LONGLEAF PINE (*Pinus palustris* Mill) ECOSYSTEM RESTORATION ON COASTAL WET PINE FLATS: DEVELOPING A MONITORING PROGRAM USING VEGETATION AND SOIL CHARACTERISTICS

By

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To my beloved wife Susana, my wonderful son Pedro, my mother, brothers and sisters

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

LONGLEAF PINE (*Pinus palustris* Mill) ECOSYSTEM RESTORATION ON COASTAL WET PINE FLATS: DEVELOPING A MONITORING PROGRAM USING VEGETATION AND SOIL CHARACTERISTICS

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Longleaf pine ecosystem restoration should include more than reforestation or the application of prescribed fire. It must include the restoration of all the major functions and processes within the forest ecosystem along with restoring overstory and understory species composition. Despite many longleaf pine restoration projects on coastal pine flats, there is no monitoring protocol in place to evaluate the success of an all-inclusive restoration effort. The goal of this study was to establish an ecological trajectory using selected indicators for wet longleaf pine flats as a monitoring framework for restoration projects.

The first specific objective was to quantify the vegetational attributes of longleaf pine flat ecosystems along a chronosequence (2-years after stand replacement to 110-years-old) of stands from within the Gulf Coast Flatwoods zone in Florida. Overstory structure and understory plant species diversity were quantified along the chronosequence. Mean diameter at breast height (dbh), height, and basal area increased until 60-70 years, and then declined. Stand volume continued to increase. Stand density decreased before reaching a steady state. Coleman rarefaction and Shannon-Wiener diversity indices for understory plants exhibited opposite trends during early stand development, but reached "equilibrium" during the mature (> 90 years) phase.

The second objective was to examine soil chemical and microbiological properties along the same chronosequence. Net nitrogen mineralization (N_{min}), soil microbial biomass carbon (C_{mb}), and fungal biomass carbon (C_{fb}) increased from the young to the mid-aged age stands and declined from the mid-aged through the mature age stands. Ammonium production dominated nitrogen cycling and ammonium enrichment occurred on these wet sites by reduction of nitrate (the DNRA pathway). The biogeochemical attributes showed that Florida's Gulf coastal pine flats reach a self-organizing threshold after 85-90 years.

The third objective was to examine the interrelationships between the structural (vegetative) and functional (soil biogeochemical) attributes. N_{min}, C_{mb} and C_{fb} increased with increases in dbh, height, basal area, and volume. Plant species diversity decreased as the FB-to-MB ratio increased. Nitrate levels and nitrifying bacteria numbers were higher in young forest soils than old forest soils. Based upon the indicators, coastal longleaf pine flats reach a steady state threshold with a lower and less variable (tighter) nitrogen cycle at 90 years.

The final objective was to determine if observed structural and functional attributes were useful for evaluating restoration projects. An ongoing restoration project at the Pt. Washington State forest was evaluated for its ecological trajectory following various restoration treatments involving herbicides. The site was determined to be a wet flatwoods based upon environmental ordination and plant species indicator analysis. Herbicide use increased soil microbial biomass carbon and net nitrogen mineralization rates. Imazapyr was the most effective herbicide treatment for this wet pine flats site based upon the level of shrub control, minimum impacts on herbaceous species diversity, and desired structural attributes of the overstory.

Key words: *Longleaf pine, reference communities, monitoring, ecological indicators, herbicides.*

CHAPTER 1
MONITORING LONGLEAF PINE RESTORATION IN COASTAL WET PINE FLAT COMMUNITIES

Longleaf Pine Ecosystems

The longleaf pine (*Pinus palustris* Mill) ecosystems that historically dominated the lower Coastal Plain from Virginia to Texas currently occupies less than 3 % of its original area (37 million ha) (Frost, 2006). This reduction in area has resulted in a great loss of habitat necessary for many plant and animal species (Wade et al. 2000; Van Lear et al. 2005). Longleaf pine ecosystems are naturally maintained by frequent fires that reduce vegetative competition during pine seedling and sapling development (Boyer, 1990). Fires, natural or prescribed, have become severely restricted, especially by urban expansion because of liability and property damage concerns (Achtemeier et al. 1998; Haines et al. 2001).

For the last thirty years, forest industries in the South preferred to replace longleaf pine stands with slash pine (*Pinus elliottii* Engelm.) on wet sites and with loblolly pine (*Pinus taeda* L.) on upland areas (Croker & Landers, 1987). Slash and loblolly pines are considered easier to regenerate and managers have little need to address the longleaf pine's unpredictable period of establishment (grass stage). Furthermore, they also reach commercial size faster than longleaf pine, which shortens the economic rotation (Outcalt, 2000).

In recent years, there has been a great deal of attention given to the restoration of the extensive and species-rich longleaf pine ecosystem. There have been attempts to restore 400,000 ha of longleaf pine in the Southeast during the past decade (WMI, 2006). This effort creates a need for monitoring protocols to be in place for evaluating the success of these restoration efforts. While established monitoring guidelines and programs are active for many of the other forest ecosystems in other parts of the U.S. (ERI, 2003), the lack of such established directives

can hinder longleaf pine restoration projects in establishing functional and self-sustaining ecosystems across its originally extensive range (Devries et al. 2003).

Monitoring Restoration Success

Three established strategies for assessing a restoration effort are direct comparison, attribute analysis, and trajectory analysis (SER, 2004). Permanent-plot studies have been used to directly identify changes over time after stand replacing harvests or other impacts. Attribute analysis uses the measurement of ecological indicators to evaluate ecosystem conditions without directly considering patterns over time. Trajectory analysis uses data collected over periodic time intervals to identify if restoration trends are toward a reference condition (SER, 2004). We used a combination of attribute analysis and trajectory analysis to monitor our restoration project. Since expensive permanent-plot studies are generally limited to less than 30 years, conducting a trajectory analysis employing a space-for-time substitution or chronosequence can be an efficient alternative for describing general trends over time or to test hypotheses based upon forest succession (Pickett, 1989). Chronosequencial studies make use of a group of sites that have similar biotic, climatic, soil biogeochemical, and historical characteristics, but differ in age since a harvest or other stand replacing disturbance (Pickett, 1989). By comparing the different aged sites, one can identify changes in composition or function between decades, centuries, or even millenniums (Williamson et al. 2005).

There is a critical requirement for the different aged sites to be subjected to the same historical conditions and have the same species available over the chronosequence to give validity for using the space-for-time substitutions. One must also deal with separating spatial variability from the variability associated with time (Veldkamp et al. 1999). A recent chronosequence study examined the relationships between stand development and understory vegetation on 15 stands ranging from 7 to 427 years in a mixed conifer forest along the

California-Oregon border. Regression analysis showed canopy openness was positively correlated with total understory cover, species richness, diversity, and composition. Surprisingly, no correlations were observed between any of the measured stand attributes. Shrub and graminoid species were negatively correlated, and forbs were positively correlated, with stand age (Jules et al. 2008). Another study used detailed forest inventory and climatic data from 43 stands along a 250-year chronosequence to assess the effects of disturbance and climate on biomass accumulation patterns across Russia. Regression analysis indicated as expected the highest biomass increments in the warmest regions and the lowest in the coldest regions. Spruce (*Picea* spp.) and birch (*Betula* spp.) forests had the highest biomass increments while larch (*Larix* spp.) and aspen (*Populus* spp.) forests had the lowest biomass accumulation. The faster growing spruce and birch forests had declines in biomass accumulation rates after 150 years whereas the slower growing larch and aspen never showed declines during the 250-year chronosequence (Krankina et al. 2005).

Monitoring Soil Characteristics

In addition to vegetative characteristics, mineral pools, and the mineralization of key elements have been identified as important attributes for evaluating restoration success in recent years (Müller et al. 2000; Müller and Lenz, 2006). During the last decade there has been a major effort at assessing the effects of different forest management practices on the long-term soil productivity of southern pine forests (Burger and Kelting, 1999), including coastal wet pine flats (Lockaby and Walbridge, 1998; Lister, 1999; Burger and Xu, 2001; Burdt, 2003). These studies have assessed treatment effects utilizing a set of soil indicators (Kelting et al. 1999) including soil pH, soil organic matter content, soil moisture content, and the mineralization levels of nitrogen, and phosphorus (Reynolds et al. 2000; Redding et al. 2004). For example, a recent chronosequence study examined the relationship between biomass accumulation and nitrogen

availability over 87 years in *Populus grandidentata* forests. Overstory biomass increment increased with stand age while understory biomass levels decreased. Net nitrogen mineralization rates were found to decrease during the first 18 years after harvest than increase over the next 70 years (White et al. 2004). In an earlier investigation, forest floor microbial biomass was studied in a chronosequence of northern hardwood forest stands ranging from 3 years after clearcut to 120 years. Microbial biomass increased during the early successional stage, decreased during the mid-aged stage, and then increased during the late successional stage. Soil organic matter followed a pattern similar to microbial biomass. There was no trend in the fungal-to-bacterial ratio along the chronosequence. Soil moisture was strongly and positively correlated with fungal biomass. Soil pH was negatively correlated with fungal biomass. Finally, ammonium (NH_4^+) production increased from the early to mid-aged stages and then decreased from the mid-aged to late successional stages (Taylor et al. 1999).

Developing a Monitoring Program

A good monitoring program should be well focused on just a few key indicators to provide for statistically sound information (Lindenmayer, 1999). The standards for restoration are obtained from measuring key environmental indicators at the restoration site and comparing them to established reference communities (SER, 2004). In ecological restoration, the pathway from the degraded condition to the restored, self-sustaining condition is called the ecological trajectory (Stanturf et al. 2001). Predicting the ecological trajectory of a longleaf pine forest is difficult because of the great variety of disturbance regimes associated with southern pine forest ecosystems along the Gulf Coast (Palik et al. 2002).

To define when a given ecological trajectory has reached a self-sustaining state it is important to establish some specific goals for the restoration project (Hobbs & Harris, 2001). Two notable standards are to restore viable populations of key native species in natural patterns

of abundance and distribution, and to sustain key geomorphologic, hydrological, ecological, biological, and evolutionary processes within the normal ranges of variation (ecological integrity; Müller et al. 2000). Forest structure and plant species composition are two of the indicators being monitored in this study to capture the successional and developmental forces. Soil chemical properties, net nitrogen mineralization, and soil microbial dynamics were also included as indicators to insure that key biogeochemical, ecological, and biological processes are also being evaluated (Harris, 2003; Müller and Lenz, 2006).

How does one determine if these goals are being achieved along the successional pattern? The normal range of variation along a spatial scale can be determined by using a series of reference communities that are evenly distributed along the distinct ecologically identified range, to compare with the restoration site (Harris, 1999). To evaluate changes in restoration along the chronosequence, each reference community had to contain stands representing distinct ages distributed evenly as possible along the 110-year scale (Müller, 1998).

In summary, the following steps have been recommended to insure that a monitoring plan functions properly: a) Set monitoring goals, b) identify the resources to monitor, c) establish threshold levels, d) develop a sampling design, e) collect and analyze data, and f) evaluate results (Block et al. 2001). The overall goal of this study was to establish an ecological trajectory using selected indicators for wet longleaf pine flats so that it can be used as a monitoring framework for restoration projects. The next four chapters will address the following four specific objectives of this study.

1. Quantify the vegetational attributes of longleaf pine flat ecosystems, along a chronosequence (2-years after a stand replacing disturbance to 110-years) of stands from within the Gulf Coast Flatwoods zone of Florida.

2. Examine soil chemical (soil organic matter content, pH, plant-available phosphorus, net nitrogen mineralization) and microbiological (microbial biomass carbon, fungal biomass carbon) properties along the same chronosequence.

3. Examine the interrelationships between the structural (vegetative) and functional (soil biogeochemical) attributes.

4. Determine if the observed structural and functional attributes could be used to evaluate restoration projects.

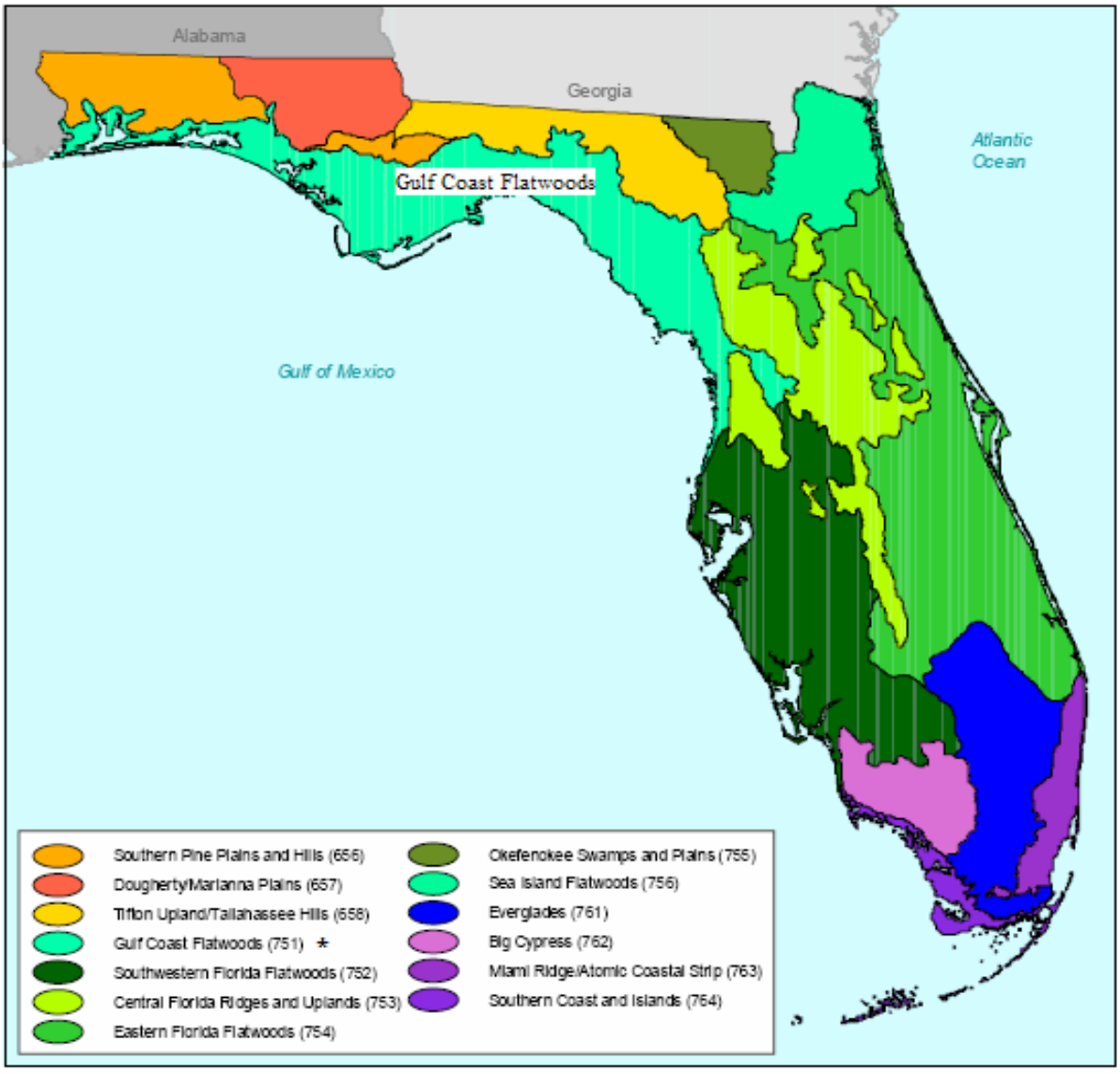


Figure 1-1. Florida's Gulf Coast Flatwoods zone where the wet pine flat sites are located (Florida DEP, 2002).

CHAPTER 2
FOREST STRUCTURE AND PLANT SPECIES DIVERSITY IN WET LONGLEAF PINE FLATS ACROSS A CHRONOSEQUENCE

Introduction

In recent years, there has been a great deal of interest in restoration of the longleaf pine ecosystem, one of the most threatened ecosystems in the United States with less than 3% of its original extent remaining. There have been attempts to restore 400,000 hectares of longleaf pine in the Southeast during the past decade alone (WMI, 2006). This situation creates a need for developing monitoring protocols to evaluate the success of these restoration efforts. While established monitoring programs are in place for many forest ecosystems in other parts of the U.S. (ERI, 2003), the lack of such established guidelines can hinder the restoration of the longleaf pine forest as a functional, self-sustaining ecosystem across its former range (Devries et al. 2003).

Community structure and species composition are two key attributes often evaluated in restoration projects (Brockway et al. 2005). However, reliable information on the ecological trajectory of longleaf pine ecosystems have hampered monitoring of restoration projects in Florida and elsewhere in the Southeast. Although past research has examined the structure and species composition of upland longleaf pine ecosystems, little information exists on the temporal patterns of forest structure and plant species diversity in wet longleaf pine flat communities located along the coastal lowlands of Florida's Gulf Coast (Michener, 1999). Wet pine flats are pine-dominated, poorly drained, broad plain wetlands (Stout and Marion, 1993; Harms et al. 1998).

In Florida, plant species richness has been found to increase with soil moisture until an ecotone between wet pine flats and cypress swamps is reached (Huck, 1986; Walker, 1993; Kirkman et al. 2001; Walker and Silletti, 2006). This ecotone is the zone where one finds wet flatwoods and wet savanna subtypes of the coastal wet pine flat (Messina and Conner, 1998). Their overstories are dominated with varying mixtures of longleaf and slash pines, but also might contain a component of Choctawhatchee sand *(Pinus clausa* var. *immuginata)* and/or pond *(Pinus serotina*) pine (Parker and Hamrick, 1996).

The environment for Florida's wet pine flats is the 1,240 km-long Gulf Coast, containing sounds, bays, and offshore islands. This coastal landscape is continuously shaped by active fluvial deposition and shore zone processes which promote and maintain the formation of beaches, swamps and wet mineral flats. The local relief ranges from 0 to 20 m in elevation. Annual precipitation ranges from 1300–1600 mm and average annual temperatures vary between 19°-21° C. Growing seasons are long, lasting 270-290 days (McNab and Avers, 1994). Soil parent material consists of marine deposits containing limestone, marl, sand, and clay. The dominant soils are Aquults, Aquepts, Aquods, and Aquents. These highly acidic soils have thermic and hyperthermic temperature regimes and an aquic moisture regime. The major forest type of this region is the longleaf-slash pine flatwoods, while water oak (*Quercus nigra*), swamp tupelo (*Nyssa sylvatica* var. *biflora*), sweetbay (*Magnolia virginiana*), and cypress (*Taxodium* sp.) are found along the major river drainages and isolated depressions (McNab and Avers, 1994). Florida's subecoregional Gulf Coast Flatwoods (Figure 2-1) covers the majority of this geographical area where both pine savannas and coastal flatwoods occur in close association with cypress ponds (Myers and Ewel, 1990; Griffith et al. 1994). Because of the growing

conditions, wet pine flats are highly productive ecosystems, and represent more than one million ha in the Southeast (Burger and Xu, 2001).

There are almost 200 rare vascular plant taxa found in the great variety of habitats classified as longleaf pine ecosystems. In addition to the majority of them being found in Florida (Collins et al. 2001), the richest sites are found in these wet pine flats and their associated wetlands (Walker, 1993). The uniqueness of Florida's wet pine flat communities make them crucial for plant species diversity and the rarity of plants to be evaluated in any monitoring plan for restoration (Walker, 1993; Collins et al. 2001; LaSalle, 2002).

One of the ways by which restoration progress can be monitored is by examining the ecological trajectory of the restored site and comparing it with the ecological trajectory of reference sites. Post-stand replacement secondary forest succession has been well studied in other ecosystems and is considered to follow four stages of development (Peet and Christensen, 1980; Oliver, 1981). *Stand Initiation* commences after a stand-replacing disturbance has occurred. Plants regenerate from sprouts, seed banks or newly dispersed seeds. Any advanced regeneration (saplings and seedlings) is released by the disturbance and commences accelerated growth. The *Stem Exclusion* stage is when the individual trees in the stand come under fierce competition for light, water, and nutrients. Canopy closure results in a great reduction of stand density as the residual stocking contains fewer, larger trees. *Understory Reinitiation* occurs when some of the dominant overstory trees begin to die forming gaps for new regeneration. Finally, the *Old Growth* or *Steady-State* stage is reached when gap dynamics dominates the landscape and the forest is now all-aged. Snags and downed logs are also found throughout the landscape (Perry, 1994). Gap dynamics is major source of regeneration in natural longleaf pine forests (Brockway and Outcalt, 1998). Steady-state is tied to the ability of a system to be self-

organizing and resilient (Müller et al. 2000; Müller and Lenz, 2006). Self-organizing forces become especially apparent during the understory reinitiation stage of forest succession when the steady-state mosaic begins to form.

When identifying patterns of succession along a chronosequence, stand changes caused by disturbance must also be considered (Frelich, 2002; Pickett and Cadenasso, 1995). The longleaf pine forest is a pyro-climax ecosystem which relies on short fire return intervals to maintain the "steady-state" stage over other woody plant species (Wade et al. 2000). In coastal wet pine flats, wind and precipitation are also major "shapers" of longleaf pine communities. Hurricanes directly affect the canopy structure of longleaf pine stands through gale-forced winds, opening them up to sunlight and changing the composition of the flora and fauna that occupy them. Hurricanes also affect longleaf pine stands by the extensive flooding that accompanies the wind. Extended flooding can cause changes in both the above and below ground productivity (Johnston and Crossley, 2002; Palik et al. 2002).

Anthropogenic effects caused by human activities in forests can also change the forest structure. Timber harvesting, grazing, and prescribed fires can cause changes in the structural complexity of forests having negative effects by exposing surface soils and reducing biodiversity (Redding et al. 2004; Van Lear et al. 2005). In addition, climatic changes such as increased atmospheric CO_2 levels may affect the soil biotic community, adding to the potential negative feedbacks toward the productivity of aboveground plant communities (Peacock, 2001; Frelich, 2002). Measuring forest structural data along a chronosequence will make it possible to evaluate change along the life cycle of coastal wet longleaf pine flats.

The objective of this study was to examine stand structural attributes and understory plant species diversity along a 110-year chronosequence. We hypothesized that stand DBH, height,

basal area (BA), and volume would increase while stand density and plant species richness and diversity would decrease through the mid-age. We expected these parameters to reach a threshold or steady-state during the mature phase when the understory reinitiation stage of succession has begun. Quantification of this ecological trajectory would help establish monitoring thresholds in terms of stand structure and plant species composition for restoration projects.

Materials and Methods

Study Sites

Three study sites were established along a wet pine flats located within three kilometers of Florida's Gulf Coast. They were Topsail Hill State Park, St. Marks National Wildlife Refuge, and Chassahowitzka Wildlife Management Area. They were found between the cities of Pensacola and Tampa Bay (Figure 2-1), a narrow zone that makes up the majority of the Natural Resource Conservation Service's Eastern Gulf Coast Flatwoods ecoregion (MLRA 152A) and the National Oceanic and Atmospheric Association's Panhandle Coast unit of the Louisianan reserve (National Estuary and River Reserve System). Both of these federal designations make this zone unique from an ecological as well as hydrological perspective. Within the Eastern Gulf Coast Flatwoods zone is the Gulf Coast Flatwoods (75I) subecoregion of Florida (Figure 2-1; Griffith et al. 1994). Any environmental variations between these sites were minimized by establishing very specifically defined spatial scales. This was accomplished by stratifying the important segments of the whole system (e.g. Florida's Gulf coastal flatwoods) down to the smallest distinct scale as possible (e.g. wet pine flats within 3 kilometers of the coast) in order to take meaningful measurements (Chertov et al. 1999; Frelich, 2002; Müller et al. 2000). The first 120 years of longleaf pine succession has been included with wet pine flats situated within 3 km of the Florida Gulf coast as the temporal and spatial scales in this study. These two scales were

determined from an in-depth preliminary survey of stand conditions found within the Gulf Coast Flatwoods zone of Florida and at the restoration site.

The herbaceous ground cover of wet longleaf pine flats is very diverse due to the warm temperatures and high rainfall. Broomsedge (*Andropogon virginicus)*, wiregrass (*Aristida stricta* var. *beyrichiana)*, witch grass *(Dichanthelium spp*.), goldenrod (*Solidago odora*), meadow beauty (*Rhexia alifanus)*, fetterbush (*Lyonia lucida)*, and aster (*Aster adnatus)* are found on both subtypes (Brewer, 1998). Pine savannas are distinguished from wet flatwoods by a greater abundance of beak sedge (*Cyperus)*, nut rush (*Scleria cilliata*), bloodroot (*Lachnanthes caroliniana)*, pitcher plants (*Sarracenia)*, and orchids (*Calopogon) or* (*Platanthera)*. Coastal flatwoods have a greater presence of titi (*Cliftonia monophylla)*, swamp tupelo, gallberry (*Ilex glabra)*, saw palmetto (*Serenoa repens*), and sweetbay. Where fire is restricted, catbrier (*Smilax pumila)* can be a prevalent vine species (LaSalle, 2002).

All three of the selected sites have a moisture gradient as represented by cypress swamps, wet pine savannas, and wet pine flatwoods. All three sites have active restoration management programs where fire has been used for more than 20 years on approximately a three-year-return interval. All of the sites are primarily managed to enhance habitat for threatened species associated with longleaf pine ecosystems, and are managed by a state or federal agency.

The southern site on the spatial gradient is the Chassahowitzka Wildlife Management Area in Hernando County, FL. It is approximately 12,140 ha, and the soils are dominated by Basinger fine sands (sandy, siliceous, hyperthermic spodic Psammaquents) and Myakka fine sands (sandy, siliceous, hyperthermic aeric alaquods) (Hyde et al. 1977; Spencer, 2004).

The St. Marks National Wildlife Refuge in Wakulla and Jefferson Counties, FL consists of 25,900 ha and the majority of the soils are mapped as the Scranton series (sandy, siliceous,

thermic humaqueptic Psammaquents) and the Leon series (sandy, siliceous, thermic aeric Alaquods) (Reinman, 1985; Allen, 1991).

Topsail Hill State Park in Santa Rosa County, FL, contains 610 ha of some of the oldest longleaf pine stands in Florida. The soils are Pickney sand series (sandy, siliceous, thermic cumulic humaquepts) and the Leon series (Overing and Watts, 1989; White, 2001).

Forest Age Classes

The 110-year chronosequence starts from the point of stand replacement to the oldest stands measured in our reference sites. The following age classes were used to stratify and analyze changes in forest structure and plant species composition within the different stands at each of the reference sites. There are 12 replicates per age class for the stand data and 48 replicates per age class for plant species data. The age classes provide a means to identify the structure of stands within specific time periods along the chronosequence and to detect changes from one time period to the next (Müller, 1998; Aravena et al. 2002). In this paper, a tree is defined as a woody plant with a diameter at breast height (DBH) of greater than 10 cm. A sapling is a woody plant with a DBH of less than 10 cm but greater than 2.5 cm. Finally, a seedling is a woody plant that is less than 91.5 cm in height (Wenger, 1984).

The young age class: A young age stand exists when the majority of the stocking (> 70%) can be found as seedlings and saplings. The average stand age should be < 20 years since replacement.

The mid-aged class: The mid-age stand should have stocking (>70%) dominated by a mixture of poles and small sawlog size trees (10-30 cm DBH). The average stand age should be between 20 and 55 years old.

The mature age class: A stand is considered within the mature age class when the majority of stocking (>70%) can be found as dominant sawlog trees (30-45 cm DBH). The stand age should be > 55 years old.

