PLANT-SOIL INTERACTIONS IN COGONGRASS (*Imperata cylindrica*)-IMPACTED SOUTHERN PINE ECOSYSTEMS

By

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To my family

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
ACU	Acaulosporaceae
AM	Arbuscular mycorrhizal
APCI	Atmospheric pressure chemical ionization
BA	Basal area
BLAST	Basic Local Alignment Search Tool
CDA	Canonical Discriminant Analysis
EcM	Ectomycorrhizal
EM	Ericoid mycorrhizal
GIG	Gigasporaceae
GIS	Geographic Information Systems
GLO	Glomeraceae
HPLC	High-performance liquid chromatography
IA	Invasive alien
M1	Mehlich-1 extractable
MS	Mass spectrometry
OM	Organic matter
OTU	Operational taxonomic unit
PAR	Paraglomeraceae
PCR	Polymerase Chain Reaction
PPM	Parts per million
SSU rRNA	Small subunit ribosomal RNA (18S)
TKN	Total Kjeldahl Nitrogen

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Major: Forest Resources and Conservation

The objective of this project was to assess how cogongrass (*Imperata cylindrica*) – an invasive alien grass considered one of the world's "worst weeds" – affects soil and ecosystem processes in southern pine ecosystems. In a greenhouse study (Chapter 2), I evaluated whether cogongrass impedes native pine savanna species through the release of allelopathic compounds. In a field study (Chapter 3), I assessed pre- and post-eradication nitrogen, phosphorus and arbuscular mycorrhizal fungal dynamics in pine sandhill stands severely impacted by cogongrass. In another field study (Chapter 4), I described the patterns (and potential drivers) of secondary succession following cogongrass eradication in these same stands.

There was an allelopathic effect of cogongrass, although it varied by species. A ruderal grass and an ericaceous shrub were unaffected by cogongrass soil leachate, while a mid-successional grass and pine were negatively affected. Chemical analyses revealed 12 putative allelopathic compounds, including a novel alkaloid, in cogongrass leachate. The concentrations of most of these compounds were significantly lower in the native leachate. Compared to a native reference treatment, cogongrass invasion had no

effect on soil chemical properties, although significant but temporary changes (increases in pH and available nitrate, decreases in available phosphorus) occurred post-eradication. While invasion resulted in the development of a novel arbuscular mycorrhizal (AM) fungal community, AM fungal community structure returned to a reference state within five years. Displaced native plant communities, however, were slower to recover following cogongrass eradication. Similar levels of plant species richness and diversity were observed by year seven, but composition remained markedly different from reference. Soil properties (*e.g.* organic matter, mycorrhizal spore counts, and pH) covaried with successional patterns.

These findings provide insight into the ecology of southern pine ecosystems impacted by cogongrass. Differences in leachate chemistry between cogongrass and native species may imply that the competitive ability of cogongrass is augmented by "novel weapons". From a restoration standpoint, the fact that soil properties return to a reference state relatively quickly following eradication is encouraging. The recovery of soil properties before native plant communities suggests that belowground processes and/or dispersal limitations may influence ecological succession following eradication.

CHAPTER 1
INTRODUCTION

Plant Invasions

"Invasive alien" (IA) plants, by one common definition, are those species that have overcome barriers to long distance dispersal and are able to persist, reproduce and spread in new areas (Richardson et al. 2000). While only a small subset of introduced plant taxa meet these criteria, these (relatively) few species greatly threaten the productivity and functioning of terrestrial ecosystems (Simberloff 2005). In the United States, some 5000 IA plant species have become established, with spread rates into forests, grasslands and other natural areas estimated at 700,000 hectares per year (Pimentel et al. 2005). In natural systems, alien plant invasions can cause dramatic shifts in plant community assembly, with their success often occurring at the expense of diverse assemblages of native species. For these reasons, it has frequently been reported that IA species (including plants) have become a leading cause of biodiversity loss, second only to habitat destruction (Simberloff 2005). Consequently, the desire among scientists to understand and predict these transformative effects has led to intense speculation on the underlying drivers and mechanisms of successful plant invasions.

What are the causes and effects of plant invasions, and why do some alien plants become invasive and others do not? These are the primary questions that have motivated IA plant research over the last two decades. Williamson and Fitter (1996), in an effort to develop a predictive framework for biotic invasions, proposed the "tens rule", which states that 1/10 of all introduced alien species escape, 1/10 of those that escape become established and 1/10 of those that become established become invasive. While

this is more of a generalization than an actual scientific rule, it illustrates that invasion is a multistep process, with many barriers that must be overcome in order for an alien plant to become invasive. Moreover, it implies that interactions between an alien plant and its environment are a major determinant of whether or not it establishes and becomes invasive. Unfortunately most studies of these interactions have focused on the primary producers (*i.e.* the plants themselves), typically viewing invasion as the end result of a plant-plant interaction in which an introduced alien species successfully outcompetes established natives to become invasive. Furthermore, most have been aboveground-centric, with few researchers attempting to elucidate the complex suite of interspecific interactions that take place in the rhizosphere (Wolfe and Klironomos 2005). As more studies are conducted, the role of belowground processes in invaded systems is becoming clearer, as are the changes to the soil community that occur following invasion, and the implications they have for ecological succession (Ehrenfeld and Scott 2001; Bais et al. 2003; Ehrenfeld 2003; Callaway and Ridenour 2004; Wolfe and Klironomos 2005).

Invasive Alien Plants and Soil Properties

A primary way that IA plants alter soil properties by differing from natives in the quantity and/or quality of biomass that they produce (Ehrenfeld et al. 2001; Ehrenfeld 2003). Since the carbon cycle is intrinsically linked to other element cycles, these changes can greatly impact the availability of soil nutrients – particularly macronutrients such as nitrogen and phosphorus which are frequently limiting (Vitousek et al. 1987; Ehrenfeld 2003). While invasive plants may or may not produce more litter than natives, most studies have found these inputs to be of higher quality (lower C:N, lignin:N and C:P ratios) This, in turn, may result in net mineralization, faster turnover rates, altered

nutrient pools and an increase in nutrient availability. The opposite trend, however, has also been observed (Ehrenfeld 2003 and citations therein). By differing from natives in terms of belowground architecture and nutrient uptake patterns, invasive species may also affect the distribution of mineral nutrients in the soil profile. An IA plant with a deep and/or highly prolific root system, for example, may act as a nutrient “mine”, effectively capturing nutrients in and depositing them at or near the soil surface as litterfall (Lambers et al. 2008; Perkins et al. 2011). The loss of a deeply rooted native species in favor of an invasive, however, would have the opposite effect.

The mechanisms behind altered nutrient cycling by invasives are not strictly limited to biomass production and litter quality. Invasives have also been shown to alter soil pH (Ehrenfeld 2003 and citations therein), which, aside from affecting the solubility of soil organic matter, has implications for nitrification, NH_4 volatilization and phosphorus complexation reactions (Brady and Weil 2002). There does not, however, appear to be a characteristic trend of pH alteration, as decreases as well as increases have been reported (Ehrenfeld 2003 and citations therein). While changes in litter quality may contribute to alterations in soil pH, differences in exudate chemistry (Bais et al. 2006), altered nitrification rates (Ehrenfeld et al. 2001) and differential uptake of nitrate vs. ammonium may also be factors (Ehrenfeld 2003).

Invasive plants can also alter the nutrient dynamics of an ecosystem indirectly through their effects on mycorrhizal communities (Pringle et al. 2009). Since many invasive plants form only weak associations with mycorrhizae, the density – and thus the efficacy – of these important mutualists may decline following invasion (Vogelsang and Bever 2009). This reduces the competitive ability of native species, and due to the

differences described above (morphology, tissue chemistry, *etc*.), leads to the alteration of nutrient cycling processes (Pringle et al. 2009). A similar pattern likely occurs when invasion results in a change in the functional group composition of a plant community (Pringle et al. 2009). The replacement of a woody species by a grass, for example, may result in a shift in mycorrhizal community structure to favor arbuscular mycorrhizae over ectomycorrhizae (Vosatka et al. 1991). Differences in root architecture between natives and invasives, coupled with differences in nutrient uptake efficiency between different types of mycorrhizae (Jones et al. 1998), may in turn affect the nutrient dynamics of an ecosystem.

The effects of altered nutrient cycling regimes are often exacerbated by the fact that invasive plant species often establish dense monocultures. This is an interesting phenomenon, considering that the same species in their native habitat typically coexist with other species (Callaway and Aschehoug 2000). This suggests that certain invasives are not only superior competitors, but are also capable of using additional mechanisms (*i.e.* "novel weapons") that exploit the lack of co-evolved tolerances among natives (Hierro and Callaway 2003). Allelopathy, the inhibition of one plant by another by the release of phytotoxic compounds (*i.e.* allelochemicals), has been suggested as such a mechanism (Hierro and Callaway 2003; Callaway and Ridenour 2004). Allelochemicals include a diverse array of secondary metabolites and can be released in various forms, including root exudates, and litter, bark and seed leachates. Some of these chemicals rapidly volatize or degrade, while others may persist in the soil (Reigosa et al. 1999). Alone or in combination, these substances can inhibit seed germination and root elongation (Hierro and Callaway 2003) and in some cases lead to

the partial or complete death of the root systems of susceptible plants (Bais et al. 2003). Many allelochemicals also have microbicidal properties, which suggests that they might impede the formation and/or efficacy of important symbioses and associations, such as those involving symbiotic nitrogen-fixing bacteria and mycorrhizal fungi (Wardle et al. 1998) and possibly mycorrhizal “helper bacteria” (Frey-Klett et al. 2007). This could be an additional disadvantage for native species, especially when phosphorus – which tends to be poorly mobile in soils – is the limiting resource (Smith and Read 1997).

The Legacy of Invasion

The body of knowledge on the effects of invasive species on belowground processes, while limited, has increased greatly in recent years. Comparatively less attention, however, has been paid to the legacies that invasives leave behind once they have been eradicated. Indeed only a handful of studies have incorporated the eradication of an IA species and subsequent monitoring of nutrient cycling processes (Maron and Jeffries 2001; Yelenik et al. 2004). This, however, is an area that deserves more consideration, as the restoration of native plant communities following the eradication of invasives is a high priority among land managers (Miller et al. 2010). Much like any other disturbance, if soil processes and properties are altered by an invader, these effects will likely persist for some time after the invader is eradicated (Corbin and D’Antonio 2004; Jordan et al. 2008). Differences in residue quality, and subsequent alteration of immobilization/mineralization processes, may further alter soil biogeochemistry following the eradication of the invasive. These changes, in turn, may have implications for the invasibility of the new community as well as its suitability for revegetation with native plant species.

A Case for Cogongrass

Cogongrass (*Imperata cylindrica* (L.) P. Beauv.) is a rapidly growing C_4 perennial grass that readily invades natural ecosystems and disturbed sites. With invasions reported on six continents, it is increasingly recognized as one of the world's most problematic invasive plant species. In total, some 500 million hectares worldwide have some degree of cogongrass infestation (MacDonald 2004) In the US, several hundred thousand hectares are infested (MacDonald 2004), with its current range overlapping much of the historic range of longleaf (*Pinus palustris* Mill.) and slash pine (*Pinus elliottii* Engelm) (Figure 1-1). The sparse canopy that is characteristic of these forests, in concert with frequent fire, allows for high levels of understory diversity, but also makes them very susceptible to transformative impacts from cogongrass (Holzmueller and Jose 2011). Since cogongrass is becoming a significant problem in forest systems of the Southeast, its ecology and management have been the subjects of considerable research interest among forest ecologists in recent years.

Perhaps the most dramatic characteristic of cogongrass invasion is the density of the resultant monoculture and the amount of biomass produced. This creates significant pressure not only for space, but also for soil resources. According to Ramsey et al. (2003), cogongrass produces over three times more foliar biomass and up to ten times more root/rhizome biomass than native vegetation growing on the same site. Fresh weights of up to 10 metric tons/ha for shoots and up to 40 metric tons/ha for rhizomes have been reported in some sites (MacDonald 2004 and citations therein). Tissue quality is also an important consideration. Daneshgar and Jose (2009a), for example, found cogongrass to be very effective at competing for soil nitrogen, but since it produced so much biomass, its tissue nitrogen concentrations were considerably lower

than those of native vegetation. Secondary organic compounds (Koger and Bryson 2004) and silica crystals (MacDonald 2004) in cogongrass tissue may also reduce its palatability to herbivores and soil microbes. Combined, these factors suggest that cogongrass invasion results in the production of recalcitrant nutrient pools, likely leading to nutrient immobilization, decreased nutrient availability and reduced nutrient pool turnover rates.

Cogongrass has been observed to alter soil chemistry in forest ecosystems. Collins and Jose (2008), for example, observed seasonal reductions in extractable NO_3-N and K, increases in Mg and decreases in pH in cogongrass-invaded pine sites, compared to non-invaded sites in the same forests. No significant differences in organic matter, P, or Ca, however, were observed between invaded and uninvaded sites. Despite these observations, however, our understanding of the effects of cogongrass invasion on soil chemistry is far from complete. The effects of invasion on the overall nitrogen cycling in a system (not simply NO_3-N), for example, deserve consideration in acidic forest soils where nitrification may be inhibited (Chapin et al. 2002). No studies have evaluated whether or not nutrient dynamics in cogongrass- invaded forest ecosystems return to pre-invasion conditions following eradication.

Cogongrass is known to form associations with AM fungi (Brook 1989), which undoubtedly contributes to its superior competitive ability in nutrient-poor soils. The formation of a cogongrass monoculture, therefore, likely results in a decrease in non-AM fungal propagule density in the soil (*i.e.* ericoid and ectomycorrhizal fungi) (Korb et al. 2003). This in turn may magnify the selective pressure against obligate non-AM plant species. Chemical eradication of cogongrass, which typically involves the use of one or

more systemic herbicides (MacDonald 2004), may depress AM fungal density as well, effectively killing the symbiont by eliminating its host. Since most plants growing in low pH, phosphorus-fixing forest soils are highly dependent on mycorrhizal symbioses (Smith and Read 1997), the recovery of the mycorrhizal community likely plays a key role in the reestablishment of the desired plant species following cogongrass eradication.

Several authors have suggested that the competitive ability of cogongrass is augmented by the production of allelopathic compounds. Putative allelochemicals (mostly phenolics) have been extracted from cogongrass tissues and from soils in the vicinity of cogongrass patches (Abdul-Wahab and Al-Naib 1972; Hussain and Abidi 1991; Inderjit and Dakshini 1991, Xuan et al. 2009) and some of these compounds have been shown to have inhibitory effects on test plants (Koger and Bryson 2004, Xuan et al. 2009). The current body of research on cogongrass allelopathy in natural systems, however, should be considered inconclusive, as single compound bioassays on weed and crop species may not be an accurate representation of the complex interaction between live plants that occurs in nature (Mallik 2000). No studies to date have assessed the effects of cogongrass allelopathy on the performance of native understory species like those that it readily displaces.

Objectives and Hypotheses

I conducted these studies to elucidate the role of belowground processes in southern pine ecosystems impacted by cogongrass and to describe the patterns of secondary succession following cogongrass eradication. The specific objectives were to:

- Assess whether or not allelopathic compounds are present in biologically significant concentrations in the cogongrass rhizosphere and to determine the effects of these compounds on a suite of species native to southeastern pine savannas.
- Analyze how invasion by cogongrass affects soil N and P dynamics and arbuscular mycorrhizal fungal communities in fire-maintained longleaf pine sandhill stands.
- Quantify soil N and P dynamics and assess changes in arbuscular mycorrhizal community assembly in the years following cogongrass eradication.
- Describe the patterns of secondary succession following the eradication of cogongrass in a longleaf pine sandhill ecosystem.

I hypothesized that rhizosphere water collected from cogongrass-invaded soils would adversely affect the growth, root morphology and mycorrhizal colonization of native species. Additionally, I expected that compounds present in the cogongrass rhizosphere would not be present in the rhizospheres of native plants, or they would be present at much lower concentrations. Cogongrass invasion was expected to decrease the availability of soil N and P, likely through reductions in pH and/or changes to the soil carbon cycle (Brady and Weil 2002). I expected these changes in N and P cycling to persist following eradication, perhaps due to the slow decomposition rates of low-quality cogongrass foliage and rhizomes after herbicide treatment (Ehrenfeld 2003). I expected that cogongrass invasion would result in the development of a novel AM fungal community, and additional modifications to AM fungal community structure would arise following eradication. I hypothesized that formerly invaded sites would, by year seven, begin to regain many of the characteristics of native reference sites. Specifically, I expected to see increases in total plant cover, increases in species richness and diversity, decreases in dominance and increases in the relative cover of desirable native species such as wiregrass (*Aristida stricta* Michx. var. *beyrichiana* Ward). Shifts in

community assembly, I hypothesized, would be associated with changes in soil resource availability and alterations to arbuscular mycorrhizal fungal community structure. I expected that the elimination of cogongrass and other competing vegetation would facilitate the establishment of longleaf pine seedlings, but would also lead to a secondary invasion of alien plant species, particularly fast growing ruderals that are readily able to take advantage to a post-eradication resource flux.

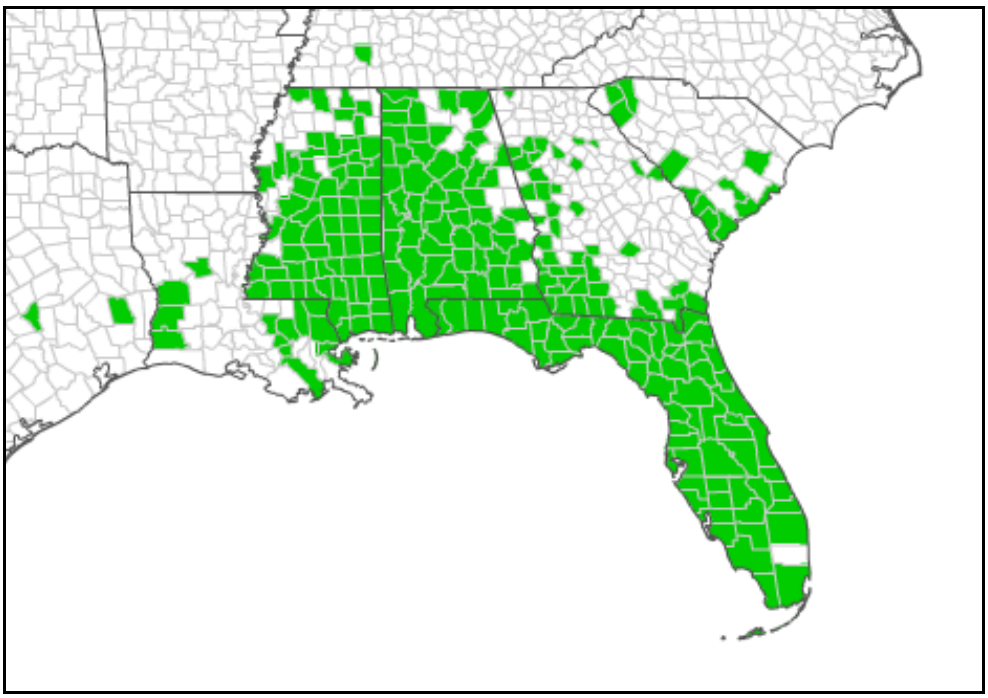

Figure 1-1. Map of the current distribution of cogongrass in the southeastern US. Adapted from EDDmapS (2012).

CHAPTER 2
NOVEL RHIZOSPHERE CHEMISTRY OF COGONGRASS: IMPLICATIONS FOR THE PERFORMANCE OF NATIVE PINE SAVANNA SPECIES IN THE SOUTHEASTERN US

Background

Numerous theories and hypotheses have been proposed to explain the success of IA plant species in their new environments, the majority of which are based on resource competition and assume that the species that "wins" is the one with the superior competitive ability (Bakker and Wilson 2001). Indeed, there is ample evidence to suggest that resource-based mechanisms such as competition play a major role in successful plant invasions. Many IA plant species, for example, grow fast and are highly efficient in the uptake, use and allocation of limiting resources (Daehler 2003) – qualities which undoubtedly help explain how they are able to cause such dramatic alterations to the community assembly of the sites they invade. The inherent competitive ability of these species is also likely augmented by the release from co-evolved specialist enemies (Maron and Vilà 2001) and in some cases may be an evolutionary response in which alien species, over time, allocate less photosynthate to defense and more to growth and reproduction (Blossey and Notzold 1995; Hänfling and Kollmann 2002).

While competition for resources likely plays a major role in most alien plant invasions, resource-based mechanisms alone may not adequately explain the success of some IA plant species (Hierro and Callaway 2003). The propensity of certain plant species to form dense monotypic stands, for example, suggests that additional interactions may also be involved (Hierro and Callaway 2003). Allelopathy, the inhibition of one plant by another via the release of phytoinhibitory chemical compounds (*i.e.* allelochemicals), is one such mechanism (Hierro and Callaway 2003; Callaway and

Ridenour 2004). Allelochemicals include a diverse array of secondary metabolites and can be released in various forms, although root exudates and litter probably constitute the primary sources (Wardle et al. 1998). Some of these chemicals rapidly volatize or degrade, while others may persist in the soil (Reigosa et al. 1999). The breakdown products of exuded allelochemicals often retain some bioactivity (Blum 1998; Blum et. al. 2000). Alone or in combination, these substances can inhibit seed germination and root elongation (Hierro and Callaway 2003) and in some cases lead to the partial or complete death of the root systems of susceptible plants (Bais et al. 2003). Many allelochemicals also have microbicidal properties, which suggests that they might impede the formation and/or efficacy of important symbioses and associations, such as those involving symbiotic nitrogen-fixing bacteria and mycorrhizal fungi (Wardle et al. 1998) and possibly "helper bacteria" that facilitate the establishment and functioning of the mycorrhizal symbiosis (Frey-Klett et al. 2007). The naivety of native communities to the novel allelochemicals produced by IA plants may make them particularly susceptible to transformative impacts (see "Novel Weapons Hypothesis", Callaway and Ridenour 2004).

While there is some compelling anecdotal evidence for the role of allelopathy in invaded natural systems, our ability to empirically assess the role of allelopathy in plant-plant interactions has been hindered by some major methodological limitations (Mallik 2000; Hierro and Callaway 2003). Conclusions about allelopathy drawn exclusively from Petri dish bioassays, in which seedlings or seeds are watered with a leachate extracted artificially from dead plant tissues (Richardson and Williamson 1988; Hierro and Callaway 2003; Gómez-Aparicio and Canham 2008), or pot studies in which plant

residues are incorporated into the soil medium (Singh et al. 2005; Norsworthy 2003), should be interpreted with some skepticism, as the chemical composition of these materials may be qualitatively or quantitatively different from the allelochemicals exuded by live plants and their litter. Additionally, biologically significant concentrations of allelopathic compounds have rarely been isolated from the rhizosphere soil of IA plants. Due to limitations such as these, evidence of allelopathic interference in most cases cannot be considered conclusive.

