention and 3) construct an N budget to assess the success of ecosystem function restoration. This study was conducted in a chronosequence of restored wetlands in the Everglades National Park.

We hypothesized that 1) the soil pools in the native community would retain more N over time than the soil pools of the restored wetland communities and 2) that the vegetation community would acquire more N from the soil in the native communities compared to the vegetation in the restored wetlands and therefore would retain more N. The basis of these two hypotheses is due to the native soil having a greater soil depth and stability due to lack of

disturbance. The restored wetlands soil depth is very shallow ranging from 0-2 cm, whereas the native community soil depth ranges from 8-20 cm.

Methods

Site Description

This study was conducted in wetland systems restored within the Hole-in-the-donut (HID) region of the Everglades National Park (ENP). Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season (Figure 6-1). The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod and control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Experimental Design

In 2006, we examined the recovery of N in field plots in the native community and the 1989, 1997, and 2003 restored wetlands. Two treatments were investigated within three replicate field plots; enriched with ^{15}N and a control, where no enriched ^{15}N was added. Each plot was 4-m^2 in size and at 0.5 m elevation above sea level. We chose plots based on a stratified random design with the following criteria: 1) presence or dominance of *Cladium jamaicense*, 2) presence or dominance of *Typha domingensis*, and 3) presence of both *C. jamaicense* and *T. domingensis*. Existing data collected by the Everglades Research Group was utilized to locate and establish these plots (O'Hare and Dalrymple 2003). Plots in the native communities were established in vegetation communities with co-dominance of *Schoenus nigricans* and *C. jamaicense*. Plots in the 1989 restored site were established in areas where both *C. jamaicense* and *T. domingensis*

were present. Plots established in the 1997 and 2003 were established in areas dominated by *T. domingensis*.

The ^{15}N tracer was applied to each field plot as a liquid form of $^{15}NH_4Cl$ (99 atom % ^{15}N, Cambridge Isotopes) at a rate of 650 mg ^{15}N m^{-2} during the dry season of January 2006. All samples were collected from the center 1-m^2 nested in the 4-m^2 plot (Figure 5-2). The outer 0.5-m^2 perimeter served as a buffer around the 1-m^2 sample plot to reduce dilution of the applied ^{15}N by the surrounding environment (Hauck et al. 1994).

Each field plot received 10 grams of $^{15}NH_4Cl$ (equivalent to 2.6 g N m^{-2}) dissolved in 7.5 liters of DI water. The solution was applied to the soil surface using a hand pumped pressurized sprayer. The solution was applied evenly over the entire 4-m^2 plot keeping the spray nozzle close to the soil surface to avoid standing vegetation and litter material.

Soil Sampling and Analysis

Soil and litter samples were collected at 24 hours after application and at days 42, 84, 168, and 365. Litter samples were collected in replicates of three by randomly clearing a 15 cm^2 area within each 1 m^2 plot. Soil samples were collected in replicates of three from each plot via a 7.6 cm diameter PVC soil core collector to the depth of 5 cm for the native soil and to bedrock in restored wetlands and stored at 4°C until laboratory analysis was performed. Before analyses were performed all litter and root material was removed from soil samples. The live roots were separated and kept for further analysis. Bulk density was calculated by determining the moisture content of each core by drying a subsample at 60°C until a constant weight was achieved. Soil depth was measured from the top of the soil to the bedrock. Organic matter content was determined as loss on ignition (LOI) by combusting 0.5 g of dry soil at 550°C for 4 hours. LOI was calculated as the percent of organic matter lost after combustion.

Within 24 to 48 hours of sample collection, each soil sample was extracted for ammonium (NH_4) with K_2SO_4 (Bundy and Meisinger 1994) and set up for incubation for potentially mineralizable nitrogen (PMN) (or biologically available N) (Keeney 1982, Bundy and Meisinger 1994, White and Reddy 2000). Ammonium and nitrate were analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 350.1 for NH_4 and EPA Method 353.2 for NO_3). A subsample of the K_2SO_4 extract was digested for total kjeldahl nitrogen (TKN) via kjeldahl block digestion and analyzed by flow injection with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 351.1). Total organic carbon (TOC) was analyzed from the extract with a Shimadzu TOC-5050A Total Organic Carbon Analyzer equipped with a ASI-5000A auto sampler. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3). Total carbon (TC) and total N were determined by dry combustion with a Thermo Electron Corporation Flash EA NC Soil Analyzer. The isotope analysis of solid soil samples was performed via isotope ratio determination with a Thermo Finnigan MAT Delta Plus XL Mass Spectrophotometer equipped with a Costech Instrument Elemental Analyzer for flash combustion of solid material for N and C analysis.

To obtain a crude estimate of potential volatilization rates within each site, in situ 0.1M HCl traps were used to capture NH_3 gas. Two liter glass containers with an affixed sample cup containing a 50 ml solution of 0.1M HCl was placed in each site. Each sample cup was collected after a 24 hour period and stored as 4°C until analysis was performed. The samples were analyzed for ammonia with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 350.1 for NH_4). Results were expressed as mg N m^{-2} d^{-1}.

Microbial biomass carbon and nitrogen (MBC and MBN) were extracted from each soil sample via the chloroform fumigation-extraction incubation method (Brookes et al. 1985). Both

the chloroform fumigated and non-fumigated samples were extracted with 0.5M K_2SO_4 solution. To determine MBC, each subsample from each extractant was acidified with concentrated ultra-pure H_2SO_4 and analyzed for TOC with a Schimadzu TOC-5050A Total Organic Carbon Analyzer equipped with a ASI-5000A auto-sampler. To determine MBN, each sample was digested for TKN via kjeldahl digestion (Brookes et al. 1985) and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 111-A rev1). The MBC and MBN were computed as the difference between the fumigated and non-fumigated samples. No correction factors were used in the calculation of this data. The ^{15}N in the soil extractions of NH_4, NO_3, and TKN (both fumigated and non-fumigated) was determined after concentrating the nitrogen in each sample onto an acidified filter paper with a diffusion technique (Stark and Hart 1996). The isotope analysis of the filter papers was performed via isotope ratio determination with a Thermo Finnigan MAT Delta Plus XL Mass Spectrophotometer equipped with a Costech Instrument Elemental Analyzer for flash combustion of solid material for N and C analysis.

Vegetation Sampling and Analysis

Subsamples of individual species were collected from each plot 168 days after the application of the ^{15}N tracer. Species were chosen for collection based on dominance in individual plots. The dominant species were: *S. nigricans* and *C. jamaicense* in the native community, *C. jamaicense* and *T. domingensis* in the 1989 site, and *T. domingensis* in the 1997 and 2003 sites. Since biomass was destructively harvested, this was not sampled at this time so as not to disrupt the final sample time at 365 days. Therefore, biomass contribution of these species was estimated as an average of biomass contribution determined during the prior years sampling (see Chapter 2) and the sampling at the 365 day.

At 365 days after the application of the tracer, above-ground biomass (separated as live and senescent) was determined. Both live and senescent plant shoots within the center 1-m^2

plots was clipped at the soil surface. In addition to being separated by live and senescent, each biomass sampling was separated into species to determine species contribution and reported as g dry weight m^{-2}. Litter production was determined within each center 1-m^{-2} plot by collecting all the litter material laying on the soil surface and reported as g dry weight m^{-2}. Root biomass was determined in each plot by collecting three replicate 7.6 cm diameter cores (Fennessy and Cronk 2001). The live roots were separated from the soil, washed and dried at 50°C and reported as g dry weight m^{-2}.

All plant samples collected were analyzed for total C and N via dry combustion with a Thermo Electron Corporation Flash EA NC Soil Analyzer for the bulk above-ground plant tissue. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Carbon, N and P ratios were calculated as C:N, C:P, and N:P. The isotope analysis of vegetation samples was performed via isotope ratio determination with a Thermo Finnigan MAT Delta Plus XL Mass Spectrophotometer equipped with a Costech Instrument Elemental Analyzer for flash combustion of solid material for N and C analysis.

Calculations

The amount of the ^{15}N tracer recovered in each of the pools analyzed was calculated as:

$$^{15}N_{mixed} = (^{15}N_{sample} - {}^{15}N_{background}) / (^{15}N_{tracer} - {}^{15}N_{background})*100, \quad (6\text{-}1)$$

where $^{15}N_{mixed}$ is the atom % of the tracer recovered in each pool, $^{15}N_{sample}$ is the atom % of ^{15}N in each sample, $^{15}N_{background}$ is the natural abundance level of ^{15}N (0.37 atom %), and $^{15}N_{tracer}$ is the amount of ^{15}N in the tracer applied (99 atom %) (Fry 2006). The individual total nitrogen pools (TN_{pool}) were calculated as mg N m^{-2}, and the pool size of the ^{15}N tracer applied recovered in the total N pool was calculated as:

$$^{15}N_{pool} = TN_{pool} * {}^{15}N_{mixed}, \quad (6\text{-}2)$$

where the $^{15}N_{pool}$ is the pool size of ^{15}N recovered in total N pool as mg ^{15}N m^{-2}. The percent of $^{15}N_{tracer}$ recovered was calculated as:

$$\%^{15}N \text{ recovered} = {}^{15}N_{pool} \text{ (mg } {}^{15}N \text{ } m^{-2}) / 650 \text{ (mg } {}^{15}N \text{ } m^{-2}) * 100, \quad (6\text{-}3)$$

The amount of ^{15}N in the microbial biomass pool was calculated with the following equation:

$$MB^{15}N = (MBN_{fum} * \text{atom \% } {}^{15}N_{fum} / 100) - (MBN_{nonfum} * \text{atom \% } {}^{15}N_{nonfum} / 100), \quad (6\text{-}4)$$

where $MB^{15}N$ is the amount of ^{15}N stored in the microbial biomass N pool, atom % $^{15}N_{fum}$ is the amount of ^{15}N in the fumigated extractions, and the atom % $^{15}N_{nonfum}$ is the amount of ^{15}N in the non-fumigated control extractions (Templer et al. 2003).

Statistical Analysis

All data were analyzed statistically using Fit Model in JMP Version 5.1 (SAS 2005). Analysis of variance (ANOVA) was performed to investigate site and species differences related to the recovery of the ^{15}N tracer applied. Regressions were performed to determine if any strong relationships existed between variables.

Results

^{15}N Retention after 24 Hours

A summary of the site characteristics before the initiation of the tracer study is found in Table 6-1 and a summary of the prior vegetation and litter characteristics are found in Table 6-2. Estimates of ^{15}N retention in the bulk soil and litter layer pools after 24hrs were the highest in the native community at 79%, whereas the in the restored sites ^{15}N retention was 46, 40, and 50% in the 1989, 1997, and 2003 sites, respectively (Table 6-3). The majority of the ^{15}N retained at this time was found in the bulk soil. Of the bulk soil parameters that we analyzed, the MBN pool contained the majority of the ^{15}N at 31, 24, 22, and 25% for the native, 1989, 1997, and 2003

sites, respectively. The percentage of ^{15}N retained in the NO_3 pool was 24%, 18, 9 and 1% in the native, 1989, 1997, and 2003 sites, respectively (Table 6-3). In all sites except the 2003 site the percentage recovered in the NO_3 pool was greater than what was recovered in the NH_4 pool.

Soil ^{15}N Retention after 365 Days

Patterns of N retention were greatly influenced by the restoration process. After 365 days, the native community ecosystem retained 42% of the initial 650 mg ^{15}N m^{-2} that was applied as compared to the restored wetland systems which retained 26, 13, and 29% in the 1989, 1997, and 2003 sites, respectively (Table 6-4). The majority of the ^{15}N recovered at this time period was found in the bulk soil pool. The organic nitrogen (ON) pool retained the majority of the ^{15}N found in the bulk soil at 22, 8, 4, and 16% in the native, 1989, 1997, and 2003 sites respectively. The MBN pool followed the ON pool in the next largest in percent recovered (Table 6-4). The 2003 restored site's litter layer retained more ^{15}N at 4% over all other sites with 1% in the native site, and 2% in the 1989 and 1997 sites.

The 1997 site's bulk soil pool retained significantly less of the ^{15}N tracer than the native communities, but the 1989 and 2003 was not significantly different than either pool ($p=0.098$, α-level = 0.1; Figure 6-3a). The litter layer in the 2003 site retained significantly more of the tracer as compared to all other sites ($p=0.03$; Figure 6-3b).

The bulk soil pool was separated into to four groups: NH_4^+, NO_3^-, ON, and MBN. The ON pool retained more of the tracer in all sites as compared to the other pools (Figure 6-4). The ON in the native community and the 2003 restored wetland retained significantly more ^{15}N over the 1989 and 1997 restored wetlands ($p=0.027$; Figure 6-4c). The ON pool was followed by the MBN pool retaining a significant amount of the ^{15}N tracer. The MBN in the native and 1989 sites retained significantly more ^{15}N than the 1997 and 2003 sites ($p=0.039$; Figure 6-4d). The

amount of ^{15}N retained in the NH_4^+ and NO_3^- pools was small compared to all other pools. The amount of the tracer remaining in the NH_4^+ pool ranges from 0.05 to 0.5 mg ^{15}N m^{-2} with no significant differences found between sites ($p=0.32$; Figure 6-4a). The NO_3^- pool ranges from 0.2 to 2.4 mg ^{15}N m^{-2} with the native community being significantly higher than all the restored communities ($p<0.0001$; Figure 6-4b).

Soil Processes

To assess microbial activity associated with mineralization of N, the potentially mineralizable N (PMN) was determined as mg NH_4^+ kg^{-1} soil day^{-1} (Figure 6-5). At all time periods except days 84 and 365, the native site has significantly lower PMN compared to the restored sites. At 84 days the native site PMN was not significantly different from the 2003 site and at 365 days the native site PMN was not significantly different form the 1997 or 2003 sites.

The results of the volatilization study indicate that the 1997 site has significantly higher potential rates of NH_3^+ volatilization compared to all other sites ($p=0.0154$; Figure 6-6). Additionally, the native community has significantly lower rates of NH_3^+ volatilization than the restored wetland systems.

Species-level ^{15}N Retention

By 168 days, selected plant species had incorporated between 4-7% of the 650 mg ^{15}N m^{-2} that was applied. Both total N and ^{15}N storage of the above-ground biomass was significantly more than the below-ground biomass for all species across all sites ($p<0.0001$; Figure 6-7, Table 6-5). The above-ground biomass N pool was significantly higher for *T. domingensis* in the 1997 and 2003 restored sites than all other species ($p<0.0001$; Figure 6-7a, Table 6-5). The above-ground N pool of *S. nigricans* was significantly higher than the N in *C. jamaicense* ($p<0.0001$; Figure 6-7a, Table 6-5). There was no significant difference in the amount of N in the below-ground biomass between species ($p=0.6699$; Figure 6-7a, Table 6-5). The above-ground biomass

of *T. domingensis* in the 1997 and *S. nigricans* in the native community retained significantly more of the tracer as compared to *C. jamaicense* and *T. domingensis* in all the other sites (p=0.0116; Figure 6-7b, Table 6-5). The below-ground biomass ^{15}N retention of *T. domingensis* in the 2003 site was significantly less than the *S. nigricans* and the *C. jamaicense* found in the 1989 site but not to any of the other species in other sites (p=0.0275; Figure 6-7b, Table 6-5). No significant differences were found between other species in other sites for ^{15}N retention in the below-ground biomass pool.

A summary of biomass (g m^{-2}), total N (mg m^{-2}), ^{15}N (mg m^{-2}) and $\%^{15}N$ for all the individual plant species found at 365 days after application of the tracer is listed in Table 6-6. The species within each site are ordered from highest to lowest in biomass production. In general, the species with the highest biomass production also had the highest total N and ^{15}N retention. Exceptions to this trend were the group of unidentified species in the 1989 site, *Sarcostemma clausum* and *Hypericum myrtifolium* in the 1997 site, and *Solidago sempervirens* in the 2003 site (Table 6-6). Of all these species only *S. sempervirens* contributed significantly to the above-ground biomass pool following *T. domingensis* in biomass production in the 2003 site. *Typha domingensis* produced almost twice the biomass and slightly more total N than S. *sempervirens*, but *S. sempervirens* retained approximately four times more ^{15}N (Table 6-6).

To take a closer look at species and site differences, the dominant species as well as Poaceae (because it occurred in large numbers in all sites) were plotted for total N and ^{15}N pools (Figure 6-8). We found that *S. nigricans* and *T. domingensis* (1997 site only) had significantly higher N pools than the other species (p=0.0001; Figure 6-8a, Table 6-5). *Cladium jamaicense* in the native and 1989 site and *T. domingensis* in the 2003 site had similar N pools and were

significantly higher than Poaceae. There was no significant difference observed for Poaceae between sites.

Schoenus nigricans had significantly higher ^{15}N retention in it's biomass as compared to all the other species (p=0.0007; Figure 6-8b, Table 6-5). *Cladium jamaicense* in the native and 1989 site and *T. domingensis* in the 1997 site had similar ^{15}N retention but were significantly higher than *T. domingensis* in the 2003 site and Poaceae. There was no significant difference observed for *T. domingensis* in the 2003 site and Poaceae between sites.

Community-level ^{15}N Retention

The above-ground biomass pool retained a greater percentage of the ^{15}N tracer than the below-ground biomass pool (Table 6-4). The amount of ^{15}N recovered in the above-ground biomass was 5.5, 3.1, 3.8, and 4.7% for the native, 1989, 1997, and 2003 sites, respectively. The below-ground biomass only retained 0.8, 1.3, 1.1, and 1.5% of the ^{15}N tracer in the native, 1989, 1997, and 2003 sites, respectively.

We found no significant differences for total N or ^{15}N pools for the above- or below-ground community level biomass across sites (Figure 6-9, Table 6-5). When comparing the total above-ground pool (live plus senescent) to the total below-ground pool (live plus dead roots), we found the above-ground biomass N was significantly higher than the below-ground biomass N in the native and 2003 sites (p= 0.0250 and 0.0326, respectively; Table 6-5) but not in the 1989 and 1997 sites (p=0.5326 and 0.0976; Figure 6-9a, Table 6-5). For ^{15}N retention we found a significant difference between the above- and below-ground biomass in the native site (p=0.0298; Table 6-5) but not in any of the other sites (Figure 6-9b). To assess relationships in ^{15}N retention, the bulk soil ^{15}N retained in each site was regressed with the ^{15}N retained in the biomass and found a positive relationship (r2 = 0.41; Figure 6-10). When the bulk soil ^{15}N was the greatest, so was the ^{15}N retention in the above-ground biomass.

In addition to investigating differences between above- and below-ground vegetation, the differences between live, senescent, litter, and root plant fractions were compared (Figure 6-11). We found no significant difference for N pools between plant fractions except for the 2003 restored site where the litter layer N pool was significantly higher than the live pool but neither were significantly different from the senescent or litter pool ($p = 0.0197$; Figure 6-11a, Table 6-5). We found no significant differences for ^{15}N retention between the plant fractions (Figure 6-11b, Table 6-5).

Seasonal Change in ^{15}N Storage and N Budget

Little change occurred in the bulk soil N pool over time except in the native community. There was a significant decrease in the amount of N stored in the native community soil during the wet season (168 days sample period) than for all other sample periods (Figure 6-12a). Additionally, the native community bulk soil stored significantly higher amounts of N as compared to all the restored sites followed by the 1989 restored wetland. The ON pool followed a similar trend as the bulk soil pool (Figure 6-12e). The amount of N stored in the 1997 and 2003 were not significantly differently. In the native and 1989 sites, the NO_3 pool resulted in an initial increase followed by a sharp decline during the wet season (Figure 6-12b).

In all sites the NH_4^+ pool gradually decreased over time with the 1989 site indicating an increase during the wet season sampling period (Figure 6-12c). In all sites, the MBN pool increased during the dry season (first three sample period) with the native community resulting in the highest increase followed by a sharp decline in the wet season (Figure 6-12d). The litter N pool resulted in the greatest variability between sites (Figure 6-12f). The 2003 site had the largest N pool and the 1989 sites had the lowest. The initial N was similar for the 1997 and native community; however, the 1997 site N was larger than the native community. All sites had an initial decline in N in the litter pool followed by and increased as time moved into the wet

season. In addition, for all sites and all parameters except NH_4, the final N pool is similar to the initial pool.

In all pools except for the ON pool, the amount of ^{15}N recovered decreased over time (Figure 6-13). Some variation, however, was observed in some sites where increases in ^{15}N occurred in later sample periods. The bulk soil pool in the 2003 site had an increase in ^{15}N after day 42 with day 1 and 84 having the same amount of ^{15}N (Figure 6-13a). Additionally, the native and 1989 sites litter layer resulted in a slight increase in ^{15}N at the 84 day sample period (Figure 6-13d). These variations could be a result in uneven distribution of the tracer during application; however, the increase observed in the 2003 site bulk soil is significant suggesting translocation of ^{15}N into bulk soil litter or vegetation exudates. The amount of ^{15}N in the ON pool increased in the first 42 to 84 days and then decreased over time (Figure 6-13e). In native community, we observed and increase in the ON ^{15}N pool in day 365 compared to what was observed at 168 days. A complete budget of N pool sizes and ^{15}N retention in each pool for each site at 365 days is found in Figure 6-14.

Discussion

Soil ^{15}N Retention

Our hypothesis that the native community soil pools would retain more N over time than the restored wetland communities was supported by this study. Soil ^{15}N retention in the native community was 2-6% greater than the ^{15}N retention in the restored communities with large differences observed in soil ^{15}N retention across sites. The seasonal variation in ^{15}N retention in the native community bulk soil indicates an increase in ^{15}N after 365 days. This increase observed could be a result in uneven distribution of the tracer during application, organic N mineralization from new litter material or translocation of ^{15}N into bulk soil litter or vegetation exudates.

Several potential losses could also contribute to the unaccounted ^{15}N applied in our study. Possible gaseous fluxes that could result in losses of the ^{15}N tracer include volatilization of NH_3, nitrification/denitrification of NO, N_2O and N_2 and possibly leaching. Since the tracer was applied during the dry season, denitrification was limited to anaerobic microsites and therefore was most likely limited until the wet season began. Furthermore, since these soils are calcareous marl wet soils with average pH values at 7.7, there is an increased potential for elevated rates of volatilization to occur. We found that the 1997 site had the potential to lose significantly more N via volatilization as compared to the other sites (Figure 6-6). Why this site has significantly higher rates of volatilization than the other sites is still unknown. All sites had similar pH, moisture content, and bulk density values and were exposed to the same climatic environment (Table 6-1) all of which are factors that can result in elevated volatilization rates (Martin and Reddy 1997). As a result of high rates of potential loss in conjunction with a dilution effect, both the native and the restored wetland soils retained significantly less ^{15}N as compared to other studies. Recovery rates as high as 38% after 7 years were found in a salt water tidal marsh (White and Howes 1994) and rates between 62-75% were found after 300 days for a forested system (Templer et al. 2005), whereas in this study, after 365 days we only recovered 13-42% of the ^{15}N applied.

Studies have shown that the microbial community can immobilize a considerable amount of ^{15}N tracers that are applied (Schimel 1988, Templer et al. 2003). Our results demonstrates that the microbial biomass communities greatly influence soil N retention in these restored wetland ecosystems via immobilization. After 24 hours of application, the microbial biomass had immobilized on average 25-30% of the ^{15}N that was applied in these systems. In addition to microbial immobilization, a considerable amount of the $^{15}NH_4^+$ was nitrified to $^{15}NO_3^-$ in the

native and 1989 sites within the first 24 hours (approximately 17-24%) demonstrating that nitrification rates are high during the dry season for these two sites (Table 6-3). This can have considerable consequences on long term retention of N in wetland systems. Nitrate is highly susceptible to leaching and denitrification; therefore N could be permanently lost from the system. Conversely, either rates of nitrification were not as high in the 1997 and 2003 sites or there was significantly more loss via leaching and denitrification in these two sites. In fact, more ^{15}N remained in the $^{15}NH_4^+$ pool than what was nitrified and stored in the $^{15}NO_3^-$ pool for the 2003 restored wetland. In the 1997 site, the elevated rates of volatilization could also be contributing to this difference observed in potential nitrification.

From estimations of N mineralization, we found that in general the restored sites had greater levels of PMN activity (Figure 6-5). This indicates that the microbial communities are actively acquiring more N in the restored wetlands than in the native communities. This is an indication that N is more limiting in the restored wetlands and in response the microbes need to mineralize more N to meet their demands. This would result in more N being transformed to labile forms and increase potential losses of the N from the system.

Plant ^{15}N Retention

Our hypothesis that plant communities in the native site will acquire more ^{15}N from the soil than plant communities in the restored systems is not fully supported. Community level biomass does not have a significant influence on total ecosystem N retention. While plant community level biomass production was different among all sites (Table 6-6), the amount of N and ^{15}N retained in the community level biomass was not significantly different (Figure 6-8). However, individual plant species are evidently important, since species level differences were found between *S. nigricans*, *C. jamaicense*, and *T. domingensis* all three of which are dominant

species in their respective sites. This indicates that shifts in species dominance could result in changes in overall N retention in the system.

In addition to differences quantified between species, we found some evidence of differences within a single species at different sites. At 168 days after application of ^{15}N, *T. domingensis* present in the 1997 site acquired approximately 4 times more ^{15}N than *T. domingensis* present in the 1989 or 2003 site indicating site influences on ^{15}N uptake at the species level. However, this difference was not as apparent for *T. domingensis* in the 1997 and 2003 sites after 365 days. Additionally, the amount of N and ^{15}N found in *T. domingensis* was significantly less during the dry season (365 days) as compared to the wet season (168 days). The wet season is when wetland plants are most active. It comes to reason that more N would be incorporated into biomass during this time period due to increased availability and plant growth.

In the 2003 site, *T. domingensis* is the dominant plant species; however, it has retained four times less ^{15}N into biomass than *S. sempervirens* which contributes approximately 50% less in biomass production. Furthermore, *T. domingensis* in the 2003 sites produced 40% less above-ground biomass compared to *T. domingensis* in the 1997 site. Consequently, the amount of ^{15}N retained in *T. domingensis* in the 2003 site was 60% less than in the 1997 restored wetland (Table 6-6). Evidently, *T. domingensis* present in the 2003 site is not as good of a competitor for N as it is in the 1997 site. While the 1997 and 2003 sites contain several shared species, the relative abundance of each is different. The two most noticeable differences are the abundance of *S. sempervirens* and *Baccharis* spp. which contribute 28 and 8% to the above-ground biomass, respectively (Table 6-6). The amount of biomass produced by *S. sempervirens* in the 2003 site is 99% greater than that in the 1997 site; additionally *Baccharis* spp. is not present in the 1997 site. Therefore, *T. domingensis* dominating the 1997 site is not competing for N against these two

species. In the 2003 site, however, both *S. sempervirens* and *Baccharis* spp. have retained more ^{15}N into biomass compared to *T. domingensis* indicating they are out competing *T. domingensis* for N.