Field Measurements

Each reference location had a cluster of three one-hectare blocks, containing stands representing each of the three previously defined age classes. Each one-hectare block was sub-divided into four randomly placed 400 m^2 measurement plots. Tree height and DBH were measured on all trees > 10 cm DBH. At least two of the dominant trees were cored at breast height to determine stand age. Stand density (trees/ha), basal area (m^2/ha) and standing volume (m^3/ha) were calculated from these data. In addition, the volume (m^3/ha) of all snags and downed woody debris (CWD) were also calculated. The equation used for tree and snag estimates was:

$V = (0.000078539816*(DBH^2))*$tree height.

The volumes of downed logs were estimated with Smalian's metric equation:

$V = [((D^2) + (d^2))*0.00003927]*$ log length (m),

where D = diameter large end (cm) and d = diameter small end (Wenger, 1984).

We adapted the system of five decomposition states for snags and downed woody debris used by Spetich et. al. (1999). The decomposition descriptions translated to five levels of decomposition deductions by percent (15, 30, 45, 60, and 75%; see Table 2-1).

Each 400 m^2 plot contained four smaller 1 m^2 subplots randomly placed within the larger plot for understory sampling (Figure 2-3). Percent cover of each species was assessed using a modified Daubenmire method incorporating eight different levels (Daubenmire, 1959). Coleman rarefaction and the Shannon-Weiner diversity indices were calculated for each stand (Koellner and Hersperger, 2004; Colwell, 2006).

Data Analysis

A three stage balanced nested design was used to integrate the indicators measured at different scales, and among sites (Figure 2-2). Hypothesis testing for differences between means was accomplished by using two-sample t-test with an alpha of 0.05 and a two-tailed confidence interval. The sampling of nine distinct reference locations produced a dataset where the assumptions for analysis of variance (ANOVA) was not ensured; therefore, non-parametric tests were used to detect any significant differences among the reference sites and among the distinct forest age class segments (SAS, 2002).

Trends over time and between variables were obtained from linear regression using the general linear model (PROC GLM) (Yang et al. 2006; SAS, 2002). Plant species indicator analysis (IndVal) was used to measure the level of relationship between a given plant species to categorical units such as pine flat subtypes or forest age classes. It calculates the indicator value d of species as the product of the relative frequency and relative average abundance in each categorical cluster. Indicator species analysis is used to attribute species to particular environmental conditions based on the abundance and occurrence of that species within the selected group. A species that is a "perfect indicator" is consistent to a particular group without fail. Indicator values range from 0 to 100, with 100 being a perfect indicator score. Because indicator species analysis is a statistical inference, a test of significance is applied to determine if species are significant indicators of the groups with which they are associated (Dufrene and Legendre, 1997). This is achieved by the Monte Carlo permutation test procedure (1000 iterations), where the significance of a P-value is determined by the number of random runs greater than or equal to the inferred value (α=0.10). Accuracy is defined from the binomial 95% confidence interval (Strauss, 1982).

Results

Overstory Stand Structure

The mean stand DBH, height, BA, and volume varied significantly among the age classes (Table 2-2). For example, the mean DBH for the young stands was 6.0 cm, 23.4cm for the mid-age stands, and 30.0cm for the mature age stands. Height, BA, and volume exhibited similar results. Snags and downed woody debris had similar values for the young and mid-age stands, but was significantly higher for the mature stands (Table 2-2). Regression analysis revealed trends over the chronosequence for stand structural variables. Except for stand density, all of the stand variables increased with forest age class. Stand density was highly variable over time and did not exhibit any specific patterns (Figure 2-3). As expected, stand DBH increased with age until 85-90 years then began to reach a steady-state (Figure 2-4). Stand height also increased over time, but reached an asymptote at 85-90 yrs. Stand basal area and volume followed similar regression curves as with DBH and height (Figure 2-4). Downed woody debris accumulation levels were highly variable, but in general increased over the 110-year chronosequence (Table 2-2; Figure 2-5). The volume of standing deadwood (snag) increased through the mid-age stands and then decreased thereafter (Figure 2-6). The level of CWD decomposition remained the same for the young and mid-age stands, but was lower for the mature age stands (Figure 2-7).

Understory

The abundance of grasses and forbs decreased, and the abundance of shrubs increased over the three forest age classes ($p < 0.05$; Figure 2-8). The Shannon-Wiener diversity index ranged from 1.28 – 2.40 for the dataset. In general, Shannon-Wiener diversity index decreased over time (Figure 2-9). Shannon-Wiener diversity index also decreased with stand density during the young age class, but increased with stand density during the mature age class (Figure 2-10). The Coleman rarefaction index ranged from 7.2-22.0 for the dataset (Table 2-9). The Coleman

rarefaction index increased with stand height during the young age class, but decreased with stand height during the mid-aged class (Figure 2-10).

Bluestem grass, blueberry (*Vaccinium* spp.), and witchgrass were the dominant plant species indicators for the young age stands ($p < 0.022$). Meadow beauty, wiregrass, and Carolina redroot were the dominant plant species indicators for the mid-aged stands ($p < 0.067$). Gallberry, running oak (*Quercus pumila*), and dangleberry were the best plant species indicators for the mature age stands ($p < 0.1$; Table 2-3).

Discussion

Overstory

The overstory variables of mean stand DBH, stand height, stand BA, and volume exhibited strong positive relationships with stand age as expected. Even downed logs and snags, heterogeneous variables among the sites and within age classes, produced a weakly positive trend with stand age ($p < 0.042$). Stand density showed no clear pattern along the chronosequence, owing to the high variability found within density across the age gradient. The data showed most of the growth variables reaching an asymptote around 85-90 years.

When the chronosequence stand data were compared to growth and yield of natural longleaf pine stands, our stands were found to have lower basal area (14 m^2 vs. 25 m^2) at age 30, but comparable stand volumes (150 m^3 vs. 130 m^3) at age 60 (Farrar, 1985). The steady-state phase for these forests is reached around 85-90 years, may be shorter than the steady state of 110 years for longleaf pine ecosystems in Texas, reported by Chapman (1909).

All of the stands over 85 years measured at our reference sites had large gaps containing saplings and some poles. This structure would indicate that the understory reinitiation stage of secondary succession was well along and the ecosystem's self-organization capacity was apparent. Of the aboveground indicators, both stand density and CWD had the greatest

heterogeneous datasets based upon statistical analysis. This great variability in stand density and CWD reflects the differences that must be evaluated when comparing "natural" versus intensively managed forest stands.

Stand growth is high during the early phase, but is slowed during the mid-aged class when the stem exclusion state is reached. Interspecific competition results in mortality of individuals and the snag accumulation rates increase. This was evident along the chronosequence when snag accumulation peaked between 60 and 80 years. During the mature phase, stand growth is slowed as the forest reaches a steady state. This indicates that the mid-aged class was the major period for CWD accumulation brought on by both competition and disturbance (Spetich et al. 1999). Downed logs and snags continued to accumulate during the mature phase, but with a decreasing rate of decomposition.

Plant Species Diversity

The species diversity exhibited interesting patterns along the chronosequence. Stand density had a strong negative relationship with the Shannon-Wiener diversity index within the young age class, but a positive relationship during the mature age class (Figure 2-10). The Coleman rarefaction index increased with stand height within the young age class then decreased during the mid-aged class (Figure 2-10). The change in response between stand height and Coleman rarefaction, and stand density with the Shannon-Wiener diversity index is due to how these two distinct indices calculate species diversity.

The Coleman rarefaction function gives more weight to the rarity than commonness of species. The function looks at the 'β' diversity where the turnover of species between two distinct species pools is measured. There was an expected number of species E(s) where Monte Carlo iterations were performed to predict which species would be more likely appear (Hurlbert, 1971). In this case, species diversity became related to habitat diversity or habitat heterogeneity

(Hersperger and Koellner, 2004). As habitats become more complex (layered) with tree growth during early stand development, rarefaction index increases with species turnover rates, and as the number of rare species increase. This approach can better assess disturbance changes to the site than the (information theory measure) Shannon-Wiener diversity index (Gotelli and Colwell, 2001).

The Shannon –Wiener diversity index responded positively to a stand with higher tree density during the mature age class. These mature higher density stands may have more habitat homogeneity than a stand with greater openness. Habitat homogeneity influences the Shannon-Wiener index's evenness function 'J'. Species evenness may be easier to attain where habitat homogeneity is greater. This is why the Shannon-Wiener index's H' value decreased as habitat heterogeneity increased during early stand development, or may increase in older stands with higher stand density (homogeneity). Since the Shannon-Wiener index's evenness function gives equal weight to rare and common species, it does not measure local patterns of assemblage where disturbance impacts could be assessed (Pianka, 1966). The Shannon-Wiener diversity index still should be included with a rarefaction index during assessments because it is an abundance-based function where the total number of species (richness S') that are found within an area are measured. In addition, a measurement of the relative abundance (N) and degree of equality among species (evenness 'J') are also calculated (Poole, 1974).

The young, mid-aged, and mature age classes varied in the abundance of grasses, forbs, and shrubs. Even with the goal of applying prescribed fire every three years at the reference sites, shrub species increased and graminoid species declined over the age classes. The mature age class changed with running oak for mesic sites and gallberry for the wetter sites. The young age class had blueberry for mesic conditions and bluestem grass for the wetter sites. In this case,

more forest structure brought on by stand maturation could represent a drying effect on soil conditions as represented by a change in plant species.

Conclusions

Stand DBH, height, and basal area increased until 85-90 years when they began to reach a steady state. Coarse woody debris accumulation levels were highly variable, but tended to increase with age. Standing deadwood also increased with age up to 60-80 years and began to decline thereafter. The decomposition levels of CWD were constant through the mid-aged class, but declined from the mid-age to the mature stage. The levels of shrub species were significantly higher in the mature sites than either the young or the mid-aged classes.

Tree growth during early stand development translates to habitat heterogeneity as partial shading brings in new groups of plant species. At this point, stand height had a strong positive relationship with Coleman rarefaction index and stand density had a strong negative relationship with Shannon-Wiener diversity index. The plant species turnover rates as indicated by the Coleman rarefaction index were high and the evenness of plant species as indicated by the Shannon-Wiener were very low. The evenness of plant species was not attained until the mature age class when competition was reduced, and the number of plant species entering the ecosystem was equal to the number of plant species leaving it. During this time period, Shannon-Wiener diversity index had a strong positive relationship with stand density and the Coleman rarefaction index had a negative relationship with stand height.

The results have shown interesting trends along the chronosequence for wet longleaf pine flat communities along the Gulf coast of Florida. The results indicate that Florida's Gulf Coastal longleaf pine flats reach the understory reinitiation condition at approximately 85-90 years. This would mean the forest is nearing a steady, self-organizing state, perhaps a threshold point for attaining restoration success in terms of structural attributes.

Three areas of this research warrant further attention. First, investigations concerning coarse woody debris in southern pine forests is lacking, probably due to a perception that any accumulation would be limited by prescribed fire. In this research, we found heavy accumulations at sites in each of the forest age classes. Secondly, experiments in plant community assemblage should be conducted to take a closer look at the relationships between the commonly used Shannon-Wiener diversity index and the Coleman rarefaction index. Coleman rarefaction was a stronger index during early stand development, but showed no advantage over the Shannon-Wiener index during later stages of stand development. Finally, our research found that shrub species dominated the mature aged stands even with aggressive fire management programs. Many of the plant species that were classified as woody do not have pioneer patterns similar to gallberry, saw-palmetto, or runner oak. They never dominated the site. There should be studies that focus on the less known woody species and their benefits to longleaf pine forest ecosystems.

Table 2-1. Qualitative classification system for downed and standing deadwood.

Characteristic	Decay Class				
	I	II	III	IV	V
Leaves	Present	Absent	Absent	Absent	Absent
Twigs	Present	Present	Absent	Absent	Absent
Bark	Present	Present	Often Present	Often Absent	Absent
Bole Shape	Round	Round	Round	Round to Oval	Oval to Flat
Wood Consistency	Solid	Solid	Semi-Solid	Partly Soft	Soft
Wood Strength	Firm	Firm	Firm	Breakable	Fragmented
Decomposition Deduction	15%	30%	45%	60%	75%

* Spetich et al. 1999; Adapted from Spies et al (1988).

Table 2-2. Forest structural and plant species diversity means among age classes.

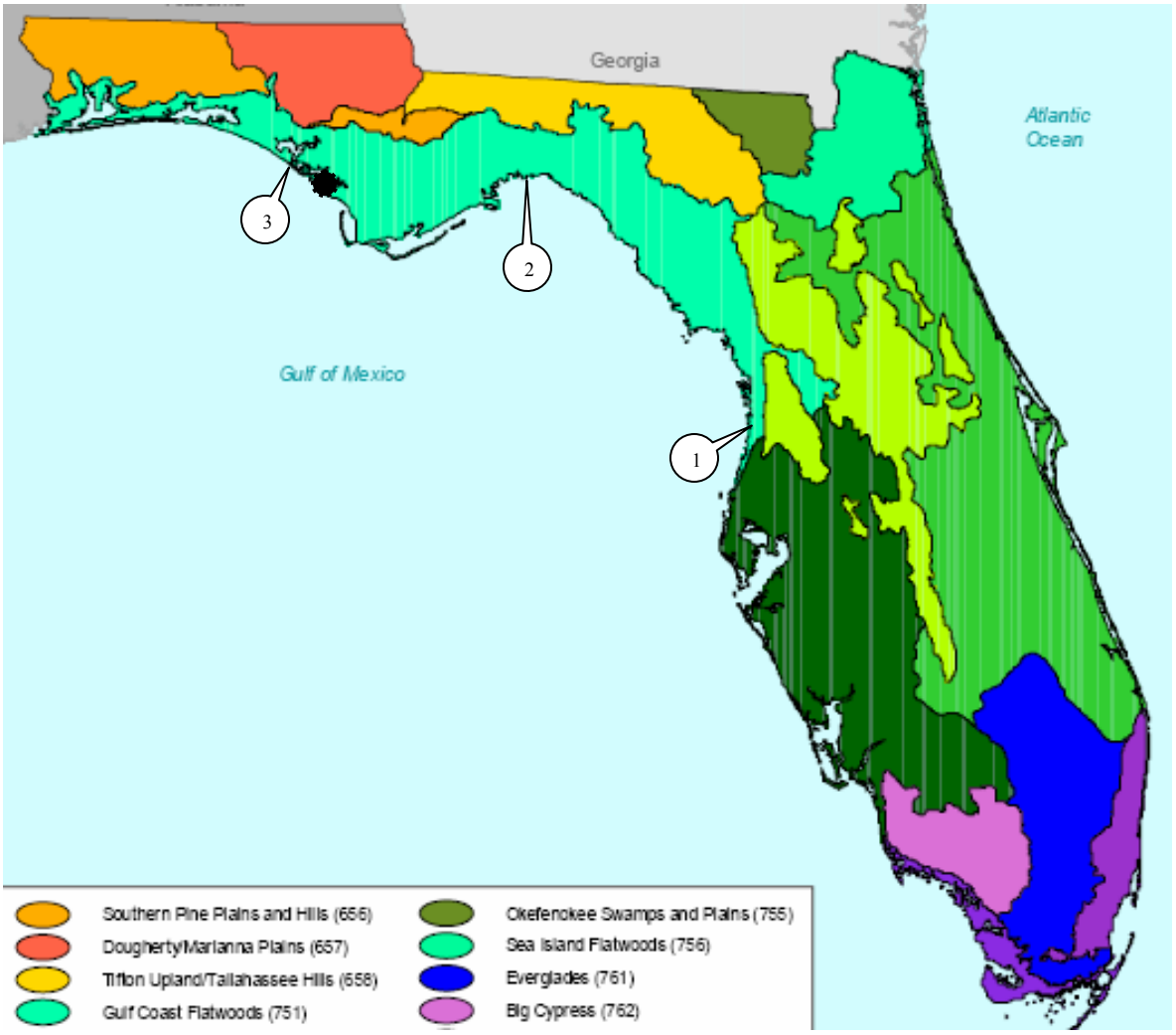
Stand Age (years)	Age Class	Stand Diameter (cm)	Stand Height (m)	Stand Basal Area (m^2/ha)	Stand density (Trees/ha)	Stand Volume (m^3/ha.)	CWD (m^3/ha)	Coleman Rarefaction ES(63)	Shannon-Wierner Diversity
6	Young	0.3	0.2	0.0	300	0.0	17.5	10.96	2.04
8	Young	0.7	2.8	0.1	200	0.0	13.3	12.93	2.24
9	Young	8.3	4.9	0.3	50	1.3	1.3	15.78	1.91
10	Young	8.5	6.0	0.3	75	1.8	2.6	15.78	1.91
17	Young	16.1	8.9	5.6	250	60.8	11.1	16.97	2.25
*	**Mean**	**6.0a**	**3.3a**	**1.3a**	**175a**	**12.9a**	**9.5a**	**14.51a**	**2.07a**
24	Mid-Aged	19.5	10.7	2.3	350	26.0	2.5	16.69	2.29
27	Mid-Aged	25.7	11.7	5.4	125	62.0	0.7	16.69	2.29
29	Mid-Aged	13.6	8.2	7.3	400	84.0	4.3	20.11	1.91
31	Mid-Aged	19.8	10.1	7.1	250	78.0	1.5	17.90	2.32
34	Mid-Aged	23.3	11.5	10.1	225	128.0	76.0	13.28	1.72
36	Mid-Aged	17.2	11.1	11.1	425	125.0	25.3	20.11	1.91
40	Mid-Aged	29.9	19.4	1.8	75	34.0	3.5	12.99	2.14
42	Mid-Aged	21.9	10.7	1.0	25	10.1	10.0	12.99	2.14
46	Mid-Aged	25.8	18.0	20.6	425	395.8	80.7	9.99	1.28
50	Mid-Aged	35.0	20.9	4.9	50	101.2	5.8	9.99	1.28
52	Mid-Aged	26.8	12.3	11.5	275	174.5	1.8	7.99	1.78
*	**Mean**	**23.4b**	**13.0b**	**7.7b**	**216a**	**112.2b**	**9.3a**	**14.41a**	**1.97a**
60	Mature	27.7	19.1	6.1	250	115.0	18.1	15.55	1.94
61	Mature	18.4	12.2	14.1	450	192.3	13.7	17.90	2.32
62	Mature	41.7	17.8	24.2	225	433.1	58.3	10.91	1.41
68	Mature	24.9	12.5	7.3	125	122.3	105.8	12.80	1.62
71	Mature	35.1	15.5	10.2	225	156.1	61.5	7.99	1.78
86	Mature	26.0	16.6	10.7	175	206.3	9.6	12.80	1.62
95	Mature	37.5	18.2	11.1	175	202.5	12.7	17.89	1.56
101	Mature	30.2	13.2	13.7	175	234.1	11.4	7.20	1.16
105	Mature	33.4	17.5	11.0	125	194.3	1.0	17.89	1.56
110	Mature	34.0	17.4	15.9	250	278.7	12.3	17.89	1.56
*	**Mean**	**30.0c**	**15.5c**	**11.8c**	**217a**	**201.7c**	**30.0b**	**14.25a**	**1.86a**

* Means followed by the same lower case letters are not significantly different (alpha=0.05). CWD represents snags and downed logs. The sample size for stand data by age class was n=12 and the understory vegetation data by age class was n=48.

Table 2-3. Indicator values for plant species in three forest age classes.

Age Class	Plant Species	Age Class					
		Young	Mid-Aged	Mature	SD	P-Value	Veg Type
Young	*Andropogan virginicus*	34	3	1	3.09	0.001	Grass
	Dichanthelium ovale	33	10	2	3.49	0.002	Grass
	Vaccinium sp.	25	2	9	3.27	0.019	Shrub
	Pteridium aquilinum	12	1	0	2.28	0.021	Forb
Mid-Aged	*Rhexia alifanus*	0	28	0	3.03	0.001	Forb
	Cyperus sp.	0	25	0	2.20	0.001	Grass
	Lachnanthes caroliana	8	25	1	2.98	0.005	Forb
	Arisitida var. *beyrichiana*	1	23	0	2.54	0.001	Grass
	Solidago odora	0	9	1	2.09	0.066	Forb
Mature	*Ilex glabra*	20	11	37	3.04	0.007	Shrub
	Quercus pumila	17	3	29	3.31	0.014	Shrub
	Gaylussacia frondosa	2	3	13	2.81	0.099	Shrub
	Licania michauxii	0	0	10	2.17	0.040	Shrub
	Kalmia hirsuta	0	0	7	1.96	0.094	Shrub

INDICATOR VALUES (% of perfect indication based on combining the values for relative abundance and relative frequency). The sample size for the understory vegetation data by age class was n=48.



Selected Reference Communities

- 1. Chassahowitzka Wildlife Management Area
- 2. St. Marks National Wildlife Refuge
- 3. Topsail Hill State Park

Figure 2-1. Locations of the Pt. Washington Longleaf Pine Restoration site (●) and the reference sites within Gulf Coast Flatwoods subecoregion of Florida (Griffith, 1994).

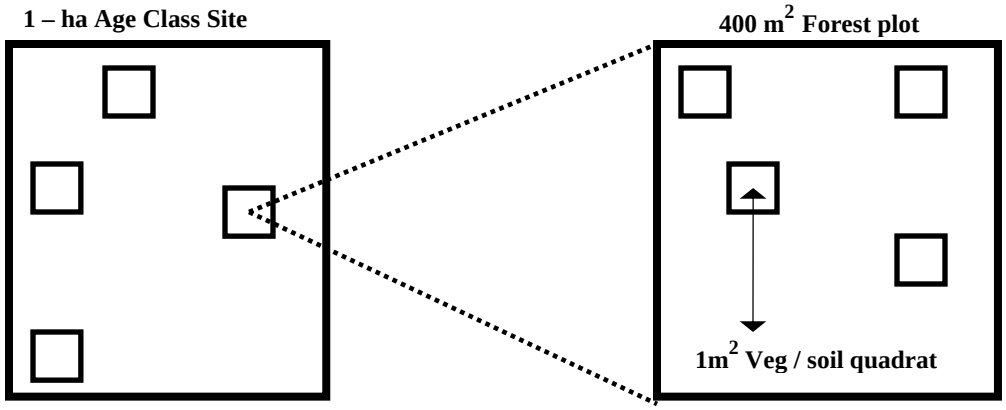


Figure 2-2. Nested plot sampling design applied at three different sites (age classes) for each reference location.

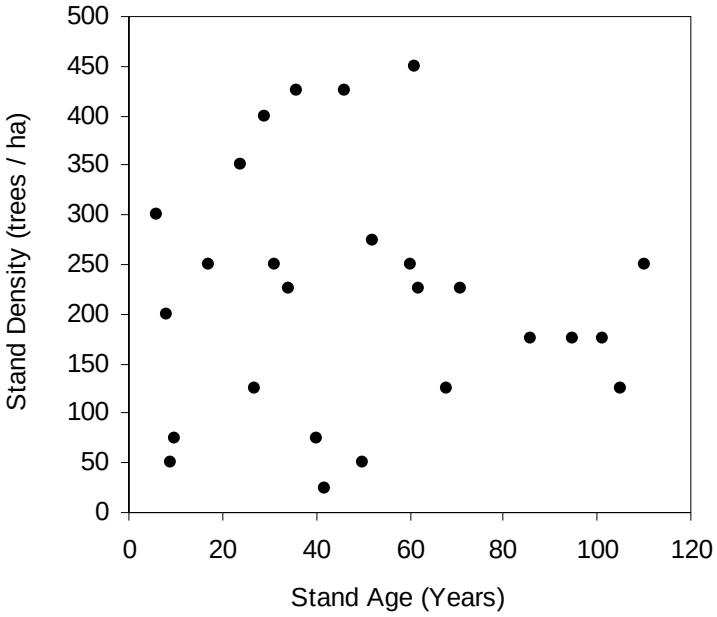


Figure 2-3. Mean stand density (trees per hectare) along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands.

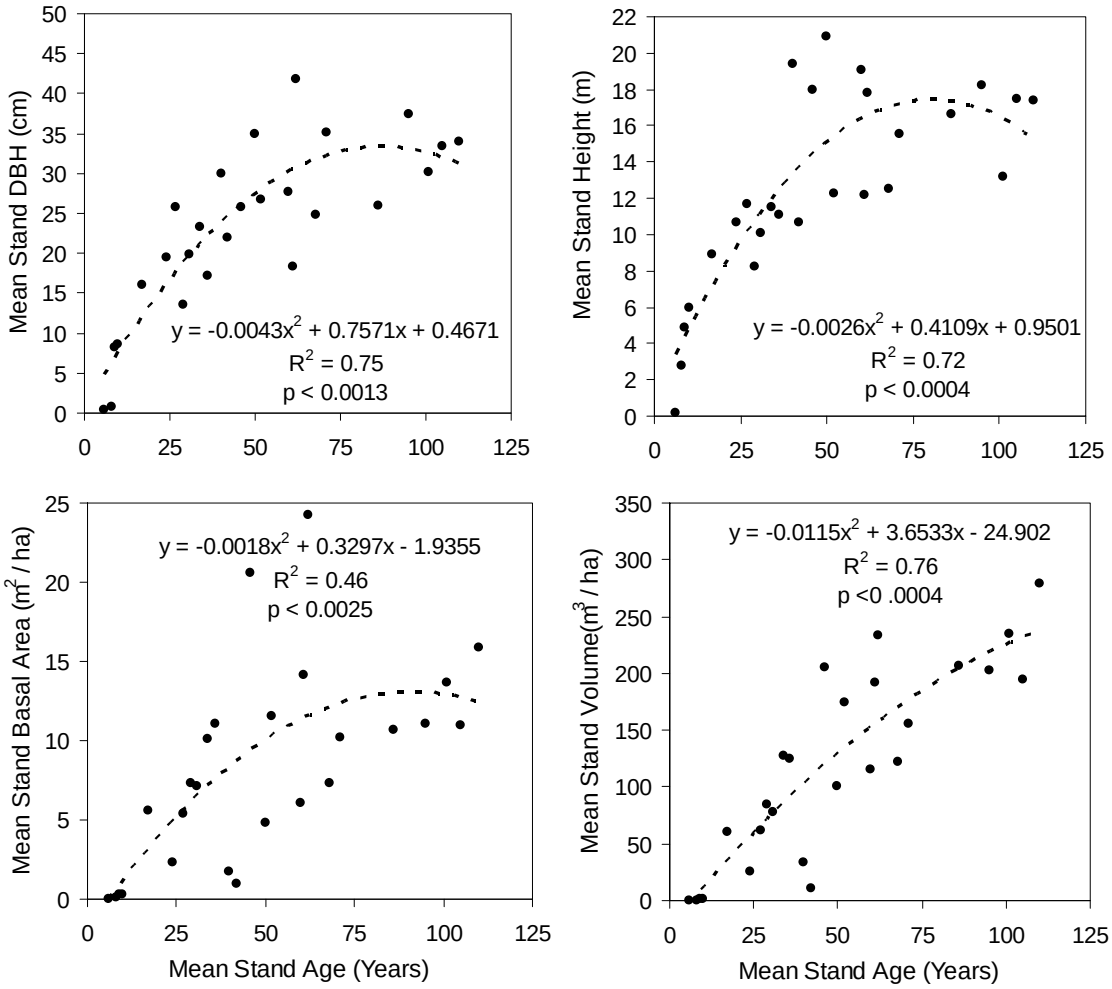


Figure 2-4. Mean stand DBH, height, BA, and volume along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands.