With invasions reported on six continents, cogongrass (*Imperata cylindrica* (L.) P. Beauv.) is increasingly recognized as one of the world's most problematic IA plants. In total, some 500 million hectares worldwide have some degree of cogongrass infestation (MacDonald 2004), with dense monotypic stands widely reported in tropical and subtropical forests, savannas, grasslands, pastures and agricultural fields (MacDonald 2004). In the southeastern US, cogongrass has been observed to dramatically alter the species and functional composition of native pine (*Pinus* spp.) ecosystems by displacing native groundcover species (Jose et al, 2002; Collins et al. 2007) and inhibiting the performance of sapling trees (Daneshgar and Jose 2009a; Holzmueller and Jose 2011). The tremendous success of cogongrass in its expanded range has been attributed, in part, to a suspected allelopathic ability (Koger and Bryson 2004; MacDonald 2004) and several putative allelopathic compounds have been isolated from cogongrass tissues and from soils in the vicinity of cogongrass patches (Abdul-Wahab and Al-Naib 1972; Hussain and Abidi 1991; Inderjit and Dakshini 1991, Xuan et al. 2009). Some of these compounds have been shown to have inhibitory effects on agricultural species (including weeds) (Koger and Bryson 2004) and other IA plants (Xuan et al. 2009). To

date, however, no studies have assessed the effects of cogongrass allelopathy on native wildland plant species. The reliance on phytotoxic compounds artificially extracted from cogongrass tissues, rather than exudates and their breakdown products, is also a limitation of previous research.

I conducted this study to assess whether or not allelopathic compounds are present in biologically significant concentrations in the cogongrass rhizosphere and to determine the effects of these compounds on a suite of plant species native to southeastern pine savannas. I hypothesized that rhizosphere water collected from cogongrass invaded soils would adversely affect the growth, root morphology and mycorrhizal colonization of native species. Additionally, I hypothesized that compounds present in the cogongrass rhizosphere would not be present in the rhizospheres of native plants, or they would be present at much lower concentrations.

Materials and Methods

Greenhouse Study

For this study, I employed a greenhouse protocol, in which seedlings of four native species were irrigated with rhizosphere water (hereafter referred to as “leachate”) collected from pot-grown monocultures of cogongrass or from polycultures of native species. The latter treatment, while not a true control, was treated as such since it consisted of conspecifics, congenerics and functionally similar species that naturally co-occur and compete with native test species in pine savannas in the southeastern US. A DI water control was used in the second season to verify that any treatment effects were due to a negative influence of cogongrass, rather than a facilitative effect from the native species. The four test species (Table 2-1) included an arbuscular mycorrhizal (AM) ruderal grass (*Andropogon arctatus* Chapm.), an AM mid-successional grass

(*Aristida stricta* Michx. var*. beyrichiana* (Trin. and Rupr.) D.B.Ward), an ericoid mycorrhizal (EM) shrub (*Lyonia ferruginea* (Walter) Nutt) and an ectomycorrhizal (EcM) tree (*Pinus elliottii* Engelm.). Predominant species in the native polycultures, in order of decreasing cover, were *A. stricta*, *Andropogon virginicus* L., *Vaccinium myrsinites* Lam., *Gaylussacia frondosa* (L.) Torr. and A. Gray, *Gaylussacia dumosa* (Andrews) Torr. and A. Gray, *Pinus elliottii* and *Smilax* spp. Three 11.4 liter pots for each of the two leachate treatments were established in March 2009 and 2010 with vegetative plugs and rhizomes obtained from local sources.

Native seedlings were planted from surface sterilized pre-germinated seeds in 200 mL Ray Leach tubes (Stuewe and Sons, Tangent Oregon). Seedlings were planted in two cohorts, with *A. arctatus* and *P. elliottii* established in June of each year, and *A. stricta* and *L. ferruginea* established in August. For both cohorts, each of three plots contained 20 tubes (five for each species x leachate treatment). Due to events beyond my control I was unable to successfully produce *L. ferruginea* seedlings in 2009. All plants, both in the leachate pots and in the seedling tubes, were grown in a Sparr fine sand (loamy, siliceous, subactive, hyperthermic Grossarenic Paleudult), one of the predominant soil series in north central Florida (United States Department of Agriculture 1985). The soil collection site was heavily vegetated with native AM, EM and EcM plant species and thus the planting medium was assumed to contain a diversity of compatible mycorrhizal inoculum. Soils were thoroughly homogenized prior to filling pots and tubes. For each species, there were three replications (blocks), each containing five seedling tubes from each of the two leachate treatments. In the second year I included an additional five seedling tubes to account for the DI water control. Tubes were arranged

in strips, randomly assigned by treatment, to prevent cross-contamination while watering.

Twice weekly, one leachate pot from each treatment was watered with 1.3.L of distilled water, which allowed me to collect approximately 1 L of raw leachate from the bottom of each pot. The other four pots were watered to field capacity (approximately 300 mL). Pots were rotated so that, under this procedure, leachate was collected from each at approximately 10 day intervals. Fresh leachates were filtered twice through Whatman #5 filter to remove debris, fungal spores and sporocarps. The first collection and application of leachate was timed to correspond with the planting of the first seedling cohort (early June). Each seedling tube received approximately 15 mL of filtered leachate from either the native or the cogongrass treatment (or DI water control). Seedlings were harvested after 8 weeks.

Upon harvest, plants were separated into their above- and belowground components. Dry weights for shoots were obtained after drying them for 48 hours at 70°C. Since fresh roots were needed for mycorrhization and root length analyses (see below), root dry weights were calculated by multiplying fresh weight by a weight conversion factor, which was determined by drying three seedlings not included in the analyses for each species x treatment combination. Root lengths were determined using the modified line intercept method described by Tennant (1975). Roots were prepped for mycorrhizal analysis using standard clearing and staining procedures (Manoharachary and Kunwar 2002) and analyzed for mycorrhizal colonization. For AM and EM species (grasses and *L. ferruginea*) root segments were assessed on each sample for the presence or absence fungal colonization as well as the degree of

colonization (*i.e.* light: 0 – 33%, moderate: 33 – 67% and heavy: 67 – 100%). Percent mycorrhizal colonization was quantified for each sample as the mean of 10 microscope fields (10X magnification) with each field assigned either 0 or the midpoint of each colonization class (*i.e.* 16%, 50% or 84%). For EcM *P. elliottii*, the line intercept method (Tenant 1975) was used to determine percent mycorrhizal colonization, using a dissecting microscope.

Isolation and Characterization of Putative Allelochemicals

After observing evidence of bioactivity in raw cogongrass leachate, steps were taken to identify active compounds and determine their concentrations. Polar fractions were separated from non-polar fractions using a chloroform extraction method and a standard lettuce seed bioassay was used to determine the bioactivity of each fraction (4 replicates of 10 per treatment, plus control) (Table 2-2). Active (polar) fractions in cogongrass leachate, along with polar fractions of native leachate, were then concentrated in an N_2 vortex evaporator. Samples were analyzed by injecting 25 µL of the concentrated extract into a Shimadzu SCL-10Avp high performance liquid chromatography system (HPLC) (Columbia, MD), compounds were separated using a silica based Columbus C8 column (4.6 mm x 250 mm, 5 µm; Phenomenex, Torrance, CA) and eluted with a two-part mobile phase gradient at a flow rate of 1 $mL \bullet min^{-1}$. Mobile phase A consisted of 0.1% H_3PO_4 buffer (pH =2.1) and mobile phase B was 100% ACN. The gradient started at 10% A, ramped linearly to 40% A at 30 min, 75% A at 40 min, 10 % A at 45 min, and was held at 10% for 14 min. The chemical profiles and concentrations of each analyte were determined by comparing the retention times of a reference library of 45 chemical standards (10 ppm) with known or suspected allelopathic properties. The concentrations of identified compounds were further

confirmed by a Thermo-Finnigan TSQ7000 triple quadrupole mass spectrometer (HPLC/MS/MS, Thermo Electron Corp., Waltham, MA) using electrospray (+ and – ionization modes) or HPLC-MS atmospheric pressure chemical ionization (APCI). For an unknown compound that appeared to be present in the cogongrass leachate at high concentrations, retention time and ion fragmentation patterns, coupled with library matching, were used to generate a tentative structure.

Statistical Analysis

The effect of cogongrass leachate on the performance of each native species was assessed via comparisons with the native leachate treatment. Statistical comparisons between these two treatments were done using the MIXED procedure in SAS 9.2 (SAS Institute 2007), within the framework of a randomized complete blocks design, replicated in time. Year and block(year) were treated as a random effects. For the different response variables (aboveground biomass, belowground biomass, total biomass, root length, specific root length, % mycorrhizal colonization and total mycorrhizal root length), differences between treatments were declared statistically significant at $P < 0.05$. Additional analyses were done with data from year two, using Dunnett's *t*-test for post-hoc comparisons with the DI water control (SAS Institute 2007). The Kenward-Roger calculation was used to estimate denominator degrees of freedom (Schaalje et al. 2002)[1]. Some non-statistically significant trends are reported in cases where there may be some ecological significance (*e.g*. when non significant relationships add evidence to inferences drawn from significant relationships). Concentrations of the various compounds (adjusted by the concentration factor) were

[1] This method can result in non-integer values for denominator degrees of freedom.

compared with the nonparametric Wilcoxon-Mann-Whitney test using the NPAR1WAY procedure (SAS Institute 2007). Differences between treatments were declared statistically significant at $P < 0.05$.

Results

Biomass Production, Allocation and Root Morphology

Allelopathic interference from cogongrass leachates had variable effects on the biomass production and allocation patterns for the four native seedlings. While no species had significant differences in total biomass between leachate treatments, aboveground biomass for *A. stricta* was 35.7 % lower in the cogongrass leachate treatment than in the native leachate treatment ($F_{(1, 40.28)} = 15.04$, $P = 0.0004$). This difference corresponded with a 22.2% reduction in total root length ($F_{(1, 41.02)} = 4.86$, P = 0.0331) and a 22.9% reduction in specific root length ($F_{(1, 41.18)} = 17.28$, $P = 0.0002$). No such effects were observed for *A. arctatus*, *P. elliottii* or *L. ferruginea* (Table 2-3). Comparisons made with the DI water control using year two data provide supporting evidence that the observed differences were due to the negative effects of cogongrass leachate. In all of the above cases where treatment effects were observed, the native leachate treatment was within 4.8% of control ($t_{(1, 21)}$ 0.35, $P = 0.9187$; $t_{(1, 21)}$ -0.12, $P = 0.9041$; $t_{(1, 21)}$ 0.14, $P = 0.8872$) for aboveground biomass, total root length and specific root length, respectively). Comparisons between the cogongrass leachate treatment and the DI water control, however, showed more substantial differences. Aboveground biomass was 37.7% lower than control ($t_{(1, 21)}$ -2.84, $P = 0.0019$), total root length was 29% lower ($t_{(1, 21)}$ -2.22, $P = 0.0695$) and specific root length was 18.1% lower ($t_{(1, 21)}$ -2.77, $P = 0.0217$).

Mycorrhizal Inoculation and Infected Root Length

Differential treatment effects were also observed for plant-mycorrhizal fungi associations. For *P. elliottii*, EcM fungal inoculation (% mycorrhizal colonization) was 19.4% lower in the cogongrass leachate treatment than in the native leachate treatment ($F_{(1, 47.54)}$ = 12.11, P = 0.0011). Reductions in total mycorrhizal root length were observed for both *A. stricta* (23.4%; $F_{(1, 41.20)}$ = 3.79, P = 0.0280) and *P. elliottii* (21.8%; $F_{(1, 47.62)}$ = 4.96, P = 0.0307). For *A. stricta*, this reduction is likely associated with the reduction in total root length observed. No such trends were observed for either *A. arctatus* or *L. ferruginea*. Percent mycorrhizal colonization and total mycorrhizal root length were higher for *A. arctatus* in the cogongrass treatment, but these differences were not statistically significant (Figure 2-1). Again, comparisons with the DI water control in year 2 suggest that these differences were due to the negative influence of cogongrass leachate. In all cases, the differences between the cogongrass treatment and control were more substantial than those between the native treatment and control. For *P. elliottii*, both EcM colonization and total mycorrhizal root length in the native leachate treatment were within 11% of control ($t_{(1, 28)}$ -1.07, P = 0.4604 and $t_{(1, 28)}$ -0.97; 0.5226, respectively). In the cogongrass leachate treatment, however, *P. elliottii* EcM colonization was 25.6% lower ($t_{(1, 28)}$ -3.24, P = 0.0059) and total mycorrhizal root length was 22.1% lower ($t_{(1, 28)}$ -1.95, P = 0.1079). Total mycorrhizal root length for *A. stricta* in the native leachate treatment was 3.6% lower than control ($t_{(1, 21)}$ -0.18, P = 0.9783), while the cogongrass treatment was 26.1% lower ($t_{(1, 21)}$ -1.30, P = 0.3461).

Chemical Profiling of Leachates

The chemical profile of cogongrass leachate was qualitatively and quantitatively different from that of native leachate. Eleven potentially allelopathic organic compounds

in the cogongrass leachate were identified in the initial HPLC analysis, most of which were found at significantly lower concentrations – or not found at quantifiable levels – in the native leachate. Phenolic acids were the predominant class of allelopathic compound in the cogongrass leachate. The phenolic compound with the highest concentration was gallic acid (3.03 ppm), followed by caffeic acid (0.85 ppm), salicilyc acid (0.61 ppm) and sinapinic acid (0.33 ppm). The other compounds, which included a carboxylic acid (benzoic acid), an anthraquinone (emodin) and a dihydroxy benzene (resorcinol) all had concentrations less than 0.16 ppm. No compounds in the native leachate had concentrations greater than 0.09 ppm. Five of the compounds (caffeic, benzoic, cinnamic, ferulic and chlorogenic acid) have been positively identified in previous studies of cogongrass allelochemistry (Abdul-Wahab and Al-Naib 1972; Hussain and Abidi 1991; Xuan et al. 2009). A complete list of the identified compounds, along with statistical comparisons of their concentrations is provided in Table 2-4.

Along with the confirmation of the compounds described above, the HPLC-MS analysis also suggested that a novel alkaloid compound was present in the cogongrass leachate. Based on fragmentation patterns and library matching, the speculated structure is hexadecahydro-1-azachrysen-8-yl ester ($C_{23}H_{33}NO_4$) (Figure 2-2). It appeared to be present at fairly high levels, although it was not possible to estimate its actual concentration due to lack of commercial reference standards. This compound was not found in the native leachate treatment.

Discussion

Uren (2007) compiled a list of over 100 secondary compounds thought to be exuded by plant tissues. This list includes an array of sugars, polysaccharides, amino acids, organic acids, fatty acids, sterols, growth factors, enzymes, flavonones,

nucleotides and other chemicals, many of which are suspected to be involved in mediating belowground interactions with plants and/or soil fauna. Effectively assessing the role of these substances on ecological processes, however, is dependent upon an understanding of their composition and significance in plant-soil systems (Mallik 2000; Uren 2007) – an area where research is sorely lacking. When using live plants in a natural soil medium as I did, however, it is undoubtedly very difficult to isolate the effects of these compounds from the confounding influences of water-extractable matrix solutes, microbes, microbial compounds, root degradation products and other substances (Uren 2007). These shortcomings, however, may be offset by the fact that test species were exposed to a biologically realistic mixture of allelochemicals and their breakdown products.

While allelopathy is commonly suspected to be a driving force behind alien plant invasions into natural areas, only a small number of studies have reported the presence and bioactivity of exudates in the rhizospheres of IA plants. The bulk of the studies of allelopathy in natural systems have focused on spotted knapweed (*Centaurea maculosa*), a species native to Europe and western Asia that has transformative effects on ecosystems throughout North America. Soils in the vicinity of this problematic invader have sometimes been shown to contain high concentrations of the flavonoid secondary metabolite (±)-catechin (Perry et al. 2007). In controlled experiments, (-)-catechin has been shown to have significant inhibitory effects on the germination, growth and overall health affected species, along with having microbicidal properties (Vivanco et al. 2004). In plants, this compound is believed to work by causing the production of reactive oxygen species at the root meristem, which initiate a series of

biochemical and genetic alterations (Bais et al. 2003, but see partial retraction 2010). A similar mechanism is suspected in closely related *C. diffusa* (Hierro and Callaway 2003).

Little is known about the exudate chemistry of cogongrass, but my findings suggest that unlike *C. maculosa*, no single compound is likely responsible for its apparent allelopathic effect. This is probably typical for most allelopathic species, as allelopathic interference is generally thought to result from combinations of allelochemicals and their breakdown products, interacting simultaneously and sometimes synergistically, with multiple physiological processes in the affected organism (Einhellig 1995). The concentrations of individual compounds are usually below a bioactivity threshold, but their effects can be additive (Chung et al. 2002). Phenolics are among the most common classes of allelopathic compounds exuded by grasses (Sánchez-Moreiras et al. 2003), and my findings suggest that cogongrass is no exception. Inderjit and Dakshini (1991) isolated 18 nonspecific phenolic fractions from cogongrass tissues and soils, but it is impossible to confirm if any of the same compounds were present in my cogongrass leachates. Some of the phenolic compounds I described have been identified and (in some cases) shown to have phytotoxic activity in studies of cogongrass tissue extracts (Abdul-Wahab and Al-Naib 1972; Hussain and Abidi 1991; Xuan et al. 2009). However, many of the phenolics identified by the above authors were not found in this study. Additionally, Xuan et al. (2009) identified several long-chain fatty acids (*e.g*. stearic acid and myristic acid) and miscellaneous compounds (coumaran and pantolactone) in cogongrass roots and rhizomes that were not present in my cogongrass leachates. A few of the non-phenolic

compounds that I identified (*e.g.* emodin, resorcinol) have not been reported in other studies of cogongrass allelopathy. Overall, despite highlighting the near-ubiquity of certain phenolics, these differences reinforce the notion that tissue extracts may not be a biologically realistic proxy for allelopathic exudates.

While the specific mechanism of action that brought about the observed reductions in growth and mycorrhization for *A. stricta* and *P. elliottii* is unclear, it has been proposed that phenolics such as cinnamic, benzoic and ferulic acids – all of which were present in the cogongrass leachate – have general toxicity and can interfere with phytohormone interactions, cell membrane structure and function, photosynthesis, enzymatic reactions and carbon flow, among other important physiological processes in plants and soil organisms (Einhellig 1995). Alkaloids have received comparatively less research attention, but common alkaloid compounds have been reported to affect DNA synthesis, respiration and electron transport (Einhellig 2002). An alkaloid similar to the one I described in this study has been identified in studies involving *Sorghum bicolor*, and appears to function as a nitrification inhibitor in soils (Chung-Ho Lin, personal communication). Emodin, an anthraquinone that is also found in the invasive plant Japanese knotweed (*Polygonum cuspidatum* Siebold and Zucc.) has been shown to reduce root and shoot growth and alter the availability of mineral nutrients in the soil (Izhaki 2002). Resorcinol, which was present in very low concentrations, does not appear to be directly phytotoxic (Seal et al. 2004) but has been shown to have antifungal properties (Suzuki et al. 1996).

An intriguing aspect of these findings is the fact that the allelopathic influence of cogongrass appears to vary by species. Others have speculated on the possible

species-specificity of allelopathic interference from IA plants (McCarthy and Hanson 1998; Abhilasha et al. 2008), including cogongrass (Xuan et al. 2009), but I believe that this is the first study that has focused on the effects of cogongrass on native species. The root length, morphology and mycorrhizal measurements provide insight into some possible explanations behind the observed differences. For *A. stricta*, reductions in total root length and concurrent decreases in specific root length suggest that allelopathic interference inhibits root elongation and/or branching. This in turn likely creates less opportunity for mycorrhizal colonization. Bluestem grasses and/or their associated belowground symbionts may be resistant to allelochemicals exuded by cogongrass, but the underlying mechanism is unclear. Among the two woody species, only the EcM tree (*P. elliottii*) appeared to be affected. Low concentrations of phenolic mixtures have been shown to inhibit EcM fungi (Souto et al. 2000); perhaps this is the mechanism at play here. Ericoid mycorrhizal fungi, like those that colonize *L. ferruginea*, may have an inherent tolerance to allelochemicals, since the root systems of these species proliferate in litter and organic soil horizons where plant exuded phenolics and their breakdown products are typically present in relatively high concentrations (Bending and Read 1997). Ericoid mycorrhizal fungi have some ability to degrade phenolics (Bending and Read 1997), which may enable access to labile organics and nitrogen formerly complexed with these compounds (Bending and Read 1996). However I observed no evidence of enhanced *L. ferruginea* growth in the cogongrass leachate treatment.

The species-specificity of the apparent allelopathic response, which was perhaps mediated by interactions with mycorrhizal fungi, may help explain the patterns of invasion that have been observed both in cogongrass-impacted pine ecosystems and in

experimental mesocosms. *Aristida stricta*, which appeared to be negatively affected by cogongrass soil leachate, rarely persists in sites invaded by cogongrass (Hagan, personal observation; Jose et al. 2002). Cogongrass invasion also inhibits pine regeneration (Daneshgar et al. 2008). While the above effects have most often been attributed to competition (Brewer 2008; Daneshgar and Jose 2009a), fire feedbacks (Lippincott 2000) and/or physical interference (Holly and Ervin 2006), my findings suggest that these interactions may be compounded by allelopathic activity as well. In the acidic nutrient-poor flatwoods soils characteristic of the environments where these species are typically found, reductions in root length and/or mycorrhizal colonization will likely represent a major fitness disadvantage for affected native species. In a mesocosm study that looked into the effects of native species diversity and identity on cogongrass invasion, broomsedge bluestem (*Andropogon virginicus* L.) performed significantly better than other native herbaceous species when grown in close association with the cogongrass (Daneshgar and Jose 2009b). Perhaps this was due in part to an inherent resistance to allelopathic interference, as my findings with closely related *A. arctatus* suggest. I know of no studies that have looked into the performance of *Lyonia* in cogongrass-invaded systems, but research on other species has suggested that the resistance to allelopathy conferred to ericaceous plants by their symbiotic fungi enhances their ability to persist in environments dominated by allelopathic invaders. Ericads, for example, are among the few species apparently capable of overcoming the powerful allelopathic influence of *Casuarina* (Reed 1989) – a commonly invasive genus known to exude phenolics and other phytotoxic compounds (Sayed et al. 2002).

Summary and Implications

This study represents the first attempt to assess the effects of allelopathy from cogongrass on native species in an ecologically relevant setting. Overall, my findings support the hypothesis that novel and potentially allelopathic compounds are present in the cogongrass rhizosphere. Substantial reductions in biomass production and/or mycorrhizal colonization, along with altered root morphology, which were observed for 2 of 4 native species treated with the cogongrass leachate, suggest that these compounds are present in biologically significant concentrations. It is likely, therefore, that the transformative nature of cogongrass in its invaded range can be attributed, at least partially, to the effects of allelopathic interference. Additional research should seek to shed light on the bioactivity of the alkaloid, as well as explore possible allelochemical tolerance mechanisms possessed by *L. ferruginea* and *A. arctatus*. It would also be beneficial to assess the presence and concentration of the observed compounds in the field.

