Microbial Communities in Cogongrass (*Imperata cylindrica* (L.) Beauv.) Invaded Commercial Loblolly Pine (*Pinus taeda* L.) Stands

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 12, 2015

Keywords: mycorrhizae, *Pinus taeda*, *Imperata cylindrica*,
allelopathy, soil microbes

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Abstract

The economy of the southeastern United States is heavily influenced by the forest products industry with a 103 billion dollar industrial output. A significant amount of this industry relies on production forestry to meet increased consumer demands on increasingly less area. To this end over 1 billion *P. taeda* seedlings are produced annually, comprising over 50 % of the dominant and co-dominant growing stock. Invasion represents a substantial threat to productivity through introduction of new primary and secondary pests as well as competitors for natural resources.

Imperata cylindrica is an invasive C4, perennial grass species from Japan that was initially introduced into the southeastern United States in 1912. *Imperata cylindrica* produces rhizomonous mats that can directly compete with native vegetation and their microbial symbionts mechanically as well as indirectly through production of potentially allelopathic compounds. In addition *I. cylindrica* can alter natural nutrient cycles, fire regimes and water availability.

In order to determine the extent to which *I. cylindrica* alters commercial *P. taeda* stands we conducted research to ascertain specific effects. We conducted measurements of percent colonization by ectomycorrhizal fungi in the top 60 cm of the soil profile as well as measurements of fine root abundance. We conducted vegetation sampling from which we applied multiple diversity indices. We measured organic carbon and nitrogen present in the

microbial biomass, as well as several other key nutrients in the soil. Finally, we analyzed the effect of individual exudate components on several ectomycorrhizal fungi found in association with *P. taeda* to help determine what the mechanism of invasion was.

We determined that some variations in mycorrhizal abundance and fine feeder roots were evident. Vegetation was significantly less diverse in invaded plots which we were able to attribute to *I. cylindrica* presence. Variations were present in microbial biomass as well as several other nutrients and no one compound in *I. cylindrica* exudate consistently resulted in reduced growth.

Acknowledgments

I took a long time in thinking about all the people that have helped me make this. Unfortunately, there are too many to list in any sort of a comprehensive way. If this thesis were a movie there would be an endless list of supporting cast listed under titles like Parkour 1, Barber, Sword Swallower 3 and Man in Dog Costume. They have provided everything from an introduction to a brief encouragement. To those innumerable people, some of whose names I don't even know thank you.

I owe a huge debt to my advisor Dr. Lori Eckhardt who was a source of encouragement, direction and momentum when I had none. Dr. Nancy Loewenstein was invaluable in her feedback and taught me more about plants than I ever thought I'd want to know. Dr. Jason Hoeksema taught me statistical analyses that I vehemently rejected over and over again until I found out, the hard way, he had been right all along. Dr. Emily Carter lightened my load while simultaneously showing me how little I knew and how much I could know.

My mentor Dr. Ryan Nadel had no idea what burden he was accepting the first time he offered me a helping hand, but to his credit not once over the course of our friendship has he ever refused to repeat this folly. My comrades in the trenches Jeff Chieppa, Andrea Cole, Tessa Bauman and Pratima Devkota stuck out the tough times right up to the end, providing me with a never-ending source of frustration and showing me what hard work looks like.

Among the undergraduate students that took time out of their “college experience” to become student workers I worked with some of the best. Cody Hartzog, Trent Williams, Seth Hunt, Jordan Heath and Nick Yashko all have bright futures ahead of them, I hope I spelled your names right. Robin Governo and Dr. B. Graeme Lockaby provided numerous consultations and gave me the opportunity to learn invaluable skills. Accolades go to my friends and family that accepted my excuses instead of my company time and again.

Last, but certainly not least, thank you to Hanna Schurman. Without her love, support and copyediting neither this document nor I would be quite right.

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Introduction and Literature Review

1.1. INVASION ECOLOGY: APPROACHING INVASION WITH A GLOBAL PERSPECTIVE

What is an invasive species and are there redundancies in invasion vernacular? There is a great deal of redundancies and a distinct lack of consensus over what an invasive species is and is not. An invasive species is introduced through human mediated actions, once introduced into a region they are capable of establishment and spread resulting in negative effects on ecosystem structure and function (Richardson *et al.* 2000). Synonyms for such species include exotic, introduced, non-indigenous and non-native (Pyšck *et al.* 2008). An invasive species is a non-native species that has the capacity to spread and overcome dispersal barriers (Richardson *et al.* 2000). The next circumstance is a naturalized species or established species, that once introduced into a new region form persistent populations (over several generations) and reproduce in the environment, not all that establish are necessarily invasive (Richardson *et al.* 2000).

With the advent of modern technologies associated with trade and transportation (shipping containers, air transport, etc), the number of organisms native to one biogeographic region that are carried to another have increased in both frequency and extent (Elton 1958, U.S. Congr. Off. Technol. Assess. 1993) over the past 100 years (Davis 2009). There are an abundance of records and examples of human mediated plant and animal introduction events, which have resulted in the total, or near collapse of native populations (Sakai *et al.* 2001). Examples include; Crazy ants (*Anoplolepis gracilipes*) on Christmas Island that have occurred on the Island since the early twentieth century, but only since the mid 1990's after a population boom have efforts been made to locate "supercolonies" or regions of high *A. gracilipes* densities (>2000 ants per m^2) (Abbott 2006). These invasive ants have killed approximately 10 - 15 million red land crabs (*Gecarcoidea natalis*), the dominant endemic consumer on the Island. The decimation of crab populations has in turn affected seedling recruitment, enhanced plant species richness, slowed plant litter breakdown and may facilitate secondary invasions (O'Dowd *et al.* 2003).

Another example is mile-a-minute weed (*Mikania micrantha* H.B.K.) that was introduced into Indonesia for use as ground cover in the 1940's and promptly spread throughout Southeastern Asia where it currently shades out native plant communities due to its vigorous growth (Zhang *et al.* 2004). In 1954 a large predatory fish the Nile perch (*Lates niloticus*) was introduced to Lake Victoria in East Africa (Goudswaard *et al.* 2008). It ultimately contributed to the loss of up to half of the over 500 species of endemic cichlid (Verschuren *et al.* 2002). Fungi, viruses, and other disease causing organisms are often overlooked taxa of invasive species whose introduction tend to have far reaching impacts and can be vectored by organisms that disperse more readily. Examples include chestnut blight (*Cryphonectria parasitica* (Murrill) Barr),

chytrid disease (*Batrachochytrium dendrobatidis* (Bd)), and avian malaria (*Plasmodium relictum*) all of which were shown to decimate if not completely driving extinct at least one species, with countless indirect effects to other species.

The process of invasion is composed of stages that are hotly contested in the literature. For the purpose of this review the authors have expanded on Catford *et al.* (2009), which states that, invasion is composed of: transport, introduction, colonization, naturalization, spread and impact.

An initial assessment of traits that predispose a given species to invasion is necessary as one of the biggest unifying questions in biology is how do we determine which species are prone to become successful invaders? There are some unifying generalizations that can be made between taxa, for example: invasive plants are not evenly distributed across phyla, have a history of invasion in species, genus, or family, reproduce vegetatively, and have low variability in seed crops (Kolar & Lodge 2001). Invasive birds, however, tended to invade ecosystems that had similar temperature or habitats to their native range and have more broods per season (Kolar & Lodge 2001). Although this may be because we are biased in the taxa we report on and geographic areas we investigate (Pyšck *et al.* 2008), or the focus we have on what predisposes a species to become an invader and a community to be invaded (Facon *et al.* 2006). There are lists of characteristics common to groups of invaders, of a specific taxa or to a particular region, that may hold for that group but will not necessarily translate universally (Lloret *et al.* 2005, Phillips *et al.* 2010). A historical list of characteristics that we associate with a group of invaders, in this case plants, is "Baker's List" (Baker 1974). While finding traits that consistently indicate a predisposition of invasion is unlikely, that does not diminish the importance of studies enumerating traits of a specific group or to a particular region.

Step one of invasion is composed of the movement of organisms to a new location. This is the first barrier that organisms face on the road to invasion and a surprising number of potential invaders do not survive transport. Examples of potential barriers to transportation include geographic distance and barriers to long-range dispersal such as water bodies, mountain ranges or intervening deserts (Theoharides & Dukes 2007). When international travel first became commonplace hundreds of years ago, trips that take hours today took months to complete. Long stretches of time in ports, waiting for supplies or weather conditions to be agreeable were commonplace. Trips in the 1700's, provided ample opportunity for the first recorded human mediated international introductions to occur such as benthic organisms picked up on the hulls of wooden ships from estuarine substrates (Lockwood *et al.* 2013). While intermittent layovers are less frequent and shorter in the 21st century, the relatively instantaneous process of travel has the potential to increase introductions by orders of magnitude. Williamson (1996) coined the "tens rule" in which he estimated that 10 % of invaders succeed from one step of invasion to the next. Flaws with this estimate include that at the time there were only considered two steps of invasion as well as that there is a lack of information on dissemination (Molnar *et al.* 2008). Similar to the problem with determining which characteristics make for a successful invader, the problem of determining which invader will cause economic harm is also difficult to predict (Lockwood *et al.* 2013). However those that belong to the 10 % that can survive transportation more frequently than others should garner more attention.

Step two includes the arrival of organism into a new location. Transport should be considered as a twofold step. The process of survival during transportation does not necessarily guarantee that an organism will invade successfully. In the case of the zebra mussel (*Dreissena polymorpha*), arguably one of the more notorious aquatic invasive species in the United States,

introduction most likely took place when a trans-oceanic vessel discharged freshwater ballast containing free-swimming larvae (Griffiths *et al.* 1991). With this introduction, simply surviving transport was not sufficient to facilitate invasion. Successful invasion required that *D. polymorpha* be in a stage that physiologically could be first discharged with ballast and second survive the process. Genetic studies have shown that the invasion of the zebra mussel was the result of numerous introductions and as such the amount of genetic diversity introduced was not reduced from its native range (Hebert *et al.* 1989). This establishment of the zebra mussel has resulted in an increase of the extinction rate of endemic mussels of which 12 % are presumed extinct and 60 % are threatened (Ricciardi *et al.* 1998). Not included in this estimate are *D. polymorpha* propensity to selectively feed, avoiding controlling populations of toxic blooming cyanobacteria (Vanderploeg *et al.* 2001). Also infrequently investigated are the 34 endosymbionts that are associated with *D. polymorpha* (Karatayev *et al.* 2003). The opposite result has been observed for the brown tree snake (*Boiga irregularis*), native to Australia and Indonesia, which is more frequently found dead on arrival rather than alive on Hawaii. Canine searches of incoming flights from Guam, where the brown tree snake is of concern are frequently performed and the snake has yet to become established (Kraus & Cravalho 2001).

Step three is colonization of an introduced organism in the new ecosystem. Barriers to survival at this stage are predominantly abiotic, will this organism be able to survive in the landscape they have arrived in (Theoharides & Dukes 2007). Here the idea that the optimal phenotype for a disperser may not be the optimal phenotype for an establisher may come into play. There are numerous examples of individuals overcoming extreme changes to their environment in order to establish themselves as invaders. To emphasize this point we looked at invasive populations that have colonized and become established in the Antarctic. There have

been several successful invasions to the Antarctic, most likely mediated by researchers who carried seeds on their gear (Chown *et al.* 2012). One example of a successful invasion is annual meadow grass (*Poa annua* L.), native to Eurasia first reported in the summer of 1985/1986. Genetic analysis has determined that the introduction has been the result of introductions from several sources (Chwedorzewska 2008). Variation found within the population was attributed to "plastic" responses to environmental conditions associated with survival in extreme conditions (Chwedorzewska & Bednarek 2012). Aside from the Arctowski research station on King George Island *P. annua* also has been observed at three other research stations: General Bernardo O'Higgins, Gabriel Gonzalez Videla and Almirante Brown (Chown *et al.* 2012). Two other vascular plants originating from South American have been discovered on Antarctica as well. The plants *Nassauvia magellanica* J.F. Gmelin and *Gamochaeta nivalis* Cabrera produce wind-dispersing seeds and were found on Desolation Island, inland from a derelict Norwegian whaling station frequented by tourists (Smith & Richardson 2011). The island is volcanic and the specimens were presumed by their discoverers to have flowered neither this season nor the previous one. One of the most recent discoveries, during the austral summer 2006/2007 was of a dipteran insect species (*Trichocera maculipennis* Meigen) widely distributed throughout the northern hemisphere also on King George Island, in the Artigas Base sewage system (Volonterio *et al.* 2013).

Step four is naturalization in which the organisms survive and reproduce allowing for a colonizing population to be self-sustaining. Once an organism has survived the initial abiotic barriers to survival, they must overcome the next hurdle: growth and establishment (Theoharides & Dukes 2007). In order to grow and reproduce the newly colonizing organism comes into direct competition with native species. Here is where propagule pressure is the most influential

factor. Propagule pressure is defined as the number of arriving individuals and their rate of arrival (Simberloff 2009). Other authors include the physiological condition of the propagules (Lockwood *et al.* 2013) or the spatial and temporal patterns of propagule arrival (Nuñez *et al.* 2011). This pressure suggests that the higher the number, rate, vigor and proximity of the propagules, the more likely they are to outcompete native species. Propagule pressure is frequently attributed to be one of the most important factors to species invasion (Lockwood *et al.* 2009). Some researchers have likened the source and sink model of conservation biology to propagule pressure in invasion biology with smaller populations being sustained in the long term with introductions from larger populations (Lockwood *et al.* 2005). Propagule pressure also is compared to the minimum viable population size in conservation biology, which states that populations are more likely to survive environmental or demographic stochasticity as more individuals are released (Lockwood *et al.* 2009). Survival also becomes more likely as genetic variation increases in the introduced population (Lockwood *et al.* 2005). Populations that have experienced a genetic bottleneck are more likely to experience extinction in the short term from inbreeding depression or in the long term from genetic drift (Simberloff 2009). A perfect example of the role of propagule pressure in the success of an invasive plant is kudzu's (*Pueraria montana* var. *lobata* (Willd.) Sanjappa & Predeep) invasion of the southeastern United States. Here the phenomenon of Kudzu invasion has occurred at a rate of 50,000 ha per year (Pappert *et al.* 2000). *Pueraria montana* has been shown to grow over thirty centimeters in a day and can form dense mats of vegetation over trees, shrubs, debris or the ground. *Pueraria montana* is spread by wind, animal and water (Miller *et al.* 2010). *Pueraria montana* was first introduced at the 1876 centennial exposition in Philadelphia. Uses for *P. montana* included planting as a government sponsored solution for erosion, as fodder for cattle as well as for use as

an ornamental (Forseth & Innis 2004). Seed and plants of kudzu were propagated in Soil Conservation Service nursery's facilitating gene exchange between different sources (Sun *et al.* 2005). Needless to say this plant has been introduced many times all over the southeastern United States and is estimated to occupy three million acres throughout the eastern United States with genetic diversity supporting this estimate (Pappert *et al.* 2000).

Step five is spread that necessitates the dispersal of propagules and the spread of populations outside the area of initial introduction. Once an organism has become established, the biggest factor limiting spread is landscape features that either hinder dispersal to new areas or the organism's ability to become established (Theoharides & Dukes 2007). For some invasive species, landscape features are more of a hindrance to spread than others. The European starling (*Sturnus vulgaris*) and the common house sparrow (*Passer domesticus*) are both examples of invasive species for which landscape features did not hinder expansion. Similarly, some species require multiple introductions spanning a spatial and temporal framework in order to become established, while other invasive species became established more easily. There is evidence to suggest that *S. vulgaris* was able to become established from a preliminary introduction of just fifty pairs, of which sixteen survived, to New York City in 1890 and 1891 (Linz *et al.* 2007). Called one of North America's most invasive birds (Sakai *et al.* 2001), by 1942 the *S. vulgaris* had reached the west coast of the United States (Cabe 1993). There are estimates that the annual loss to agriculture due to *S. vulgaris* feeding is US $800 million per year (Pimintel *et al.* 2000). In addition, the European starling has been shown to contribute to declines observed in native cavity-nesting birds (Koenig 2003), increased bird aircraft strikes (Linz *et al.* 2007), and the transmission of harmful human pathogen, such as *E. coli* (LeJeune *et al.* 2008). The European starling is a strong flier with migration distances of 1,000 to 1,500 km (Lintz *et al.* 2007). These

dispersal events, as noted by Sakai *et al.* (2001), are a source of genetic variation, which could result in the spread of more invasive genotypes. Another aggressively dispersing organism, which physical barriers did not hinder is the common house sparrow, *Passer domesticus*. Evidence suggests, through review of historical documentation, that the establishment of *P. domesticus*, was likely a consequence of an initial introduction of just sixteen birds to New York City in 1851 (Moulton *et al.* 2010). Since introduction, *P. domesticus* has been found as far north as Alaska and south as Panama. This wide-scale expansion may be due to the ability of *P. domesticus* to locate and utilize novel food sources compared to established bird populations (Martin & Fitzgerald 2005). This example of behavioral (feeding) plasticity, that may be genetically linked, could explain this species ability for invasion.

Step six, the final step, is a perspective step, impact. Here impacts of a species either ecologically or economically are observed. The impact of non-native species on indigenous ecosystems continue to yield losses of approximately 5 % of the global gross domestic product (Pimentel 2002), with this figure taking into account human diseases such as HIV/AIDS and tuberculosis. The number of publications concerning non-native species invasions into new areas has increased exponentially since the 1990's as shown when searching the Web of Knowledge database using the keywords Invas* and Ecolog* (Richardson & Pyšck 2008; Figure 1.1).

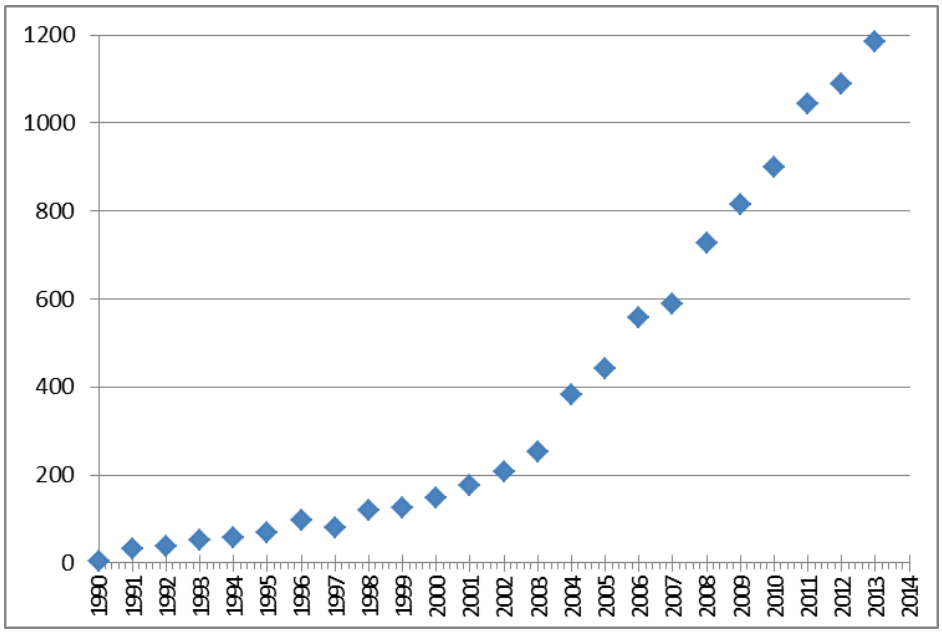


Figure 1.1. Searches returned from the ISI Web of Science search engine between 1990 and 2013 incorporation the keywords "invas*" and "ecolog*."

1.1.1. Novel Weapons Hypothesis and Alternatives:

There are a number of hypotheses to explain the successful invasions relevant to different taxa and ecosystems. Catford *et al.* (2009) synthesized twenty-nine of the leading hypotheses in order to create a unified theoretical framework by eliminating redundancies. They concluded that a "top-down" approach that examines the most influential factors: propagule pressure along with abiotic and biotic characteristics would maximize research efficiency.

The novel weapons hypothesis, relevant to the world's most economically important invasive species, suggests that the success of invasive plant species may be due to the production of biochemicals, or novel weapons, that native species have not yet encountered (Callaway & Ridenour 2004). This hypothesis explains some cases in which a relatively minor species in their

native range can become aggressive invaders when introduced elsewhere. Callaway *et al*. (2008) examined the reason why garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grand) is such a successful invader in North America. They found that *A. petiolata* has a far stronger inhibitory effect on North American competitor's mycorrhizae than competitor's mycorrhizae in its native European range. Variation in suppression could be explained, as in the case of spotted knapweed (*Centaurea maculosa* Lam.) used in Vivanco *et al*. (2004), by a plant exuding compounds to which soil biota in its native range had become accustom.

Contrary to the novel weapons hypothesis are the far more common consumer-based hypotheses such as the enemy release hypothesis. The enemy release hypothesis states that invasive plant species experience a decrease in predation, and therefore regulation, by herbivory, that results in an increase in abundance (Keane & Crawley 2002). These theories have evolved further, such as the evolution of increased competitive ability hypothesis, wherein competition will favor genotypes with improved competitive abilities and reduced resource allocation to defense (Blossy & Notzold 1995). However, as Callaway & Ridenour (2004) suggests, the relative impact of consumers is often minimal and no study is yet to explicitly link greater size to reallocation of resources.

1.2. COGONGRASS: A FRAME-BY-FRAME ANALYSIS OF INVASION

Cogongrass (*Imperata cylindrica* (L.) Beauv.) is recognized as the seventh worst weed in the world (Holm *et al.,* 1977). *Imperata cylindrica* was accidently introduced from Japan to the United States in 1912, then intentionally introduced as a forage crop in 1920 (Holzmueller & Jose 2011). However, due to the high silica content of cogongrass, the plant was unsuccessful as a forage crop (MacDonald 2004). *Imperata cylindrica* has established populations in Florida, Georgia, Alabama and Mississippi with scattered invasions in South Carolina, east Texas and

Louisiana (Miller *et al.* 2011). In the southeastern United States the plant has, in recent years, been moved unintentionally by moving forage across state lines for livestock and soil for construction (MacDonald *et al.* 2002).

1.2.1. Physiology:

Imperata cylindrica is a C_4, rhizome producing perennial plant that can reach heights of three meters, but typically grows to heights of 1.2 meters (Koger & Bryson 2004). *Imperata cylindrical* spreads sexually, through seeds, and asexually, through rhizomes (Daneshgar *et al.* 2008). Seed production can be described as prolific with many seeds being produced by a single plant (Figure 1.2), that are generally dispersed within fifteen meters of the plant (Daneshgar *et al.* 2008).

Figure 1.2. *Imperata cylindrica* is a prolific seed producer although many seeds are inviable and the majority of the seeds are dispersed locally.

Low germination rates (Shilling *et al.* 1997) and short seed viability (Dozier *et* al. 1998) have been observed as a barrier to sexual reproduction (Jose *et al.* 2002). More frequently *I. cylindrica,* will reproduce vegetatively. Rate of spread of *I. cylindrica* patches vary by habitat: Collins *et al.* (2007) found that in forest-harvested sites *I. cylindrica* will spread 1.63 m^2 per year while non-harvested sites spread less at 1.14 m^2 per year. *Imperata cylindrica* can grow on a variety of soils, from nutrient-poor, coarse sands to nutrient-rich, sandy loam soils (Jose *et al.* 2002). The plant is particularly difficult to eradicate because *I. cylindrica* produces vast networks of underground rhizomes from which the plant regenerates (Dozier *et al.* 1998). Some

investigators have found the density of rhizomes in the soil to be 89 meters per square meter of soil (MacDonald 2004).

1.2.2. Changes in Ecosystem Processes:

Within a forest setting, *I. cylindrica* has the potential to stress both natural and commercial forests by increasing the intensity and frequencies of fire regimes (Lippincott 2000), physical injury (MacDonald 2004), and production of allelopathic compounds (Koger & Bryson 2009; Holzmueller & Jose 2011; Hagen *et al.* 2013). Brewer (2008) found a significant loss of native herbaceous plants in longleaf pine (*Pinus palustris* Mill.) stands and attributed the loss to increased shade (by up to 99 %) (Brewer 2008). Burning *I. cylindrica* grasslands can result in loss of soil nitrogen and actually facilitate *I. cylindrica* regeneration (Chapin III *et al.* 2000). Additionally, *Imperata* increases soil compaction, resulting in nutrient deficiencies and altered hydrology within a stand (D'antonio & Meyerson 2002). A combination of any number of these factors may vary in intensity and harm to the native plant ecosystem.