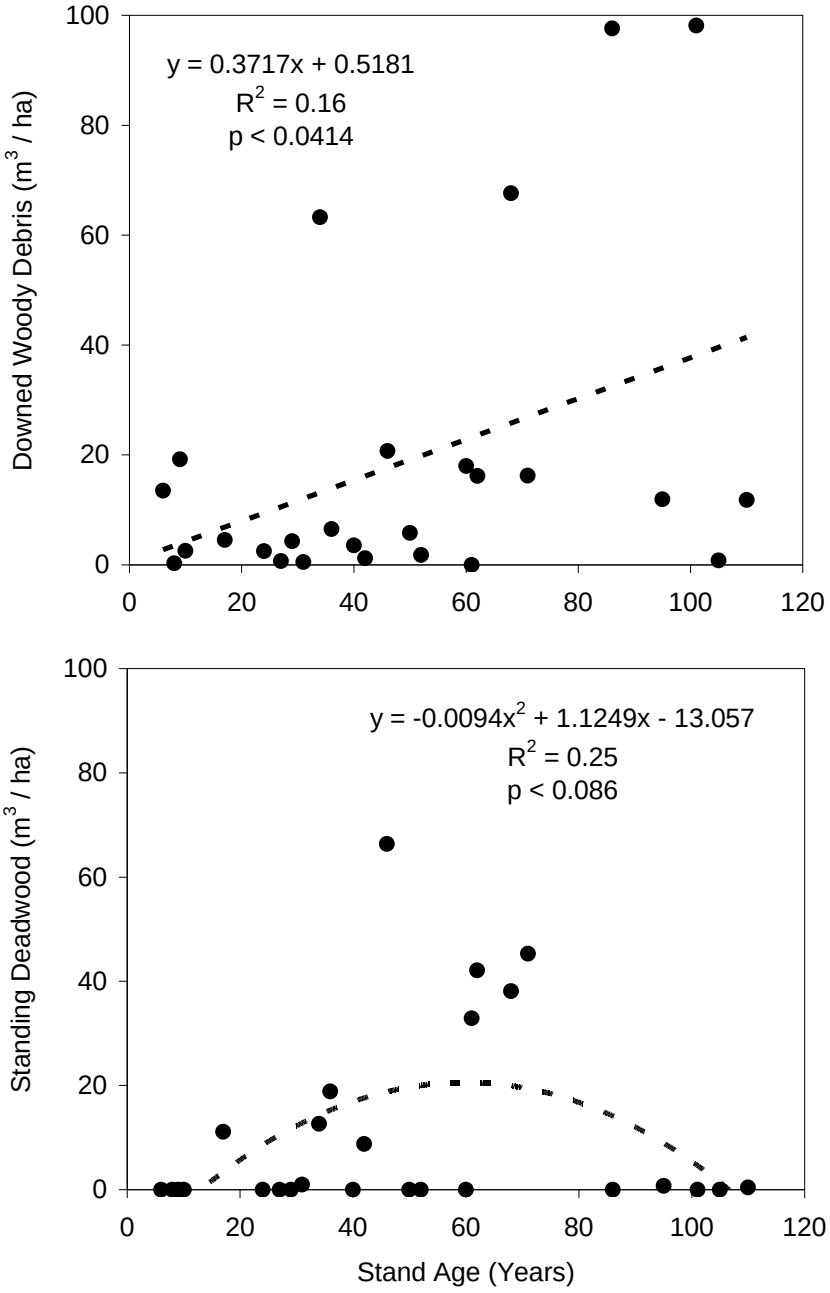


Figure 2-5. Downed woody debris and standing deadwood (snag) accumulations along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands.

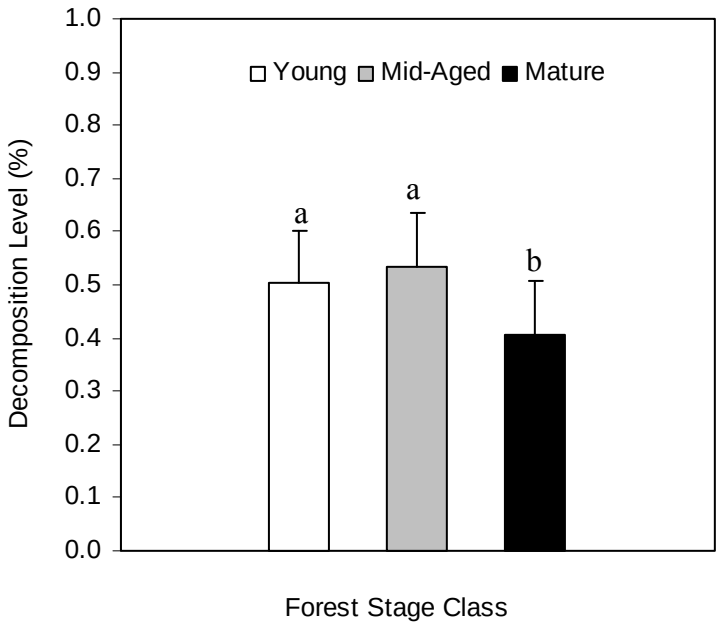


Figure 2-6. Decomposition levels by forest age class as measured from 26 differently aged stands. Percent levels represent the amount of decay.

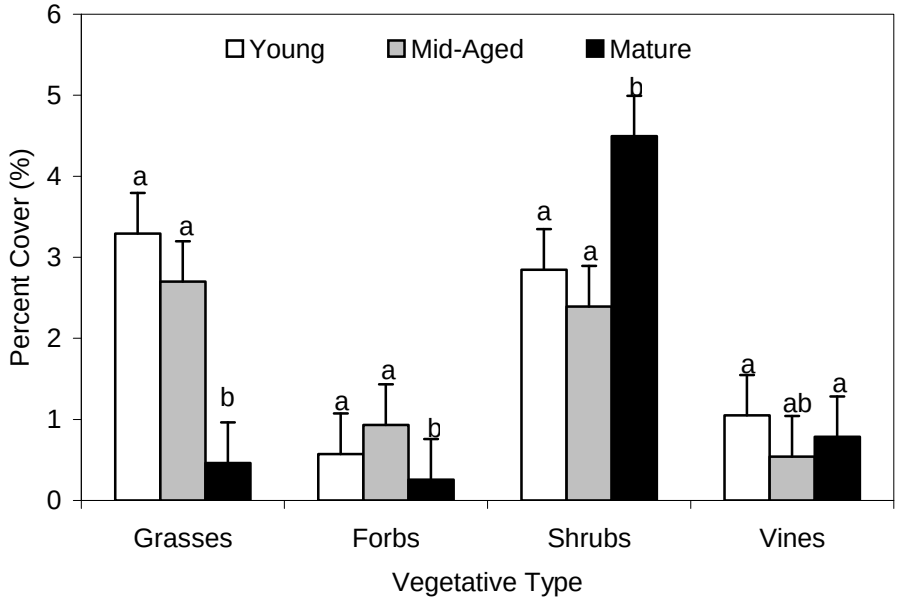


Figure 2-7. Composition of understory vegetation by forest age class.

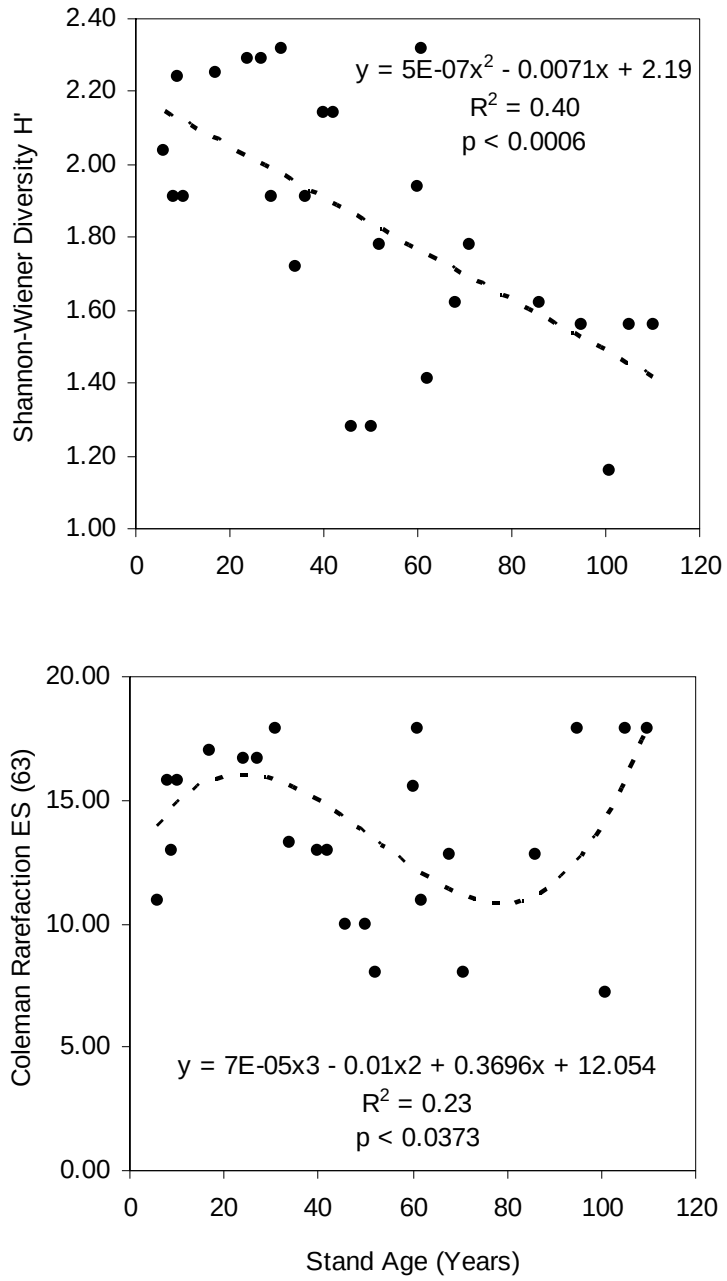


Figure 2-8. Shannon-Wiener Diversity and Coleman Rarefaction indices along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands.

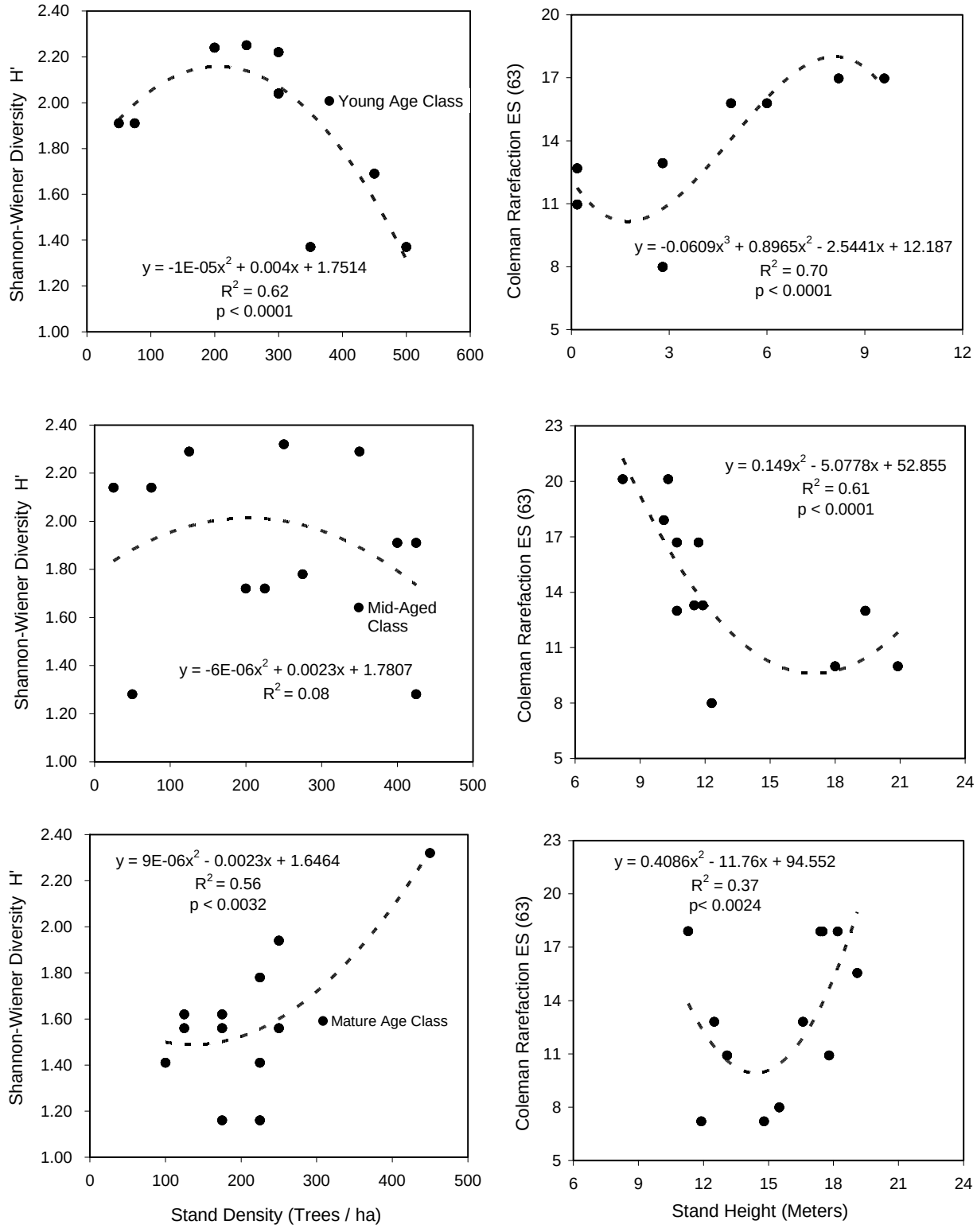


Figure 2-9. Mean stand density versus the Shannon-Wiener Diversity index and mean stand height versus the Coleman Rarefaction index as measured from the young, mid-age and mature age longleaf pine stands.

CHAPTER 3
PATTERNS OF SOIL CHEMICAL AND MICROBIAL PROPERTIES ALONG A CHRONOSEQUENCE IN WET LONGLEAF PINE FLATS OF FLORIDA

Introduction

Soil nutrient dynamics and their relationship to forest stand development have been under investigation for some time (Odum, 1969; Vitousek and Reiners, 1975). Studies describing soil nutrient status, in particular nitrogen, and its influence on stand productivity and canopy nutrient dynamics have focused mostly on plantations (Morris and Boerner, 1998; Kirkman et al. 2001; Allen and Schlesinger, 2003), but research conducted in natural stands are also found in the literature (Zak et al. 1990; Vance and Entry, 2000; Aravena et al. 2002; Chapman et al. 2003; White et al. 2004). Similar studies are rare in the longleaf pine ecosystem, one of the most threatened ecosystems in the United States (Wilson et al. 2002). For example, patterns of nitrogen mineralization, the relationship between nitrogen levels and soil microbes, and how this relationship changes over time, have not been given much attention (Johnston and Crossley, 2003). Such information could aid the efforts of restoration professionals who are interested in not only restoring the structural attributes of the longleaf pine ecosystem, but its functional attributes as well.

One way to study the relationship between forest stand development and soil microbial dynamics is to use a chronosequence of similar stands having different ages since stand replacement (Pickett, 1989; Williamson et al. 2005). In an earlier investigation, Taylor et al. (1999) studied forest floor microbial biomass of northern hardwood forest stands ranging from 3 years after clearcut to 120 years. These authors reported an increasing trend in microbial biomass with age during the early successional stage. However, microbial biomass decreased with age during the mid-aged stage, but increased again during the late successional stage. Soil organic matter followed a pattern similar to microbial biomass. They further reported that fungal biomass

was positively correlated soil moisture and negatively correlated with soil pH. Finally, ammonium (NH_4^+) production increased from the early to mid-aged stages and decreased from the mid-aged to the late successional stage (Taylor et al. 1999). In another study, investigators wanted to detect the effects of plant diversity on the levels of fungal microbes by measuring the populations of fungal-feeding nematodes. As the plant community succeeded toward late successional conditions, there was little effect on the numbers of fungal-feeding nematodes (Kardol et al. 2005). The relationship between fungal growth and nematode populations was more complex than the investigators surmised.

Recently, increased emphasis has been placed on examining soil microbial communities during soil assessments, especially when monitoring restoration projects (Harris, 2003; Johnston and Crossley, 2003). Some of the measured soil biotic variables have included microbial biomass carbon and nitrogen (Vance and Entry, 2000; Wilson et al. 2002), most probable numbers (MPN) of microbial functional groups (Schmidt and Belser, 1982), fungal biomass estimates (Montgomery et al. 2000), and complete community profiling (Bailey et al. 2002).

Studies from other parts of the U.S. and the world have also contributed to our understanding of the soil community relationships. For example, a growing number of studies have indicated that soil microbial communities with distinct functional groups inhabit different forest types (Pennanen et al. 1999). A black pine (*Pinus nigra*) forest in Austria was found to have higher relative amounts of fungi and actinomycetes in the soil microbial biomass than were found in a neighboring oak-beech (*Quercus petrea - Fagus sylvatica*) hardwood forest (Hackl et al. 2005). Researchers conducting a study in England found that soil moisture, pH, and microbial biomass levels decreased along a successional gradient from moorland to grassland to mature pine forest (Chapman et al. 2003). Researchers in Finland found great variability within soil

microbial populations when measured beyond a 3-4 m radius (Pennanen et al. 1999). This result could imply soil microbial measurements farther than 4 meters apart may need to be considered as independent samples and not replications. A recent study in a longleaf pine ecosystem found that nitrogen mineralization rates declined significantly along a moisture gradient from xeric sandhills through the pine scrub to wet pine flatwoods, but the levels of soil carbon, nitrogen, microbial biomass carbon, and aboveground net primary productivity (ANPP) had reverse trends (Wilson et al. 2002). These studies illustrate the strong interactions that exist between soil biogeochemical properties and vegetative changes in the aboveground cover type.

Fires are important agents of natural disturbance in many forest types, and especially in maintaining southern pine forests (Outcalt, 2000). Fires have variable effects on soil microbial biomass in forest soils where fire intensity, season, and weather play a role (Wilson et al. 2002). Hurricanes, another prominent disturbance along the Gulf coast, can have major impacts on forest structure by strong winds (Palik et al. 2002), but the associated flooding may have bigger impacts on soil productivity, biogeochemistry and soil microbial populations (Lockaby and Walbridge, 1998).

Anthropogenic disturbance effects from human activities in forests can also have effects on the functioning of forest soils. In a military installation study focused on the effects of different levels of vehicle traffic on the soil microbial community within a longleaf pine forest, investigators found that increasing levels of traffic produced a decrease in the fungal biomass (Peacock et al. 2001). Fertilization can have negative feedbacks. In a grassland restoration study, fertilizer additions caused increases in the number of bacteria, but a decrease in the fungal population. Sites with no fertilizer had larger fungal biomass levels, a greater number of legumes, and higher plant species richness than the fertilized areas (Smith et al. 2003). Changes

in soil microbial functional groups caused by natural or human-induced disturbances can have negative impacts on long term soil nutrient cycles.

Ecosystem health has been described in terms of nutrient retention or the ability of an ecosystem to prevent nutrient loss (Odum, 1969). Ecosystems have been identified as "leaky" where nitrate (NO_3^-) was found in higher concentrations, or as nitrogen-limited or having a "tight" nutrient cycle where the less mobile ammonium (NH_4^+) ion was in higher concentrations (Davidson, 2000). The steady-state development stage of succession (Oliver, 1981) has been described as the time period of forest succession when the ecosystem's nutrients are held tightly within (Odum, 1969). Vitousek and Reiners (1975) concluded the "tightest" period of nutrient retention was during the mid-aged period when nutrients are brought into short supply by heavy competition. They further concluded that nutrient retention in an ecosystem actually reflects biomass accumulation patterns. They suggested that differences between net nitrogen input and output were proportional to the rate nitrogen was incorporated into net biomass increment. Biogeochemical equilibrium would be signified when the differences between nitrogen inputs and outputs would be equal to zero, or during the period of late succession when net biomass accumulation is close to zero (Zak et al. 1990). Given the relationship between biomass accumulation and nutrient retention, the biogeochemical thresholds should be found when the ecosystem is self-organizing or during the understory reinitiation stage of succession (Oliver, 1981).

Recent investigations have found an internal mechanism by which excessive nitrate is conserved in wet forested ecosystems. In upland forested environments, examined within both the temperate and tropical zones, investigators have discovered that dissimilatory nitrate reduction to ammonium (DNRA) was a major pathway for transforming nitrate to ammonium,

and preventing losses from leaching or by the denitrification pathway (Silver et al. 2001; Huygens et al. 2007). Through ^{15}N tracing, investigators discovered the majority of any surplus nitrate was reduced by DNRA, rather than reduced by denitrification or immobilized by soils. The common conditions found at the research sites were wet soils, high organic carbon, and normally nitrogen-limited environments.

We conducted our studies within longleaf pine ecosystems located along the Gulf Coast Flatwoods zone. This coastal region is found between the panhandle community of Pensacola and Tampa Bay, Florida. Various ecological studies have investigated the changes in plant community composition along soil moisture gradients within the Gulf Coast Flatwoods zone, but none have examined the soil chemical and microbial properties along a chronosequence. Previous research has concluded that plant species richness increases along a soil moisture gradient until an ecotone between mesic flatwoods and cypress swamps is reached (Huck, 1986; Walker, 1993; Kirkman et al. 2001). This ecotone is the interface where one would find the wet flatwoods and wet pine savanna subtypes of the coastal longleaf-slash pine flat (Messina and Conner, 1998). There are almost 200 rare vascular plant taxa found in the great variety of habitats classified as longleaf pine ecosystems. In addition to the majority of them being found in Florida (Collins et al. 2001), the richest sites are found in these wet pine flats and their associated wetlands (Walker, 1993). Wet pine flats represent more than 1 million ha in the Southeast (Burger and Xu, 2001). Plant species richness of wet longleaf pine communities has been positively correlated with soil productivity (Kirkman et al. 2001), and specific soil properties (Wilson et al. 2002). Soil characteristics need to be included with plant species richness in any restoration assessment of coastal wet longleaf pine flat ecosystems to couple functional with structural attributes (Johnston and Crossley, 2002).

Our main objective was to measure soil pH, moisture content, organic matter content (SOM), plant-available phosphorus, soil nitrogen mineralization rates (N_{min}), soil microbial biomass carbon (C_{mb}) and fungal biomass (C_{fb}) along a 110-year chronosequence to determine the ecological trajectory in terms of soil chemical and microbial characteristics for longleaf pine in coastal wet pine flat communities. We specifically tested our hypothesis that this group of soil biogeochemical indicators measured along a 110-year chronosequence would follow a pattern similar to the biomass accumulation curve of forest succession (Vitousek and Reiners, 1975). In response to rapid increase in growth during the early years of stand establishment, we predicted a similar increase in net nitrogen mineralization rates, microbial biomass and fungal biomass levels. We hypothesized that these variables would decrease at some point during the late mid-aged phase and reach a threshold some time during the mature phase.

Materials and Methods

Study Areas

Three representative locations along Florida's Gulf Coast Flatwoods zone (720 km) were selected for this study. The three locations were Topsail Hill State Park, St. Marks National Wildlife Refuge, and the Chassahowitzka Wildlife Management Area of the Florida Fish and Wildlife Conservation Commission. At each location, four 400 m^2 plots representing each of early, mid, and mature age classes of longleaf pine stands were laid out for vegetation (reported in Chapter 1) and soil sampling. The different successional ages (age classes) represented a chronosequence of 110-years.

Soil Sampling and Preparation

Soils samples (> 500 g) were taken from four ($1m^2$) quadrats taken in each of the 400 m^2 plots during September of 2005 and 2006 for general analysis. The samples were taken from the upper 10 cm of the 'A' horizon, not including the organic layers. An additional sampling was

conducted in August, 2005, at which time a paired-soil sample was buried in place for incubation (Eno, 1960; described later). The incubated samples were taken out during the September, 2005 sampling period. All samples were immediately stored at 4ºC until analysis. A sub-sample (20g) from all of the samples was used for determining moisture content after oven drying at 105ºC for 72 hours.

Soil Chemical Analysis

The soil samples were analyzed at the University of Florida soil testing laboratory (UF Analytical Research Laboratories), Gainesville, Florida. Soil water pH was determined from prepared slurries using a soil-to-water ratio of 1-to-2 (EPA method 150.1). Plant-available phosphorus was determined with the use of Mehlich-1 extractant (H_2SO_4 & HCL) and measured on an inductively coupled plasma (ICP) spectrophotometer (EPA method 200.7; Nelson et al. 1953). Soil organic matter content (SOM %) was determined by the Walkley-Black method (Walkley, 1947).

Net Nitrogen Mineralization

Net nitrogen mineralization was determined by the buried bag technique (Eno, 1960). Forty eight samples were collected from each reference location and the restoration site for a total of 144. In general, one bag was buried in situ for incubation during August 2005 (Eno, 1960) and the other bag taken to the soil lab for analysis. The incubated bags were collected and analyzed after 30 days. Mineral nitrogen was extracted from 20 g of both soil samples with 60 ml 2N KCL and placed in a shaker for one hour. They were then filtered through # 42 Whatcom filter papers into 20 ml scillination bottles. The samples were analyzed by the University of Florida's Analytical Research Laboratory for ammonium (EPA method 350.1) and nitrate (EPA method 353.2) with a continuous auto-flow analyzer. Net mineralization was calculated as the

difference between incubated-N and initial-N (corrected for soil moisture) (Keeney & Nelson, 1982).

Microbial Biomass

Soil microbial biomass C was determined by chloroform fumigation-extraction (Vance et. al., 1987), with the following modifications. Twelve grams of soil were sieved from soil samples stored at 4° C and then placed in 50 ml centrifuge test tubes. Matching 12 g soil samples were set aside in additional 50 ml centrifuge tubes as the control. The soil samples were fumigated in a desiccator with 40 ml of alcohol-free chloroform placed into a center beaker, an additional 0.5 ml of chloroform was placed into each centrifuge tube. The top of the desiccator was pressure sealed and vacated until the chloroform began to boil. The tubes were then incubated for 24 hours at 25°C. The dessicator was then opened, resealed, and after the chloroform was reboiled, incubated for an additional 24 hours.

The control and fumigated samples were extracted with 36 ml of 0.05 M K_2SO_4, shaken (360 rpm) on an orbital shaker for 1 hour, and centrifuged @ 6000 rpm for 15 minutes. The supernatant was then filtered through # 42 Whatcom filter papers into 20 ml scintillation vials and frozen until analysis. Levels of total organic carbon (TOC) were determined on a Shimadzu TOC-VCSH analyzer (Vance & Entry, 2000). Microbial biomass carbon was calculated as: [(fumTC – ConTC) / 0.51] / (Soil Wt.) = mg C kg^{-1} dry wt. soil (Joergensen, 1996). The value of 0.51 is the conversion factor equal to the extractable portion of microbial biomass in a forest soil. Fumigated and non-fumigated blanks were measured to correct for the chloroform and potassium persulfate.

Soil fungal biomass levels were determined by a physical disruption method for extraction of ergosterol from soil samples (Gong et al. 2001) with the following modifications. Six grams of soil were mixed with 9 ml of 0°C methanol and 1.9 g of glass beads into 20 ml scintillation

vials. The vials were vortexed for 30 seconds, shaken (360 rpm) on an orbital shaker for 1 hour, and refrigerated over night. An aliquot of 1.8 ml was placed into 2 ml micro-centrifuge tubes and centrifuged @ 11,000 rpm for 20 minutes. After extraction, the samples needed to be filtered before running through a High Performance Liquid Chromatography (HPLC) computerized machine. A syringe was used to remove 1.5 ml of the supernatant from the micro-centrifuge and filtered through a 0.20 μm filter into amber colored 2 ml glass HPLC vials. The HLPC vials were covered with aluminum foil and stored in the dark at 0°C until ready to inject into the HPLC.