Table 2-1. Native pine savanna species used in a study of the allelopathic effects of cogongrass leachate.

Scientific name	Common name	Family	Symbiont[a]
Aristida stricta Michx. var*. beyrichiana* (Trin. and Rupr.)	Wiregrass	Poaceae	AM
Andropogon arctatus Chapm.	Pinewoods bluestem	Poaceae	AM
Lyonia ferruginea (Walter) Nutt.	Rusty lyonia	Ericaceae	EM
Pinus elliottii Engelm.	Slash pine	Pinaceae	EcM

[a]Denotes the mycorrhizal fungal symbiont (AM, arbuscular mycorrhizal; EM, ericoid mycorrhizal; EcM, ectomycorrhizal).

Table 2-2. Effects of aqueous and chloroform extracts of leachates (native, cogongrass, DI water control) on the germination (%) of lettuce seeds. Four replicates of each treatment x extract combination, each with 10 seeds, were used. Comparisons with the control were made with Dunnett's post-hoc t-test. Pairs of means with asterisks are significantly different at $P < 0.05$.

Extract	Native	Cogongrass	Control
Aqueous	90	75*	92.5*
Chloroform	85	87.5	87.5

Table 2-3. Species-wise comparisons (means and standard errors) of the effects of a cogongrass leachate treatment vs. the effects of a native leachate treatment on biomass production, allocation, root length and specific root length for four native pine savanna species. Pairs of means with asterisks are significantly different at $P < 0.05$.

Species	Treatment	Aboveground (g)	Belowground (g)	Total (g)	Root length (cm)	cm root/g
A. stricta	Cogon	0.009 (0.006)*	0.035 (0.010)	0.044 (0.015)	45.70 (13.92)*	1349.75 (58.33)*
A. stricta	Native	0.014 (0.006)*	0.036 (0.010)	0.049 (0.015)	58.71 (13.99)*	1751.96 (65.56)*
A. arctatus	Cogon	0.031 (0.005)	0.111 (0.017)	0.143 (0.017)	103.90 (9.38)	1064.02 (123.33)
A. arctatus	Native	0.023 (0.006)	0.109 (0.020)	0.140 (0.026)	93.61 (8.11)	1071.23 (125.50)
P. elliottii	Cogon	0.159 (0.008)	0.231 (0.017)	0.389 (0.020)	37.44 (6.25)	169.96 (21.52)
P. elliottii	Native	0.184 (0.008)	0.269 (0.017)	0.451 (0.020)	37.88 (6.24)	142.64 (21.47)
L. ferruginea	Cogon	0.005 (0.001)	0.012 (0.002)	0.017 (0.003)	7.62 (1.30)	662.11 (36.16)
L. ferruginea	Native	0.005 (0.001)	0.013 (0.002)	0.017 (0.003)	7.84 (1.28)	701.60 (29.13)

Table 2-4. Mean chemical composition of leachates (ppm) collected from the rhizosphere of greenhouse-grown cogongrass monocultures and native polycultures. Compounds identified in previous studies are denoted, along with the source of the extract.

Compound	Family	Previously reported?	Retention (min)	Concentration (ppm)		Wilcoxon-Mann-Whitney*
				Cogon	Native	
Gallic acid	Phenolic acid		2.50	3.03	0.09	< 0.05
Caffeic acid	Phenolic acid	1 SH	3.77	0.85	0.03	< 0.05
Salicylic acid	Phenolic acid		12.41	0.61	0.05	< 0.05
Sinapinic acid	Phenolic acid		5.60	0.33	0.01	< 0.05
Benzoic acid	Carboxylic acid	3 RH, RO	10.30	0.16	0.03	NS
Emodin	Anthraquinone		24.51	0.16	BQ	< 0.05
Cinnamic acid	Phenolic acid	3 RO,	15.92	0.12	0.01	< 0.05
Ferulic acid	Phenolic acid	1 SH, 3 RH, RO	6.10	0.11	0.01	NS
4-hydroxyphenylacetic acid	Phenolic acid		4.09	BQ	BQ	--
Cholorogenic acid	Phenolic acid	1 SH, 2 RO	2.50	BQ	BQ	--
Resorcinol	Dihydroxy benzene		4.13	BQ	BQ	--

[1]Abdul-Wahab and Al-Naib (1972); [2]Hussain and Abidi (1991); [3]Xuan et al. (2009); SH = extracted from shoots; RH = extracted from rhizomes; RO = extracted from roots; *< 0.05, indicates that differences between treatments were statistically significant; NS, indicates that differences between means were not statistically significant; BQ, below limits of quantification; NS --, indicates that comparisons not possible due to concentrations in both leachates being below limits of quantification.

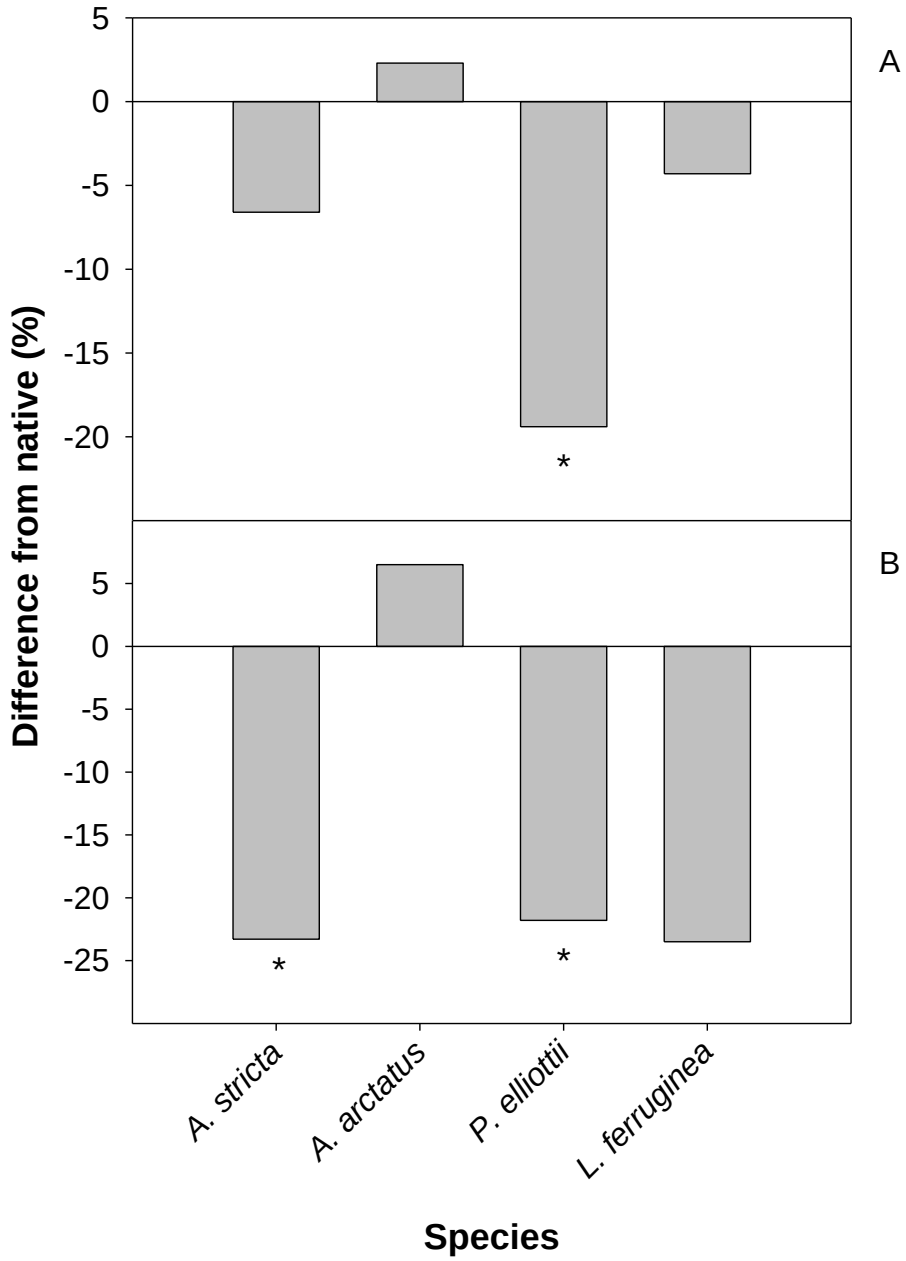

Figure 2-1. Difference in percent mycorrhizal colonization (A) and total mycorrhizal root length (B) for four native species watered with cogongrass leachate, relative to those watered with leachate from native species. Means and standard errors. Differences with asterisks are statistically different at $P < 0.05$.

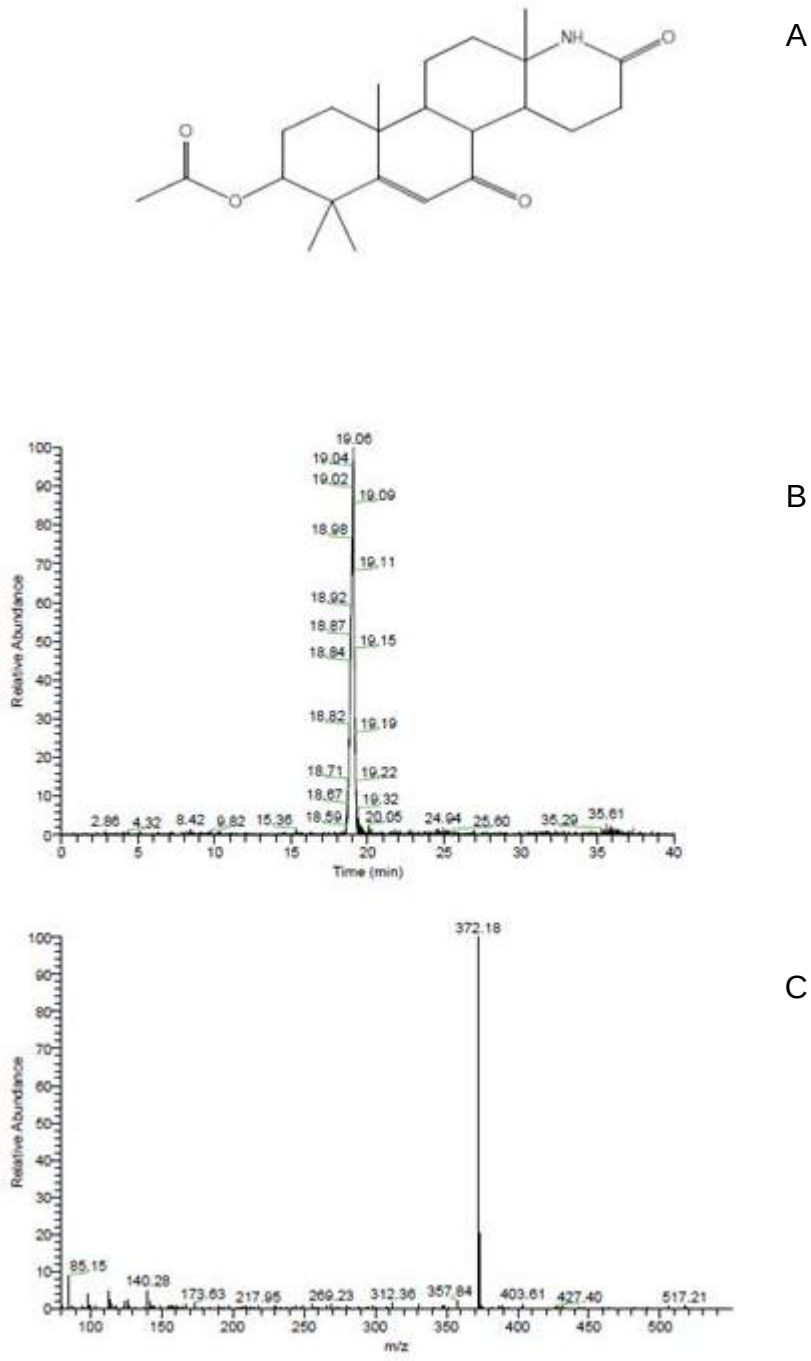

Figure 2-2. Speculated chemical structure of a novel alkaloid (hexadecahydro-1-azachrysen-8-yl ester) identified in cogongrass leachate (A), ion chromatography of the alkaloid, indicating the retention time (19.06 minutes) (B) and the mass spectrum (m/z 372.18) (C).

CHAPTER 3
COGONGRASS INVASION AND ERADICATION: IMPLICATIONS FOR SOIL BIOGEOCHEMICAL PROPERTIES IN A FIRE-MAINTAINED FOREST ECOSYSTEM

Background

Invasive alien (IA) plants typically grow fast, rapidly colonize new sites, compete favorably with native species and have few natural enemies outside of their home range. While theories abound as to the specific mechanisms alien plants use, or traits they possess, that enable them to be successful invaders (Davis et al. 2000; Maron and Vilà 2001; Bakker and Wilson 2001; Callaway and Ridenour 2004), a near-universal characteristic is their ability to alter – often dramatically – the species composition of plant communities (D'Antonio and Vitousek 1992; Gordon 1998; Hejda et al. 2009). Differences in resource uptake patterns, as well as the changes in litter quality and quantity that accompany these vegetative shifts, in turn, can greatly alter the nutrient cycling dynamics of an ecosystem (Kourtev et al. 2002; Ehrenfeld 2003; Allison and Vitousek 2004). The extent of change depends on how different the invader is from the species that it replaces with respect to traits such as life history, physiology, size, above- and belowground architecture, tissue chemistry, photosynthetic pathways, symbiotic relationships and other factors (Ehrenfeld 2003).

The body of knowledge on the effects of IA species on soil nutrient cycling processes, while limited, has increased greatly in recent years (Ehrenfeld 2003). Less attention, however, has been paid to post-eradication effects (Maron and Jeffries 2001; Yelenik et al. 2004), although researchers have speculated on the potential for legacy factors to impede restoration efforts (Corbin and D'Antonio 2004; Renz and Blank 2004; Yelenik et al. 2004). This, clearly, is an area that deserves more consideration, as the restoration of native plant communities following the eradication of IA species is a high

priority among land managers (Zavaleta et al. 2001; Hartman and McCarthy 2004; Miller et al. 2010). Much like any other disturbance, if soil processes and properties are altered by an invader, these effects will likely persist for some time after the invader is removed (Corbin and D'Antonio 2004; Jordan et al. 2008). The decomposition of plant biomass following treatment might cause further alterations to soil properties through its effects on soil organic matter (OM), pH and the mineralization and immobilization of nitrogen (N) and phosphorous (P). These alterations may in turn affect nitrification rates (Raison 1979; Chapin et al. 2002), or phosphorus complexation reactions (Brady and Weil 2002). Novel mycorrhizal fungal communities may also persist (or develop) following eradication, but this is an area that has yet to receive much research attention. In concert, these changes might impede the re-establishment of desirable native species and/or increase the potential for re-invasion by either the same or new alien species (Kourtev et al. 2003).

An improved understanding of how IA plant species alter soil properties, and how novel soil properties persist/develop following eradication, is essential in order to develop effective long-term restoration strategies for invaded forest communities. Toward these ends, I undertook this study to assess nutrient dynamics in forest stands severely impacted by cogongrass (*Imperata cylindrica* (L.) P. Beauv.) – a C_4 rhizomatous invader that affects tropical and subtropical ecosystems on six continents (MacDonald 2004). I chose a fire-maintained longleaf pine (*Pinus palustris* Mill.) sandhill ecosystem as the study site, as these forests are frequently targeted in restoration efforts (Walker and Silletti 2006) and are commonly invaded by cogongrass (Jose 2002; Daneshgar and Jose 2009a). Since nitrogen and/or phosphorus availability often drive

ecological succession following disturbance (Tilman 1985; Vitousek et al. 1993), I focused on processes that affect their availability and uptake. The three primary objectives of this study were:

- Analyze how invasion by cogongrass affects soil N and P pools, fluxes and associated processes in fire-maintained longleaf pine sandhill stands.
- Quantify soil N and P dynamics in the years following cogongrass eradication.
- Assess the effects of cogongrass invasion and eradication on arbuscular mycorrhizal (AM) fungal communities

I hypothesized that cogongrass invasion would decrease the availability of soil N and P, likely through reductions in pH and/or changes to the soil carbon cycle. I also expected these changes in N and P cycling to persist following eradication, perhaps due to the slow decomposition rates of low-quality cogongrass foliage and rhizomes after herbicide treatment. Additionally, I hypothesized that cogongrass invasion would result in the development of a novel AM fungal community, and that additional modifications to AM fungal community structure would arise following cogongrass eradication.

Materials and Methods

Study Area

The study area was an uneven-aged, naturally regenerated longleaf pine forest in the Croom Tract of Withlacoochee State Forest in Hernando County, Florida (28°36'19.99"N, 82°16'19.73"W). The tract is near one of the original points of cogongrass introduction in the United States and has a long history of invasion. Efforts in recent years to chemically eradicate most cogongrass infestations in the tract have been successful and there are numerous areas in various stages of recovery throughout, although some untreated cogongrass patches remain. The uninvaded areas are characterized by high levels of understory species richness and diversity, as is

typical of an actively managed, frequently burned longleaf pine sandhill community. Predominant understory species in uninvaded areas were wiregrass (*Aristida stricta* Michx. *var. beyrichiana* (Trin. and Rupr.) D.B.Ward), along with various native trees, graminoids, forbs, shrubs and vines (Chapter 4). Soils in the study area were predominantly deep, well-drained to excessively drained sands of the Lake and Candler series (hyperthermic coated Typic Quartzipsamments and hyperthermic uncoated Lamellic Quartzipsamments, respectively). Small inclusions of the Arredondo series (Loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults) – comprising less than 20% of the total area – were also present (US Department of Agriculture 1977). Mean overstory basal area for the study site was 10.7 m^2 ha. Longleaf pine constituted approximately 88% of total basal area.

Experimental Design

I used invaded and uninvaded sites across 4 longleaf pine sandhill stands in the study area to assess the effect of cogongrass invasion on soil N and P dynamics. Additionally, since some sites within these stands had cogongrass eradicated in previous years, I established a "recovery chronosequence" to measure temporal changes in N and P cycling following eradication. Sites selected for the chronosequence treatments were treated in the late summer/early fall – approximately three, five and seven years prior, respectively – with a tank mix solution (sprayed to the point of runoff) consisting of 2% Roundup Pro™ (41% glyphosate plus surfactant) and 0.4% Arsenal™ (28.7% imazapyr). Glyphosate and imazapyr tank mixes such as this are among the most common and effective methods of chemical control for cogongrass (MacDonald

2004)[1]. All sites, hereafter referred to as plots, were identified and selected using Geographic Information Systems (GIS) and with the help of state forest personnel. Native reference plots, randomly selected using GIS from the surrounding uninvaded area, were ground-truthed to verify that they were not currently invaded and did not fall on disturbed or degraded sites (*e.g.* roads, bicycle trails, abandoned rock mines, formerly invaded sites). Plots with live cogongrass were estimated to be 1-2 years old, which is approximately the same age that those in the recovery chronosequence were when they were treated. Cogongrass was the dominant species in these plots.

The study was laid out as a complete blocks design, replicated twice. Each replicate consisted of an adjacent pair of 259 ha stands (blocks) with similar, but asynchronous burn histories (both burned approximately every 4 years, but usually staggered 2 years apart). One block in each replicate was burned last in June 2009 and the other was burned in June 2007. Each block typically contained 2-3 plots from each of the five treatments: reference (*i.e.* uninvaded), invaded, three years since eradication, five years since eradication, and seven years since eradiation (Table 3-1). Within each plot, three subplots were randomly selected, each being at least 2.5 meters from the other, at least 8 m from the edge and distant from any cogongrass re-sprouts (where applicable) (Figure 3-1). Additionally, 4 recently treated cogongrass patches were selected as locations for a litterbag decomposition study (described below).

[1] A single herbicide treatment does not always completely eradicate a cogongrass patch. However, for young (≤ 2 year old) patches in the Croom tract, > 95% control is typical. For the purposes of this study, all such patches were considered “eradicated”.

Soil Chemistry and Nutrient Pools

Soil samples from the top 15 cm of the profile were collected from subplots in June 2010 using a standard 1-piece soil probe. Each sample was a composite of five subsamples – one taken at plot center and the remaining four collected one meter away at each of the four cardinal directions. Samples were transported to the lab in a cooler and then moved to a freezer, where they remained at minus 4°C until analysis. Soil OM content was determined by acid digestion and pH was measured using a 1:2 soil:water ratio. Total N (TKN method) and potentially available P were quantified using an Apkem autoanalyzer and a Mehlich-1 (M1) extraction, respectively (Mylavarapu 2002).

Soil Nutrient Availability

The availability of N and P in the different treatments was assessed with mixed cation-anion exchange resin bags, incubated *in situ* (Standish et al. 2004; Harpole and Tilman 2007) during the 2010 growing season. By integrating microenvironmental factors (*e.g.* water availability, flow and plant uptake) during the incubation period (Binkley 1984), this method provides additional information about N and P cycling in terrestrial systems (Binkley et al. 1986; Gibson 1986; Feller et al. 2003). Prior to incubation, resins were washed in sodium chloride (NaCl) and sodium hydroxide (NaOH) per the procedure outlined in Thiffault et al. (2000). Bags consisted of approximately 10 g (moist weight) of washed resin (Dowex Marathon MR-3), cinched in a square of acid-washed nylon-lycra mesh with a plastic zip tie to make a firm, spherical bag (Thiffault et al. 2000). In early May and September of 2010, a bag was buried at a depth of five cm in each subplot. Bags were removed after 33 day incubation periods. Upon return to the lab, they were gently rinsed in de-ionized (DI) water to remove adhering soil particles, and then shaken in a 2 M NaCl solution for two hours. Extracts

were analyzed using an autoanalyzer for total adsorbed nitrite+nitrate-N (NO_2+NO_3) and ammonium-N (NH_4) and by inductively coupled plasma mass spectrometry (ICP-MS) for total adsorbed P (Thiffault et al. 2000; Harpole and Tilman 2007).

Litter Decomposition and Nutrient Mineralization

Initial nutrient cycling following cogongrass eradication may be affected by the decomposition of dead biomass. Therefore, to determine the rates of cogongrass decomposition and nutrient mineralization/immobilization, 40, 1 mm fiberglass mesh litterbags (20 filled with five g of air dried cogongrass rhizomes and 20 filled with five g of air dried cogongrass foliage) (Ashton et al. 2005) were incubated in clusters of five in four random locations in recently treated (fall 2009) cogongrass patches scattered across the two blocks. These tissues were collected from an adjacent area that was treated two weeks prior with the glyphosate and imazapyr tank mix. In December 2009, rhizome bags were buried to a depth of five cm and foliage bags were left on the soil surface. After 31, 90, 192, 373 and 544 days (approximately 1, 3, 6, 12 and 18 months), eight bags were collected (one per tissue type, per location) and transported to the lab, where their contents were carefully removed, freed from adhering soil and dried at 65° C. Subsamples were ground to <1 mm and analyzed for total C, N and P, per the procedure outlined in Bray et al. (2005). Mass loss and nutrient mineralization/immobilization, relative to pre-incubation values, were then determined for each sampling date (Allison and Vitousek 2004).