1.2.3. Allelopathy:

Imperata cylindrica is of particular relevance to commercially valuable timber species because the plant produces exudates that may have an allelopathic effect (Hussain & Abidi 1991; Koger & Bryson 2004; Holzmueller & Jose 2011; Hagen *et al.* 2013). Compounds suspected of being allelopathic include: Gallic acid, Caffeic acid, Salicylic acid, Sinapinic acid, Benzoic acid, Cinnamic acid, Emodin, Ferulic acid, 4-hydroxyphenylacetic acid, Chlorogenic acid, and Resorcinol (Hagen *et al.* 2013). Altogether, the list includes seven phenolic acids, two aromatic acids, one trihydroxy anthraquinone, and one meta dihydroxyl phenol. While the allelopathic compounds are known to be detrimental to plant growth, studies to examine the effect of these compounds on mycorrhizae are few. One study reported that four phenolic acids

disproportionally decreased the growth of, *Cenoccum geophilum* Fr. and *Laccaria laccata* (Scop.) Cooke (Boufalis *et al.* 1994).

1.3. FORESTRY IN A RAPIDLY HOMOGENIZING ECOSYSTEM

In the southeastern United States the industrial output for the forest products industry totaled US$103 billion (Tilley & Munn 2007). In today's market that equates to the forest products industry being at number twenty-one on the Fortune 500 list. There are a variety of dominant species and groups that compose the abundance of forestry in the southeast.

1.3.1. *Loblolly pine* (Pinus taeda *L.*):

Pinus taeda is the leading timber species in the United States, comprising over 50 % of the dominant and co-dominant growing stock in commercial forests (Schultz 1997). In the 2012-2013 season over 700,000,000 *P. taeda* seedlings were produced in the southern United States alone (Enebak 2013). In Alabama the amount of loblolly pine is higher than the southern average, with 83 % of commercial stands growing *P. taeda* (Schultz 1997). Historically *P. taeda* was a minor species in southeastern forests but 17th century settlers realized early on the value of this species for farm and ship building (Schultz 1997). *Pinus taeda* was harvested in both North and South Carolina in the 1860s after the supply of longleaf pine (*Pinus palustris* Mill.) decreased. Together with shortleaf pine (*Pinus echinata* Englem.), loblolly pine became a mainstay of the southeastern United States in the early 1900s (Wahlenberg, 1960). Beginning in 1951 trees began to be selected for superior quality, factors such as rapid growth, good form, and durable wood properties. Fewer than one in 100,000 trees examined being selected for clonal and seed orchard production (Schultz, 1997).

Pinus taeda is most productive on moderately acidic soils with imperfect to poor surface drainage, a deep medium textured surface layer, and fine textured subsoil (Baker & Balmer 1983). Loblolly pine is intolerant to moderately tolerant to shade, moderately drought tolerant, and can typically survive at least one growing season of continuous flooding (Baker & Balmer 1983). *Pinus taeda* grows particularly well in areas with 6 to 10 frost free months per year, 40-50 inches average rainfall, and enough humidity to create a favorable precipitation-evaporation ratio (Carmean 1947). Soil type determines the general development of *P. taeda* root systems (Wahlenberg 1960) with roots growing throughout the year (Schultz 1997).

1.3.2. *Longleaf pine* (Pinus palustris *Mill.*):

Pinus palustris a once dominant feature within the Atlantic and Gulf coast plains, Piedmont, ridge and valley, Cumberland plateau and blue ridge physiographic region of the southeastern United States landscape (Varner and Kush 2004) occupying an estimated thirty-seven million ha (Frost 1993). It has been replaced in large part due to the faster growth rate found in *P. taeda* and *P. elliottii* (Perry 1998) and therefore cannot economically compete (Landers *et al.* 1995). Varner & Kush estimated in 2004 that only 5095 ha of old growth longleaf pine exist, which represents 0.004 % of extant area. Many game species thrive in well maintained *P. palustris* stands (Landers *et al.* 1995) and it is known to host an abundance of non-game diversity "unparalleled" outside of the tropics (Varner & Kush 2004).

1.3.3. *Slash pine* (Pinus elliottii *Englem.*):

There are a number of varieties of *P. elliottii*, but the true slash pine (var. elliottii) is found in the coastal plains as far north as southern South Carolina south to central Florida and west to southeastern Louisiana (Little & Dorman 1952). As a result of the fire suppression of the 1900s

most forest regeneration took the form of mixed *P. elliottii* and *P. palustris* (Gholz *et al.* 1985). As recently as 2007, *P. elliottii* forests totaled 5.3 million ha (Smith *et al.* 2009), 69 % of which was deemed intensively managed with rotation times of thirty years or less (Barnett & Sheffield 2004).

The trees that readily hybridize with slash pine include *P. taeda*, *P. clausa* (Chapm. Ex Engelm.) Vasey ex Sarg. and *P. palustris* (Moore & Walker-Wilson 2006). It also has in the past been confused with *Pinus caribaea* W.H.G. Barrett & Golfari which is native to the West Indies and Central America (Little & Dorman 1952).

1.3.4. *Mixed Hardwood*:

Traditionally hardwood species occupied the role of late successional dominant species in southeastern United States forests, (Quarterman & Keever 1962). In these forests, canopy stratification is dictated by relative shade tolerance (Cain & Shelton 1994). Mixed hardwood forests provide a variety of ecosystem services such as: water storage, enhanced water quality, nutrient cycling, erosion control, and wildlife habitat (Kellison & Young 1997). Due to their relatively slow growth rate when compared to the pioneer forests comprised of conifers, mixed hardwood forests are normally not managed. As much as 90 % of timberlands in the south are privately owned (Kellison & Young 1997). Since much of this land is managed and harvested very little is selectively managed for hardwood. Though they have the potential to be productive given selective removal of mature trees they are of little relevance to this review.

1.3.5. Forest Pests and Pathogens in the Southeastern United States:

Forest pathology is the study of tree diseases, nested within plant pathology, as a crop oriented science that serves the public interest by applying scientific principles to the prevention

and control of diseases (Tainter & Baker 1996). A pathogen, the smallest unit relevant to forest pathology is a biotic agent that incites disease and thus selectively eliminates the less vigorous or genetically unfit individuals of a population (Castello *et al.* 1995).

Forest diseases play a vital role in succession and maintenance of a genetically fit forest. The logical conclusion to be drawn is that many pests also fit into this paradigm, and from Manion (2003) definition of a disease as "any agent that may negatively impact the survival of an individual tree." This is not universally acceptable when invasive organisms are concerned. Non-native, invasive organisms, may act to remove entire species from the national landscape such as the American chestnut (*Castanea dentate* [Marsh.] Borkh.), American elm (*Ulmus americana* L.), and someday soon, ash (*Fraxinus* spp. L.) and redbay (*Persea borbonia* [L.] Spreng.).

1.3.6. *Fusiform Rust* (Cronartium quercuum *[Berk.] Miyabe ex. Shirai f. sp*) *fusiforme*:

A good example of how the restructuring of a forest species can result in unexpected outcomes is in the case of the causal agent of fusiform rust, *Cronartium quercuum f.sp fusiforme*. Unusually high densities of susceptible hosts lead to more tangible consequences of the reduced growth, quality and survival commonly associated with fusiform rust (Kayihan *et al.* 2005). Baseline incidences of infection reported at 5 % in the early 1900s have increased to levels as high as 20 to 50 % with further increases of 3 % per year (Tainter & Baker 1996). Majority of infections occur prior five years after planting, at which time if plantations are showing 50 % or more infection they are typically clearcut (Price 2001).

1.3.7. *Annosum Root Disease* (Heterobasidion irregulare *[Fr.] Bref*):

Robert Hartig described this disease in the 1870s as the cause of circular patterns of dead trees in conifers (Tainter & Baker 1996). Losses associated with annosum root disease caused by *Heterobasidion irregulare* (formerly known as *Heterobasidion annosum* (Fr.) Bref) have been particularly severe in the southeastern United States (Tainter & Baker 1996). Of particularly high risk in the southeast are sandy or sandy loam soils with no high seasonal water table (Price 2001). Signs of the disease include irregularly shaped annual conks forming at the base of the tree and symptoms such as thin unhealthy crowns, resinous roots, with infections appearing in groups (Tainter & Baker 1996).

1.3.8. Southern Pine Decline:

Diseases, along with insect and plant pests, including non-native invasive fungal species are responsible for reductions in forest productivity and stand regeneration of *P. taeda* (Daneshgar & Jose 2009, Enebak & Carey 2000, Oak & Tainter 1988). In the southeastern United States reports of forest declines and mortality have been increasing for 20 years (Eckhardt *et al.* 2007). One such decline, southern pine decline is an emerging forest health issue in the southeastern United States (Eckhardt *et al.* 2010) and is characterized by a variety of different stand stresses (Brown & McDowell 1968). The symptoms (short chlorotic needles, sparse crowns, and reduced radial growth followed by eventual mortality) are similar to littleleaf disease of *P. echinata* (Eckhardt *et al.* 2007) caused by the oomycete *Phytophthora cinnamomi* Rands. Southern pine decline is associated with root-feeding bark beetles that are attracted to stressed trees, vectoring ophiostomatoid fungi to the root system (Matusick *et al.* 2013).

1.4. THE ROLE OF MYCORRHIZAE IN RHIZOSPHEREIC COMMUNITIES ASSOCIATED WITH INVADED ECOSYSTEMS

Mycorrhizae's role in plant growth and defense is well documented (Marx & Bryan 1971; Trappe 1977; Perrin 1990). There are unknown interactions that are not so well understood. Oftentimes a mutualism involves more than simply a mycorrhizal fungi and a partner. Almost always, this interaction occurs in tandem with influences from other taxa and occasionally, the symbiosis includes those addition taxa. Agents in this symbiosis can include endophytes, non-mycorrhizal fungi, rhizobacteria, phages and in more extreme environments, archaea (Bomberg *et al.* 2003, Bomberg & Timonen 2007, Bomberg *et al.* 2010). Actively absorbing plant rootlets are connected to the surrounding soil through an interface called the mycorrhizosphere (Johansson *et al.* 2004) the soil zone under the influence of both roots and mycorrhizal fungi (Bonfante & Anca 2009). This is in contrast to the rhizosphere, which is defined as the area under influence only of the root (Lugtenberg & Kamilova 2009). The mycorrhizosphere area is larger in volume than the rhizosphere and has several physical and biochemical differences such as modified soil structure by the mycorrhizae, root exudation, and structure of bacterial communities (Frey-Klett *et al.* 2005).

Endophytes are organisms growing within plant tissue, which may be either symbiotic or parasitic. Endophytes can be either fungi or bacteria but are required to live asymptomatically (Herre *et al.* 2007). Rodriguez *et al.* (2009) makes the distinction that endophytes may emerge to sporulate at plant or host tissue senescence. Almost all vascular plants examined to date have been found to be associated with endophytic bacteria and/or fungi (Tan & Zou 2001). With a few exceptions, most fungal endophytes are ascomycetes (Carrol 1988). These endophytes are ubiquitous within the plant kingdom; several to hundreds of endophyte species can be associated with a single organism. At least one of these endophytic species is usually host specific, and populations of endophytes are usually dependent on host species and location (Tan & Zou 2001).

Endophytes are traditionally classified as either clavicipitaceous (c-endophytes) which infect some grasses or nonclavicipitaceous (NC-endophytes) which are found in asymptomatic nonvascular plants, ferns, conifers and angiosperm tissue (Rodriguez *et al.* 2009). Endophytes have been further subdivided by Rodriguez *et al.* (2009) to four categories dictated by host range, tissue colonized, *in planta* colonization and diversity, method of transmission and fitness benefits.

While many studies focus on the plethora of medically relevant metabolites (Tan & Zou 2001, Strobel & Daisy 2003, Strobel 2003) some studies have examined the ecological roles of fungal endophytes. Keenen *et al*. (2008) examined the interaction between an invasive plant (*Schedonorus phoenix* Scop), an aboveground fungal endophyte (*Neotyphodium coenophialum* [Morgan-Jones and Gams] Glenn, Bacon and Hanlin)) and belowground arbuscular mycorrhizae. They found that while the mycorrhizal fungi treatment had no significant impact on the endophyte and plant biomass, the endophyte increased plant biomass.

Non-mycorrhizal fungi play a large role in nutrient availability, the bacterial communities in the rhizosphere, and the ecological fitness of their hosts. The saprotrophic fungi would be included and encompass both yeasts and filamentous fungi across all terrestrial phyla (Buee *et al*. 2009). Cellulose, the most abundant organic compound on the planet, is the energy source whose utilization allows fungi to determine composition of rhizosphere communities (de Boer *et al.* 2005). Likewise, lignin also provides an abundant energy source for fungi and endophytic organisms. One of the most vital contributors to this ecosystem is the plant root itself, whose exudates are an important factor in soil fungal populations (Broeckling *et al.* 2008). Bacteria compete with fungi for fungal-derived organic solutes. Depending on the bacterial species present, they may inhibit their growth just as fungi produce antibacterial compounds (de Boer *et*

al. 2005). It has been suggested that the reason soil fungi dominate soil bacteria, is the inability of bacteria to bridge empty space such as fungal hyphae are more adapted to doing (de Boer *et al.* 2005). In addition to saprobic fungi there also are non-obligate plant pathogenic fungi growing as saprobes in the rhizosphere (Buee *et al.* 2009). The growth of which is facilitated by the large intercellular spaces and "leaky" nature of cortical cells in the outer cortex (Tainter & Baker 1996).

According to Enebak *et al.* (1998) plant growth promoting rhizobacteria (PG-PR) occur naturally in the soil and are able to aggressively colonize roots and stimulate plant growth. The diversity of soil bacteria is staggering, with some studies finding numerous sequences belonging to clusters with previously uncultured bacterial divisions (Kuske *et al.* 1997). Among the variety of functional roles bacteria occupy are niches as mycorrhizal helper bacteria, which improve mycorrhizal and plant growth (Burke *et al.* 2006). In addition mycorrhizae exert selective pressure on bacterial communities, indirectly affecting the host plant (Frey-Klett *et al.* 2005).

Rhizobacteria have been observed to be highly specific in their interactions and, depending on the species of fungi and bacteria involved, either positive or negative on plant growth (Garbaye 1994). Garbaye & Bowen (1989) postulated that helper bacteria should be adapted to living in association with mycorrhizae. Their studies have discovered that 80 % of the bacteria embedded in the outer mantle were beneficial to mycorrhizal establishment and only 20 % either neutral or inhibitory (Garbaye & Bowen 1989). Soil clustered in the ped, organic soil held together by fungal hyphae, were sampled in *P. taeda* stands and found to have lower levels of diversity on viable tips than decaying tips (Burke *et al.* 2006). This may suggest that bacteria associated with viable roots are symbiotic and once the tip dies saprophytes move in.

1.4.1. Mycorrhizae:

Mycorrhizae are a ubiquitous group of organisms, with 95% of vascular plants belonging to families that are characteristically mycorrhizal (Trappe 1977). Fungi associated with this group are known to provide numerous benefits to their symbiotic partners such as nutrient absorption (Trappe 1977), reduced predation (Ingham 1988), and disease resistance (Perrin 1990). These fungi are distributed non-randomly throughout the root system with few fungal species making up the majority of the community (Horton & Bruns 2001). The mycorrhizal fungi are found predominantly on fine root tips of host species in the topsoil layers rather than mineral soil (Brundrett *et al.* 1996). Plants and mycorrhizal fungi have seasonal fluctuations in their growth and activities. The fungal species present and fungal activity is dependent upon time of the year (Brundrett *et al.,* 1996).

1.4.2. Ectomycorrhizae:

Ectomycorrhizae are a functional group of mycorrhizal fungi that span Ascomycota and Basidiomycota that have evolved many times independently (Allen *et al.* 2003). This group of mycorrhizae is characterized by a fungal sheath or mantle enclosing the root, the presence of a Hartig net, and an outwardly growing system of hyphal elements (Smith & Read 2010). Ectomycorrhizal fungi grow between the root cells of their symbionts, rather than within root cortex cells, as in the case of vesicular-arbuscular mycorrhizae (Brundrett *et al.* 1996).

Ectomycorhizal fungi reproduce through sexually produced spores created by their fruiting bodies (both above and below ground), sclerotia, and through the mycelial network (Brundrett *et al.* 1996). Hyphal fronts are typically denser than those of arbuscular-mycorrhizal fungi, and respond to both organic material and inorganic nutrients with increased growth (Olsson *et al.* 2002).

1.4.3. Mycorrhizae of Pines:

The nature of root systems to be composed of several different types of roots is particularly pronounced in pines; with shorter roots being completely colonized by mycorrhizae and longer roots being sporadically colonized (Richardson 2000). These fungi depend on their mutualism with pine roots for carbohydrates, the majority of exchange taking place on fine roots. These fine roots have a variety of longevity based on the fine root size (i.e. < 1mm, < 2mm, etc.) and the pine species. The genus *Pinus* is composed of species that are considered to be predominantly ectomycorrhizal associated (Brundrett *et al.* 1996). These fungi have a variety of roles and niches that leads to some genera being associated with varying successional stages (Richardson 2000). Visser (1995) found, in a study of ectomycorrhizal fungi associated with jack pine (*Pinus banksiana* Lamb.) in a variety of age classes that while ectomycorrhizal colonization did not decrease with age there were discrete groups of early and late successional ectomycorrhizal fungi.

1.4.4. *Case Studies with Allelopathy: Garlic Mustard* (Allaria petiolata *[M. Bieb.] Cavara & Grande*)

Invasions by plant species can disrupt many natural processes such as niche displacement, resource competition, allelopathy, alteration of nutrient cycling, hydrology, fire regimes and disruption of mutualistic relationships (Castellano & Gorchov 2012). However, case-by-case examples of the impacts of a single species at the community level are rare.

One example of an invasion in which sufficient information is known to fully outline the effects of a single species is garlic mustard (*Allaria petiolata*). *Allaria petiolata* was first introduced to the United States from Europe in the 1800s and was noted as an escaped weed on Long Island, New York in 1868 (Clapman *et al.* 1952, Miller *et al.* 2011). As early as 1990 the

plant had spread to three Canadian provinces, 27 midwest and northeastern states, the District of Columbia, and several western States (Roberts & Anderson 2001). Genetic testing suggests that based on the dissimilarity among North American populations of *A. petiolata* it had likely been introduced many times and from a variety of European seed sources (Meekins *et al.* 2001).

Distinguishable by the plants erect and pubescent stems that reach heights of 1.25 m, deltoid and coarsely toothed small leaves and a characteristic garlic odor (Cavers *et al*. 1979). The plant occurs less frequently under dense canopies and is more common at forest edges and openings, with seed ballistic dispersal capabilities up to ten feet (Miller *et al.* 2010). Of the 9,500 to 10,700 seeds/m^2 that this plant can produce, the majority remain dormant for one to two years, occasionally up to 5, with only 2 to 4 % surviving to flower (Cavers *et al.* 1979, Castellano & Gorchov 2012).

A variety of studies have examined the effect of *A. petiolata* on invaded systems. *Allaria petiolata* like other members of the Cruciferae produces glucosinolates, which when degraded can inhibit the germination of arbuscualar mycorrhizae and reduce the mycorrhizal inoculum potential of soil (Roberts & Anderson 2001). Non-mycorrhizal species may recover from *A. petiolata* removal more quickly than mycorrhizal species (Anderson *et al.* 2010). Castellano & Gorchov (2012) found that between two sites, one which had been invaded by *A. petiolata* and one which hadn't, there was a difference in colonization of ectomycorrhizal fungi on Northern red oak, of 7 and 26%, respectively. Furthermore they found a higher level of pH and organic fractionation in densely invaded sites. There have also been reports that *A. petiolata* can cause declines of mycorrhizal colonization on mature roots of canopy trees. Decline of mycorrhizae may interfere with normal tree functions caused by nutrient disruption (Wolfe *et al.* 2008). More information on how the lack of mycorrhizae in an infested forest is affecting tree growth is

needed, as well as how the variety of chemicals and microorganisms that come with introduced organisms affect their new ecosystems.

In order to more effectively understand how commercially important species may interact with invasive species, particularly those that produce allelopathic compounds, we must assess the "unseen" aspects of tree health. There are various degrees to this interaction: invasion, forestry and microbiology that sometimes interact in counter intuitive or contrasting ways. An economically imperative group of organisms involved in every aspect of this interaction, and largely influential on production, are mycorrhizae. It is difficult to estimate how production will be affected in a plantation environment by an invasive species without this knowledge. This simplistic ecosystem has the potential to greatly contribute to science and a commercial industry that totaled US$103 billion in the southeastern United States alone. It is therefore necessary to investigate in detail the effect of *Imperata cylindrica* on microbial mycorrhizal communities associated with *Pinus taeda* in order to determine whether yield is being reduced, to what degree, and if this reduction can be counteracted in a cost effective manner.

1.5. OBJECTIVES

The major goal of this research is to describe the interactions between soil microbial communities, with specific focus on mycorrhizae, and *I. cylindrica*. To this end three objectives were addressed:

1. To examine the effects of *I. cylindrica* invasion on abundance of mycorrhizal fungi;
2. To examine the effects of *I. cylindrica* invasion on microbial abundance through organic nutrient turnover and abiotic soil characteristics; and
3. To examine the effect of individual components of *I. cylindrica* exudate on individual species of mycorrhizal fungi.

1.6. REFERENCES

Abbott, K.L. 2006. Spatial Dynamics of Supercolonies of the Invasive Yellow Crazy Ant, Anoplolepis Gracilipes, on Christmas Island, Indian Ocean. *Diversity & Distributions* 12 (1): 101–10.

Allen, M.F., W. Swenson, J.I. Querejeta, L.M. Egerton-Warburton, and K.K. Treseder. 2003. Ecology of Mycorrhizae: A Conceptual Framework for Complex Interactions Among Plants and Fungi. *Annual Review of Phytopathology* 41 (1): 271–303.

Anderson, R.C., M.R. Anderson, J.T. Bauer, M. Slater, J. Herold, P. Baumhardt, and V. Borowicz. 2010. Effect of Removal of Garlic Mustard (Alliaria Petiolata, Brassicaeae) on Arbuscular Mycorrhizal Fungi Inoculum Potential in Forest Soils. *Open Ecology Journal* 3: 41–47.

Baar, J., T.R. Horton, A.M. Kretzer, and T.D. Bruns. 1999. Mycorrhizal Colonization of Pinus Muricata from Resistant Propagules after a Stand-Replacing Wildfire. *New Phytologist* 143 (2): 409–18.

Baker, H.G. 1974. The Evolution of Weeds. *Annual Review of Ecology and Systematics* 5 (1): 1–24.

Baker J., and Balmer W.E. 1983. Loblolly Pine [Pinus Taeda, Ecology, Management, Production, United States]. *Agriculture Handbook U.S. Dept. of Agriculture*.

Barnett, J.P., and R.M. Sheffield. 2004. Slash Pine: Characteristics, History, Status, and Trends.

Blossey, B., and R. Notzold. 1995. Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. *Journal of Ecology* 83 (5): 887–89.

Boer, W.de, L.B. Folman, R.C. Summerbell, and L. Boddy. 2005. Living in a Fungal World: Impact of Fungi on Soil Bacterial Niche Development. *FEMS Microbiology Reviews* 29

(4): 795–811.

Bomberg, M., G. Jurgens, A. Saano, R. Sen, and S. Timonen. 2003. Nested PCR Detection of Archaea in Defined Compartments of Pine Mycorrhizospheres Developed in Boreal Forest Humus Microcosms. *FEMS Microbiology Ecology* 43 (2): 163–71.

Bomberg, M., and S. Timonen. 2007. Distribution of Cren- and Euryarchaeota in Scots Pine Mycorrhizospheres and Boreal Forest Humus. *Microbial Ecology* 54 (3): 406–16.

Bomberg, M., L. Montonen, and S. Timonen. 2010. Anaerobic Eury- and Crenarchaeota Inhabit Ectomycorrhizas of Boreal Forest Scots Pine. *European Journal of Soil Biology* 46 (6): 356–64.

Bonfante, P., and I.A. Anca. 2009. Plants, Mycorrhizal Fungi, and Bacteria: A Network of Interactions. *Annual Review of Microbiology* 63 (1): 363–83.

Boufalis, A., F. Pellissier, and L. Trosset. 1994. Responses of Mycorrhizal Fungi to Allelopathy: Cenococcum Geophilum and Laccaria Laccata Growth with Phenolic Acids. *Acta Botanica Gallica* 141 (4): 547–50.

Brewer, S. 2008. Declines in Plant Species Richness and Endemic Plant Species in Longleaf Pine Savannas Invaded by Imperata Cylindrica. *Biological Invasions* 10 (8): 1257–64.