This behavior was not observed for *C. jamaicense* and *S. nigricans*. Both species had similar amount of N during both the wet and dry season. Furthermore, *C. jamaicense* acquired comparable amounts of ^{15}N in both the native and 1989 sites. This indicates that the 1989 site plant community is shifting to a functionally similar plant community found in the native site. Not only is *C. jamaicense* the dominant species in the 1989 site, no signs of *T. domingensis* was found at the end of this study (day 365) (Table 6-6). *Typha domingensis* was present during the wet season sampling but the overall contribution to above-ground biomass and ^{15}N retention was small (Figure 6-6).

Plant species influence on above-ground biomass ^{15}N retention did not influence community level biomass ^{15}N retention. Plant community structure did not impact community level influences on ecosystem N retention. But rather community level above-ground biomass was influenced by soil ^{15}N retention (Figure 6-10). Several studies have reported that plant species richness results in greater ecosystem N retention (Naeem et al. 1994, Tilman et al. 1996). However, in this study we found that the 1997 site had the highest species richness but the lowest ecosystem ^{15}N retention. The 1997 site had the highest above-ground biomass production and biomass N storage above all other sites. The biomass in the 1997 site was approximately 1.5 times greater than the native community, yet we found 1.5 times less ^{15}N retained in the biomass in this site. This implies that species richness had no influence on ecosystem ^{15}N retention. This is further supported by a similar study conducted in grassland ecosystems where they found that

communities with greater functional group diversity yielded lower ^{15}N retention than the communities with only one functional group present (Epstein et al. 1998).

Conclusions

Our results indicate that soil processes have a greater influence on ecosystem N retention than does the plant community composition. The soil in the native community retained significantly more ^{15}N compared to the restored wetland ecosystems. This evidence supported our first hypothesis which stated that the native community soil pools would store more N over time than the restored wetland soil pools. This was not surprising since the restoration technique employed by the ENP to remove all the soil was a severely destructive means of restoration. As a result, the restored wetlands had considerably less N and decreased N availability. Consequently, the microbial activity in the restored wetlands was elevated relative to the native community which demonstrated that the microbial biomass communities greatly influence soil N retention in these restored wetland ecosystems.

Vegetation community level ^{15}N retention differences were insignificant across sites indicating that regardless of soil ^{15}N retention the community level vegetation was able to acquire similar amounts of N from the soil. The plant communities inhabiting the restored wetlands benefited from the elevated microbial activities which resulted in higher levels of mineralized N. Accordingly, the vegetation communities in the restored sites were not more N-limited than the community in the native site.

However, species level effects on ^{15}N retention were significant. The amount of biomass produced by *S. sempervirens* in the 2003 site is 99% greater than that in the 1997 site; additionally *Baccharis* spp. is not present in the 1997 site. *Typha domingensis* dominated the 2003 site, however, both *S. sempervirens* and *Baccharis* spp. (which contributed significantly

less to above-ground biomass production) have retained more ^{15}N into biomass compared to *T. domingensis* indicating they are out competing *T. domingensis* for N.

There are indications of restoration success in the 1989 restored wetland. *Cladium jamaicense* acquired comparable amounts of ^{15}N in both the native and 1989 sites. This indicates that the 1989 site plant community is shifting to a functionally similar plant community found in the native site. Additionally, *C. jamaicense* the dominant species in the 1989 site, and no signs of *T. domingensis* was found in this site at the end of the study.

Table 6-1. Summary of soil physio-chemical parameters at the start of the tracer study. (n=10)

	Native	1989	1997	2003
Parameter	Ave SE	Ave SE	Ave SE	Ave SE
Physical				
pH	7.6 (0.03)	7.6 (0.02)	7.7 (0.02)	7.6 (0.03)
Moisture Content (%)	46.7 (0.40)	56.2 (0.84)	51.4 (0.93)	40.4 (0.82)
Bulk Density (g cm^{-3})	0.4 (0.03)	0.3 (0.02)	0.3 (0.01)	0.3 (0.01)
Soil Depth (cm)	5.0	2.7 (0.13)	1.0 (0.08)	1.3 (0.08)
LOI (%)	18.5 (0.64)	23.9 (1.01)	22.4 (0.76)	17.0 (0.46)
Chemical				
Total C (g kg^{-1})	155.6 (0.68)	169.9 (2.83)	158.2 (2.72)	151.5 (2.13)
Total N (g kg^{-1})	9.5 (0.13)	10.4 (0.28)	9.4 (0.26)	7.8 (0.19)
Total P (g kg^{-1})	0.2 (0.01)	0.6 (0.02)	0.7 (0.05)	0.8 (0.01)
TOC (mg kg^{-1})	528.6 (21.01)	858.2 (28.09)	940.5 (63.25)	971.8 (69.84)
Extractable TKN (mg kg^{-1})	115.1 (5.93)	184.4 (10.33)	180.1 (14.35)	191.3 (23.05)
NO_3-N (mg kg^{-1})	19.7 (0.66)	32.8 (2.69)	68.3 (5.36)	7.7 (0.53)
NH_4-N (mg kg^{-1})	25.7 (2.81)	38.7 (7.21)	44.8 (6.14)	79.3 (15.96)

Table 6-2. Summary of vegetation community and litter layer chemical parameters at the start of the tracer study. (n=10)

	Native		1989		1997		2003	
Parameter	Ave	SE	Ave	SE	Ave	SE	Ave	SE
Above-ground								
Biomass (g m^{-2})	253.3	(6.8)	186.4	(8.5)	221.9	(10.8)	136.9	(6.1)
Total C (g kg^{-1})	435.6	(20.5)	431.5	(11.6)	417.6	(17.5)	432.9	(23.1)
Total N (g kg^{-1})	9.4	(1.1)	10.6	(0.1)	11.0	(2.2)	6.2	(0.9)
Total P (g kg^{-1})	0.3	(0.01)	0.3	(0.01)	0.4	(0.02)	0.4	(0.02)
C:N	46.9	(6.8)	41.3	(5.4)	39.9	(1.0)	70.6	(9.1)
C:P	1723.4	(103.6)	1500.2	(79.0)	1382.9	(54.0)	1200.4	(49.6)
N:P	37.7	(2.3)	35.8	(1.6)	34.8	(1.0)	16.8	(0.6)
Litter Layer								
Pool Size (g m^{-2})	96.9	(26.5)	54.1	(4.1)	100.4	(5.1)	157.7	(6.6)
Total C (g kg^{-1})	374.1	(2.8)	363.9	(2.5)	361.0	(2.2)	368.1	(6.5)
Total N (g kg^{-1})	11.2	(0.2)	13.3	(0.2)	12.9	(0.1)	13.1	(0.2)
Total P (g kg^{-1})	0.06	(0.003)	0.2	(0.004)	0.2	(0.009)	0.2	(0.008)

Table 6-3. Percent of total ^{15}N as NH_4Cl recovered after 24 hours of application. (n=3)

Parameters (%)	Native	1989	1997	2003
Bulk Soil	73.1	44.1	36.4	41.5
Soil Pools				
NO_3-N	23.5	17.7	9.1	1.1
NH_4-N	16.4	1.4	4.5	3.3
MBN	31.4	24.2	22.2	25.0
Org-N	1.8	0.8	0.6	12.2
Litter Pool	5.5	2.3	3.5	8.5
Vegetation Pools*	n.d.	n.d.	n.d.	n.d.
Percent Recovered	78.7	46.4	39.9	50.0
Un-accounted	21.3	53.6	60.1	50.0

*n.d. - Not determined for 24 hours after application

Table 6-4. Percent of total ^{15}N as NH_4Cl recovered after 365 days of application. (n=3)

Parameters (%)	Native	1989	1997	2003
Bulk Soil	33.9	19.6	5.7	18.5
Soil Pools				
NO_3-N	0.37	0.09	0.02	0.02
NH_4-N	0.07	0.04	0.01	0.08
MBN	11.3	11.5	1.2	2.3
Org-N	22.2	8.0	4.4	16.1
Litter Pool	1.4	1.6	1.9	4.3
Vegetation Pools				
Above-ground Biomass	5.5	3.1	3.8	4.7
Below-ground Biomass	0.8	1.3	1.1	1.5
Percent Recovered	41.5	25.6	12.5	29.0
Un-accounted	58.5	74.4	87.5	71.0

Table 6-5. Summary of results from a two-way ANOVA test with dependant variables of total nitrogen (N) and ^{15}Nitrogen (^{15}N) pools.

Test	Variable	d.f.	F-stat	P-value
Sites Differences				
Species-level (168 days)				
	Total N			
	Above-ground	3	36.8	<0.0001
	Below-ground	3	2.9	0.0790
	^{15}N			
	Above-ground	3	3.8	0.0389
	Below-ground	3	6.9	0.0066
Community-level (365 days)				
	Total N			
	Above-ground	3	2.4	0.1429
	Below-ground	3	1.4	0.3221
	^{15}N			
	Above-ground	3	0.7	0.5564
	Below-ground	3	1.2	0.3624
Above- vs Below-ground				
	Total N			
	Native	3	12.2	0.0250
	1989	3	0.5	0.5326
	1997	3	4.6	0.0976
	2003	3	10.3	0.0326
	^{15}N			
	Native	3	10.9	0.0298
	1989	3	1.4	0.3062
	1997	3	7.0	0.0567
	2003	3	5.2	0.0855
Plant Fractions				
	Total N			
	Native	3	0.4	0.7401
	1989	3	3.8	0.0582
	1997	3	1.8	0.2211
	2003	3	5.9	0.0197
	^{15}N			
	Native	3	2.8	0.1055
	1989	3	0.2	0.9140
	1997	3	2.4	0.1424
	2003	3	2.2	0.1718

Table 6-6. A summary of individual species biomass, nitrogen and ^{15}N data for each site. Species are ordered from most to least abundant. (n=3)

Species	Biomass ($g\ m^{-2}$)	SE	Total N ($mg\ m^{-2}$)	SE	Total ^{15}N ($mg\ m^{-2}$)	SE	% Applied ^{15}N % ^{15}N	SE	% Plant Total % ^{15}N
Native									
Schoenus nigricans	153.1	(15.3)	1033.3	(67.4)	22.7	(2.0)	3.50	(0.31)	63.8
Cladium jamaicense	78.2	(20.3)	542.1	(142.1)	10.6	(3.0)	1.63	(0.46)	29.7
*Poaceae (10)	15.0	(6.4)	105.2	(40.2)	2.1	(0.8)	0.32	(0.13)	5.9
Cassytha filiformis	1.5	(0.8)	10.6	(6.1)	0.1	(0.1)	0.02	(0.01)	0.3
Centella asiatica	0.6	(0.3)	6.9	(4.0)	0.1	(0.1)	0.02	(0.01)	0.3
Total	248.3		1698.0		35.6		5.48		
1989									
Cladium jamaicense	69.4	(20.4)	472.2	(123.4)	13.37	(5.4)	2.06	(0.83)	65.6
*Poaceae (3)	21.7	(1.0)	172.2	(12.5)	2.56	(0.1)	0.39	(0.02)	12.6
Centella asiatica	5.7	(0.9)	93.6	(14.2)	1.29	(0.1)	0.20	(0.02)	6.3
Eupatorium capillifolium	5.3	(3.1)	96.3	(55.6)	1.31	(0.8)	0.20	(0.12)	6.4
Sagittaria lancifolia	2.7	(1.5)	31.4	(18.1)	0.80	(0.5)	0.12	(0.07)	3.9
Sarcostemma clausum	2.2	(0.3)	36.7	(8.7)	0.42	(0.1)	0.06	(0.02)	2.0
Ludwigia repens	2.0	(0.7)	21.1	(6.8)	0.26	(0.1)	0.04	(0.01)	1.3
Axonopus furcatus	1.6	(0.5)	8.0	(5.3)	0.08	(0.1)	0.01	(0.01)	0.4
Unidentified	1.4	(0.6)	22.3	(8.1)	0.30	(0.2)	0.05	(0.03)	1.5
Total	111.9		953.8		20.38		3.14		
1997									
Typha domingensis	199.8	(40.4)	853.2	(176.9)	9.8	(1.5)	1.5	(0.23)	39.9
Andropogon spp.	57.9	(33.4)	397.4	(229.5)	8.0	(4.6)	1.2	(0.71)	32.9
*Poaceae (3)	23.5	(6.4)	182.9	(49.9)	2.3	(0.6)	0.3	(0.09)	9.2
Unidentified	19.9	(1.4)	145.8	(14.3)	2.0	(0.2)	0.3	(0.03)	8.0
Sarcostemma clausum	14.0	(4.3)	180.3	(56.9)	1.4	(0.6)	0.2	(0.10)	5.6
Sagittaria lancifolia	3.0	(1.7)	39.8	(23.0)	0.4	(0.2)	0.06	(0.04)	1.7
Centella asiatica	1.9	(1.1)	37.0	(21.3)	0.4	(0.2)	0.06	(0.04)	1.6
Ludwigia peruviana	1.6	(0.9)	15.1	(8.7)	0.07	(0.04)	0.01	(0.01)	0.3
Ludwigia repens	1.2	(0.3)	10.3	(2.5)	0.08	(0.02)	0.01	(0.003)	0.3
Hypericum myrtifolium	0.8	(0.5)	5.0	(2.9)	0.03	(0.02)	0.01	(0.003)	0.1
Axonopus furcatus	0.7	(0.2)	6.7	(1.4)	0.03	(0.005)	0.005	(0.001)	0.1
Solidago sempervirens	0.4	(0.2)	3.8	(2.2)	0.03	(0.02)	0.004	(0.003)	0.1
Hydrocotyle umbellata	0.2	(0.1)	2.1	(1.2)	0.01	(0.01)	0.002	(0.001)	0.05
Eupatorium capillifolium	0.2	(0.1)	2.2	(1.2)	0.02	(0.01)	0.003	(0.001)	0.07
Total	325.0		1881.5		24.5		3.8		
2003									
Typha domingensis	126.7	(23.0)	543.5	(95.4)	4.0	(0.7)	0.62	(0.1)	13.4
Solidago sempervirens	78.6	(14.9)	484.4	(86.0)	15.6	(4.2)	2.40	(0.6)	51.7
*Poaceae (2)	35.4	(13.2)	266.3	(72.1)	4.4	(1.2)	0.68	(0.2)	14.6
Baccharis spp.	21.6	(1.5)	131.1	(2.5)	4.3	(0.8)	0.66	(0.1)	14.2
Ludwigia peruviana	5.2	(3.0)	50.5	(29.2)	0.2	(0.1)	0.02	(0.01)	0.5
Ludwigia repens	4.9	(2.5)	49.3	(23.9)	0.8	(0.4)	0.12	(0.06)	2.5
Sagittaria lancifolia	3.4	(1.9)	29.5	(17.0)	0.4	(0.2)	0.06	(0.03)	1.2
Fuirena breviseta	2.3	(0.8)	11.7	(3.5)	0.1	(0.06)	0.02	(0.01)	0.5
Axonopus furcatus	1.7	(1.0)	12.4	(7.2)	0.2	(0.09)	0.02	(0.01)	0.5
Mikania scandens	1.4	(0.3)	14.4	(2.4)	0.2	(0.02)	0.03	(0.003)	0.6
Juncus megacephalus	0.9	(0.5)	6.0	(3.5)	0.06	(0.03)	0.01	(0.01)	0.2
Eupatorium capillifolium	0.4	(0.1)	3.9	(0.3)	0.03	(0.0004)	0.01	(0.0001)	0.1
Total	282.5		1603.0		30.2		4.6		

*Grasses could only be indentified to the family level. The number in ( ) is the number of difference species found.

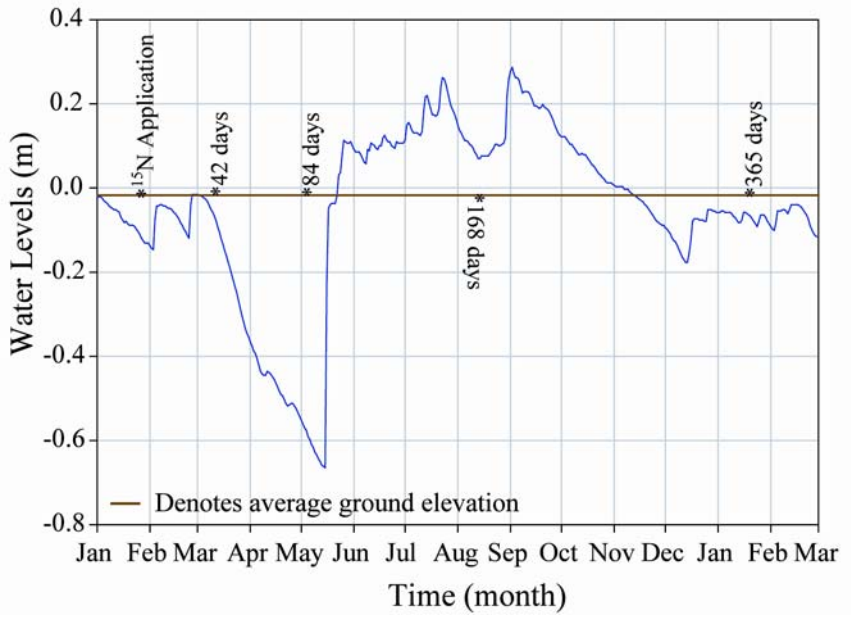

Figure 6-1. Hole-in-the-Donut hydroperiod for 2006 reported as groundwater level above NAVD 1988 (obtained from USGS National Water Information System).

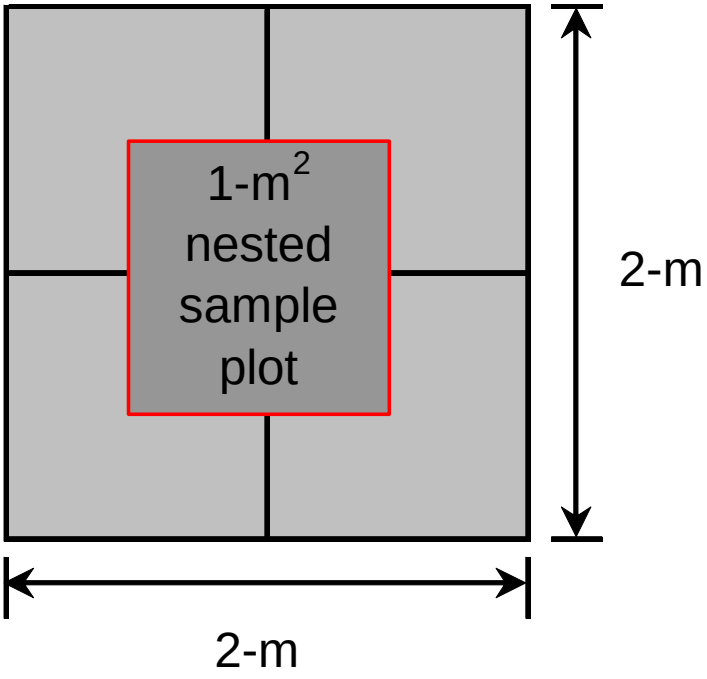

Figure 6-2. Plot layout for the application and sampling of ^{15}N tracer study.

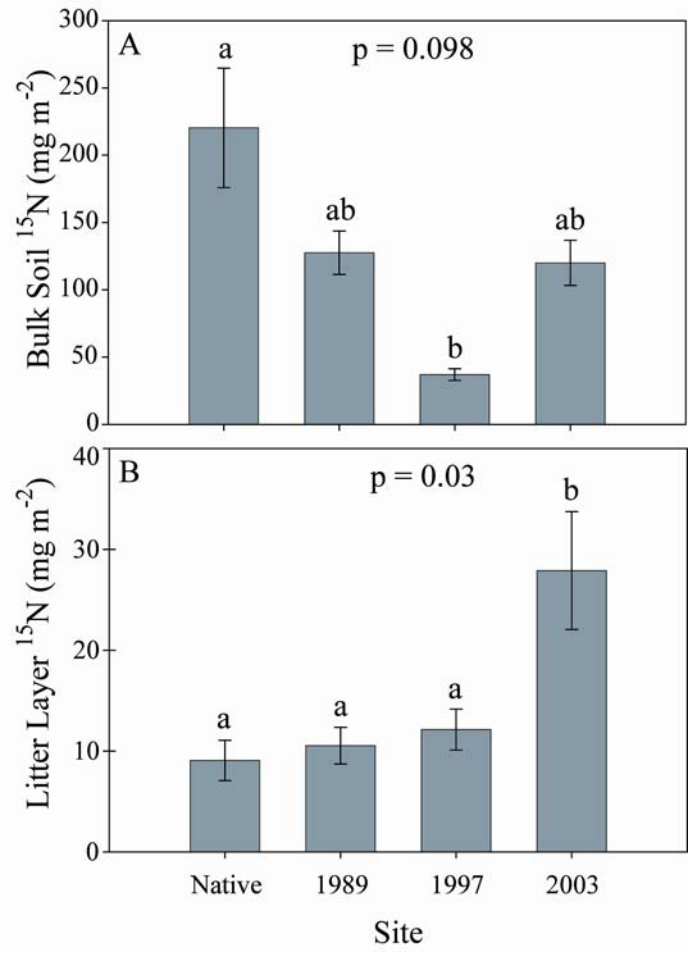

Figure 6-3. Total amount of ^{15}N applied remaining after 365 days. A) Bulk soil pool; d.f.=3, F=2.96, p=0.0975. B) Litter layer pool; d.f.=3, F=3.8, p=0.0300. Amount of ^{15}N remaining from initial 650 mg m^{-2} applied. (n=3)

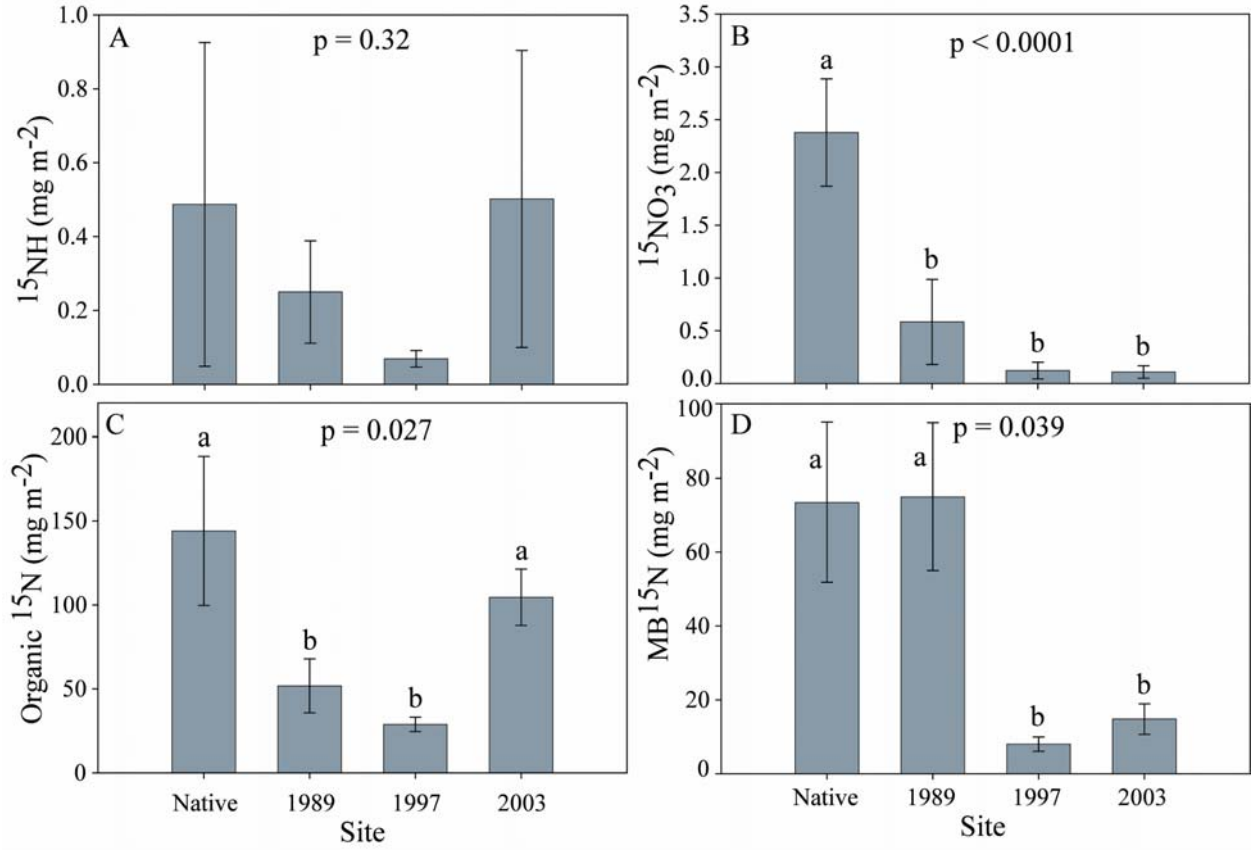

Figure 6-4. Total amount of ^{15}N applied remaining after 365 days. A) Ammonium (NH_4); d.f.=3, F=1.4. B) Nitrate (NO_3); d.f.=3, F=32.2. C) Organic nitrogen (ON); d.f.=3, F=10.8. D) Microbial biomass nitrogen (MBN); d.f.=3, F=4.1. Amount of ^{15}N remaining from initial 650 mg m^{-2} applied. (n=3)