AM Fungal Spore Quantification

Arbuscular mycorrhizal spores from soil samples (approximately five g) taken from each plot were isolated using a standard wet-sieving, decanting and glucose centrifugation technique (Daniels and Skipper 1982). The final product was transferred

to a test tube and brought to a volume of 5 mL. A 0.5 mL aliquot was transferred to a piece of filter paper cut to the size of a microscope slide for spore quantification. Spore counts were converted to spores/mL, then spores/gram for statistical analysis.

Soil AM Fungal DNA Extraction, PCR, Cloning and Sequencing

Composite soil samples (two per treatment = 10 total) were prepared by thoroughly homogenizing four samples from randomly selected plots (one plot per block) from each of the five treatments. An exception was made for the invaded treatment, since one of the blocks contained only one invaded plot. Two of the samples in this composite sample came from the same block. Each of the four samples in a composite sample was, in itself, a composite of soil samples from three subplots in a plot. Soil DNA was extracted from composite samples according to the manufacturer's instructions using a MO BIO UltraClean® Soil DNA Isolation Kit. This DNA was used in a PCR reaction using the AM fungal SSU rRNA specific primers AML1 (5-ATC AAC TTT CGA TGG TAG GAT AGA-3) and AML2 (5-GAA CCC AAA CAC TTT GGT TTC C-3) (Lee et al. 2008) using the following regime: 15 min initial denaturation at 94°C, 36 cycles at 94°C for 30 sec, 58°C for 40 sec, 72°C for 55 sec and a final extension at 72°C for five min. PCR products (10 µL) were verified by gel electrophoresis and purified, as needed, with a MO-BIO Ultra Clean® PCR clean up kit, following the manufacturer's instructions. Purified PCR products were cloned according to the manufacturer's instructions using an Invitrogen TOPO® TA Cloning® kit with One Shot® electrocompetent cells and kanamycin selective plates. Colonies were incubated for overnight at 37°C. Selected colonies were then transferred to 96 well plates in 200 µL of kanamycin selective LB medium and incubated at 37°C for an additional 24 hours.

Clones were sequenced on an Applied Biosystems Model 3130 Genetic Analyzer using the T7 and R24 sequencing primers.

Sequence Processing and Analysis

Sequences were aligned using CLUSTALX2 (Larkin et al. 2007) and a consensus neighbor-joining phylogenetic tree was generated with Mega V. 5 (Tamura et al. 2011), using default settings, 1000 bootstrap replications and representative sequences from Genbank. The MOTHUR program (v. 1.23.0) was used to assign sequences to operational taxonomic units (OTUs) at a 3% cutoff, using the cluster (furthest neighbor algorithm) and bin.seqs commands (Schloss et al. 2009). A BLAST search was conducted on representative sequences from each OTU using the nr/nt nucleotide database. Since the different AM fungal families may have different functional strategies or ecological niches (Lekberg et al. 2007), BLAST hits were binned by their respective families, by treatment.

Fungal OTU richness (Chao1 richness estimator) for each sample was estimated using the summary.single command in group mode in MOTHUR. Summary.single was also used to calculate two common measures of OTU diversity. The Shannon-Wiener index (*H'*) index was calculated by MOTHUR as follows:

$$H_{shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} ln \frac{n_i}{N}$$

where S_{obs} is the number of observed OTUs, n_i is the number of individuals in OTU *i* and *N* is the total number of individuals in the community (Schloss et al. 2009). The Simpson index (*D*) was calculated by MOTHUR as follows:

$$D_{simpson} = \frac{\sum_{i=1}^{S_{obs}} n_i (n_i - 1)}{N (N - 1)}$$

where n_i is the number of OTUs with *i* individuals (all other parameters are the same as in *H'*). The Jaccardian pairwise similarity index (summary.shared; MOTHUR) was used to determine the proportion of individuals between each combination of treatments that belong to shared OTUs (Schloss et al 2009). A Mantel test, which tests the null hypothesis of no relationship between matrices (McCune and Grace 2002), were used to assess the relationship between a matrix of fungal OTUs and the soil variables reported in this study.

Statistical Analysis

The three subplot values from the soil, resin bag and spore analyses were averaged to obtain plot-level estimates. For the resin bags, plot-level estimates for the May and September deployments were also averaged to generate single growing season estimates of NO_2+NO_3, NH_4 and P availability. The data were analyzed using the MIXED procedure in SAS 9.2 (SAS Institute, Inc.). Replicate and block(replicate) were treated as random effects. The Kenward-Roger calculation, a preferred method for unbalanced mixed models (Spilke et al. 2005), was used to estimate denominator degrees of freedom[2]. Differences between means were declared statistically significant at $P < 0.05$ and Tukey's post-hoc test was used for pairwise comparisons. Some non-statistically significant trends are reported in cases where there may be some ecological significance (*e.g*. when non significant relationships add evidence to inferences drawn from significant relationships). Decomposition rates (*k*-coefficients) for cogongrass rhizomes and foliage were calculated based on a negative exponential model following Bray (2005). Treatment means (Chao1, Shannon-Wiener and Simpson) for mycorrhizal

[2] This method can result in non-integer values for denominator degrees of freedom.

analyses were compared using the GLM procedure in SAS 9.2. The weighted UniFrac Significance Test, a Monte Carlo procedure (100 permutations), was used to compare the community structures of the five treatments. This method measures the fraction of branch length in a phylogenetic tree that is unique to each treatment, and accounts for the proportional representation of each treatment in each branch (Schloss 2008). It generates *P* values, which are used to assess if the differences in genetic distance between pairs of communities is greater than would be expected by chance alone (Lozupone et al. 2007; Schloss 2008). Differences between treatments with UniFrac *P* values ≤ 0.05 were declared statistically significant. A principal components analysis (PCA) biplot was generated in UniFrac to help visualize treatment separation in variable space (Lozupone et al. 2007).

Results

Organic Matter and pH

Like many of the other measured soil properties, soil OM was highly variable among the five treatments, ranging from 0.83 to 3.17% with a mean of 1.84%. Differences between treatments were significant ($F_{(4, 43.94)} = 2.71$, $P = 0.0417$), with OM contents being highest in invaded plots (2.22%) and lowest in plots where cogongrass was eradicated seven years prior (1.62%) (Figure 3-2) Soil pH in the different treatments ranged from 4.87 to 6.00 with a mean of 5.44. Differences in pH between treatments were significant ($F_{(4, 43.47)} = 7.34$, $P = 0.0001$), with pH in five year plots (5.63) being significantly higher than that of the native reference plots (5.40) and currently invaded plots (5.23) (Figure 3-3).

Nitrogen

Total N contents ranged from 666.7 to 1400.0 mg/kg, with a mean of 990.1 mg/kg. Differences between treatments were not significant ($F_{(4, 43.52)} = 1.38$, $P = 0.2557$). Resin-adsorbed NH_4 followed a similar pattern, ranging from <0.01 to 0.08 mg/bag, with a mean of 0.02 mg/bag and no significant differences between treatments ($F_{(4, 43.38)} = 0.26$, $P = 0.9000$). Resin-adsorbed NO_2+NO_3, did however vary significantly between treatments ($F_{(4, 43.15)} = 12.81$, $P < 0.0001$). Contents ranged from <0.01 to 0.10 mg/bag, with a mean of 0.02 mg/bag. Soil NO_2+NO_3 levels were highest three years after eradication (0.05 mg/bag) and lowest in the reference and invaded treatments (0.01 and 0.01 mg/bag, respectively. Levels decreased after this initial spike and were not significantly different from the reference treatment seven years following cogongrass eradication (Figure 3-4).

Phosphorus

Soil M1-extractable P contents ranged from 50.8 to 347.3 mg/kg with a mean of 120.7 mg/kg. Differences between treatments were significant ($F_{(4, 43.92)} = 3.76$, $P = 0.0102$), with M1-P contents being lowest three and five years after cogongrass eradication (98.2 and 102.3 mg/kg, respectively), and highest in cogongrass invaded plots (164.4 mg/kg). Resin-adsorbed P ranged from 0.02 to 0.78 mg/bag with a mean of 0.20 mg/bag. It followed a similar pattern as M1-extractable P ($F_{(4, 44.04)} = 3.06$, $P = 0.0263$), with the highest values being found in invaded plots (0.33 mg/bag) and the lowest values found in plots where cogongrass was eradicated three and five years prior (0.13 and 0.14 mg/bag, respectively) (Figure 3-5).

Tissue Quality, Decomposition and Mineralization

Cogongrass rhizomes and foliage differed with respect to tissue chemistry and they exhibited different patterns of mass loss during the 18 month field incubation period. Decomposition rates (*k*-coefficients) were 1.01 and 0.44 for rhizomes and foliage, respectively (Table 3-2). Nutrient mineralization occurred fairly rapidly for cogongrass foliage, with 43.7 and 20.5% of initial N and P remaining, respectively, after 18 months. A different trend, however, was observed for rhizome tissues. Following an initial spike in immobilization, in which tissue N levels were more than 2.5 times greater than initial values, tissue N dropped to 70.0% of initial levels after 18 months. In contrast with N, rapid P mineralization occurred for cogongrass rhizomes, with 15.4% remaining after 18 months (Figure 3-6).

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal spore counts ranged from 1.89 to 33.93 spores/g with a mean of 12.72 spores/g. There were no significant differences between chronosequential treatments ($F_{(4, 43.65)} = 0.72$, $P = 0.5838$) nor were there any apparent trends or patterns.

The PCR protocol generated the expected ca. 800 base pair amplicons. At the 97% cutoff, clone library coverage averaged 92% and was highest in the seven year treatment (97%) and lowest in the invaded treatment (84%). The 304 sequences were classified into 31 OTUs. BLAST searches suggested that nine OTUs were in the Acaulosporaceae family, five were Gigasporaceae, twelve were Glomeraceae and five were Paraglomeraceae (Appendix A). Across treatments, Chao1 richness ranged from 13.33 to 21.75, *H*' from 1.84 to 2.05 and 1-*D* from 0.80 to 0.87. None of these indices differed significantly between treatments ($F_{(4,5)} = 0.89$, $P = 0.53$; $F_{(4,5)} = 0.86$, $P = 0.54$;

and $F_{(4,5)}$ = 1.76, P = 0.27) for Chao1, *H*' and 1-*D*, respectively), and there were no clearly evident trends, but there was considerable between-treatment variability (Figure 3-7). Many fungal OTUs were shared between treatments, as indicated by high similarity indices (mean 72.8, range 56.6 – 80.4). The Mantel test did not indicate a strong relationship between fungal and soil matrices (*P* = 0.270).

A condensed phylogenetic tree (Figure 3-8), generated from a subset of aligned sequences, was consistent with AM fungal SSU trees constructed previously (Redecker 2002; Redecker and Raab 2006). Pairwise comparisons made using the UniFrac Significance Test (using a phylogenetic tree made from all sequences) indicated significant differences in the community structure between the different treatments. The reference treatment was significantly different from the three year and invaded treatments (*P* = 0.00 and 0.00, respectively). The three year treatment was also significantly different from the seven year treatment (*P* = 0.01) and the invaded treatment was significantly different from the five year treatment (*P* = 0.05) (Table 3-3). A plot of the first two axes of the weighted UniFrac PCA, which accounted for >67% of total variation between treatments, illustrated a similar pattern. While there was substantial within-treatment variability, points for the five and seven year treatments generally were closer to reference than were the points from the three year and invaded treatments (Figure 3-9).

Discussion

My findings suggest that no substantial changes in soil properties occurred directly as a result of cogongrass invasion in the longleaf pine sandhill ecosystem. This does not support my first hypothesis and it stands contrary to the findings of other researchers, who have suggested that cogongrass alters soil chemistry in southern pine

ecosystems (Collins and Jose 2008; Daneshgar and Jose 2009a). Since changes in soil nutrient cycling due to cogongrass invasion were not evident, the persistence of such effects – which I proposed in hypothesis 2 – was not possible. Some temporary alterations to N and P dynamics did, however, develop in the years following eradication. These trends, I propose, are most readily explainable by decomposition, mineralization and nitrification processes, in concert with differences in pH along the experimental chronosequence. Invasion and eradication had substantial – but again temporary – implications for AM fungal community structure.

Influence of Cogongrass on Soil Chemistry

Like many IA plants, cogongrass grows faster and produces more biomass than the native understory species that it displaces (Jose et al. 2002). Cogongrass is also suspected to have high nutrient use efficiency (Daneshgar and Jose 2009a), which contributes to the production of low quality tissues that decompose slowly (Bray 2005). Since the carbon cycle is intrinsically linked to other elemental cycles, alterations in biomass production may affect the cycling of soil nutrients – particularly macronutrients such as N and P that are frequently limiting. These differences in biomass production and tissue chemistry would seemingly lead to elevated soil OM levels (Ehrenfeld 2003), although this was not clearly evident in this study. It is difficult to assess why the expected trends were not observed, but it is possible that the invaded plots used for this study had not been impacted long enough for substantial alterations to occur.

There does not appear to be a characteristic trend of pH alteration in invaded systems, as studies show both decreases (Gremmen et al. 1998; Grierson and Adams 2000; Collins and Jose 2008) and increases (Hector et al. 1999; Ehrenfeld et al. 2001). The apparent lack of effect of cogongrass invasion on soil pH in this study, however, is

contrary to the findings of other cogongrass researchers. Collins and Jose (2008), for example, reported pH values in cogongrass invaded forest sites to be nearly one quarter unit lower than in uninvaded sites. While differential NH_4 and NO_3 uptake is commonly cited as an explanation for such effects (Ehrenfeld et al. 2001; Hewins and Hyatt 2010), the fact that I did not observe differences in pH, NO_2+NO_3 or NH_4 availability between invaded and reference plots suggests that this may not be the case for cogongrass. Cogongrass tissues have been shown to contain or exude a variety of different organic acids (Inderjit and Dakshini 1991; Koger and Bryson 2004, Chapter 2) that may increase soil acidity, but again this was not evident in this study.

While the superior competitive ability of cogongrass (Collins and Jose 2008; Daneshgar and Jose 2009a; Holzmueller and Jose 2011) would seemingly lead to reductions in soil nutrient levels in invaded areas, total N, M1-P and resin-adsorbed N and P in this study were not lower in invaded plots relative to reference plots. In the case of N, it is likely that both systems have highly conservative cycles, in which inputs are limited (due to frequent fire and the preponderance of non-N-fixing vegetation) and that intense competition for this frequently limiting resource leads to internalized N cycling, and the maintenance of low levels of soil N (Chapin et al. 2002). It is possible that cogongrass more effectively captures soil P than do native species, perhaps by “mining” it from deeper horizons and/or by an enhanced scavenging ability, as has been observed with other species (Lambers et al. 2008; Perkins et al. 2011). However my findings, which showed no significant difference in soil P between invaded and reference plots, do not support this assertion.

The Post-Eradication Legacy of Cogongrass on N and P Cycling

The different patterns of N and P mineralization that I observed in the litter bag study can be attributed to differences in tissue chemistry. The decomposition rates of dead plant tissues are largely controlled by their C:N ratios (*i.e.* tissue quality) (Chapin et al. 2002). For low quality tissues (*e.g.* C:N >25:1), N immobilization occurs, for a time, and slow decomposition rates may cause litter to accumulate. For higher quality tissues, N mineralization occurs and decomposition is more rapid (Ehrenfeld 2003). Carbon:phosphorus ratios have less of an effect on decomposition rates, but like N, organic P immobilization and mineralization are governed by a tissue quality threshold (between 200 and 300:1) (Brady and Weil 2002). While P mineralized quite rapidly, for both above- and belowground tissues, a substantial amount of N taken up by cogongrass remained immobilized after 18 months. Daneshgar and Jose (2009a) proposed that cogongrass establishes and maintains dominance in forest ecosystems by monopolizing the soil N pool and storing it in belowground tissues, which constitute the bulk of total biomass. My findings partially support this claim, and further suggest that much of this N remains sequestered for an extended period of time following eradication. The burning of cogongrass “thatch” following treatment, as is often done, releases previously immobilized N into the atmosphere, potentially magnifying an N limitation (Daneshgar and Jose 2009a), although there may be a temporary increase in available forms of soil N (Certini 2005).

Since the early stages of succession are often driven by N availability (Vitousek et al., 1993), an increased N limitation due to immobilization in cogongrass biomass (and atmospheric losses from burning) could affect the establishment and growth of desirable nitrophilic species immediately after eradication. The spike in NO_2+NO_3 at three years is

probably an example of an “Assart flush” (Li et al. 2003) as the various factors that promote nitrification (*e.g.* OM mineralization, elevated pH and temperature) along with decreases in fine root biomass (Attiwill and Adams 1993), likely resulted in a temporary increase in net nitrification. The subsequent decline was likely due to a combination of leaching, plant uptake and the development of conditions less conducive to nitrification. The rapid decline in M1- and resin-adsorbed P in the first three years after eradication is enigmatic, given the poor mobility of P in most soils (Chapin et al. 2002), but it could be explained by the exploitation of this newly available pool by overstory pines in the absence of most competing understory vegetation.

While little is known about post-eradication nutrient cycling processes in invaded systems, it can be assumed that they are strongly tied – following the decomposition of dead tissues – to the effects of soil OM (Attiwill and Adams 1993; Tiessen et al. 1994). In terrestrial systems, the OM pool is constantly turning over, with measurable OM contents representing the balance between inputs (*e.g.* litter) and outputs (*e.g.* carbon mineralization) at a given point in time (Chapin et al. 2002). In the sandy soils typical of longleaf pine systems, soil OM constitutes a major pool of potentially available nutrients (Wilson et al. 1999). My findings suggest that soil OM levels either were not significantly affected by invasion and eradication, or they equilibrated to near reference levels within three years of eradication.

In highly leached, acidic forest soils of humid regions, slight changes in pH can greatly alter the chemical form, solubility and mobility of N and P (Attiwill and Adams 1993). Soil pH has been shown to vary predictably with changes in vegetation during forest succession, with the highest pH values typically found in intermediate

successional seres (Christensen and Peet 1984). In this study, soil pH increased for five years following cogongrass eradication before declining to near-reference levels by seven years. Since nitrification is inhibited at low pH (Chapin et al. 2002), the significant increases in pH observed for five years following eradication could have contributed to the elevated levels of resin-adsorbed NO_2+NO_3 observed across plots. Perhaps this increase in pH is also associated with burning (Raison 1979), as all five year plots had been burned at least once since cogongrass eradication.

Arbuscular Mycorrhizal Fungal Dynamics

While cogongrass has been shown to form associations with AM fungi (Brook 1989), its mycorrhizal characteristics (*e.g.* host/symbiont specificity, dependence) relative to the native species that it displaces is not known. Because of this, It was difficult to make informed assumptions about the effect that cogongrass invasion might have on AM fungal spore availability. Research on other IA plants has shown that invasion can result in a reduction in AM fungal inoculum (Roberts and Anderson 2001; Vogelsang and Bever 2009; Busby et al. 2012), but this was not evident in this study. If reductions occurred following eradication, these effects were apparently short-lived. Since mycorrhizal fungal spores are readily transported by a variety of abiotic and biotic vectors (Sylvia 1986; Warner et al. 1987), spore counts in the recovery chronosequence may indicate rapid re-dispersal following eradication. This would support the findings of Anderson et al. (2010), who found that AM fungal inoculum potential rebounded soon after the eradication of IA plant populations. Spore longevity for AM fungi is not well known, but it is also possible that a persistent spore bank remained following eradication, even when host vegetation was largely absent (Chapter 4).

Additionally, my findings suggest that the richness and diversity of the AM fungal community were unaffected by cogongrass invasion. This is contrary to the findings of Busby (2011), who reported substantial reductions in AM fungal richness and diversity in semi-arid steppe communities invaded by cheatgrass (*Bromus tectorum* L.). To my knowledge, this study is the first to use molecular methods to assess post-eradication effects on AM fungal richness and diversity in ecosystems impacted by IA plants. If there was an effect of eradication, it was only temporary, as differences in richness and diversity were not evident by year three. While the invaded and three year treatments were characterized by novel assemblages of AM fungal sequences, structural convergence occurred by year five. The diversity and richness of the plant community in these same treatments, however, converged later (year seven) and remained markedly different from the reference in terms of species composition and community structure (Chapter 4). The fact that the mycorrhizal community, along with other soil properties, returned to a reference state prior to the plant community doing so suggests that belowground recovery might be a prerequisite for aboveground recovery (Anderson et al. 2010). Indeed, it is increasingly accepted that mycorrhizal fungi can play a significant role in terrestrial plant community succession (Janos 1980; Hartnett and Wilson 1999), particularly in resource poor soils (Gange et al. 1993).

Summary and Implications

This study represents one of the first attempts to assess the effects of both invasion and eradication of alien grasses on soil nutrient cycling processes. With the exception of alterations to AM fungal community structure, my findings suggest that the effects of cogongrass invasion on soil properties in longleaf pine sandhill ecosystems are not substantial. However, considerable – albeit temporary – alterations to soil N and

P cycling, as well as additional changes to the AM fungal community did occur in the years following eradication. Since the restoration of formerly invaded sites is a high priority among land managers, a logical next step is to determine the ecological significance of these effects as they pertain to the re-establishment – either naturally or artificially – of desirable native plant species within an acceptable time frame. This includes improving our understanding of how altered soil properties affect the potential for re-invasion by cogongrass, as well as other problematic IA plant species.

Table 3-1. Number of study plots in each in each treatment x block x replication combination in longleaf pine sandhill communities in Hernando County, FL, USA.

Treatment	Replicate 1		Replicate 2	
	Block 1	Block 2	Block 1	Block 2
Reference	3	3	3	3
Invaded	3	3	1	3
3 years	2	3	3	2
5 years	3	2	3	3
7 years	3	3	3	3

Table 3-2. Initial mean tissue chemistry (standard deviations in parentheses) of herbicide-treated cogongrass rhizomes and foliage, along with calculated *k*- coefficients for mass loss and N and P mineralization over time.

Tissue	%N	%P	%C	C:N	C:P	$k_{biomass}$
Foliage	0.94(0.29)	0.22(0.05)	43.43(0.56)	48.70(12.38)	207.70(44.72)	0.44(0.14)
Rhizomes	0.43(0.14)	0.29(0.04)	45.06(0.60)	122.73(64.25)	147.91(5.76)	1.01(0.09)

Table 3-3. Pairwise treatment comparisons of AM fungal community structure generated using the weighted UniFrac significance test. Differences with *P* values < 0.05 are statistically significant.