Broeckling, C.D., A.K. Broz, J. Bergelson, D.K. Manter, and J.M. Vivanco. 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. *Applied and Environmental Microbiology* 74 (3): 738–44.

Brown, H., and W. McDowell. 1968. *Status of Loblolly Pine Die-Off on the Oakmulgee District, Talladega National Forest, Alabama*. 69-2-28.

Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. Working with Mycorrhizas in Forestry and Agriculture.

Buée, M., W.De Boer, F. Martin, L. van Overbeek, and E. Jurkevitch. 2009. The Rhizosphere Zoo: An Overview of Plant-Associated Communities of Microorganisms, Including Phages, Bacteria, Archaea, and Fungi, and of Some of Their Structuring Factors. *Plant & Soil* 321 (1/2): 189–212.

Buée, M., M. Reich, C. Murat, E. Morin, R. H. Nilsson, S. Uroz, and F. Martin. 2009. 454 Pyrosequencing Analyses of Forest Soils Reveal an Unexpectedly High Fungal Diversity. *New Phytologist* 184 (2): 449–56.

Burke, D.J., A.M. Kretzer, P.T. Rygiewicz, and M.A. Topa. 2006. Soil Bacterial Diversity in a Loblolly Pine Plantation: Influence of Ectomycorrhizas and Fertilization. *FEMS Microbiology Ecology* 57 (3): 409–19.

Cabe, P.R, American Ornithologists' Union, and Academy of Natural Sciences of Philadelphia. 1993. *European Starling: Sturnus Vulgaris*. [Washington, DC; Philadelphia, PA: American Ornithologists' Union ; Academy of Natural Sciences.

Cain, M.D., and M.G. Shelton. 2014. Indigenous Vegetation in a Southern Arkansas Pine-Hardwood Forest after a Half Century without Catastrophic Disturbances.

Callaway, R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson, and J. Klironomos. 2008. Novel Weapons: Invasive Plant Suppresses Fungal Mutualists in America but Not in Its Native Europe. *Ecology* 89 (4): 1043–55.

Callaway, R.M., and W.M. Ridenour. 2004. Novel Weapons: Invasive Success and the Evolution of Increased Competitive Ability. *Frontiers in Ecology and the Environment* 2 (8): 436–43.

Carmean, W.H. 1947. The Effects of Clear-Cutting in Patches on the Reproduction of Old Field Loblolly Pine in the Duke Forest.

Castellano, S.M., and D.L. Gorchov. 2012. Reduced Ectomycorrhizae on Oak Near Invasive Garlic Mustard. *Northeastern Naturalist* 19 (1): 1–24.

Catford, J.A., R. Jansson, and C. Nilsson. 2009. Reducing Redundancy in Invasion Ecology by Integrating Hypotheses into a Single Theoretical Framework. *Diversity & Distributions* 15 (1): 22–40.

Cavers, P.B., M.I. Heagy, and R.F. Kokron. 1979. The Biology of Canadian Weeds.: 35. Alliaria Petiolata (M. Bieb.) Cavara and Grande. *Canadian Journal of Plant Science* 59 (1): 217–29.

Chapin III, F. Stuart, E.S. Zavaleta, V.T. Eviner, R.L. Naylor, P.M. Vitousek, H.L. Reynolds, D. U. Hooper, et al. 2000. Consequences of Changing Biodiversity. *Nature* 405 (6783): 234–42.

Chown, S.L., Ad H.L. Huiskes, N.J.M. Gremmen, J.E. Lee, A. Terauds, K. Crosbie, Y. Frenot, et al. 2012. Continent-Wide Risk Assessment for the Establishment of Nonindigenous Species in Antarctica. *Proceedings of the National Academy of Sciences* 109 (13): 4938–43.

Chwedorzewska, K.J. 2008. Poa Annua L. in Antarctic: Searching for the Source of Introduction. *Polar Biology* 31 (3): 263–68.

Chwedorzewska, K.J., and P.T. Bednarek. 2012. Genetic and Epigenetic Variation in a Cosmopolitan Grass Poa Annua from Antarctic and Polish Populations. *Polish Polar Research* 33 (1): 63–80.

Daneshgar, P., and S. Jose. 2009. Imperata Cylindrica, an Alien Invasive Grass, Maintains Control over Nitrogen Availability in an Establishing Pine Forest. *Plant and Soil* 320 (1-2): 209–18.

Daneshgar, P., S. Jose, A. Collins, and C. Ramsey. 2008. Cogongrass (Imperata Cylindrica), an Alien Invasive Grass, Reduces Survival and Productivity of an Establishing Pine Forest. *Forest Science* 54 (6): 579–87.

D'Antonio, C., and L.A. Meyerson. 2002. Exotic Plant Species as Problems and Solutions in Ecological Restoration: A Synthesis. *Restoration Ecology* 10 (4): 703–13.

Davis, M.A. 2009. *Invasion Biology*. Oxford University Press.

Dozier, H., J.F. Gaffney, S.K. McDonald, E.R.R.L. Johnson, and D.G. Shilling. 1998. Cogongrass in the United States: History, Ecology, Impacts, and Management. *Weed Technology : A Journal of the Weed Science Society of America*.

Eckhardt, L.G., A.M. Weber, R.D. Menard, J.P. Jones, and N.J. Hess. 2007. Insect-Fungal Complex Associated with Loblolly Pine Decline in Central Alabama. *Forest Science* 53 (1): 84–92.

Eckhardt, L.G., M.A. Sword-Sayer, and D. Imm. 2010. State of Pine Decline in the Southeastern United States. *Southern Journal of Applied Forestry* 34 (3): 138–41.

Elton, C.S., 1958. The Ecology of Invasions by Animals and Plants. — European Environment Agency (EEA). 2014. Rationale Reference.

Enebak, S.A., and W.A. Carey. 2000. Evidence for Induced Systemic Protection to Fusiform Rust in Loblolly Pine by Plant Growth-Promoting Rhizobacteria. *Plant Disease* 84 (3): 306–8.

Enebak, S.A., G. Wei, and J.W. Kloepper. 1998. "Effects of Plant Growth-Promoting Rhizobacteria on Loblolly and Slash Pine Seedlings." *Forest Science* 44 (1): 139–44.

Enebak, S. 2013. Forest Tree Seedling Production in the South for the 2012–2013 Planting Season. Auburn University Southern Forestry Nursery Management Cooperative.

Facon, B., B.J. Genton, J. Shykoff, P. Jarne, A. Estoup, and P. David. 2006. A General Eco-Evolutionary Framework for Understanding Bioinvasions. *Trends in Ecology & Evolution* 21 (3): 130–35.

Forseth, I.N., and A.F. Innis. 2004. Kudzu (Pueraria Montana): History, Physiology, and Ecology Combine to Make a Major Ecosystem Threat. *Critical Reviews in Plant Sciences* 23 (5): 401–13.

Frey-Klett, P., M. Chavatte, M.L. Clausse, S. Courrier, C.L. Roux, J. Raaijmakers, M.G. Martinotti, J.C. Pierrat, and J. Garbaye. 2005. Ectomycorrhizal Symbiosis Affects Functional Diversity of Rhizosphere Fluorescent Pseudomonads. *New Phytologist* 165 (1): 317–28.

Frost, C. 1993. "Four Centuries of Changing Landscape Patterns in the Longleaf Pine Ecosystem." *Proceedings of the Tall Timbers Fire Ecology Conference* 18.

Garbaye, J. 1994. Tansley Review No. 76 Helper Bacteria: A New Dimension to the Mycorrhizal Symbiosis. *New Phytologist* 128 (2): 197–210.

Garbaye, J., and G.D. Bowen. 1989. Stimulation of Ectomycorrhizal Infection of Pinus Radiata by Some Microorganisms Associated with the Mantle of Ectomycorrhizas. *New Phytologist* 112 (3): 383–88.

Gardes, M., and T.D. Bruns. 1993. ITS Primers with Enhanced Specificity for Basidiomycetes - Application to the Identification of Mycorrhizae and Rusts. *Molecular Ecology* 2 (2): 113–18.

Gholz, H.L., R.F. Fisher, and W.L. Prichett. 1985. Nutrient Dynamics in Slash Pine Plantation Ecosystems. *Ecology* 66 (3): 647–59.

Goudswaard, K., F. Witte, and E.F.B. Katunzi. 2008. The Invasion of an Introduced Predator,

Nile Perch (Lates Niloticus, L.) in Lake Victoria (East Africa): Chronology and Causes. *Environmental Biology of Fishes* 81 (2): 127–39.

Griffiths, R.W., D.W. Schloesser, J.H. Leach, and W.P. Kovalak. 1991. Distribution and Dispersal of the Zebra Mussel (Dreissena Polymorpha) in the Great Lakes Region. *Canadian Journal of Fisheries and Aquatic Sciences* 48 (8): 1381–88.

Hagan, D.L., S. Jose, and C.H. Lin. 2013. Allelopathic Exudates of Cogongrass (Imperata Cylindrica): Implications for the Performance of Native Pine Savanna Plant Species in the Southeastern US. *Journal of Chemical Ecology* 39 (2): 312–22.

Hebert, P.D.N., B.W. Muncaster, and G.L. Mackie. 1989. Ecological and Genetic Studies on Dreissena Polymorpha (Pallas): A New Mollusc in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 46 (9): 1587–91.

Herre, E.A., L.C. Mejía, D.A. Kyllo, E. Rojas, Z. Maynard, A. Butler, and S.A. Van Bael. 2007. Ecological Implications of Anti-Pathogen Effects of Tropical Fungal Endophytes and Mycorrhizae. *Ecology* 88 (3): 550–58.

Holm, L.G., D.L. Plucknett, J.V. Pancho, and J.P. Herberger. 1977. "The World's Worst Weeds.," 609 pp.

Holzmueller, E.J., and S. Jose. 2011. Invasion Success of Cogongrass, an Alien C4 Perennial Grass, in the Southeastern United States: Exploration of the Ecological Basis. *Biological Invasions* 13 (2): 435–42.

Horton, T.R. 2002. Molecular Approaches to Ectomycorrhizal Diversity Studies: Variation in ITS at a Local Scale. In *Diversity and Integration in Mycorrhizas*, edited by Sally E. Smith and F. Andrew Smith, 29–39. Developments in Plant and Soil Sciences 94. Springer Netherlands.

Horton, T.R., and T.D. Bruns. 2001. The Molecular Revolution in Ectomycorrhizal Ecology: Peeking into the Black-Box. *Molecular Ecology* 10 (8): 1855–71.

Hussain, F., and N. Abidi. 1991. Allelopathy Exhibited by Imperata Cylindrica (L.) P. Beauv. *Pakistan Journal of Botany (Pakistan)*, June.

Ingham, R.E. 1988. Interactions between Nematodes and Vesicular-Arbuscular Mycorrhizae. *Agriculture, Ecosystems & Environment*, Proceedings of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth, 24 (1–3): 169–82.

Johansson, J.F., L.R. Paul, and R.D. Finlay. 2004. Microbial Interactions in the Mycorrhizosphere and Their Significance for Sustainable Agriculture. *FEMS Microbiology Ecology* 48 (1): 1–13.

Jose, S., J. Cox, D.L. Miller, D.G. Shilling, and S. Merritt. 2002. Alien Plant Invasions: The Story of Cogongrass in Southeastern Forests. *Journal of Forestry* 100 (1): 41–44.

Kayihan, G.C., Huber, D.A., Morse, A.M., White, T.L., and Davis, J.M. 2005. Genetic Dissection of Fusiform Rust and Pitch Canker Disease Traits in Loblolly Pine. *Theoretical and Applied Genetics*, *110*(5), 948-958.

Karatayev, A.Y, S.E. Mastitsky, L.E. Burlakova, D. P. Molloy, and G.G. Vezhnovets. 2003. Seasonal Dynamics of Endosymbiotic Ciliates and Nematodes in Dreissena Polymorpha. *Journal of Invertebrate Pathology* 83 (1): 73–82.

Keane, R.M., and M.J. Crawley. 2002. "Exotic Plant Invasions and the Enemy Release Hypothesis." *Trends in Ecology & Evolution* 17 (4): 164–70.

Kellison, R.C., and M.J. Young. 1997. The Bottomland Hardwood Forest of the Southern United States. *Forest Ecology and Management*, Harvesting Impacts on Bottomland Hardwood

Ecosystems, 90 (2–3): 101–15.

Koenig, W.D. 2003. "European Starlings and Their Effect on Native Cavity-Nesting Birds." *Conservation Biology* 17 (4): 1134.

Koger, C.H., and C.T. Bryson. 2004. Effect of Cogongrass (Imperata Cylindrica) Extracts on Germination and Seedling Growth of Selected Grass and Broadleaf Species. *Weed Technology* 18 (2): 236–42.

Kolar, C.S., and D.M. Lodge. 2001. Progress in Invasion Biology: Predicting Invaders. *Trends in Ecology & Evolution* 16 (4): 199–204.

Kraus, F., and D. Cravalho. 2001. The Risk to Hawai'i from Snakes. *Pacific Science* 55 (4): 409–17.

Kuske, C.R., S.M. Barns, and J.D. Busch. 1997. Diverse Uncultivated Bacterial Groups from Soils of the Arid Southwestern United States That Are Present in Many Geographic Regions. *Applied and Environmental Microbiology* 63 (9): 3614–21.

Landers, J.L., D.H. Van Lear, and W.D. Boyer. 1995. The Longleaf Pine Forests of the Southeast: Requiem or Renaissance? *Journal of Forestry* 93 (11): 38–44.

LeJeune, J., J. Homan, G. Linz, and D.L. Pearl. 2008. Role of the European Starling in the Transmission of E. Coli O157 on Dairy Farms. *Proceedings - Vertebrate Pest Conference*.

Linz, G.M., H.J. Homan, S.M. Gaulker, L.B. Penry, and W.J. Bleier. 2007. European Starlings: A Review of an Invasive Species with Far-Reaching Impacts. *Managing Vertebrate Invasive Species*, August.

Little, E.L., and K.W. Dorman. 1952. Slash Pine (Pinus Elliottii), Its Nomenclature and Varieties. *Journal of Forestry* 50 (12): 918–23.

Lloret, F., F. Médail, G. Brundu, I. Camarda, E. Moragues, J. Rita, P. Lambdon, and P.E. Hulme. 2005. Species Attributes and Invasion Success by Alien Plants on Mediterranean Islands. *Journal of Ecology* 93 (3): 512–20.

Lockwood, J.L., P. Cassey, and T. Blackburn. 2005. The Role of Propagule Pressure in Explaining Species Invasions. *Trends in Ecology & Evolution*, Special issue: Invasions, guest edited by Michael E. Hochberg and Nicholas J. Gotelli, 20 (5): 223–28.

Lockwood, J.L., P. Cassey, and T.M. Blackburn. 2009. The More You Introduce the More You Get: The Role of Colonization Pressure and Propagule Pressure in Invasion Ecology. *Diversity & Distributions* 15 (5): 904–10.

Lockwood, J.L., M.F. Hoopes, and M.P. Marchetti. 2013. *Invasion Ecology*. John Wiley & Sons.

Lugtenberg, B., and F. Kamilova. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology* 63 (1): 541–56.

MacDonald, G.E., D.G. Shilling, B.J. Brecke, J.F. Gaffney, K.A. Langeland, and J.T. Ducar. 2002. Weeds in the Sunshine: Cogongrass (Imperata Cylindrica (L.) Beauv.) Biology, Ecology and Management in Florida. *University of Florida IFAS*.

MacDonald, G.E. 2004. Cogongrass (Imperata cylindrica)—Biology, Ecology, and Management. *Critical Reviews in Plant Sciences* 23 (5): 367–80.

Manion, P.D. 2003. Evolution of Concepts in Forest Pathology. *Phytopathology* 93 (8): 1052–55.

Martin, L.B., and L. Fitzgerald. 2005. A Taste for Novelty in Invading House Sparrows, Passer Domesticus. *Behavioral Ecology* 16 (4): 702–7.

Marx, D.H., and W.C. Bryan. 1971. Influence of Ectomycorrhizae on Survival and Growth of Aseptic Seedlings of Loblolly Pine at High Temperature. *Forest Science* 17 (1): 37–41.

Matusick, G., R.D. Menard, Y. Zeng, and L.G. Eckhardt. 2013. Root-Inhabiting Bark Beetles

(Coleoptera: Curculionidae) and Their Fungal Associates Breeding in Dying Loblolly Pine in Alabama. *Florida Entomologist* 96 (1): 238–41.

Meekins, J.F., H.E. Ballard Jr., and B.C. McCarthy. 2001. Genetic Variation and Molecular Biogeography of a North American Invasive Plant Species (Alliaria Petiolata, Brassicaceae). *International Journal of Plant Sciences* 162 (1): 161–69.

Miller, J.H., E.B. Chambliss, and N.J. Loewenstein. 2011. *Field Guide for the Identification of Invasive Plants in Southern Forests*. DIANE Publishing.

Molnar, J.L, R.L. Gamboa, C. Revenga, and M.D Spalding. 2008. Assessing the Global Threat of Invasive Species to Marine Biodiversity. *Frontiers in Ecology and the Environment* 6 (9): 485–92.

Moore, L., and W. Walker-Wilson. 2006. Slash Pine. *USDA NRCS*.

Moore, W.C. *Flora of the British Isles,*. Vol. 35.

Moulton, M.P., W.P. Cropper Jr, M.L. Avery, and L.E. Moulton. 2010. The Earliest House Sparrow Introductions to North America. *Biological Invasions* 12 (9): 2955–58.

Nuñez, M.A., A. Moretti, and D. Simberloff. 2011. Propagule Pressure Hypothesis Not Supported by an 80-Year Experiment on Woody Species Invasion. *Oikos* 120 (9): 1311–16.

Oak, S.W., and F.H. Tainter. 1988. Risk Prediction of Loblolly Pine Decline on Littleleaf Disease Sites in South Carolina. *Plant Disease (USA)*, April.

O'Dowd, D.J., P.T. Green, and P.S. Lake. 2003. Invasional 'meltdown' on an Oceanic Island. *Ecology Letters* 6 (9): 812–17.

Olsson, P.A., I.M. van Aarle, W.G. Allaway, A.E. Ashford, and H. Rouhier. 2002. Phosphorus Effects on Metabolic Processes in Monoxenic Arbuscular Mycorrhiza Cultures. *Plant*

Physiology 130 (3): 1162–71.

Pappert, R.A., J.L. Hamrick, and L.A. Donovan. 2000. Genetic Variation in Pueraria Lobata (Fabaceae), an Introduced, Clonal, Invasive Plant of the Southeastern United States. *American Journal of Botany* 87 (9): 1240–45.

Perrin, R. 1990. Interactions between Mycorrhizae and Diseases Caused by Soil-Borne Fungi. *Soil Use and Management* 6 (4): 189–94.

Perry, D.A. 1998. The Scientific Basis of Forestry. *Annual Review of Ecology and Systematics* 29 (1): 435–66.

Phillips, B.L., G.P. Brown, and R. Shine. 2010. Life-History Evolution in Range-Shifting Populations. *Ecology* 91 (6): 1617–27.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and Economic Costs of Nonindigenous Species in the United States. *BioScience* 50 (1): 53–65.

Pyšek, P., D.M. Richardson, J. Pergl, V. Jarošík, Z. Sixtová, and E. Weber. 2008. "Geographical and Taxonomic Biases in Invasion Ecology." *Trends in Ecology & Evolution* 23 (5): 237–44.

Quarterman, E., and C. Keever. 1962. Southern Mixed Hardwood Forest: Climax in the Southeastern Coastal Plain, U.S.A. *Ecological Monographs* 32 (2): 167–85.

Ricciardi, A., R.J. Neves, and J.B. Rasmussen. 1998. Impending Extinctions of North American Freshwater Mussels (Unionoida) Following the Zebra Mussel (Dreissena Polymorpha) Invasion. *Journal of Animal Ecology* 67 (4): 613–19.

Richardson, D.M. 2000. *Ecology and Biogeography of Pinus*. Cambridge University Press.

Richardson, D.M., and P. Pyšek. 2008. "Fifty Years of Invasion Ecology – the Legacy of Charles Elton." *Diversity & Distributions*, March, 161–68.

Richardson, D.M., P. Pysek, M. Rejmanek, M.G. Barbour, F. Dane Panetta, and C.J. West. 2000. "Naturalization and Invasion of Alien Plants: Concepts and Definitions." *Diversity & Distributions* 6 (2): 93.

Roberts, K.J., and R.C. Anderson. 2001. Effect of Garlic Mustard [Alliaria Petiolata (Beib. Cavara & Grande)] Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi. *The American Midland Naturalist* 146 (1): 146–52.

Rodriguez, R.J., J.F. White Jr, A.E. Arnold, and R.S. Redman. 2009. Fungal Endophytes: Diversity and Functional Roles. *New Phytologist* 182 (2): 314–30.

Sakai, A.K., F.W. Allendorf, J.S. Holt, D.M. Lodge, J. Molofsky, K.A. With, S. Baughman, et al. 2001. "The Population Biology of Invasive Species." *Annual Review of Ecology and Systematics* 32 (1): 305–32.

Schultz, R.P. 1997. Loblolly Pine: The Ecology and Culture of Loblolly Pine (Pinus Taeda L.).

Shilling, D.G., T. Bewick, J. Gaffney, S. McDonald, Chase, and E. Johnson. 1997. Ecology, Physiology, and Management of Cogongrass (Imperata Cylindrica).

Simberloff, D. 2009. The Role of Propagule Pressure in Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics* 40 (1): 81–102.

Smith, R.I.L., and M. Richardson. 2011. Fuegian Plants in Antarctica: Natural or Anthropogenically Assisted Immigrants? *Biological Invasions* 13 (1): 1–5.

Smith, S.E., and D.J. Read. 2010. *Mycorrhizal Symbiosis*. Academic Press.

Smith, W.B., P.D. Miles, C.H. Perry, and S.A. Pugh. 2009. Forest Resources of the United States, 2007: A Technical Document Supporting the Forest Service 2010 RPA Assessment. *General Technical Report - USDA Forest Service*, no. WO-78: vii + 337 pp.

Strobel, G.A. 2003. Endophytes as Sources of Bioactive Products. *Microbes and Infection* 5 (6):

535–44.

Strobel, G., and B. Daisy. 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. *Microbiology and Molecular Biology Reviews* 67 (4): 491–502.

Sun, J.H., Z.C. Li, D.K. Jewett, K.O. Britton, W.H. Ye, and X.J. Ge. 2005. Genetic Diversity of Pueraria Lobata (kudzu) and Closely Related Taxa as Revealed by Inter-Simple Sequence Repeat Analysis. *Weed Research* 45 (4): 255–60.

Tainter, F.H., and F.A. Baker. 1996. *Principles of Forest Pathology*. John Wiley & Sons.

Tan, R.X., and W.X. Zou. 2001. Endophytes: A Rich Source of Functional Metabolites (1987 to 2000). *Natural Product Reports* 18 (4): 448–59.

Theoharides, K.A., and J.S. Dukes. 2007. Plant Invasion across Space and Time: Factors Affecting Nonindigenous Species Success during Four Stages of Invasion. *New Phytologist* 176 (2): 256–73.

Tilley, B., and I.A. Munn. 2007. 2001 Economic Impacts of the Forest Products Industry in the South. *Southern Journal of Applied Forestry* 31 (4): 181–86.

Trappe, J.M. 1977. Selection of Fungi for Ectomycorrhizal Inoculation in Nurseries. *Annual Review of Phytopathology* 15 (1): 203–22.

United States Congress. 1993. *Harmful Non-Indigenous Species in the United States: Summary*. Washington, DC: The Office.

Vanderploeg, H.A, J.R. Liebig, W.W. Carmichael, M.A. Agy, T.H. Johengen, G.L. Fahnenstiel, and T.F. Nalepa. 2001. Zebra Mussel (Dreissena Polymorpha) Selective Filtration Promoted Toxic Microcystis Blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 58 (6): 1208–21.

Varner, J.M. III, and J.S. Kush. 2014. Remanat Old-Growth Longleaf Pine (Pinus Palustris Mill.)

Savannas and Forests of the Southeastern USA: Status and Threats.

Verschuren, D., T.C. Johnson, H.J. Kling, D.N. Edgington, P.R. Leavitt, E.T. Brown, M.R. Talbot, and R.E. Hecky. 2002. History and Timing of Human Impact on Lake Victoria, East Africa. *Proceedings of the Royal Society B: Biological Sciences* 269 (1488): 289–94.

Visser, S.. 1995. Ectomycorrhizal Fungal Succession in Jack Pine Stands Following Wildfire."*New Phytologist* 129 (3): 389–401.