Each sample was quantified on a Beckman Coulter HPLC equipped with an UV detector, a pump, an auto-sampler, and through a C-18 reverse-phased analytic column (4.6 x 250 mm). The UV detector was set at 282 nm and pure methanol was used as the mobile phase at a flow rate of 1 ml per minute. Extracts (100 μl) were injected while the column pressure was maintained at 1000 psi. Pure ergosterol (Sigma) was recrystalized in pure methanol at different concentrations to establish a set of standards. The standard curve was constructed from a linear regression relationship between peak area and ergosterol concentration**.**

Ergosterol recoveries were calculated from the difference between spiked and non-spiked paired samples divided by the amount of ergosterol added**.** Under such conditions, an isolated peak was identified from field samples at approximately 13 minutes, based upon the peaks obtained from the ergosterol standards. Established from results of previous investigations, an average conversion factor for 3.65 μg ergosterol per mg of soil is converted to fungal biomass (mg^{-1} /g^{-1} soil) when multiplied by 220 (Montgomery et al. 2000). Fungal-to-microbial biomass ratios were determined using a ratio of the calculated soil fungal biomass carbon and the soil microbial biomass carbon for each sample.

Coastal wet longleaf pine flats experience long periods of standing water (Harms et al. 1998). This flooding causes changes in the biogeochemical cycling of nutrients. These forested wetlands also contain highly acidic soils that require modifications to standard soil biochemical analysis techniques normally used in moderate pH (6.0) wetlands. The following modifications were necessary in order to produce good laboratory results.

The microbial biomass carbon was extracted from soils using a lower 0.05 M K_2SO_4 extractant instead of the standard molarity (0.5 M) for improved efficiency in these low pH soils (Haney et al. 2001). The samples were centrifuged before filtering to reduce the high amount of woody material found present in the soil samples. A relatively new ergosterol extraction method by physical disruption was utilized to simplify the process for analyzing fungi in a large number of soil samples (Gong et al. 2001). A lower conversion factor for fungal biomass was used to account for the flooded conditions on soil fungal growth (Montgomery et al. 2000).

Data Analysis

A three stage balanced nested design was used to integrate the indicators measured at different scales and among sites. Hypothesis testing for differences between means was accomplished by using two-sample t-test with an alpha of .05 and a two-tailed confidence interval. Since the monitoring of the restoration site with nine distinct reference locations produced a dataset where the assumptions for analysis of variance (ANOVA) were not ensured, non-parametric tests were used to detect any significant differences among the reference sites and among the distinct age class segments (SAS, 2002).

Correlations between soil moisture, soil chemical and microbial abundances were determined using Spearman's rank (*r*) correlations (Dumortier et al. 2002; SAS, 2002; Spyreas and Mathews, 2006). Trends between variables were obtained from linear regression using the general linear model (PROC GLM) (Yang et al. 2006; SAS, 2002). The chronosequencial trends

were enhanced by incorporating moving average smoothing (MA model) as a data filter to reduce cyclical and seasonal variations found in the datasets for a number of the indicators affected by climate **(**Platt and Denman 1975; Kumar et al. 2001; Ittig, 2004). The trend analysis was followed by $\log_{10}$ data transformations where necessary to stabilize variances prior to analysis. Partial Canonical Correspondence Analysis with multivariate regression (proc CANCORR) was used to determine the relative contributions of the different variables to the relationship (SAS, 2002; Fortin and Dale, 2005).

Results

Soil Types, Soil Organic Matter, and Soil pH

All three reference sites contained taxonomically equivalent soil types. All of the soils had similar soil properties (sandy, acidic, thermic, aquic). The soils were also found to be functionally equivalent even when compared by drainage class (Table 3-1). Soil organic matter content (SOM) was found to increase from 1% to 4.5% as gravimetric soil moisture increased from 20% to 60% of soil weight (Figure 3-1). Soil pH decreased from a pH of 5.0 to 4.0 as SOM increased from 1% to 4.5% (Figure 3-2). The plant-available phosphorus tests produced too many non-detectable samples for any meaningful results (Table 3-2).

Net Nitrogen Mineralization

Net nitrogen mineralization rates (N_{min}) increased during the young age class, peaked during the mid-age class, and then decreased after 60 years (Figure 3-3). Mean N_{min} rates were 12 mg N / kg soil / month for the young age stands, 14 mg N / kg soil / month during the mid-aged class, and 8 mg N / kg soil / month during the mature age class for the reference sites (table 3-2). The pattern for N_{min} rates followed microbial biomass levels (C_{mb}) over the 110-year chronosequence (Figure 3-4). N_{min} rates increased from 5 mg N / kg soil / month to 20 mg N / kg soil / month as C_{mb} increased from 100 mg^{-1} C / kg soil to 1000 mg^{-1} C / kg soil (Figure 3-5).

Nitrate production was 13.8 mg-1 NO_3 / kg soil during April 2002 and 135.7 mg^{-1} NO_3 / kg soil during August 2002. In comparison, Nitrate production was 4.7 mg^{-1} NO_3 / kg soil during April 2005 and 1.9 mg^{-1} NO_3 / kg soil during August 2005 (Table 3-3; Figure 5-3, Chapter 5). Ammonium production was 13.4 mg^{-1} NH_4 / kg soil during April 2002 and 104.7 mg^{-1} NH_4 / kg soil during August 2002. During 2005, ammonium production was 8.9 mg^{-1} NH_4 / kg soil during April and 9.6 mg^{-1} NH_4 / kg soil during August (Table 3-3).

N_{min} was positively related to ammonification (NH_4^+) ($r > 0.810$; $p < 0.0001$) during all three age classes, but not correlated with nitrification. N_{min} became positively correlated with soil moisture and SOM ($r > 0.460$ ($p < 0.01$) during the mid-aged class and remained so through the mature age class (Table 3-4). Ammonium production was negatively related to nitrate production (NO_3^-) during the mid-age and mature ($r = 0.470$; $p < 0.001$) age classes (Table 3-3).

Microbial Properties

Mean soil microbial biomass carbon (C_{mb}) levels were 275 (mg C / kg soil) for the young age stands, 416 (mg C / kg soil) during the mid-aged class, and 339 (mg C / kg soil) during the mature age class for the reference sites (Table 3-2). Mean soil fungal biomass carbon (C_{fb}) levels were 102 (mg C / kg soil) for the young age class, 163 (mg C / kg soil) for the mid-aged stands, and 125 (mg C / kg soil) during the mature age class at the reference sites (Table 3-2). Fungal biomass carbon increased during the first 60 years (~200 mg C / kg soil), then decreased down to 110 years (~100 mg C / kg soil; Figure 3-6). The fungal-to-microbial biomass ratio (FB-to-MB) decreased from a mean value of 0.4 to 0.2 during the first fifteen years after establishment, and then increased to 0.8 at 50 years (Figure 3-7). Microbial biomass (C_{mb}) had a negative relationship ($r > 0.400$ ($p < 0.01$) with soil pH during the mid-aged and mature age classes (Table 3-3).

Discussion

The nitrogen mineralization (N_{min}) process in high soil moisture conditions was dominated by ammonium production (NH_4^+), with low concentrations of nitrate being measured. The net nitrification rates represented 50% of the production during 2002 and less than 25% during 2005. The net nitrogen mineralization rates were 10 magnitudes greater during 2002 compared to 2005 (Table 3-3). Similar results between NO_3^- and NH_4^+ were measured in a study comparing xeric, mesic, and wet longleaf pine sites in southern Georgia (Wilson et al. 2002).

When N_{min} became positively correlated with soil moisture and SOM during the mid-age and mature age classes, nitrate levels (NO_3^-) became negatively correlated to ammonium (NH_4^+) production. The dynamics indicates a portion of the NO_3^- was converting to NH_4^+ during saturated conditions. This condition might be indicating the dissimilatory nitrate-reduction-to-ammonium (DNRA) process is taking place during flooded conditions. Little dinitrogen (N_2) gas is lost to the atmosphere or NO_3^- by leaching when the DNRA pathway is dominant.

Flooding causes a lower redox potential ($Eh < 0.6$), and with a sufficient supply of NO_3^- and labile carbon, DNRA became the preferred pathway over denitrification, resulting in the enriched pool of NH_4^+ (Stevens et al. 1998). Investigators examined the changes in nitrogen and phosphorus availability in longleaf pine sites from wetlands through an ecotone to upland sites, and they measured higher levels of nitrate and phosphorus taken from soils in the middle of wetland sites than found in the ecotone or upland sites. However, the upland sites had higher amounts of labile nitrate than the wetter sites (Craft and Chiang, 2002).

The anaerobic conditions and a high supply of non-labile nitrate in wet longleaf pine sites are conducive to DNRA. During anaerobic conditions, the DNRA pathway provides NH_4^+ to plants and microbes, requiring less energy to assimilate than NO_3^- assimilation (Silver et al. 2001). The characteristics favoring DNRA over denitrification are high rainfall, a high C:N ratio,

and a forested ecosystem that is naturally N-limited during dry conditions. The DNRA pathway has been determined to be less sensitive to higher soil temperatures and a lower pH than denitrification. The DNRA pathway is now considered a major nitrogen conservation mechanism in humid forest ecosystems (Silver et al. 2001; Huygens et al. 2007).

One of the reasons why the nitrogen mineralization patterns closely followed microbial biomass changes over the chronosequence (Figure 3-4) was because the nitrogen mineralization data did not contain significantly high levels of nitrate during the 2005 growing season. Heterotrophic bacteria and fungi that dominate the soil microbial biomass produce ammonium from organic nitrogen. Only a few chemoautotrophic bacteria produce the majority of nitrite and nitrate (Richards, 1987).

Soil nitrogen mineralization rates increased during stand establishment, but eventually decreased after canopy closure as longleaf pine stands entered the stem exclusion phase of stand development (Oliver, 1981). Other studies in hardwood forests have found increases in nitrogen mineralization rates after stand replacing harvests up to 60 years, then declining to a constant range (Zak et al. 1990).

Investigators evaluating the affects of ponderosa pine restoration treatments on mycorrhizal fungi, determined treatments promoting graminoid and herbaceous ground cover had a positive relationship to levels of arbuscular mycorrhizal (AM) fungi (Korb et al. 2003). These authors also discovered a positive relationship between stand BA and levels of ectomycorrhizal (EM) fungi (Korb et al. 2003). In an earlier experiment in a slash pine forest, Sylvia and Jarstfer (1997) reported a strong competition that exists between AM weeds and EM pine roots (Sylvia and Jarstfer, 1997). The implication would be that AM fungal levels were high at harvest, declined during the first 15 years as growing EM trees crowded out the AM

groundcover. Eventually the AM fungi were replaced with EM fungi, and the overall fungal biomass levels increased after 15 years. This pattern is similar to our results (Figure 3-7).

Phosphorus availability was very limited in these sites as indicated by the poor results. Similar results have been reported in loblolly pine plantations throughout the South (Martin and Jokela, 2004). Fertilization can dramatically improve biomass accumulation, but unless it is maintained, nutrient-deficient soils can result from the fast pine growth (Adegbidi et al. 2005). Phosphorus levels were found to be higher and P-mineralization rates lower in wet southern pine forests (Grierson et al. 1999; Craft and Chiang, 2002). In our study, soil organic matter content (SOM) was found to increase with soil moisture, and increased levels of SOM caused decreases in soil pH. As soil organic matter increases, it forms complexes with Mg^{2+} and Ca^{2+}cations in solution, releasing H^{+} ions into soil solution from organic acids (Brady and Weil, 2002). This relationship was confirmed by a negative relationship between SOM and soil pH. A lower soil pH usually leads to lower nitrogen mineralization rates (Morris and Boerner, 1998). Active bacterial respiration and microbial biomass levels substantially decline below a soil pH threshold of 5.0, resulting in lower rates of nitrogen mineralization (Baath and Anderson, 2003). Lower mineralization rates results in higher organic matter accumulation.

Conclusions

Nitrogen cycling was dominated by ammonium production during the wet 2005 growing season when compared to a drier 2002. There was ammonium enrichment at the cost of nitrate levels. This probably indicates that the dissimilatory-nitrate reduction-to-ammonium (DNRA) pathway was prominent during the flooded 2004-2005 growing seasons. The net nitrogen mineralization rates, microbial biomass carbon, and fungal biomass carbon increased between the young and mid-aged classes, then decreased between the mid-aged and mature age classes. The FB-to-MB ratios increased dramatically up to 60 years, then decreased to 110 years. Finally,

soil organic matter content (SOM), increased with soil moisture. Based upon the results, this group of soil biogeochemical indicators follows biomass accumulation patterns and will attain biogeochemical equilibrium after a stand age of approximately 60-70 years. The threshold would be during the mature age class after the understory reinitiation phase of forest succession has started.

Soil biogeochemical studies require a great amount of resources and equipment to conduct an ecosystem-level analysis. The research could have been improved if a series of soil samples were analyzed over a two-year period, at 3-month intervals instead of annual sampling. However, the cost of running net nitrogen mineralization, microbial biomass and ergosterol determinations would be quite high. Our research has shown some interesting results, but additional research is required to explore the biogeochemistry of wet longleaf pine flats. This would include exploring the soil organic matter accumulation vs. flooding cycles in facultative wetland pine sites, the relationship between tree root mass and fungal biomass during longleaf succession, and the effects of competition between mycorrhizal and saprophytic fungi during longleaf pine development.

Table 3-1. Soil and stand properties between reference sites.

LOCATION	SOIL GREAT GROUPS	SOIL TEXTURE (Top 10 cm)	MOISTURE REGIME	TEMPERATURE REGIME	DRAINAGE CLASS
Chassahowitzka Wildlife Management Area					
	Psammaquent	Sandy	Aquic	Hyperthermic	Very poorly drained
	Alaquod	Sandy	Aquic	Hyperthermic	Poorly drained
St. Marks National Wildlife Refuge					
	Psammaquent	Sandy	Aquic	Thermic	Very poorly drained
	Alaquod	Sandy	Aquic	Thermic	Poorly drained
Topsail Hill State Preserve					
	Humaquept	Sandy	Aquic	Thermic	Very poorly drained
	Alaquod	Sandy	Aquic	Thermic	Poorly drained

STAND BASAL AREA AND SOIL BIOCHEMICAL PROPERTIES (Mean Values*)					
DRAINAGE CLASS	STAND BASAL AREA (m^2 / ha)	pH [H+]	NET NITROGEN MINERALIZATION RATES (mg N / kg soil / month)	MICROBIAL BIOMASS CARBON (mg C / kg soil)	FUNGAL BIOMASS CARBON (mg C / kg soil)
Very poorly drained	6.5a	4.4a	11.6a	374.3a	133.8a
Poorly drained	8.3a	4.5a	9.9a	356.1a	135.3a

* Means followed by the same lower case letters are not significantly different. (alpha=0.05)

Table 3-2. Soil chemical and microbial biomass means between age classes.

Stand Age (years)	Age Class	Net N_{min} (mg^{-1}/kg^{-1} Soil / year)	C_{mb} (mg^{-1} / kg^{-1} / soil)	SOM Content (%)	Soil pH [H+]	Plant Avail. P (mg/kg soil)	C_{fb} mg/kg soil	FB to MB Ratio
6	Young	2.4	92.2	2.75	4.10	0.40	29.0	7.08
8	Young	8.3	33.7	1.56	4.70	ND	31.8	6.76
9	Young	23.6	253.0	1.89	4.50	0.04	57.3	12.73
10	Young	22.3	448.6	1.96	4.60	ND	72.8	15.82
17	Young	5.3	674.5	1.69	4.30	ND	102.2	23.77
*	**Mean**	**12.0a**	**275.4a**	**1.97a**	**4.44a**	*	**101.5a**	**14.48a**
24	Mid-Aged	21.8	1169.5	0.83	4.50	ND	39.8	0.03
27	Mid-Aged	9.6	703.6	1.29	4.70	ND	24.1	0.03
29	Mid-Aged	40.8	402.1	3.55	4.40	0.02	34.0	0.08
31	Mid-Aged	9.2	762.6	2.09	4.30	ND	83.2	0.11
34	Mid-Aged	2.2	285.1	3.02	4.60	ND	191.1	0.67
36	Mid-Aged	17.0	403.8	2.49	4.30	ND	83.5	0.21
40	Mid-Aged	-0.7	30.3	1.03	4.60	ND	98.5	1.00
42	Mid-Aged	3.5	98.6	1.03	4.80	ND	41.9	0.43
46	Mid-Aged	16.4	321.0	1.16	4.00	1.28	117.1	0.36
50	Mid-Aged	11.4	131.8	1.29	5.30	ND	95.7	0.73
52	Mid-Aged	19.1	275.8	3.95	4.10	0.14	154.4	0.56
*	**Mean**	**14.0a**	**416.3b**	**1.98a**	**4.51a**	*	**162.9b**	**14.45a**
60	Mature	0.8	33.4	1.36	4.90	0.8	38.4	1.00
61	Mature	10.7	519.6	0.70	4.50	ND	92.9	0.18
62	Mature	32.6	753.6	3.62	4.10	ND	59.9	0.08
68	Mature	10.0	511.5	3.55	4.00	ND	189.3	0.37
71	Mature	-0.4	132.5	4.54	4.00	ND	36.6	0.28
86	Mature	6.4	241.8	0.90	4.40	ND	53.9	0.22
95	Mature	7.7	1.0	1.63	4.30	0.0	40.3	1.00
101	Mature	5.4	570.2	1.49	4.30	ND	88.0	0.15
105	Mature	6.0	83.0	1.49	4.50	ND	28.5	0.34
110	Mature	3.7	82.7	1.63	4.70	0.5	85.9	1.00
*	**Mean**	**8.4b**	**338.7ab**	**2.02a**	**4.44a**	*	**125.3ab**	**14.25a**

* Means followed by the same lower case letters are not significantly different (alpha=0.05). The sample size for the soil data by age class was n=48.

Table 3-3. Soil nitrogen mineralization means (Nmin) for dry season 2002 and wet season 2005.

Date	Nmin (mg N / kg soil)	Nmin (mg NO_3 / kg soil)	Nmin (mg NH_4 / kg soil)
Apr-02	27.2b	13.8b	13.4b
Aug-02	240.4a	135.7a	104.7a
Sep-02	189.1a	128.4a	60.7a
Apr-05	7.6b	4.7b	8.9b
Aug-05	11.5b	1.9b	9.6b

*Means followed by the same lower case letters are not significantly different (alpha=0.05). The sample size for the 2002 soil data was n=180 and for the 2005 soil data was n=144.

Table 3-4. Differences in soil biogeochemical relationships based upon Spearman rank correlations *r* as stratified by forest age class (n = 48).

Prob > |r| under H0: Rho=0							
Young Age Class (6-20)							
	Net N_{min}	NH_4^+ Min	NO_3^- Min	Moisture	Soil pH	SOM	C_{mb}
Net Nmin		0.885****			0.333*		
NH_4^+ Min					0.342*		
Mid-Aged Class (25-55)							
	Net N_{min}	NH_4^+ Min	NO_3^- Min	Moisture	Soil pH	SOM	C_{mb}
Net Nmin		0.805****		0.367*		0.468**	
NH_4^+ Min			-0.310*	0.513***			
NO_3^- Min							
Soil pH							-0.411**
Mature Age Class(60-110)							
	Net N_{min}	NH_4^+ Min	NO_3^- Min	Moisture	Soil pH	SOM	C_{mb}
Net Nmin		0.860****		0.471***	-0.413**	0.470**	
NH_4^+ Min			-0.528***	0.449**	-0.329*		
Soil pH							-0.412**

Significance of the Spearman rank correlation test: blank: non-significant, *$0.05 < p \leq 0.01$, **$0.01 < p \leq 0.001$, ***$0.001 < p \leq 0.0001$, **** $p < 0.0001$.

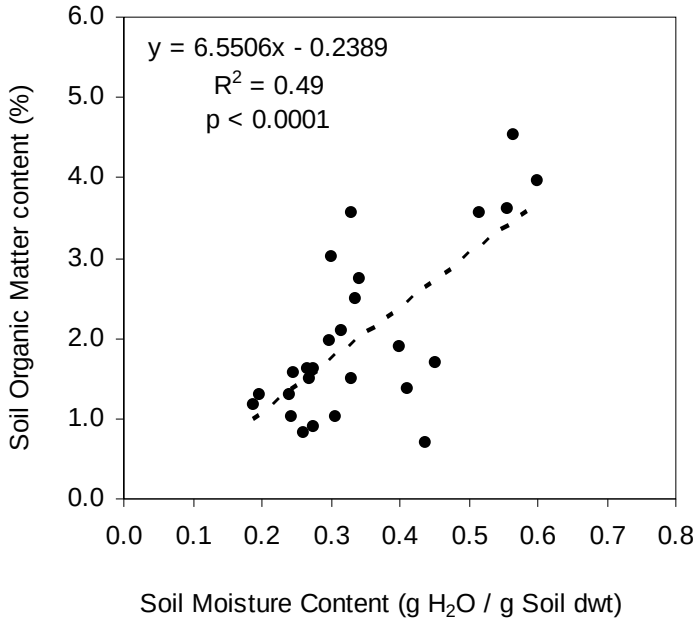


Figure 3-1. Soil organic matter content versus soil moisture as measured from 26 differently aged stands.

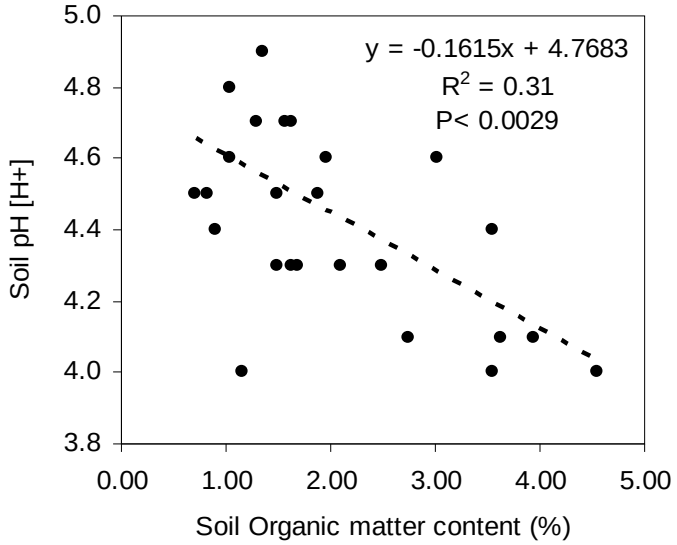


Figure 3-2. Soil pH versus soil organic matter content (percent) as measured from 26 differently aged stands.

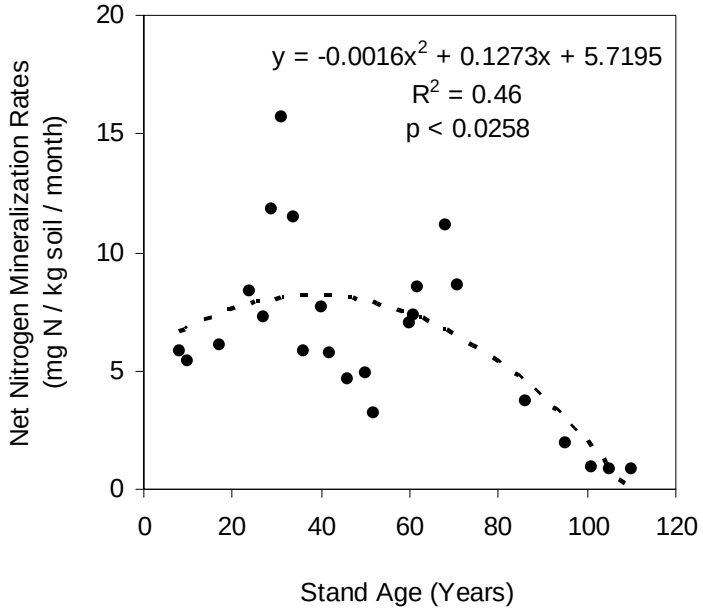


Figure 3-3. Total net nitrogen mineralization, ammonification and nitrification rates (mg $^{-1}$ nitrogen / kg $^{-1}$ soil / month $^{-1}$) along a 110-year chronosequence as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

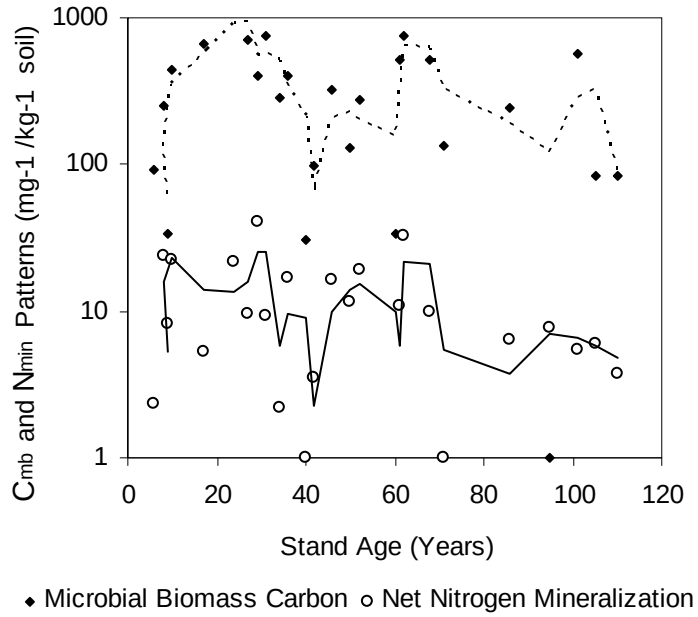


Figure 3-4. Trends for microbial biomass carbon (C_{mb}) and net nitrogen mineralization rates (N_{min}) along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

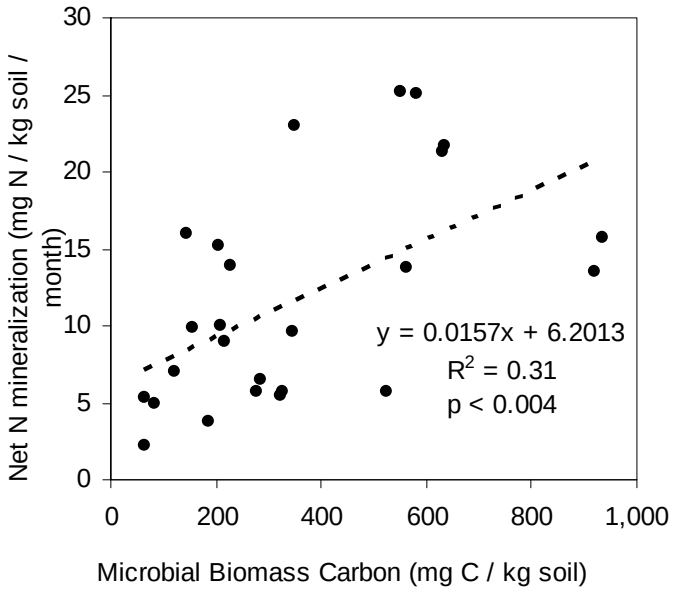


Figure 3-5. Microbial biomass carbon versus net nitrogen mineralization rates as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

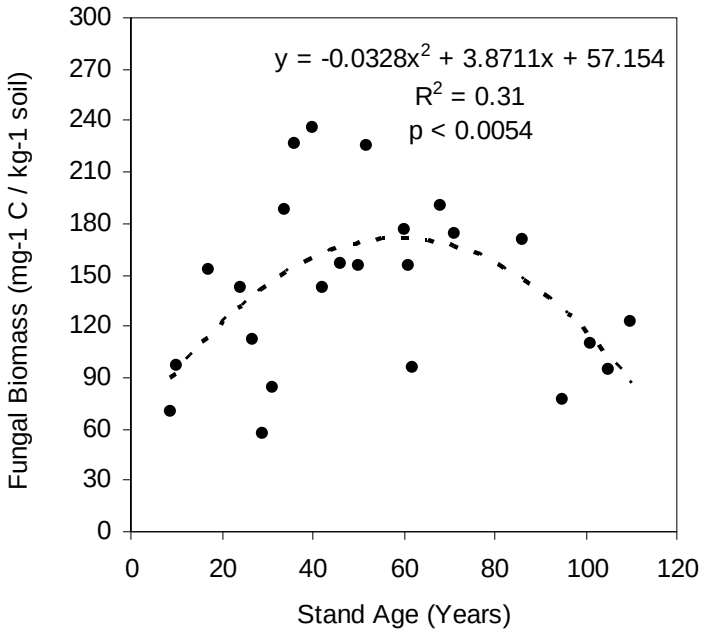


Figure 3-6. Fungal biomass carbon (C) along a 110-year longleaf pine chronosequence as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

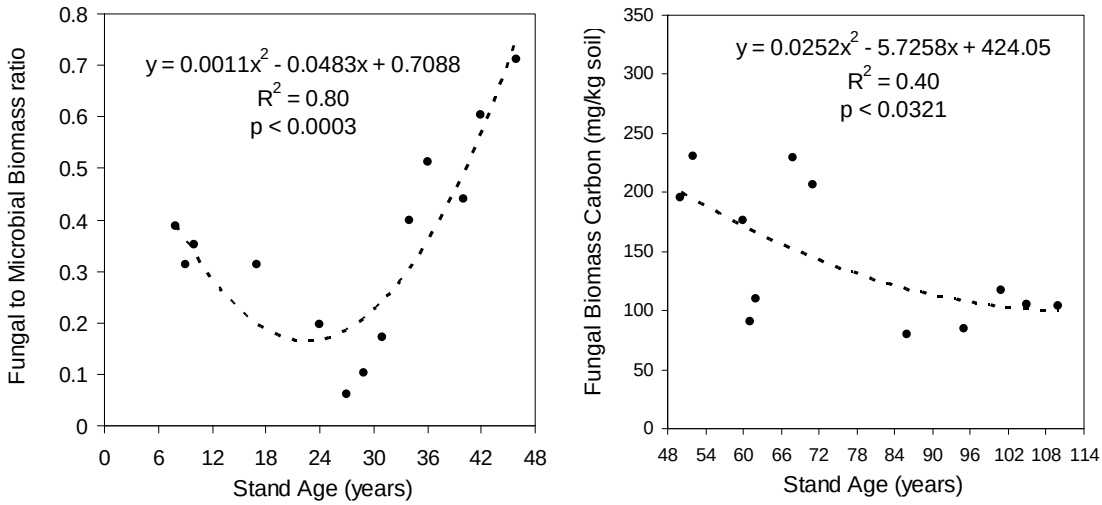


Figure 3-7. The fungal-to-microbial biomass ratio and fungal biomass carbon levels (means) during the earlier and later portions of chronosequence respectively, as measured from 26 differently aged stands along the 110-year longleaf pine chronosequence.