	Reference	Invaded	3yr	5yr	7yr
Reference					
Invaded	0.00				
3 yr	0.00	0.11			
5 yr	0.06	0.05	0.07		
7 yr	0.34	0.36	0.01	0.59	

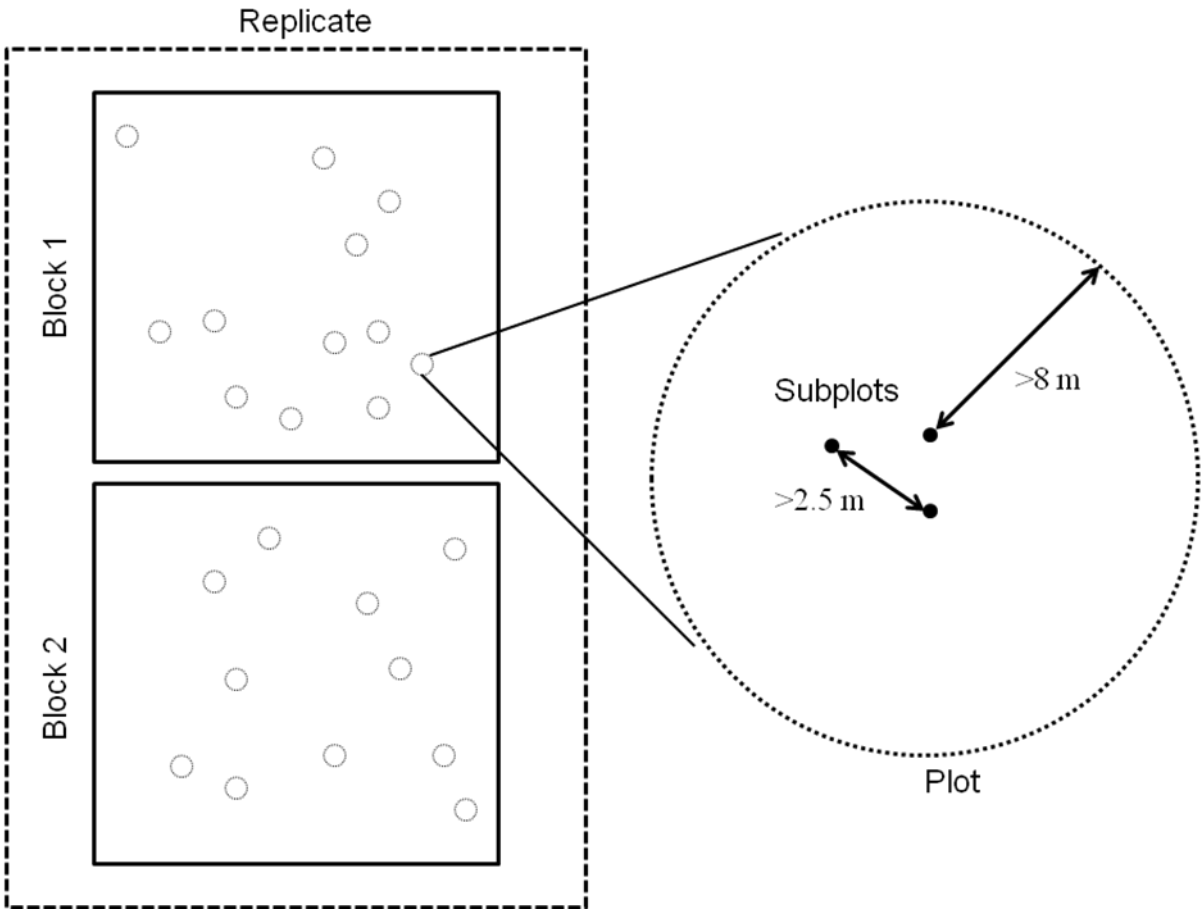

Figure 3-1. Schematic diagram of a replicate (not to scale), with formerly and currently invaded plots indicated as dashed circles. Reference plots, three per block, are randomly scattered in the uninvaded area among the other plots. Close-up view of a plot indicates the location and arrangement of subplots. Blocks are located in longleaf pine sandhill communities in Hernando County, FL, USA.

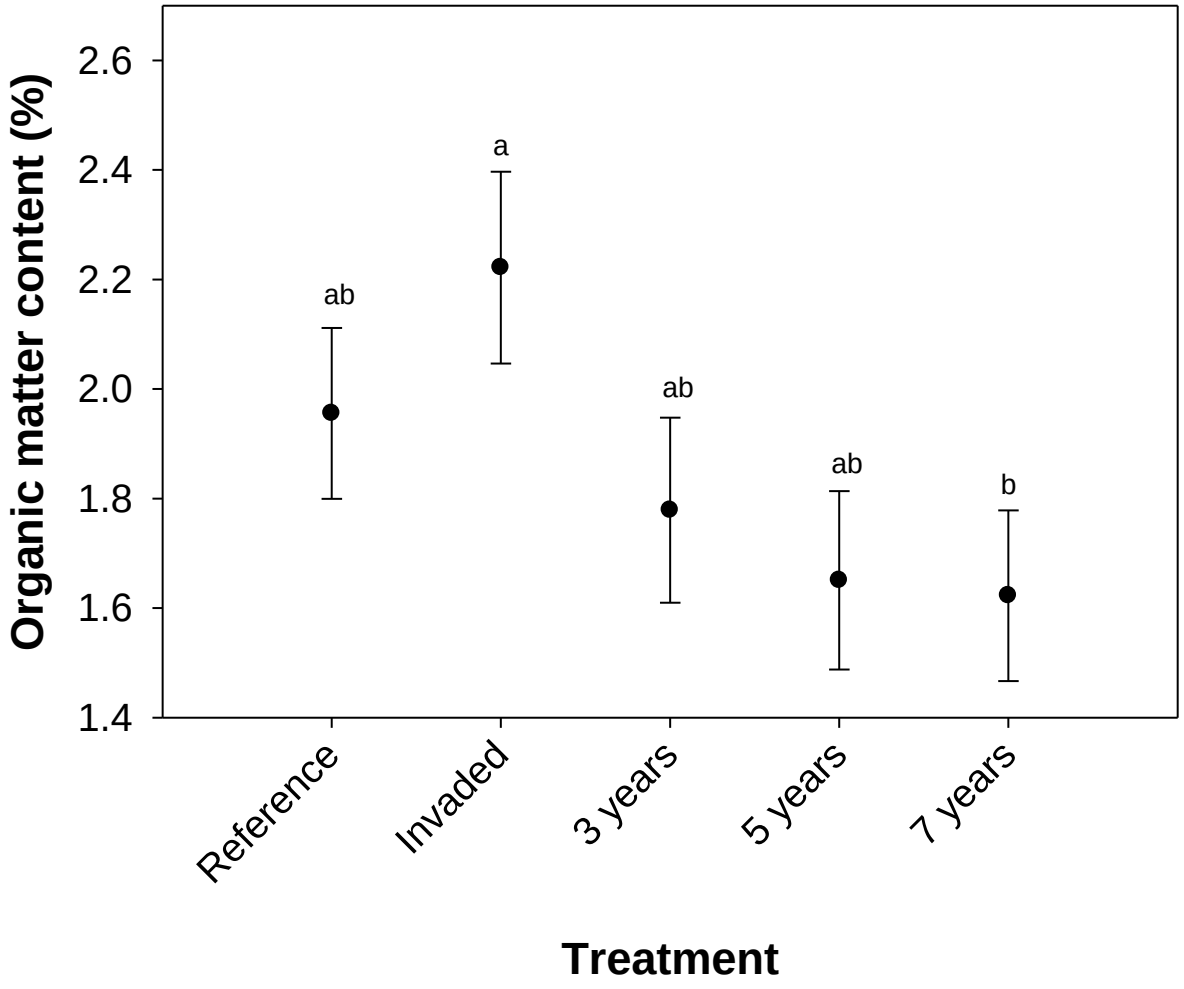

Figure 3-2. Mean soil organic matter content (%) in native reference plots, plots currently invaded by cogongrass and plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill communities in Hernando County, FL, USA. Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

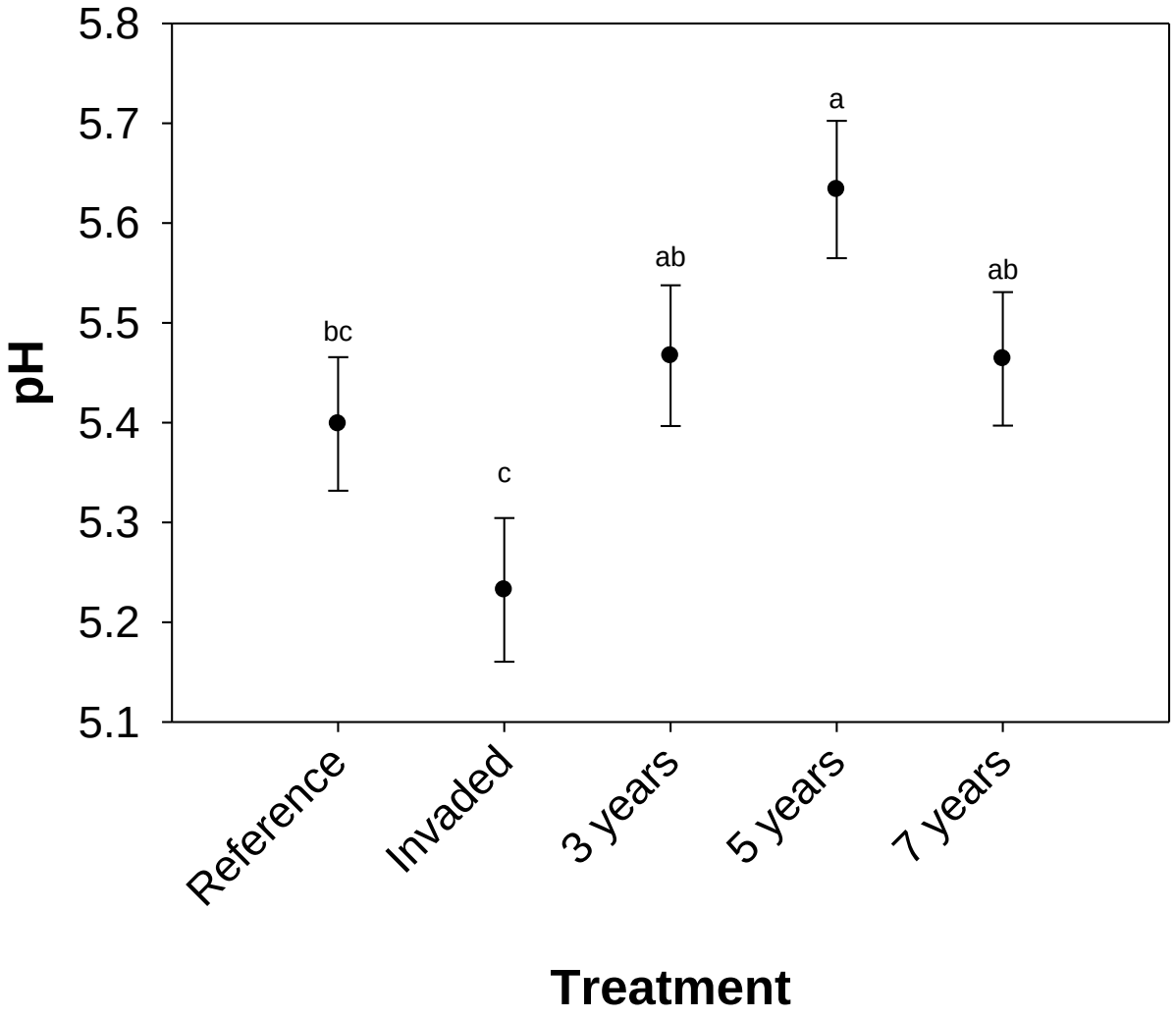

Figure 3-3. Mean water extractable soil pH in native reference plots, plots currently invaded by cogongrass and plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill communities in Hernando County, FL, USA. Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

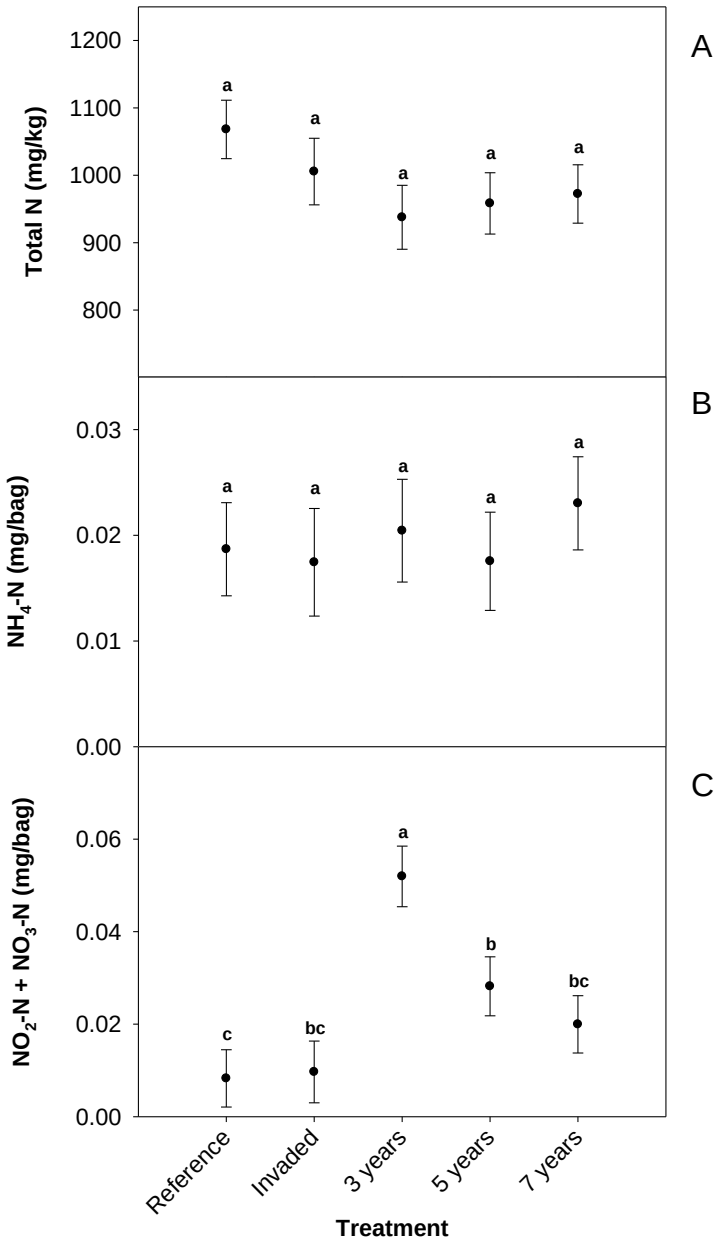

Figure 3-4. Mean total soil nitrogen (TKN method) (A), resin-adsorbed soil ammonium (B) and nitrite+nitrate (C) in native reference plots, plots currently invaded by cogongrass and plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill communities in Hernando County, FL, USA.. Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

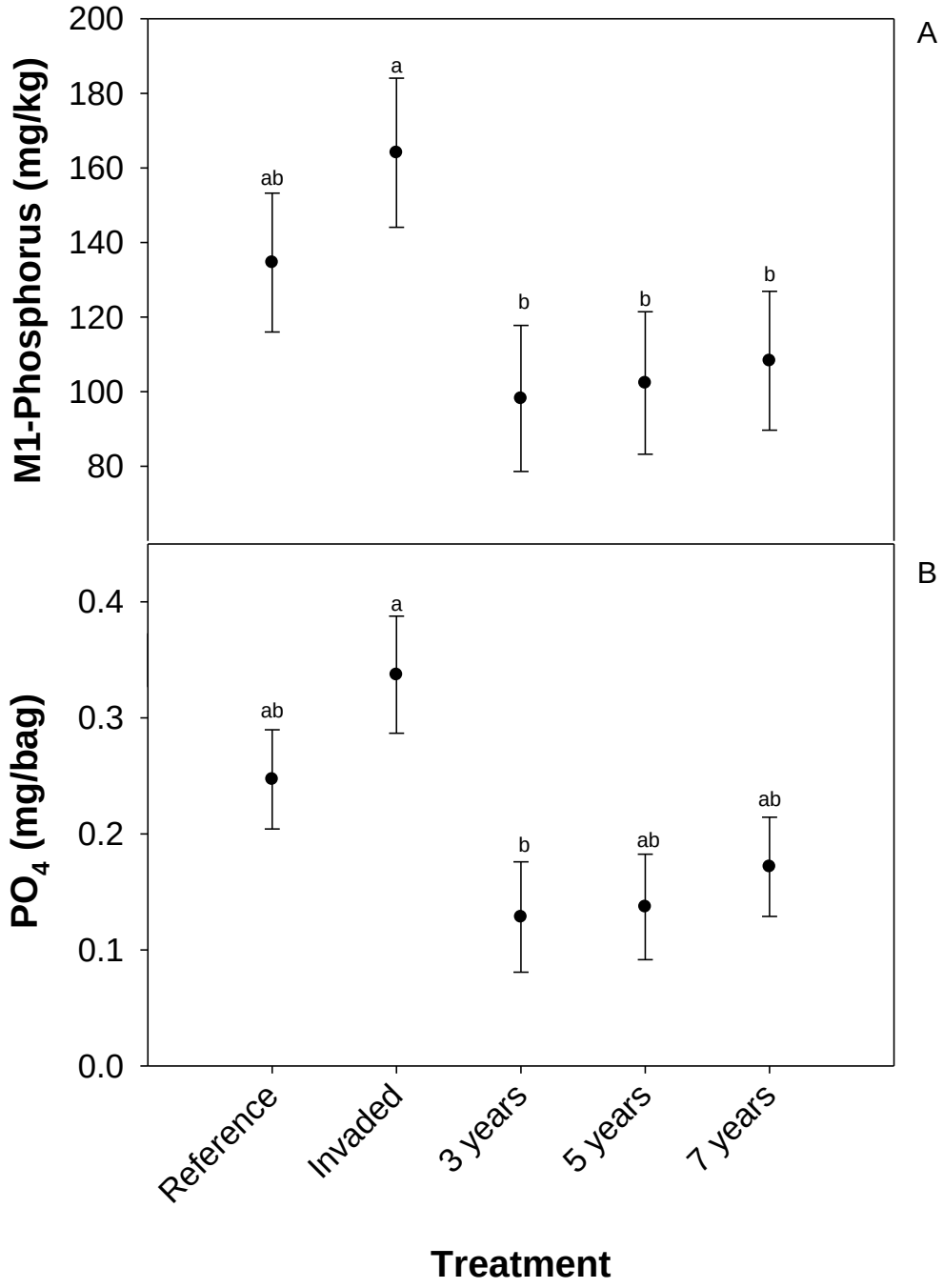

Figure 3-5. Mean Mehlich-1 (M1) extractable phosphorus (A) and resin-extracted soil phosphorus (B) in native reference plots, plots currently invaded by cogongrass and plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill communities in Hernando County, FL, USA. Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

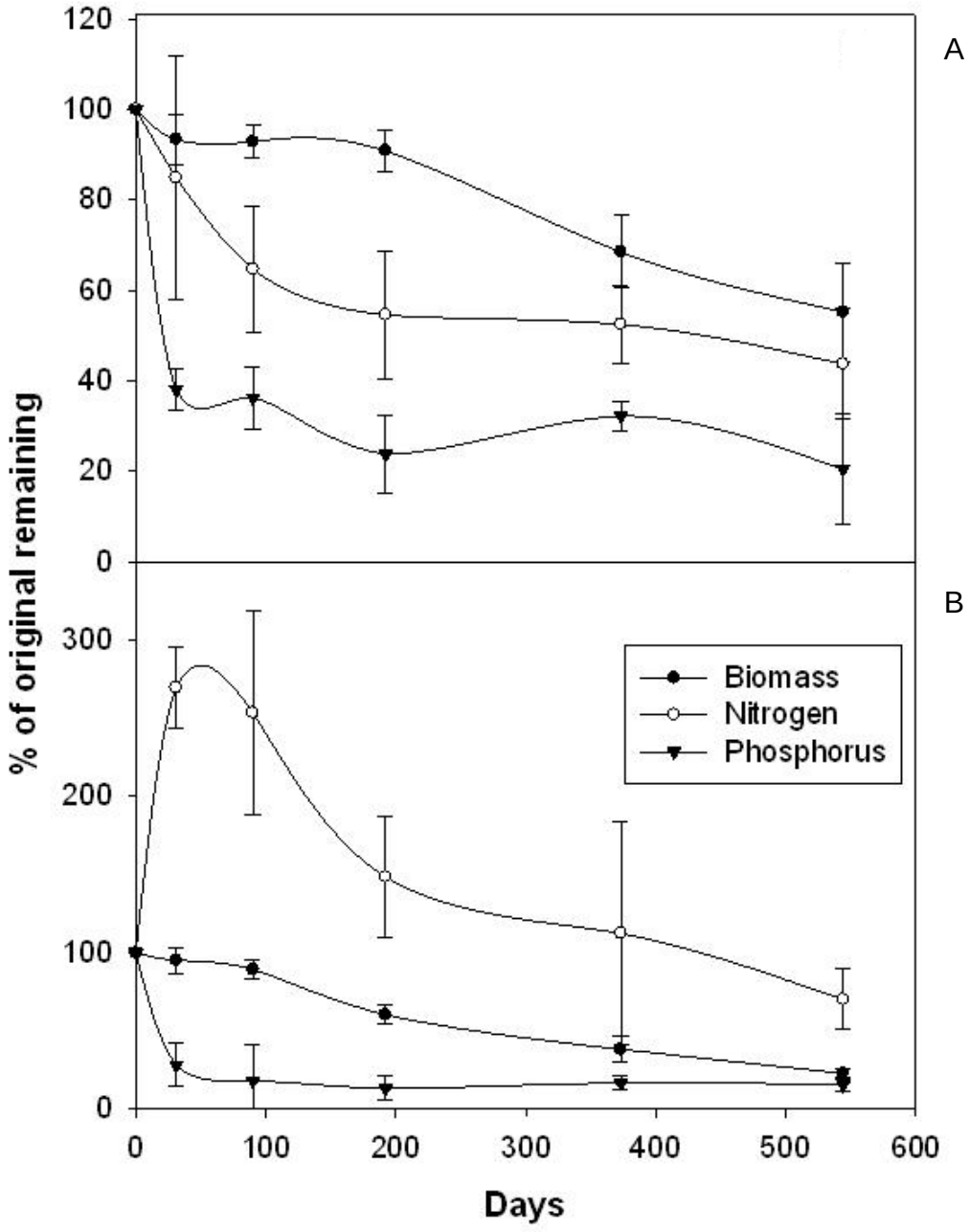

Figure 3-6. Patterns of mass loss and N and P mobilization/immobilization for foliage (A) and rhizomes (B) of cogongrass treated with glyphosate and imazapyr herbicides. Means and standard deviations.