Vivanco, J.M., H.P. Bais, F.R. Stermitz, G.C. Thelen, R.M. Callaway, and M. Schwartz. 2004. REPORT Biogeographical Variation in Community Response to Root Allelochemistry: Novel Weapons and Exotic Invasion. *Ecology Letters* 7 (4): 285–92.

Volonterio, O., R.P. de León, P. Convey, and E. Krzemińska. 2013. First Record of Trichoceridae (Diptera) in the Maritime Antarctic. *Polar Biology* 36 (8): 1125–31.

Wahlenberg, W.G. 1960. *Loblolly Pine. Its Use, Ecology, Regeneration, Protection, Growth and Management.*

Williamson, M., and A. Fitter. 1996. The Varying Success of Invaders. *Ecology* 77 (6): 1661–66.

Wolfe, B.E., V.L. Rodgers, K.A. Stinson, and A. Pringle. 2008. The Invasive Plant Alliaria Petiolata (garlic Mustard) Inhibits Ectomycorrhizal Fungi in Its Introduced Range. *Journal of Ecology* 96 (4): 777–83.

Zhang, L.Y, W.H. Ye, H.L. Cao, and H.L. Feng. 2004. Mikania Micrantha H. B. K. in China – an Overview. *Weed Research* 44 (1): 42–49.

Chapter 2

Imperata cylindrica impacts colonization by mycorrhizal fungi on *Pinus taeda* in commercial stands

2.1. ABSTRACT

Cogongrass (*Imperata cylindrica*), an invasive grass species native to Asia, has been shown to reduce tree vigor in loblolly pine (*Pinus taeda*) plantations, which comprise over 50 % of growing stock in commercial forests of the United States. *Imperata cylindrica* produces exudates with possible allelopathic effects that may influence abundance of *P. taeda* symbionts such as soil microbes and ectomycorrhizal fungi. Soil microbial communities and root colonization by mycorrhizal fungi were sampled in intensively managed *P. taeda* stands in Greene County, Mississippi, in *I. cylindrica* present and absent plots. *Imperata cylindrica* present plots had a reduction in abundance of ectomycorrhizal colonization of pine fine feeder roots in the top 40 cm of soil in comparison to *I. cylindrica* absent plots. Abundance of pine fine feeder roots in the 21–40 cm and 41–60 cm layers of the soil profile was also reduced in *I. cylindrica* present plots. Vegetative diversity was negatively correlated with *I. cylindrica* abundance (% cover) which probably contributed to the reduced microbial diversity in *I.*

cylindrica present plots. Due to the variety of roles these microorganisms play, the changes associated with the invasion of *I. cylindrica* in commercial forestry plantations are likely to alter nutrient cycling and reduce *P. taeda* growth as well as site productivity.

2.2. INTRODUCTION

Economic losses attributable to the nearly 50,000 non-indigenous species in the United States are estimated to be US$ 120 billion per year (Pimentel *et al.* 2005). A large portion of the impacts of invasive species is ecological in nature and often unnoticed, although they can contribute to ecosystem or community transformation over time (Simberloff *et al.* 2013). In addition to direct impacts on above ground flora and fauna, effects on below ground symbionts and other organisms that co-occur with native species can magnify the impact of an invasive species. While a few instances of compounding effects of co-invasion by plants and their symbionts have been well documented (Richardson *et al.* 2000; Pasternak *et al.* 2007; Dickie *et al.* 2010), principles that outline how an invader can affect pre-existing communities of mutualists are poorly defined.

Cogongrass (*Imperata cylindrica* (L.) Beauv) is classified as a noxious weed in over 70 countries and represents a tangible threat to biodiversity and resource management (Burrell et al. 2015; Estrada & Flory 2015). In areas where it has successfully invaded it often forms dense monotypic stands (MacDonald 2004). *Imperata cylindrica* was introduced accidentally from Japan to the United States in 1912, but later was intentionally introduced for forage crop trials and erosion control (Bryson & Carter 1993; Holzmueller & Jose 2011). Due to its high silica content, it was ultimately not selected for use as a forage crop (MacDonald 2004). *Imperata cylindrica* is a C_4, rhizome producing, perennial plant that can reach heights of 3 m but typically grows to heights of 1.2 m (Koger & Bryson 2004). It spreads both sexually through seeds and

asexually through rhizomes. Seed production can be prolific with most seeds generally dispersed within 15 m of the plant (Daneshgar *et al.* 2008). *Imperata cylindrica* can grow on a variety of soils, from nutrient-poor, coarse sands to nutrient-rich, sandy loam soils (Jose *et al.* 2002). It is particularly difficult to eradicate because it produces a vast network of underground rhizomes from which the plant regenerates. Rhizomes can reach densities of 89 $m.m^{-2}$ of soil (MacDonald 2004).

Daneshgar & Jose (2009) found that competition for nitrogen (N) between *I. cylindrica* and loblolly pine (*Pinus taeda* L.) seedlings resulted in a greater reduction in pine seedling growth than competition with native vegetation. *Imperata cylindrica* also may impact growth of timber species such as *P. taeda* through production of exudates that may have an allelopathic effect (Hagen *et al.* 2013; Holzmueller & Jose 2011; Koger & Bryson 2004; Hussain & Abidi 1991). Compounds isolated from exudates of *I. cylindrica* with possible allopathic effects include seven phenolic acids, two aromatic acids, one trihydroxy anthraquinone, and one meta dihydroxyl phenol (Hagen *et al.* 2013). In addition to direct impacts on plant growth, compounds present in *I. cylindrica* exudates may affect growth through influence on mycorrhizae and other soil microbial taxa (Roberts & Anderson 2001; Kourtev *et al.* 2002). Boufalis *et al.* (1994) found that the growth of two ectomycorrhizal fungal species, *Cenococcum geophilum* and *Laccaria laccata*, were affected by the applications of several phenolic acids similar to those produced by *I. cylindrica*. For this study, we hypothesized that *I. cylindrica* present *P. taeda* plots would have reduced mycorrhizal and fine *P. taeda* feeder root abundance, a reduction in microbial biomass (N) ($mg.L^{-1}$) and *P. taeda* growth rate ($mm.yr^{-1}$), as well as reduced vegetation diversity when compared to *I. cylindrica* absent plots.

2.3. MATERIALS AND METHODS

2.3.1. Site Description:

The research site was located east of Leakesville, Greene County, Mississippi, an area under commercial *P. taeda* production. The area receives an average annual rainfall of 1,677 mm (www.idcide.com). The dominant mid-story vegetation includes yaupon (*Ilex vomitoria* Ait.), scrub oak (*Quercus* spp.), and *P. taeda*. Much of the area has also been invaded by *I. cylindrica*. The soil series was identified as Benndale sandy loam on 8 - 15% slopes, and Benndale sandy loam or McLaurin sandy loam on 2 - 5% slopes. Benndale soils are classified as a coarse-loamy, siliceous, semi-active, thermic Typic Paleudults and McLaurin soils are classified as a coarse-loamy, siliceous, sub-active, thermic Typic Paleudult. These soils are well drained and generally nutrient poor due to low amounts of organic matter and clay (Soil Survey Staff 2012).

Eight study plots (16 m X 16 m) total were established within two similar stands of *P. taeda* that have received slightly different management regimens (Table 2.1). The plots were situated within the northeast quadrant of larger plots (30 m x 30 m), which were initially established for a study in 2010 (Brunson 2013). Four plots were located within areas of the stands that were not invaded with *I. cylindrica*, the remaining four in areas that were invaded with *I. cylindrica*.

Table 2.1. The site conditions and past treatments listed by plot. Plots in which no *I. cylindrica* were observed have the prefix N, plots with <50 % *I. cylindrica* have the prefix P and plots in which *I. cylindrica* was present >50 % have the prefix A. DAP was applied in 2006 at a rate of 280 kg.ha^{-1} and Urea applied in 2010 at a rate of 224 kg.ha^{-1}. DAP applied in 2006 but at a rate of 140 kg.ha^{-1} and Urea applied in 2007 at a rate of 224 kg.ha^{-1}.

	Imperata cylidrica absent			*Imperata cylindrica* present (<50% cover)		*Imperata cylindrica* abundant (>50% cover)		
Plot number	N1	N2	N3	P1	P2	A1	A2	A3
Imperata cylindrica cover 2011 (%)	0	0	0	0	86	94	97	64
Stand Age	16	16	22	21	21	16	16	22
Basal Area (m²/Ha)	20.9	21.6	24.8	25.3	25.3	21.6	20.9	24.8
Trees Per Acre	227	244	266	243	243	244	227	266
Percent Stocking	70		80		80	70		80
GPS	31.14842, -88.482	31.15198, -88.48292	31.14825, -88.48052	31.14714, -88.4763	31.14804, -88.47518	31.14896, -88.48191	31.1479, -88.4818	31.14785, -88.48038
Urea	2010		2007		2010		2007	
Diammonium phosphate application (DAP)	2006		2006		2006		2006	
Thinned	2009		Prior to 2006		2009		Prior to 2006	
Burned	X		Cool season, 2009		X		Cool season, 2009	

Over the course of this study, *I. cylindrica* invaded one of the non-*I. cylindrica* plots and it was determined that *I. cylindrica* density in one of the *I. cylindrica* invaded plots was lower than in the original larger plot as a whole. Therefore, for analyses the plots were characterized by whether *I. cylindrica* was present (< 50% cover, n=2), abundant (>50% cover, n=3), or absent (n=3) (Figure 2.1).

Figure 2.1. (**A**.) An example of a plot where *I. cylindrica* was abundant (>50%); (**B**.) An example of a plot where *I. cylindrica* was absent.

Sites were all in close proximity and represent a high degree of homogeneity with the exception of *I. cylindrica* being present.

2.2.2 Mycorrhizae:

Field sampling of roots for mycorrhizae was performed in November 2014 and May 2015. Preliminary studies have shown that May is the most reliable time to survey active mycorrhizal communities in this region (J. Hoeksema pers. obs.). Another sampling in November was included to account for seasonal variation (Brundrett *et al.* 1996). At each plot, nine evenly distributed soil cores (10 cm dia x 60 cm ht) were removed across each of the eight

plots using a pneumatic coring device. This method of soil core sampling assured that both the O and A soil horizons were sampled for mycorrhizae. As it is unclear how deep *I. cylindrica* exudates may leach into the soil, an analysis of multiple soil layers was necessary. Each soil core was taken approximately 2.5 m from the next core and each plot contained several trees. Due to limitations in space and accessibility more sampling was not feasible. Individual cores were sealed and immediately stored at 4°C until processing.

Soil cores were cut into 20 cm increments and roots were gently washed free of soil with water through a 0.5 mm sieve (Horton & Bruns 1998; Roberts & Anderson 2001). Roots were pooled within each plot by soil core increment (0–20, 21–40 and 41–60 cm). Pine roots were separated and kept in a 2% cetyl trimethyl ammonium bromide (CTAB) lysis buffer (*Tedersoo et al.* 2006). As ectomycorrhizal tips are abundant (7–72 x $10^4.m^{-2}$) (Taylor 2002) we arbitrarily sampled 100 1-cm root segments for a total of 100 cm of fine roots per 20 cm soil core increment except when there were not enough roots present, in which case we examined all roots (Anderson *et al.* 2010) to measure the percent of fine root segments that were colonized. The length of fine feeder roots (<2 mm) was measured (cm) for all plots. Mycorrhizal structures on subsampled roots were identified and quantified at 10–40x magnification using the gridline intercept method (Brundrett *et al.* 1996).

2.2.3 Vegetation Survey:

A vegetation analysis was undertaken in July 2014 to quantify percent cover of vegetation on *I. cylindrica* invaded and non-invaded plots (Table 2.2). Vegetation was assessed using a 10% scale by the line transect method, where ten adjacent 1 m^2 quadrats were sampled on a diagonal transect within each plot (Krebs 1989). Plants that were present but in lower

abundance than 10% were given a classification of 5% to ensure they were accounted for in diversity estimates. Vegetation that could not be identified in the field was collected and pressed for later identification by plant taxonomists at Auburn University.

2.2.4 Microbial Biomass N:

Four separate soil samples were collected from each plot to determine organic N levels (mg/L) that were used to calculate microbial biomass N. Soil samples were collected 2 m apart in each cardinal direction, in the southwest corner of each plot or the center of the original plot. Prior to sampling, the top layer of loose organic debris (or duff) was removed. As target microbial communities are more abundant higher in the soil profile, only the upper layer of soil (≤10 cm) was sampled. Samples were placed in plastic bags and transported within eight hours to the laboratory where they were refrigerated. All tests were performed within three days.

Table 2.2. Species present indexed by site. Values represent percent cover averaged across all sub-plots. Species are divided by type.

	Imperata cylindrica absent			*Imperata cylindrica* present				
Species	**N1**	**N2**	**N3**	**P1**	**P2**	**A1**	**A2**	**A3**
Bare ground	46	10	50	36	28	2	3	22
Grass								
Diacanthelium aciculare	0	0.5	0	0	0	0	0	0
Imperata cylindrica	0	0	0	21	12	92	97	53
Muhlenbergia scheberi	0.5	0.5	3.5	0	0	0	0	0
Unknown sp 1	2	21	0	0	0	0	0	0
Unknown sp 2	0	0	0.5	0	0	0	0	0
Herbaceous								
Chamaecrista fasiculata	0	6	0	0	0	0	0	0
Conya canadensis	0.5	2	1	1	0	0	0	0
Elephantopus tomentosus	0	1.5	0	0	0	0	0	0
Eupatorium capillifolium	0	2.5	0	0	0	0	0	0
Eupatorium rotundifolium	28.5	11.5	33	8.5	0.5	1	0	5
Lathyrus venosus	0	14	0.5	0	0	0	0	0
Oxalis stricta	0	0	0.5	0	0	0	0	0
Tephrosia virginiana	0	0	0	0.5	2	0	0	0
Tragia smallii	0	0	0	0	0	0	0.5	0
Shrub								
Callicarpa americana	2.5	3	0	4	0	0	0	2.5
Hypericum hypericoides	0	0	0	0	10	0	0	0
Ilex glabra	0	0	0	1	30	0	0	0
Illex vomitoria	6	3.5	3	6	8	3	0	0
Rubus sp.	2.5	21	0	1.5	3	0.5	0	0.5
Vaccinium stamineum	0.5	0	0	0	0.5	2	0	0
Tree								
Halesia diptera	8	0.5	0	0	0.5	0	0	0
Morella cerifera	2	0	0	0	0	0	0	0
Pinus taeda	3	2	0	2	1	0	0	3
Quercus sp.	1	3	0	0	4	0	0	0
Rhus copallinum	0	0	0	0	0	0.5	0	0
Vine								
Gelsenium sempervirens	2	0.5	12	18	3	0	0	14
Parthenocissus quinquefolia	0	5	0	0	0	0	0	0
Smilax pumila	0	0	0	0	0	1	0	0
Smilax sp.	2.5	1.5	0.5	1	0	1	0	0.5
Toxicodendron radicans	0	0	0	1.5	0	0	0	2.5

Soil samples were sieved using a 2 mm sieve. Each soil sample was separated into two 18.5 g samples and was processed using the chloroform-fumigation extraction (CFE) technique (Vance *et al.* 1987). This method measures only the organic N in microbial organisms in the soil by lysing cell walls in one sample (using chloroform) and not disturbing the second sample. Organic N levels were measured utilizing a TNM-1 Total N measuring Unit (Shimadzo Scientific Instruments, Columbia, MD). The organic N levels from the non-disturbed samples are subtracted from the organic N levels in the fumigated sample. The subtracted value, divided by the efficiency of extraction constant, yielded the microbial biomass N. These values for the four soil samples collected per plot were averaged prior to plot comparisons.

2.2.5 Tree Radial Growth:

Within each of the original 30 m x 30 m plots, six dominant or co-dominant trees closest to plot center were evaluated for tree growth rate. An increment borer was used to remove a tree core at breast height (1.37 m). Cores were mounted onto blocks and sanded down to show clear annual growth rings. Total radial growth over the last five and ten years was determined by measuring from the early wood of the fifth and tenth annual rings, respectively, to the bark. In order to account for differences in growth due to relative density of trees on each plot, growth was expressed as a function of percent stocking. Percent stocking standardizes available growing space across plots with trees of various ages and sizes and differing basal area (Gingrich 1967).

2.2.6 Statistical Analysis:

Analyses examining mean percent colonization and mean fine root length as response variables were conducted in R® version 3.0.2, using lmerTest package. Graphs were constructed

in STATISTICA® (StatSoft 2013). Mean values were calculated from multiple cores (9) in each plot on each sampling date. We performed split-plot, repeated measures mixed model ANOVAs in which *I. cylindrica* abundance was a whole plot between-subjects factor, sampling depth was a split-plot between-subjects factor, sampling date was a within-subject factor, and plot was a random factor. These models included Treatment, Depth, Month, and all two-way interactions between them as fixed factors. The three-way interaction among all three was excluded from final models, as it was always highly non-significant.

Models were fit with the *lmer* function using REML likelihood. Degrees of freedom and P-values were estimated using the Satterthwaite method. Marginal means and standard errors for significant predictors were obtained using the *lsmeans* function, and pairwise significant differences between means were estimated using the *difflsmeans* function. For significant interactions of Treatment with Depth or Month, we only conducted pairwise tests between levels of Treatment within each Depth or Month, to minimize the number of pairwise tests conducted. As such, and due to our low replication for the Treatment effect, the significance of all p-values was assessed at $\alpha = 0.05$.

SAS® version 9.3 (SAS Institute Inc. 2010) and STATISTICA® (StatSoft 2013) were used for one-way analysis of variance (ANOVA) on microbial biomass with *I. cylindrica* invasion as the predictor variable. In addition, a factorial ANOVA examining the effect of *I. cylindrica* presence and loblolly stocking percentage was conducted on radial growth measurements. Post hoc Tukey's HSD test ($\alpha = 0.05$) was performed to compare means in pairwise tests. Residuals were tested for normality and response variables log transformed when found to be non-normally distributed.

Species richness and diversity for each plot was calculated using both the Shannon-Wiener and Simpson's nonparametric measurements of diversity (Magurran 2004; Colwell 2013). A student's t-test was undertaken to compare mean richness and diversity across invasion category. Percent cover of *I. cylindrica* was regressed against both the Shannon-Wiener and Simpson's diversity index to determine if the two were correlated.

2.3 RESULTS

2.3.1 Mycorrhizae:

Mycorrhizal fungi colonization was affected by a significant interaction between *I. cylindrica* abundance and month ($F_{(2,29)}$=5.53, p=0.009) (Figure 2.2). *Imperata cylindrica* exercised a different influence on mycorrhizal fungi colonization in November than May. Overall there is a trend towards decreased colonization by mycorrhizal fungi in the presence of *I. cylindrica,* but in November mycorrhizal colonization was significantly lower in *I. cylindrca* present plots compared to *I. cylindrica* abundant plots. In May, the comparison of mycorrhizal colonization in plots with no *I. cylindrica* versus *I. cylindrica* present plots was nearly significant ($p<0.1$), despite very low replication (n=3 plots each). Mycorrhizal fungi colonization was also significantly different across depth classes (Figure 2.3). Specifically, fine roots at depths of 0-20 cm were more heavily colonized than those 21-40 cm ($p<0.0001$) or 41-60 cm ($p<0.0001$). Similarly, roots in the 21-40 cm depth class were more heavily colonized than those in the 41- 60 cm depth class (p=0.041).

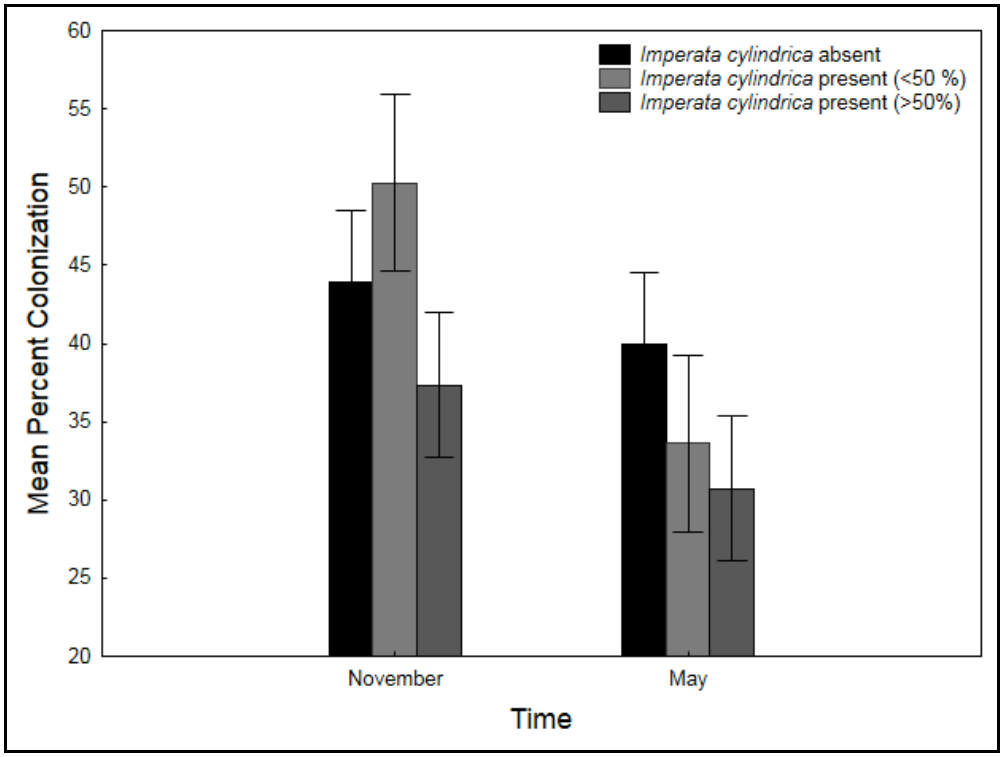


Figure 2.2. Mean values of percent colonization of mycorrhizal fungi on fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent standard error. Letters represent statistically significant different pairwise comparisons between *I. cylindrica* abundances, not between sampling seasons.

Amount of fine roots recovered was significantly affected by an *I. cylindrica* abundance by month interaction. In both November and May *I. cylindrica* absent plots yielded significantly more *P. taeda* fine roots than *I. cylindrica* present (p=0.038 and p<0.0001) and *I. cylindrica* abundant plots(p<0.0001 and p=0.005) (Figure 2.4).

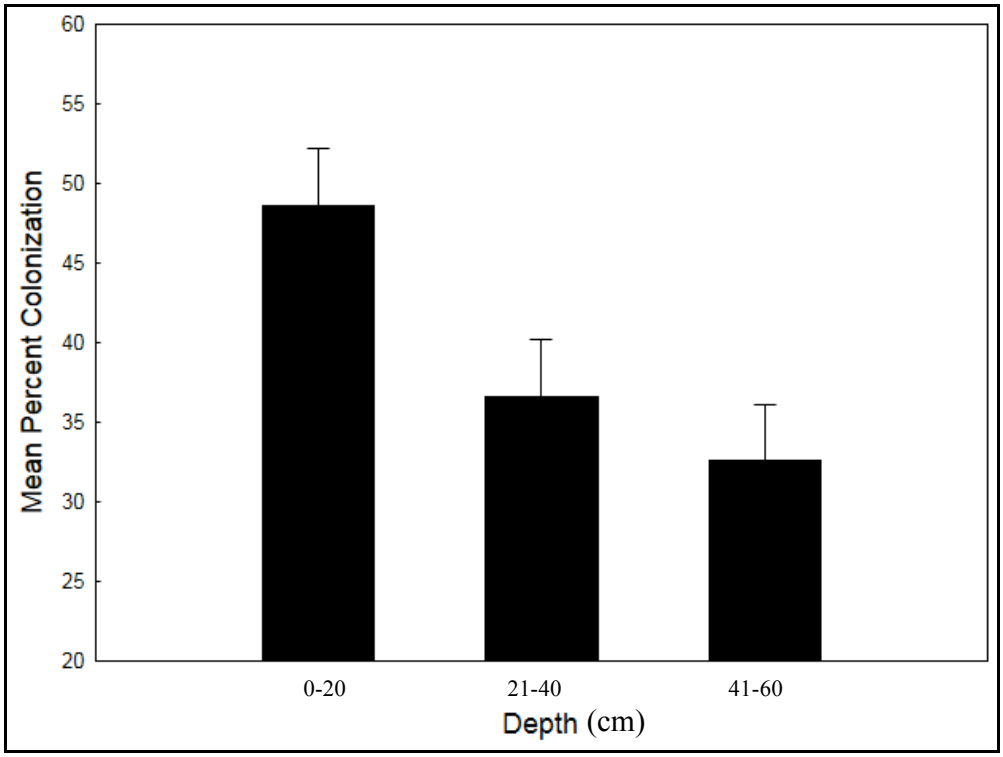


Figure 2.3. Mean values of percent colonization of mycorrhizal fungi on fine feeder roots of *P. taeda* at each of the three depth classes over the course of two field seasons. Error bars represent standard error. Letters indicate statistically significant differences.