CHAPTER 4
RELATIONSHIP BETWEEN VEGETATION AND SOIL CHARACTERISTICS IN WET LONGLEAF PINE FLATS ALONG FLORIDA'S GULF COAST

Introduction

There have been many efforts on assessing the inter-relationships between community structure, plant species composition and soil biochemical attributes of forested ecosystems around the globe (Goebel et al. 2001; Peacock et al. 2001; Wilson et al. 2002; Allen and Schlesinger, 2003). For example, researchers in northwest Spain found that specific herbaceous species assemblages were indicators for soil pH, soil organic matter levels, C: N ratios, and high or low levels of soil nitrogen, phosphorus, potassium, calcium, and magnesium (Zas and Alonso, 2002). South American researchers compared mature natural alerce (*Fitzroya cupressoides*) forests with mature mixed beech (*Nothofagus-Podocarpus*) forests in the Chilean Andes and found that the mixed beech-conifer forests that contained greater tree and plant species biodiversity, had significantly higher soil nitrogen mineralization rates (Perez et al. 1998). These and other studies have greatly contributed to our understanding of the soil-vegetation community relationships for various ecosystems in the U.S. and other parts of the world (Vance and Entry, 2000; Reynolds et al. 2000; Chapman et al. 2003; Korb et al. 2003; Hackl et al. 2005). However, the reasons why soil nutrient and microbial dynamics influence community structure and composition of the longleaf pine ecosystem of the southeastern U.S. still remains unexplored. Longleaf pine ecosystem is one of the most threatened ecosystems in the U.S. Knowledge about the interrelationships among soil chemical, microbial and vegetational characteristics of the longleaf pine ecosystem may aid in restoring it to a healthy, functional ecosystem across its range.

The concept of using soil chemical and microbial properties in combination with vegetation attributes for monitoring restoration projects has gained momentum in the recent past.

For example, researchers monitoring the restoration of ponderosa pine (*Pinus ponderosa*) forests in Arizona explored the relationship between mycorrhizal and plant functional groups (Korb et al. 2003). They discovered that arbuscular mycorrhizal (AM) fungi were highly positively correlated with increases in grasses and forbs, and negatively correlated with tree cover and pine litter. Ectomycorrhizal (EM) fungi had no response to the restoration treatments, but had a high positive correlation to stand basal area (Korb et al. 2003). A companion study found that as plant species richness increased primarily due to an increase in legumes and stress tolerant plants, there was a corresponding increase in soil fungi and an abundance of fungi relative to bacteria (Smith et al. 2003).

A growing number of studies have indicated that soil microbial communities with distinct functional groups inhabit different forest types (Pennanen et al. 1999). A black pine forest in northeastern Austria was found to have higher relative amounts of fungi and actinomycetes in the soil microbial biomass than were found in a neighboring oak-beech hardwood forest (Hackl et al. 2005). Chapman et al. (2003), investigating native woodland expansion in England, found that soil moisture, pH, and microbial biomass levels decreased along a successional gradient from moorland to grassland to mature pine forest, but the fungal component increased. In beech (*Fagus* sp.) forests of Denmark, researchers found that different fractions of coarse woody debris supported distinct fungal species. Larger trees parts contained more fungal species, smaller pieces had higher densities of a few species, and snags were species-poor. They concluded that coarse woody debris should be left as whole trees compared to smaller or larger pieces to insure high species richness in the fungal community of the forest floor (Heilmann-Clausen and Christensen, 2004). These studies illustrate the strong interactions that exist between soil biogeochemical properties and aboveground cover type.

The objective of this study was to examine the relationships between key soil chemical and microbial properties and the overstory and understory plant characteristics of a wet longleaf pine flat community in the Gulf Coastal Plain of Florida. We hypothesized stand volume will show a positive relationship with soil nitrogen mineralization, which, in turn, will be driven by the microbial community dynamics in the soil. We also hypothesized that the fungal biomass will increase as coarse woody debris accumulated on the forest floor and the standing stock increased over time**.**

Materials and Methods

Study Areas

Three reference site locations along a spatial gradient from within the Coastal Flatwoods subecoregion of Florida (Chapter 1), sub-divided into three one-hectare blocks, representing young, mid-aged, and mature age classes, were used in this study. The different successional age classes represented a chronosequence of 110-years across a moisture gradient containing mesic flatwoods, wet flatwoods, wet savannas, and bordered by cypress ponds. The three hectares established at each reference location was scaled to match the three hectares established at the restoration site (Chapter 5). The three locations are Topsail Hill State park, St. Marks National Wildlife Refuge, and the Chassahowitzka Wildlife Management Area of the Florida Fish and Wildlife Commission (Chapter 2).

Field Measurements

Each site had a cluster of three one-hectare blocks, containing stands representing each of the three previously defined age classes. Each one-hectare block was sub-divided into four randomly placed 400 m^2 measurement plots. To assess the forest structure, tree height and diameter-at-breast height (DBH), were measured on all trees ≥ 10cm DBH. At least two of the dominant trees were cored to determine stand age. Stand density (trees/ha), basal area (m^2/ha)

and volume (m^3/ha) were calculated from this data. In addition, the volume (m^3/ha) of all snags and coarse woody debris (CWD) were also measured (Spetich et al. 1999).

Each 400 m^2 plot contained four randomly placed 1 m^2 subplots for understory sampling (Chapter 2; Figure 2-2). Percent cover of each species was assessed using the Daubenmire method modified to estimate eight levels of percent cover (Daubenmire, 1959). Coleman rarefaction and the Shannon-Weiner diversity indices were calculated for each stand based upon four sub-samples (Colwell, 2006; Koellner and Hersperger, 2004).

Soil Sampling and Preparation

Soils were sampled (> 500 grams) from within the vegetation survey ($1m^2$) quadrats taken in the top 10cm of the 'A' horizon. The sampling took place during August and September of 2005, and September of 2006, at each of the reference sites and the restoration test site. They were immediately stored at 4ºC until analysis. A sieved and oven dried (105ºC) sub-sample (20g) was used for determining moisture content.

Soil Chemical Analysis

The soil samples were analyzed at the University of Florida Soil Testing Laboratory (UF ARL), Gainesville, Florida. Soil water pH was determined from prepared slurries using a soil-to-water ratio of 1-to-2 (EPA method 150.1). Plant-available phosphorus was determined with the use of Mehlich-1 extractant (H_2SO_4 & HCL) and measured on an inductively coupled plasma (ICP) spectrophotometer (EPA method 200.7). Soil organic matter content (SOM %) was determined by the Walkley-Black method. The gravimetric soil water content was determined in 2005 and 2006 for all of the samples analyzed.

Mineral Nitrogen Fluxes

Net nitrogen mineralization was determined by comparing collected paired soil samples contained in plastic bags. Forty eight samples were collected from each reference location for a total of 144. One bag was buried in situ for incubation during August, 2005 (Eno, 1960) and the other bag taken to the soil lab for analysis. The incubated bags were collected and analyzed after 30 days. Mineral nitrogen was extracted from 20 g of both soil samples with 60 ml 2N KCL and placed in shaker for one hour. They were then filtered through # 42 Whatcom filter papers and analyzed by the UF ARL for ammonium (EPA method 350.1) and nitrate (EPA method 353.2) with a continuous auto-flow analyzer. Net Mineralization was calculated as the difference between incubated-N and initial-N (corrected for soil moisture) (Keeney & Nelson, 1982).

Bacterial Abundance and Microbial Dynamics

Enumeration of nitrifying bacteria was determined by the most probable numbers (MPN) method for densities of ammonium and nitrite oxidizing bacteria using a five tube dilution (Schmidt and Belser, 1982). The ammonium oxidizing bacteria were incubated in a medium of di-ammonium sulfate, and the nitrite oxidizing bacteria were incubated in potassium nitrite. The tubes were incubated for 8 weeks for the first readings and 16 weeks for the final readings. A pH indicator of bromothymol blue was used to determine pH changes caused by increased respiration of the ammonium oxidizing bacteria. Positive readings for the nitrite oxidizing bacteria were determined from a nitrate test reagent of diphenylamine in sulfuric acid solution (Schmidt and Belser, 1982).

Soil microbial biomass C was determined by chloroform fumigation-extraction (Vance et. al., 1987), with the following modifications. Sieved 12 grams of soil were taken from soil samples stored at 4° C and then placed in 50 ml centrifuge test tubes. Matching 12 gram soil samples were set aside in additional 50 ml centrifuge tubes as the control. The soil samples were

fumigated in 24 tubes per desiccator with 40 ml of alcohol-free chloroform placed into a center beaker and an additional 0.5 ml of chloroform was placed into each centrifuge tube. The top of the desiccator was pressure sealed and vacated until the chloroform began to boil. The tubes were then incubated for 24 hours at 25°C. The dessicator was then opened, resealed, and after the chloroform was reboiled, incubated for an additional 24 hours. The control and fumigated samples were extracted with 36 ml of 0.05 M K_2S0_4, shaken (360 rpm) on an orbital shaker for 1 hour, and centrifuged @ 6000 rpm for 15 minutes. The supernatant was then filtered through # 42 Whatcom filter papers into 20 ml scintillation vials and frozen until analysis. Levels of total organic carbon (TOC) were determined on a Shimadzu TOC-VCSH analyzer (Vance & Entry, 2000). Microbial biomass carbon was equal to [(fumTC – ConTC) / 0.51] / (Soil Wt.) = mg C kg dry wt. Soil $^{-1}$ (Joergensen, 1996). The value of 0.51 is the conversion factor equal to the extractable portion of microbial biomass in a forest soil. Fumigated and non-fumigated blanks were measured to correct for the chloroform and potassium persulfate.

Soil fungal biomass levels were determined by a physical disruption method for extraction of ergosterol from soil samples (Gong et al. 2001); with the following modifications. Weighed 6 grams of soil were mixed with 9 ml of 0°C methanol and 1.9 grams of glass beads into 20 ml scintillation vials. The vials were vortexed for 30 seconds, shaken (360 rpm) on an orbital shaker for 1 hour, and refrigerated over night. An aliquot of 1.8 ml was placed into 2 ml micro-centrifuge tubes and centrifuged @ 11,000 rpm for 20 minutes. A syringe was used to remove 1.5 ml of the supernatant from the micro-centrifuge tubes and filtered through a 0.20 µm filter into amber colored 2 ml glass HPLC vials. The HLPC vials were covered with aluminum foil and stored in the dark at 0 degrees C until ready to inject into the HPLC. Each sample was quantified on a Beckman Coulter HPLC equipped with an UV detector, a pump, an auto-

sampler, and through a C-18 reverse-phased analytic column (4.6 x 250 mm). The UV detector was set at 282 nm and pure methanol was used as the mobile phase at a flow rate of 1 ml per minute. Extracts (100 µl) were injected while the column pressure was maintained at 1000 psi. Pure ergosterol (Sigma) was recrystalized in pure methanol at different concentrations to establish a set of standards. The standard curve was constructed from on a linear regression relationship between peak area and ergosterol concentration**.** Ergosterol recoveries were calculated from the difference between spiked and non-spiked paired samples divided by the amount of ergosterol added**.** Under such conditions, an isolated peak was identified from field samples at approximately the 13 minutes, based upon the peaks obtained from the ergosterol standards. An averaged conversion factor for 3.65 µg ergosterol per mg of soil translates to a fungal biomass (mg /g $^{-1}$ soil) when multiplied by (220) (Montgomery et al. 2000). Fungal: microbial biomass ratios were represented by a ratio of the calculated soil fungal biomass, and the soil microbial C biomass levels for each sample.

Experimental Design and Analysis

A three stage balanced nested design was used to integrate the indicators measured at different scales, and between sites. Since the monitoring of the restoration site with nine distinct reference locations produced a dataset where the assumptions for analysis of variance (ANOVA) were not ensured, non-parametric tests were used to detect any significant differences between the reference sites and between the distinct forest age classes (SAS, 2002).

Inter-relationships between forest structural variables, understory species diversity indices, and the soil biogeochemical variables were determined by Spearman's rank (*r*) correlations using SAS 8.2 (Dumortier et al. 2002; SAS, 2002; Spyreas and Mathews, 2006). Trends between variables were obtained from linear regression using the general linear model (PROC GLM)

(Yang et al. 2006; SAS, 2002). These trends were enhanced by incorporating moving average smoothing (MA model) as a data filter to reduce seasonal variations found in the datasets for a number of the indicators affected by climate **(**Platt and Denman 1975; Kumar et al. 2001; Ittig, 2004). The trend analysis was followed by $\log_{10}$ data transformations where necessary.

Results

Nitrifying Bacteria and Nitrogen Mineralization

Young forest soils at one of the reference sites, St. Marks, had numbers of ammonium oxidizing bacteria (AOB) that were 34 times greater (14,690 / g soil) than that found in soils from the mature sites (427 / g soil) (Table 4-1). The higher AOB numbers in the young forest soils corresponded to lower ammonium production (0.14 mg NH_4^+ / kg soil/month) and higher nitrate production (Table 4-2). Topsail Hill State Preserve also had numbers of ammonium oxidizing bacteria that were 60 times greater in the young forested soils (240 / g soil) than found in soils from the mature sites (4 / g soil) (Table 4-1). However, the young wet pine savanna had very high ammonium production compared to nitrification (Table 4-2). The mesic mature forest soils at Topsail had lower ammonium levels than the wet young forest soils (Table 4-2). The numbers of AOB at St. Marks (14,690) were significantly larger compared to the numbers measured at Topsail Hill (240). The higher AOB numbers in the soil under the young forest at St. Marks resulted in lower ammonification 0.14 mg NH_4^+ / kg soil/month compared to the soil from the young forest at Topsail Hill 17.9 mg NH_4^+ / kg soil/month. St. Marks had larger numbers of AOB in the old forest soils (427 vs. 4.0), but the level of ammonium production was smaller 2.98 vs. 4.98 mg NH_4^+ / kg soil/month when compared with the soils from the Topsail old forest site (Table 4-2). The numbers of nitrite oxidizing bacteria (NOB) showed differences between the age groups (427 / g^{-1} soil, in young soil vs. 4 / g^{-1} soil, in old soil), but not between the sites

(Table 4-1). The nitrate production levels in young (2.39 vs. 1.74 mg NO_3^- / kg soil/month) and old (1.57 vs. 0.9 mg NO_3^- / kg soil/month) soils were similar between the sites (Table 4-2).

Overstory

Stand volume increased with net nitrogen mineralization (N_{min}) until the volume reached 200 m^3 / ha, when N_{min} decreased substantially (Figure 4-1). All of the overstory stand variables were positively correlated with microbial biomass carbon (C_{mb}) during the young age class, but mean stand DBH and height were negatively correlated with C_{mb} during the mid-aged and mature age classes (Table 4-3). Similar to C_{mb}, all of the forest structural variables were positively correlated with fungal biomass carbon (C_{fb}) during the young age class, but remained positively correlated with C_{fb} during the mid-aged class (Table 4-3). FB-to-MB ratios increased with stand height during the mid-aged and mature age classes, when the mean stand height was greater than 7.5 m (Figure 4-2). C_{fb} increased by more than 130% as stand BA approached 10 m^2 / ha. However, C_{fb} declined by 30 % as the stand BA grew from 10 m^2 / ha to 20 m^2 / ha (Figure 4-3). Coarse woody debris CWD was positively correlated with C_{fb} during the mid-aged and mature age class (Table 4-3). C_{fb} increased by more than 45 % as CWD increased from 1 to 55 m^3 / ha (Figure 4-4). Stand density had a positive relationship with soil organic matter content (SOM) during the young and mid-aged class, but not during the mature age (Table 4-3).

Understory

Coleman rarefaction was positively correlated with C_{mb} during the young and mid-aged class, and negatively correlated to C_{mb} during the mature age class (Table 4-3). The Coleman rarefaction index decreased by 50% and Shannon-Wierner diversity index by 25% as the FB-to-MB ratio approached 1.0 (Figure 4-5; Figure 4-6) The Coleman rarefaction index and the Shannon-Wiener diversity index were also negatively correlated with soil organic matter content (SOM), during the young age and mature age classes (Table 4-3).

Discussion

Net nitrogen mineralization declined at a stand volume of 200 m^3 / ha which corresponds to a stand age of 90 years (Chapter 2; Figure 2-9). This could be a stand volume threshold where fungi and actinomycetes have become the major decomposers in the microbial community due to lignin concentrations (Richards, 1987). Even in high soil moisture conditions, the forest soils from young longleaf pine stands had significantly higher levels of nitrifying bacteria than soils from mature pine sites. The nitrifying bacteria data confirmed that nitrification rates were higher during the young age class than measured in the mature aged stands. The AOB numbers were highly variable between sites, but the NOB numbers were similar. Nitrate levels were lower and ammonium levels were higher in the soils from the mature forest sites compared to the soils from the young forests. The higher levels of ammonium and lower levels of nitrate in mature forest soils could be an indication of a nitrogen conserving (tighter) ecosystem (Davidson, 2000). There was an exception with the wet young Topsail pine savanna soil that had higher ammonification levels than the mesic mature Topsail soil. Higher ammonium levels and lower nitrification levels have been measured in wet longleaf pine sites when compared to more xeric sites (Wilson et al. 2002). Ammonium production was higher and nitrate production was lower in the soils from the unburned Topsail Hill sites compared to St. Marks. The larger numbers of nitrifying bacteria measured at St. Marks NWR compared to Topsail Hill State Park were probably due to the higher frequency of prescribed fire implemented at St. Marks. Higher nitrification rates after prescribed fire have been measured in a number of studies (Cookson et al. 2007; Hart et al. 2005; Wilson et al. 2002). In addition, researchers studying disturbance in a Norway spruce (*Picea abies*) forest measured large enumerations of ammonium oxidizing bacteria (AOB) in sites recently harvested, but only detected very small numbers (< 10 / gm) in mature undisturbed sites (Paavolainen and Smolander, 1998).

The effect of forest growth on the environment represents more than creating a preference for shade tolerant plant species or the creation of a multi-layered architecture. It also represents the evolution of soil organic matter (SOM) inputs from an easily decomposed substrate to a SOM complex having a higher portion of recalcitrant material. As the inputs to the soil change, there is a corresponding change in the soil microbial community as ectomycorrhizal and saprophytic fungi play greater roles. This relationship between the aboveground component and the belowground biological community is important in shaping the ecological trajectory of ecosystems (Hackl et al. 2005).

The positive relationship between stand density and soil organic matter (SOM) through the mid-aged class illustrates the effect of site quality on stand productivity. Stand BA and volume had strong positive relationships with C_{mb} up to the mature age class (60 years+). Correlations also showed strong positive relationships between most of the forest growth variables (DBH, height, BA) and C_{fb} levels, again up to the mature age class (60 years+). These two relationships reinforce how the rate of stand volume growth is interdependent on the rate of organic matter decomposition and nutrient cycling (Vitousek and Reiners, 1975).

Regression analysis produced a trend showing that C_{fb} increased dramatically as stand basal area decreased. A ponderosa pine restoration study produced similar results showing positive relationships between increases in forest basal area and higher levels of ectomycorrhizal (EM) fungi (Korb et al. 2003). The relationship between the fine root biomass of trees and EM fungal levels has also been well established (Hendricks et al. 2006; Wallander et al. 2001; Sylvia and Jarstfer, 1997). C_{fb} was also found to increase with higher accumulations of CWD. Researchers in Demark determined that a combination of larger DBH logs and the greater surface area of smaller diameter CWD, promoted the highest level of fungal species richness

(Heilmann-Clausen and Christensen, 2004). Whether the increase in C_{fb} during longleaf pine succession was due to the size of a tree's root system (mycorrhizal fungi) or in part due to increases in coarse woody debris accumulation (saprophytic fungi), fungal biomass (C_{fb}) increased as the average stand height increased. These results indicate that both C_{mb} and C_{fb} are important soil variables for longleaf pine flat development, but the C_{fb} portion of the biomass becomes more important over time as the ecosystem requires the decomposition of larger amounts of CWD and the improved cycling of nutrients (Hackl et al. 2005; Leckie et al. 2004; Pennamen et al. 1999).

The relationship between the FB-to-MB ratio and the Coleman rarefaction index was similar to Shannon-Wiener diversity index, species diversity decreased as the fungal component increased. Both Coleman Rarefaction and Shannon-Wiener diversity H' indices were also negatively related to SOM during the young and mature age classes. Through a restoration study in England, researchers also found a negative relationship between native plant species richness and soil fertility. Never the less, in contrast to our results, they found a positive relationship with plant species richness and FB-MB ratios. The investigators attributed this positive relationship to a greater presence of legumes in the lower fertile soils (Smith et al. 2003).

Conclusions

The majority of the soil biogeochemical indicators influenced longleaf pine stand growth, and as stands developed, changes in aboveground vegetation influenced the soil biogeochemical indicators. Net nitrogen mineralization increased with stand volume until a threshold of 200 m^3 / ha (stand age = 90 years). Nitrate was found to be in higher concentrations in the young forest soils than the mature forest soils. Populations of nitrifying bacteria (AOB + NOB) were also found to be higher in the young forest soils. At Topsail Hill, ammonium levels were found to be higher in the wet young pine savanna soils than the mesic mature soil. Higher soil moisture

translates to lower nitrification levels. The relationships between fungi and increases in stand height or coarse woody debris accumulation indicate a strong continual relationship between the soil biogeochemical indicators and longleaf pine stand development. The dynamics of this relationship might be better understood if the measured fungal biomass could have been identified as arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi, or saprophytic fungi along the chronosequence. The dominance of fungi negatively affected the Coleman Rarefaction and Shannon-Wiener diversity indices. This may have indicated a decrease in species richness, but the functional redundancy component of ecosystem resilience has probably been strengthened. The strong relationships between forest biomass accumulation and soil biogeochemistry should be assessed in any monitoring event. Nitrogen cycling appears to become tighter in mature forests at a threshold of 90 years. This condition is dependent on mycorrhizal and saprophytic fungi dominating the soil microbial biomass.

Table 4-1. MPN enumerations of nitrifying bacteria in young and old longleaf pine forest soils.

Enumerations (MPN - g^{-1})		
Site Locations	Ammonium Oxidizers	Nitrite Oxidizers
St. Marks - Seedling site (6 yrs.)	1.4690×10^4 (0.278, 6.318)	0.4273×10^3 (0.103, 1.385)
St. Marks - Mature site (100 yrs.)	0.0427×10^4 (0.103, 1.385)	0.0040×10^3 (0.005, 0.123)
Topsail Hill - Sapling site (19 yrs.	0.0240×10^4 (0.047, 0.965)	0.4273×10^3 (0.103, 1.385)
Topsail Hill - Mature site (100 yrs	0.0004×10^4 (0.005, 0.123)	0.0036×10^3 (0.005, 0.123)

*****All values are expressed in units of MPN per gram (wet weight) of 0 to 10-cm soil and are averages of three replicates. Lower and upper limits in parentheses reflect 95% confidence intervals.

Table 4-2. Ammonification and nitrification in young and old longleaf pine forest soils.

Site Locations	Ammonification (mg NH_4 / kg soil / month)	Nitrification (mg NO_3 / kg soil / month)
St. Marks - Seedling site (6 yrs.)	0.14	2.39
St. Marks - Mature site (100 yrs.)	2.98	1.57
Topsail Hill - Sapling site (19 yrs.)	17.9	1.74
Topsail Hill - Mature site (100 yrs.)	5.98	0.9

Values expressed as mean monthly rates and based on the dry weight of soil.

Table 4-3. Soil biogeochemical relationships with stand attributes based upon Spearman Rank correlations *r* as stratified by forest age class (n = 48).

Prob > |r| under H0: Rho=0

Regeneration Time Interval (6 - 20)				
	C_{mb}	SOM	C_{fb}	FB-to-MB ratio
Stand Height	0.524****		0.543****	
Stand Density		0.394**		
Stand DBH	0.513****		0.510****	
Stand BA	0.542****		0.593****	
Stand Volume	0.540****		0.564****	
CWD				
Shannon Diversity		-0.584****		
Coleman Rarefaction	0.464***	-0.363**		
Mid-Aged Time Interval (25- 55)				
	C_{mb}	SOM	C_{fb}	FB-to-MB ratio
Stand Height	-0.345*		0.296*	0.476***
Stand Density	0.509***	0.581****		-0.358*
Stand DBH	-0.401**	-0.365**	0.319*	0.539****
Stand BA	0.465***	0.585****	0.348*	
Stand Volume	0.360*	0.502***	0.457**	
CWD			0.326*	0.339*
Shannon Diversity			-0.396**	-0.290*
Coleman Rarefaction	0.351*	0.302*	-0.322*	-0.517***
Mature Time Interval (60 - 110)				
	C_{mb}	SOM	C_{fb}	FB-to-MB ratio
Stand Height	-0.646****		-0.289*	0.446**
Stand Density				
Stand DBH	-0.429**	0.422**		
Stand BA				
Stand Volume				
CWD	0.326*	0.648****	0.293*	
Shannon Diversity		-0.439**		
Coleman Rarefaction	-0.348*	-0.565****		

Significance of the Spearman rank correlation test: blank: non-significant,
*$0.05 < p \leq 0.01$, **$0.01 < p \leq 0.001$, ***$0.001 < p \leq 0.0001$, **** $p < 0.0001$.