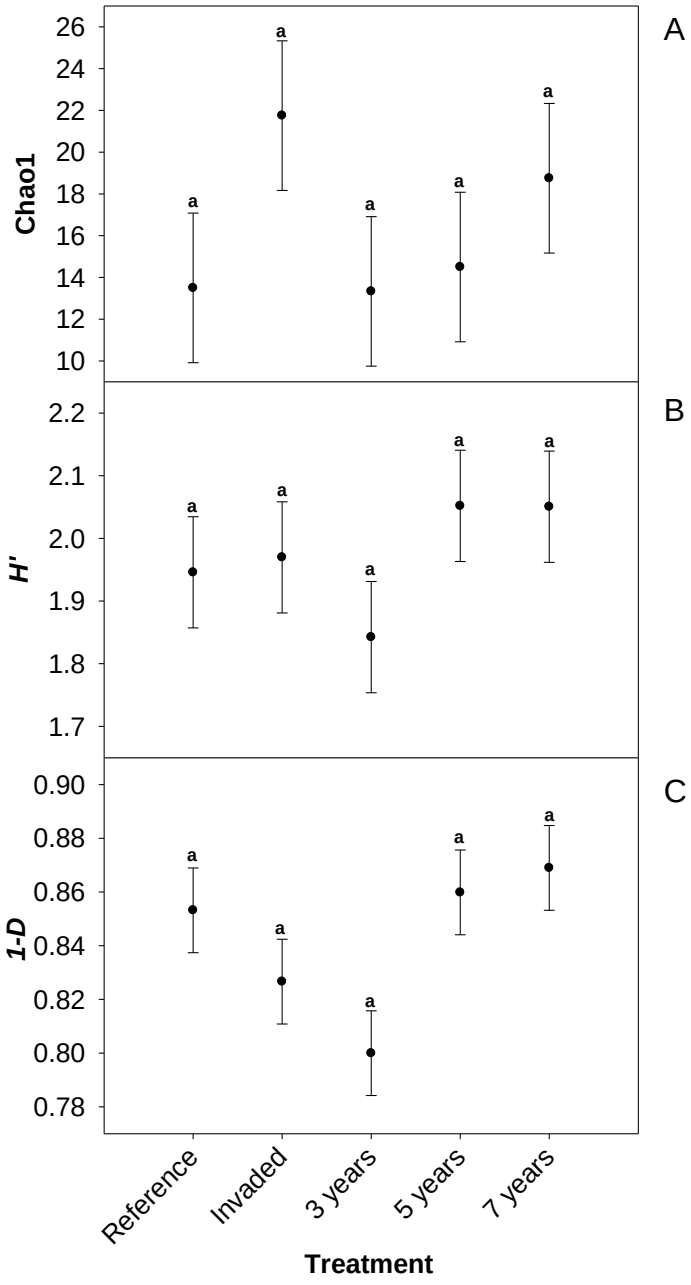

Figure 3-7. Summary statistics (Chao1 richness, Shannon index, 1-Simpson's index), for arbuscular mycorrhizal fungal communities generated in native reference plots, plots currently invaded by cogongrass and plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill communities in Hernando County, FL, USA. Estimates were generated using the summary.single command in MOTHUR (cutoff = 0.03): Chao1 Richness (A), Shannon-Wiener index (B) and 1-Simpson's index (C). Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

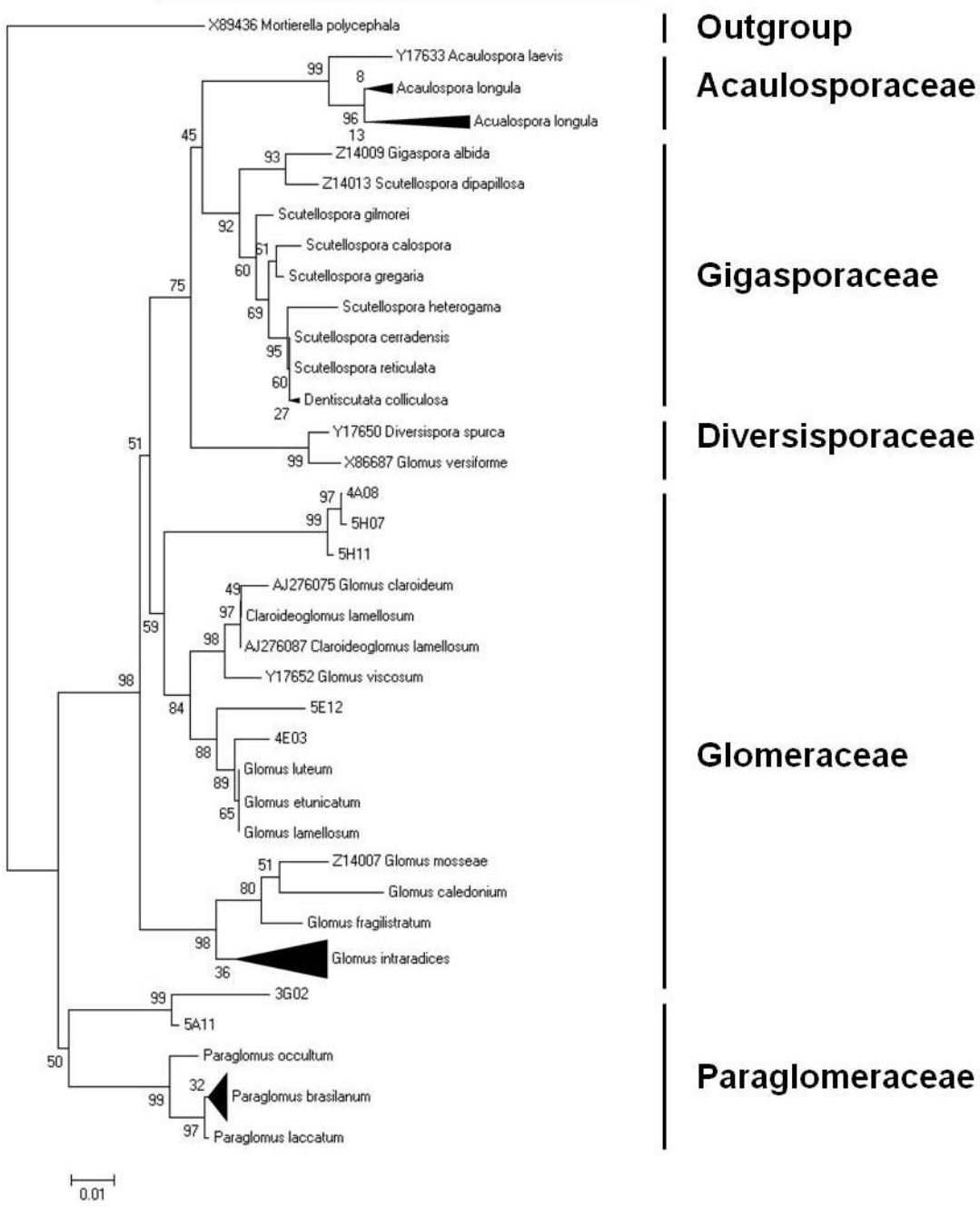

Figure 3-8. Condensed phylogenetic tree of arbuscular mycorrhizal fungal SSU rRNA genes, based on a subset of 61 randomly selected sequences. Interior nodes with bootstrap values less than 50 were collapsed. Sequences from Genbank (preceded with accession numbers) included for reference. Sequences not assigned a genus and specific epithet had < 97% similarity with sequences in Genbank.

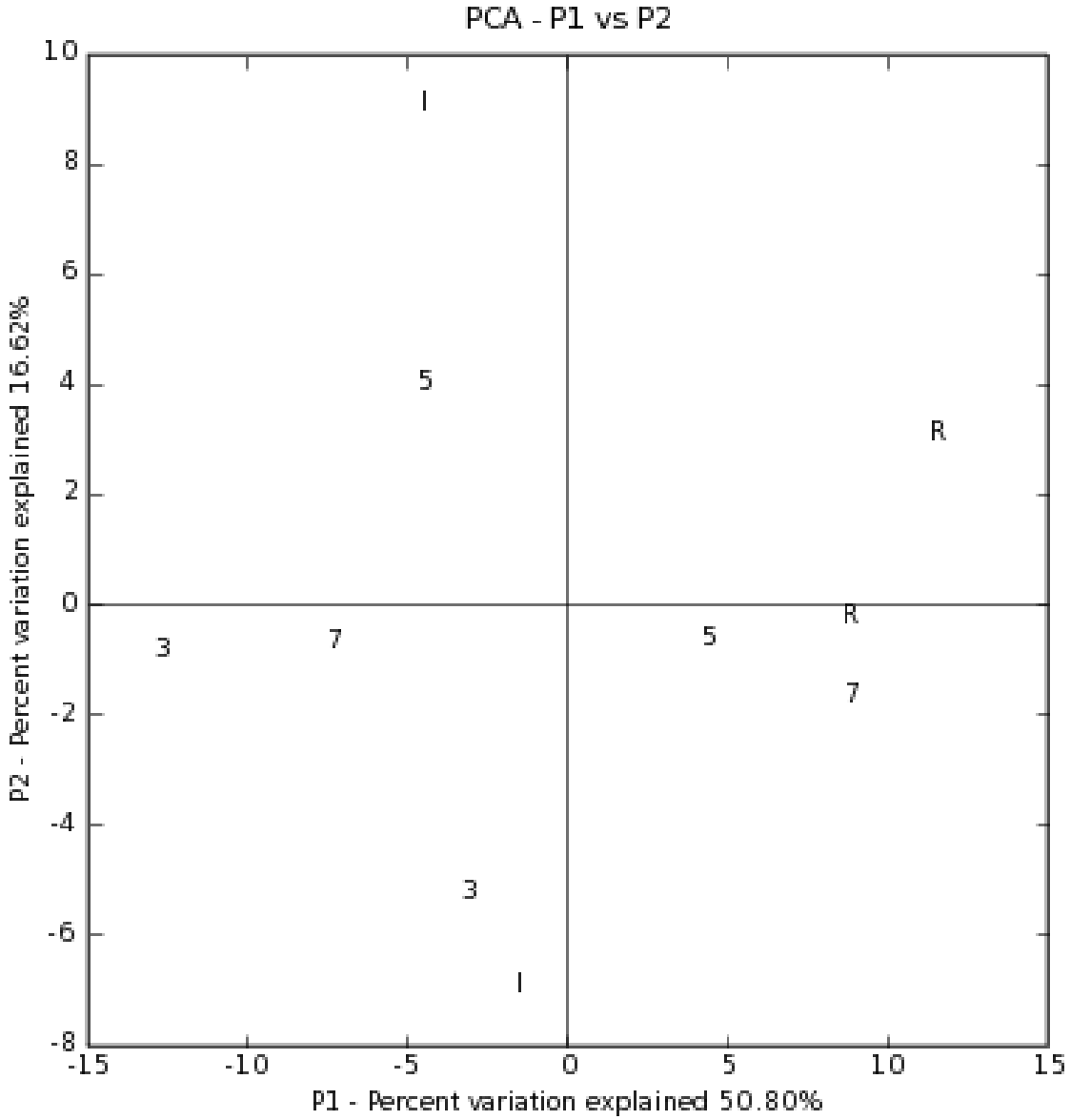

Figure 3-9. Weighted UniFrac PCA biplot illustrating separation in variable space between the mycorrhizal fungal community structure of 2 composite soil samples from each of the five treatments (reference = R; invaded = I; three year = 3; five year = 5; seven year = 7) in longleaf pine sandhill communities in Hernando County, FL, USA.

CHAPTER 4
PATTERNS OF SECONDARY SUCCESSION FOLLOWING COGONGRASS ERADICATION IN A LONGLEAF PINE SANDHILL ECOSYSTEM

Background

The effects of IA plants on natural communities have been well documented in the ecological literature. These impacts include both alterations to plant community assembly and the disruption of important ecological processes (D'Antonio and Vitousek 1992; Gordon 1998; Mack et al. 2001; Hejda et al. 2009). Considerable research attention has also been paid to the species traits that confer invasiveness (Rejmanek and Richardson 1996) and the community characteristics that impart resistance or susceptibility to invasion (Elton 1958; Davis et al. 2000). On the management side, advancements in herbicide chemistry, coupled with research and field trials on integrated approaches have contributed to the development of highly effective species-specific control strategies (Miller et al. 2010). Relatively little attention, however, has been given to the recovery of native plant communities following the removal of problematic IA plant populations from the landscape.

Successional theory suggests that the manipulation or reintroduction of natural processes that control disturbance, colonization and species performance can promote the development of robust native communities following the eradication of IA plant populations (Sheley et al. 2006; Sheley and Krueger-Mangold 2011). This is a critical assumption, because the control of IA plants in natural areas is merely the first step in a restoration process that should also include the re-establishment of desirable native plant species (Ogden and Rejmanek 2005, Miller et al. 2010). In some cases, recruitment of desirable species may occur with little to no assistance. Assuming there are no barriers to establishment, temporal shifts in resource availability should cause

early colonizers such as ruderals and legumes to ultimately give way to later successional species (Tilman 1985) like those that are typically targeted in restoration efforts. Unfortunately, empirical evidence to suggest that ecosystems can recover on their own following the eradication of an invasive plant species remains scant. To explain this, authors have suggested that legacy factors such as altered soil chemical or biological properties may lead to novel successional trajectories following the eradication of IA species (Yelenik et al. 2004; Wolfe and Klironomos 2005; Malcolm et al. 2008; Pyšek and Richardson 2010). The recovery of certain desirable species may be also hindered by dispersal limitation (Seabloom et al. 2003), particularly where seed longevity is short and/or remnant native populations are lacking (Clark et al. 2007).

Cogongrass (*Imperata cylindrica* (L.) P. Beauv.), a pyrogenic C_4 rhizomatous grass, is widely recognized as one of the world's most problematic IA plants (Holm et al. 1977). In total, some 500 million hectares worldwide have some degree of cogongrass infestation. In the US, several hundred thousand hectares are infested (MacDonald 2004), with the current range overlapping much of the historic range of longleaf (*Pinus palustris* Mill.) and slash pine (*Pinus elliottii* Engelm). The sparse canopy that is characteristic of these forests, in concert with frequent fire, allows for high levels of understory diversity, but also makes them very susceptible to transformative impacts from cogongrass (Holzmueller and Jose 2011). Within a few years of invasion, near-monocultures of cogongrass can dominate longleaf pine understories where species richness previously exceeded 20/m^2 (Hagan personal observation). Cogongrass also impedes pine regeneration (Richardson et al. 2007; Daneshgar et al. 2008).

Standard rates of common forestry herbicides have proven effective at controlling cogongrass in longleaf pine systems (Jose 2002), but it is not known if desirable native species will recolonize formerly invaded sites in the years following eradication. In a study of potential legacy effects (Chapter 3), I found that alterations to soil N and P cycling processes develop following cogongrass eradication and persist for up to five years. Concurrent with these changes, I also observed changes in the assembly of the arbuscular mycorrhizal fungal community. Secondary invasions are also cause for concern following eradication (Symstad 2004; Yelenik et al. 2004).

The objective of this study was to assess the patterns and possible drivers of secondary succession following the eradication of cogongrass in a longleaf pine sandhill ecosystem. Specifically, I sought to answer the following four questions:

- Do native species recolonize formerly invaded sites within seven years, or do novel community characteristics persist?
- What soil and environmental factors covary with the observed successional patterns?
- How does cogongrass eradication affect longleaf pine regeneration?
- Are formerly invaded sites susceptible to invasion by other alien plant species in the years following cogongrass eradication?

I hypothesized that formerly invaded sites would, by year seven, begin to regain many of the vegetative characteristics of native reference sites. Specifically, I expected to see increases in total plant cover, increases in species richness and diversity, decreases in dominance and increases in the relative cover of desirable species such as wiregrass (*Aristida stricta* Michx. var. *beyrichiana* Ward) and other pyrogenic native plants. Shifts in community assembly, I hypothesized, would be associated with changes in soil resource availability. I expected that the elimination of cogongrass and

other competing vegetation would facilitate the establishment of longleaf pine seedlings, but would also lead to a secondary invasion of alien plant species, particularly fast growing ruderals that are readily able to take advantage to a post-eradication resource flux.

Materials and Methods

Study Area

The study area was an uneven-aged, naturally regenerated longleaf pine forest in the Croom Tract of Withlacoochee State Forest in Hernando County, Florida (28°36'19.99"N, 82°16'19.73"W). The tract is adjacent to one of the original points of cogongrass introduction in the United States and has a long history of cogongrass invasion. Efforts in recent years to chemically eradicate most cogongrass infestations in the tract have been successful and at the time of this study there were hundreds of areas in various stages of recovery throughout. The uninvaded matrix was characterized by high levels of understory species richness and diversity, as is typical of an actively managed, frequently burned longleaf pine sandhill community. Soils in the study area were predominantly deep, well-drained to excessively drained sands of the Lake and Candler series (hyperthermic coated Typic Quartzipsamments and hyperthermic uncoated Lamellic Quartzipsamments, respectively). Small inclusions of the Arredondo series (Loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults) – comprising less than 20% of the total area – were also present (US Department of Agriculture, 1977). Mean basal area for the study area was 10.02 m^2 ha. Longleaf pine constituted approximately 89% of total basal area.

Experimental Design

Across 4 stands in the study area, I used sites where cogongrass was eradicated in previous years as a recovery chronosequence to assess temporal changes in plant community assembly following eradication. Uninvaded native understory sites, randomly selected from across these same stands, were used as a reference treatment. Sites selected for the chronosequence treatments were treated in the late summer/early fall – approximately three, five and seven years prior, respectively – with a tank mix solution (sprayed to the point of runoff) consisting of 2% Roundup Pro™ (41% glyphosate plus surfactant) and 0.4% Arsenal™ (28.7% imazapyr). Glyphosate and imazapyr tank mixes such as this are among the most common and effective methods of chemical control for cogongrass (MacDonald 2004)[1]. Study sites, hereafter referred to as plots, were identified using Geographic Information Systems (GIS) and with the help of state forest personnel. Native reference plots were ground-truthed to verify that they were not currently invaded and did not fall on disturbed or degraded sites (*e.g.* roads, bicycle trails, abandoned rock mines, formerly invaded sites).

The study was laid out as a complete blocks design, replicated twice. Each replicate consisted of an adjacent pair of 259 ha stands (blocks) with similar, but asynchronous burn histories (both burned approximately every 4 years, but usually staggered 2 years apart). One block in each replicate was burned last in June 2009 and the other was burned in June 2007. Each block contained 2-3 plots from each of the 4 treatments: reference (*i.e.* uninvaded), three years since eradication, five years since

[1] A single herbicide treatment does not always completely eradicate a cogongrass patch. However, for young (≤ 2 year old) patches in the Croom tract, > 95% control is typical. For the purposes of this study, all such patches were considered “eradicated”.

eradication, and seven years since eradiation (Table 4-1). Within each plot, three 3 m^2 vegetation sampling subplots were randomly selected, each being at least 2.5 meters from the others, at least 8 m from the edge of the plot and distant from any cogongrass re-sprouts (where applicable)[2].

Sampling Protocol

In September 2010, plants in each vegetation sampling subplot were identified and the cover of each species was visually estimated. Coverage values were then used to compute relative cover (P_i) for each species. Relative cover is defined as the percentage that a species contributes to the total cover of all species in a given location (Bazzaz 1975). Additionally, I quantified longleaf pine stem density (seedlings + saplings) in each subplot. Cover data were also used to determine species richness (mean number of species in three 3 m^2 subplots) and to calculate commonly used indices of community diversity and evenness. The Shannon-Wiener diversity index (*H'*), was calculated as follows:

$$-\sum P_i \ln(P_i)$$

The Simpson's index (*D*) was calculated as follows:

$$1/(\sum P_i^2/S)$$

For both indices, *S* represents the total number of species (Wilsey and Potvin 2000). For the purposes of this study, I expressed the Simpson index as 1-*D*, so that higher values (*i.e.* approaching 1) indicate greater species evenness (Jones et al. 2009).

[2] Vegetation sampling subplots were centered on the soil sampling subplots from Chapter 3.

Species were classified by functional group (*i.e.* forb, graminoid, tree, shrub, vine) using the growth habit categories from the USDA Plants Database (US Department of Agriculture 2012). The same database was also used to determine the nativity status and persistence of the identified species. Plot values for plant cover (including associated indices, described below), along with longleaf pine seedling and sapling stem counts, were calculated as the mean of the three subplots. Longleaf pine stem counts were converted to stems/m^2 for analysis. Data that met parametric assumptions were analyzed in SAS 9.2 (SAS Institute, Inc.), using the MIXED procedure. Replicate and block(replicate) were treated as random effects. The Kenward-Roger calculation, a preferred method for unbalanced mixed models (Spilke et al. 2005), was used to estimate denominator degrees of freedom[3]. For the five treatments, differences between means were declared statistically significant at $P < 0.05$ and Tukey's post-hoc test was used for pairwise comparisons. Due to their non-parametric nature (*i.e.* the preponderance of zero or one values), comparisons of longleaf pine seedling/sapling stem counts, annual cover and nonnative cover were done with the Kruskal-Wallis test, using the Wilcoxon-Mann-Whitney test ($P < 0.05$) for pairwise comparisons (SAS 9.2; NPAR1WAY procedure). A Mantel test (PC-ORD 5; McCune and Grace 2002) was used to compare a matrix of species P_i values to a secondary matrix of selected soil and environmental variables (Table 4-2) from Chapter 3. This procedure tests the null hypothesis of no relationship between matrices (McCune and Grace 2002). After the Mantel test indicated a relationship ($P = 0.025$), I compared plant community assembly between treatments using a canonical discriminant analysis (CDA; JMP 9) based on the

[3] This method can result in non-integer values for denominator degrees of freedom.

P_i values of the predominant species ($P_i \geq 1\%$) in reference plots. Variables from the secondary matrix were included in this analysis. The CDA is an eigenanalysis technique in which variables are used to predict group membership (McCune and Grace 2002). In this case, the groups were the four treatments. Dominant variables (standardized scoring coefficients > |1|) that influenced the two most important canonical axis are reported. For a detailed description of the soil sampling and analysis protocol for the variables used in the Mantel test and CDA, see Chapter 3.

Results

Cover, Richness and Diversity

Total percent plant groundcover showed a significant increase ($F_{(3, 35.07)} = 15.62$, $P < 0.0001$) in the years following cogongrass eradication. Mean cover averaged 20.2% after three years, and rose to 36.9% and 44.9% after five and seven years, respectively. By year seven, cover was not significantly different from the reference treatment (52.3%). Shannon-Wiener diversity indices (*H'*) showed a pattern of increasing understory plant diversity with increasing time since cogongrass eradication ($F_{(3, 35.31)} = 23.35$, $P < 0.0001$). Specifically, values were significantly lower than reference (2.01) three and five years post eradication (1.32 and 1.39, respectively) before recovering to near reference levels by seven years (2.07). The distinct increase in *H'* that occurred between years five and seven was statistically significant. A similar trend was observed for the Simpson's index (1-*D*), with the values observed after three and five years (0.64 and 0.64, respectively) being significantly lower (less evenness) than the seven year (0.80) and reference (0.77) treatments ($F_{(3, 35.35)} = 11.10$, $P < 0.0001$). Species richness also increased with time, again being lowest at three and five years (6.02 and 7.14 species/plot) and increasing significantly by year seven (13.05 species/plot) ($F_{(3,35.10)} =$

53.35, $P < 0.0001$). The latter was not significantly different from the reference treatment (14.71) (Table 4-3).