Fine root abundance was also affected by a significant interaction between depth class and *I. cylindrica* abundance. In the 21-40 cm depth class *I. cylindrica* absent plots yielded more fine roots than either *I. cylindrica* present or abundant plots ($p<0.0001$ and $p<0.0001$, respectively). Similarly, in the 41-60 cm depth class *I. cylindrica* absent plots yielded more fine roots than *I. cylindrica* present or abundant plots ($p=0.008$ and $p<0.0001$, respectively) (Figure 2.5).

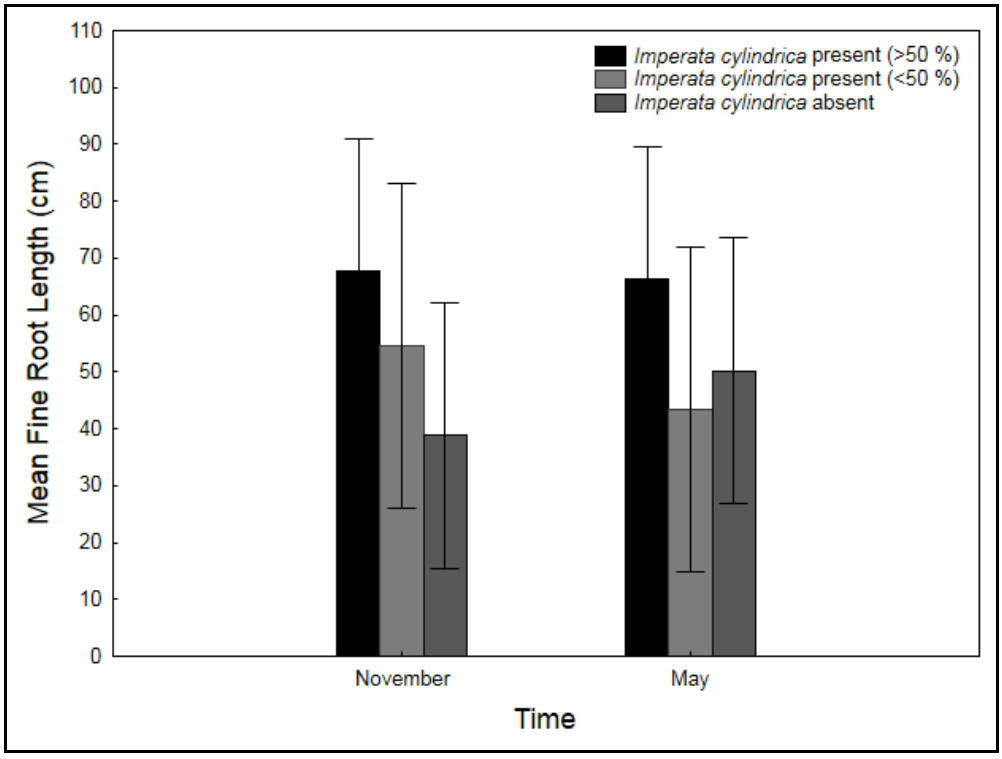


Figure 2.4. Mean values of recovered fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent standard error. Letters represent statistically significant differences between *I. cylindrica* abundances, not between sampling seasons.

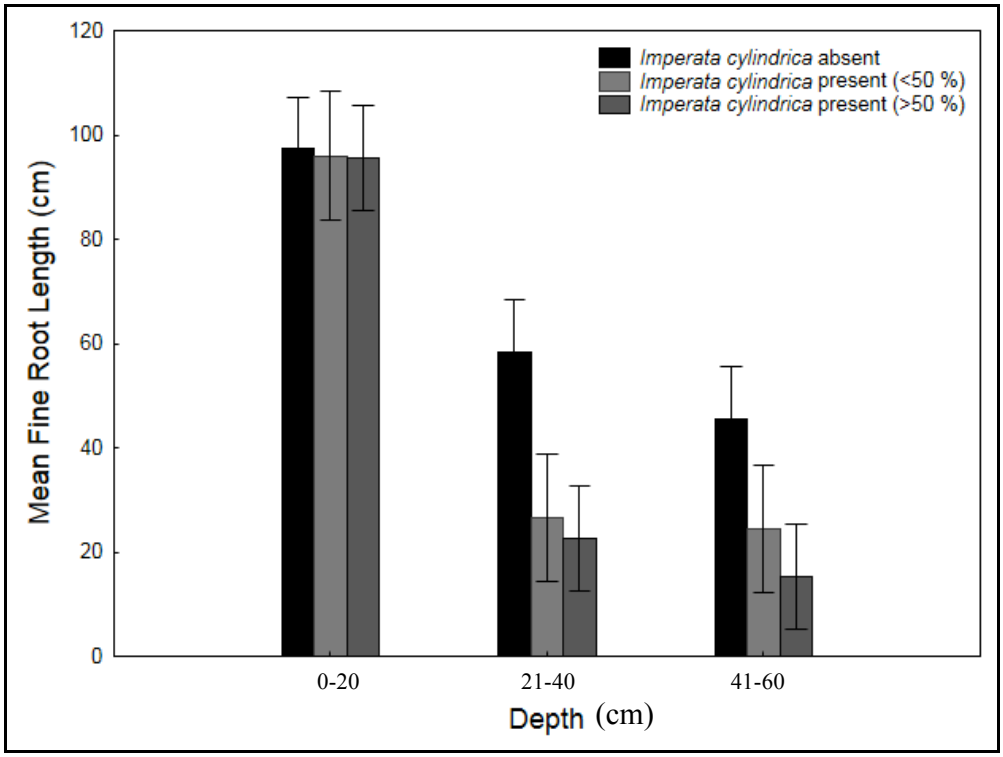


Figure 2.5. Mean values of recovered fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent 95 % confidence intervals. Letters represent statistically significant differences between depth classes, not between sampling seasons.

2.3.2 Vegetation survey:

Plant species richness and diversity differed between the *I. cylindrica* abundant and *I. cylindrica* absent plots for both Shannon-Wiener (p=0.049) and Fisher's alpha (p=0.042) indices, but not Simpson's diversity (p=0.120) (Table 2.3).

Table 2.3. Percent cover of *Imperata cylindrica* and bare ground on plots, as well as species richness and measures of diversity.

	Imperata cylidrica absent			*Imperata cylindrica* present		*Imperata cylindrica* abundant		
Plot	N1	N2	N3	P1	P2	C1	C2	C3
Imperata cylindrica (% cover)	0	0	0	22	12	92	97	53
Bare ground (% cover)	46	10	50	36	28	2	3	22
Species richness	13	18	9	11	12	7	2	7
Shannon-Wiener	1.79	2.31	1.21	1.78	1.84	0.4	0.03	1.03
Simpson index	3.62	7.49	2.36	4.49	4.44	1.17	1.01	1.99

A correlation matrix was constructed to examine the relationship between individual species and the observed diversity indices (Table 2.4).

We determined that no species were significantly negatively correlated with measures of diversity with the exception of *I. cylindrica*. An inverse correlation was also observed in a linear regression between *I. cylindrica* percent cover and Shannon-Wiener diversity index (y=1.8926-0.0172x, r= -0.9027, p=0.0021, r^2=.815) (Figure 2.6a) as well as *I. cylindrica* and Simpson's diversity index (y=4. 6486-0.0385x, r=-0.7299, p=0.0398, r^2=.533) (Figure 2.6b).

2.3.3 Microbial Biomass N:

Table 2.4. Correlation coefficients of each plant species present at the study site. Bolded values are significant at $p<.05$, N=8.

	Diversity Index	
Species	Shannon-Weiner	Simpson's
Bare ground	0.481	0.142
Grass		
Diacanthelium aciculare	0.522	**0.779**
Imperata cylindrica	**-0.903**	**-0.730**
Unknown sp 1	0.551	**0.791**
Unknown sp 2	-0.046	-0.180
Muhlenbergia scheberi	0.067	-0.062
Herbaceous		
Eupatorium rotundifolium	0.351	0.135
Elephantopus tomentosus	0.522	**0.779**
Tephrosia virginiana	0.343	0.265
Chamaecrista fasiculata	0.522	**0.779**
Conya canadensis	0.666	**0.788**
Oxalis stricta	-0.046	-0.180
Lathyrus venosus	0.523	**0.776**
Eupatorium capillifolium	0.522	**0.779**
Tragia smallii	-0.655	-0.432
Shrub		
Rubus sp.	0.622	**0.851**
Callicarpa americana	0.603	0.578
Vaccinium stamineum	-0.337	-0.342
Ilex glabra	0.289	0.217
Hypericum hypericoides	0.280	0.209
Illex vomitoria	0.684	0.530
Tree		
Halesia diptera	0.308	0.119
Rhus copallinum	-0.464	-0.402
Morella cerifera	0.254	0.056
Quercus sp. 1	0.279	0.209
Quercus sp. 2	0.602	**0.791**
Pinus taeda	0.629	**0.839**
Vine		
Gelsenium sempervirens	0.174	-0.028
Toxicodendron radicans	0.009	-0.107
Parthenocissus quinquefolia	0.522	**0.779**
Smilax sp.	0.470	0.386

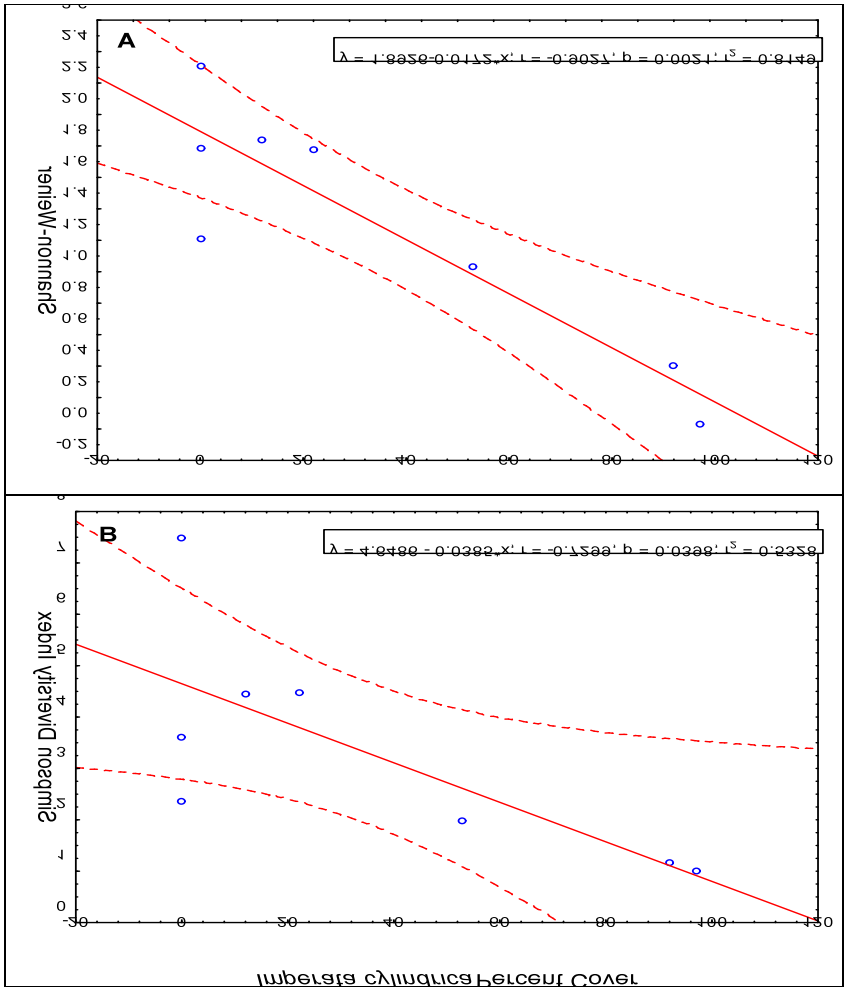


Figure 2.6. Linear regression of (**A**) Shannon-Weiner index and *Imperata cylindrica* percent cover (n=8); (**B**) Simpson diversity index and *Imperata cylindrica* percent cover (n=8).

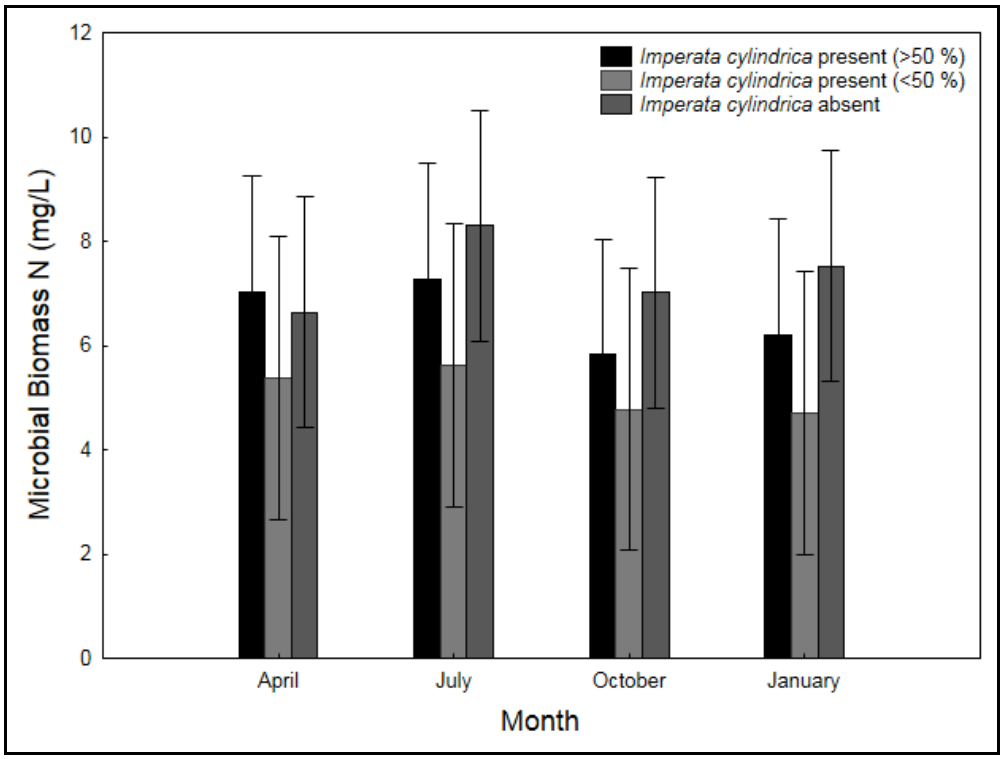


Figure 2.7. Mean values of microbial biomass N are listed for *I. cylindrica* present and absent plots by season. Error bars represent standard error, n=8.

A significant interaction was not observed between *I. cylindrica* and month of sampling. Furthermore no significant differences were observed in either *I. cylindrica* abundance or time on microbial biomass N. There is a consensus in the literature that microbial biomass is related to soil moisture (Wardle 1992, Devi and Yadava 2006). We didn't find this relationship with microbial biomass N and soil moisture ($y=4.8233+0.1803x$; $r=0.2919$; $p=0.1050$; $r^2=0.0852$).

2.3.3 Radial Growth:

No significant differences in radial growth were observed between *I. cylindrica* present and absent plots (Figure 2.8a and 2.8b).

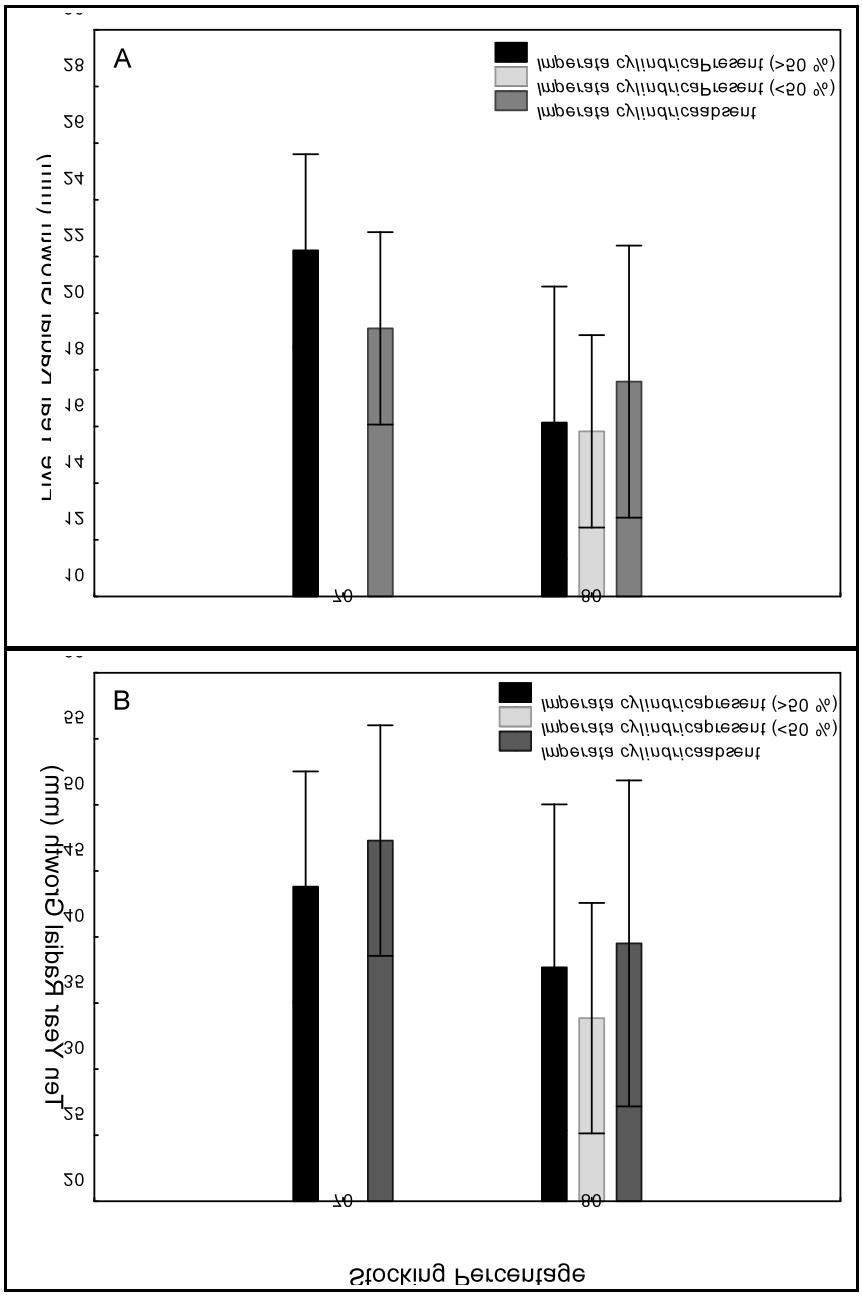


Figure 2.8. Mean values of (**A**) five year radial growth (**B**) ten year radial growth (mm) of *P. taeda* is listed by stocking percentage.

No *I. cylindrica* present plots were located in stands with lower than70 % stocking (Table 2.1). Plots in stands with lower stocking densities tended to have higher five and ten year growth rates than higher stocked stands.

2.4 DISCUSSION

Mycorrhizae are responsible for many plant functions, in particular nutrient uptake. With a significant reduction in colonization by ectomycorrhizal fungi, as observed here in *P. taeda* plots with high abundance of *I. cylindrica* during the November sampling (Fig. 2.2), the volume of soil the plant is able to exploit is reduced, potentially resulting in reduced uptake of essential nutrients. Although the magnitude of reduction in mycorrhizal colonization density (percent colonization of fine feeder roots) is not dramatic, the reduced abundance of fine feeder roots also observed on the invaded sites, especially deeper in the soil profile and in the November field sampling period (Figs. 2.4 and 2.5), likely compounds the effect of reduced mycorrhizal colonization in more heavily invaded sites, and hampers the ability of trees to uptake nutrients directly. Although significant differences in mycorrhizal colonization were only noted between *I. cylindrica* present and *I. cylindrica* abundant in November, we noted a trend towards increasing cover of *I. cylindrica* leading to decreased mycorrhizal colonization in both field seasons. In particular, the *I. cylindrica* absent and *I. cylindrica* abundant plots showed a nearly significant difference (p=0.066), despite very low replication (n=3).

We found that there was no significant difference in plant diversity indices between *I. cylindrica* present, abundant and absent plots. However, we did see a significant difference among *I. cylindrica* abundant and *I. cylindrica* absent plots. We also found that *I. cylindrica* percent cover was negatively related to vegetation diversity. It is well documented that diverse

plant communities are more microbially active (Kowalchuk *et al.* 2002, Zak *et al.* 2003, Eisenhauer *et al.* 2010). Microorganisms play an imperative role in nutrient cycling, in particular through mineralization and competition for nutrients (van der Heijden *et al.* 2008). Since dense monocultures of *I. cylindrica* reduce plant diversity associated microorganisms as well as non-associated beneficial microorganisms are likely being extripated simultaneously, disrupting nutrient cycling. However, it is difficult to determine the exact effects of *I. cylindrica* on the resident microbial population without long-term and physiological research. Regardless of what the specific effects may be, they will doubtless have implications on resource management and conservation of biodiversity.

Microbial biomass N was highly variable in all plots and no significant differences were evident between plots; either by treatment or over time. We attributed this observation to the limited number of replications employed. A combination of observed effects including reduced plant diversity associated with *I. cylindrica*, increased bare space in *I. cylindrica* present plots and physical differences observed by other researchers (such as reduced light) may have a significant effect on the relationship between microbial biomass C:N. Microbial processes are linked with nutrient cycling, fertilization and organic matter transformation (Černý *et al.* 2003), and microbial biomass is often used as an indicator of soil quality. However, high levels of variability in microbial biomass, even among nearby areas, can make generalizations difficult (Xu *et al.* 2013). This 'natural' heterogeneity likely contributed to the variation observed between plots and may have been more influential as a whole than the influence of *I. cylindrica*.

Significant differences in *P. taeda* growth were not observed between *I. cylindrica* percent cover by stocking percentages. Although lower growth rates would be expected in the stands with higher stocking levels, growth of trees in the *I. cylindrica*-present plots in these

stands was not significantly reduced. While competitive effects of *I. cylindrica* were expected, some invasive plants may produce a short-term fertilization effect due to increased net primary productivity, and production of litter with higher decomposition rates than native vegetation (Ehrenfeld 2003). In addition, variability in the time since *I. cylindrica* first appeared may be responsible for this discrepancy.

2.5 CONCLUSION

Abundance of mycorrhizal fungi (in November) and fine feeder roots (especially in November and deeper in the soil) were significantly reduced in plots where *I. cylindrica* was present. Vegetative communities also were less diverse in *I. cylindrica* present plots. These factors likely contribute to lower overall microbial diversity and a reduction in nutrient cycling. Although impacts on growth were not observed it is expected that over time, an impact may be seen. Furthermore, these changes may lead to communities that are more susceptible to disease and secondary invasion. Therefore, it is important to both production of *P. taeda* and conservation of biodiversity to limit area affected and invaded by *I. cylindrica* as effectively as possible by prompt and repeated treatment with effective herbicides.

2.6 REFERENCES

Roberts K.J., Anderson R.C. 2001. Effect of garlic mustard [Alliaria petiolata (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist*, 146:146-152.

Boufalis A., Pellissier F., Trosset L. 1994. Responses of mycorrhizal fungi to allelopathy: Cenococcum geophilum and Laccaria laccata growth with phenolic acids. *Acta Botanica Gallica* 141:547–550. doi:10.1080/12538078.1994.10515197

Brundrett M., Bougher N., Dell B., Grove T., Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture.

Brunson B., Eckhardt L.G. 2012. Assessing the impacts of cogongrass (Imperata cylindrica (L.) Beauv) on root-feeding bark beetle populations associated with Southern Pine Decline. *Phytopathology* 102:1-2

Bryson C.T., Carter R. 1993. Cogongrass, Imperata cylindrica, in the United States. *Weed Technology*, 1005-1009.

Cerny J., Balik J., Pavlikova D., Zitkova M., Sykora K. 2003. The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long-term experiments with maize. *Plant, Soil and Environment* - UZPI (Czech Republic).