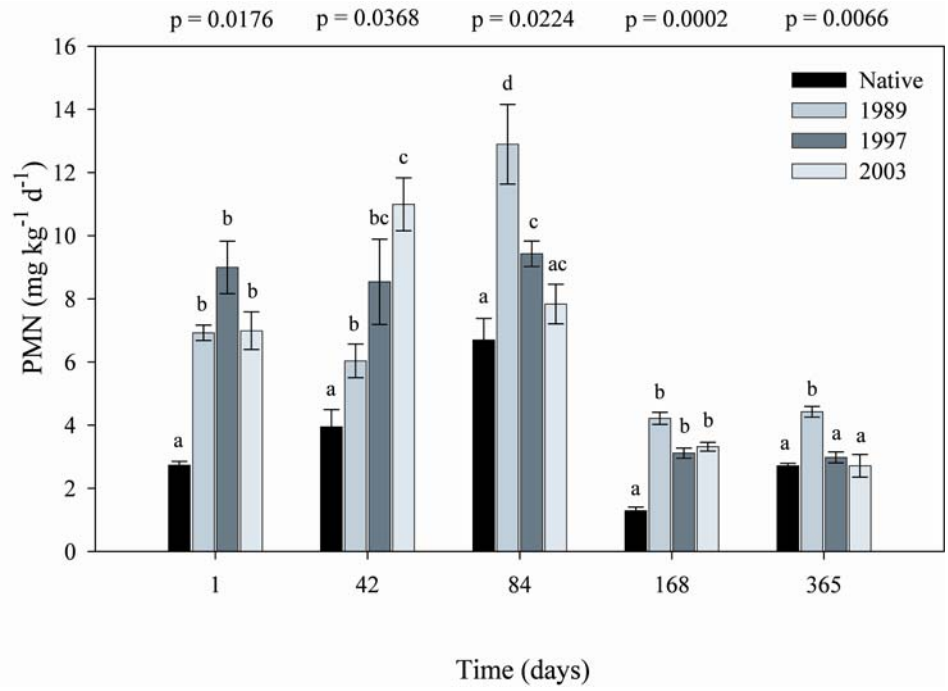

Figure 6-5. Potentially mineralizable nitrogen (PMN) reported as mg NH_4 kg^{-1} soil day^{-1} for each sample period and each site. The lower case letters indicate significant difference between sites within a given time periods. Appropriate p-values are given above the figure for each time; 1 – d.f.=3, F=4.3; 42 – d.f.=3, F=3.8; 84 – d.f=3, F=11.8, 168 – d.f.=3, F=10.6; 365 – d.f.=3, F=6.9. (n=6)

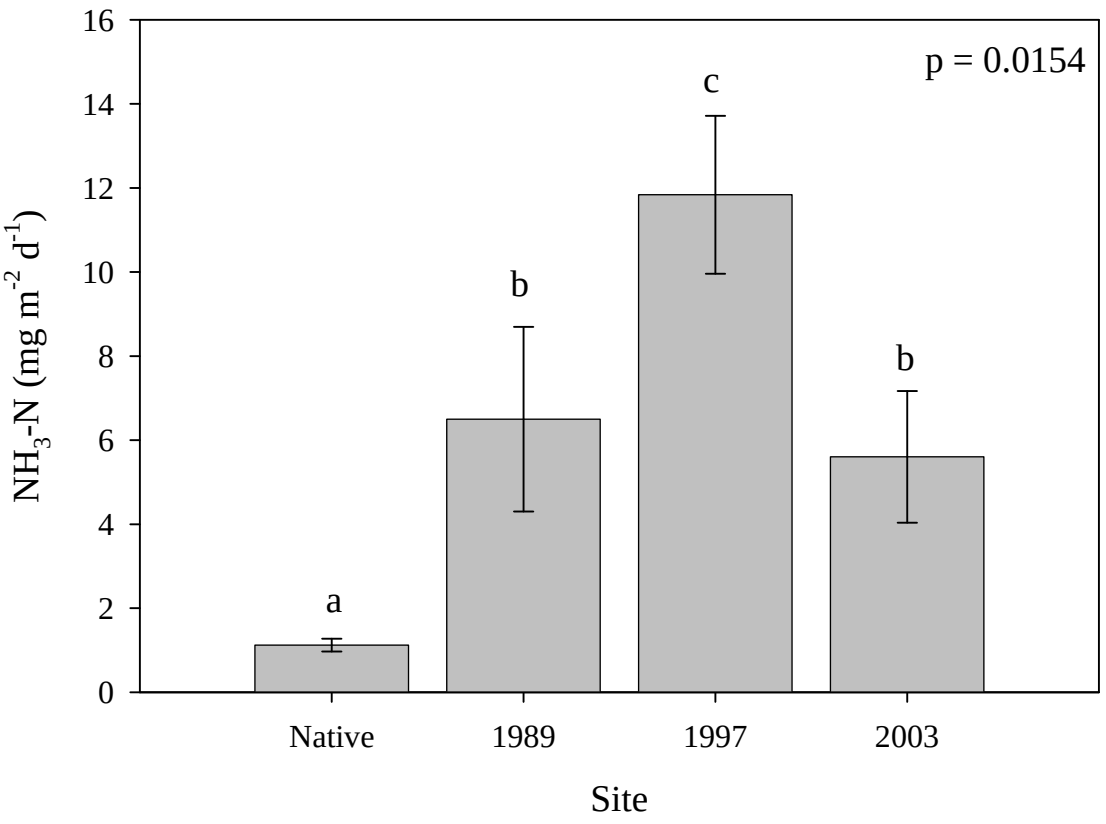

Figure 6-6. Estimates of volatilization rates of NH_3-N in native and restored wetland communities; d.f.=3, F=5.9. (n=3)

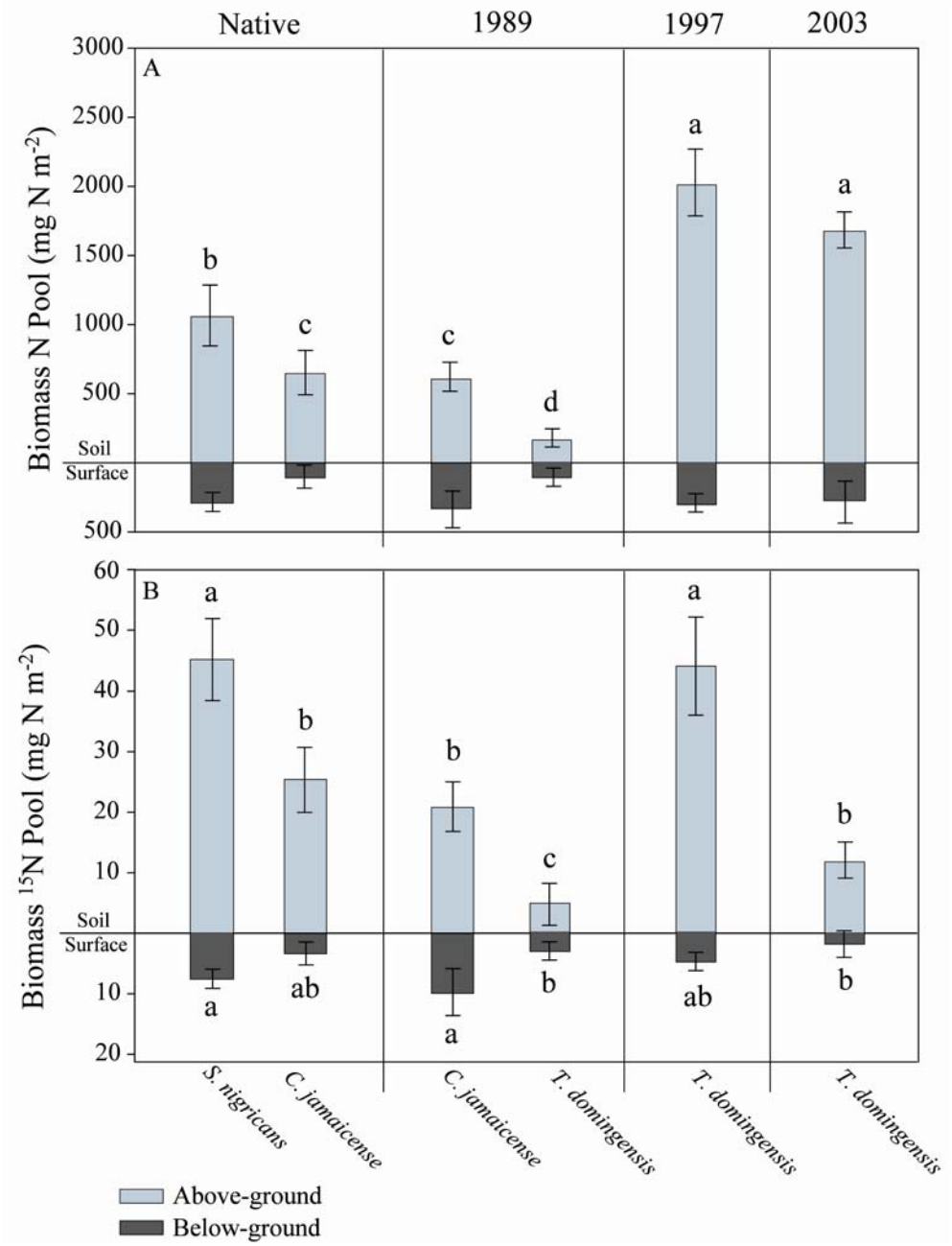

Figure 6-7. Amount of ^{15}N recovered in *Cladium jamaicense* and *Typha domingensis* in each site at 168 days after application. A) Species level nitrogen pool. B) Species level ^{15}Nitrogen pool; see Table 6-5 for statistics. (n=3)

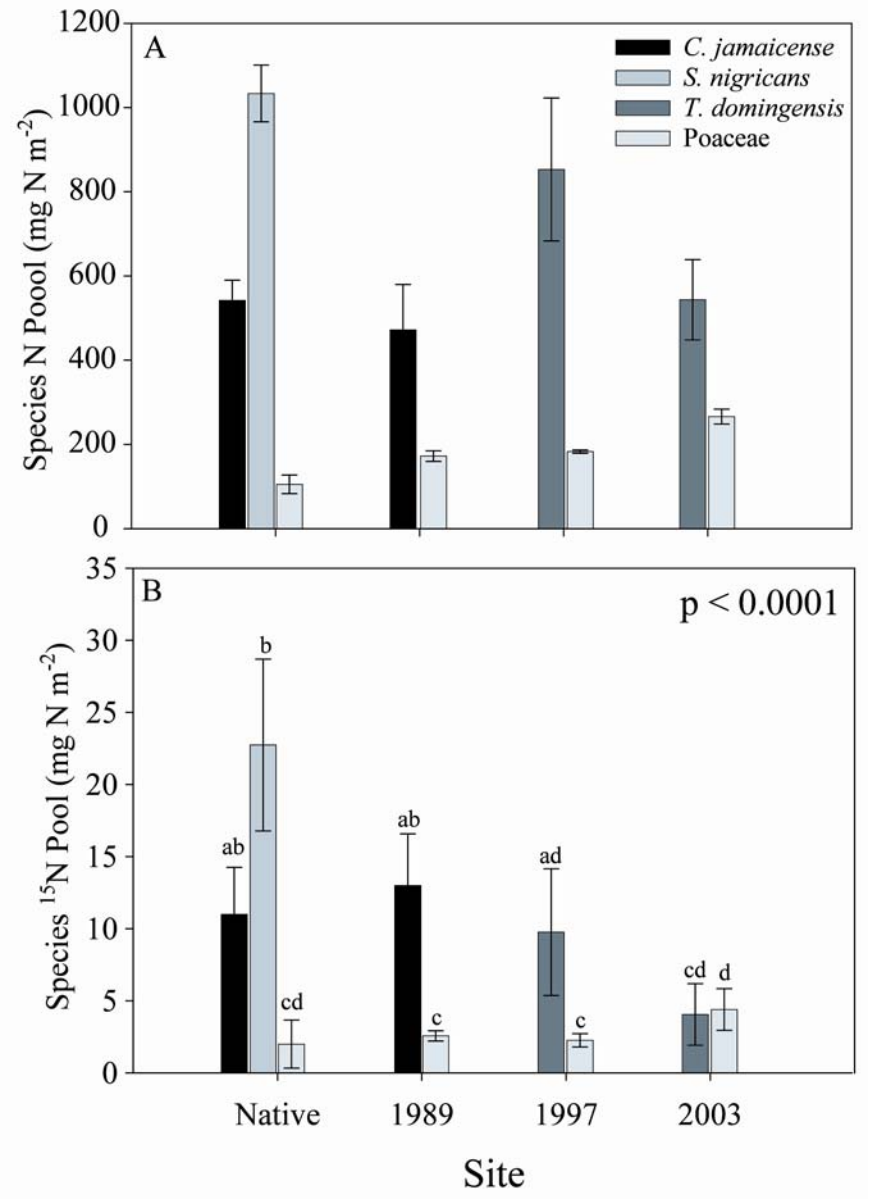

Figure 6-8. Amount of ^{15}N recovered in dominant vegetation species in each site after 365 days of application. A) Species biomass nitrogen pool. B) Species biomass ^{15}Nitrogen pool; d.f.=8, F=16.0. (n=3)

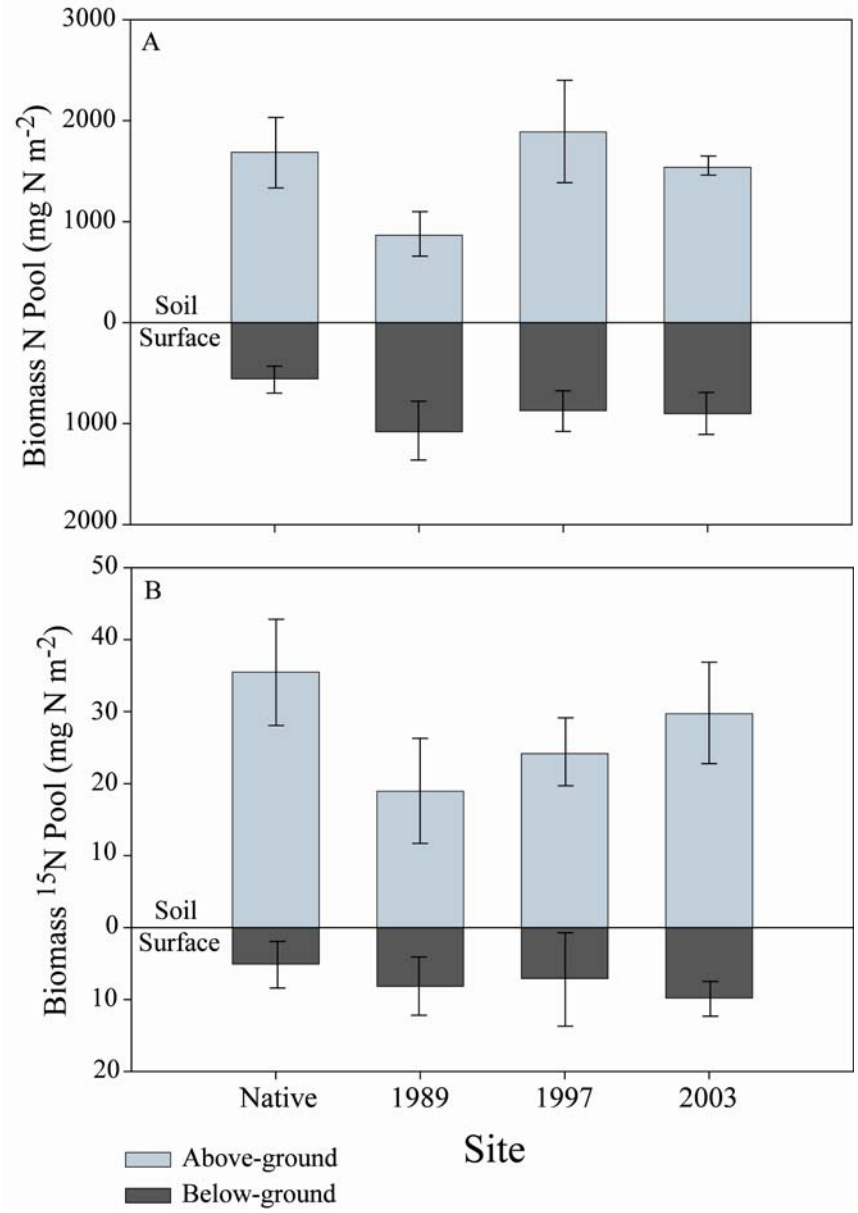

Figure 6-9. Amount of nitrogen stored in above- (live plus senescent) and below-ground biomass in each site at 365 days of the study. A) Nitrogen storage. B) ^{15}Nitrogen storage. (n=3)

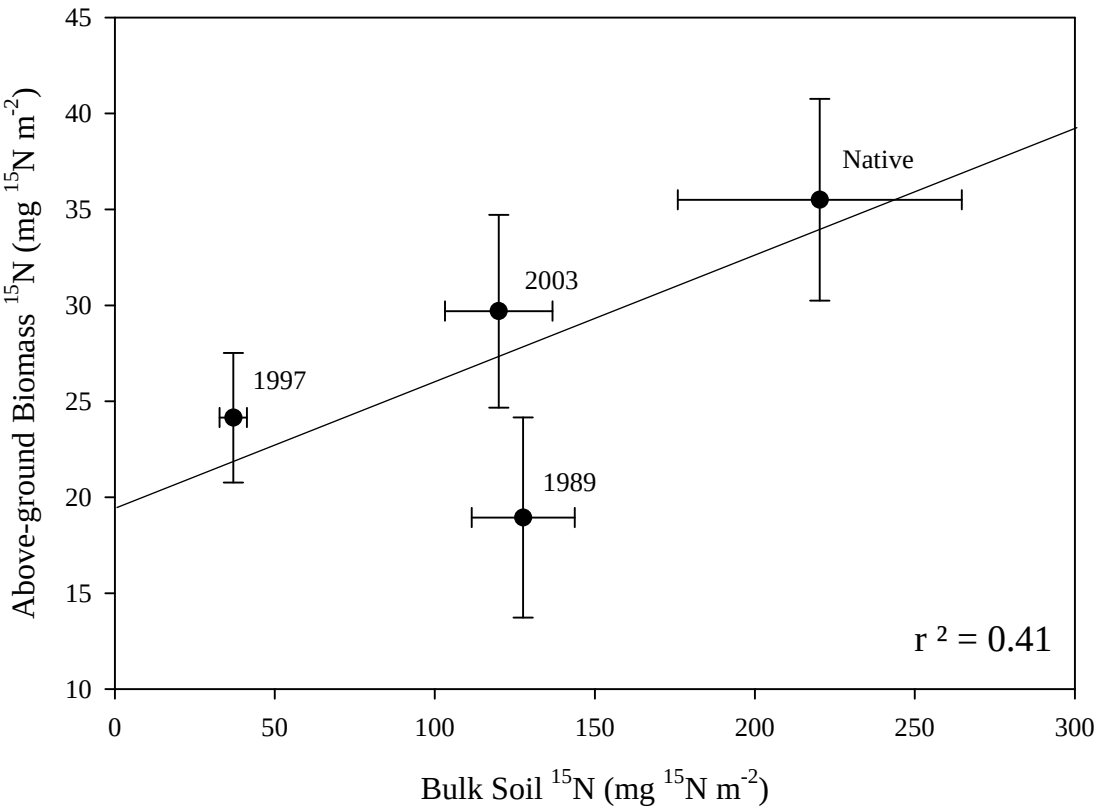

Figure 6-10. Relationship between the bulk soil ^{15}N retention and the above-ground biomass ^{15}N retention for each site. Values are plotted as averages in order to compare relationship between sites as well as the two parameters; d.f.=3, F=1.4, p=0.3597. (n=3)

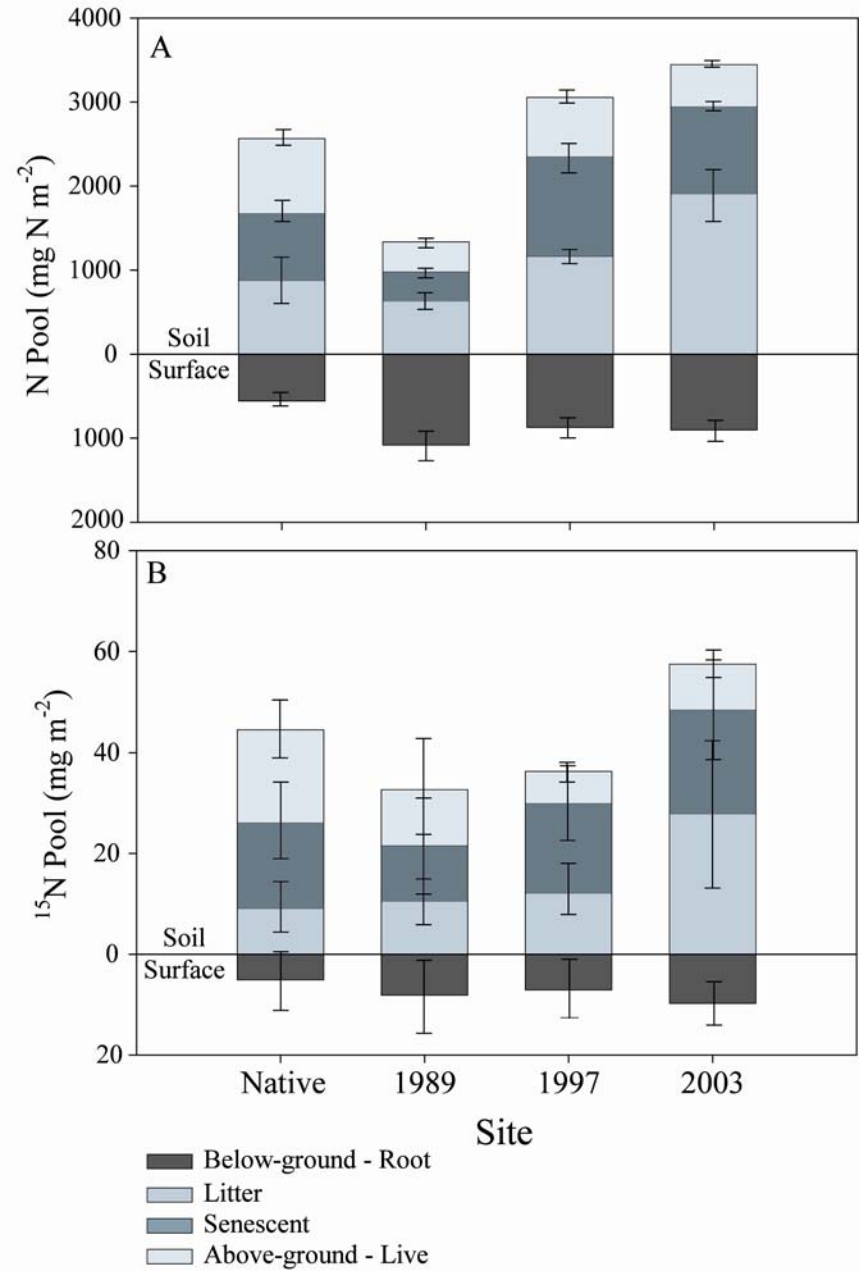

Figure 6-11. Amount of nitrogen stored in the vegetation community pools in each site after 365 days. A) Nitrogen pool. B) ^{15}Nitrogen pool. (n=3)

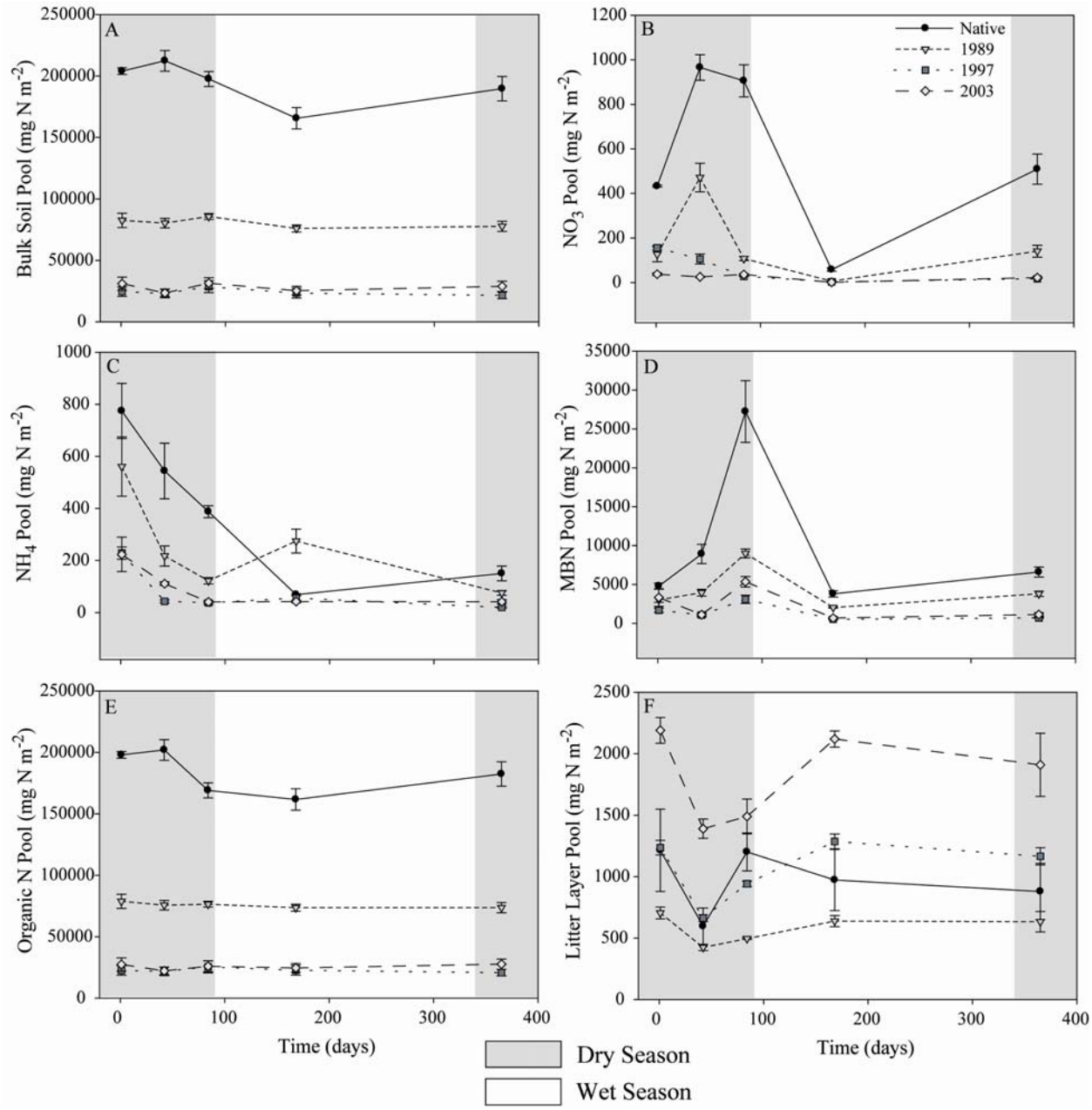

Figure 6-12. Changes in total nitrogen storages during the year long ^{15}N tracer study for each pool. A) Bulk soil nitrogen pool. B) Nitrate (NO_3) pool. C) Ammonium (NH_4) pool. D) Microbial biomass nitrogen (MBN) pool. E) Soil organic nitrogen (ON) pool. F) Litter layer pool. (n=3)