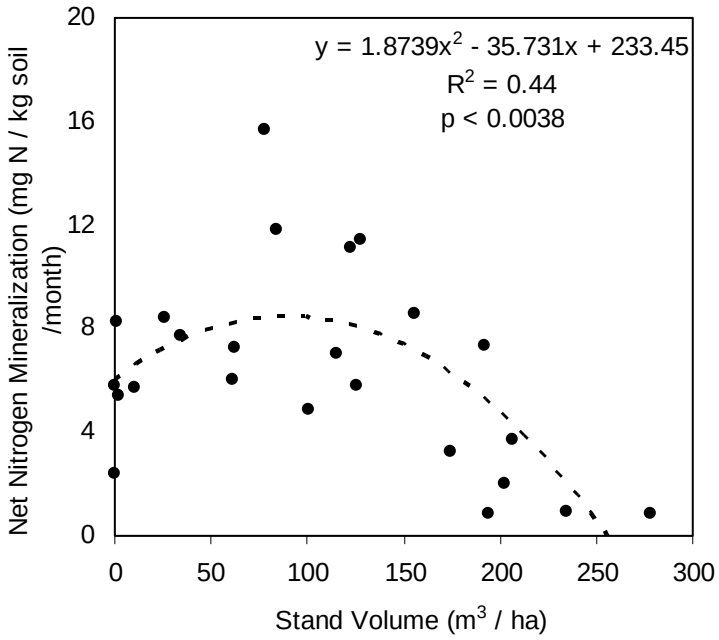


Figure 4-1. Net nitrogen mineralization versus stand volume as measured from 26 differently aged stands.

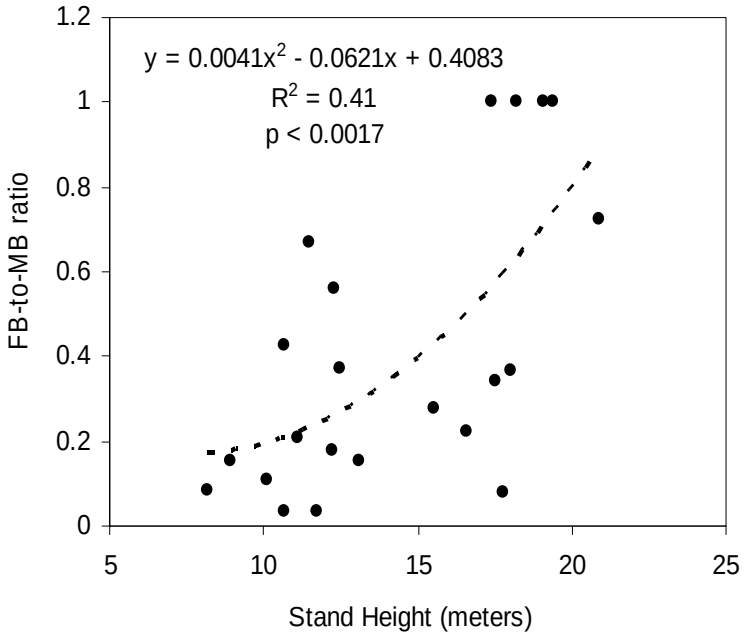


Figure 4-2. The fungal biomass (FB)-to-microbial biomass (MB) ratio versus stand height as measured from 26 differently aged stands.

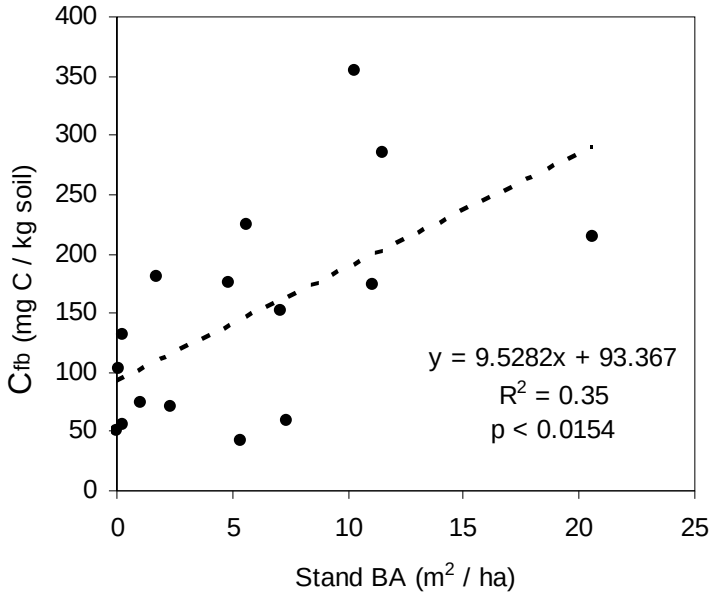


Figure 4-3.Fungal biomass carbon (C_{fb}) versus stand basal area (BA) as measured from stands grouped within the mid-aged and mature age classes only.

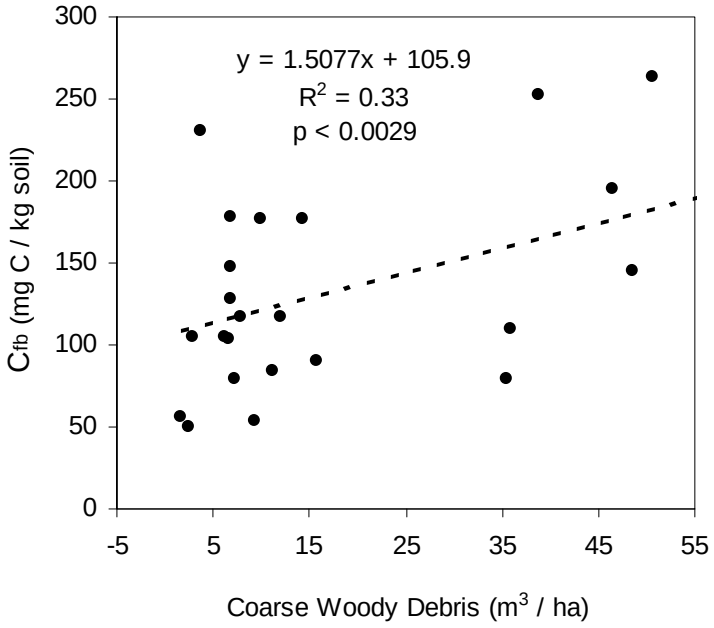


Figure 4-4. Coarse woody debris accumulation versus fungal biomass carbon (C_{fb}) as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

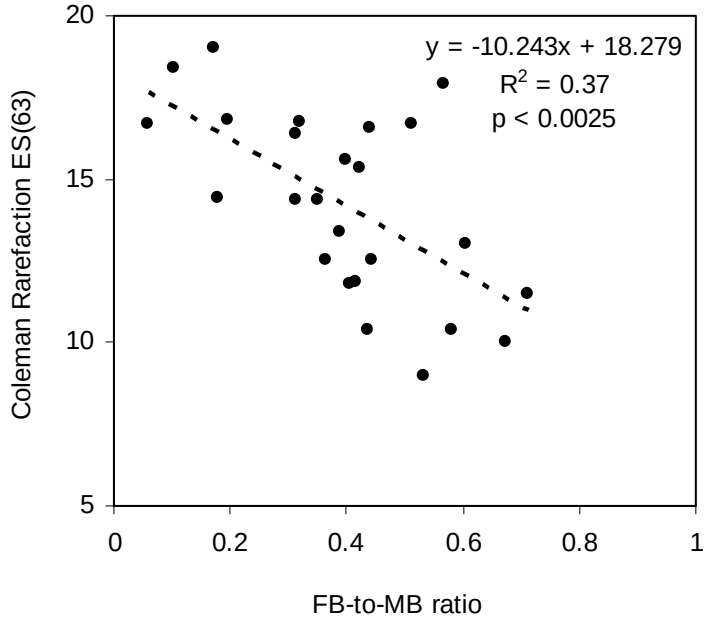


Figure 4-5.Coleman Rarefaction index versus the fungal biomass (FB)-to-microbial biomass (MB) ratio as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal and cyclic effects.

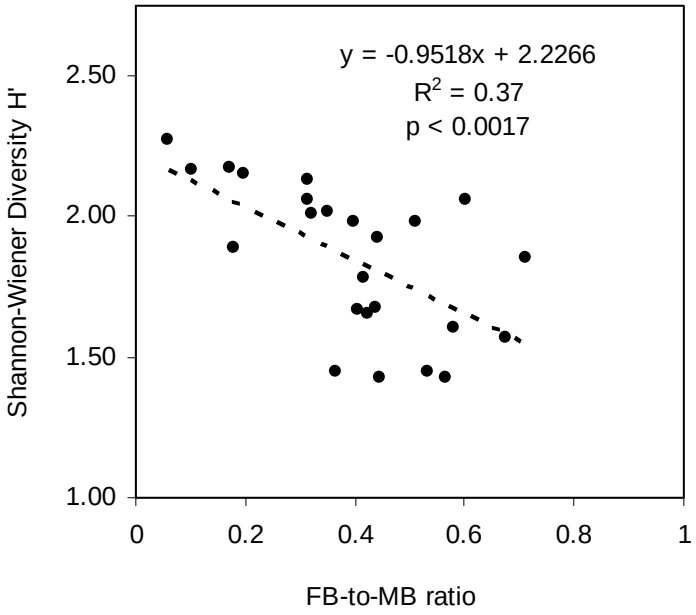


Figure 4-6. Shannon-Wiener diversity H' index versus the fungal biomass (FB)-to-microbial biomass (MB) ratio as measured from 26 differently aged stands. The data was filtered with moving average smoothing to remove seasonal effects.

CHAPTER 5
MONITORING RESTORATION SUCCESS USING VEGETATION AND SOIL AS KEY INDICATORS: CASE STUDY OF A WET LONGLEAF PINE FLATS RESTORATION PROJECT

Introduction

Ecosystems ecology requires the integration of structural and functional characteristics for developing a holistic understanding of ecological change caused by natural or anthropogenic disturbance. However, these two characteristics of ecosystems have generally been studied separately along vegetative and geochemical gradients (Muller, 1998). Soil chemical and biotic properties need to be included as indicators with forest structural and vegetative compositional measurements for the integration to take place (Johnston and Crossley, 2002). Soil microbial community analysis also provides a means to measure how responsive soils are to disturbance and restoration treatments (Harris, 2003). Additionally, the inter-relationships between vegetation and soil characteristics have also been identified and used to assess site quality. In pine plantation research, specific soil properties have been found to be associated with the growth of specific tree and plant species. Similarly, certain groups of plant species may indicate specific soil conditions (Burger and Kelting, 1999; Zas and Alonzo, 2002). This combination of above and below ground data can also be used to ecologically verify if a restoration site falls within the spatial gradient of the reference sites (Goebel et al. 2001).

The research reported in Chapter 3 determined that net nitrogen mineralization rates increased until 90 years. It was also determined that the soil fungal-to-microbial biomass ratio increased with stand growth and total woody debris accumulation. Finally, soil fungal biomass increased with mean stand height (Chapter 4). These results show strong relationships exist between stand development and soil biochemical dynamics. This paper examines a case study of a restoration project, hereafter referred to as the Pt. Washington restoration project in Florida.

The Pt. Washington restoration project was initiated in 2001 to convert a slash pine plantation to a longleaf pine ecosystem. The effects of low-level herbicide applications on longleaf pine development and understory species richness were evaluated. The central goal of this experimental application of herbicides was to determine which herbicide, as a substitute for fire, would produce the best results for longleaf pine seedling survival and growth, understory plant species richness and composition, and soil nitrogen mineralization.

Herbicides are currently being used in restoration projects throughout the United States for promoting the establishment of native grasslands, assisting in the control of exotic invasive species as part of integrated pest management programs, for weed control during early forest stand development, and to combat eutrophication from unwanted plant growth in aquatic ecosystems (Sigg, 1999). Yet, many environmentally-sensitive managers and scientists are hesitant to support the use of herbicides making it imperative that the correct herbicide is used in the proper environment, with the lowest feasible application rates (Murphy, 1999; Sigg, 1999).

What primary factors make the restoration of coastal longleaf pine flats unique compared to other pine ecosystems? First, longleaf pine regeneration is dependent on a grass stage when the pine seedlings are able to survive light surface fires and during fierce vegetative competition (Boyer and Peterson, 1983; Boyer, 1990). Longleaf pine seedlings have been known to stay in the grass stage from 5-20 years. This protective state can make the growth rates of longleaf pines unpredictable (Haywood, 2000). Secondly, although many longleaf pine ecosystems are found on a variety of upland sites (Peet and Allard, 1993), coastal wet pine flats are unique because they are located on low, rain-fed coastal terraces where weather patterns maintain high soil moisture conditions for extended periods during the growing season (Messina and Conner, 1998).

The longleaf pine grass stage becomes the critical factor in the amount of time the pine remains in the seedling stage when prescribed fire is restricted. One study found that the application of a hexazinone herbicide helped to release longleaf pine seedlings from fierce vegetative competition, enhancing conditions for leaving the grass stage (Haywood, 2000). A recent study of herbicides in an old field restoration project found a higher first-year seedling survival rate and a higher percentage of seedlings out of the grass stage (2^{nd} year) than with no herbicide treatment (Ramsey et al. 2003). There have also been studies to control fuel loads by different fuel reduction techniques, including herbicide applications. An earlier study found that fire and mechanical removal of fuels had immediate but short-term impacts on reducing fireline intensity levels, but herbicide applications had longer term affects on reducing fuel levels, starting in the second year after treatment and lasting up to six years (Brockway et al. 1998). Herbicides can be the short-term substitute for fire when applied in the correct manner, and within the correct environment.

In 2004, the Pt. Washington restoration project was expanded to include the development of a monitoring program, the addition of bio-indicators for evaluation including soil microbial dynamics, and the use of reference sites for establishing a chronosequence for the restoration site evaluation. We predict the overstory, understory, and soil biogeochemical indicators will be useful for ecologically classifying the Pt. Washington restoration site as a mesic flatwoods, wet flatwoods, or wet savanna. We will use them for trying to detect differences among the four herbicide treatment effects applied on the restoration site. Finally, we will use them to predict the development or ecological trajectory in wet longleaf pine flat restoration. The predicted values will be presented with pine growth results on the effects of herbicide treatments applied in the second year after planting compared to first year only, consecutive herbicide treatments (1^{st} &

2nd Year), and whether an early or late spring application changes the effects (McCaskill data, 2006).

Materials and Methods

Pt. Washington Restoration Site

The longleaf pine restoration project is located on the Point Washington State Forest ($30^0$20'16.04" N, 86^0 4'19.22" W) in southern Walton County, Florida. This coastal wet pine flats site was approximately a 4-ha, 26 year-old slash pine plantation having a basal area of 1.85 m^2 / ha and an average dbh of 19.1 cm as measured in 1991. It contained scattered residual longleaf pine saplings and poles as part of the stand's stocking. The adjacent area makes up approximately 15 ha of mixed slash and longleaf pine surrounding a cypress dome, and contained within the greater 6800 ha Pt. Washington State Forest. The understory plant community was dominated by broomsedge, a smaller component of wiregrass, and a group of shrub species highlighted by gallberry, saw palmetto, running oak, and dangleberry (*Gaylussacia frondosa*). The annual precipitation averages 1500 mm with most of it occurring during the late summer. The soil belongs to the Leon series and classified as sandy, siliceous, thermic aeric Alaquods. This soil series signifies that they are very poorly drained soils (Jokela and Long, 1999). Since this pine flats forest is found very close to the coast (within 3 kilometers), its soils were formed on sandy quaternary parent material derived from marine deposits (Stout and Marion 1993). These soils are described as highly weathered, acidic, infertile substrates (LaSalle, 2002).

The surrounding area consists of wet pine savannas and wet flatwoods sites that are found within Florida's Gulf Coast Flatwoods zone (Chapter 1; Griffith, 1994). Florida's Gulf coastline is continuously shaped by active fluvial deposition and shoreline processes which promote and maintain the formation of beaches, swamps and mineral flats. The local relief is less than 20 m in

elevation. The annual precipitation ranges from 1300–1,600 mm, and the average annual temperatures vary between 19° to 21° C. The growing season is long, lasting 270-290 days. The parent material consists of marine deposits containing limestone, marl, sand, and clay. The dominant soils are Aquults, Aquepts, Aquods, and Aquents. These acidic soils have thermic and hyperthermic temperature regimes and an aquic moisture regime. The soils are poorly drained, deep, and moderately textured. The dominant vegetative cover consists of longleaf-slash pine forests with a smaller component of Choctawhatchee sand and/or pond pine (McNab and Avers, 1994; Parker and Hamrick, 1996).

As the first step towards restoring the Pt. Washington site back to longleaf pine, the overstory of slash pine was clearcut during August 2001. The site was roller chopped once and prescribed burned in October 2001. There was no existing bedding or any other hydrological-modifying practice applied. A randomized complete-block design (RCB) with six blocks was used to measure the effects of four vegetation-control chemical mixtures on the dynamics of the understory plant species and pine growth and survival. Five plots were randomly located within each of the six blocks. All treatment plots were 26.6m x 24.4 m, and included at least a 3-m buffer strip between plots. The six blocks with buffers make up approximately 3.5 ha within the 4 ha clearcut.

In December 2001, one-year-old containerized longleaf pine seedlings were hand-planted at 3.1 x 1.8 m spacing. Seedlings were planted in rows to facilitate the application of herbicides. In March 2002, four herbicide treatments Sulfometuron methyl (methyl 2-[[[[(4,6-dimethyl-2 pyrimidinyl)amino]carbonyl] amino]sulfonyl]benzoate) at 0.26 ai kg ha^{-1}, Hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) at 0.56 ai kg ha^{-1}, Sulfometuron (0.26 ai kg ha^{-1}) + Hexazinone (0.56 ai kg ha^{-1}) mix, and Imazapyr (4,5-dihydro-

4methyl-4(1-methylethyl)-5-oxo-1-H-imidazol2-yl-3 pyridinecarboxylic acid) at 0.21 ai kg ha^{-1}, were applied in a 1.2 m band over the top of seedlings using a knapsack sprayer. In each block, one treatment plot was kept herbicide-free as a control plot (Ranasinghe, 2003).

Pine Survival and Growth

Pine survival and growth (root collar diameter and height) were monitored at the end of the growing season every year, through 2006. Seedling height was measured using a ruler, from the soil surface to the top of the bud. Root collar diameter (RCD) was measured using a digital caliper. Stem volume index (SVI) was calculated with the measured RCD and height data.

Vegetation Sampling

A preliminary vegetation survey was conducted (June 2001) prior to overstory harvest and site preparation to assess the initial percent cover of understory species. After study establishment and herbicide application, four vegetation surveys were conducted. Two randomly selected $1m^2$ quadrats were sampled within each treatment plot and the same location was revisited for subsequent surveys. In every survey, all plants found within the quadrat were identified to species and assigned to shrub, graminoid, forb, or fern vegetation classes. Percent cover was ocularly estimated by species using the modified Daubenmire scale (Daubenmire, 1959). In addition to percent cover, the number of stems and average stem height were collected for the woody understory species. These plant surveys were conducted concurrently at the reference sites (described below) during the 2004 growing season. Coleman rarefaction and the Shannon-Weiner diversity indices were calculated for each stand (Colwell, 2006; Koellner and Hersperger, 2004). The assemblage pathway for the plant community was determined from these measurements over time using Canonical Correspondence Analysis (CCA) ordination (Palmer, 1993).

Reference Sites

Three representative locations along a spatial gradient from Pensacola to Tampa Bay (720 km) sub-divided into three one-hectare blocks, representing young, mid-aged, and mature age class; were used in this study. The different stages (age classes) represented a chronosequence of 110-years. The three locations are Topsail Hill State Park, St. Marks National Wildlife Refuge, and the Chassahowitzka Wildlife Management Area of the Florida Fish and Wildlife Commission.

A three stage balanced nested design was used to integrate the indicators measured at different scales, and between sites. Each reference site had a cluster of three one-hectare blocks containing stands that represent young, mid-aged, and 100^{+} year-old age class. Each one-hectare block was sub-divided into four randomly placed 400 m^2 measuring plots where forest structure and coarse woody debris (CWD) were determined. Within each 400 m^2 subplot, vegetation was inventoried on four randomly placed 1 m^2 quadrat using the same modified Daubenmire scale method utilized at the restoration site.

Soil Sampling and Preparation

Soils were sampled from within the vegetation survey quadrats taken from the top 10cm, at each of the reference sites and the restoration test site during August of 2005, and September of 2005 and 2006. They were stored at 4º C until analysis. Sub-samples were sent to the University of Florida soil testing lab for analysis of soil pH by prepared slurries using a soil-to-water ratio of 1-to-2 (EPA method 150.1), organic matter content (%) by the Walkley-Black method, and plant-available phosphorus by the use of Mehlich-1 extractant (H2SO4 & HCL) and measured on an inductively coupled plasma (ICP) spectrophotometer (EPA method 200.7). Soil microbial biomass was determined by chloroform fumigation-extraction extraction (Vance et. al., 1987). Net nitrogen mineralization rates were estimated from in-situ incubation of soil samples (Eno,

1960). Fungal biomass levels were determined by soil ergosterol analysis (Gong et al. 2001). Fungal-to-microbial biomass ratios were calculated along the gradient (Montgomery et al. 2000). A sieved and dried (105ºC) sub-sample was used to determine moisture content.

Data Analysis

Pine survival and growth

Pine survival, RCD, and height data collected during five growing seasons were analyzed using analysis of variance (ANOVA) within the framework of a randomized complete block design (RCBD) using JMP IN version 5 (SAS Institute, Inc.). Height and RCD comparisons were made separately for seedlings in the grass stage (GS) and out of the grass stage (OOGS) using a threshold height of 12 cm (Haywood, 2000). The study addressed only the main effects of herbicide treatment, and tests of these effects were not dependent on the assumption of no treatment x block interaction. Block effects were therefore treated as random effects in a univariate ANOVA model with two independent variables: 'treatment' with 'Block&Random' as a covariate. Data were log-transformed where necessary to meet the assumptions of ANOVA. Significant differences between treatments were separated with the Tukey-Kramer HSD test. . Following a prescribed fire in February of 2007, post-fire seedling mortality was assessed in June 2006. Post-fire survival was analyzed with ANCOVA, using pre-fire survival as a covariate (Freeman, 2008).

Understory

The effects of treatments on stem counts and heights of the major shrub species were also analyzed using ANOVA for a randomized complete block design. The study addressed only the main effects of herbicide treatment, and tests of these effects were not dependent on the assumption of no treatment x block interaction. Block effects were therefore treated as random effects in a univariate ANOVA model with two independent variables: 'treatment' with

'Block&Random' as a covariate. Data were log-transformed where necessary to meet the assumptions of ANOVA. Significant differences between treatments were separated with Tukey's HSD or Hsu's MCB. Post-fire treatment differences were analyzed with ANCOVA, using pre-fire distributions as a covariate (Freeman, 2008; Ranasinghe, 2003).

Biogeochemical indicators

A three stage balanced nested design was used to integrate the indicators measured at different scales, and between sites. Significant treatment effects on the biogeochemical indicators (α=0.05) were also compared with the control using Dunnett's t-test for multiple means comparison. Hypothesis testing for differences between means was accomplished by using two-sample t-test with an alpha of .05 and a two-tailed confidence interval. Since the monitoring of the restoration site with nine distinct reference locations produced a dataset where the assumptions for analysis of variance (ANOVA) was not ensured, non-parametric multiple and linear regression, and multivariate Canonical Correspondence Analysis (CCA) tests (ter Braak, 1994) were used to analyze for similarities and differences between the reference sites and between the distinct age class segments using SAS version 8.2 (SAS, 2002). For identifying which variables contribute the most to a given relationship, partial Canonical Correspondence Analysis using univariate multiple regression (PROC CANCORR) was used to determine the relative contributions of each indicator (Fortin and Dale, 2005; SAS, 2002).

Trend analysis was enhanced by incorporating moving average smoothing (MA model) as a data filter to reduce cyclical and seasonal variations found in the datasets for a number of the indicators affected by climate (Platt and Denman 1975; Kumar et al. 2001; Ittig, 2004). The trend analysis was followed by log10 data transformations where necessary.

CCA multivariate analysis functions by relating a primary matrix of plant species abundance data with a secondary matrix of environmental or soil data. PC-ORD, a PC-based program (McCune and Meffrod, 1999) containing an algorithm for Canonical Correspondence Analysis (CCA), was used to examine the overall spatial structure of the individual reference sites, the restoration site with the understory plant species along vectors (gradients) for soil chemical, net nitrogen mineralization, and soil microbial values found among the study sites (Heady and Lucas, 2004) (Palmer, 1993). Linear combinations of environmental variables are used to maximum the separation of plant species along four dimensional axes. Site scores are derived from the weighted averages of the associated species scores. The sites are located in the biplot where the center of the associated species cluster exists. Community structure is illustrated by the influence of different environmental variables on its ordination (ter Braak, 1994).

Plant species indicator analysis (IndVal) was used to measure the level of relationship between a given plant species to categorical units such as pine flat subtypes or age class. It calculates the indicator value d of species as the product of the relative frequency and relative average abundance in each categorical cluster. Indicator species analysis was used to attribute species to particular environmental conditions based on the abundance and occurrence of that species within the selected group. A species that was a "perfect indicator" was consistent to a particular group without fail. Indicator values range from 0 to 100 with 100 being a perfect indicator score. Because indicator species analysis is a statistical inference, a test of significance was applied to determine if species are significant indicators of the groups to which they are associated (Dufrene and Legendre, 1997). This was achieved by the Monte Carlo permutation test procedure (1000 runs) where the significance of a P-value was determined by the number of

random runs greater than or equal to the inferred value (α=0.1). Accuracy was defined from the binomial 95% confidence interval: p +/- accuracy (Strauss, 1982).

Growth predictions were determined from linear regression using the general linear model (PROC GLM) (Yang et al. 2006). The multiple regression model selection procedures R-squared, Backward Elimination, and Mallow Cp were used to determine the combination of indicators for prediction of each variable. The results from regression analysis were based on best model selection criteria of minimizing Mallow Cp and maximizing R2 and included only those indicators having a biological significance level of $p < 0.05$ (SAS, 2002).

Results

Ecological Classification

The Pt. Washington restoration site can be classified ecologically as a wet pine flatwoods subtype of the coastal pine flat based upon the results from Canonical Correspondence Analysis (CCA) ordination, indicator species analysis (IndVal), and pre-harvest stand data. Canonical Correspondence Analysis ordination indicated the majority of the plots measured at Pt. Washington fall in between the environmental patterns (moisture05, pH, SOM) (Table 5-1) for mesic flatwoods and wet flatwoods measured at the reference sites (Figure 5-1). Indicator species analysis produced results showing that gallberry was the indicator for both wet flatwoods and the Pt. Washington restoration site (Table 5-2). When the data were analyzed by age class, the ordination produced the same vectors of moisture05, soil pH, and soil organic matter content (SOM), but with stronger results.(Table 5-3). CCA ordination did not show any clear separation along age class (Figure 5-2). Indicator species analysis did produce results showing that the restoration site had similar plant species as the young age class of the reference sites (Table 5-4). Witch grass and blue stem grass were species indicators for the young age class, while witch grass and wiregrass were found to be the species indicators of the Pt. Washington restoration site

(Table 5-4). The means for each of the soil biogeochemical variables measured at the restoration site were found to be within the range of mean values measured at the reference sites, except for the significantly higher soil microbial biomass levels (C_{mb}) (Table 5-5). The seasonal trend for nitrogen mineralization fluxes over a 14 month period was for the rates to increase from winter through spring, to peak during the middle of August, and to decline through the fall and winter (Figure 5-3).