Colwell R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application published at: http://purl.oclc.org/estimates

Daneshgar P., Jose S. 2009. Imperata cylindrica, an alien invasive grass, maintains control over nitrogen availability in an establishing pine forest. *Plant Soil* 320:209–218. doi:10.1007/s11104-008-9886-8

Daneshgar P., Jose S., Collins A., Ramsey C. 2008. Cogongrass (Imperata cylindrica), an alien invasive grass, reduces survival and productivity of an establishing pine forest. *Forest Science* 54:579–587.

Devi NB, Yadava PS (2006) Seasonal dynamics of soil microbial biomass c, n and p in a mixed oak forest ecosystem of Manipur, north-east india. Applied Soil Ecology 31:220-227.

Dickie, I.A., Bolstridge, N., Cooper, J.A., Peltzer, D.A. 2010. Co-invasion by Pinus and its mycorrhizal fungi. *New Phytologist* 187, 475–484.

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6:503–523.

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W.W., Scheu, S. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91, 485–496.

Gingrich, S.F. 1967. Measuring and evaluating stocking and stand density in upland hardwood forests in the Central states. *Forest Science* 13:38-53.

Hagan D.L., Jose S., Lin C.H. 2013. Allelopathic exudates of cogongrass (Imperata cylindrica): Implications for the performance of native pine savanna plant species in the southeastern *US. J Chem Ecol* 39: 312–322.

Holm L.G., Plucknett D.L., Pancho J.V., Herberger J.P. 1977. The world's worst weeds. 609 pp.

Holzmueller E.J., Jose S. 2011. Invasion success of cogongrass, an alien C4 perennial grass, in the southeastern United States: exploration of the ecological basis. *Biol Invasions* 13:435–442.

Horton T.R., Bruns T.D. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (Pseudotsuga menziesii) and bishop pine (Pinus muricata). *New Phytologist* 139:331–339.

Hussain F., Abidi N. 1991. Allelopathy exhibited by Imperata cylindrica (L.) P. Beauv. Pakistan *Journal of Botany* (Pakistan).

Jose S, Cox J, Miller DL, Shilling DG, Merritt S (2002) Alien plant invasions: The story of cogongrass in southeastern forests. *Journal of Forestry* 100:41–44.

Koger C.H., Bryson C.T. 2004. Effect of Cogongrass (Imperata cylindrica) Extracts on germination and seedling growth of selected grass and broadleaf species. *Weed Technology* 18:236–242.

Kowalchuk G.A., Buma D.S., Boer W., Klinkhamer P.G.L., Veen J.A. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek* 81:509–520.

Kourtev P.S., Ehrenfeld J.G., Häggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83:3152-3166.

Krebs C. 1989. Ecological methodology. Harper Collins Publishers.

MacDonald G.E. 2004. Cogongrass (Imperata cylindrica)—Biology, ecology, and management. *Critical Reviews in Plant Sciences* 23:367–380.

Magurran A.E. 2013. Measuring biological diversity. John Wiley and Sons.

Pasternak Z., Diamant A., Abelson A. 2007. Co-invasion of a Red Sea fish and its ectoparasitic monogenean, Polylabris cf. mamaevi into the Mediterranean: observations on oncomiracidium behavior and infection levels in both seas. *Parasitol Res* 100:721–727.

Pimentel D., Zuniga R., Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics, Integrating Ecology and Economics in Control Bioinvasions* IEECB S.I. 52:273–288.

Richardson D.M., Allsopp N., D'antonio C.M., Milton S.J., Rejmánek M. 2000. Plant invasions — the role of mutualisms. *Biological Reviews* 75:65–93.

Roberts K.J., Anderson R.C. 2001. Effect of Garlic Mustard [Alliaria petiolata (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist* 146:146–152.

Simberloff D., Martin J.L., Genovesi P., Maris V., Wardle D.A., Aronson J., Courchamp F., Galil B., García-Berthou E., Pascal M., Pyšek P., Sousa R., Tabacchi E., Vilà M. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology and Evolution 28:58–66.

Soil Survey Laboratory Staff. 2012. Soil survey laboratory methods manual. Soil Surv. Invest. Rep. 42. Version 4.0. Natl. Soil Surv. Ctr., Lincoln, NE.

Taylor A.F. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Diversity and Integration in Mycorrhizas* 244:19-28.

Tedersoo L., Suvi T., Larsson E., Kõljalg U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research* 110:734–748.

USDA Forest Service. 1982. Service foresters handbook. SE Area, State and Priv. For., Atlanta, GA

Vance E.D., Brookes P.C., Jenkinson D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil biology and Biochemistry*, 19:703-707.

Van Der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11:296–310.

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67:321-358.

Xu X., Thornton P.E., Post W.M. 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography* 22:737–749.

Zak D.R., Holmes W.E., White D.C., Peacock A.D., Tilman D. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84:2042–2050.

Chapter 3

The impact of invasion by *Imperata cylindrica* on microbial abundance and abiotic soil factors in *Pinus taeda* plantations

3.1 ABSTRACT

Cogongrass (*Imperata cylindrica*) is a significant pest in plantation forestry, agriculture and right-of ways. In belowground communities, which are notoriously difficult to quantify, *I. cylindrica* may have wide reaching effects that largely go unnoticed. We quantified carbon and nitrogen present in the mass of all living microorganisms in the soil. We also measured pool size of several key nutrients in the top 10 cm of both *I. cylindrica* present and absent plots. We analyzed these variables across spatial and temporal scales to account for seasonal variations. There were no significant reductions in soil moisture in any plot types. Ratio of microbial biomass C:N was significantly not significantly different in any *I. cylindrica* present plots. Finally, we determined that differences between nutrient pools existed between *I. cylindrica* present and absent plots. These factors have the potential to significantly affect the microbial

communities. In order to determine what long term consequences will be to nutrient cycles and native plant communities additional research is necessary.

3.2 INTRODUCTION

Cogongrass (*Imperata cylindrical* (L.) Beauv.) is an invasive grass species native to Asia. In its invaded range of the southeastern United States, it influences soil moisture and is more robust on acidic soils (Bryson & Carter 1993; Jose *et al.* 2002). *Imperata cylindrica* is more effective at locating and exploiting nutrient patches, altering fire regimes and altering light resources compared to native grass species (Lippincott 1997). Daneshgar & Jose (2009) found that due to severe competition for nitrogen, compared to native vegetation, *I. cylindrica* reduced the growth of loblolly pine (*Pinus taeda* L.). In general, invasive plant species tend to be more dominant in an invaded range through a variety of mechanisms including the production of novel "biochemical weapons" (Callaway & Ridenour 2004). This dominance has the potential to drastically alter plant community composition.

Plant community composition along with several other factors such as availability of resources and land use determine fine scale spatial and temporal distribution of soil microbes (Stotzky 1997; Zak *et al.* 2003; Bossio *et al.* 2005). Soil microbial organisms largely are averse to growing in culture (Amann *et al.* 1995). The underground effect of an invader may, therefore, be difficult to quantify and go unnoticed. Since microorganism functions are an integral component of ecosystem functions such as nutrient cycling, fertilization and organic matter transformation, altered microorganism communities may have far-reaching effects (Černý *et al.* 2003). To quantify these differences we investigated how *I. cylindrica* alters some of these below ground functions. We expected that differences would be observed in soil physical

properties, nutrient availability and microbial biomass of stands invaded by *I. cylindrica* due to the presence of *I. cylindrica* and the compounds it produces.

3.3 MATERIALS & METHODS

3.3.1 Site Description:

Twenty plots (30 m x 30 m) were established on production loblolly pine stands in Greene County, Mississippi in 2010. Ten plots had *I. cylindrica* present and ten plots did not contain *I. cylindrica*. In 2011 percent cover of *I. cylindrica* was assessed. Plots were divided into *I. cylindrica* present (n=10) and absent (n=10), with all invaded plots exhibiting over 50 % cover of *I. cylindrica*. The sites were re-examined in 2013 and a smaller subset of plots (16 m x 16 m) were reclassified by cover of *I. cylindrica*. Plots were divided into sites that had less than 50 % cover (n=2), sites that had greater than 50 % cover (n=3) and sites in which *I. cylindrica* was absent (n=3) (referred to as present, abundant, and absent, respectively). The soil series on which these plots are located was identified as Benndale sandy loam on 8 to 15% slopes, and Benndale sandy loam or McLaurin sandy loam on 2 to 5% slopes.

3.3.2 Soil Moisture:

Field sampling was conducted during April 2014, July 2014, October 2014 and January 2015. Four samples were collected from each site, 1 m in each cardinal direction from plot center. The top 10 cm of soil was sampled with a shovel and placed in plastic bags, samples were then stored at 4°C until lab processing. Samples were passed through a 2-mm sieve in order to remove coarse organic debris. Soil moisture was determined by calculating the percentage of weight loss in each sample after being subjected to 105 °C for a 72 hour period.

3.3.3 Microbial Biomass:

Samples were also stored at 4°C until microbial biomass was measured using the chloroform-fumigation extraction technique as described in detail by Ricker & Lockaby (2014) and Vance *et al.* (1987). Samples were divided into two subsamples, one of which was fumigated with chloroform. Soil fumigation occurred in complete darkness for a 24 h period, following which 125 ml of 0.5 M K_2SO_4 was added. The solution was shaken for 30 minutes and vacuum filtered through No. 5 Whatman filter paper before being frozen. The unfumigated samples were extracted and frozen using the same protocol.

After a minimum of 3 days samples were thawed and analyzed, using a Shimadzu TOC-V and N combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD.) for total organic C (TOC) and N (TON). Organic C and N from unfumigated samples were subtracted from fumigated samples and the product used to calculate the microbial biomass C and N with an extraction efficiency coefficient of 0.45 (Jenkinson 1988; Wu *et al.* 1990).

3.3.4 Nutrient Analysis:

Soil samples were collected in November 2011. Four soil cores (5 x 60 cm) were removed from each plot at approximately 1 m from plot center in each cardinal direction. The Soil Characterization Laboratory at the University of Missouri performed sample analyses by combustion analyzer or Mehlich-3 procedure (Mehlich 1984; Bremner 1996). Values were reported in concentrations from which pool size was derived for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) with bulk density values.

3.3.5 Statistical Analysis:

R® version 3.0.2 and STATISTICA® (StatSoft 2013) were used for statistical analyses on mean soil moisture and mean microbial biomass using ImerTest package. Means were calculated from multiple samples in each plot (4) per sampling date (4). We performed repeated measure mixed model ANOVAs in which *I. cylindrica* abundance was a whole plot between subjects factor, sampling date was a within subject factor and plot was a random factor. These models included Treatment and Month and all two-way interactions between them as fixed factors.

Models were fit with the "lmer" function using REML likelihood and degrees of freedom. P-values were estimated using the Satterthwaite method. Marginal means and standard errors for significant predictors were obtained using the "lsmeans" function, and pairwise significant differences among means were estimated using the "difflsmeans" function. For significant interactions of Treatment with Depth or Month, we only conducted pairwise tests between levels of Treatment within each Depth or Month, to minimize the number of pairwise tests conducted. As such, and due to our low replication for the Treatment effect, the significance of all p-values was assessed at $\alpha = 0.05$.

SAS® version 9.3 (SAS Institute Inc. 2010) and STATISTICA® (StatSoft 2013) were used for statistical analyses of soil nutrients. A multivariate analysis of variance (MANOVA) was undertaken for N, P and K nutrient pool size and select cation pool size. Post hoc Tukey tests (α=0.05) were performed for all pairwise comparisons. Residuals were tested for normality and log transformed when data sets were found to be non-normally distributed.

3.4 RESULTS

3.4.1 Soil Moisture:

A significant treatment effect on soil moisture was not found to be present across *I. cylindrica* absent, present and abundant plots ($F_{(2,5)}$=0.506, p=0.631) (Figure 3.1). Soil moisture was significantly different over time ($F_{(2,15)}$=15.477, p<0.0001) indicating that across treatments seasonal variations are present in the soil moisture. A significant interaction between treatment and month was not present ($F_{(6,15)}$=1.571, p=0.223).

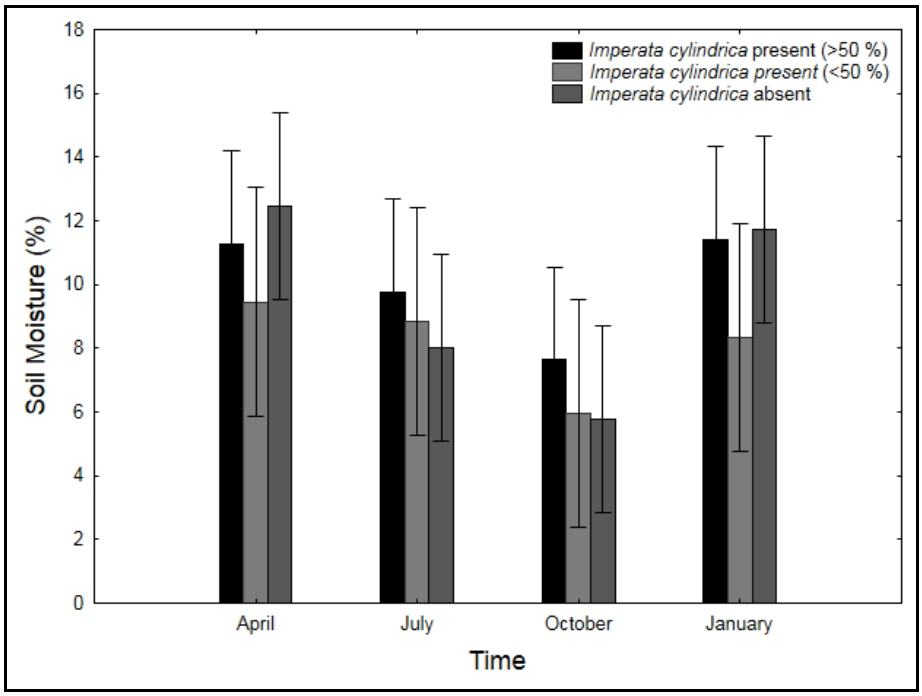


Figure 3.1. Mean values of soil moisture in *P. taeda* stands over the course of four collection periods. Comparisons were made between sites within the same collection period. Error bars represent standard error; n=8.

3.4.2 Microbial Biomass:

A significant treatment effect on soil microbial biomass was not found to be present across treatments (($F_{(2,5)}$=3.661, p=0.104). A significant time effect was present ($F_{(3,15)}$=5.371,

p=0.010) indicating that across treatments microbial biomass C:N varies between seasons. No significant interaction was observed between treatment and month ($F_{(6,15)}$=2.026, p=0.125)

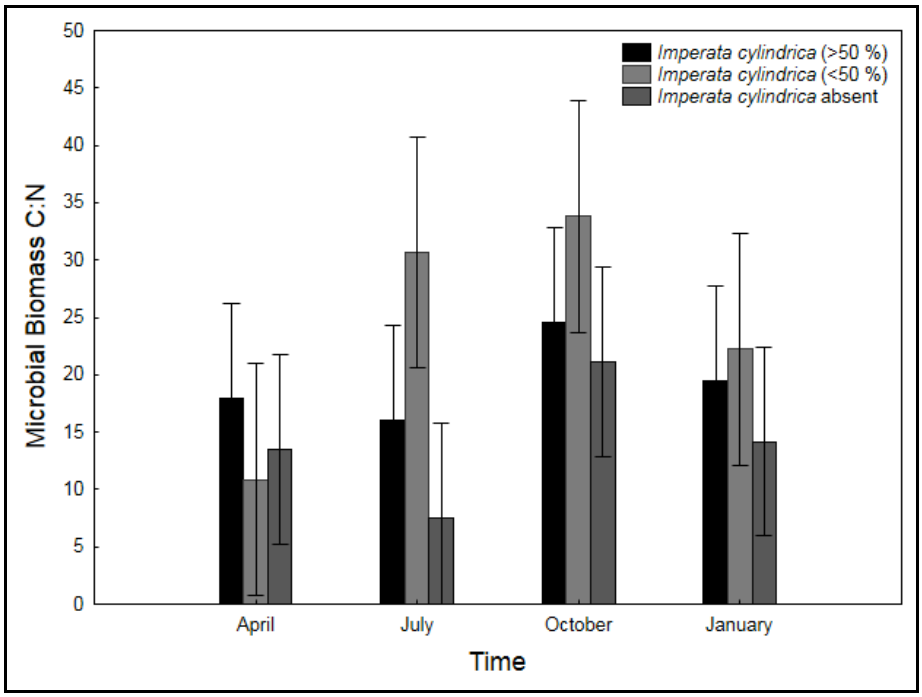


Figure 3.2. Mean values of microbial biomass C:N ratio measured during four collection periods. Comparisons were made between sites within the same collection period. Error bars represent confidence intervals; n=8.

3.4.3 Nutrient Analysis:

Composition of N, P and K pools varied significantly between invaded and non-invaded plots ($F_{(3,16)}$=6.05, p=0.006) (Figure 3.3). Individual comparisons showed that there was no significant difference between *I. cylindrica* abundant and absent plots for the total N pool ($F_{(1,18)}$=2.88, p=0.11) or P pool ($F_{(1,18)}$=0.40, p=0.53). *Imperata cylindrica* abundant plots had significantly higher levels of total K pools compared to *I. cylindrica* absent plots ($F_{(1,18)}$=17.23,

p=0.0006). Additional soil structure related cations (Ca, Mg and Na) pools did not vary significantly between invaded and non-invaded plots ($F_{(3,16)}$=2.21, p=0.127). Individual analyses did, however, reveal that the Na pool significantly varied between *I. cylindrica* present and absent plots where it was found at higher levels ($F_{(1,18)}$=5.80, p=0.03) (Figure 3.4).

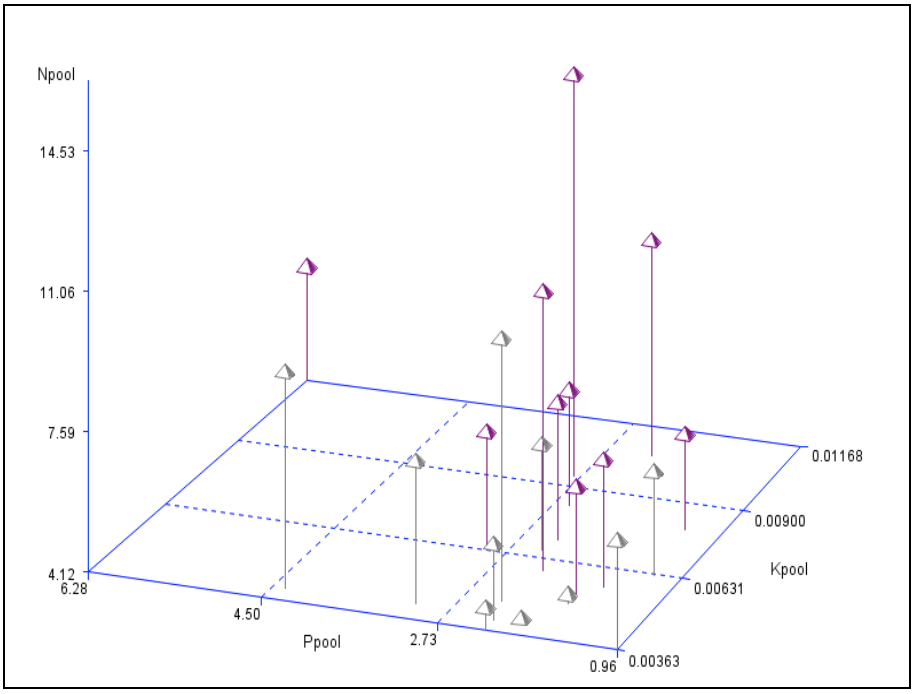


Figure 3.3. A 3 dimensional scatter plot of N, P and K pools. Darker shaded points are sites in which *I. cylindrica* was present and lighter shaded points are sites in which *I. cylindrica* was absent.

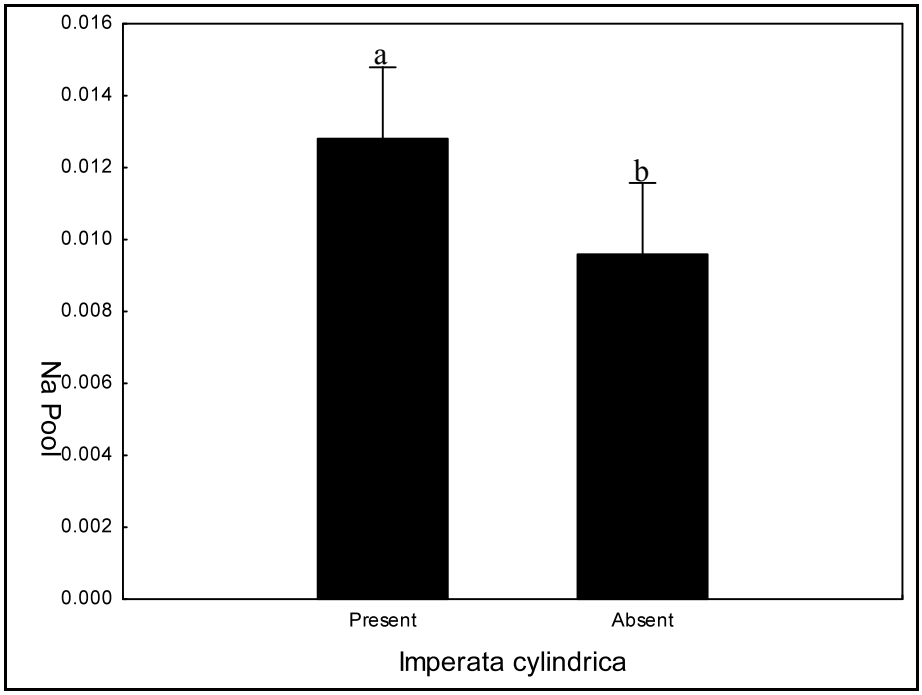


Figure 3.4. Mean pool size of Na found in *I. cylindrica* present absent sites. Significant differences are shown with Tukey variables, error bars represent significant error; n=20.

The pool size for both Ca ($F_{(1,18)}$=1.83, p=0.19) and Mg ($F_{(1,18)}$=3.41, p=0.08) were not statistically different between *I. cylindrica* present and absent sites.

3.5 DISCUSSION

No significant treatment effects were observed for either soil moisture or microbial biomass C:N. Significant seasonal differences do, however, exist in both soil moisture and microbial biomass C:N. Considering the extremely low replication size (n=3 in *I. cylindrica* absent and abundant plots and n=2 in *I. cylindrica* present plots) in conjunction with a near significant treatment effect on microbial biomass (p=.105) we recommend a more complete sampling regime during the summer months (June, July and August), when microbial biomass C:N was most variable, in order to get a more complete analysis of belowground processes.

Carbon and N cycles encompass several trophic levels and these measurements provide a snapshot of availability of C and N to each respective group. Due to the importance of these nutrients, the variety of factors that affect them and their high spatial variability additional testing is warranted (Blagodatskaya & Kuzyakov 2008; Eisenhauer *et al.* 2010).

Composition of nutrient pools of N, P and K varied significantly between invaded and non-invaded plots. Pool size of N, P and K were analyzed together because utilization of these macronutrients by plants are linked in plant usage. Higher levels of nutrients were typically found in invaded plots, contrary to our hypothesis. This is, however, not unprecedented. Some invasive plants are known to, initially and temporarily, increase biomass, net primary productivity (NPP) and produce litter that decomposes more readily relative to native plants (Ehrenfeld 2003).

Difference existed between cation pools in different plot types (*I. cylindrica* present and absent). Sodium was more prevalent at sites on which *I. cylindrica* was present ($F_{(1,19)}$=5.80, p=0.03). In addition a near significant difference was observed in Mg with higher levels observed in *I. cylindrica* present sites ($F_{(1,19)}$=3.41, p=0.08), especially considering the low replication rate (n=20). The excess of Na relative to Ca and Mg may result in more dispersed soil, due to Na role as a poor flocculator relative to Ca and Mg.

A possible explanation for the abundance of K and Na may exist in some of the compounds produced by *I. cylindrica*. Hagan *et al* 2013 found that several compounds were found in plots invaded by *I. cylindrica* at higher concentrations than in plots without *I. cylindrica*, including the compound emodin. Emodin has been known to be produced by members of the family poacea and has a worldwide distribution (Izhaki 2002). It has been

observed that emodin is consistent with increases in K and Na as well as decreases in Mg (Inderjit & Nishimura 1999).

3.6 CONCLUSION

Several chemical incongruities were noted between *I. cylindrica* present and absent plots that have the potential to impact tree and other vegetative growth. Differences were not observed between soil moisture in the top ten cm of the soil profile in *I. cylindrica* present plots compared to *I. cylindrica* absent and *I. cylindrica* abundant plots. The levels of organic C and organic N in microbial biomass were also not significantly different across the sampled invasion gradient of *I. cylindrica*, during any time. Different pool sizes of vital nutrients N, P and K were present as were some cation pool sizes; but not cations in general. From this data we are able to confidently conclude that there are some differences in inorganic nutrient pools consistent with *I. cylindrica* invasion.