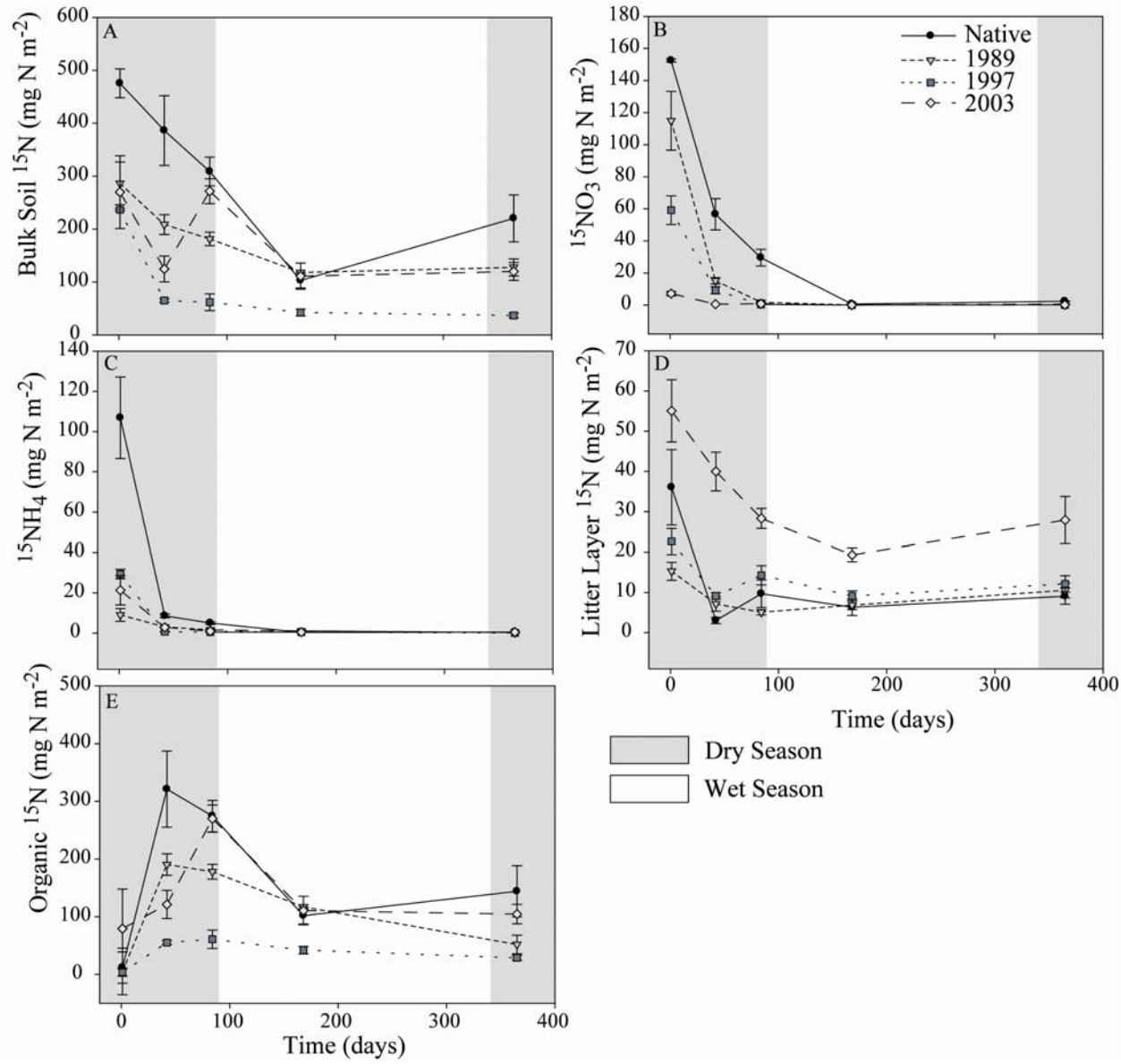

Figure 6-13. Changes in total ^{15}N nitrogen retention during the year long ^{15}N tracer study for each pool. A) Bulk soil nitrogen pool. B) Nitrate (NO_3) pool. C) Ammonium (NH_4) pool. D) Microbial biomass nitrogen (MBN) pool. E) Soil organic nitrogen (ON) pool. (n=3)

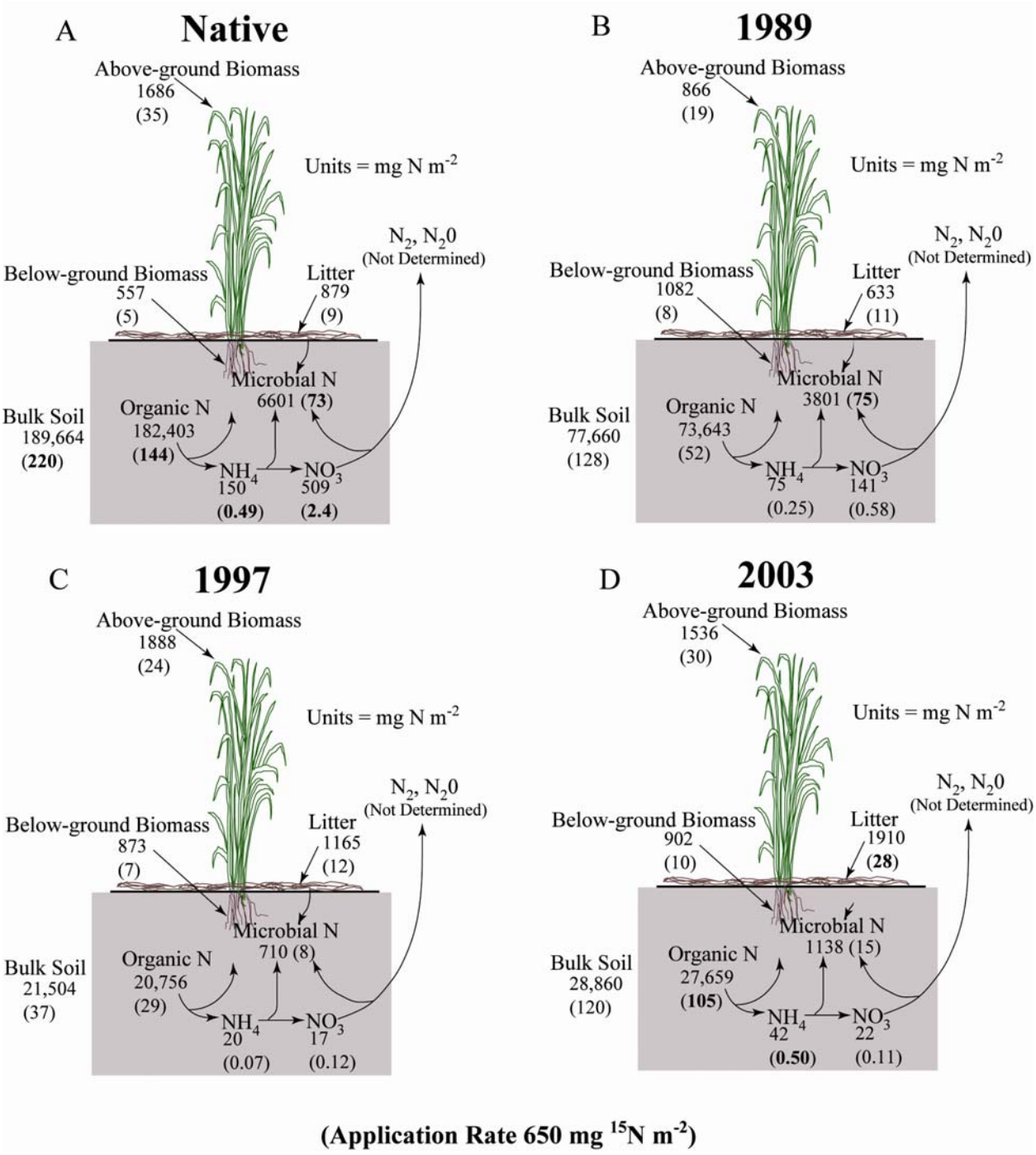

Figure 6-14. Total nitrogen and ^{15}N budget for each component of this study. The number in parenthesis is the amount of ^{15}N retained in each pool from the initial 650 mg m^{-2} applied. A) Native site. B) 1989 site. C) 1997 site. D) 2003 site.

CHAPTER 7
VARIATIONS IN THE NATURAL ABUNDANCE OF ^{15}N IN RESTORED SUBTROPICALWETLAND VEGETATION COMMUNITIES

Introduction

In most terrestrial systems, nitrogen (N) is the element that most limits plant productivity (Chapin et al. 2002). Many studies have been conducted which show relationships between N content in plants and their corresponding δ^{15}N value. If we can gain insight on the mechanisms controlling whole-plant and foliar N isotopic composition, we will advance our knowledge of plant N acquisition and allocation (Evans 2001). Very little work has been done to date which has investigated the relationship of δ^{15}N values of the vegetation under extremely P-limited conditions.

The δ^{15}N values of plant communities and individual species has been used to determine plant N assimilation and translocation (Handley and Scrimegeour 1997, Evans 2001). Past research has attempted to use δ^{15}N signatures of the plants and soil N pools to determine the N source to the plant communities (Fry 2006, Templer et al. 2007). Additionally, numerous studies have shown that plant δ^{15}N values and soil N pools or process rates are strongly correlated (Garten 1993, Jones et al. 2004, Templer et al. 2007). It has been argued that these correlations are found due to the microbial discrimination against the heavier isotope ^{15}N. The microbial community will discriminate against ^{15}N during several soil N processes such as nitrification (Nadelhoffer and Fry 1994, Högberg 1997), denitrification (Piccolo et al. 1996), and mineralization or decomposition (Nadelhoffer and Fry 1994). When discrimination occurs, the amount of ^{15}N in the product is relatively lighter than the amount of ^{15}N in the substrate form which it was formed. As a result, the substrate becomes enriched relative to the product produced.

Fractionation occurs during N processes in which the heavier isotope ^{15}N is discriminated against. Commonly studied soil processes like mineralization of organic N and nitrification of NH_4^+ will result in fractionation rates of 0-5 and 15-35 ‰, respectively (Robinson 2001). Denitrification cans result in fractionation rates between 28-33 ‰ while NH_4^+ volatilization fractionation rates can be as high as 60 ‰ (Robinson 2001). Microbial assimilation of NH_4^+ and NO_3^- can have fractionation rates of 14-20 and 13 ‰, respectively. The fractionation NH_4^+ and NO_3^- that occurs during plant assimilation ranges from 9-18 and 0-19 ‰, respectively (Robinson 2001).

As a result of the numerous possibilities for fractionation during the cycling of N, it becomes very difficult to use the natural abundance of ^{15}N as a tracer to determine N sources for plant uptake and assimilation. Therefore, it has been suggested that we cannot infer that the isotopic signatures of various ecosystem pools relate to each other (Handley and Scrimegeour 1997). In spite of these shortcomings, we believe we can utilize seasonal and temporal changes in plant $\delta^{15}N$ values along with changes in soil N pools (i.e. NH_4^+ and NO_3^- pools sizes) and what we know about fractionation rates to better understand how shifts in N cycling potentially affect plant N availability and uptake.

This paper reports the natural abundance of ^{15}N of the vegetation at the community and species level for restored wetland communities and how they compare to the native undisturbed communities. We also investigated seasonal and temporal variations in $\delta^{15}N$ values for plant communities and how they relate to changes in soil N pool sizes (i.e. NH_4^+ and NO_3^-). Additionally, we investigated whether natural abundance ^{15}N was driven by a potential P-limitation which persists in the native community and how the altered nutrient dynamics in the resorted wetlands may contribute to differences in $\delta^{15}N$ values of the vegetation.

Methods

Site Description

This study was conducted in wetland systems restored within the Hole-in-the-donut region of the Everglades National Park. Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season (Figure 7-1). The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

Soil Analysis

In April (dry season) and July (wet season) of 2005, soil and vegetation samples were collected with a 7.6 cm diameter PVC core from 10 plots randomly distributed throughout sites restored in 1989 (16), 1997 (8), 2001 (4), 2003 (2), and 2004 (1) as well as the surrounding native communities. The number in parentheses indicates the number of years since restoration was completed at the time of this study. Elevation was kept constant at 0.5 m to eliminate hydrology differences as a driving factor of nutrient availability.

The soil cores were transported to the laboratory and stored at 4°C until analysis. Before analyses were performed all rocks, roots, and litter material was removed for the soil samples. Within 24 to 48 hours of sample collection, each soil sample was extracted for ammonium (NH_4^+) and nitrate (NO_3^-) with K_2SO_4 (Bundy and Meisinger 1994) and set up for incubation for potentially mineralizable nitrogen (PMN) (or biologically available N) (Keeney 1982, Bundy and Meisinger 1994, White and Reddy 2000). Soil extracts were analyzed via flow injection analysis

with a Bran Luebbe Auto Analyzer 3 Digital Colorimeter (EPA Method 350.1). A subsample of the K_2SO_4 extract was digested for total kjeldahl nitrogen (TKN) via kjeldahl block digestion and analyzed by flow injection with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 351.1). A subsample of each soil was dried at 60°C for 3 days then ground with a ball grinder to a fine powder for total N and P analysis. Dry soil samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

Nitrogen and P were determined for both the composite biomass (community level) and selected individual plant species (species level) within 10, 1 m^2 plots in each site. To determine nutrients in composite biomass, all the vegetation in a 1 m^2 plot was cut at the soil surface, separated by live and senescent plant tissue, bulked and dried at 70°C until all moisture was removed. Once dry, all vegetation from each plot was passed through a Wiley Mill tissue grinder equipped with a 2-mm mesh screen to achieve homogeneity. A subsample was ball ground to a fine powder for N and P analysis. Individual plant species of *Cladium jamaicense*, *Schoenus nigricans, Typha domingensis,* and *Sagittaria lancifolia* were collected from each site near each plot when available. In some cases, these species were not always present. Plant fractions of live, senescence, and litter were collected for each species. The samples were dried at 70°C until all moisture was removed and ground with a Wiley Mill tissue grinder equipped with a 2-mm mesh screen. A subsample was ball ground to a fine powder. All plant samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+

Automated Discrete Analyzer (EPA Method 119-A rev3). Nitrogen and P ratios were calculated on a mass basis as N:P.

The isotope analysis of soil and vegetation samples was performed via isotope ratio determination with a Thermo Finnigan MAT Delta Plus XL Mass Spectrophotometer equipped with a Costech Instrument Elemental Analyzer for flash combustion of solid material for N and C analysis. The $\delta^{15}N$ results were reported in δ-notation as the deviation from the international standards (air):

$$\delta^{15}N\ (‰) = [(R_{sample} / R_{standard}) - 1] * 1000 \qquad (7\text{-}1)$$

where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratio of the samples and standard, respectively. Replicate anlaysis of both random soil and vegetation samples resulted in a precision of <0.1 ‰. Reference materials from the National Institute of Standards and Technology (NIST) were used for accuracy (ammonium sulfate standards N1 and USGS25) as well as NIST standard material peach.

Statistical Analysis

All data collected were analyzed statistically using Fit Model in JMP Version 5.1 (SAS 2005). Analysis of variance (ANOVA) and regressions were performed to investigate site and seasonal relationships in $\delta^{15}N$ values and plant N, P and N:P ratios and differences in soil NH_4^+ and NO_3^-. Stepwise regressions were performed to determine which variables most significantly explained variability in $\delta^{15}N$ values of the community level vegetation. Variables included in stepwise regression were soil and plant N, P, N:P ratios, soil extracts of NH_4^+, NO_3^-, TKN, and PMN. Multiple regressions were performed with the variables determined significant from the stepwise regression.

Results

The $\delta^{15}N$ values of the soil during the wet season ranged from -1 to 3 for all the sites. A relationship was not found between the N:P ratios and the $\delta^{15}N$ values for the soil ($r^2 = 0.0009$; Figure 7-2). However, the native community had a higher N:P ratio compared to the restored wetlands indicating that the native community would be more P-limited.

The $\delta^{15}N$ values for the community level vegetation during the dry season was similar to the $\delta^{15}N$ values during the wet season, suggesting little change in N use with seasonal variability ($r^2 = 0.80$; Figure 7-3). Additionally, the native community level vegetation had significantly higher $\delta^{15}N$ values compared to the community level vegetation present in the restored wetlands. When combining all sites together, a relationship between the N:P and $\delta^{15}N$ values of the community level vegetation in the dry or wet season was not observed ($r^2 = -0.08$ and -0.24, respectively; Figure 7-4). However, when the sites were separated and investigated individually, we did find relationships between N:P ratios and $\delta^{15}N$ values in some of the sites. The $\delta^{15}N$ of the vegetation community during the wet season in the native, 1989, 1997, and 2004 sites were negatively correlated with N:P ratios ($r^2 = -0.74$, -0.38, -0.60, and -0.31, respectively; Figure 7-5). During the dry season a relationship between $\delta^{15}N$ and N:P ratios of the vegetation community of individual sites was only observed in the 2004 site ($r^2 = - 0.77$, Figure 7-6).

Individual plant species collected during he wet season from each site varied considerably in $\delta^{15}N$ values (Figure 7-7). The relationship between N and P content was the same for most species. *Schoenus nigricans* (a native plant species) had the lowest $\delta^{15}N$ value at -2.6 along with the lowest N (5.3 g kg^{-1}) and P (0.08 g kg^{-1}) content (Table 7-1, Figures 7-7a). *Sagittaria lancifolia* had the highest $\delta^{15}N$ value at 1.6 and her highest N and P content at 10.9 and 0.59 g kg^{-1}, respectively (Table 7-1, Figure 7-7a and b). *Cladium jamaicense, T. domingensis*, and *J.*

megacephalus all had similar $\delta^{15}N$ signatures and N content (Table 7-1), The P content was lower for *C. jamaicense* at 0.20 g kg-1 where *T. domingensis* and J. *megacephalus* had similar P content at 0.40 and 0.37 g kg^{-1}, respectively (Table 7-1). Poaceae and *Andropogon* spp. had similar N and P content and similar $\delta^{15}N$ values (Table 7-1).

Ammonium concentrations were significantly lower during the dry season for all sites except the 2004 restored wetland (Figure 7-8a). During the wet season, the 1989, 1997, 2001, and 2003 restored wetlands had significantly higher NH_4^+ concentrations than the native site, whereas the 2004 site had significantly lower NH_4^+ concentrations (Figure 7-8a). Nitrate concentrations were non-detectable during the wet season. During the dry season, the 1997 and 2001 site had significantly higher NO_3^- concentration as compared to all other sites (Figure 7-8b). The native and 1989 site had similar NO_3^- concentrations and the 2003 and 2004 site had similar NO_3^- concentrations that were significantly lower than all other sites (Figure 7-8b). TKN concentrations were not significantly different between sites during the dry season (Figure 7-8c). In general, TKN concentrations during the wet season were lower than dry season concentrations. During the wet season the 2004 site had significantly lower TKN concentrations than all other sites except for the native site (Figure 7-8c).

A stepwise regression resulted in plant P and soil NH_4^+ as the variables that most significantly predicted $\delta^{15}N$ values of the community level vegetation during the dry season (plant P p-value=0.005, r^2=0.89; NH_4^+ p-value=0.10, r^2=0.96). The $\delta^{15}N$ values of the wet season community level vegetation were most significantly predicted by plant P and soil TKN (plant P p-value=0.04, r^2=0.92; TKN p-value=0.07; r^2=0.89). Multiple regressions were performed with the variables that were selected from the stepwise regressions. The multiple regressions performed on the dry season data resulted in plant P and NH_4^+ explaining 96% of the

variation in $\delta^{15}N$ values (Figure 7-9a), while the wet season variables (plant P and TKN) explained 92% of the variation (Figure 7-9b).

Discussion

The variability in isotopic signatures for both the community level and species level vegetation indicate that plant species within and across sites potentially access different sources of N and their use of N may result in different fractionation rates during N assimilation, transfer, and translocation processes. While differences were observed between sites, wet and dry seasonal variation in the community level vegetation $\delta^{15}N$ values was minimal. This indicates that community level vegetation isotopic signature of N was relatively unchanged with changes in hydrology. Since the presence of different chemical species of N (i.e., NH_4^+ and NO_3^-) is controlled by hydrology in seasonally flooded wetlands (Martin and Reddy 1997, Hefting et al. 2004, Troxler Gann and Childers 2006), this indicates that the source of N to the vegetation may remain unchanged throughout the year even though NH_4^+ and NO_3^- concentrations may vary.

The non-detectable limits of NO_3^- concentrations during the wet season can be expected due to favorable conditions for denitrification (Figure 7-8). Competition for NO_3^- in wetlands is high and any NO_3^- present will quickly be taken up by microbes or plants (Jackson et al. 1989, Olsson and Falkengren-Grerup 2000, Henry and Jefferies 2002). The stepwise regression performed with this data indicated that the $\delta^{15}N$ signatures of the vegetation communities in this study were not dependent on NO_3^- concentrations found during the dry season. It has been shown that if nitrification is high then the $\delta^{15}N$ of the plants will be dependent upon NO_3^-, whereas if nitrification is low no relationship between plant $\delta^{15}N$ and NO_3^- will be observed (Koba et al. 2003). This indicates that the nitrification rates in the HID may be low and that the microbes may out-compete the plants for available NO_3^-.

Nitrification has been shown to be limited in wetland systems resulting in NH_4^+ being a more important source of N to plant communities (Högberg 1997). In this study, we found that the community level vegetation $\delta^{15}N$ was strongly dependent upon NH_4^+ during the dry season (r^2=0.96, p=0.10), whereas during the wet season, $\delta^{15}N$ was dependent on TKN (r^2=0.92, p=0.04). Mineralization can be limiting in wetland systems due to anaerobic conditions limiting microbial activity (Oomes et al. 1997, Bridgham and Richardson 2003), therefore during the wet season, NH_4^+ may be limiting due to decreased mineralization rates and as a result $\delta^{15}N$ would be less dependent on NH_4^+ alone. The strong relationship found between $\delta^{15}N$ and TKN indicates the vegetation may be utilizing organic forms of N during the wet season. Under conditions where inorganic forms of N are limiting, plants have been shown to preferentially take up amino acids as a source of N (Streeter et al. 2000, Henry and Jefferies 2003b, a, Schimel and Bennett 2004, Weigelt et al. 2005). However, the majority of these studies have been performed in artic, boreal systems with little application in subtropical systems; therefore limited information is available on the importance of organic forms of N in subtropical wetlands.

In this study, we found the $\delta^{15}N$ signature of the community level vegetation resulted in a 6 ‰ decrease in sites with higher N:P ratios (increased P-limitation). This change in $\delta^{15}N$ signature was most pronounced between the vegetation community in the 2004 site (highest P availability) and native site (lowest P availability). Additionally, this same relationship between $\delta^{15}N$ and N:P ratios was found in all sites except the 2001 and 2003 sites during the wet season (Figure 7-5). During the dry season, however, this relationship was only found within the 2004 site (Figure 7-6). Other studies have also shown that the $\delta^{15}N$ signature of plant biomass is affected by the availability of P. It has been observed that red mangroves can result in a 5 ‰ decrease in $\delta^{15}N$ under P-limited conditions as compared to N-limited conditions (McKee et al.

2002), lake macrophytes nave been shown to vary in $\delta^{15}N$ values by 4 ‰ under differing N availability (Jones et al. 2004), and in a comparison of *Cladium jamaicense* in *Typha domingensis* across a P nutrient gradient a 8 and 4 ‰ increase, respectively, in $\delta^{15}N$ signature was found when P levels were enriched (Inglett and Reddy 2006).

Increased levels of P can result in increases in organic N mineralization and NH_4^+ flux by stimulating the microbial activity associated with these processes (White and Reddy 2000). It has been proposed that the process of organic matter mineralization will result in the preferential mineralization of the lighter isotope thus resulting in an increase in the $\delta^{15}N$ of the remaining organic matter (Fogel and Tuross 1999, Novak et al. 1999). From a previous study, we found that organic N mineralization was higher in the restored sites (see Chapter 6) thus potentially resulting in organic matter with increased $\delta^{15}N$ values and in turn could result in a vegetation community with increased $\delta^{15}N$ values. However, while this explanation may seem likely, it may only partially contribute the increase in vegetation $\delta^{15}N$ in the restored sites. The bulk soil N (~95% organic N) only resulted in a ~3 ‰ change in $\delta^{15}N$ within the sites as compared to the vegetation 6 ‰ change. Additionally, there is no indication that this change in bulk soil $\delta^{15}N$ was driven by a P-limitation (Figure 7-2).

Additional contributions to this shift in community level $\delta^{15}N$ values could be a result of mechanistic controls of individual species. A limitation in P availability could result in differing demands and use of N by plant species as well as controls on vegetation community structure. Previous research has shown that *C. jamaicense* is well adapted to and prefers P-limited environments (Newman et al. 1996, Craft and Richardson 1997, Richardson et al. 1999), whereas, *T. domingensis* prefers nutrient enriched environments and will rapidly take up both N and P under enriched conditions (Miao et al. 2000, Lorenzen et al. 2001). In a previous study,

we found that the nutrient-use efficiency of N (NUE-N) for *C. jamaicense* and *T. domingensis* were not significantly different at 0.17±0.03 and 0.15±0.02 g biomass mg^{-1} N, respectively (see Chapter 4). As a result of similar N use, their corresponding $\delta^{15}N$ values are also similar, 0.31±0.08 and 0.21±0.07 ‰, respectively (Table 7-1). *Schoenus nigricans* also had a similar NUE-N at 0.18±0.02 but had a much lower $\delta^{15}N$ signature at -2.5±0.1 ‰. In contrast, the NUE-N of *S. lancifolia* is 0.07±0.03 g biomass mg^{-1} N (see Chapter 4) and its corresponding $\delta^{15}N$ signature is 1.56±0.13 ‰ (Table 7-1). These results suggest that species that are less efficient with the use of N (those which require less N per unit of biomass produced) may discriminate less against the heavier isotope than the species which have significantly higher NUE-N.

Nitrogen and P content also contributes to the $\delta^{15}N$ signatures of both the community and species level vegetation. At the species level, *S. lancifolia* had the highest N and P content at 10.9 and 0.59 g kg^{-1}, respectively, and the highest $\delta^{15}N$ signature at 1.56±0.13 ‰, while *S. nigricans* had the lowest N and P content at 5.3 and 0.08 g kg^{-1}, respectively, and the lowest $\delta^{15}N$ signature at -2.5±0.1 ‰. *Cladium jamaicense* and *T. domingensis* which had similar $\delta^{15}N$ signatures also had similar N content at 7.0±0.2 and 6.6±0.1 g kg^{-1}, respectively; however the P content of *C. jamaicense* was 50% less than that of *T. domingensis* at 0.20±0.1 as compared to 0.40±0.2 g kg^{-1}, respectively (Table 7-1). The difference in P content did not affect the $\delta^{15}N$ signature of either species, indicating that their similarities in N content and NUE-N outweigh the limitation of P in *C. jamaicense*. This was not the case with *S. nigricans*. Not only did it have lower N content, its P content was ~60% less than *C. jamaicense* and 80% less than *T. domingensis*.