Pine Growth and Vegetation Control

A concurrent study produced results from five years of pine growth and four years of vegetation surveys showing imazapyr and sulfometuron-hexazinone herbicide treatments significantly reduced longleaf pine seedling survival after four growing seasons. Imazapyr, followed by hexazinone treatments produced significantly higher numbers of pine seedlings in the out-of-the-grass stage when compared to the other treatments. Imazapyr also produced significantly taller pines in the out-of-the-grass stage compared to the other trees. Imazapyr treatments also resulted in the best control of the overall cover (%) and stem counts of the major shrub species, while producing the highest level of herbaceous richness (Freeman, 2008). From the same four years of pine data (2002-2006) both imazapyr and hexazinone produced better pine growth when applied during the second growing season compared to the first (Table 3-6). They both had higher survival rates as indicated by higher stand densities. Imazapyr produced the best pine growth of all the treatments when applied during April instead of March. Hexazinone produced better pine growth when applied in March (Table 3-6; McCaskill data, 2006).

Treatment Effects-Biogeochemical Indicators

Imazapyr produced the highest monthly mean nitrogen mineralization rates while sulfometuron methyl treatments produced the lowest monthly rates. Most of the herbicides increased the nitrogen mineralization rates, but imazapyr was the only treatment to produce

statistically significant higher levels of net nitrogen mineralization when compared to the control (Figure 5-4). This difference was more pronounced for the ammonification data (Figure 5-5). The sulfometuron methyl mixed with hexazinone treatment produced a higher mean than imazapyr for the nitrification data (Figure 5-6). Only the sulfometuron methyl treatment produced significantly lower microbial biomass levels when compared to the control (Figure 5-7). Two years of herbicide applications resulted in a significant increase in the soil microbial biomass carbon when compared to a single year application (Figure 5-8). The mean microbial biomass carbon levels were higher at the Pt. Washington restoration site than any of the reference sites (Table 5-2; Figure 5-9). Sulfometuron methyl also produced the lowest levels of fungal biomass, although not significantly different (Figure 5-10). Fungal biomass carbon (C_{fb}) levels failed to detect significant differences among any of the treatments (Figure 5-10).

The predicted values for mean stand DBH, stand density, and stand basal area, were close to the actual values (Table 5-7). Predicted values involving stand height were different than the actual restoration site.

Discussion

The vegetative and soil biogeochemical variables collected from the reference sites were effective for ecologically classifying the restoration site at Pt. Washington. They were able to determine the pine subtype and the age class. The environmental gradients as evaluated by the soil biogeochemical indicators were stronger determinants of ecosystem conditions than was age (Figure 5-1; Figure 5-2). The power of the soil indicators can be realized by the results of the CCA ordination of the sites along the environmental axes and the plant species indicator analysis (Figure 5-1; Table 5-2; Table 5-4).

An analysis of all of the treatment effects indicated that Imazapyr produced the best improvements in pine seedling development and vegetative control while having the smallest

impact on the natural patterns of understory herbaceous richness. These gains are offset by Imazapyr producing one of the lowest pine seedling survival rates. The survival rate can be improved if the herbicide is applied at the beginning of the second growing season during April instead of March (Table 5-6).

Most herbicides are readily broken down by soil microbes causing an increase in their numbers and activity (Haney et al. 2002). Some researchers have found certain herbicides cause a reduction in microbial biomass accompanied by an increase in nitrogen mineralization rates (Busse et al. 2006). The decrease in microbial biomass was attributed to a corresponding decrease in organic matter inputs from the vegetative control, and not from direct microbial mortality. In any case, the general response following the application of herbicides has been an increase in the soil nitrogen mineralization rates (Li et al. 2003).

If Imazapyr, a leucine, and isoleucine protein inhibitor, was the only treatment to produce significantly higher net nitrogen mineralization rates when compared to the control, then some factor must have partially interfered with the affects of the other chemical treatments on nitrogen cycling. The factor of interference may be tied to Imazapyr being the only chemical amongst this group of herbicides to be currently registered for use in aquatic systems (Langeland et al. 2006). The Leon soil series found on this restoration site has a moderate soil leaching rating and a high soil runoff rating for pesticide selection (Obreza and Hurt, 2006). The concerns for hexazinone, an photosystem II quinone inhibitor, are mobility in soils and persistence in water. It was also found to inhibit ammonification and promote denitrification, dominant transformations during flooding events (Vienneau et al. 2004).

Sulfometuron methyl, an acetolactate synthase inhibitor, has been found to quickly move off-site when applied to sites in contact with wetlands (Michael et al. 2006). The chemical has

also been found to be toxic in low concentrations to many strains of pseudomonas, a major heterotrophic bacteria commonly found in forest soils (Boldt and Jacobsen, 1998). This mortality was attributed to the acetolactate synthase inhibition (ALS) property of sulfometuron methyl (Whitcomb, 1999). This finding might explain the reduction in microbial biomass found in our experiment from applying sulfometuron methyl.

The chemical properties of hexazinone and sulfometuron methyl limited the treatment effects on this coastal wet pine flat when flooding and the associated high water tables were present. Nitrification was impacted more than ammonification by excessive water from flooding. This condition might explain why the sulfometuron methyl-hexazinone treatment had a significant difference with the control in the nitrification data, but not the net nitrogen mineralization or ammonification data. The effect of soil moisture content on herbicides was observed when comparing the ammonification treatment data with the nitrification results (Figure 5-3; Figure 5-5; Figure 5-6). The results show microbial biomass measurements are able to detect differences between sites where herbicides have and have not been applied. These C_{mb} measurements were also sensitive to the number of herbicide applications used on a given site. Fungal biomass carbon did not detect any differences among the treatments. Previous studies have failed to detect any herbicide effects on fungal communities (Busse et al. 2004).

A primary reason for the fungal biomass measurements failing to detect statistically significant differences between the treatments may be attributed to the time since the treatments were applied. Most of the individual effects of herbicides on microbial biomass levels are greatly reduced beyond two years after application (Li et al. 2003). With the exception of the Pt. Washington nitrogen studies, the soils were collected and analyzed for fungal and microbial biomass 40 months after the second year treatments. The predictions were generally good except

for height and volume estimates (Table 5-6). Mean stand height values were skewed due to a group of the 400 m^2 forest structure plots measured within the young age class containing naturally regenerated stands. These naturally regenerated stands are dominated with larger saplings, poles and some small sawlog-size trees, causing the predicted values for height and volume in a 6-year old stand to be exaggerated.

Why was Imazapyr more effective than the other herbicide treatments in reducing vegetation competition without significantly impacting natural patterns of understory succession within this wet flatwoods site? The answer to this question goes back to achieving the central goal of this experiment. The Point Washington State Forest restoration site suffers from extensive seasonal flooding and drought, which adds to pine seedling mortality and complicates the selection of the proper herbicide for vegetation control. Imazapyr is a broader spectrum herbicide (less selective) and more effective at controlling perennial woody species. These properties are critical in mimicking fire effects. Secondly, Imazapyr is more persistent in wet sandy soils than the other herbicide treatments. This is also a critical factor in wet longleaf pine flat sites where the water table is constantly near the surface and the effects of herbicide treatments can be reduced by flooding.

Conclusions

The Pt. Washington restoration site contains elements of mesic flatwoods, wet flatwoods, and wet savannas. However, based upon CCA environmental ordination, plant species indicator analysis, and pre-harvest stand data it is a wet flatwoods site. These multivariate techniques were also useful in determining similarities between the Pt. Washington restoration site and the young age class data of the reference sites. Imazapyr was the best herbicide treatment for this site based on its ability to control shrubs and remain effective during flooding events. In general, herbicide use increased nitrogen mineralization rates, but imazapyr was the only treatment to produce

statistically significant higher levels of net nitrogen mineralization when compared to the control. Both imazapyr and the sulfometuron methyl-hexazinone treatments had a significant difference with the control in the nitrification data. The herbicide-treated restoration site had higher soil microbial biomass carbon levels than the reference sites. Two years of herbicide applications increased soil microbial biomass carbon over a single application. There was an indication that sulfometuron methyl treatments caused soil microbial mortality. Higher nitrogen mineralization rates at Pt. Washington were negatively correlated with both of the species diversity indices. The net nitrogen mineralization data proved effective at detecting differences between the herbicide treatments. Soil microbial biomass carbon was sensitive to the amount of herbicide applied. The predictions were generally good except for height and volume estimates. Mean stand height values were skewed due to a group of the 400 m^2 forest structure plots measured within the young age class containing naturally regenerated all-aged stands.

Table 5-1. Correlations and biplot scores for the biogeochemical variables by pine flat type.

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Moisture 05	-0.772	0.245	-0.194	***-0.351***	0.082	-0.058
Soil pH	0.358	-0.875	0.272	0.163	***-0.292***	0.081
SOM	-0.835	0.136	-0.477	***-0.380***	0.045	-0.142
NetNmin	-0.087	-0.297	-0.349	-0.039	-0.099	-0.104
MBc	-0.048	0.018	-0.026	-0.022	0.006	-0.008
FBc	-0.291	0.124	0.539	-0.132	0.042	0.160
FBc:MBc	0.445	0.144	-0.302	0.202	0.048	-0.090

*The Pearson correlations are "intraset correlations of ter Braak (1986).

Table 5-2. Plant Indicator Values (IndVal) (percent of perfect indication) with associated biogeochemical variable by pine flat type. P-values represent the proportion of randomized runs (1000) equal to or less than observed values (α=0.1).

Pine Subtype	Plant Species	Pine Subtype					
		Mesic	Wet Flatwoods	Wet Savanna	SD	P-Value	Veg Type
Mesic	*Smilax pumila*	25	1	5	4.69	0.038	Vine
	Hypericum hypericoides	17	1	0	3.08	0.024	Forb
	Gaylussacia frondosa	16	0	4	3.30	0.057	Shrub
	Pteridium aquilinum	12	0	1	3.00	0.066	Fern
Wet Flatwoods	*Lachnanthes caroliana*	0	52	4	3.57	0.001	Forb
	Arisitida beyrichiana	0	36	0	3.51	0.001	Grass
	Dichanthelium ovale	6	36	7	4.41	0.007	Grass
	Cyperus	1	11	1	2.67	0.088	Grass
Wet Savanna	*Ilex glabra*	19	13	38	3.55	0.009	Shrub
	Scleria	17	3	29	3.31	0.014	Grass
Pt. Washington		Blocks 1&2	Blocks 3&4	Blocks 5&6	SD	P-Value	Veg Type
Blocks 1 & 2	*Arisitida beyrichiana*	34	10	7	5.97	0.039	Grass
	Tragia urens	13	0	0	2.11	0.016	Forb
Blocks 3 & 4	*Smilax pumila*	0	25	0	5.58	0.001	Vine
	Pteridium aquilinum	2	18	0	3.42	0.013	Fern
Blocks 5 & 6	*Scleria*	0	3	25	6.16	0.078	Grass
	Lachnanthes caroliana	0	0	25	4.82	0.024	Forb

INDICATOR VALUES (% of perfect indication based on combining the values for relative abundance and relative frequency) n=48

Table 5-3. Correlations and biplot scores for the biogeochemical variables by forest age class.

CORRELATIONS AND BIPLOT SCORES (7 Biogeochemical Variables)						
	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Moisture 05	-0.772	0.245	-0.194	**-0.521**	0.142	-0.106
Soil pH	0.358	-0.875	0.272	0.242	**-0.505**	0.148
SOM	-0.835	0.136	-0.477	**-0.563**	0.079	-0.260
NetNmin	-0.087	-0.297	-0.349	-0.058	-0.171	-0.190
MBc	-0.048	0.018	-0.026	-0.032	0.011	-0.014
FBc	-0.291	0.124	0.539	-0.196	0.072	0.294
FBc:MBc	0.445	0.144	-0.302	0.300	0.083	-0.164

*The Pearson correlations are "intraset correlations of ter Braak (1986).

Table 5-4. Plant Indicator Values (IndVal) (percent of perfect indication) with associated biogeochemical variable by forest age class. P-values represent the proportion of randomized runs (1000) equal to or less than observed values (α=0.1). Species codes are found in Appendix A.

Species	AgeGroup	IndVal	p-value
Dich-Anvi	Regen	32.9	0.0010
Rhal	MidAged	27.8	0.0010
Ilgl	Mature	28.4	0.0160
Dich-Arbe	Pt Wash	36.4	0.0010

Table 5-5. The means for soil biogeochemical variables between reference site locations and the Pt. Washington restoration site.

Site	Time Interval (years)	Stand Age (years)	Soil Moisture 2005	Soil Moisture 2006	Net Nmin (mg^{-1}/kg^{-1} Soil / year)	C_{mb} (mg/kg/ soil)	SOM Content (%)	Soil pH [H+]	Plant Avail-P (mg^{-1}/kg-1 soil)	C_{fb} (mg/kg /soil)	FB to MB Ratio
St. Marks	Seedling	6	0.34	0.22	2	115	2.8	4.3	0.24	51	0.44
St. Marks	Mid-Aged	36	0.26	0.28	15	589	1.4	4.6	-0.24	75	0.13
St. Marks	Mature	110	0.25	0.11	5	49	1.5	4.6	0.31	87	1.66
Chassahow	Sapling	9	0.44	0.07	11	186	2.9	4.3	-0.09	105	0.57
Chassahow	Mid-Aged	45	0.23	0.08	8	145	1.1	4.7	-0.04	161	1.11
Chassahow	Mature	71	0.57	0.22	17	369	4.6	4.1	0.23	156	0.42
Topsail Hill	Sapling	19	0.45	0.10	20	524	2.9	4.3	-0.36	171	0.33
Topsail Hill	Mid-Aged	49	0.31	0.10	5	559	1.9	4.5	-0.50	179	0.32
Topsail Hill	Mature	101	0.32	0.09	7	490	2.0	4.2	-0.40	190	0.39
Pt Wash	**Seedling**	6	0.27	0.07	2	**1198**	1.4	4.6	-0.27	126	0.16

Table 5-6. Pt. Washington actual vs. predicted indicator values.

	Predicted Values	Pt. Washington Actual Values (2006)						
Age-6	Reference Sites	Control	Velpar 1st Year Only	Velpar 2nd Year Only	Velpar March Application	Arsenal 1st Year Only	Arsenal 2nd Year Only	Arsenal April Application
DBH (cm)	**3.73**	**2.87**	**3.03**	**3.14**	**3.31**	**3.26**	**3.23**	**3.62**
$R^2 = 0.81$ $p < 0.0001$								
Height (m)	**2.09**	**0.17**	**0.18**	**0.25**	**0.19**	**0.26**	**0.29**	**0.34**
$R^2 = 0.82$ $p < 0.0001$								
Density (trees/ha)	**265.57**	**259**	**268**	**298**	**302**	**224**	**244**	**218**
$R^2 = 0.1$ $p < 0.0083$								
BA (m^2/ha)	**0.09**	**0.17**	**0.19**	**0.23**	**0.26**	**0.19**	**0.20**	**0.22**
$R^2 = 0.56$ $p < 0.0001$								
Volume (m^3/ha)	**17.50**	**0.03**	**0.03**	**0.06**	**0.05**	**0.05**	**0.06**	**0.07**
$R^2 = 0.52$ $p < 0.0001$								

Predicted DBH = [(-0.00510*Age 2) + (0.82861* Age) - 1.06278], Predicted Height = [(-0.00288*Age2) + (0.45277*Age) - 0.52632], Predicted Density = [(-0.83301*Age) + 270.56934], Predicted Basal Area = [(-0.00190*Age2) + (0.3323*Age) - 1.95528], Predicted Volume = [(2.46264*Age) + 2.72759]

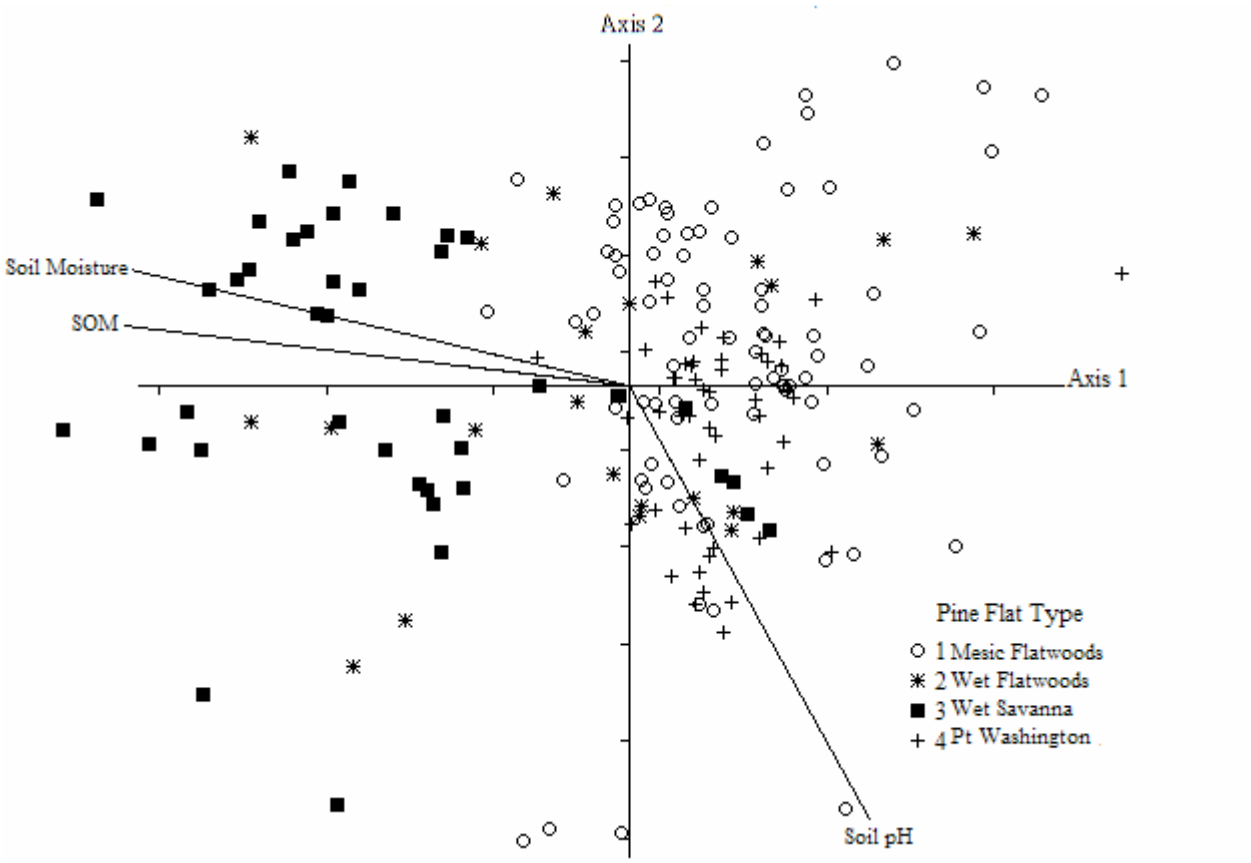


Figure 5-1. Pine flat type determined by a three-dimensional ordination biplot derived from Canonical Correspondence Analysis (CCA) of 192 plots using understory plant species abundance and soil biogeochemical data including the Pt. Washington restoration site.

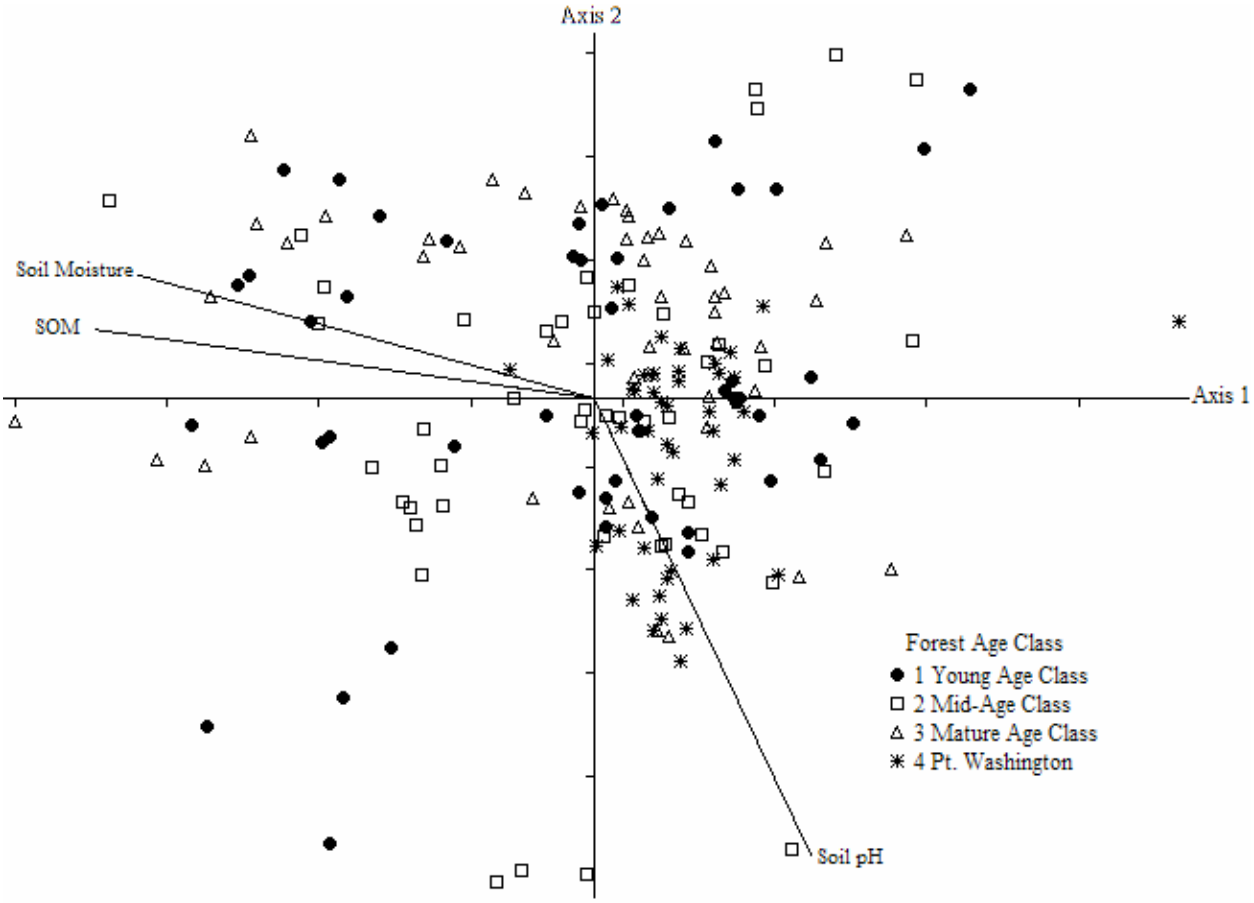


Figure 5-2. A three-dimensional ordination biplot derived from Canonical Correspondence Analysis (CCA) of 192 plots using understory plant species abundance and soil biogeochemical data collected within the young, mid-aged, mature age class, and the Pt. Washington restoration site.

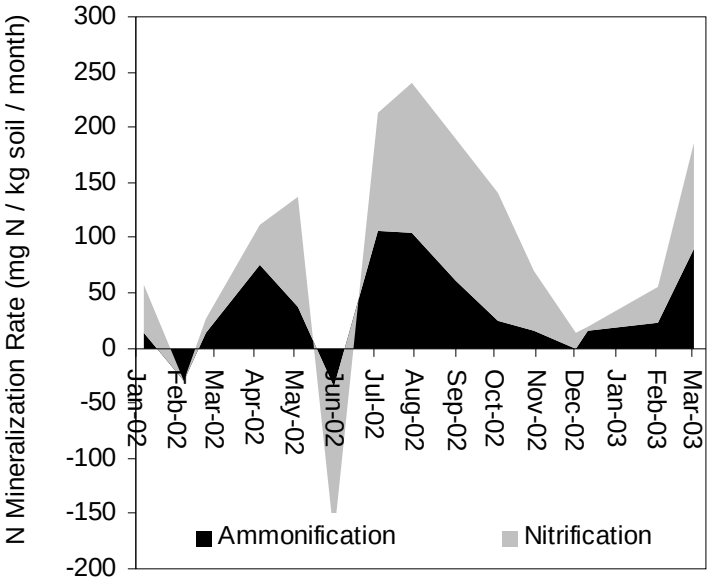


Figure 5-3. Monthly variation of total nitrogen mineralization, ammonification and nitrification rates (mg^{-1} kg^{-1} $month^{-1}$) obtained from field incubation of soils (untreated) during 14 months before and after the 2002 treatments.

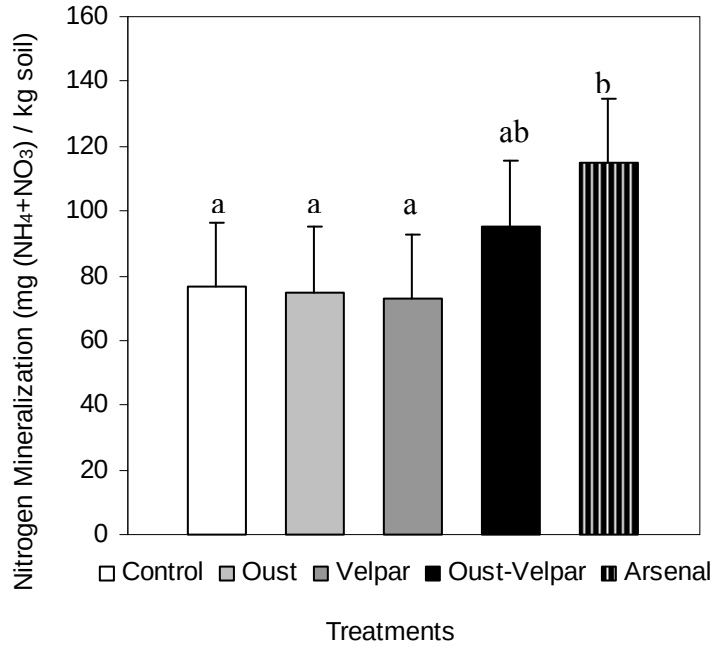


Figure 5-4. Net nitrogen mineralization means mg ($NH_4^+ + NO_3^-$) / kg^{-1} soil / month for the control, Oust: sulfometuron methyl, Velpar: hexazinone, sulfometuron methyl-hexazinone mix and Arsenal: imazapyr. Results are from soil samples collected during 14 months before and after the 2002 treatments.

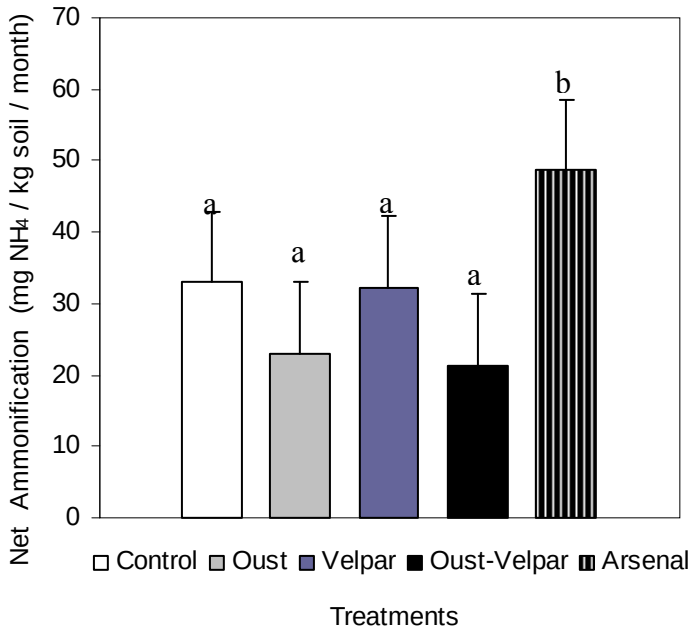


Figure 5-5. Net ammonification mean monthly rates (mg^{-1} NH_4^+ / kg^{-1} soil / month) for the control, Oust: sulfometuron methyl, Velpar: hexazinone, sulfometuron methyl-hexazinone mix and Arsenal: imazapyr. Results are from soil samples collected during 14 months before and after the 2002 treatments.