Relative Groundcover by Growth Habit and Persistence

Forb cover, as a percent of total cover, was highest five years after eradication (61.8%), followed by three years (51.2%), seven years (38.2%) and reference (24.8%) ($F_{(3, 35.10)} = 12.80$, $P < 0.0001$). Relative graminoid cover was highest after three years (35.8%), followed by seven years (36.5%), five years (22.7%) and reference (12.8%) ($F_{(3, 35.09)} = 8.27$, $P = 0.0003$). Understory tree cover, on the other hand, showed a different trend, increasing from 3.8% at three years to 12.1% and 14.9% and five and seven years, respectively. All of these coverage values, however, were significantly lower than reference (48.0%) ($F_{(3, 35.21)}$, = 36.27, $P < 0.0001$). Shrub cover also increased after eradication (4.5%, 2.9% and 8.4% for three five and seven year plots, respectively; ($F_{(3, 35.18)} = 3.89$, $P = 0.0168$), the latter not being significantly different from reference (12.7%). Vine cover averaged 1.9% and did not vary significantly between treatments (Figure 4-1). Relative cover of annual vegetation was lower in the native reference plots (2.2%) than in formerly invaded plots (8.1, 8.2 and 6.7% in three, five and seven year plots, respectively), but these differences were not statistically significant ($chi^2 = 4.954$, $P = 0.1752$).

Dominant Species

A total of 100 plant species were identified in the understory of the study plots. Seventy eight of these species were found in the native reference plots. Of these 78, 23 had relative groundcovers greater than or equal to 1%. Ten of these species were forbs, five were graminoids, five were trees and three were shrubs. All 23 species were perennials. Bluejack oak (*Quercus incana* W. Bartram) was the most dominant species

in reference plots (Table 4-4). The majority of the dominant species in formerly invaded plots were graminoids and forbs, one of which (S*etaria corrugata* (Elliot) (prevalent in three, five and seven year plots) was an annual. Dogfennel (*Eupatorium capillifolium* (Lam.) Small ex Porter and Britton) was the most dominant species three and five years following eradication and *S. corrugata* was the dominant species after seven years (Table 4-5).

Multivariate Analyses

The CDA analysis was quite robust (0% treatment misclassification) and indicated considerable variability between treatments ($P < 0.0001$). The first two canonical axes cumulatively accounted for 94.7% of the total variation (77.0 and 17.7% respectively). For axis 1, the most influential variables, based on standardized discriminant function coefficients, were *Quercus incana* (-2.14), organic matter (1.78), *Q. laurifolia* (-1.52), TKN (-1.43), *Desmodium* sp. (1.21), *Q. margaretta* (-1.11), pH (1.05) and M1-P (-1.03). For axis 2, the most influential variables were arbuscular mycorrhizal spores (-1.90), pH (1.50), *Polygala* sp. (-1.20), *Diospyros virginiana* (1.19) and M1-P (1.16). Overall, there was no clear indication that formerly invaded plots begin to approach a reference state over time (Figure 4-2A). A different pattern, however, was observed when woody species (which managers may consider undesirable) were eliminated from the analysis. This modification still resulted in a high degree of treatment separation ($P < 0.0001$), but distance between treatments – particularly between reference plots and formerly invaded plots – was much less distinct. Axes 1 and 2 cumulatively accounted for 93.4% of total variation (70.6 and 22.8%, respectively). For axis 1, the most influential variables were *Polygala* sp. (1.58), arbuscular mycorrhizal spores (1.57), M1-P (-1.48), *Paspalum*

sp. (1.37) and organic matter (1.05). For axis 2, the most influential variable was *Eupatorium capillifolium* (1.07) (Figure 4-2B).

Longleaf Pine Regeneration

Longleaf pine regeneration (seedling and sapling stems/m^2) varied greatly among the four treatments. Stem counts were highest five and seven years following cogongrass eradication (0.37 and 0.26/m^2, respectively) and lowest in reference plots (0.02). These differences were statistically significant (chi^2 = 17.84, P = 0.0005). Stem counts in three year plots (0.14) were not significantly different from the other treatments (Figure 4-3).

Nonnative Species

All 23 of the most prevalent species in the study plots were native, but nonnative plant species were found in all treatments. A total of two nonnative plant species, both legumes (white clover (*Trifolium repens* L.) and hairy indigo (*Indigofera hirsuta* L.)), were identified. Relative nonnative cover (both species combined) was 1.4% in treated plots and 0.1% in reference plots. Mean relative cover of *T. repens* ranged from 0 to 11.9%, with a mean of 0.7%. While *T. repens* cover was highest in plots where cogongrass was eradicated three years prior (1.9% vs. 0.7, 0.5 and 0.01% for five year, seven year and reference plots, respectively), these differences were not statistically significant (chi^2 = 3.5214, P = 0.2678). For *I. hirsuta*, relative cover ranged from 0 to 6.3% with a mean of 0.3%. This species was only found in seven year and reference plots (0.1% and trace, respectively) and differences between these treatments were not statistically significant (chi^2 = 0.4943, P = 0.4820).

Discussion

Understory Community Assembly

The late summer/early fall application of a glyphosate + imazapyr herbicide tank mix effectively eliminated nearly all vegetation (including remnant natives) from cogongrass invaded longleaf pine sandhill sites (Hagan, personal observation). It can be assumed, therefore, that the plants observed in treated plots originated from the soil seed bank, recruitment/encroachment from adjacent unimpacted areas, or the persistence of scattered individuals not killed as a by-product of cogongrass treatment. Steady increases in plant cover following eradication can be attributed to these factors, along with the growth and spread of newly established individuals (Huston and Smith 1987). The fact that total understory plant cover for the first five years remained significantly lower than in the reference treatment suggests that there was still available space for additional recruitment and expansion. It is also arguably the most readily observable indication that the effects of cogongrass invasion and eradication on native sandhill plant communities persist for several years.

Along with the availability of sites for establishment, species availability and species performance are ultimately what dictate which species colonize following disturbance (Pickett et al. 1987). Typically, early successional seres are characterized by low species diversity and richness and high species dominance (low evenness) (Huston and Smith 1987). This is consistent with the post-eradication findings from the first five years of recovery. The significant increase in diversity by year seven was associated with an increase in species richness and an increase in evenness, but the ecological explanation for this spike is unknown. Similar levels of diversity, evenness and richness between the seven year and reference plots suggest that the complexity –

though not composition – of formerly invaded sites approaches reference levels over time.

Patterns and Environmental Covariates of Species Colonization

Successional theory suggests that shifts in understory composition in the years following cogongrass eradication reflect differences in plant functional strategies and changes in resource availability (Tilman 1985; Grime 1985). The graminoids and forbs which dominated the early stages of succession were likely the species that were best suited to rapidly capitalize on a post-eradication resource flux. After seven years, many of these species were still dominant, perhaps due to multiple burns which helped to maintain a subclimax state. While some recruitment of trees and shrubs occurred following eradication, the significant reductions in woody species cover in formerly invaded plots – particularly non-pyrogenic species like oaks – may be viewed favorably by restoration ecologists (Provencher et al. 2001; Walker and Silletti 2006). The relative lack of wiregrass cover in treated plots (cover < 1.1% three, five and seven years post-eradication), however, is problematic, since it is considered a keystone species for the longleaf pine ecosystem (Noss 1989; Mulligan et al. 2002). Reductions in fuel connectivity, caused by the decreased herbaceous component and reductions in total groundcover, may have altered the behavior of the low intensity ground fires that are considered essential for the maintenance of these systems (Landers 1989). Additionally the loss of nutrient-rich woody browse may be detrimental to populations of native ungulates (Pearson and Sternitzke 1976).

Multivariate analytical techniques such as CDA provide a useful index to visually and quantitatively assess complex ecological questions and have proven useful in chronosequence studies (Matthews 1979; Stylinski and Allen 1999; Frouz et al. 2008).

In this study, while there was distinct separation between the four treatments, there was little evidence that formerly invaded plots began to approach a reference state in the first seven years following eradication, especially when woody species are included in the model. Stylinski and Allen (1999) reported a similar trend in severely degraded shrubland ecosystems in California. These authors attributed the lack of native species recovery to the severity of disturbance (a combination of anthropogenic soil disturbance and alien plant invasions) that their study sites had experienced. The passing of a resistance threshold, they proposed, lead to the development of an alternative stable state characterized by novel species assemblages. While my study plots had not been subject to severe disturbance, perhaps the invasion – and subsequent eradication – of a functionally novel grass had a similar effect on successional processes. Indeed, substantial, but temporary, alterations to soil biogeochemistry were shown to develop in the years following cogongrass eradication (Chapter 3) and the results of the multivariate analyses suggest that these alterations to the soil environment may play a role in determining successional trajectories.

Most studies on longleaf pine regeneration have focused on the effects of competition from overstory trees (Brockway and Outcalt 1998; McGuire et al. 2001). In uninvaded areas throughout the Croom Tract, large numbers of longleaf pine seedlings and young saplings can typically only be found in canopy gaps where substantial reductions in basal area have occurred due to lightning or disease. My longleaf pine stem counts, however, highlight the role of understory competition. High seedling and sapling stem counts for longleaf pine in formerly invaded plots, relative to reference plots, suggest that the conditions suitable for regeneration are enhanced following

cogongrass eradication. Since juvenile longleaf pines are known to be poor competitors for water, nutrients and light (Jose et al. 2003), it is likely that the elimination of cogongrass and most other competing vegetation helped facilitate the establishment of this desirable overstory species.

No evidence of cogongrass re-invasion was observed in the study plots or in other formerly invaded sites across the Croom Tract. Relative covers of other nonnative species were generally (albeit not significantly) higher in treated plots than in reference plots, but this does not appear to constitute a secondary invasion. Both *T. repens* and *I. hirsuta* are naturalized throughout Florida (US Department of Agriculture 2012) and neither is listed by the Florida Exotic Pest Plant Council as species that is likely to substantially alter native plant communities (FLEPPC 2009). Both species were introduced to the study area many years prior as forage crops (Vincent Morris, personal communication) and hardseededness and the presence of dispersal agents (*e.g.* deer, horses, *etc*.) likely permitted their spread outside of the original areas of cultivation (Sulas et al. 2000). Their presence in formerly invaded plots may simply reflect the successional status of these plots, as legumes are one of the functional groups most characteristic of early secondary succession in longleaf pine systems, partly due to a nitrogen limitation exacerbated by frequent fire (Lajeunesse et al. 2006). A companion study (Chapter 3), however, showed little evidence of a post eradication nitrogen limitation. This could indicate that dispersal limitations impede the reestablishment of nitrophilic plant species.

Summary and Implications

Cogongrass is becoming a major threat to the ecological integrity of longleaf pine ecosystems in the southeastern US. As such, there is a growing need to develop

effective strategies for the restoration of native understory communities following cogongrass eradication. By shedding light on the successional dynamics of longleaf pine sites formerly invaded by cogongrass, the seven year post-eradication chronosequence provides valuable information about the feasibility of passive regeneration as a restoration option. However, the results of this study only partially support the original hypotheses. While the diversity and complexity of formerly invaded plots increased in the years following eradication, the species composition remained markedly different from that of native reference plots. If recovery is occurring, it is likely proceeding at a slower-than-desirable pace. The substantial reductions in woody species cover observed following cogongrass eradication may, however, be viewed favorably by some land managers. As expected, differences in community assembly were, to an extent, associated with variability in soil properties. The presence of large numbers of longleaf pine seedlings and saplings in formerly invaded plots is a positive sign and suggests that the removal of cogongrass (and most other understory vegetation) alleviates a substantial recruitment limitation. The lack of a secondary invasion, which is contrary to one hypothesis, is also encouraging. Future studies should seek to determine the specific soil and environmental factors that limit the dispersal or recruitment of desirable native species. Manipulative studies and a longer-term chronosequence would also be beneficial.

Table 4-1. Number of study plots in each in each treatment x block x replication combination in longleaf pine sandhill communities in Hernando County, FL, USA.

Treatment	Replicate 1		Replicate 2	
	Block 1	Block 2	Block 1	Block 2
Reference	3	3	3	3
3 years	2	3	3	2
5 years	3	2	3	3
7 years	3	3	3	3

Table 4-2. Landscape and soil variables for a canonical discriminant analysis (CDA), used along with the relative covers of the 23 most dominant plant species in reference plots, to assess the patterns secondary succession in plots formerly invaded by cogongrass. Abbreviations provided.

Variable	Abbreviation	Mean (SE)
Basal area (m^2)	BA	10.02 (0.63)
Soil pH	pH	5.49 (0.03)
Total soil P (mg/kg)	M1-P	113.18 (4.67)
Total soil N (mg/kg)	TKN	955.25 (22.31)
Soil organic matter (%)	OM	1.67 (0.08)
Mycorrhizal inoculum (spores/g)	Spores	12.54 (1.22)

Table 4-3. Mean values for the Shannon-Wiener (*H'*) and Simpson's (1-*D*) Indices and species richness (mean number of species in three 3 m^2 subplots) in plots where cogongrass was eradicated three, five and seven years prior, along with uninvaded native reference plots, in longleaf pine sandhill stands in Hernando County, FL, USA. Means and standard errors. Means having different lowercase letters are statistically different at $P < 0.05$.

Treatment	*H'*	1-*D*	Richness	Total cover (%)
3 years	1.33 a	0.64 a	6.02 a	20.20 a
	(0.08)	(0.03)	(0.64)	(3.62)
5 years	1.39 a	0.64 a	7.15 a	36.91 b
	(0.08)	(0.03)	(0.61)	(3.46)
7 years	2.07 b	0.80 b	13.05 b	44.92 bc
	(0.08)	(0.03)	(0.58)	(3.29)
Reference	2.01 b	0.77 b	14.71 c	52.33 c
	(0.08)	(0.03)	(0.58)	(3.29)

Table 4-4. Mean relative cover of the 23 most prevalent understory species (relative cover > 1%) in reference plots, compared to their relative covers in plots where cogongrass was eradicated three, five and seven years prior, in longleaf pine sandhill stands in Hernando County, FL, USA. CDA abbreviations provided.

Species	CDA	Growth habit	Treatment			
			3 yr	5 yr	7 yr	Ref
Quercus incana W. Bartram	QuI	Tree	0.00	1.37	3.88	20.40
Quercus margaretta Ashe ex Small	QuM	Tree	0.00	0.57	1.58	10.95
Quercus laurifolia Michx.	QuA	Tree	1.35	1.49	2.78	10.04
Morella cerifera L.	MoC	Shrub	0.74	0.00	2.84	6.65
Pteridium aquilinum (L.) Kuhn var. pseudocaudatum (Clute)Clute	PtA	Forb	0.48	3.43	5.45	5.22
Quercus virginiana Mill.	QuV	Tree	0.40	0.73	0.92	4.29
Aristida stricta Michx. var. *beyrichiana* Ward	ArS	Graminoid	0.15	0.73	1.08	2.65
Rubus argutus Link	RuA	Shrub	2.06	0.25	1.66	2.30
Dichanthelium laxiflorum (Lam.) Gould	DiL	Graminoid	4.09	1.53	3.10	2.15
Paspalum sp.	Pas	Graminoid	9.01	2.99	5.68	2.11
Elephantopus carolinianus Raeusch.	ElC	Forb	2.32	0.51	1.92	1.97
Eupatorium capillifolium (Lam.) Small ex Porter and Britton	EuC	Forb	13.68	36.62	9.82	1.89
Dyschoriste oblongifolia (Michx.) Kuntze	DyO	Forb	1.49	1.07	1.59	1.81
Dichanthelium aciculare (Desv. ex Poir.) Gould and C.A.Clark	DiA	Graminoid	1.38	0.85	2.41	1.65
Diospyros virginiana L.	DiV	Tree	0.00	1.25	0.78	1.57
Rhus copallinum L.	RhC	Shrub	0.12	1.75	0.31	1.34
Desmodium sp.	Des	Forb	0.31	0.87	1.36	1.31
Stillingia sylvatica L.	StS	Forb	0.20	0.17	0.83	1.23
Sorghastrum secundum (Elliott) Nash	SoS	Graminoid	0.44	2.81	1.18	1.18
Eupatorium pilosum Walter	EuP	Forb	7.36	0.24	0.55	1.16
Desmodium floridanum Chapm.	DeF	Forb	0.14	0.19	0.23	1.06
Polygala sp.	Pol	Forb	2.31	0.54	0.53	1.02
Galactia regularis (L.) Briton et al.	GaR	Forb	1.56	0.23	0.83	1.01

Table 4-5. Top 5 dominant species in plots where cogongrass was eradicated three, five and seven years prior in longleaf pine sandhill stands in Hernando County, FL, USA. Abbreviations and relative covers (%) in parentheses. See Table B-1 (Appendix) for full names.

Rank	Treatment		
	3 years	5 years	7 years
1	*Eupatorium capillifolium* (F, P) (13.68)	*Eupatorium capillifolium* (F, P) (36.62)	*Setaria corrugata* (G, A) (10.03)
2	*Paspalum* sp. (G, P) (9.01)	*Pinus palustris* (T, P) (6.11)	*Eupatorium capillifolium* (F, P) (9.82)
3	*Setaria corrugata* (G, A) (7.93)	*Setaria corrugata* (G, A) (4.57)	*Andropogon arctatus* (G, P) (8.11)
4	*Eupatorium pilosum* (F, P) (7.36)	*Rhynchosia michauxii* (F, P) (3.81)	*Paspalum* sp. (G, P) (5.68)
5	*Andropogon arctatus* (G, P) (5.61)	*Pteridium aquilinum* (F, P) (3.43)	*Pteridium aquilinum* (F, P) (5.48)

A = annual; F = forb; G = graminoid; P = perennial; T = tree

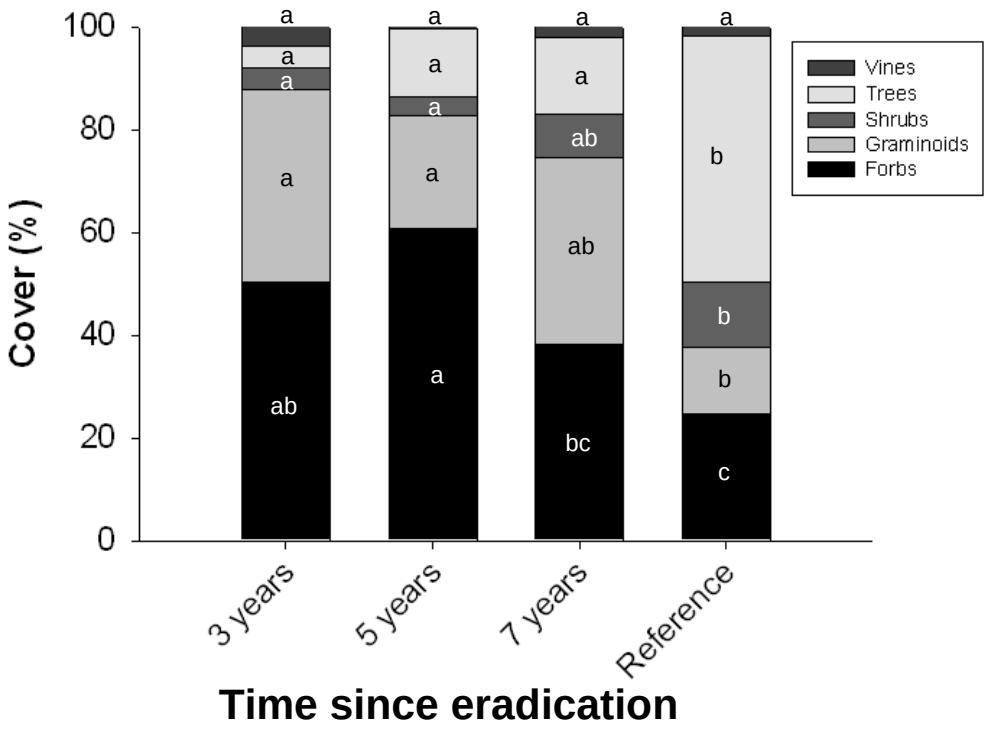

Figure 4-1. Mean relative cover (%) by growth habit type in plots where cogongrass was eradicated three, five and seven years prior, along with uninvaded native reference plots, in longleaf pine sandhill stands in Hernando County, FL, USA. Means having different lowercase letters are statistically different at $P < 0.05$.

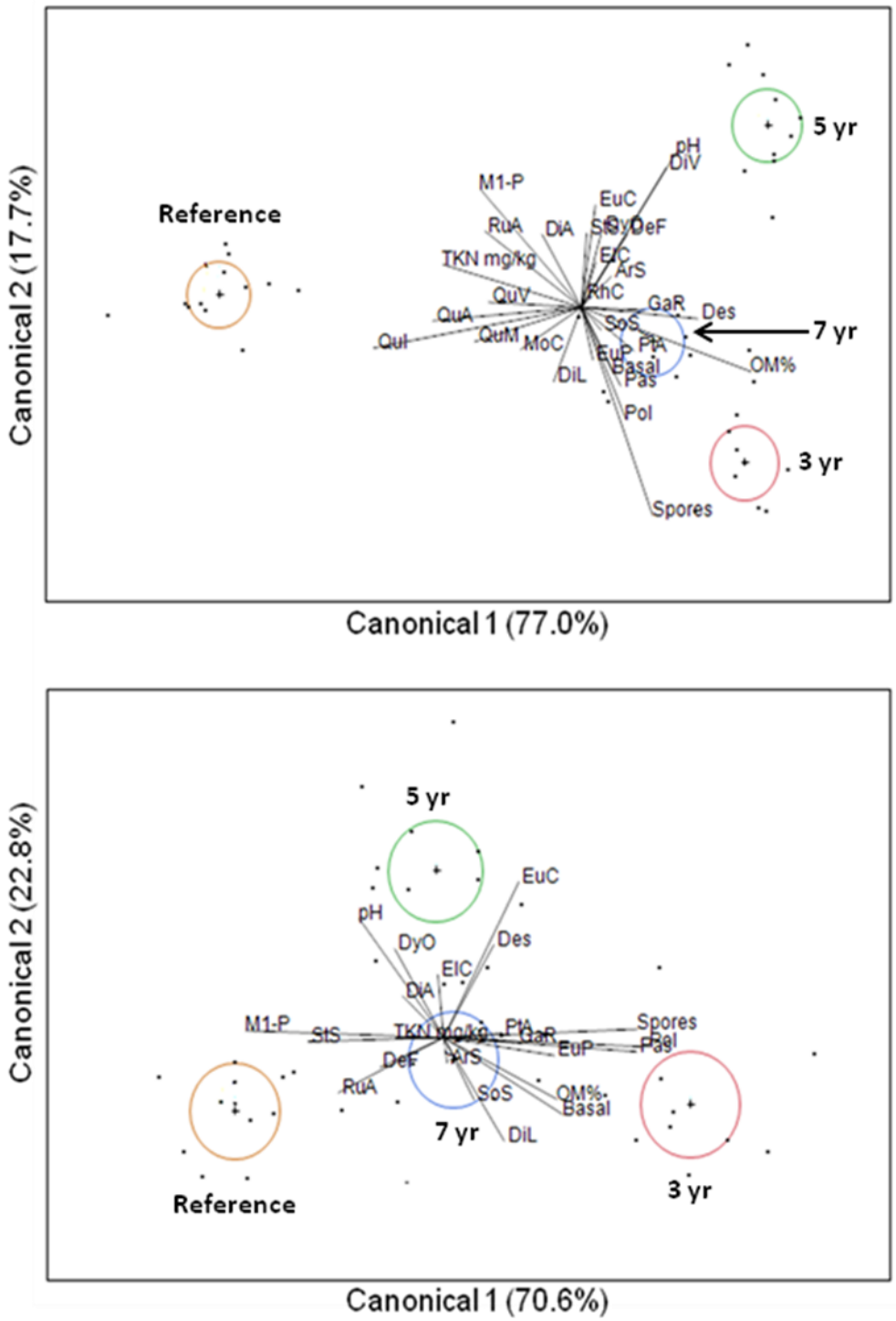

Figure 4-2. Canonical discriminant analysis (CDA) biplot (with 95% confidence circles) of the patterns of compositional similarity between native reference plots and plots where cogongrass was eradicated three, five and seven years prior (A). Additional CDA with woody species removed (B). Circles represent the 95% confidence intervals for each treatment. Each point represents one plot. Abbreviations are provided in Tables 4-2 and 4-4.