The potential for vegetation P content to affect $\delta^{15}N$ signature is further supported by strong relationship of the community level $\delta^{15}N$ signature with plant P (Figure 7-9). During both

the dry and wet season, plant P content account for 80 and 92% of the variation in community level $\delta^{15}N$. The same stepwise regression indicated that plant N did not significantly contribute to the variability in community level $\delta^{15}N$, suggesting that plant P is a more important determinant than plant N. These results on the affects of N and P content on $\delta^{15}N$ signatures is further supported by a study on *C. jamaicense* and *T. domingensis* indicating similar correlations with $\delta^{15}N$ (Inglett and Reddy 2006).

Conclusions

The use of natural abundance ^{15}N signatures can be a useful tool in assessing soil N cycling and availability and N uptake in plant communities. Additionally, it is important to investigate both species level $\delta^{15}N$ values as well as community level. Large differences were observed between the community level and species level $\delta^{15}N$ values in this study, indicating that individual species will respond differently to changes in both N and P availability.

In this study we found that NH_4^+ was an important source of N to the vegetation community as compared to NO_3^-. Additionally, during the wet season the $\delta^{15}N$ signatures were dependent on TKN concentrations in the soil indicating that organic forms of N may be a source of N to plant communities under anaerobic conditions. Little research has been performed on the importance of organic N as a source of N to plants in subtropical wetlands. Additional research is needed on the preferential uptake of NH_4^+, NO_3^- and organic N to determine the importance of each form of N to plant communities in subtropical wetlands.

The availability of P also had some controls over the $\delta^{15}N$ of the plant communities. At both the species and community levels, the $\delta^{15}N$ was dependent on the plant P content. Plants inhabiting restored wetlands that were rich in P compared to the native system resulted in higher plant P content and $\delta^{15}N$ signatures. Additionally, plant mechanisms like NUE-N may affect

$\delta^{15}N$ signatures of plants. Plant species like *S. nigricans, C. jamaicense* and *T. domingensis* were more efficient with their use of N as compared to *S. lancifolia* and as a result they had much lower $\delta^{15}N$ signatures. Little research has been done to compare the NUE-N and the $\delta^{15}N$ signature of plants to definitively link this mechanism to N use. Additional research is needed to test this potential mechanistic control on $\delta^{15}N$ signatures of plant species.

Table 7-1. Chemical and isotopic values for selected dominant species from each site.

Species	Site*	N (g kg^{-1}) Average	SE	P (g kg^{-1}) Average	SE	N:P Average	SE	$\delta^{15}N$ Average	SE
Cladium jamaicense	Native, 1989	7.0	(0.2)	0.20	(0.1)	35.8	(2.9)	0.31	(0.08)
Schoenus nigricans	Native	5.3	(0.05)	0.08	(0.01)	66.2	(1.3)	-2.51	(0.10)
Sagittaria lancifolia	1989, 1997, 2003	10.9	(0.4)	0.59	(0.3)	18.4	(2.8)	1.56	(0.13)
Typha domingensis	1997, 2003, 2004	6.6	(0.1)	0.40	(0.2)	16.6	(1.1)	0.21	(0.07)
Juncus megacephalus	2003, 2004	7.8	(0.2)	0.37	(0.2)	20.9	(1.0)	0.32	(0.25)
Andropogon spp.	1997, 2001, 2003	7.6	(0.2)	0.45	(0.3)	16.8	(3.0)	-0.31	(0.19)
Poaceae	All	8.0	(0.1)	0.44	(0.2)	18.3	(9.5)	-0.99	(0.03)

*all values reported are an average for each species in all sites. No significant differences were found for species present in different sites.

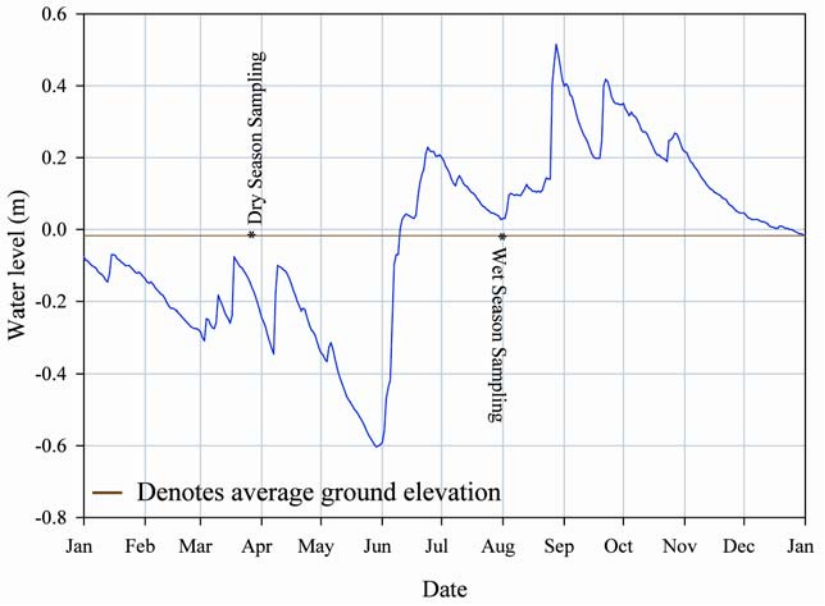

Figure 7-1. Hole-in-the-Donut hydroperiod for 2005 reported as groundwater level above NAVD 1988 (obtained from USGS National Water Information System).

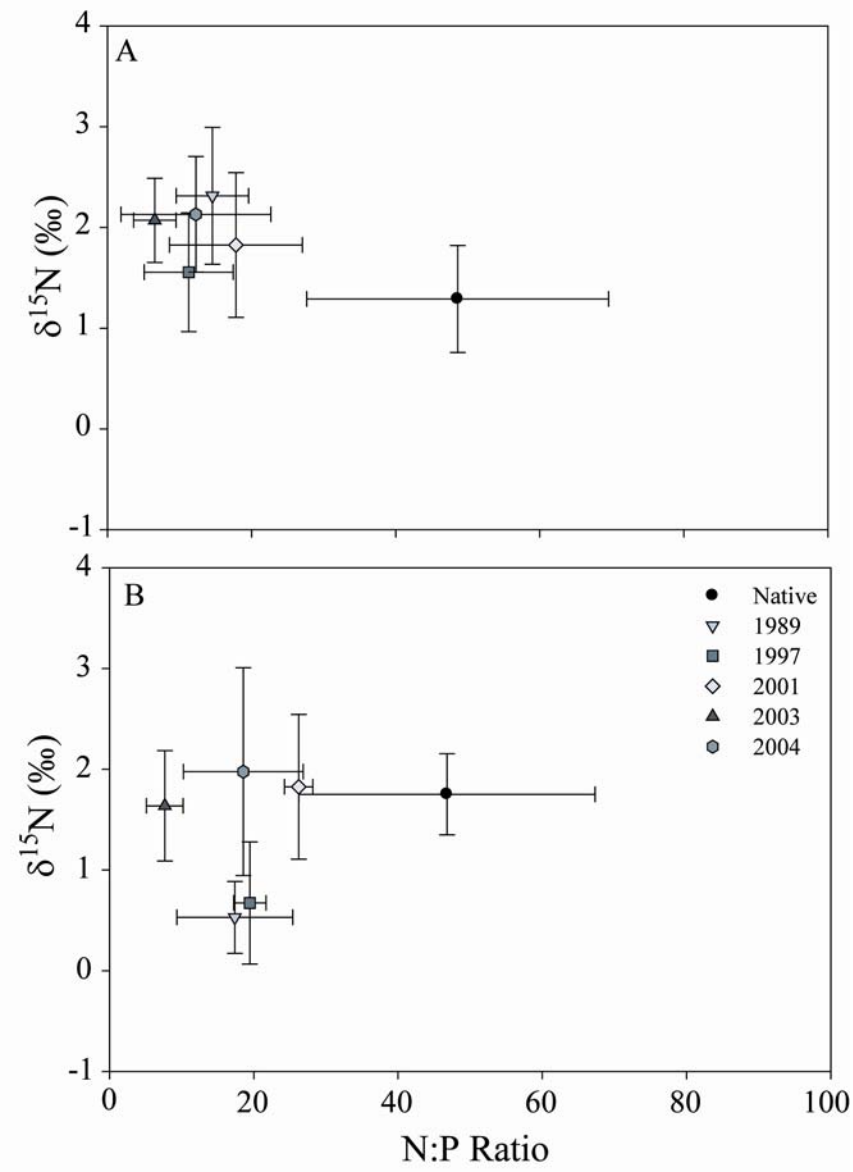

Figure 7-2. Relationship between $\delta^{15}N$ values and N:P ratios of the soil in each restored wetland and the native community. A) All data points plotted. B) Averages of plots from each site. (n=10 for each site)

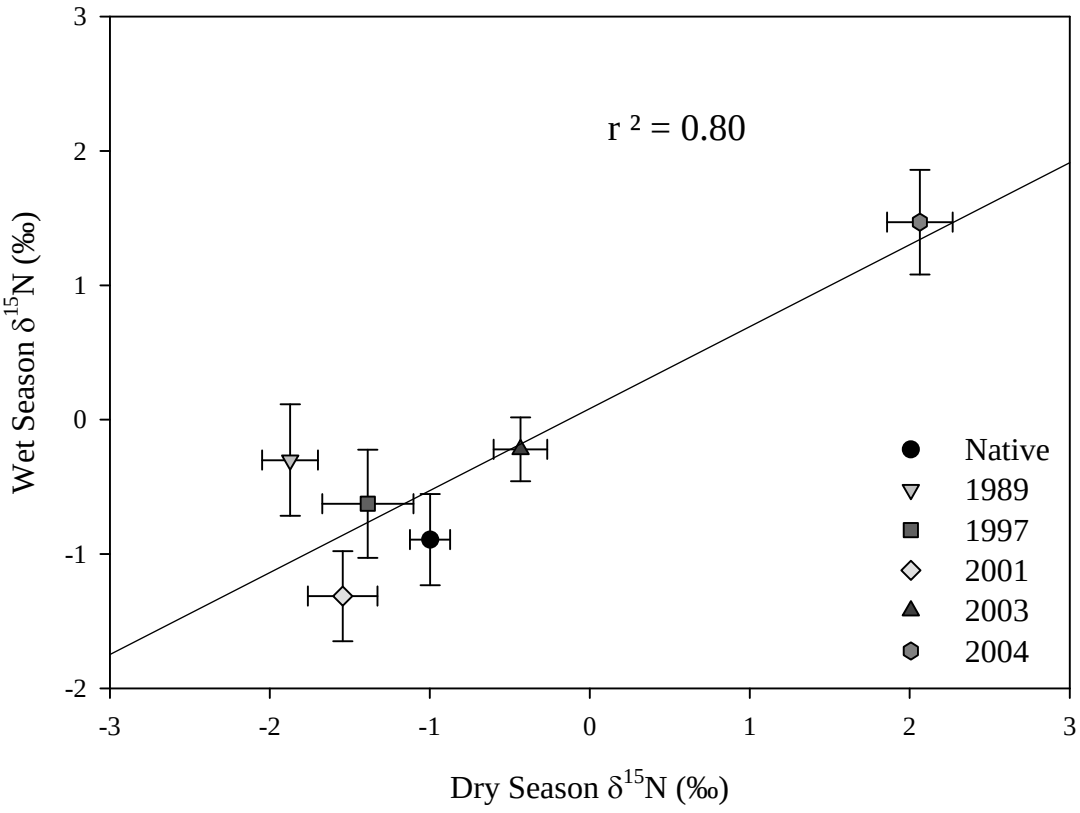

Figure 7-3. Relationship between community level plant $\delta^{15}N$ values measured during the dry season (April 2005) and those measured during the wet season (late July 2005). (n=10 for each site)

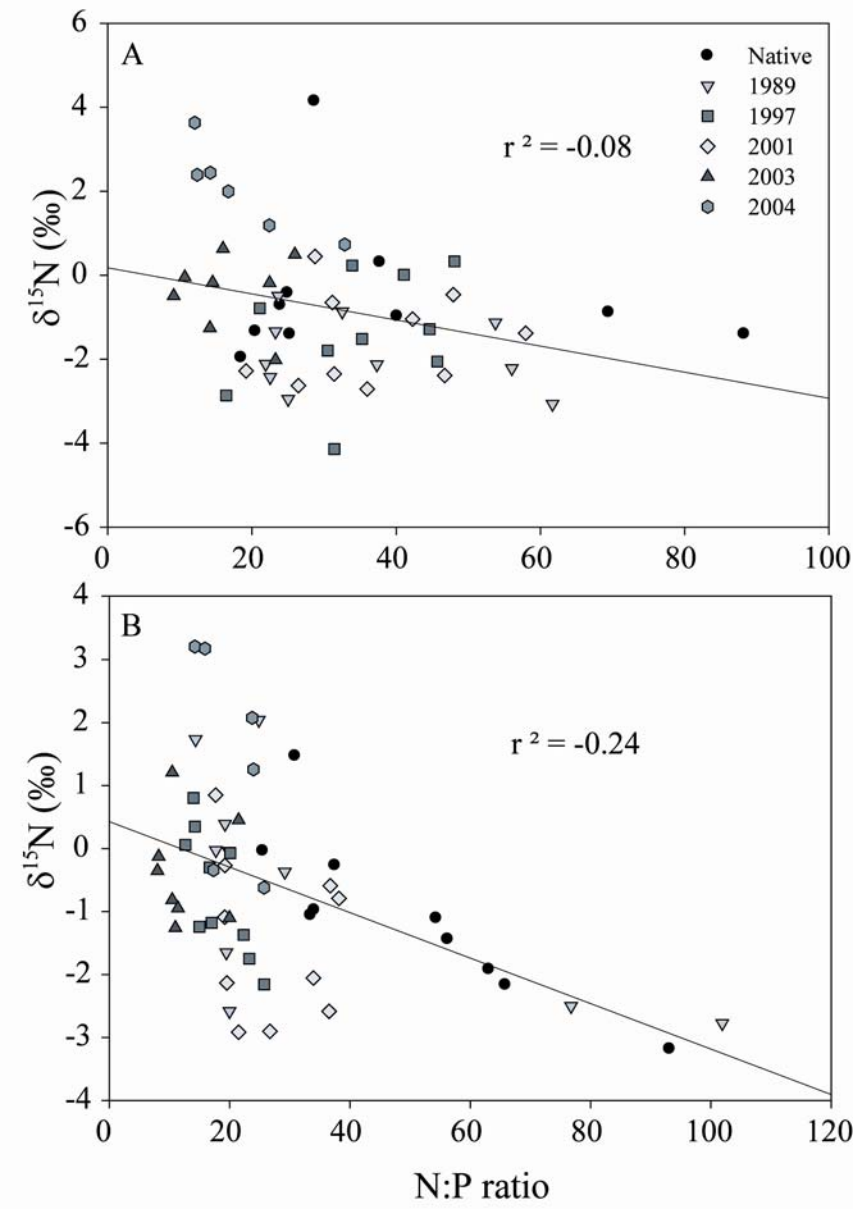

Figure 7-4. Relationship between community level plant $\delta^{15}N$ values and the N:P ratios which correspond to each value. Each site is coded to indicate site relationships. A) Dry season. B) Wet season. (N=60)

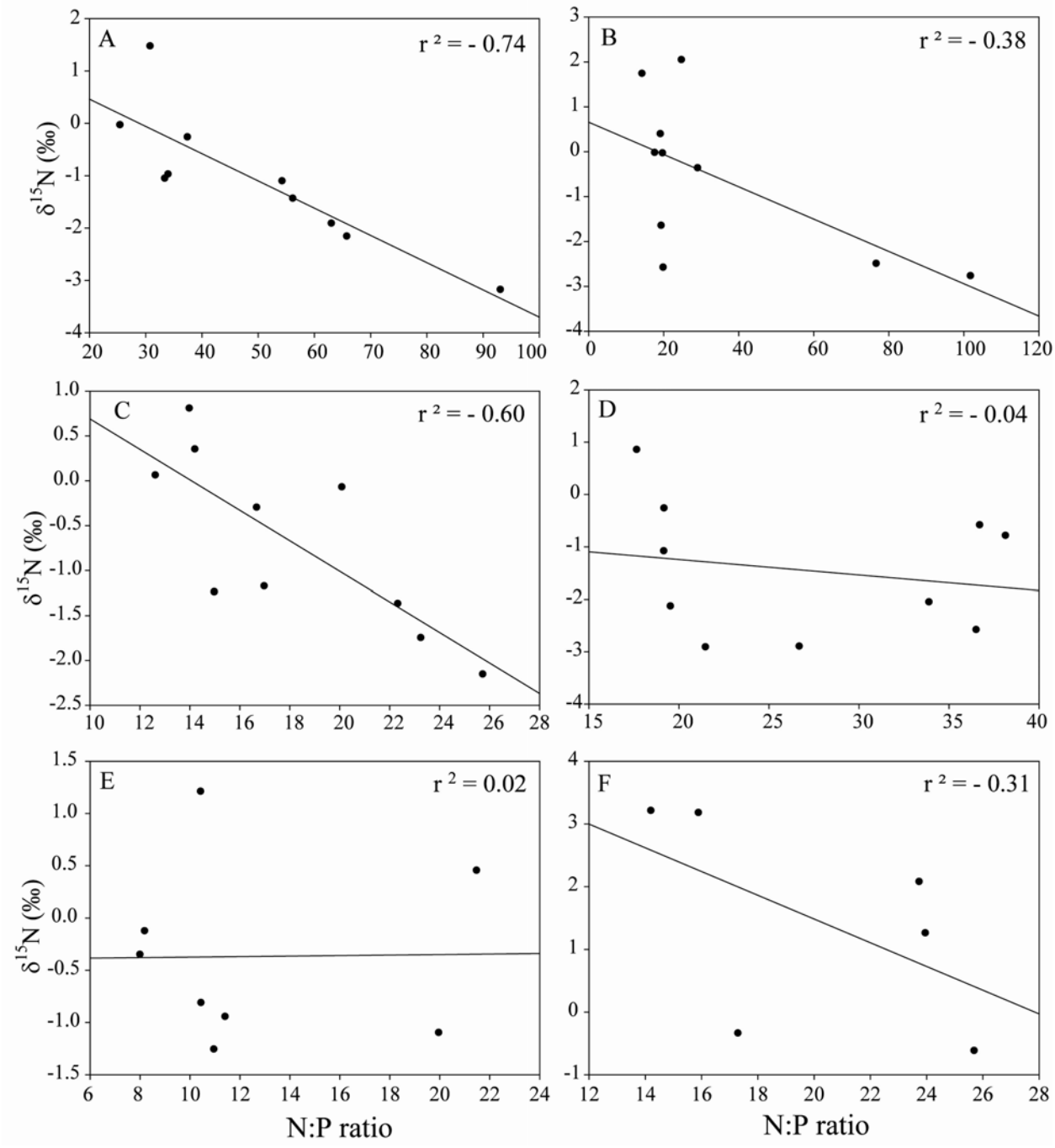

Figure 7-5. Relationship between the wet season community level plant $\delta^{15}N$ values and the N:P ratios which correspond to each value. A) Native. B) 1989. C) 1997. D) 2001. E) 2003. F) 2004. (N=10)

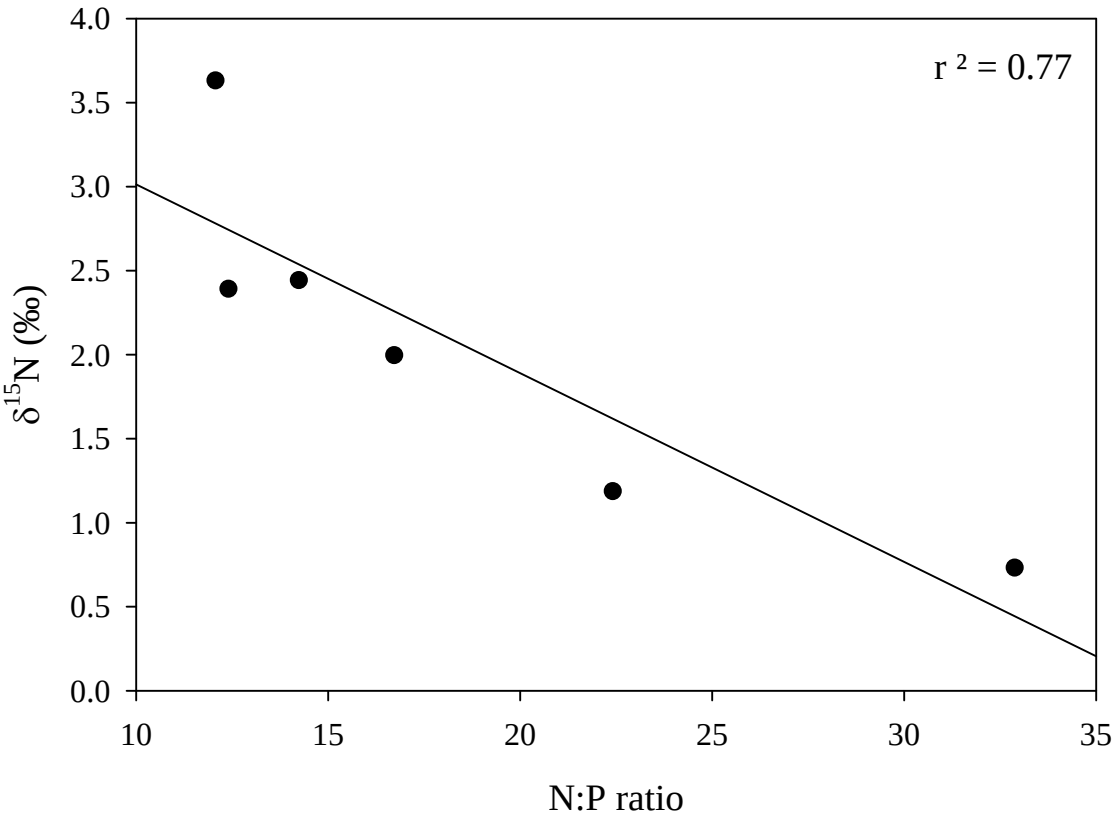

Figure 7-6. Relationship between the dry season community level plant $\delta^{15}N$ values and the N:P ratios which correspond to each value for the 2004 restored wetland community. Individual graphs for other sites not shown due to lack of relationship: native (r^2=0.02), 1989 (r^2=0.06), 1997 (r^2=0.16), 2001 (r^2=0.03), and 2003 (r^2=0.0018). (N=10)

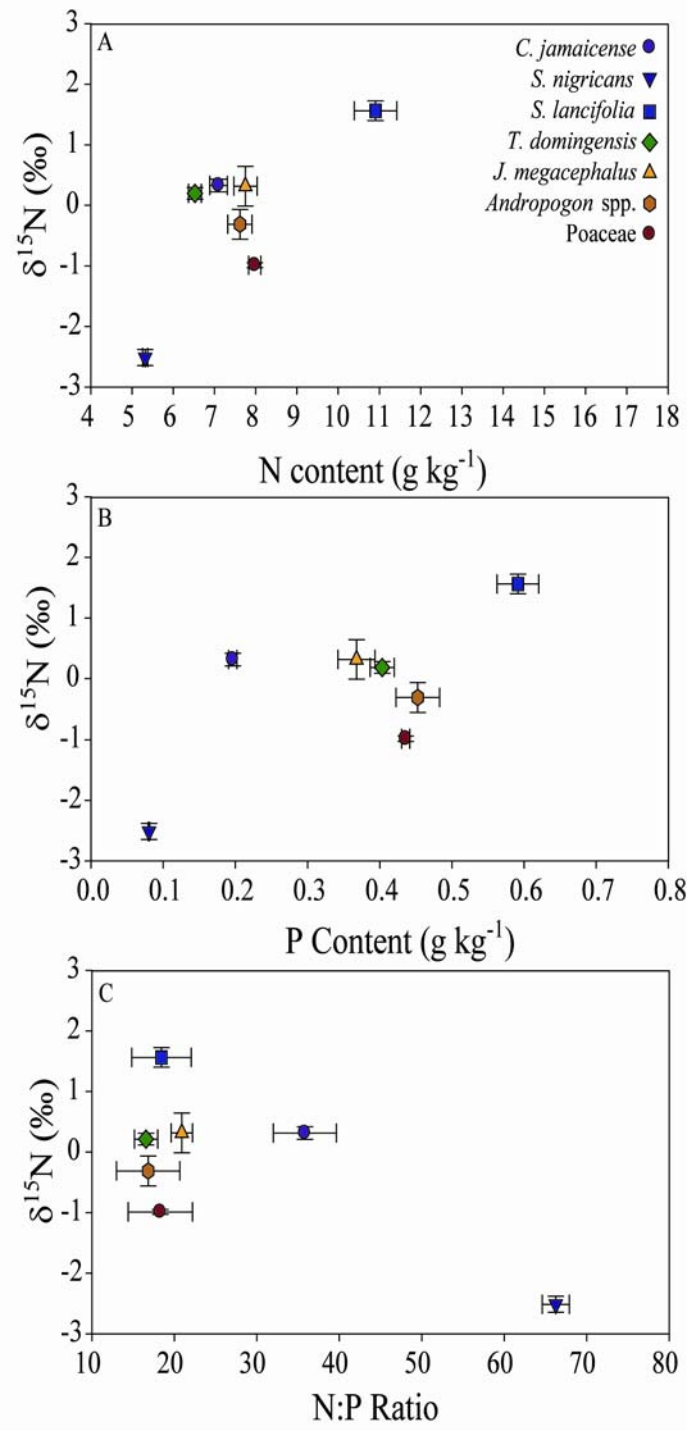

Figure 7-7. Relationship between species level $\delta^{15}N$ values and nitrogen, phosphorus and N:P ratios of dominant species. A) N Content. B) P content. C) N:P content. (N=10)