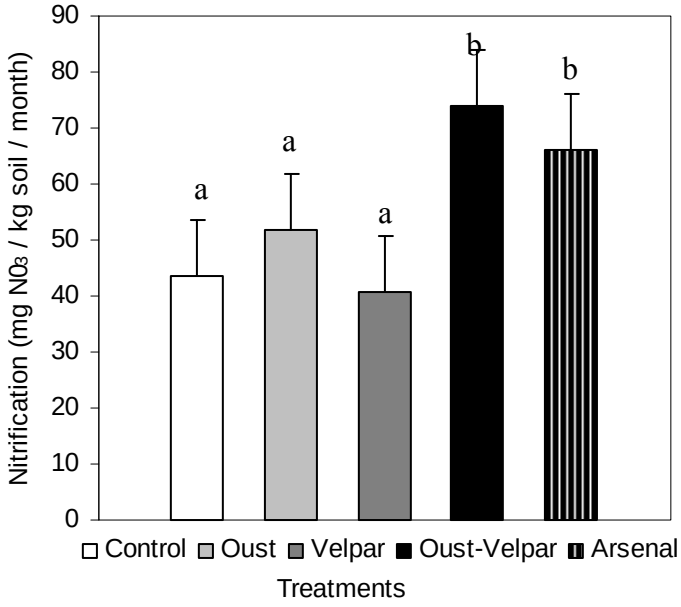


Figure 5-6. Net nitrification mg $^{-1}$ $N0_3^-$ / kg $^{-1}$ soil / month; for the control, Oust: sulfometuron methyl, Velpar: hexazinone, sulfometuron methyl- hexazinone mix, and Arsenal: imazapyr; applied in different growing seasons, frequencies, and time of year. Results are from soil samples collected during 14 months before and after the 2002 treatments.

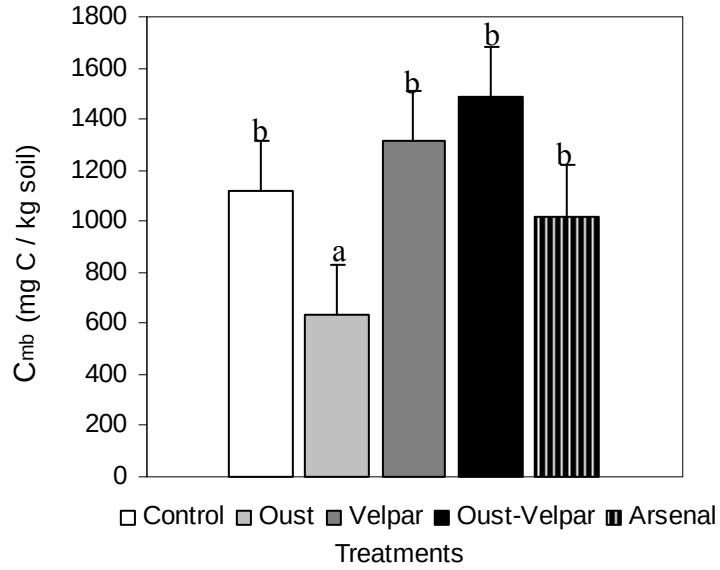


Figure 5-7. Microbial biomass carbon (C_{mb}) mg $^{-1}$ C / kg $^{-1}$ soil; for the control, Oust: sulfometuron methyl, Velpar: hexazinone, sulfometuron methyl- hexazinone mix, and Arsenal: imazapyr. Results are 40 months after second treatment (2006).

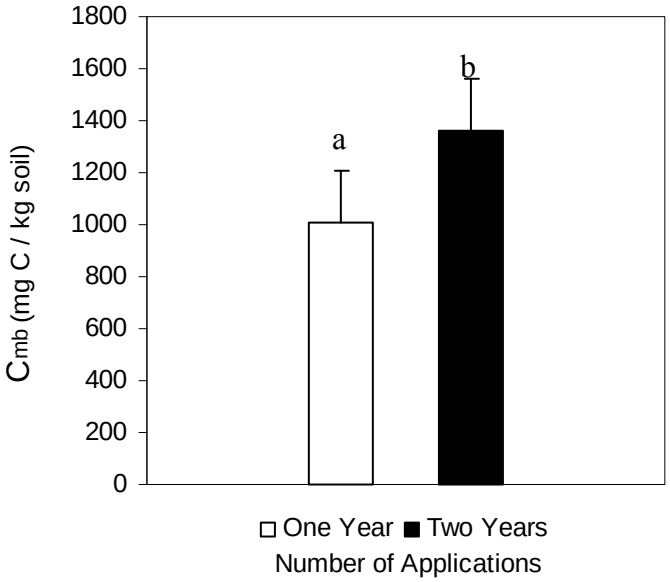


Figure 5-8. Effects of one year and two consecutive years of herbicide applications on microbial biomass carbon (C_{mb}) mg^{-1} carbon / kg^{-1} soil from soils. Results are forty months after second treatment (2006).

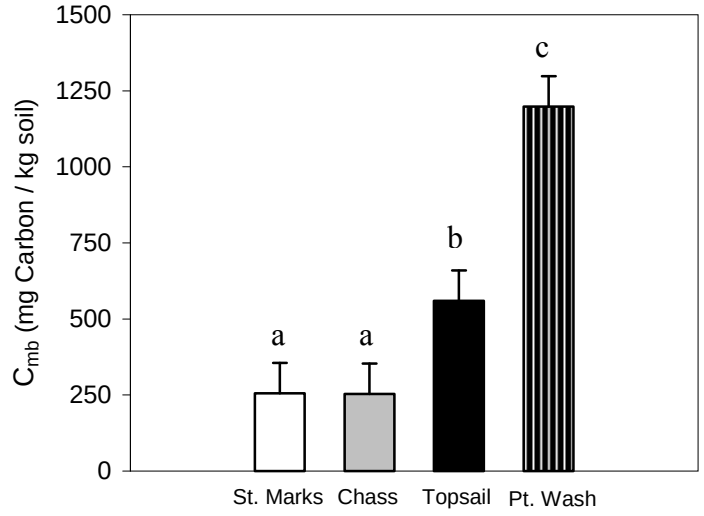


Figure 5-9. Soil levels of Microbial biomass carbon (C_{mb}) mg $^{-1}$ carbon / kg $^{-1}$ measured at the reference sites and the Pt. Washington restoration site. Results are forty months after second treatment (2006).

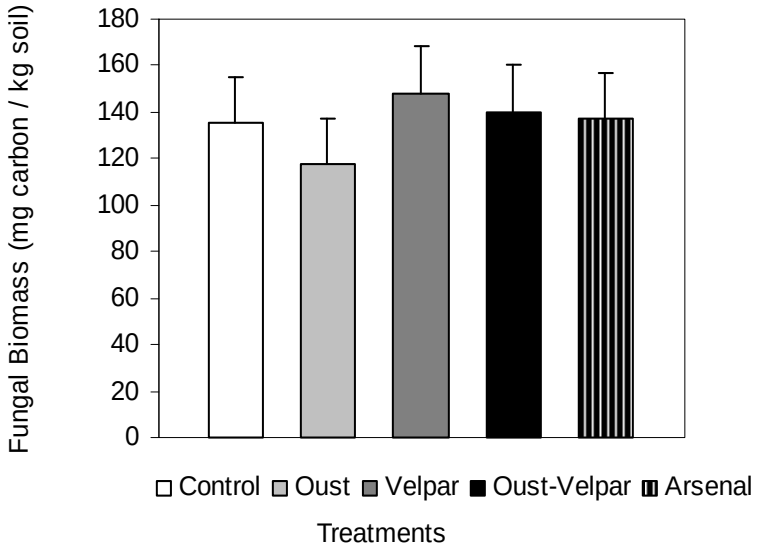


Figure 5-10. Fungal biomass carbon mg $^{-1}$ carbon / kg $^{-1}$ soil; for the control, Oust: sulfometuron methyl, Velpar: hexazinone, sulfometuron methyl- hexazinone mix, and Arsenal: imazapyr. Results are four years after second treatment (2007).

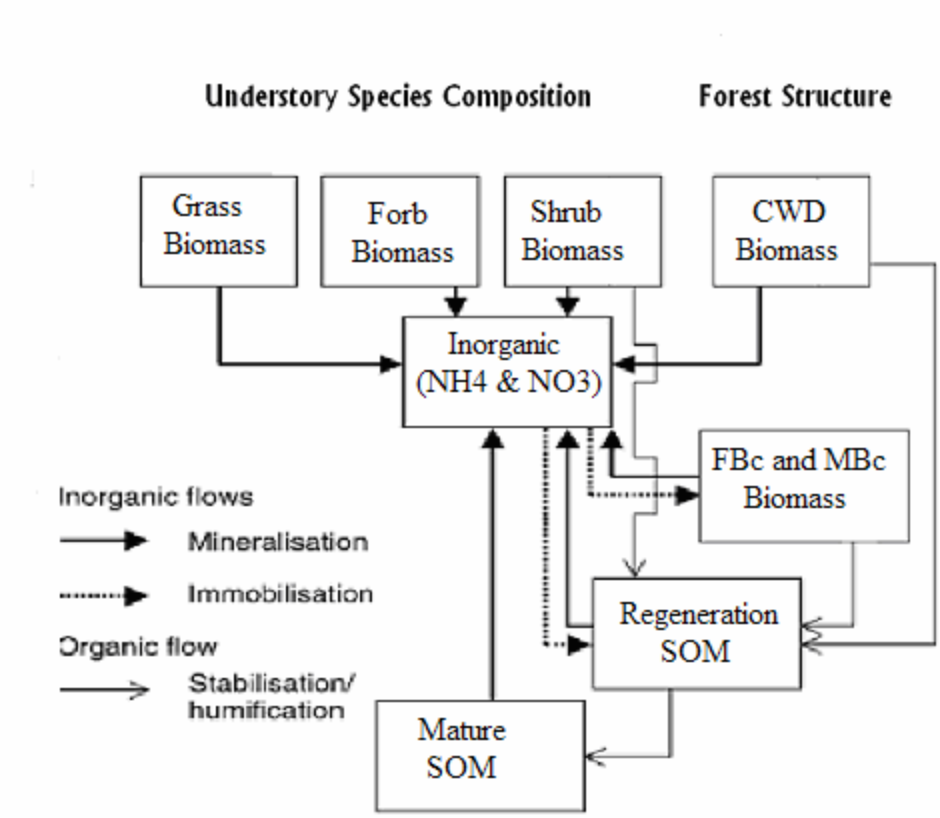


Figure 5-11. Pools and fluxes of nitrogen in the RESDYN restoration model. MP, metabolic pool; grass&forbs, holocellulose pool; shrubs, lignocellulosic pool; and CWD, woody pool. There are distinctive stabilization coefficients for microbial biomass, young soil organic matter (Y-SOM), and old soil organic matter (SOM) (adapted from Corbeels et al. 2005).

CHAPTER 6
SUMMARY AND CONCLUSIONS

Ecosystem restoration requires a good monitoring system that allows for the tracking of success by measuring key ecological indicators at the restoration site and comparing those results with reference communities. The measured ecological indicators must include monitoring changes belowground as well as in the aboveground vegetation for the coupling of functional and structural attributes. The overall objective of this study was to examine stand structure, understory species composition, and soil chemical and microbial properties along a chronosequence in longleaf pine wet flats along Florida's Gulf Coast in an attempt to develop an ecological trajectory for this community. Such an ecological trajectory would serve as the basis for developing a monitoring framework for restoration projects in the southern Gulf Coastal Plain.

We selected three reference sites within the Gulf Coast Flatwoods subecoregion to accomplish our objective. Within each reference site we sampled a total of 12 plots, 4 plots each in the early, mid and mature age classes. This experimental design resulted in 29 different age groups representing a chronosequence of 2 to 110-year-old stands.

The selected reference locations not only represented the highest quality sites that could be found in Florida, but were also located within the specific range for coastal wet longleaf pine flats found along Florida's Gulf coast. Monitoring this very specific biogeographical area (Gulf Coast Flatwoods subecoregion of Florida) created a spatial gradient pertinent to the restoration site that we wanted to evaluate. The time scale was limited to the oldest available longleaf pine stands (110-year old) distributed along the specified spatial range.

The major focus in Chapter 2 was to examine overstory stand structure data and understory plant species composition along the 110–year chronosequence. As expected, stand DBH, height,

and basal area increased with age, but reached a steady state plateau around 80-90 years. when they began to decline. Coarse woody debris accumulation levels were highly variable, but tended to increase with age. The decomposition levels of CWD were constant through the mid-aged class, but declined from the mid-age to the mature age class. The level of shrub species was significantly higher in the mature sites than found in either the young or the mid-aged classes.

Stand growth during early development translates to habitat heterogeneity as partial shading brings in new groups of plant species. At this point, stand height had a strong positive relationship with the Coleman rarefaction index and stand density has a strong negative relationship with the Shannon-Wiener diversity index. The plant species turnover rates as indicated by Coleman rarefaction values were high and the evenness of plant species as indicated by Shannon-Wiener was very low. The evenness of plant species was not attained until the mature stage when the number of plant species entering the ecosystem was equal to the number of plant species leaving it. At this point, Shannon-Wiener diversity values had a strong positive relationship with stand density and the Coleman rarefaction index had a negative relationship with stand height. The equilibrium between Coleman rarefaction and Shannon-Wiener diversity indices at this stage indicates a steady state in the overstory. Based upon the chronosequencial trends, Florida's Gulf Coastal longleaf pine flats reach the understory reinitiation stage at approximately 90 years. This would mean the forest is self-organizing, a threshold point for restoration.

In Chapter 3, Our main objective was to measure soil pH, moisture content, organic matter content (SOM), plant-available phosphorus, soil nitrogen mineralization rates (N_{min}), soil microbial biomass carbon (C_{mb}) and fungal biomass (C_{fb}) along the same 110-year chronosequence for determining the ecological trajectory in terms of soil chemical and microbial

characteristics of longleaf pine in coastal wet pine flat communities. We specifically tested our hypothesis that this group of soil biogeochemical indicators measured along the chronosequence would follow a pattern similar to the biomass accumulation curve for forest succession (Vitousek and Reiners, 1975). In response to rapid increase in growth during the early years of stand establishment, we predicted a similar increase in net nitrogen mineralization rates, microbial biomass and fungal biomass levels. We hypothesized that these variables would decrease at some point during the mid-aged stage and reach a threshold steady-state some time during the early mature stage when the understory reinitiation process of forest succession has begun.

Nitrogen cycling was dominated by ammonium production during the wet 2005 growing season when compared to a drier 2002. Nitrification represented 50% of the production during 2002 and less than 25% during 2005. There was ammonium enrichment by nitrate reduction. This probably indicates that the dissimilatory-nitrate reduction-to-ammonium (DNRA) pathway was prominent during the flooded 2004-2005 growing seasons. The net nitrogen mineralization rates, microbial biomass carbon, and fungal biomass carbon increased between the young and mid-aged classes, then decreased between the mid-aged and mature age classes. The FB-to-MB ratios increased dramatically up to 60 years, then decreased to 110 years. Finally, soil organic matter content (SOM), increased with soil moisture. Based upon the results, this group of soil indicators follows biomass accumulation patterns and will attain biogeochemical equilibrium after a stand age of approximately 60-70 years. The threshold would be during the mature age class after the understory reinitiation phase of forest succession has started.

The objective of Chapter 4 was to examine the relationships between key soil chemical and microbial properties and the overstory and understory characteristics of a wet longleaf pine flat community in the Gulf Coastal Plain of Florida. We hypothesized stand volume will show a

positive relationship with soil nitrogen mineralization, which, in turn, will be driven by the microbial community dynamics in the soil. We also hypothesized that the fungal biomass will increase as coarse woody debris accumulated on the forest floor and the standing stock increased over time**.**

The majority of the soil biogeochemical indicators influenced longleaf pine stand growth, and as stands developed, changes in aboveground vegetation influenced the soil biogeochemical indicators. Net nitrogen mineralization increased with stand volume until a threshold of 200 m^3 / ha (stand age = 90 years). Nitrate was found to be in higher concentrations in the young forest soils than the mature forest soils. Populations of nitrifying bacteria (AOB + NOB) were also found to be higher in the young forest soils. At Topsail Hill, ammonium levels were found to be higher in the wet young pine savanna soils than the mesic mature soil. Higher soil moisture translates to lower nitrification levels. The relationships between fungi and increases in stand height or coarse woody debris accumulation indicate a strong continual relationship between the soil biogeochemical indicators and longleaf pine stand development. The dynamics of this relationship might be better understood if the measured fungal biomass could have been identified as arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi, or saprophytic fungi along the chronosequence. The dominance of fungi negatively affected the Coleman Rarefaction and Shannon-Wiener diversity indices. This may indicate a decrease in species richness, but the functional redundancy component of ecosystem resilience is probably being strengthened. The strong relationships between forest biomass accumulation and soil biogeochemistry should always be studied in any monitoring event. Nitrogen cycling appears to become tighter in mature forests at a threshold of 90 years. This condition is dependent on mycorrhizal and saprophytic fungi dominating the soil microbial biomass.

The objectives of Chapter 5 were to use the indicator data to ecological classify the Pt. Washington restoration site as a mesic flatwoods, wet flatwoods or wet savanna. Secondly, to use the soil biogeochemical indicators for trying to detect differences among the four herbicide treatment effects applied on the restoration site. Finally, we will use both the vegetative and soil biogeochemical data to predict the development or ecological trajectory in wet longleaf pine flat restoration. The predicted values will be presented with pine growth results on the effects of herbicide treatments applied in the second year after planting compared to first year only, consecutive herbicide treatments (1^{st} & 2^{nd} Year), and whether an early or late spring application changes the effects (McCaskill data, 2006).

The Pt. Washington restoration site contains elements of mesic flatwoods, wet flatwoods, and wet savannas. However, based upon CCA environmental ordination, plant species indicator analysis, and pre-harvest stand data it is a wet flatwoods site. These multivariate techniques were also useful in determining similarities between the Pt. Washington restoration site and the young age class data of the reference sites. Imazapyr was the best herbicide treatment for this site based on its ability to control shrubs and remain effective during flooding events. In general, herbicide use increased nitrogen mineralization rates, but imazapyr was the only treatment to produce statistically significant higher levels of net nitrogen mineralization when compared to the control. Both imazapyr and the sulfometuron methyl-hexazinone treatments had a significant difference with the control in the nitrification data. The herbicide-treated restoration site had higher soil microbial biomass carbon levels than the reference sites. Two years of herbicide applications increased soil microbial biomass carbon over a single application. There was an indication that sulfometuron methyl treatments caused soil microbial mortality. Higher nitrogen mineralization rates at Pt. Washington were negatively correlated with both of the species

diversity indices. The net nitrogen mineralization data proved effective at detecting differences between the herbicide treatments. Soil microbial biomass carbon was sensitive to the amount of herbicide applied. The predictions were generally good except for height and volume estimates. Mean stand height values were skewed due to a group of the 400 m^2 forest structure plots measured within the young age class containing naturally regenerated all-aged stands.

Research Implications in Coastal Wet Longleaf Pine Flats Restoration

The monitoring study proved effective at evaluating our restoration site with a set of indicators that integrated the structural and functional attributes of the wet longleaf pine ecosystem. The aboveground vegetative variables and the soil biogeochemical measurements produced similar threshold periods. The selection process for the reference sites also proved fruitful based upon the sites having similar stand, soil properties, and common understory plant species among the locations. It was critical to restrict the location of the reference sites to within the 3 kilometers of the Gulf coast.

Our set of reference sites were selected to evaluate southern coastal pine communities that are directly affected by tropical storms. The restoration of Gulf coastal wet longleaf pine flats is distinct from other longleaf pine communities. Flooding caused by active hurricane seasons can leave these sites inundated for more than two years. This condition causes two major results in the biogeochemistry of these pinelands. First, extended flooding causes the nitrogen cycle to be dominated by ammonium production. When ammonium becomes scarce, nitrate is converted to ammonium through the DNRA pathway conserving nitrogen losses. Secondly, long term flooding results in the accumulation of soil organic matter, causing the pH of the soil medium to drop. This condition favors fungi and anaerobic bacteria over the aerobes. When the conditions become dry, there is a great flush of growth in both the overstory and understory vegetation. The effects of this flooding cycle are greater on younger forests than mature forests

where nitrate is in greater demand because of stand growth requirements. This demand was expressed by the nitrate levels and numbers of nitrifying bacteria being significantly higher in soils from the young stands compared to the mature stands. When prescribing fire in these sites, it is critical not to burn them during a flooding cycle before the flush of growth is completed. Based upon the conditions at our four sites, that can take 12-14 months after the drying process has started.

The understory vegetation was also distinct in these wet pine flats. There are higher densities of facultative wetland grasses and forbs and fewer hardwoods, especially the oaks. Very few oaks were measured on any of our sites other than the creepers (running oak). Some of these sites have not been burned in over 5 years. The implication here is the fire return-intervals can be extended well beyond 2-3 years if flooding conditions exist. The mesic mature sites had a higher composition of shrub species than the young mesic stands, even under fire return-intervals of 3 years. Soil moisture in the terms of extended flooding can enrich wet longleaf pine flat soils, conserve their nitrogen supply, and prevent invasion by shrub species. The flooding cycle can provide as many benefits to coastal wet pine ecosystems as fire does.

In summary, monitoring needs to include indicators that measure the functions as well as structural attributes of a given ecosystem. This proved to be extremely important in Gulf coastal pine communities where soil conditions are distinct from inland ecosystems. It was also important to restrict the sites to within the Gulf Coast Flatwoods subecoregion of Florida and to within 3 kilometers of the coast for insuring the same climatic effects that occurred at the restoration site occurred at each of the reference sites. One result of these stratifications was that all of the sites had 63 understory plant species in common. This may not have been attainable had the spatial scale been broader. This set of indicators and the time scale for the

chronosequence can be utilized at other environments where longleaf pine ecosystems are found. The chronosequence approach is strengthened by having as many replications as economically feasible at each of the differently aged sites. A difficult and important aspect to monitoring is selecting the spatial scale for the reference sites. If one is looking to monitor longleaf pine in mountain terrain found in the northern limit of its range, it would be more effective to restrict the reference sites to within that environment in order to capture the ecological differences found within the local climatic and soil conditions. A key direction for future research is to conduct investigations for improving our understanding of the biogeochemical dynamics that take place in facultative pine wetlands (i.e., wet pine-dominated mineral flats). This research would need to include molecular analysis for the identification species that change in soil microbial community between wet and dry conditions.

APPENDIX
SPECIES CODE LIST

Table A-1. Species list.

Scientific name	Code	Common name
Shrubs		
Asimina incana	Asin	Wooly paw paw
Cyrilla racemiflora	Cyra	Titi
Gaylussacia dumosa	Gadu	Drawf huckleberry
Gaylussacia frondosa	Gafr	Dangleberry
Ilex coriacea	Ilca	Large gallberry
Ilex glabra	Ilgl	Gallberry
Ilex vomitoria	Ilvo	Yaupon
Kalmia hirsuta	Kahi	Hairy wicky
Licania michauxii	Limi	Gopher apple
Lyonia lucida	Lylu	Fetterbush
Magnolia virginiana	Mavi	sweet bay
Myrica cerifera	Myce	Wax myrtle
Photinia pyrifolia	Phpy	Red choke berry
Quercus pumila	Qupu	Running oak
Serenoa repens	Sere	Saw palmetto
Stillangia sylvatica	Stsy	Queens delight
Vaccinium spp	*Vacc*	Blueberry spp
Grasses		
Andropogan virginicus	Anvi	Bluestem grasses
Aristida stricta var. *beyrichiana*	Arbe	Wiregrass
Calamovilfa curtissii	Cacu	Curtis sandgrass
Ctenium aromaticum	Ctar	Toothache grass
Cyperus	Cype	Sedge spp
Eragrostis spectabilis	Ersp	Purple lovegrass
Dichanthelium ovale	Dich	Eggleaf witch grass
Panicum - Dichanthelium	Pani	Panicum spp
Dichanthelium erectifolium	Paer	Erect leaf witchgrass
Panicum laxiflorum	Pala	Velvet Witchgrass
Scleria	Scle	Nutrush spp
Xyris caroliniana	Xyca	Yellow eyed grass
Forbs		
Asclepias viridula	Asvi	Southern milkweed
Aster adnatus	Asad	Scaleleaf aster
Aster eryngiifolius	Aser	Thistleleaf aster
Aster reticulatus	Asre	White top aster
Aster tortifolius	Asto	Dixie aster

Table A-1. Continued

Carphephorous pseudoliatris	Caps	Bristleleaf chaffhead
Carphephorus odoratissimus	Caod	Deer tongue
Chrysopsis	Chry	Silkgrass spp
Conyza canadensis	Coca	Canadian horseweed
Coreopsis linifolia	Coli	Texas tickseed
Desmodium rotundifolium	Dero	Tricklyfoil
Drosera capillaris	Drca	Pink sundew
Elephantopus tomentosus	Elto	Devils grandmother
Eupatorium capillifolium	Euca	Dog fennel
Eupatorium compositifolium	Euco	Yankee weed
Eupatorium mohrii	Eumo	Mohr's thoroughwort
Eupatorium pilosum	Eupi	Rough Boneset
Euthamia graminifolia	Eugr	Flat top goldenrod
Gelsemium sempervirens	Gese	Yellow jessamine
Gratiola hispida	Grhi	Rough Hedgehyssop
Hypericum hypericoides	Hyhy	St. Andrews cross
Hypoxis sessilis	Hyse	Glossyseed yellow stargrass
Hypoxis spp	Hypo	Stargrass spp
Lachnanthes caroliniana	Laca	Carolina redroot
Lechea	Lech	Pineweed spp
Lechea pulchella	Lepu	Leggett's pineweed
Liatris gracilis	Ligr	Slender gayfeather
Liatris tenuifolia	Lite	Shortleaf gayfeather
Mimosa quadrivalvis	Miqu	Sensitive brier
Oenothera fruticosa	Oefr	Evening primrose
Opuntia humifusa	Ophu	Prickly pear
Pityopsis graminifolia	Pigr	Silkgrass
Pterocaulon pycnostachyum	Ptpy	Blackroot
Rhexia alifanus	Rhal	Meadow beauty
Rhexia petiolata	Rhpe	Fringed meadow beauty
Sabatia brevifolia	Sabr	Shortleaf Rosegentian
Seymeria cassioides	Seca	Yaupon Blacksenna
Smilax laurifolia	Smla	Laurel green brier
Smilax pumila	Smpu	Green brier
Solidago odora	Sood	goldenrod
Stylisma patens	Stpa	Coastal plain dawn flower
Tragia urens	Trur	Wavyleaf noseburn
Verbena brasiliensis	Vebr	Brazilian vervain
Viola septemloba	Vise	Blue violet
Vitis rotundifolia	Viro	Muscadine

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BIOGRAPHICAL SKETCH

George McCaskill started his doctoral study at the School of Forest Resources and Conservation, University of Florida in January, 2003. Before joining the University of Florida, he was Associate Faculty teaching multiple courses at the College of the Redwoods in Eureka, California. Prior to that he worked as a Bilingual Forestry instructor at Mt. Hood Community College. For three years, he was a State Lands Timber Sales Forester for the Washington Department of Natural Resources. He spent 3.5 years in the U.S. Peace Corps serving as an Environmental Program Specialist evaluating Chilean forest practices as applied to their Monterey pine plantations and their native Nothofagus forests. While working with the Chilean Forestry Corporation, he served as interpreter/translator/editor during the Sixth Congress on Criteria and Indicators for the Conservation and Sustainable Management of Temperate and Boreal Forests. Also known as the Montréal Protocol, he helped to finalize the treaty where all the Pacific Rim countries signed the document. In 1990, Mr. McCaskill completed his Masters program at California Polytechnic in San Luis Obispo, California. He is a Registered Professional Forester in California.