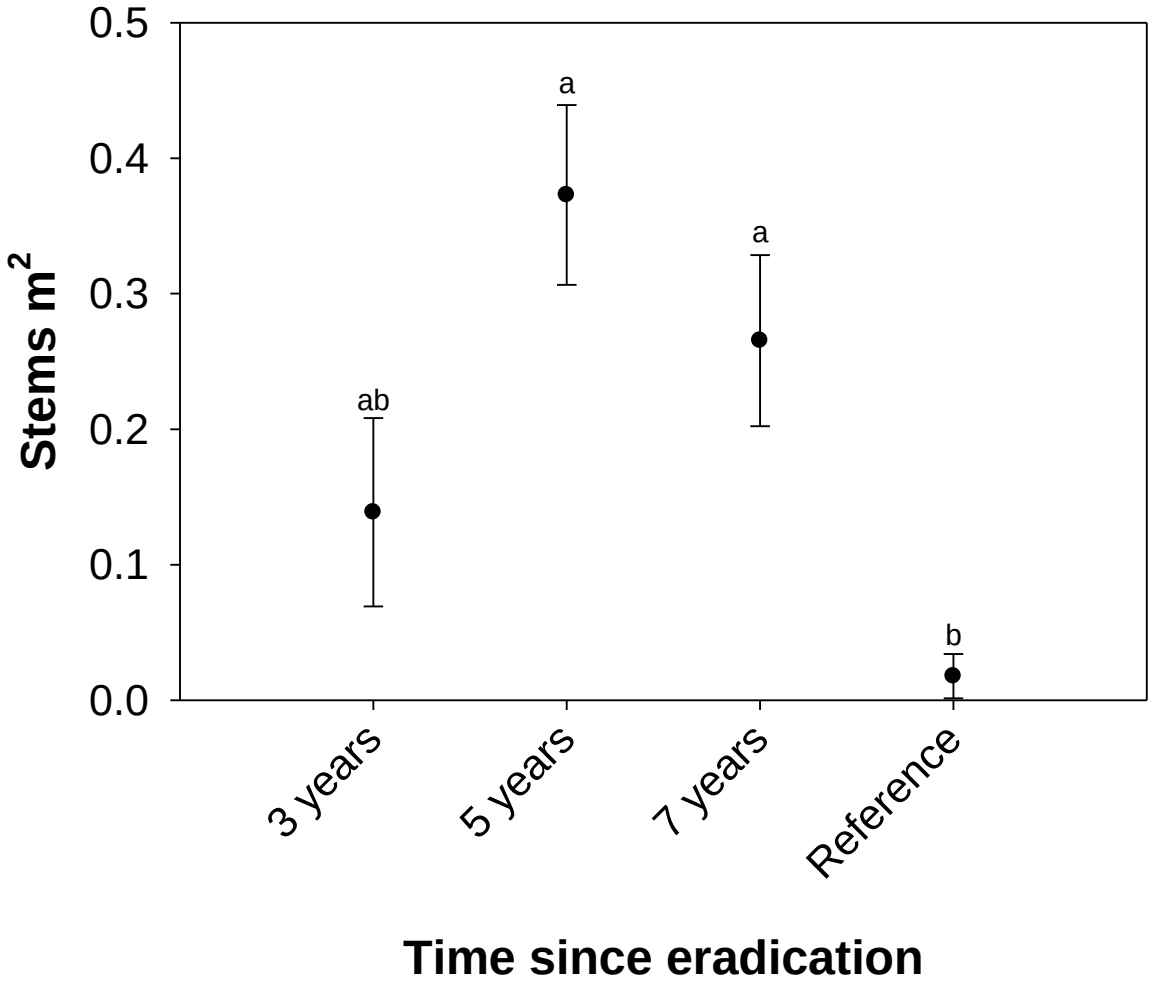

Figure 4-3. Longleaf pine stems per m^2 in plots where cogongrass was eradicated three, five and seven years prior, along with uninvaded native reference plots, in longleaf pine sandhill stands in Hernando County, FL, USA. Means and standard errors. Lowercase letters denote the results of the post-hoc Wilcoxon-Mann-Whitney pairwise comparisons. Pairs with different lowercase letters are statistically different at $P < 0.05$

CHAPTER 5
CONCLUSIONS

Cogongrass (*Imperata cylindrica* (L.) P. Beauv.) invasion is a significant and growing threat to the integrity of pine ecosystems in the southeastern United States. This fast-growing C_4 rhizomatous grass readily displaces native understory forbs, graminoids and shrubs (Daneshgar et al. 2008), impedes the regeneration of commercially and ecologically valuable overstory trees (Daneshgar et al. 2009a) and alters fire behavior (Lippincott 2000). Little is known, however, about the belowground mechanisms that might help explain the transformative success of this species. Our understanding of post-eradication legacy factors and successional processes is also lacking. In light of these deficiencies, I conducted a series of studies to bolster our understanding on the effects of cogongrass invasion and eradication on soil properties and to assess the patterns and possible drivers of native plant community recovery following eradication.

Cogongrass is suspected to have allelopathic properties (Abdul-Wahab and Al-Naib 1972; Hussain and Abidi 1991; Inderjit and Dakshini 1991; Koger and Bryson 2004; Xuan et al. 2009) but the specific compounds it produces, and their mechanisms of action on susceptible plants, were previously unknown. In Chapter 2, a greenhouse study, I hypothesized that rhizosphere leachate collected from cogongrass pot cultures would adversely affect the growth, root morphology and mycorrhizal colonization of native species (relative to leachate collected from mixed natives). Additionally, I hypothesized that compounds not present in a native savanna rhizosphere would be present in the cogongrass rhizosphere. My results indicated an apparent allelopathic effect from cogongrass, although it varied by species. A ruderal grass (*Andropogon*

arctatus Chapm.) and ericaceous shrub (*Lyonia ferruginea* (Walter) Nutt.) were unaffected by the cogongrass leachate, while mid-successional grass (*Aristida stricta* Michx. *var. beyrichiana* (Trin. and Rupr.) D.B.Ward) and the tree (*Pinus elliottii* Engelm.) were negatively affected. For *A. stricta*, I observed a 35.7% reduction in aboveground biomass, a 22.2% reduction in total root length, a 22.9% reduction in specific root length and a 23.4% reduction in total mycorrhizal root length, relative to the native leachate treatment. For *P. elliottii*, there was a 19.4% reduction in percent mycorrhizal colonization and a 21.8% reduction in total mycorrhizal root length. Comparisons made with a DI water control in the second year support the notion that the observed differences were due to the negative effects of cogongrass leachate. My chemical analyses identified 12 putative allelopathic compounds (mostly phenolics) in the cogongrass leachate. The concentrations of most of these compounds were significantly lower, if they were found at quantifiable levels, in the native leachate. One compound was a novel alkaloid. The speculated structure was hexadecahydro-1-azachrysen-8-yl ester ($C_{23}H_{33}NO_4$) and it appeared to be present at fairly high levels. This compound was not found in the native leachate treatment.

Researchers have suggested that cogongrass alters soil nutrient dynamics in southern pine ecosystems (Collins and Jose 2008; Daneshgar and Jose 2009a). In Chapter 3, I assessed pre- and post-eradication soil biogeochemical dynamics in longleaf pine sandhill stands severely impacted by cogongrass. Across a seven-year post-eradication “recovery chronosequence”, which also included untreated cogongrass and native reference plots, I analyzed soils for total N (TKN), potentially available P (Mehlich-1), pH and organic matter content. I also used a resin bag technique to assess

fluxes of plant available N and P in the soil solution. Since nutrient cycling following eradication may be influenced by the turnover of herbicide-treated biomass, I used litterbags to monitor the decomposition and nutrient mineralization patterns of rhizomes and foliage. I also used spore counts and molecular techniques (PCR, cloning and sequencing) to characterize changes to the AM fungal community. My results indicate similar total N and M1-P contents in invaded and reference plots, with levels of M1-P being lower than in invaded plots for five years following eradication. Soil organic matter content was highest in cogongrass-invaded plots and lowest seven years following eradication. Resin bag analyses suggest that cogongrass invasion did not affect soil nitrate availability, although an apparent "Assart flush" of NO_2+NO_3 occurred in the first three years following eradication. No such trends were observed for ammonium. Resin-adsorbed PO_4 was lowest three years following eradication and pH was highest five years following eradication. The litterbag study showed that approximately 55% of foliar biomass and 23% of rhizome biomass remained 18 months after herbicide treatment. Substantial N immobilization was observed in rhizomes for the first 12 months, with slow mineralization occurring thereafter. Rapid P mineralization occurred for both tissues, with 15.4 and 20.5% of initial P remaining after 18 months in rhizomes and foliage, respectively. Neither cogongrass invasion nor eradication affected AM fungal diversity, richness or spore counts. Substantial alterations to AM fungal community assembly, however, occurred due to invasion, with novel community characteristics persisting for an additional three years following eradication. I suggest that future research should assess the extent to which the sum of these changes affect the re-establishment of

desirable native species, as well as the potential for re-invasion by cogongrass or other IA plant species.

The re-establishment of native plant cover following IA plant removal is considered essential for the long-term control of cogongrass in natural areas (Miller et al. 2010). Natural regeneration, if effective, may be an attractive option for many land managers. In Chapter 4, I used the post-eradication chronosequence to assess patterns of secondary succession following cogongrass eradication. I hypothesized that the plant community assembly of formerly invaded plots would begin to approach reference state within seven years. Results revealed a general pattern of increasing richness, diversity and evenness in the years following eradication. For all three measures, there was a distinct and statistically significant change between years five and seven. By year seven, cover, diversity and richness were not statistically different from the native reference treatment, Despite this apparent recovery, there was no clear evidence that the composition of formerly invaded plots – even after seven years – approached a reference state, unless woody species were removed from the analysis. Soil properties (*e.g.* organic matter, mycorrhizal spores, and pH) appeared to correlate with successional patterns. Dogfennel (*Eupatorium capillifolium* (Lam.) Small ex Porter and Britton) was the most dominant species three and five years following eradication and *Setaria corrugata* (Elliott) was the dominant species after seven years. Bluejack oak (*Quercus incana* W. Bartram) was the most dominant species in reference plots. Longleaf pine regeneration was enhanced following eradication (0.37 and 0.26 stems/m^2 five and seven years post-eradication vs. 0.02 in reference). Nonnative

legumes were found in all treatments, but it does not appear that a secondary invasion occurred following cogongrass eradication.

My findings provide insight into the ecological dynamics of southern pine ecosystems impacted by cogongrass. The differences in leachate chemistry between cogongrass and native species, coupled with the negative effects observed on wiregrass and slash pine, suggest that allelopathy contributes to the alterations in plant community assembly that have been observed in cogongrass invaded southern pine ecosystems. The fact that soil chemical and arbuscular mycorrhizal properties return to a reference state within five to seven years of cogongrass eradication is encouraging, as it indicates that post-eradication legacy effects are short-lived. The recovery of soil properties before native plant communities suggests that belowground processes may influence ecological succession following eradication. Dispersal limitation of desirable species, however, is a possibility that should be addressed in the future, perhaps via manipulative studies that evaluate the performance of reintroduced native plants.

APPENDIX A
RELATIVE ABUNDANCE OF EACH OF THE 31 AM FUNGAL OTUS, BY TREATMENT

Table A-1. Relative abundance of each of the 31 arbuscular mycorrhizal fungal OTUs (operational taxonomic units) identified from soils collected from plots where cogongrass was eradicated 3, 5 and 7 years prior, currently invaded plots and uninvaded native reference plots in longleaf pine sandhill stands in Hernando County, FL, USA.

	ACA*	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	GIG	GIG	GIG	GIG	GIG	GLO	GLO	GLO	GLO	GLO	GLO	GLO	GLO	GLO	GLO	GLO	PAR	PAR	PAR	PAR	PAR
	8**	9	10	11	12	13	14	15	16	27	28	29	30	31	17	18	19	20	21	22	23	24	25	26	32	33	1	2	3	4	7
Reference	0.00	0.02	0.02	0.00	0.15	0.02	0.02	0.00	0.00	0.00	0.12	0.00	0.00	0.17	0.12	0.00	0.00	0.00	0.06	0.06	0.00	0.02	0.00	0.00	0.03	0.02	0.00	0.18	0.00	0.00	0.02
Invaded	0.03	0.00	0.03	0.03	0.07	0.00	0.00	0.00	0.00	0.03	0.14	0.00	0.03	0.04	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.07	0.04	0.00	0.40	0.00	0.03	0.00
3 yr	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.17	0.02	0.03	0.02	0.02	0.00	0.02	0.03	0.00	0.05	0.00	0.05	0.02	0.40	0.00	0.03	0.00
5yr	0.00	0.00	0.00	0.00	0.11	0.02	0.00	0.02	0.02	0.00	0.15	0.02	0.00	0.08	0.11	0.00	0.03	0.00	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.28	0.02	0.06	0.00
7yr	0.00	0.00	0.00	0.00	0.13	0.02	0.01	0.00	0.00	0.00	0.08	0.00	0.00	0.05	0.15	0.01	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.05	0.05	0.02	0.28	0.00	0.04	0.00

*Family abbreviation: ACU=Acaulosporaceae, GIG=Gigasporaceae, GLO=Glomeraceae, PAR=Paraglomeraceae
**Numeric OTU ID provided by MOTHUR

APPENDIX B
SPECIES LIST

Table B-1. Complete list of all plant species identified in plots where cogongrass was eradicated 3, 5 and 7 years prior and uninvaded native reference plots in longleaf pine sandhill stands in Hernando County, FL, USA.

Species	Family	Growth habit
Aeschynomene viscidula Michx.	Fabaceae	Forb
Ambrosia artemisiifolia L.	Asteraceae	Forb
Andropogon arctatus Chapm.	Poaceae	Graminoid
Andropogon virginicus L. var. *glaucus* Hack.	Poaceae	Graminoid
Aristida stricta Michx. var. *beyrichiana* Ward	Poaceae	Graminoid
Asclepias tuberosa L.	Apocynaceae	Forb
Asimina pygmea (W.Bartram) Dunal	Annonacee	Shrub
Astragalus obcordatus Elliott	Fabaceae	Forb
Astragalus villosus Michx.	Fabaceae	Forb
Baccharis halimifolia L.	Asteraceae	Tree
Balduina angustifolia (Pursh) B.L.Rob.	Asteraceae	Forb
Chamaecrista fasciculata (Michx.) Greene	Fabaceae	Forb
Clitoria fragrans Small	Fabaceae	Forb
Cnidoscolus stimulosus (Michx.) Engelm. and A.Gray	Euphorbiaceae	Forb
Crotalaria rotundifolia Gmelin	Fabaceae	Forb
Croton argyranthemus Michx.	Euphorbiaceae	Forb
Croton michauxii G.L.Webster	Euphorbiaceae	Forb
Desmodium floridanum Chapm.	Fabaceae	Forb
Desmodium paniculatum (L.) DC.	Fabaceae	Forb
Dichanthelium aciculare (Desv. ex Poir.) Gould and C.A.Clark	Poaceae	Graminoid
Dichanthelium laxiflorum (Lam.) Gould	Poaceae	Graminoid
Diospyros virginiana L.	Ebenaceae	Tree
Dyschoriste oblongifolia (Michx.) Kuntze	Acanthaceae	Forb

Table B-1. Continued

Species	Family	Growth habit
Elephantopus carolinianus Raeusch.	Asteraceae	Forb
Eupatorium capillifolium (Lam.) Small ex Porter and Britton	Asteraceae	Forb
Eupatorium pilosum Walter	Asteraceae	Forb
Galactia regularis (L.) Britton et al.	Fabaceae	Vine
Galium pilosum Aiton	Rubiaceae	Forb
Gelsemium sempervirens (L.) Aiton F.	Gelsemiaceae	Vine
Helianthus hirsutus Raf.	Asteraceae	Forb
Hieracium megacephalon Nash	Asteraceae	Forb
Houstonia procumbens (J.F. Gmelin)	Rubiaceae	Forb
Hypericum hypericoides (L.) Crantz	Clusiaceae	Shrub
Hypericum punctatum Lam.	Clusiaceae	Shrub
Ilex opaca Aiton	Aquifoliaceae	Tree
Indigofera hirsuta L.	Fabaceae	Forb
Itea virginica L.	Iteaceae	Shrub
Lespedeza hirta (L.) Hornem.	Fabaceae	Shrub
Licania michauxii Prance	Chrysobalanaceae	Shrub
Lobelia homophylla E.Wimm.	Campanulaceae	Forb
Lygodesmia aphylla (Nuttall) de Candolle	Asteraceae	Forb
Mimosa quadrivalvis L. var. *angustata* (Torr. and A.Gray) Barneby	Fabaceae	Vine
Morella cerifera L.	Myricaceae	Shrub
Opuntia humifusa (Raf.) Raf.	Cactaceae	Shrub
Parthenocissus quinquefolia (L.) Planch.	Vitaceae	Vine
Passiflora incarnata L.	Passifloraceae	Vine
Pinus clausa (Chapm. ex Engelm.) Vasey ex Sarg.	Pinaceae	Tree
Pinus palustris Mill.	Pinaceae	Tree
Pityopsis graminifolia (Michx.) Nutt.	Asteraceae	Forb
Plantago major L.	Plantaginaceae	Forb

Table B-1. Continued

Species	Family	Growth habit
Pseudognaphalium obtusifolium (L.) Hilliard and B.L.Burtt	Asteraceae	Forb
Pteridium aquilinum (L.) Kuhn var. *pseudocaudatum* (Clute) Clute ex A.Heller	Dennstaedtiaceae	Fern
Pterocaulon pycnostachyum (Michx.) Elliott	Asteraceae	Forb
Quercus incana W. Bartram	Fagaceae	Tree
Quercus laevis Walter	Fagaceae	Tree
Quercus laurifolia Michx.	Fagaceae	Tree
Quercus margaretta Ashe ex Small	Fagaceae	Tree
Quercus nigra L.	Fagaceae	Tree
Quercus sp.	Fagaceae	Tree
Quercus virginiana Mill.	Fagaceae	Tree
Rhus copallinum L.	Anacardiaceae	Shrub
Rhynchosia michauxii Vail	Fabaceae	Forb
Rubus argutus Link	Rosaceae	Shrub
Rudbeckia hirta L.	Asteraceae	Forb
Ruellia caroliniensis (J.F.Gmel.) Steud.	Acanthaceae	Forb
Setaria corrugata (Elliott) Schult.	Poaceae	Graminoid
Sisyrinchium angustifolium Mill.	Iridaceae	Forb
Smilax spp.	Smilacaceae	Vine
Solanum chenopodioides Lam.	Solanaceae	Forb
Sorghastrum nutans (L.) Nash	Poaceae	Graminoid
Sorghastrum secundum (Elliott) Nash	Poaceae	Graminoid
Sporobolus junceus (P.Beauv.) Kunth	Poaceae	Graminoid
Stillingia sylvatica L.	Euphorbiaceae	Forb
Trichostema setaceum Houtt.	Lamiaceae	Forb
Trifolium repens L.	Fabaceae	Forb
Unknown *Andropogon*	Poaceae	Graminoid
Unknown aster	Asteraceae	Forb

Table B-1. Continued

Species	Family	Growth habit
Unknown Carex	Cyperaceae	Graminoid
Unknown Cyperus 1	Cyperaceae	Graminoid
Unknown *Cyperus* 2	Cyperaceae	Graminoid
Unknown *Cyperus* 3	Cyperaceae	Graminoid
Unknown *Cyperus 4*	Cyperaceae	Graminoid
Unknown *Desmodium* sp.	Fabaceae	Forb
Unknown grass 1	Poaceae	Graminoid
Unknown grass 2	Poaceae	Graminoid
Unknown *Hypericum* sp.	Clusiaceae	Shrub
Unknown *Ipomoea* sp.	Convolvulaceae	Vine
Unknown *Paspalum* sp.	Poaceae	Graminoid
Unknown *Polygala* sp.	Polygalaceae	Forb
Unknown *Pseudognaphalium* sp.	Asteraceae	Forb
Unknown *Rhynchospora* sp.	Cyperaceae	Graminoid
Unknown *Scleria* sp.	Cyperaceae	Graminoid
Unknown *Solidago* sp.	Asteraceae	Shrub
Vaccinium arboreum Marshall	Ericaceae	Shrub
Vaccinium darrowii Camp	Ericaceae	Shrub
Vaccinium myrsinites Lam.	Ericaceae	Shrub
Vaccinium stamineum L.	Ericaceae	Shrub
Vitis cinerea (Engelm.) Engelm. ex Millardet var. *floridana* Munson	Vitaceae	Vine
Vitis rotundifolia Michx.	Vitaceae	Vine

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BIOGRAPHICAL SKETCH

Donald Hagan's interest in ecology was cultivated by a childhood spent exploring the sandhills, swamps and bayous of his native Florida Panhandle. In 2002, He earned a bachelor's degree in environmental studies from the University of West Florida. From 2004 to 2006, he served as a Peace Corps agroforestry extensionist in Ecuador, where he worked with landowners to preserve some of the last remnants of coastal dry tropical forest. Upon returning to the U.S., he enrolled in the interdisciplinary ecology master's program at the University of Florida, graduating in 2008. From 2008 to 2012 he was a Ph.D. Alumni Fellow in the School of Forest Resources and Conservation at the University of Florida.