3.7 REFERENCES

Abbasi, M. Kaleem, and A. Khizar. 2012. "Microbial Biomass Carbon and Nitrogen Transformations in a Loam Soil Amended with Organic–inorganic N Sources and Their Effect on Growth and N-Uptake in Maize." *Ecological Engineering* 39 (February): 123–32. doi:10.1016/j.ecoleng.2011.12.027.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. "Phylogenetic Identification and in Situ Detection of Individual Microbial Cells without Cultivation." *Microbiological Reviews* 59 (1): 143–69.

Blagodatskaya, E., and Y. Kuzyakov. 2008. "Mechanisms of Real and Apparent Priming Effects and Their Dependence on Soil Microbial Biomass and Community Structure: Critical Review." *Biology and Fertility of Soils* 45 (2): 115–31.

Bossio, D.A., M.S. Girvan, L. Verchot, J. Bullimore, T. Borelli, A. Albrecht, K.M. Scow, A.S. Ball, J.N. Pretty, and A.M. Osborn. 2005. "Soil Microbial Community Response to Land Use Change in an Agricultural Landscape of Western Kenya." *Microbial Ecology* 49 (1): 50–62.

Bryson, C.T., and R. Carter. 1993. "Cogongrass, Imperata Cylindrica, in the United States." *Weed Technology* 7 (4): 1005–9.

Callaway, R.M., and W.M. Ridenour. 2004. "Novel Weapons: Invasive Success and the Evolution of Increased Competitive Ability." *Frontiers in Ecology and the Environment* 2 (8): 436–43.

Černỳ, J., J. Balík, D. Pavlíková, M. Zitková, and K. Sỳkora. 2003. "The Influence of Organic and Mineral Nitrogen Fertilizers on Microbial Biomass Nitrogen and Extractable Organic Nitrogen in Long-Term Experiments with Maize." *Plant Soil Environ* 49: 560–64.

Daneshgar, P., and S. Jose. 2009. "Imperata Cylindrica, an Alien Invasive Grass, Maintains Control over Nitrogen Availability in an Establishing Pine Forest." *Plant and Soil* 320 (1-2): 209–18.

Ehrenfeld, J.G. 2003. "Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes." *Ecosystems* 6 (6): 503–23.

Eisenhauer, N., H. Beßler, C. Engels, G. Gleixner, M. Habekost, A. Milcu, S. Partsch, et al. 2010. "Plant Diversity Effects on Soil Microorganisms Support the Singular Hypothesis." *Ecology* 91 (2): 485–96. doi:10.1890/08-2338.1.

Hoorman, J.J., and R. Islam. 2010. *Understanding Soil Microbes and Nutrient Recycling*. Fact Sheet SAG-16-10. Agriculture and Natural Resources. Ohio State University Extension. http://low.sare.org/content/download/68290/968447/file/Understanding%20Soil%20Microbes%20and%20Nutrient%20Recycling%20SAG_16_10_june2.pdf.

Jenkinson, D.S. and J.N. Ladd. 1981. Microbial biomass in soil: Measurement and turnover. p. 415-471

Jose, S., J. Cox, D.L. Miller, D.G. Shilling, and S. Merritt. 2002. "Alien Plant Invasions: The Story of Cogongrass in Southeastern Forests." *Journal of Forestry* 100 (1): 41–44.

Lippincott, C.L. 1997. "Ecological Consequences of Imperata Cylindrica (cogongrass) Invasion in Florida Sandhill." Gainesville, FL: University of Florida.

https://ia601702.us.archive.org/27/items/ecologicalconseq00lipp/ecologicalconseq00lipp.pdf.

McGuire, K. L., and K.K. Treseder. 2010. “Microbial Communities and Their Relevance for Ecosystem Models: Decomposition as a Case Study.” *Soil Biology and Biochemistry* 42 (4): 529–35.

Mehlich, A. 1984. “Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant.” *Communications in Soil Science and Plant Analysis* 15 (12): 1409–16.

Miltner, Anja, Petra Bombach, Burkhard Schmidt-Brücken, and Matthias Kästner. 2011. “SOM Genesis: Microbial Biomass as a Significant Source.” *Biogeochemistry* 111 (1-3): 41–55.

Nannipieri, P., J. Ascher, M.T. Ceccherini, L. Landi, G. Pietramellara, and G. Renella. 2003. “Microbial Diversity and Soil Functions.” *European Journal of Soil Science* 54 (4): 655–70.

Needham, T.D., Burger, J.A., & Oderwald, R.G. 1990. “Relationship between diagnosis and recommendation integrated system (DRIS) optima and foliar nutrient critical levels.” *Soil Science Society of America Journal*, *54*(3), 883-886.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. “Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States.” *Ecological Economics*, Integrating Ecology and Economics in Control Bioinvasions IEECB S.I., 52 (3): 273–88.

Ricker, M.C., and B.G. Lockaby. 2014. “Soil Biogeochemical Processes across a Lateral Toposequence in an Old-Growth Floodplain Forest.” *Soil Science Society of America Journal* 78 (6): 2100–2111.

Rowell, D. L. 2014. *Soil Science: Methods & Applications*. Routledge.

Staley, J.T., and A. Konopka. 1985. “Measurement of in Situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats.” *Annual Review of Microbiology* 39 (1): 321–46.

Stotzky, G. 1997. “Soil as an Environment for Microbial Life.,” 1–20.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. “An Extraction Method for Measuring Soil Microbial Biomass C.” *Soil Biology and Biochemistry* 19 (6): 703–7.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. “Quantifying Threats to Imperiled Species in the United States.” *BioScience* 48 (8): 607–15.

Wolfe, B.E., and J.N. Klironomos. 2005. “Breaking New Ground: Soil Communities and Exotic Plant Invasion.” *BioScience* 55 (6): 477–87.

Wu, J., R.G. Joergensen, B. Pommerening, R. Chaussod, and P.C. Brookes. 1990. “Measurement of Soil Microbial Biomass C by Fumigation-Extraction—an Automated Procedure.” *Soil Biology and Biochemistry* 22 (8): 1167–69.

Zak, D.R., W.E. Holmes, D.C. White, A.D. Peacock, and D. Tilman. 2003. “Plant Diversity, Soil Microbial Communities, and Ecosystem Function: Are There Any Links?” *Ecology* 84 (8): 2042–50.

Chapter 4

Response of ectomycorrhizal fungi associated with *Pinus taeda* to *Imperata cylindrica* exudate constituents

4.1 ABSTRACT

Imperata cylindrica represents a significant threat to communities of native vegetation in its invaded range. As part of the invasion process *I. cylindrica* produces compounds that inhibit the germination and growth of some plant species, including *Pinus taeda,* a commercially important timber species. These compounds may also inhibit the growth of symbiotic microbial organisms associated with *P. taeda*. We applied treatments of individual components of *I. cylindrica* exudate to plates of modified Melkin-Norans (MMN) agar on which several species of mycorrhizal fungi were grown. We analyzed the effect of these treatments on mean area of individual fungi relative to a control, several times over the course of 8 weeks. In addition, we performed analyses to determine if two isolates from the same fungal species would react similarly to the exudate components. All fungi demonstrated a significant time by treatment interaction that did not have a consistent causal agent. There were also significant differences between how isolates of the same species reacted to the same treatment. This research

demonstrates that several compounds consistent with *I. cylindrica* invasion will reduce area of mycorrhizal fungi at concentrations found in natural conditions.

4.2 INTRODUCTION

Cogongrass (*Imperata cylindrica* (L.) Beauv.) is a rhizome producing, C4, perennial grass with a multifaceted and dynamic invasion process (MacDonald 2004). *Imperata cylindrica* is a prolific seed producer and reproduces vegetatively from established plants, which makes it an effective disperser that thrives on disturbance (King & Grace 2000; MacDonald 2004). Once present *I. cylindrica* can outcompete native vegetation through several strategies such as shading out, altered fire regimes, and production of potentially allelopathic compounds (Lippencott 2000; Koger & Bryson 2009; Holzmueller & Jose 2011). These compounds were enumerated by Hagen *et al.* 2013 and demonstrated to be present in the soil in higher concentrations in *I. cylindrica* present soil than *I. cylindrica* absent soil.

During a 27 month study Daneshgar *et al.* 2008 showed that *Pinus taeda* L. seedlings grown in plots with dense *I. cylindrica* cover had lower rates of survival, displayed reduced growth and root collar diameter. Similarly, Koger & Bryson 2004 found that treatment with *I. cylindrica* extract resulted in reduced germination and growth of some grass and broadleaf species, concluding that *I. cylindrica* extract may contain allelochemicals. Phenolic acids, comparable to those found in *I. cylindrica* exudate were found to reduce growth of two common mycorrhizal fungi *Cenococcum geophilum* Fr. and *Laccaria laccata* (Scop. ex Fr.) Berk and Br., although the effect varied (Boufalis *et al.* 1994). Changes to soil-based ecosystem processes may establish positive feedback loops that further facilitate invasion; affecting native soil microbes, like mycorrhizal fungi (Ehrenfeld *et al.* 2001).

Ectomycorrhizal fungi (ECM) perform functions vital to healthy plant growth and development, in particular, exchange of N and P for excess plant C, increase nutrient uptake, heavy metal tolerance and disease resistance (Smith & Read 2010; Allen *et al.* 2003; Ingham 1988). Using individual species, without confounding factors, we established a study to measure individual components of *I. cylindrica* exudate on various mycorrhizal species in culture. We hypothesized that these compounds would have varying effects and severity on growth of individual species.

4.3 MATERIALS & METHODS

4.3.1 Cultures:

Fungal specimens were collected from multiple points around Oxford, Mississippi. Specimens were preferentially selected if they were in an early developmental stage and less likely to have been contaminated by insect feeding. Individual fruiting bodies were split open and internal pieces of cap or stalk context tissue were excised and plated on modified Melin-Norkrans (MMN) agar under aseptic conditions. Identities of successfully cultured fungi were subsequently identified utilizing Sanger sequencing of the ITS regions 1 and 2 (as in Hoeksema et al. 2012). Cultures were maintained by sub-culturing when cultures neared plate edge. The following nine isolates of ectomycorrhizal fungi were cultured from fruiting bodies and used in this experiment: *Amanita muscaria* (L.: Fr.) Hooker, *Laccaria laccata*, *Lactarius paradoxus* Beardslee & Burlingham, *Rhizopogon roseolus* (Corda) T.M. Fries, *Suillus brevipes* (Pk.) Kuntze, *Suillus hirtellus* (Peck) Snell (2 isolates, A and B), *Suillus salmonicolor* (Frost) Halling (2 isolates, A and B).

4.3.2 Plate study:

Modified Melin-Norkrans plates were inoculated with one of six components of *I. cylindrica* exudate (hereby referred to as treatments) found by Hagen *et al.* 2013 to be present in the soil in significantly higher abundances in the presence of *I. cylindrica* compared to control plots. Each treatment was mixed to the exact concentration reported in Hagen *et al.* 2013 in autoclaved deionized water using high purity chemicals. Treatments were applied in 500 µl increments with control plates receiving 500 µl of autoclaved deionized water. Treatments were applied evenly over the surface of the plate with a sterile implement and the autoclaved deionized water was allowed to evaporate. Each fungus was plated with each treatment 25 times and all fungi were plated within the span of 3 days. Control plates were inoculated the following week, also within 3 days. Plates were stored in complete darkness in an incubator at room temperature (25° C).

Measurement of growth was conducted every two weeks with a Lasico series 1281 Area/Length Meter (Lasico, Los Angeles, California). Measurements were taken and recorded in cm^2. Initial measurements were calculated for consistency. Fungal colonies that reached plate edge on all sides were also calculated for consistency. Plates contaminated by ambient microbes were marked and discarded if the contaminant physically interacted with the fungal growth. Measurements were taken until the first fungus reached the edge of any plate, to a maximum of eight weeks. Seven fungal isolates were measured for the full trial period, but the two isolates of *S. salmonicolor* reached the edge of their plates two weeks early during the six week measurement period.

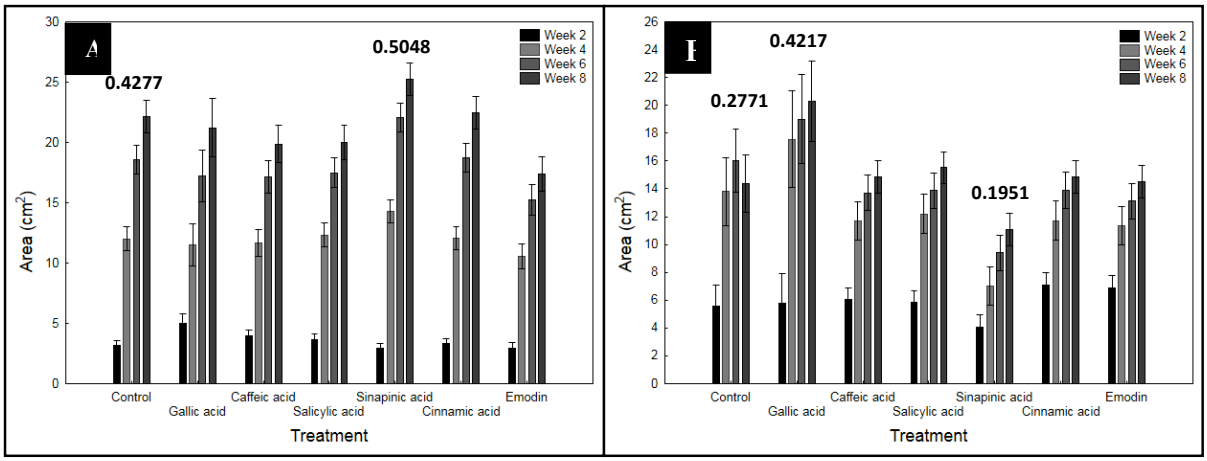
4.3.4 Statistical analysis:

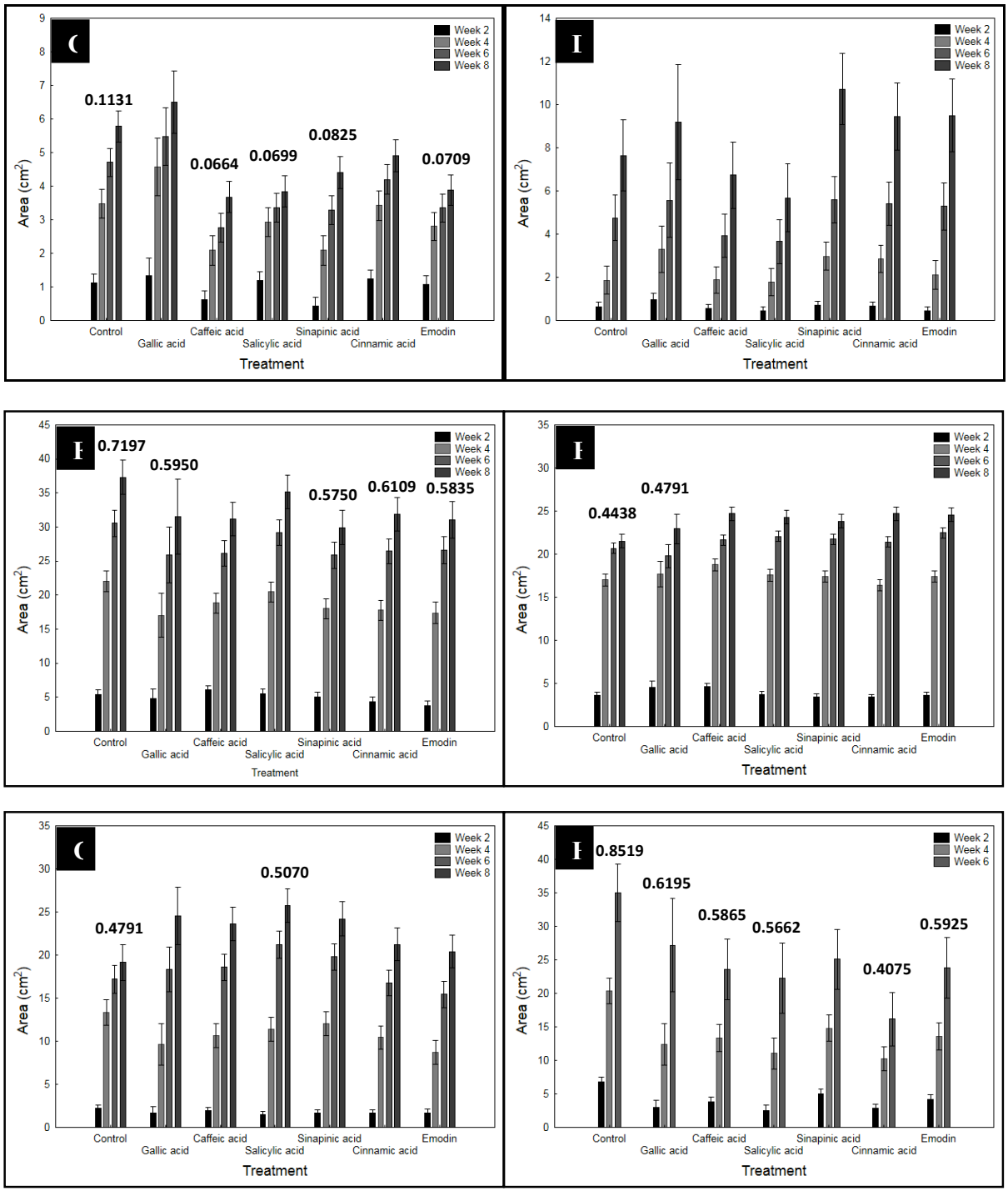
SAS® version 9.3 (SAS Institute Inc. 2010) and STATISTICA® (StatSoft 2013) were used for all statistical analyses. Separate mixed models were constructed for each fungal isolate separately, to determine if differences in area existed across treatments. Pairwise Tukey HSD comparisons (α=0.05) were also carried out between every treatment and the corresponding control, when significant treatment effects were observed. In these models, meanarea was the response variable and treatment and time were predictor variables.

Factorial ANOVAs were utilized to determine if final area was significantly different between isolates of the same species. Pairwise Tukey comparisons (α=0.05) were also carried out to determine which treatments, if any, were significantly different.

4.4 RESULTS

Amanita muscaria demonstrated a significant time by treatment interaction ($F_{(6,608)}$=13.12, p<0.0001) (Figure 4.1A). Sinapinic acid elicited an increase in growth compared to control treatments ($F_{(2,174)}$=4.88, p=0.0002) in pairwise comparisons. *Laccaria laccata* also demonstrated a significant time by treatment interaction ($F_{(6,627)}$=11.35, p<0.0001) (Figure 4.1B). In *L. laccata* cultures treatment with gallic acid resulted increased growth ($F_{(2,169)}$=4.77, p<0.0001), while treatment with sinapinic acid resulted in significantly decreased growth ($F_{(2,169)}$=3.61, p=0.007) in pairwise comparison. *Lactarius paradoxus* demonstrated a significant time by treatment interaction ($F_{(6,630)}$=11.34, p<0.0001) (Figure 4.1C).




C
Week 2
Week 4
Week 6
Week 8
0.1131
0.0664
0.0699
0.0825
0.0709
Area (cm2)
Control
Gallic acid
Caffeic acid
Salicylic acid
Sinapinic acid
Cinnamic acid
Emodin
Treatment
I
Week 2
Week 4
Week 6
Week 8
Area (cm2)
Control
Gallic acid
Caffeic acid
Salicylic acid
Sinapinic acid
Cinnamic acid
Emodin
Treatment
F
Week 2
Week 4
Week 6
Week 8
0.7197
0.5950
0.5750
0.6109
0.5835
Area (cm2)
Control
Gallic acid
Caffeic acid
Salicylic acid
Sinapinic acid
Cinnamic acid
Emodin
Treatment
F
Week 2
Week 4
Week 6
Week 8
0.4438
0.4791
Area (cm2)
Control
Gallic acid
Caffeic acid
Salicylic acid
Sinapinic acid
Cinnamic acid
Emodin
Treatment
C
Week 2
Week 4
Week 6
Week 8
0.4791
0.5070
Area (cm2)
Control
Gallic acid
Caffeic acid
Salicylic acid
Sinapinic acid
Cinnamic acid
Emodin
Treatment
F
Week 2
Week 4
Week 6
0.8519
0.6195
0.5865
0.5662
0.4075
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Emodin
Treatment

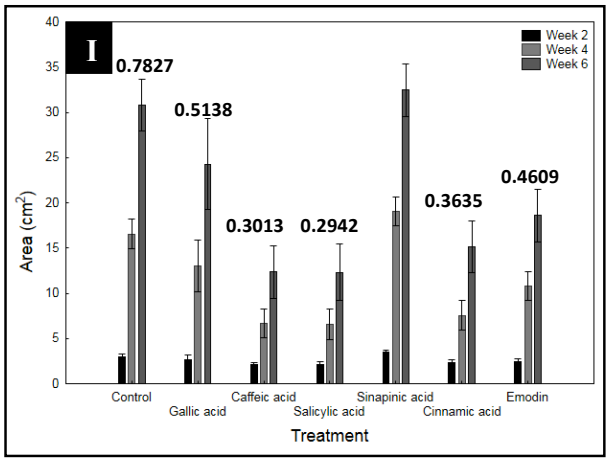

Figure 4.1. Mean values of fungal colony area measured across four collection periods ((a) *Amanita muscaria*, (b) *Laccaria laccata*, (c) *Lactarius paradoxus*, (d) *Rhizopogon roseolus*, (e) *Suillus brevipes*, (f) *Suillus hirtellus* A, (g) *Suillus hirtellus* B) or two collection periods ((h) *Suillus salmonicolor* A, (i) *Suillus salmonicolor* B), each two weeks apart. Pairwise comparisons (α=0.05) were made between individual treatments and controls in the same collection period, not among collection periods. Significant differences are denoted by growth rates (cm/day), bars denote 95 % standard error.

In *L. paradoxus* cultures treatment with caffeic acid ($F_{(2,169)}$=6.27, p<0.0001), salicylic acid ($F_{(2,169)}$=4.69, p=0.0001), sinapinic acid ($F_{(2,169)}$=4.99, p<0.0001) and emodin ($F_{(2,169)}$=4.88, p<0.0001) all resulted in negative growth according to pairwise comparisons. *Rhizopogon roseolus* likewise yielded a time by treatment interaction ($F_{(2,639)}$=11.63, p<0.0001) (Figure 4.1D), although no treatment differed significantly from the control. *Suillus brevipes* demonstrated a time by treatment interaction ($F_{(6,629)}$=5.19, p<0.0001) (Figure 4.1E). Gallic acid ($F_{(2,171)}$=3.55, p=0.009), sinapinic acid ($F_{(2,171)}$=3.73, p=0.005), cinnamic acid ($F_{(2,171)}$=3.12, p=0.034) and emodin ($F_{(2,171)}$=4.05, p=0.002) were correlated with decreased growth in pairwise comparisons.

Two isolates of *Suillus hirtellus* were examined. Specimen A (*S. hirtellus* A) didn't demonstrated a significant time by treatment interaction ($F_{(6,620)}$=1.20, p=0.307) (Figure 4.1F). No treatment decreased growth of *S. hirtellus* A but caffeic acid ($F_{(2,171)}$=3.49, p=0.011) was consistent with increased growth. Specimen B (*S. hirtellus* B) had a significant time by treatment interaction ($F_{(6,625)}$=6.81, p<0.0001) (Figure 4.8G). Again no treatment was consistent with decreased growth but salicylic acid ($F_{(6,170)}$=3.43, p=0.013) was consistent with increased growth. Final growth of *S. hirtellus* A and *S. hirtellus* B were compared and it was determined that a significant difference existed between isolate growth ($F_{(6,292)}$=4.29, p=0.0004) (Figure 4.2). Specifically, a difference was observed in *S. hirtellus* response to cinnamic acid (p=0.048) and emodin (p=0.006).