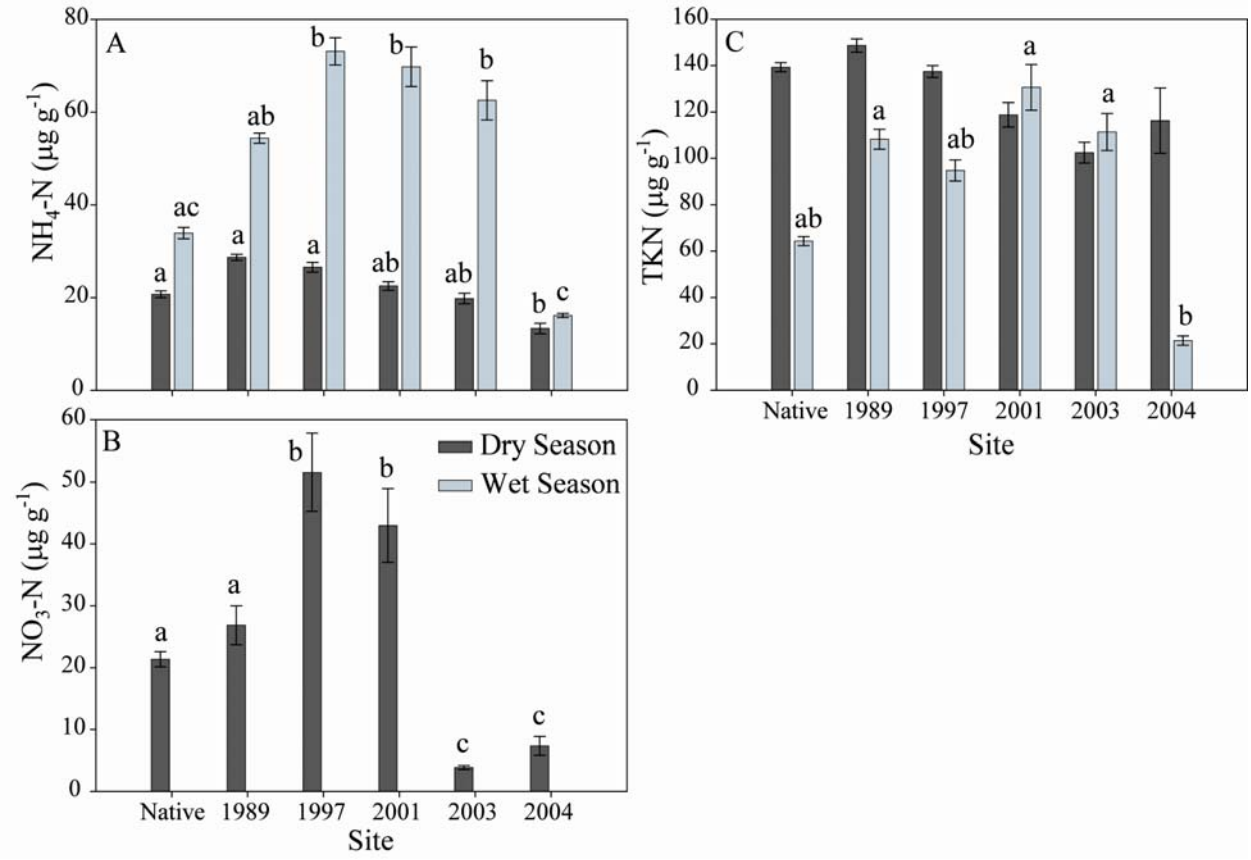

Figure 7-8. Inorganic nitrogen in each site in the HID during the wet and dry seasons. A) NH_4-N; dry season F=2.92 and p=0.02, wet season F=6.29 and p=0.0001. B) NO_3-N; dry season F=1.9 and p=0.010. C) TKN; dry season F=1.07 and p=0.39, wet season F=4.5 and p=0.002. During the wet season NO_3-N levels were non-detectable (B). (N=10)

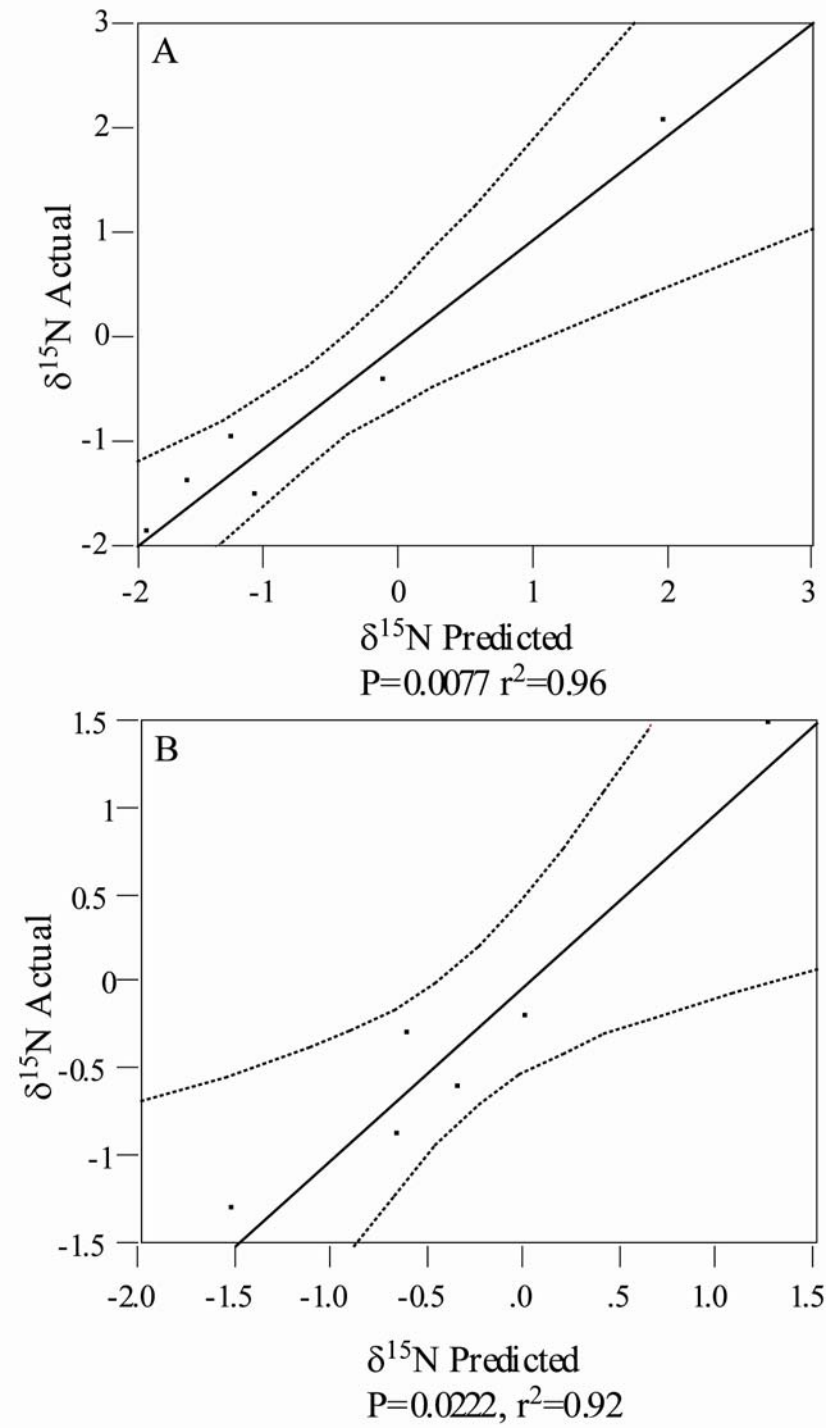

Figure 7-9. Relationship between the community level vegetation $\delta^{15}N$ to the inorganic nitrogen and phosphorus in each restored site. A) Actual and predicted $\delta^{15}N$ to NH_4-N and plant P during dry season; F=36.9, p=0.008, d.f.=5. B) Actual and predicted $\delta^{15}N$ to TKN and plant P during wet season; F=17.5, p=0.02, d.f.=5. (N=6)

CHAPTER 8
NUTRIENT MEMORY IN INVADED ABANDONED FARMLAND WITHIN THE EVERGLADES NATIONAL PARK: CONSIDERATIONS AND IMPLICATIONS FOR RESTORATION

Introduction

It has long been understood that anthropogenic alterations of nutrient dynamics can result in shifts in plant community structure. These ecosystem alterations can result in substitution one or more native plant species with an exotic species (Ehrenfeld 2003). While the visual effect of the replacement of native plant species by the invasion of exotics species is apparent, the consequences of invasion on soil processes is less obvious. Numerous studies have been conducted in natural communities showing the links between species composition on ecosystem function (Hector et al. 1999, Diaz and Cabido 2001, Loreau et al. 2001, Catovsky et al. 2002, Hooper et al. 2005), therefore, it can reasonably be deduced that the invasion of exotics will have consequent effects on ecosystem function including but not limited to: nutrient processes, decomposition, storage pools, productivity and microbial activities.

Ecosystem disturbance is often followed by the invasion of a non-native plant species. The sudden change in the resources available to plant communities often times result in the loss of biodiversity which weakens the stability of plant community structure allowing for the ease of invasion (Meekins and McCarthy 2001). Because invasion is not foreseen, most studies are conducted after the invasion has occurred making it difficult to determine the direct cause of initial entry into the system (Wiser et al. 1998). Previous farming in the Hole-in-the-Donut (HID) region of the Everglades National Park (ENP) which altered approximately 4000 ha of continuous natural vegetation and soil has resulted in drastic nutrient dynamic shifts and aggressive colonization by exotic species *Schinus terebinthifolius* (Dalrymple et al. 2003).

Schinus terebinthifolius, Raddi (Brazilian pepper or Florida holly) is a Category I "most invasive plant species" in the state of Florida. It has invaded many disturbed ecosystems where the soil and vegetation community has been drastically altered due to anthropogenic activities. *Schinus terebinthifolius* is a small escaped ornamental tree (typically 10 feet in height but can reach 40 feet) native to Brazil and Paraguay (Clark 1997). First introduced in 1840s, it is now abundant in disturbed moist to mesic sites in the southern half of the Florida peninsula. The canopy of *S. terebinthifolius* forms a closed, dense thicket which excludes native vegetation via shading and chemical inhibition of their growth, and provide relatively poor wildlife habitat (Clark 1997). *Schinus terebinthifolius* is related to poisonwood, poison oak, poison ivy, mango, and pistachio, etc. and the berries it produces have been found to have narcotic or toxic effects on birds and other wildlife.

Schinus terebinthifolius is moderately salt tolerant, withstand flooding, fire, drought, and quickly re-sprout after being cut (Clark 1997). It is considered a serious threat to natural Florida ecosystems and therefore its control and eradication is critical. Attempt to control *S. terebinthifolius* in the ENP have included mechanical removal, fire management, herbicide treatments and biological control agents all of which have been unsuccessful in this region of the park (Dalrymple et al. 2003). Restoration effects led to a cooperative agreement between the ENP and Miami-Dade County via use if mitigation funds to restore the HID region by implementing a "scraping" method to remove all vegetation and soil down to the calcium-limestone bedrock.

The effect of previous management and invasion has created an ecosystem nutrient legacy which could impede ecosystem restoration. The goal of this study was to evaluate the environmental conditions of the invaded abandoned farmland and how the invasion if *Schinus*

terebinthifolius has potential impacted ecosystem nutrient dynamics. The objectives of this study were to: 1) determine differences in soil characteristics and relationships between the invaded, native, and 2003 restored wetland communities, 2) determine the nutrient and cellular fractionation of *S. terebinthifolius* and the dominant plant species in the native community (*Schoenus nigricans*) and the 2003 restored wetland (*Typha domingensis*), and 3) evaluate functional differences in nutrient regeneration and storage between the invaded, native, and 2003 restored communities.

Methods

Site Description

This study was conducted in wetland systems restored within the HID region of the ENP. Past farming and management practices in the areas that were restored left these systems open to invasion by *Schinus terebinthifolius* (Brazilian pepper). The nutrient enriched soil, higher elevation (resulting in short hydroperiods) and subtropical conditions of Florida made these disturbed areas an ideal location for invasion by *S. terebinthifolius*. The natural surrounding marl prairie wetlands are inundated for approximately six months of the summer season. The goal of the restoration of the HID was to remove the enriched soil and lower the elevation to increase the hydroperiod to control *S. terebinthifolius* re-invasion (see Chapter 1 for a more detailed site description).

To determine the legacy of invasion and previous farming practices within HID the methodology from chapters 4-5 were applied to areas that have yet to be restored. A summary of the methodology is provided here, however, detailed methodology is found in previous chapters. Site comparisons will be made between the invaded and native communities as well as the 2003 restored wetland community.

Sample Collection and Analysis

In April 2005 (dry season) and July 2005 (wet season), soil samples were collected with a 7.6 cm PVC core from 10 plots randomly distributed throughout the sites The soil cores were transported to the laboratory and stored at 4°C until analysis. Within 24 to 48 hours of sample collection, each soil sample was extracted for ammonium (NH_4^+) with K_2SO_4 (Bundy and Meisinger 1994) and set up for incubation for potentially mineralizable N (PMN) (or biologically available N) (Keeney 1982, Bundy and Meisinger 1994, White and Reddy 2000). Ammonium extracts were analyzed via flow injection analysis with a Bran Luebbe Auto Analyzer 3 Digital Colorimeter (EPA Method 350.1). A subsample of each soil was dried at 60°C for 3 days then ground with a ball grinder to a fine powder for total N and P analysis. Dry soil samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

To determine nutrient content, random live and senescent samples were collected, bulked and dried at 70°C until all moisture was removed. Once dry, all vegetation was passed through a Wiley Mill tissue grinder equipped with a 1-mm mesh screen to achieve homogeneity. A subsample was ball ground to a fine powder for N and P analysis. All plant samples were analyzed for total N with a Thermo Electron Corp. Flash EA 1112 Series NC Soil Analyzer. Total P was determined via HCl ash extraction and analyzed with a Seal AQ2+ Automated Discrete Analyzer (EPA Method 119-A rev3) (Anderson 1976). Nitrogen and P ratios were calculated on a mass basis as N:P.

Decomposition Experiment

We used the same in situ litterbag decomposition experiment to estimate mass loss and nutrient regeneration potentials *Schinus terebinthifolius, Schoenus nigricans* and *Typha domingensis* as described in Chapter 5. All samples were analyzed for extra-cellular enzyme activities (EEA) and microbial biomass nitrogen and carbon analysis (MBN and MBC). Additionally, samples were analyzed for total C, N and P analysis and soluble cellular content (sugars, carbohydrates, lipids, etc.), hemi-cellulose, α-cellulose, and lignin. See Chapter 4 for detailed description of methodology.

Assessment of Litter Quality

We classified *S. terebinthifolius*, *S. nigricans*, and *T. domingensis* as high or low quality based on percent lignin, C:N and C:P ratios and a decomposition rate constant. Rates of N mineralization or immobilization are highly influenced by lignin content and C:N ratios (Figure 5-2) (Brady and Weil 1999). Litter with high lignin content and high C:N ratios are considered poor in quality and would have slow rates of decomposition. Lignin contents of 20-25% and C:N ratios greater than 30 would be considered high (poor quality). Differing amounts of lignin in combination with varying C:N ratios can effect rates of N regeneration from litter material.

To determine a decomposition constant, k, for *S. terebinthifolius*, *S. nigricans*, and *T. domingensis* we assumed an exponential rate of mass loss for both species. The following equation was utilized to calculate k;

$$M_f = M_i e^{-kt} \quad (8\text{-}1)$$

where M_i is the initial mass of the litter, M_f is the final mass of the litter and t is the time at M_f. The decomposition constant, k, was determined both litter at each site. The mean residence time, or time required for the litter to decompose under steady state, was calculated as 1/k (Chapin et al. 2002).

^{15}N Tracer Experimental Design

In 2006, we examined the retention of N via the use if 15N stable isotope techniques in three replicate field plots in the invaded and native communities and the 2003 restored wetland (see Chapter 6 for methodology and experimental design). Soil and vegetation samples were collected at time 24 hours after application an on days 42, 84, 168, and 365. Samples were analyzed for total N, NH_4, NO_3, MBN, ON, and ^{15}N in previously mentions pools as well as PMN, and volatilization (see Chapter 5 for detailed methodology).

Statistical Analysis

All data collected were analyzed statistically using Fit Model in JMP Version 5.1 (SAS 2005). Analysis of variance (ANOVA) was performed to investigate site and seasonal differences in soil. Regressions were performed to determine relationships between variables. Multiple comparisons were made using the Least Square Means test and to determine Pearson's correlation coefficients between all variables.

Results

Soil Characteristics

Differences were observed between many of the soil physical, chemical and biological parameters analyzed (Table 8-1). A few notable differences include %LOI, NO_3^-, carbon (C), N, P, C:P and N:P ratios, and PMN. The invaded sites had higher %LOI (34%) compared to the native community (22%) and the 2003 restored site (16%). Non-detectable limits of NO_3^- were found in the 2003 and native communities whereas 6.33 $\mu g\ g^{-1}$ was detected in the invaded sites. The invaded soil had the highest levels of C (214 $g\ kg^{-1}$) followed by the native community soil (188 $g\ kg^{-1}$). Small differences were found between the N content in the invaded, 2003, and native sites, 8.8, 6.2, and 11 $g\ kg^{-1}$, respectively. However, the range of P content was large for all three sites; invaded (1.6 $g\ kg^{-1}$), 2003 (0.87 $g\ kg^{-1}$), and native (0.33 $g\ kg^{-1}$). The C:P and N:P

ratios were significantly greater in the native community soil as compare to the invaded and 2003 soil. Finally, the PMN in the soils were higher in the invaded site (20.2 mg kg^{-1} d^{-1}) as compared to the native (10.3 mg kg^{-1} d^{-1}) and the 2003 site (14 mg kg^{-1} d^{-1}).

The principal components analysis revealed that there are differences among soil environmental parameters in the invaded, native, and 2003 restored communities. Principal component 1 described 37% of the variation and principal component 2 described 35% of the variation (Figure 8-1). This analysis has factored the native community out from the invaded and 2003 restored site based on soil environmental characters. The invaded and 2003 sites are grouped closely together with overlapping error bars indicating that there are similarities between the two sites. The C:P and N:P ratios were highly correlated with the native community and primarily responsible for the distinct separation (Table 8-2 and Figure 8-2c). Additionally, the N:P ratios were positively correlated with the MBC suggesting that with increases in N:P ratios (as found in the native community) the MBC increases. Additional correlations can be found in Table 8-2.

Distinct differences were not visible in soil N content across sites, but the soil P content is clearly greater in the invaded community as compared to the native community (Figure 8-2a and b). The P content in the 2003 sites soil falls between the invaded and native sites concentrations. A similar trend is demonstrated by the PMN data with the invaded site having the greatest PMN activity and the native site the lowest (Figure 8-2d).

Vegetation Characteristics

The above-ground live, root and senescent tissue N content for *S. terebinthifolius* was higher than what was found for *T. domingensis* and *S. nigricans* whereas the litter layer for all species had similar values for N content (Figure 8-3a). The tissue P content was considerably higher in *S. terebinthifolius* as compared to the other two species (Figure 8-3b). In *S.*

terebinthifolius plant tissue, little difference is found in P content for the above-ground live, roots, and senescent whereas the litter layer had approximately 90% less P than the live fraction. For both *T. domingensis* and *S. nigricans*, the root contained more P than the above-ground live portion of the plant and the senescing tissue had significantly less P than the live. However, *T. domingensis* contained greater amounts of P in all plant parts as compared to *S. nigricans* (Figure 8-3b).

The C:P and N:P ratios for *S. nigricans* was much greater in all plant parts than for either *T. domingensis* or *S. terebinthifolius* (Figure 8-3d and e). Additionally, both the C:P and N:P ratios of the senescent material for *S. nigricans* was considerably higher than other plant parts. The cellular fractionation of the senescent plant material of each species shows that *S. terebinthifolius* consists of approximately 75% lignin and that *T. domingensis* and *S. nigricans* are only 5 and 17% lignin, respectively (Figure 8-4a).

Ecosystem Functions

Typha domingensis and *S. nigricans* had similar decomposition rates of mass loss (Table 8-3 and Figure 8-5). The turnover time for each species was 0.86 and 0.89 years, respectively. The decay rate of *S. terebinthifolius* was much faster with a turnover rate of 0.44 years (Table 8-3 and Figure 8-5). After 365 days of decomposition the cellular fractionation of each species was very different than the initial. *Schinus terebinthifolius* litter consisted of approximately 50% soluble cellular content and only 30% lignin indicating a 60% loss in initial lignin content (Figure 8-4b). A larger portion of the remaining *T. domingensis* litter consisted of lignin than what was present in the initial analysis whereas the fraction of lignin in *S. nigricans* remained unchanged (Figure 8-4b).

The amount of P and N regenerated via mineralization from *S. terebinthifolius* was considerably greater than that of *T. domingensis* and *S. nigricans* (Figure 8-6a and b). After 365

days of decomposition *S. terebinthifolius* regenerated approximately 50 mg N g^{-1} litter material whereas *T. domingensis* only regenerated about 10 mg N g^{-1} litter and *S. nigricans* regenerated 30 mg N g^{-1} litter (Figure 8-6a). For P regeneration, *S. terebinthifolius* released 12 mg P g^{-1} litter and *S. nigricans* only regenerated about 0.4 mg P g^{-1} litter (Figure 8-6b). *Typha domingensis* did not regenerate any P during the 365 day study, but resulted in 0.4 mg P g^{-1} litter being immobilized.

The βGA associated with the decomposition of *S. terebinthifolius* was similar to that associated with *T. domingensis* up to the 365 days sampling period. After 365 days, the βGA was significantly for *S. terebinthifolius* than for *T. domingensis* (Figure 8-7a). Initially, the βGA association with *S. nigricans* was considerably higher than for the other two species. After 168 and 365 days the activities were low for all species (Figure 8-7a). The L-LAA associated with each litter type was very similar to the association of βGA (Figure 8-7b). Additionally, the activities of L-LAA were significantly lower than the activities of βGA. The enzyme activity for APA was only determined at 365 days. The APA activity associated with *S. nigricans* is significantly greater than that of *T. domingensis* and *S. terebinthifolius* (Figure 8-8). The APA associated with *S. terebinthifolius* was near zero at 365 days of decomposition.

The native site retained more N from the ^{15}N tracer study after 24 hours at 72% than either the invaded (50%) or the 2003 restored wetland (41%) (Figure 8-9a). At 24 hours, more of the ^{15}N was in the MBN pool compared to the other pools for all three sites. After 365 days the native soil retained 33% of the initial ^{15}N tracer that was applied closely followed by the invaded site at 28% (Figure 8-9b). The 2003 restored site only retained 18% in the soil of the initial ^{15}N applied. After 365 days, most of the ^{15}N was recovered in the ON pool (Figure 8-10a). The $MB^{15}N$ pool was the highest in the native community and the lowest in the 2003 restored

wetland (Figure 8-10b). The amount of ^{15}N retained in the NH_4^+ pool was small and the same for all sites (Figure 8-10c). The total amount of ^{15}N remaining in the NO_3^- pool was small, however, the native community retained significantly more as compared to the invaded and 2003 sites (Figure 8-10d).

Discussion

The effects of previous farming practices and invasion of *Schinus terebinthifolius* on ecosystem function has resulted in drastic alterations of the soil nutrient dynamics. The disturbance from the farming and invasion resulted in highly enriched levels of P relative to the native community. The outcome of increased P concentrations has resulted in a shift away from the historical oligotrophic P-limited conditions found in the native communities.

The elevated levels of P found in the invaded communities have resulted in high plant tissue P content in *S. terebinthifolius* as compared the native *S. nigricans* (Figure 8-3). Above-ground live tissue P content is approximately 95% greater in *S. terebinthifolius* compared to *S. nigricans*. This difference in tissue content between these species has demonstrated direct effects on the mineralization of P. The amount of P regenerated from *S. terebinthifolius* is about 97% greater than the amount regenerated from *S. nigricans* (Figure 8-6). The majority of the studies on invasion and soil processes have focused on or have found greatest significance on soil C and N processes, however some herbaceous species have been shown to result in increases in both soil and plant P (Ehrenfeld 2003, Vanderhoeven et al. 2006). More comparably, an exotic tree in Hawaii was found to release significant amounts of both N and P during decomposition as compared to the native tree species (Rothstein et al. 2004).

This investigation found that the soil P content in the 2003 restored wetland was about 50% less than what was found in the invaded soils and about 60% greater than what is indicative of the native communities (Table 8-1). This indicates that the restoration process was able to

remove half of the P legacy from farming and invasion. The consequences of the additional P remaining above the native levels could hinder the colonization of native plant communities. Several studies on native plant species of the ENP have indicated that the native plants will not thrive when the natural oligotrophic conditions are altered (Newman et al. 1996, Richardson et al. 1999) leaving the system open to invasion by undesirable species like *T. domingensis* (Urban et al. 1993, Craft et al. 1995, Craft and Richardson 1997). In addition to the destructive restoration technique, the residual P present in the 2003 site could be contributing to the domination by *T. domingensis*.

The dominance *T. domingensis* also has long term effects on nutrient availability in the restored ecosystems. After 365 days of decomposition, *T. domingensis* immobilized approximately 5 mg P g^{-1} litter (Figure 8-6b). The rate of immobilization coupled with the slower decay rates of *T. domingensis* demonstrate that the residual P could become permanently buried in the soil profile making it unavailable for further uptake. If this pattern in nutrient decomposition continued over a long period of time, the nutrient dynamics could become similar to the native oligotrophic P-limited condition ideal for native plant community development.

In addition to alterations in P dynamics, there are indications that the legacy of farming and invasion has altered the processes of N cycling. *Schinus terebinthifolius* litter material is a source of N to the microbial community. Both the above-ground live and senescent plant tissue of *S. terebinthifolius* is greater than the live and senescent tissue of *T. domingensis* and *S. nigricans* (Figure 8-3). As a result, this produced different N regeneration patterns for each species. Both *S. terebinthifolius* and *S. nigricans* resulted in N regeneration; however, the contribution from *S. terebinthifolius* was considerably higher (Figure 8-6). Due to this difference in litter decomposition, more N is made available for further microbial or vegetation uptake.

This effect of invasion on alterations in N regeneration has been demonstrated in other studies. It has been demonstrated that invasive grasses can increase N regeneration as compared to native woodland species (Mack et al. 2001) as well as in several herbaceous species (Ehrenfeld 2003).

In contrast, N was immobilized into the litter of *T. domingensis* during the majority of the decomposition study. Mineralization of N did not occur until the final 6 months of the study (Figure 8-6a). While the total soil N content was not significantly difference in the 2003 site compared to the invaded and native soils, the amount of N stored in the 2003 restored site soil was lower (Table 8-1 and Figure 8-2). This small difference, however, could be significant enough to result in N-limitations to the vegetation community growing in the 2003 restored wetland. A previous study indicated a potential N-limitation to the vegetation community found in the 2003 restored wetland (see chapter 4 for more information on nutrient limitation). In response to an N-limitation, the litter material produced by the vegetation found in the 2003 restored wetland would also be N-limited. Therefore, limiting the microbial communities ability to regenerate N.

The total N storage capacity of the soil impacted by farming and invasion was not significantly different from the soil in the native community (Figure 8-10e). In contrast, the 2003 stored significantly less N over the duration of the ^{15}N tracer study. This concludes that long term total N storage capabilities are not altered by farming or invasion in this area however; the restoration technique has altered total N storage capacity. While the total N storage capacity has not been affected by farming and invasion, the processes of N transformation are altered. Differences in rates of nitrification are apparent from the comparison of the $\%^{15}N$ retained in the NO_3 pool across sites (Figures 8-9 and 8-10d). The amount of ^{15}N nitrified to NO_3 in the invaded site within the first 24 hours was less than half of what it was in the native community

(Figure 8-9). Therefore, nitrification rates are lower has a result of farming and invasion. In a review of the effects of exotic species on soil processes, select species were shown to result in decreases in nitrification while the majority resulted in increases in nitrification (Ehrenfeld 2003). Additionally, in grassland systems nitrification rates were found to be significantly higher in invaded grasses as compared to native woodland areas (Mack and D'Antonio 2003).

Nitrification is a microbial mediated process and changes in rates of nitrification indicate that the microbial communities found in the invade communities have also been altered. In addition to altered nitrification rates, the MBC and the enzyme activities responsible for nutrient regeneration and OM decomposition have also been altered in the invaded communities. As a result of increased N and P concentrations in the *S. terebinthifolius* litter material the amount of enzyme activity associated with decomposition is significantly lower than that for both *T. domingensis* and *S. nigricans* (Figure 8-7 and 8-8). With higher levels of nutrient content the microbial community does not need to put energy into producing enzymes for hydrolysis (Chróst 1991).

Conclusions

The memory of enriched P lingering in the invaded sites will be a challenge to overcome. The elevated levels of soil and plant P found in the invaded communities have resulted in high P availability as compare to the oligotrophic conditions of the native communities. Previous farming and the consequent invasion by *S. terebinthifolius* has resulted in increase P availability, altered microbial activities and reduced N transformation rates. Additionally, the effect of the restoration method utilized will also carry with it a legacy which the native plant species will have to over come in order to colonize these sites.

Table 8-1. Summary of physical, chemical, and biological parameters for the invaded, native and 2003 restored communities. (n=10)

	Invaded		2003		Native	
Parameter	Ave	SE	Ave	SE	Ave	SE
Physical						
Moisture (%)	38.24	(0.65)	59.52	(0.91)	61.19	(0.45)
Bulk Density (g cm^{-3})	0.31	(0.004)	0.30	(0.01)	0.41	(0.01)
LOI (%)	34.53	(1.87)	15.47	(0.51)	21.99	(0.63)
Chemical						
NO_3-N (mg kg^{-1})	6.33	(0.47)	n.d.*		n.d.*	
NH_4-N (mg kg^{-1})	53.21	(3.36)	62.53	(4.23)	33.88	(1.22)
TKN (mg kg^{-1})	103.59	(9.99)	111.36	(7.97)	64.30	(1.97)
TOC (mg kg^{-1})	1818.76	(140.09)	1185.44	(36.19)	1069.90	(23.37)
DON (mg kg^{-1})	44.05	(6.83)	48.83	(4.05)	30.42	(1.31)
C (g kg^{-1})	214.52	(7.43)	152.83	(0.89)	187.52	(1.75)
N (g kg^{-1})	8.77	(0.39)	6.19	(0.14)	11.04	(0.22)
P (g kg^{-1})	1.59	(0.03)	0.87	(0.02)	0.33	(0.03)
C:N	25.21	(0.23)	25.48	(0.41)	17.32	(0.19)
C:P	153.54	(11.80)	190.88	(6.67)	865.09	(44.26)
N:P	6.33	(0.53)	7.60	(0.27)	48.27	(2.10)
Biological						
PMN (mg NH_4 kg^{-1} d^{-1})	20.15	(0.93)	13.95	(1.62)	10.29	(0.34)
MBC (mg kg^{-1})	3775.79	(164.43)	6255.62	(274.09)	5589.40	(99.55)
MBN (mg kg^{-1})	502.64	(26.78)	495.36	(19.55)	492.03	(16.79)
MBC:N	8.25	(0.28)	13.18	(0.50)	11.92	(0.21)

*non-detectable limits

Table 8-2. Correlation coefficients from a principal components analysis on soil environmental characteristics for the invaded, native, and 2003 restored wetland communities. The table abbreviations are: BD = bulk density, LOI = loss on ignition, TOC = total organic carbon, P = phosphorus, N = nitrogen, C = carbon, PMN = potentially mineralizable nitrogen, and MBC and N = microbial biomass carbon or nitrogen. Boldface indicates a correlation coefficient >0.70.

Parameter	Site	Moisture	BD	LOI	NH4	TKN	TOC	P	N	C	C:N	C:P	N:P	PMN	MBC	MBN
Moisture	-0.27															
BD	-0.50	0.28														
LOI	-0.73	-0.18	0.73													
NH4	0.37	0.37	-0.60	0.46												
TKN	0.28	0.32	-0.75	0.61	0.93											
TOC	0.15	-0.10	0.14	0.89	0.65	0.81										
P	0.57	-0.64	-0.22	0.28	0.19	0.73	0.16									
N	-0.59	0.33	0.37	0.75	0.33	0.46	0.60	-0.24								
C	-0.17	-0.80	0.13	0.98	0.42	0.62	0.90	0.11	0.81							
C:N	0.78	-0.63	-0.38	-0.21	-0.16	-0.17	-0.79	0.52	-0.76	-0.28						
C:P	-0.75	0.32	0.21	-0.12	-0.29	-0.15	-0.10	-0.79	0.29	0.29	-0.53					
N:P	-0.82	0.37	0.24	-0.11	-0.29	-0.16	-0.12	-0.80	0.36	0.42	-0.62	0.99				
PMN	0.22	0.76	-0.14	0.62	0.75	0.73	0.65	0.30	0.40	0.56	-0.14	-0.25	-0.25			
MBC	0.25	0.82	0.14	0.17	0.75	0.67	0.36	-0.34	0.42	0.20	-0.53	0.63	0.99	0.53		
MBN	0.12	0.41	0.24	0.65	0.68	0.70	0.70	-0.24	0.63	0.65	-0.35	-0.58	-0.32	0.56	0.67	
MBC:N	-0.47	0.41	-0.14	-0.39	0.92	0.82	-0.20	-0.38	-0.14	-0.33	-0.17	0.17	0.17	-0.83	0.33	-0.39

Table 8-3. Summary of the decomposition constants, k (yr^{-1}), and turnover rates, 1/k (yr), as determined by the mass loss from a field decomposition study.

	Decompostion Rate (k)		Turnover (1/k)	
Species	Average	SE	Average	SE
Schinus terebinthifolius	2.31	(0.19)	0.44	(0.04)
Schoenus nigricans	1.12	(0.04)	0.89	(0.03)
Typha domingensis	1.22	(0.29)	0.86	(0.19)

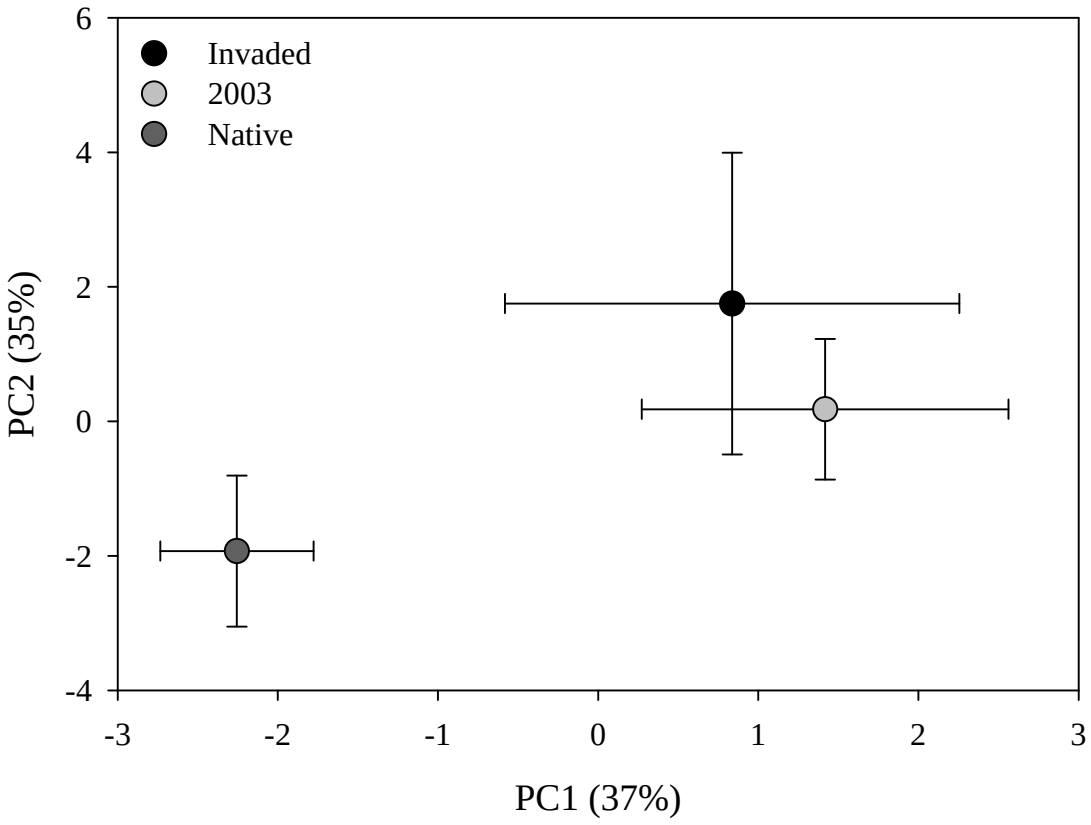

Figure 8-1. Principal component analysis for soil environmental characteristics in the invaded, native, and 2003 restored wetland communities. The first two principal components explained 72% of the total variance in the soil environment (n=20).

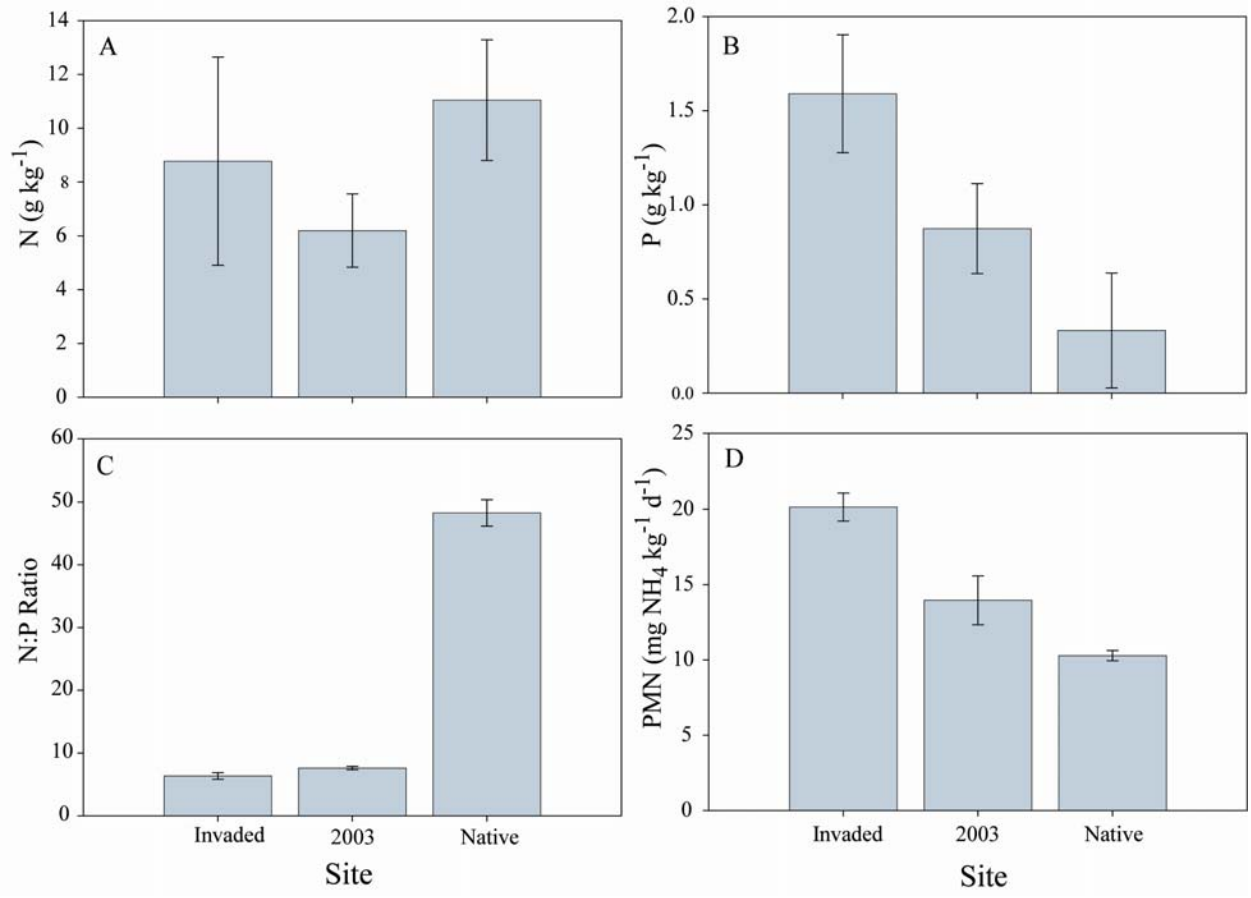

Figure 8-2. A comparison of soil nutrients for the invaded, native, and 2003 restored wetland communities. A) Nitrogen content. B) Phosphorus content. C) N:P ratios. D) Potentially mineralizable nitrogen (PMN). (n=10)

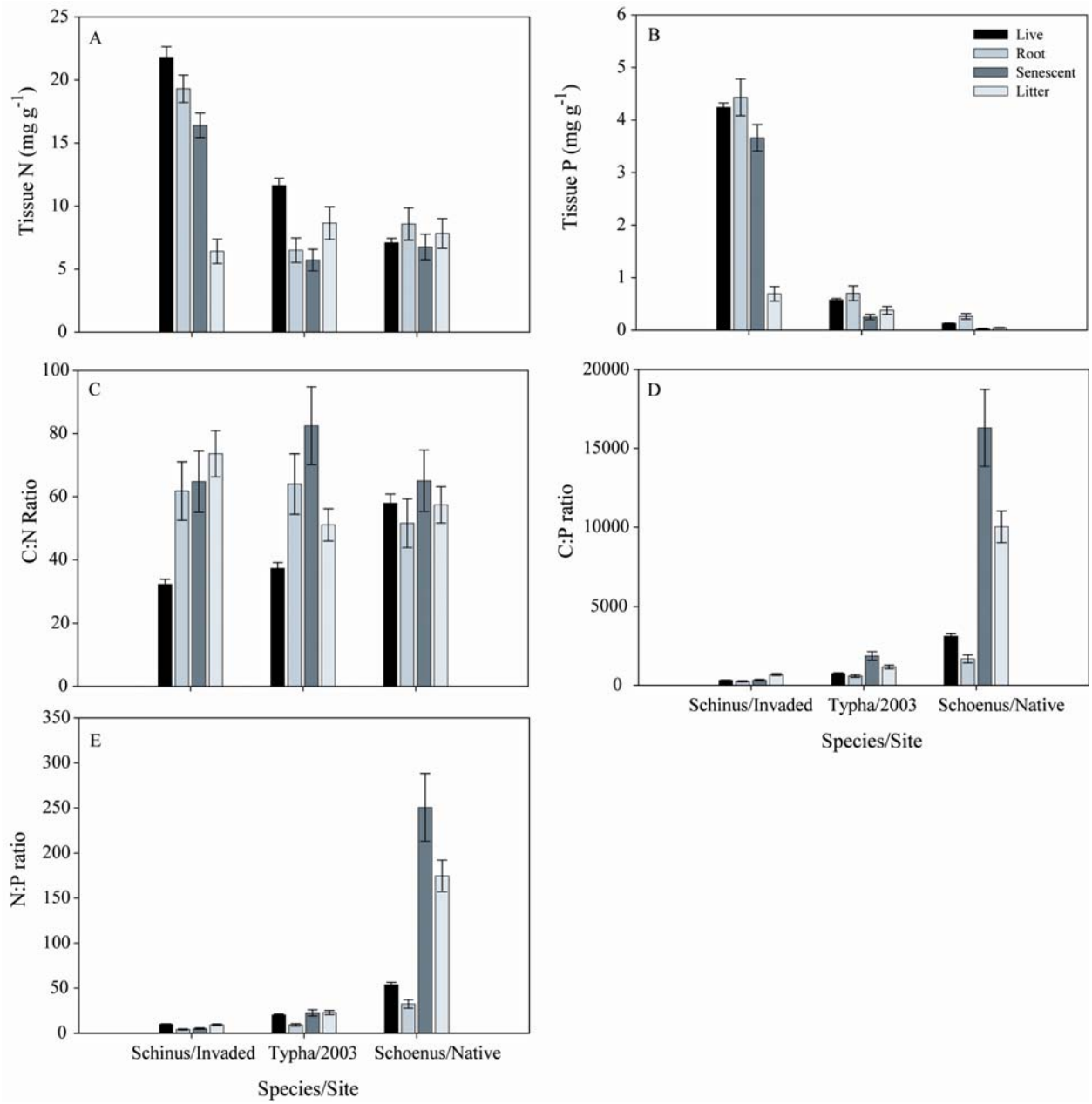

Figure 8-3. A comparison of plant tissue chemistry for *S. terebinthifolius*, *T. domingensis*, and *S. nigricans* for the invaded, native, and 2003 restored wetland communities. A) Nitrogen content. B) Phosphorus content. C) C:N ratios. D) C:P ratios. D) N:P ratios. (n=10)

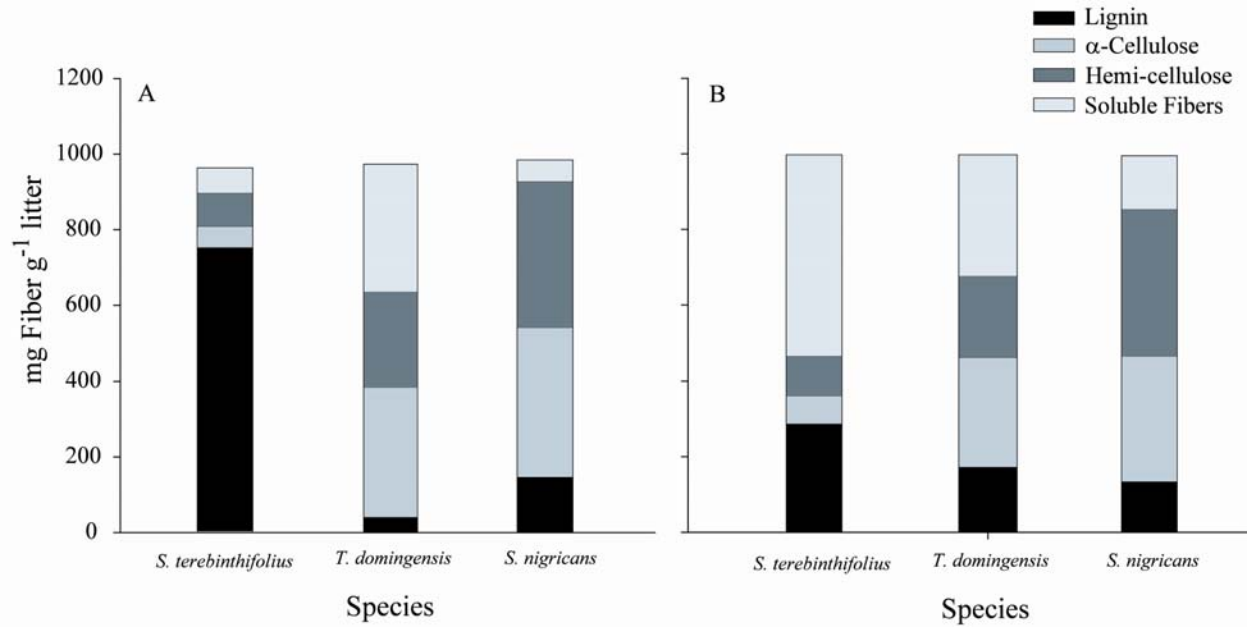

Figure 8-4. Litter cellular fractionation after 365 days of decomposition for *S. terebinthifolius*, *T. domingensis*, and *S. nigricans*. A) Initial content. B) Final content.

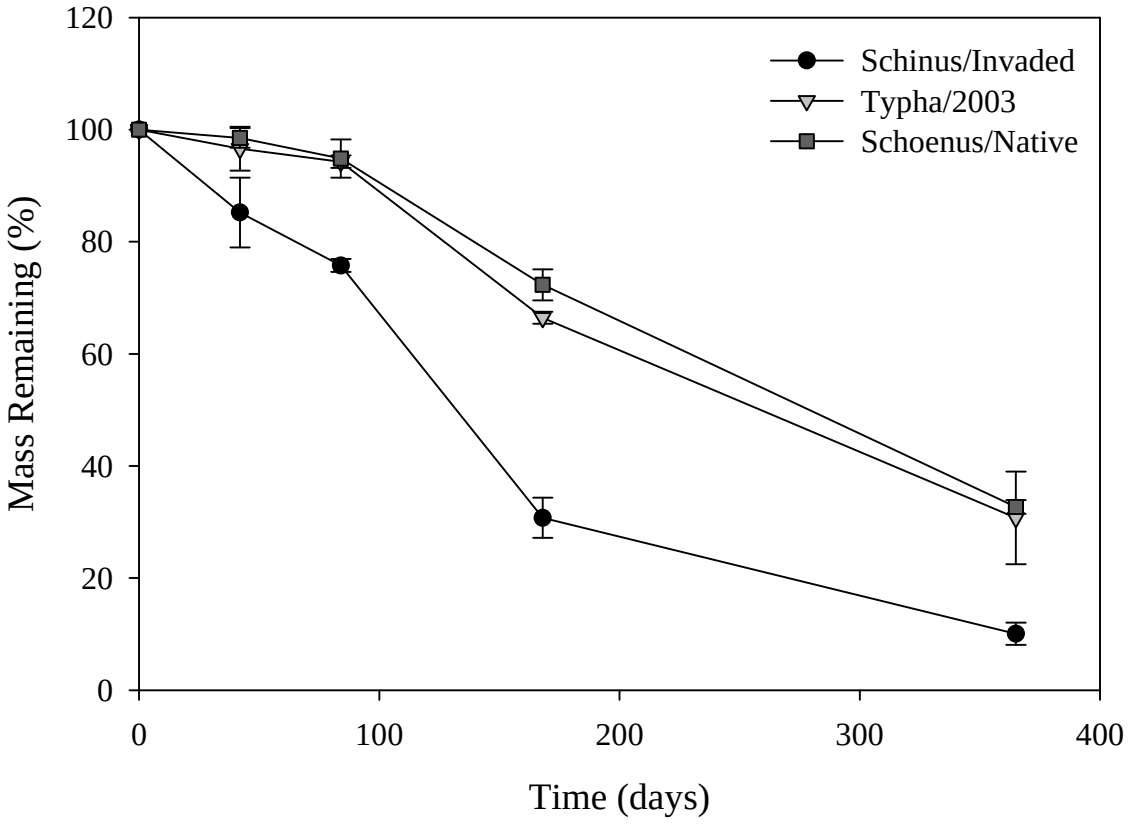

Figure 8-5. Percent mass remaining for *Schinus terebinthifolius*, *Typha domingensis*, and *Schoenus nigricans* from time zero to 365 days.

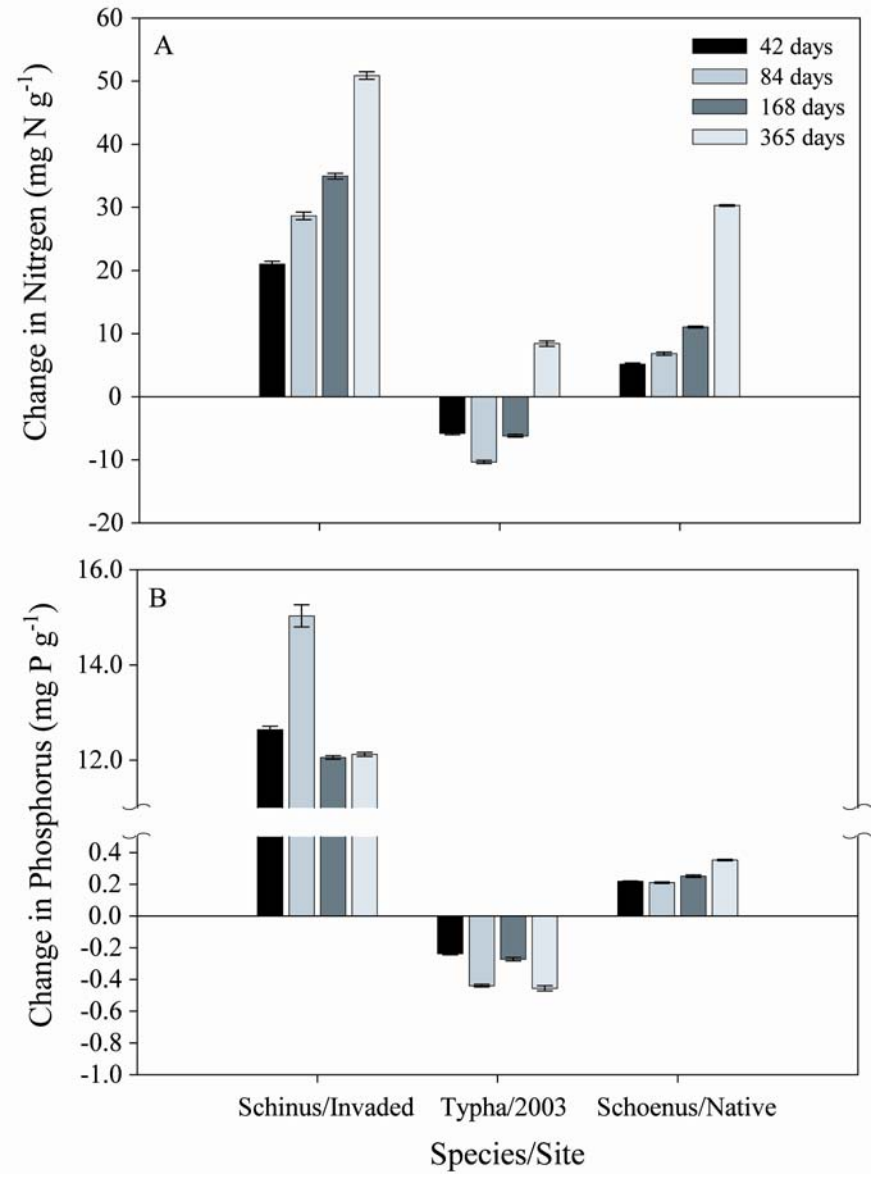

Figure 8-6. Change in nutrients for *Schinus terebinthifolius*, *Typha domingensis*, and *Schoenus nigricans* for each time period analyzed. A) Nitrogen. B) Phosphorus. Positive numbers indicate nutrient mineralization and negative numbers indicate nutrient immobilization.