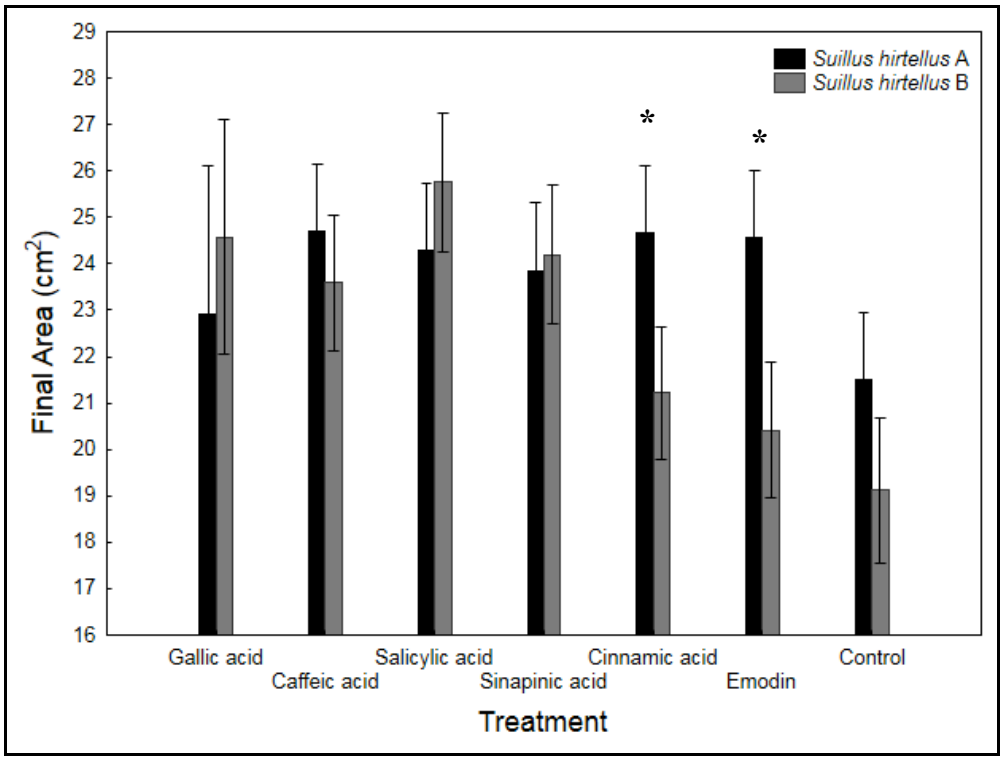

Figure 4.2. Mean final area of fungal colonies is compared across like treatments of different specimens of the same fungal species *Suillus hirtellus*. Significant differences (α=0.05) are denoted with asterisks.

Two isolates of *Suillus salmonicolor* were also incorporated. Specimen A (*S. salmonicolor* A) displayed significant time by treatment interaction ($F_{(6,460)}$=11.21, p<0.0001) (Figure 4.1H). In this specimen all treatments resulted in significantly decreased growth: gallic acid ($F_{(2,177)}$=3.15, p=0.031), caffeic acid ($F_{(2,177)}$=3.48, p=0.011), salicylic acid ($F_{(2,177)}$=3.82, p=0.003), cinnamic acid ($F_{(2,177)}$=5.95, p<0.0001) and emodin ($F_{(2,177)}$=3.15, p=0.031). Specimen B (*S. salmonicolor* B) had a time by treatment interaction ($F_{(6,476)}$=49.46, p<0.0001) (Figure 4.1I). Gallic acid ($F_{(2,170)}$=4.75, p<0.0001), caffeic acid ($F_{(2,170)}$=8.93, p<0.0001), salicylic acid ($F_{(2,170)}$=8.81, p<0.0001), cinnamic acid ($F_{(2,170)}$=7.75, p<0.0001) and emodin ($F_{(2,170)}$=5.95, p<0.0001) treatments all resulted in decreased growth relative to the control. Final growth of *S. salmonicolor* A and *S. salmonicolor* B was compared and a significant difference was present between similar treatments of different isolates ($F_{(6,286)}$=5.42, p<0.0001) (Figure 4.3). *Suillus salmonicolor* had two treatments that were significantly different between specimens of the same species: caffeic acid (p=0.003) and salicylic acid (p=0.010). In total gallic acid and emodin were the two most common sources of reduced area (Table 4.1)

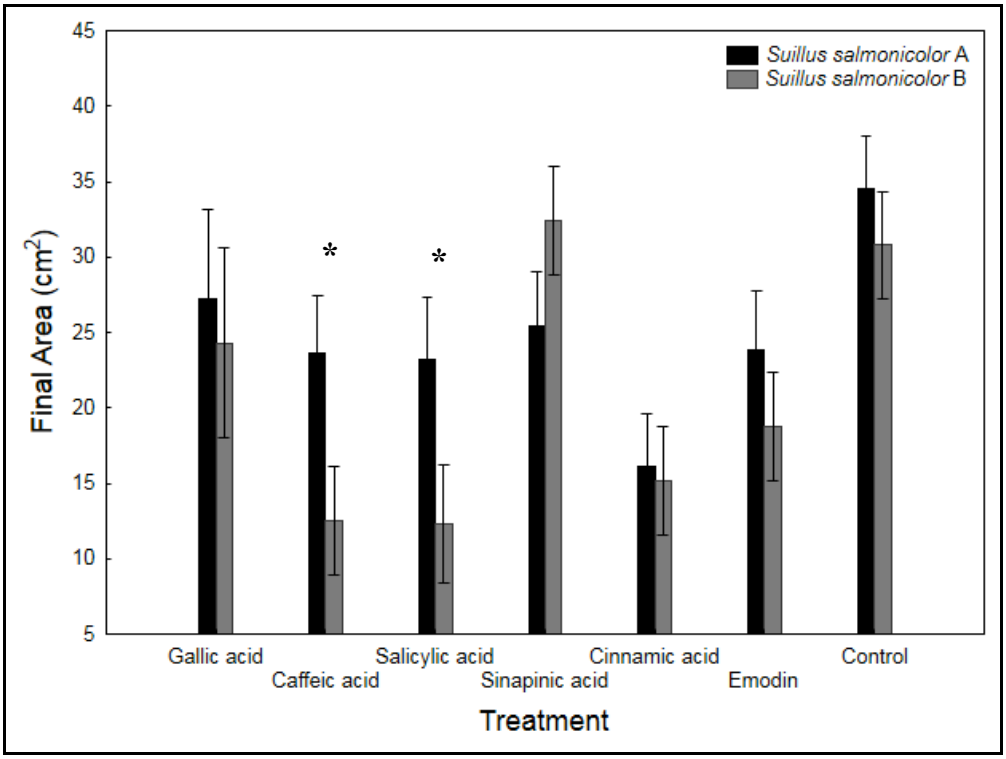

Figure 4.3. Mean final area of fungal colonies is compared across like treatments of different specimens of the same fungal species *Suillus salmonicolor*. Significant differences (α=0.05) are denoted with asterisks.

Table 4.1. Effects of individual components of *I. cylindrica* exudate are summarized across fungal species of isolates of the same species, differentiated with the variable "A" or "B." Cells with "+" indicate that mean area was greater than control. Cells with "-" indicate that growth was retarded compared to control. Cells with "0" denote no difference was observed between treatment and control.

	Gallic acid	Caffeic acid	Salicylic acid	Sinapinic acid	Cinnamic acid	Emodin
Amanita muscaria	0	0	0	+	0	0
Laccaria laccata	+	0	0	-	0	0
Lactarius paradoxus	0	-	-	-	0	-
Rhizopogon roseolus	0	0	0	0	0	0
Suillus brevipes	-	0	0	-	-	-
Suillus hirtellus A	0	+	0	0	0	0
Suillus hirtellus B	0	0	+	0	0	0
Suillus salmonicolor A	-	-	-	0	-	-
Suillus salmonicolor B	-	-	-	0	-	-

4.5 DISCUSSION

Emodin most frequently resulted in reduced growth when applied to plates on which mycorrhizal fungi were grown (4 out of 9). Emodin is found across 17 families in a diversity of growth forms worldwide (Izhaki 2002). In experiments on a variety of media and taxa emodin has been found to reduce the growth of several plant species and soil bacteria, in some cases at minimal concentrations (Inoue *et al.* 1992, Hasan 1998, Izhaki 2002). Emodin has also been recorded to decrease availability of Mn^{2+} and increase the availability of Na^{+} and K^{+} (Inderjit & Nishimura 1999). The preponderance of these findings was consistent with conditions observed in other *I. cylindrica* invaded plots (See chapter 3).

All treatments but cinnamic acid elicited a statistically significant response of some kind in 4 out of 9 fungi. The effect we observed was not, however, consistent; some species increased in growth in the presence of an exudate constituent that decreased or had no effect on other species. From these results we determined that although almost every fungal species demonstrated a time by treatment interaction, no one compound was singularly responsible. Instead it is far more likely that compounds act in tandem to exclude some individual species of mycorrhizal fungi and facilitate others *in situ*.

In addition, within the confines of our limited replications (n=2) we observed a divide in the effect of compounds on growth of two isolates of the same species, although these results were also inconsistent. To this end we conclude that isolates within the same species responds similarly, within the confines of our experimental design, but not the same and that additional research is necessary to provide more strength to this trend.

Few other researchers have investigated the effects that invasive species utilizing allelopathic compounds may have on belowground mutualists. Those that have largely confirm our findings that invaders may disrupt some aspects of these mutualisms. Garlic mustard (*Allaria petiolata* [M. Bieb.] Cavara & Grande) may produce compounds that inhibit germination and reduce inoculum of arbuscular mycorrhizal fungi in the soil (Roberts & Anderson 2001, Stinson *et al.* 2006). Anderson *et al.* 2010 found that mycorrhizal fungi recovered from removal of *A. petiolata* more slowly than non-mycorrhizal species. Beyond the role of mycorrhizal fungi, disturbance to belowground communities may affect biogeochemical cycling, other soil microbial organisms that have direct effects on plant growth as well as soil structure (Wolfe & Klironomos 2005).

4.6 CONCLUSION

A significant time by treatment effect was observed in every mycorrhizal fungi. No single compound was the sole cause of decreased area. We therefore conclude that different compounds provoke different responses in mycorrhizal fungi and that almost all compounds produce both positive and negative effects to area. When multiple isolates of the same species were exposed to the same treatments a few treatments produced different responses. We attribute this to variations among a species and note that for neither of the two species with more than one specimen did the control treatments vary significantly.

4.7 REFERENCES

Allen, M.F., W. Swenson, J.I. Querejeta, L.M. Egerton-Warburton, and K.K. Treseder. 2003. "Ecology of Mycorrhizae: A Conceptual Framework for Complex Interactions Among Plants and Fungi." *Annual Review of Phytopathology* 41 (1): 271–303.

Anderson, R.C., M.R. Anderson, J.T. Bauer, M. Slater, J. Herold, P. Baumhardt, and V. Borowicz. 2010. "Effect of Removal of Garlic Mustard (Alliaria Petiolata, Brassicaeae) on Arbuscular Mycorrhizal Fungi Inoculum Potential in Forest Soils". *Open Ecology Journal* 3: 41–47.

Boufalis, A., F. Pellissier, and L. Trosset. 1994. "Responses of Mycorrhizal Fungi to Allelopathy: Cenococcum Geophilum and Laccaria Laccata Growth with Phenolic Acids." *Acta Botanica Gallica* 141 (4): 547–50.

Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. "Working with Mycorrhizas in Forestry and Agriculture."

Daneshgar, P., S. Jose, A. Collins, and C. Ramsey. 2008. "Cogongrass (Imperata Cylindrica), an Alien Invasive Grass, Reduces Survival and Productivity of an Establishing Pine Forest." *Forest Science* 54 (6): 579–87.

Ehrenfeld, J.G., P. Kourtev, and W. Huang. 2001. "Changes in Soil Functions Following Invasions of Exotic Understory Plants in Deciduous Forests." *Ecological Applications* 11 (5): 1287–1300.

Hagan, D.L., S. Jose, and C. Lin. 2013. “Allelopathic Exudates of Cogongrass (Imperata Cylindrica): Implications for the Performance of Native Pine Savanna Plant Species in the Southeastern US.” *Journal of Chemical Ecology* 39 (2): 312–22.

Hasan, H.A.H. 1998. “Studies on toxigenic fungi in roasted foodstuff (Salted seed) and halotolerant activity of emodin-producingAspergillus wentii”. *Folia microbiologica*, *43*(4), 383-391.

Holzmueller, E.J., and S. Jose. 2011. “Invasion Success of Cogongrass, an Alien C4 Perennial Grass, in the Southeastern United States: Exploration of the Ecological Basis.” *Biological Invasions* 13 (2): 435–42.

Hoeksema, J. D., J.V. Hernandez, D.L. Rogers, L.L. Mendoza, and J.N. Thompson. 2012. “Geographic divergence in a species-rich symbiosis: interactions between Monterey pines and ectomycorrhizal fungi”. *Ecology*,*93*(10), 2274-2285.

Horton, T.R., and T.D. Bruns. 2001. “The Molecular Revolution in Ectomycorrhizal Ecology: Peeking into the Black-Box.” *Molecular Ecology* 10 (8): 1855–71.

Inderjit., H. Nishimura. 1999. Effect of the anthraquinones emodin and physcion on availability of selected soil inorganic ions”. *Annals of applied biology*, *135*(1), 425-429.

Ingham, R.E. 1988. “Interactions Between Nematodes and Vesicular-Arbuscular Mycorrhizae.” *Agriculture, Ecosystems & Environment*, Proceedings of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth, 24 (1–3): 169–82.

Inoue, M., H. Nishimura, H.H. Li, and J. Mizutani. 1992. “Allelochemicals fromPolygonum sachalinense Fr. Schm.(Polygonaceae).” *Journal of Chemical Ecology*, *18*(10), 1833-1840.

Izhaki, I. 2002. “Emodin–a secondary metabolite with multiple ecological functions in higher plants”. *New Phytologist*, *155*(2), 205-217.

King, S.E., and J.B. Grace. 2000. “The Effects of Soil Flooding on the Establishment of Cogongrass (Imperata Cylindrica), a Nonindigenous Invader of the Southeastern United States.” *Wetlands* 20 (2): 300–306.

Koger, C.H., and C.T. Bryson. 2004. “Effect of Cogongrass (Imperata Cylindrica) Extracts on Germination and Seedling Growth of Selected Grass and Broadleaf Species.” *Weed Technology* 18 (2): 236–42.

Lippincott, C.L. 1997. “Ecological Consequences of Imperata Cylindrica (cogongrass) Invasion in Florida Sandhill.” Gainesville, FL: University of Florida. https://ia601702.us.archive.org/27/items/ecologicalconseq00lipp/ecologicalconseq00lipp.pdf.

MacDonald, G.E. 2004. “Cogongrass (Imperata cylindrica)—Biology, Ecology, and Management.” *Critical Reviews in Plant Sciences* 23 (5): 367–80.

Peay, K.G., M. Garbelotto, and T.D. Bruns. 2010. “Evidence of Dispersal Limitation in Soil Microorganisms: Isolation Reduces Species Richness on Mycorrhizal Tree Islands.” *Ecology* 91 (12): 3631–40.

Peay, K.G., M.G. Schubert, N.H. Nguyen, and T.D. Bruns. 2012. “Measuring Ectomycorrhizal Fungal Dispersal: Macroecological Patterns Driven by Microscopic Propagules.” *Molecular Ecology* 21 (16): 4122–36.

Roberts, K.J., and R.C. Anderson. 2001. “Effect of Garlic Mustard [Alliaria Petiolata (Beib. Cavara & Grande)] Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi”. *The American Midland Naturalist* 146 (1): 146–52.

Smith, S.E., and D.J. Read. 2010. *Mycorrhizal Symbiosis*. Academic Press.

Stinson, K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D.Prati, and J.N. Klironomos. 2006. "Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms." *PLoS biology, 4(5), 727.*

Chapter 5

Summary and Conclusions

5.1 INVASION IN PRODUCTION FORESTRY SYSTEMS

It is estimated that more than 50,000 invasive species are present in the United States causing an estimated US$120 billion/yr in direct and indirect damage (Pimentel *et al.* 2000, Pimentel *et al.* 2005). The southeastern United States is referred to as the "wood basket" due to its highly productive forest products industry (Schultz 1997). Areas with lower species diversity are usually more susceptible to disease and invasion (Keesing *et al.* 2006, Kennedy *et al.* 2002). Therefore production forestry may be particularly susceptible to disease and invasion due to the low species diversity. Loblolly pine is the most utilized tree in this region comprising over 50 % of the dominant and co-dominant growing stock, with average production over one billion seedlings annually (Enebak 2013, Schultz 1997). The forest products industry has an industrial output of US$103 billion annually (Tilley & Munn 2007). Any reduction in production, as a result of reduced tree growth, can result in damages in the millions of dollars.

5.2 *IMPERATA CYLINDRICA* IN THE SOUTHEASTERN UNITED STATES

Cogongrass (*Imperata cylindrica* (L.) Beauv.) is a perennial rhizome producing grass in the Poaceae (Graminae) (Bryson & Carter 1993, Dozier *et al.* 1998). This species is highly aggressive growing grass and has been designated as the seventh worst weed in the world (Holm *et al.* 1997). *Imperata cylindrica* is currently established in Florida, Georgia, Alabama and Mississippi with scattered populations occurring in South Carolina, east Texas and Louisiana (Miller *et al.* 2010). This grass was originally introduced into the United States for forage crop trials in 1920, they were found to have little utility due to the high silica content of its leaves and propensity to injure livestock with sharp rhizomatous growths (Coille & Shilling 1993, Holzmueller & Jose 2011). *Imperata cylindrica* is particularly difficult to eradicate with some researchers finding up to 89 meters of rhizome per sq. m^2 of soil.

5.3 FINAL RESEARCH SUMMARY AND POTENTIAL RESEARCH

Plant accessible N, P, and K were measured concurrently due to their codependent roles in plant function. Nitrogen was not found to be significantly different between *I. cylindrica* abundant (>50 %) and *I. cylindrica* absent plots. Phosphorus levels also did not vary significantly between plot types. Potassium, however, was found in significantly different abundances. This indicated that other base cations should be examined in order to determine if this represented a trend. No other base cation with the exception of Na was found in differing quantities. The presence of some compounds in *I. cylindrica* exudates should be investigated as a potential cause to this trend. In addition, nutrients with linked functions should be examined in conjunction.

During the November sampling season a significant difference was observed in mean percent colonization of fine roots by mycorrhizal fungi between plots with >50% cover of *I. cylindrica* and those with less *I. cylindrica*. Differences were observed with very low replication and so a more broad scale comprehensive approach should be taken in the future to better understand the processes at work. A significant reduction in mean mycorrhizal colonization existed in some *I. cylindrica* present plots in different depth classes. In addition, a significant reduction was observed in retrieved fine feeder root quantity in 21-40 cm and 41-60 cm depth classes across treatments in both November and May sampling periods. This difference across treatments was also observed, over all, between *I. cylindrica* absent and *I. cylindrica* present (both <50 % and >50 %). Mycorrhizal fungi have a vital role in healthy plant growth and development. Specifically they contribute to area of soil a tree may uptake nutrients from, improve metal resistance and perform disease suppression functions (Smith & Read 2002). Mycorrhizal fungi are a hyperdiverse taxa and are suspected to be highly functionally diverse as well (Cairney 1999, Smith & Read 2002). Furthermore they play a unique and important role in function of rhizospere community dynamics and diversity (Marks 1973). The loss of any mycorrhizal species may represent a loss of function from the soil community. Reduced colonization of mycorrhizal fungi and substrate on which they interact with their plant host, represents an increased possibility of loss of functional diversity.

Soil moisture content was only found to be significantly different in the winter collection at which time *I. cylindrica* invaded stands (<50 % cover) had less soil moisture than *I. cylindrica* absent. Contrary to our hypothesis there was no significant difference between *I. cylindrica* invaded (>50 % cover) and *I. cylindrica* absent. Differences in the C:N ratio of microbial

biomass were found along a seasonal gradient. The season in which microbial communities are most active found a difference between the *I. cylindrica* <50 % cover plots and the *I. cylindrica* >50 % cover plots. Microbes are integral to nutrient cycling and organic matter transformation. Microbial biomass is a measure of the abundance of microbes in the soil determined by the amount of C and N in their tissue. By taking a ratio of these values we can determine the relative proportion of C and N that is being utilized by microorganisms and will become available for plant use. Higher C:N indicates that more organic C is available relative to organic N. This appears to be consistent with the findings of Daneshgar & Jose (2009) that *I. cylindrica* has lower concentration of N than native vegetation but more N per ha. The researchers cite this as the reason for the seven fold higher quantity of below ground biomass compared to native plant species.

Research was also conducted to determine which component of *I. cylindrica* exudate was responsible for the observed disparity in abundance of mycorrhizal fungi. We determined that although sinapinic acid and emodin most often resulted in reduced area relative to a control this effect was not consistent. Several additional compounds inhibited additional fungi, with some overlap. We would expect to observe a disparity in the species of mycorrhizal fungi found in *I. cylindrica* present and absent stands.

It is suggested that further research in this field should examine how individual components of *I. cylindrica* exudates influence the growth rate of more species of mycorrhizal fungi. This research may better elucidate the mechanism behind functional loss in mycorrhizal communities. Additionally it is important to determine if different mycorrhizal fungi responses

vary to different plant produced compounds. Another beneficial variable to examine is whether different geographic sources of *I. cylindrica* leachate have different effects on different mycorrhizal fungi in order to determine if additional sources of introduction may cause additional damage. Further research into cycling of C, N and P would be useful, including N-mineralization, a week-by-week analysis of microbial biomass C and N as well as information on microbial biomass P.

5.4 REFERENCES

Bremner, J.M. 1996. Nitrogen-total. In D.L. Sparkes et al., Eds. Methods of Soil Analysis, Part 3-Chemical Methods. *Soil Science Society of Ameica Journal*., American Society of Agronomy, Madison, WI, pp-1085-1121.

Bryson, C.T.; Carter, R. 1993. Cogongrass, Imperata cylindrica, in the United States. *Weed Technology* 7: 1005-1009.

Cairney, J.W.G. 1999. Intraspecific Physiological Variation: Implications for Understanding Functional Diversity in Ectomycorrhizal Fungi. *Mycorrhiza* 9: 125-35.

Callaway, R.M.; Ridenour, W.M. 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436-43.

Daneshgar, P., Jose, S. 2009. *Imperata cylindrica*, an alien invasive grass, maintains control over nitrogen availability in an establishing pine forest. *Plant and Soil* 320: 209-18.

Dozier, H., Gaffney, J.F., McDonald, S.K., Johnson, E.R.R.L., Shilling, D.G. 1998. *I. cylindrica* in the United States: History, ecology, impacts, and management. *Weed Technology* 12:737-743.

Enebak, S. 2013. Forest tree seedling production in the south for the 2012–2013 planting season. Auburn University Southern Forestry Nursery Management Cooperative.

Hagan, D.L., Jose, S., Lin, C. 2013. Allelopathic exudates of *I. cylindrica* (*Imperata cylindrica*): Implications for the performance of native pine savanna plant species in the southeastern US. *Journal of Chemical Ecology* 39: 312-322.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P.. 1977. The World's Worst Weeds. 609 pp.

Holzmueller, E.J., Jose, S. 2011. Invasion success of *I. cylindrica*, an alien c4 perennial grass, in the southeastern United States: Exploration of the Ecological Basis. *Biological Invasions* 13: 435-42.

Keesing, F., Holt, R.D., Ostfeld, R.S. 2006. Effects of species diversity on disease risk. *Ecology Letters* 9: 485-498.

Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M.H., Tilman, D., Reich, P. 2002. Biodiversity as a barrier to ecological invasion. *Nature* 417: 636-638.

Marks, G.C. 1973. Ectomycorrhizae: Their Ecology and Physiology. Academic Press, University of California, 444 p.

Mehlich, A. 1984. Mehlich 3 Soil test extractant: A modification of mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 15: 1409-1416.

Miller, J.H., Chambliss, E.B., Loewenstein, N.J. 2010. Field Guide for the Identification of Invasive Plants in Southern Forests. USDA, Forest Service, Southern Research Station, Ashville, NC, 126 p.

Coile, N., Shilling, D. 1993. *I. cylindrica, Imperata cylindrica(L.) Beauv.: A good grass gone bad!*. Botany Circular 28. Florida Department of Agriculture and Consumer Services.

Pimentel, D., Lach, L., Zuniga, R., Morrison, D. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50: 53-65.

Pimentel, D., Zuniga, R., Morrison , 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics, Integrating Ecology and Economics in Control. *Bioinvasions* 52: 273-288.

Schultz, R. P. 1997. Loblolly pine: The ecology and culture of loblolly pine (Pinus Taeda L.)., no. 713: xiv + 494 p.

Smith, S.E., Read, D.J. 1997. Mycorrhizal Symbiosis. Academic Press. San Diego, CA, 605 p.

Tilley, B., Munn, I.A. 2007. 2001 Economic impacts of the forest products industry in the south. *Southern Journal of Applied Forestry* 31: 181-186.

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19: 703-707.