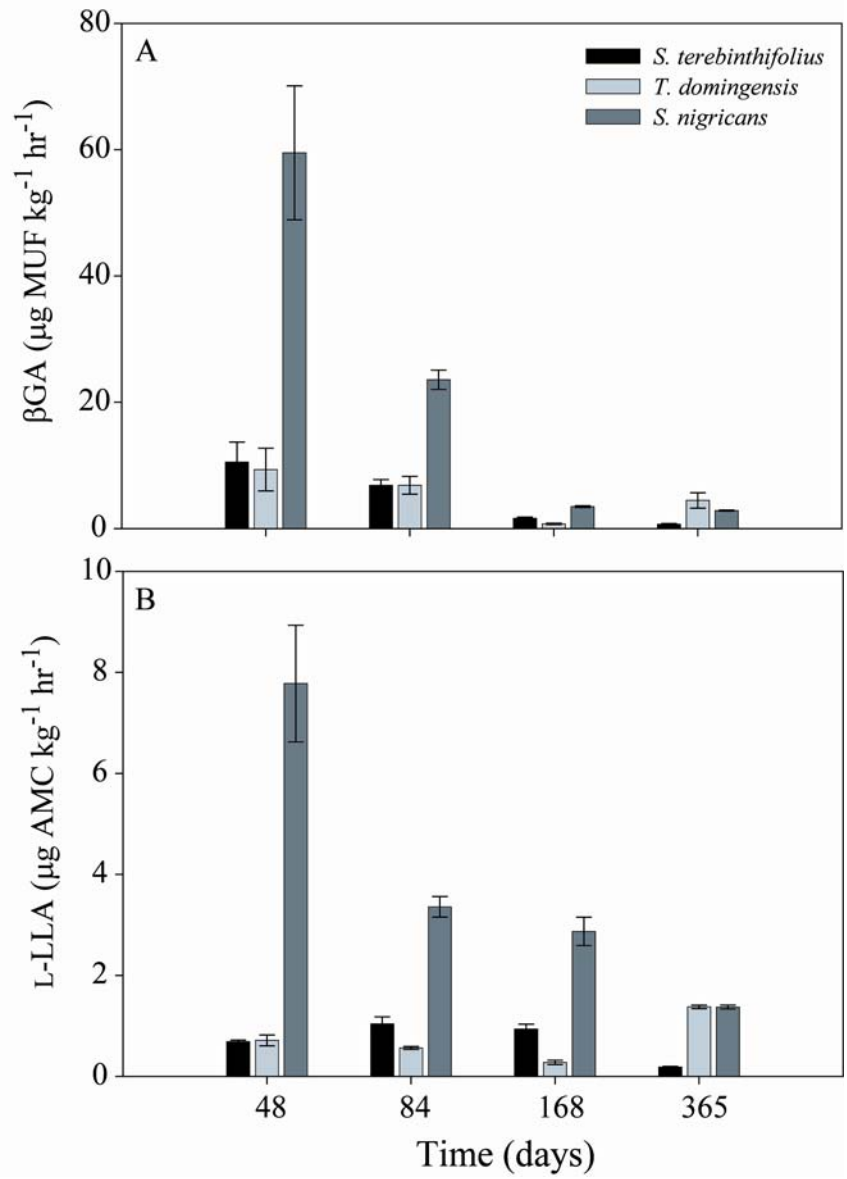

Figure 8-7. Enzyme activities associated with *Schinus terebinthifolius*, *Typha domingensis*, and *Schoenus nigricans* during decomposition. A) β-glucosidase (βGA). B) L-leucine-aminopeptidase (L-LLA).

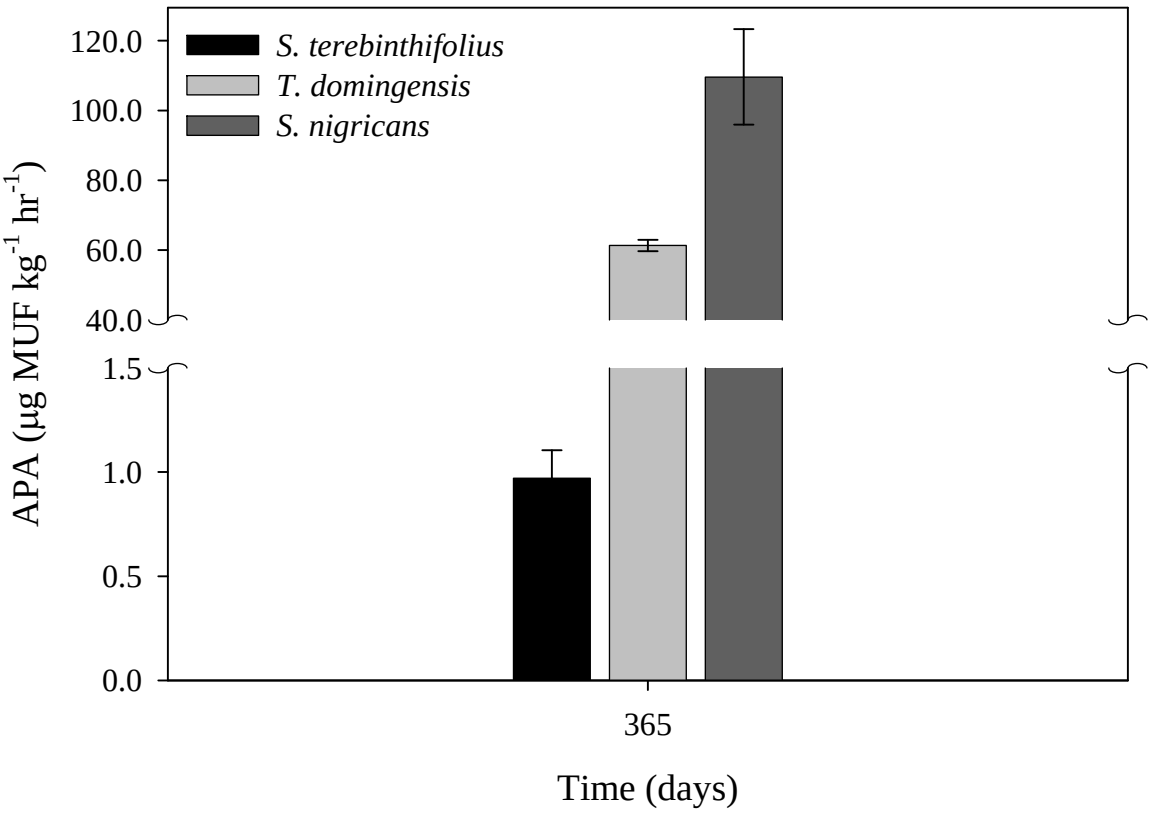

Figure 8-8. Alkaline phosphatase enzyme activity (APA) associated with *Schinus terebinthifolius*, *Typha domingensis*, and *Schoenus nigricans* during decomposition.

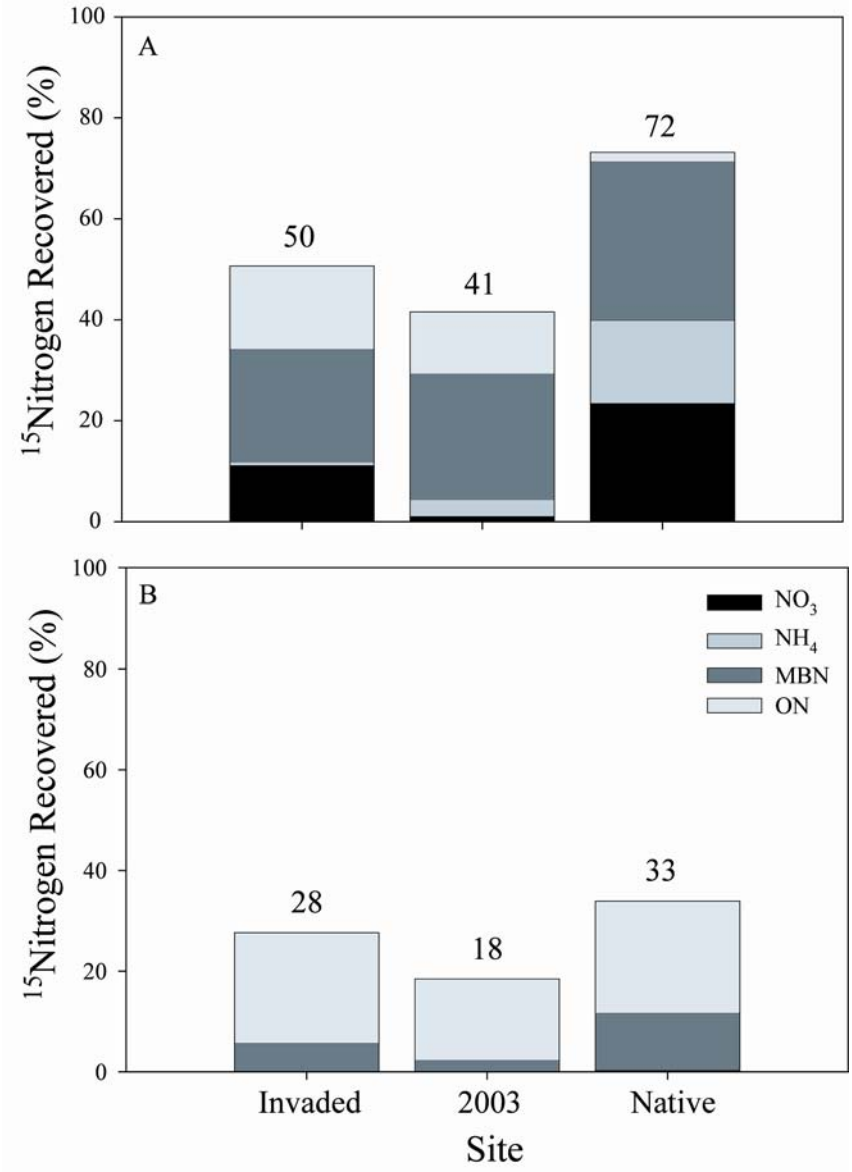

Figure 8-9. The percent ^{15}N recovered in each soil pool fraction from the initial 650 mg ^{15}N m^{-2} that was applied. A) 24 hours. B) 365 days.

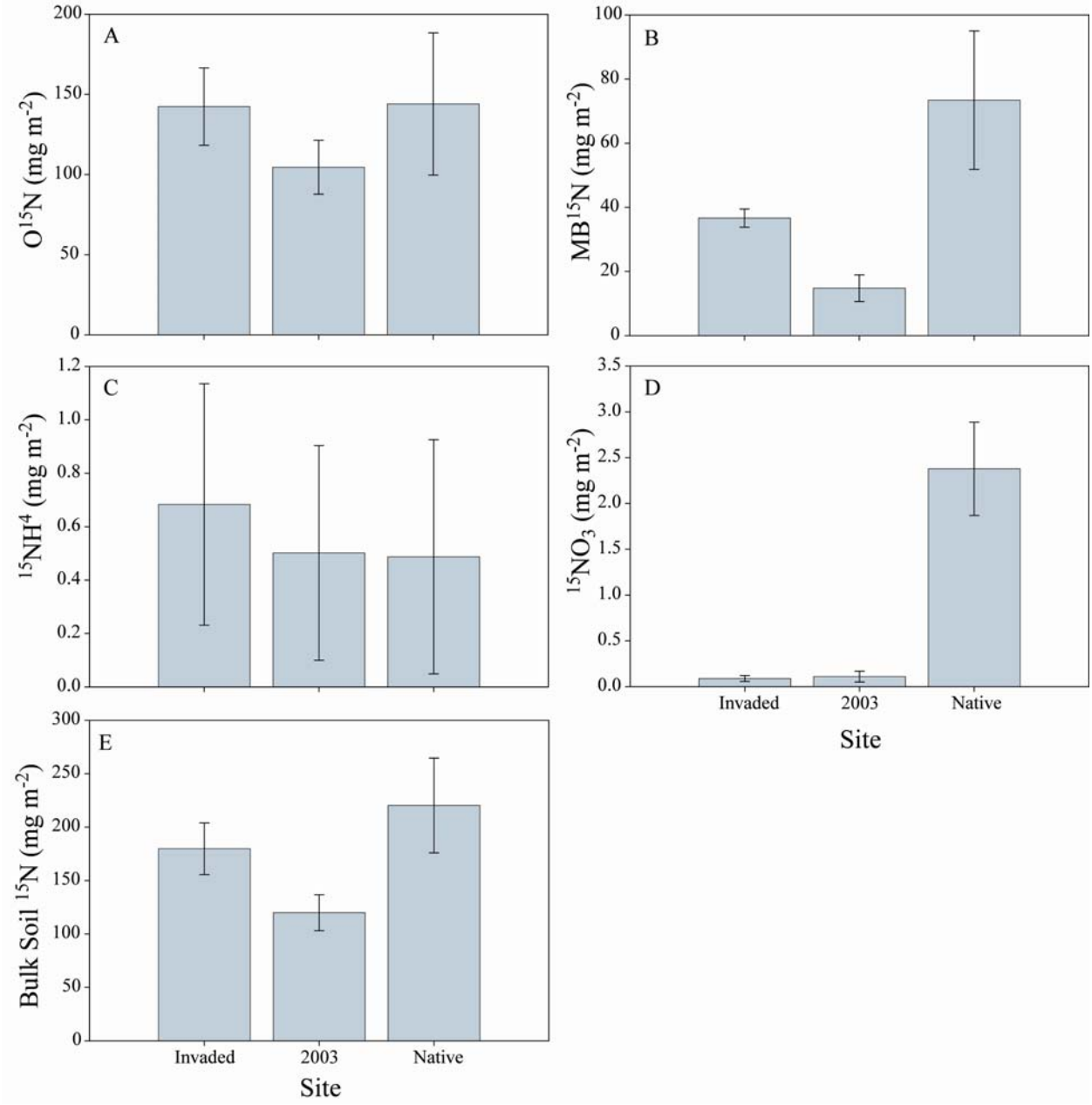

Figure 8-10. The amount of ^{15}N recovered after 365 days in each soil pool fraction from the initial 650 mg ^{15}N m^{-2} that was applied. A) Organic ^{15}N pool. B) $MB^{15}N$ pool. C) $^{15}NH_4$ pool. D) $^{15}NO_3$ pool. E) Bulk soil ^{15}N pool.

CHAPTER 9
SYNTHESIS AND CONCLUSION

One important question in the successional development of the HID is 'what are colonization patterns for *Cladium jamaicense* and *Schoenus nigricans* and why are they not dominating the restored sites?' *Schoenus nigricans* has not been found to repopulate any of the restored wetland communities. This brings up some interesting life history question on the required conditions in order for *S. nigricans* to successfully propagate and survive. *C. jamaicense*, however, is dominant in the 1989 site and starting to become more abundant in more recently restored sites, indicating that with time it will inhabit these wetland areas even if the environmental conditions are not restored to native conditions.

Nitrogen and Phosphorus Limitations Drive Community Development

The vegetation communities which have developed in the restored wetlands of the HID are very different than the surrounding desired native plant communities. Several factors can control re-vegetation patterns after disturbances. In this study we evaluated potential nutrient limitations as a control over vegetation community structure and restoration success.

The links between diversity and function during the successional development of the wetlands in this study has implications to the management of restored ecosystems. Landscape and watershed alterations can result in severe degradation of wetland systems which result in species compositional changes and loss of biodiversity. Wetland systems are driven predominantly by hydrology and many plant species will respond differently to fluctuations or changes in water level and flow. To maximize species diversity and composition development similar to a native (or reference) system, it is important to understand the factors governing the native system. For this restoration project, the native site is a P-limited system with extremely low levels of N and P. To enhance the potential for native plant species in colonize, the nutrient

rich soil was complete removed to eliminate the effects from previous farming practices. While this restoration method was destructive and labor intensive, the result is the development of herbaceous wetland plant communities that, with time (i.e., the 1989 site), have developed a species composition similar to the native plant communities. However, more time is needed for development of ecosystem function.

The NMS ordination performed in this study indicated there is a clear differentiation between the restored and native sites in ordination space. The most distinct contributing factor to this difference in ordination analysis is due to the presence of *S. nigricans* in the native community. The dominance of this plant species in the native community and the lack of its presence in any restored wetland system outweigh any similarities found between the native and 1989 site when analyzed statistically.

By comparing the native and 2003 communities (the two extremes), we found that the native communities are P-limited and that the 2003 restored site may be N-limited within the first few years after restoration. The soil and vegetation in the native community indicates a P-limited system (N:P ratios of 48.3 and 37.7, respectively), whereas the soil and vegetation in the 2003 site indicates a possible N-limited system (N:P of 7.6 and 16.8). It has been suggested that a N:P < 14 results in an N-limitation and a N:P > 16 results in a P-limitation (Koerselman and Meuleman 1996). However, more importantly is the significant difference between the N:P ratios from the native and 2003 sites. The much lower ratio in 2003 site suggests that either more phosphorus and/or less nitrogen are available in the soil. The differences in ratios are a result of the native site having the highest TN and the lowest TP values and the 2003 site having the lowest TN and the highest TP values.

We found that soil N and P content varied considerably within the restored sites but the N:P ratios were less variable. The N:P ratios of the native plant community were two to three times greater than the ratios found in the restored communities. Additionally, the soil P and soil N:P ratios exhibited controls on the plant and plant N:P at both the community and species level. No such conclusion can be made in terms of soil N.

At the vegetation community level, the native plant community has N:P ratios and NUE-P that are two to four times greater than that of the restored plant communities. The species level N:P ratios and NUE-P of the native communities were also two to four greater than the species found in the restored communities. This concludes that at the community and species level, the native site is more P-limited than the restored sites.

We can say with certainty that a P-limitation is prevalent in the native communities as well as most of the restored communities. Little evidence was found to support a N-limitation in any of the sites. The N:P ratios of the site restored in 2003 imply that it is N-limited not P-limited, however, no other data collected at the community or species level indicates a N-limitation is prevalent. At the community level, we find no differences in the NUE-N between sites and the differences observed in species level NUE-N appear to be due to plant traits not a N-limitation.

Due to differences in the level of P-limitations in the restored sites verses the native communities, the increased levels of soil P could influence the re-vegetation patterns of the restored wetlands. At these higher levels of P, the desired vegetation composition (*Cladium jamaicense* and *Schoenus nigricans* co-dominance) is not achieved. A greater understanding of the lasting impacts of the residual P in the restored wetlands is necessary to determine if the desired native vegetation species will inhabit these wetlands with time.

Furthermore, without conducting a N and P fertilization study directly, it is difficult to say with certainty that a N- or P-limitation exists (Vitousek 2004). Over the past decade, several review papers of fertilization studies in terrestrial systems have been conducted to develop critical N:P ratios which allow us to make inferences about nutrient limitations without conducting a fertilization study (Koerselman and Meuleman 1996, Güsewell and Koerselman 2002, Güsewell 2004). These studies focused primarily on community level limitations that did not consider multi-species interactions. Therefore, it is difficult to make conclusions about species level nutrient limitations solely by considering their N:P ratios. The use of additional tools, such as the nutrient-resorption efficiency (NRE) and nutrient-use efficiency (NUE), could be used in conjunction with the N:P ratios of individual species to potentially determine species level nutrient limitations (Vitousek 1982, Vitousek 1984, Berendse and Aerts 1987, Aerts and Decaluwe 1994, Feller et al. 2003, Güsewell 2005).

Nutrient Regeneration

The potential for N and P to be regenerated from the dominant plant species (*Cladium jamaicense* and *Typha domingensis*) found in he HID was investigated via a in situ litter bag decomposition study. *Cladium jamaicense* and *T. domingensis* had different initial nutrient contents and differences in litter quality throughout the decomposition study; however, their decay rates and coefficients were the same. Regardless of the path each litter type followed, the end result in terms of organic matter input into each system was the same.

In regards to the nutrient regeneration, *C. jamaicense* indicated a much greater potential to release N and P for further utilization as compared to *T. domingensis* regardless of site location. This would indicate that in the restored communities where *T. domingensis* is dominating fewer nutrients would be recycled and available for future plant or microbial uptake. This has severe implications in terms of native plant community establishment in restored wetlands. It is clear

that *T. domingensis* has an impact on both N and P cycling which could prohibit native plant communities from colonizing these areas. Competition studies for uptake of N and P is needed to determine how *C. jamaicense* and *T. domingensis* interact for nutrients to determine what effect *T. domingensis* litter decay may have.

Under native conditions, both the microbial communities and *C. jamaicense* are thriving under nutrient limited conditions. In response the microbes produce extra-cellular enzymes to acquire needed nutrients from litter associated with *C. jamaicense*. A greater understanding of this plant-microbe interaction is needed to gain more insight to why the microbial communities across all sites are putting more energy into nutrient acquisition from a litter source of inferior quality.

Long Term Nitrogen Storage

The results of this study indicated that soil processes have a greater influence on ecosystem N dynamics than does the plant community composition. With the use of and ^{15}N tracer study, we found that the soil in the native community stores significantly more N compared to the restored wetland ecosystems. This was not surprising since the restoration technique employed by the ENP to remove all the soil was a severely destructive means of restoration. As a result, the restored wetlands had considerably less total N and decreased N availability. Consequently, the microbial activity in the restored wetlands was elevated relative to the native community which demonstrated that the microbial biomass communities greatly influence soil N dynamics in these restored wetland ecosystems.

Vegetation community level N retention differences were insignificant across sites indicating that regardless of soil N retention the community level vegetation was able to acquire similar amounts of N from the soil. The plant communities inhabiting the restored wetlands benefited from the elevated microbial activities which resulted in higher levels of mineralized N.

Accordingly, the vegetation communities in the restored sites were not more N-limited than the community in the native site.

Applications to Wetland Restoration and Mitigation

It has been suggested that planting desired plant species and diversity levels is needed to facilitate vegetation composition development in restored wetland communities (Zedler 1993, Kellogg and Bridgham 2002, Callaway et al. 2003). In large-scale restoration applications, planting desired plant species is not always feasible. Therefore it is necessary to gain a better understanding of the natural recruitment of plant species from primary succession and the development of ecosystem function to increase the success of large-scale restoration projects. In this study, we found that natural recruitment would result in increases in species richness with time, and that the species composition would develop similarly to the native community provided enough time has past. We saw an immediate recruitment of a diverse plant community consisting of 38 individual plant species within six months of restoration. While additional increases in species diversity were slow, the diversity did increase significantly after 8 years. Additionally, it took between 8 to 16 years before the plant community developed into one representative of the native community. However, the ecosystem function was not restored in this time period, indicating that more time is needed for development of native ecosystem function.

The species composition changed significantly from site to site indicating that the plant communities are very dynamic and unstable. Understanding the seed dispersal, recruitment mechanisms, propagation requirements, and growth and survival rates of the native plant species could aid in the success of restoration efforts. If a desired native plant species has a limited seed dispersal mechanism, it could take several years before that species colonizes or dominates a restored wetland. For example, the seed dispersal mechanism of *T. domingensis* is relatively fast

compared to *C. jamaicense* (van der Valk and Rosburg 1997) which could contribute to the slower colonization patterns observed for *C. jamaicense* in this study.

The results of this study offers evidence that, with time, a diverse plant community similar to a native wetland community can develop without human intervention. However, more time is clearly needed to restore ecosystem function to the level of the native system. The key here is time. Unfortunately, wetland mitigation laws require that wetlands created or restored that serve as mitigation projects are only required 5-10 years of monitoring (Clean Water Act, Section 404). This study along with many others provides ample evidence that this monitoring time period is may not be long enough to restore and maintain plant community structure or ecosystem function (Whigham 1999, Brinson and Malvarez 2002, Kellogg and Bridgham 2002, Callaway et al. 2003, Dalrymple et al. 2003, Seabloom and van der Valk 2003, Polley et al. 2005).

Future Recommendations

It is still unclear of the long lasting effects of this residual P on vegetation community structure in the restoration of the HID. The legacy effect of enriched P lingering in the invaded sites will be a challenge to overcome. The elevated levels of soil and plant P found in the invaded communities have resulted in high P availability as compare to the oligotrophic conditions of the native communities. Previous farming and the consequent invasion by *S. terebinthifolius* has resulted in increase P availability, altered microbial activities and reduced N transformation rates. Additionally, the effect of the restoration method utilized will also carry with it a legacy which the native plant species will have to over come in order to colonize these sites.

The combination of field and laboratory research has provided an indication of the influence of nutrient availability and limitations on macrophyte diversity, dominance and

composition. The results have helped elucidate links between macrophyte diversity and ecosystem functions (i.e., productivity, decomposition, nutrient availability, and nutrient-use efficiency). The knowledge gained from this research can be used to inform scientists and policy makers about the importance of macrophyte diversity in freshwater wetlands as it relates to wetland mitigation and restoration.

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BIOGRAPHICAL SKETCH

Angelique Marie Keppler is the second oldest of seven siblings, she grew up in the hills of southern Ohio in the small Village of Beaver. Angelique spent her childhood days playing in the woods that surrounded a small privately own lake. Her childhood surroundings and her love for nature help guide her future educational goals. After graduating with honors from Eastern High School in 1993, she attended Ohio University studying chemical engineering for a brief period. Her time as a chemical engineering student only helped her to realize even more how much in need our environment was of protecting. After 4 years, Angelique chose to change her educational focus to natural resources and environmental science.

In 2000, Angelique began her environmental science education at The Ohio State University where she graduated with Distinction in Environmental Science in 2002. Upon completion of her bachelor's degree, she immediately began work on her masters at The Ohio State University. Her master's work was focused in ecological engineering. Her research involved the use of ecological treatment tanks to treat highly concentrated manure wash water from the universities dairy milk house. Angelique was awarded her masters degree in 2004.

Angelique's next stop in life took her to the University of Florida in Gainesville where she continued her path in environmental science focusing her research on the ecology and biogeochemical processes of wetlands systems with a minor in botany. Her research was focused in a chronosequence of restored wetlands in the Everglades National Park. The overall goal of her project was to gain a greater understanding of the controls on plant composition and species diversity in relation to coupled biogeochemical processes of nitrogen.

Her future plans include obtaining a position as a professor continuing to conduct research in natural systems. She hopes to develop a research program that focuses on plant species diversity conservation with an emphasis on multi-tropic level relationships and coupled

biogeochemical processes related to carbon, nitrogen, and phosphorus that could potentially alter plant species composition. Additionally, she looks forward to starting a teaching program that will benefit both higher education and K-12 level students.