Cogongrass (Imperata cylindrica (L.) Beauv.) in Louisiana: Cause and Consequence

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COGONGRASS (*IMPERATA CYLINDRICA* (L.) BEAUV.) IN LOUISIANA: CAUSE AND CONSEQUENCE

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Biological Sciences

by

Lorissa Radunzel-Davis
B.S., University of Wisconsin-Madison, 1996
A.A.S., Argosy University, 2005
August 2019
I would like to dedicate this to my husband Jeff and sons William and Matthew Davis. They have all been very supportive of my endeavor and have taken on additional tasks enabling me to focus on finishing my degree.
ACKNOWLEDGEMENTS

This research could not have been done without the assistance of many people. I have had this opportunity because Dr. Erik Aschehoug decided to take me on has his student and Dr. Jim Cronin took me in when circumstances changed. Thank you also to Dr. Rodrigo Diaz for serving on my committee. The Department of Biological Sciences support staff also answered my questions and took care of the many small things that often go unnoticed.

There are many others I would like to thank. Dr. Haley Dozier directed me to Lee Memorial Forest as a source of cogongrass. Without her, I would never have met the manager Joe Nehlig who spent 2 days taking me throughout the property to mark cogongrass patches and opened his files to me. Denise McKinney pointed me to cogongrass patches at Bogue Chitto State Park. Chapter 2 could not have happened without the help of Caryn Davis collecting, drying and shipping soil from Japan. I appreciate how responsive the Louisiana State University greenhouse staff was to all my requests. My labmate Rachel Harman helped me tremendously by being there to discuss items both related and unrelated to my work. I want to thank Honza Čuda for being a sounding board and company in the lab for a semester. Finally, thank you to undergraduates Daniel, Celine and Marshall along with Honza, Rachel and Joe Johnston for their help during harvest and clean-up times. Many hands made light work.
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ABSTRACT

Cogongrass (*Imperata cylindrica* (L.) Beauv.), an invasive species from East Asia, is found worldwide and is problematic in several countries. In the United States, it grows primarily in the Southeast, reducing biodiversity by growing in dense patches and potentially causing mortality and reducing value of native and planted pinelands due to a high burning temperature. Using Lee Memorial Forest, a Louisiana State University AgCenter property in Washington Parish as a study site, this thesis explores cogongrass in Louisiana with emphasis on soil microbes and soil legacy effects on native plant species. Cogongrass populations at Lee Memorial Forest were more likely to occur in management units with a prescribed burn, found primarily in evergreen forest on both soil types on the property and have a clustered distribution.

Soil microbial community effects on cogongrass growth were compared in soil collected from the native range in Japan and in soil collected from Louisiana. There was an initial release from soil microbial pathogens with 1.4 times more aboveground biomass in live versus sterile soil from the invaded range. In the native-range soil, 1.4 times more aboveground biomass was produced in sterile soil than in live soil, indicating the presence of pathogens. Live soils were reused to test how cogongrass induced alterations in microbial community affected subsequent cogongrass growth. Aboveground biomass production in sterile soil was always greater than in live soil, 1.2 times in Japanese soil and 2.2 times in Louisiana soil, indicating increased pathogens. Cogongrass from two Louisiana populations responded similarly in aboveground biomass production, however there were differences in the allocation of that biomass to leaves or additional height, indicating variable invasive potential.

The second study investigated the cogongrass legacy remaining in soil after its removal and the impact on subsequent plants. Two native plants, *Schizachyrium scoparium* and...
Arnoglossum ovatum, were grown in soil either previously containing cogongrass or having no previous plant growth. Schizachyrium scoparium accumulated 2.9 times and A. ovatum 1.3 times more total biomass in cogon-free soil compared to cogon-exposed soil. Soil mitigation techniques due to cogongrass soil legacy may be needed for optimal restoration success.
CHAPTER 1. INTRODUCTION TO COGONGRASS WITH EMPHASIS ON LEE MEMORIAL FOREST

INTRODUCTION

Cogongrass (*Imperata cylindrica* (L.) Beauv.), is a clonal C4 perennial grass species that has strong negative effects on native plant communities worldwide and is listed as one of the world’s top 100 worst invasive species (Estrada and Flory 2015). There are five varieties of cogongrass, with at least one variety successfully established on all continents except Antarctica (Hubbard et al. 1944, Dozier et al. 1998). It is found on a wide variety of soil types, from heavy clay to sandy soils and in a wide range of nutrient conditions (Jose et al. 2002, Bryson et al. 2010). It is native to Southeast Asia and was introduced into the United States in 1912 in Alabama as packing material and to Mississippi and Florida in 1921 for study as a possible forage crop (Hubbard et al. 1944, Tabor 1952, Dozier et al. 1998). By 1952, it was recognized as a concern and, although a study and an eradication plan were suggested, little was done (Dickens 1974). Now cogongrass is found across the Southeast United States (Dozier et al. 1998).

Cogongrass has strong negative ecological and agricultural impacts throughout the world (Hubbard et al., 1944, Brook, 1989, Chikoye et al., 2005, Dozier et al., 1998). When left unmanaged, it quickly becomes the dominant species, growing in tall, dense patches, and outcompeting native species, with the help of allelopathic chemicals (Estrada and Flory 2015, Hagan et al. 2013, Xuan et al. 2009, Brewer 2008). It has a dense mat of roots and pointed rhizomes that can penetrate other root systems (Holly and Ervin, 2006). Cogongrass causes significant decreases in pine root growth, height and biomass compared to areas with native or no understory vegetation (Daneshgar et al. 2008). Pine seedlings were smaller and had less nitrogen content in areas where cogongrass was growing than seedlings growing without
cogongrass (Daneshgar and Jose 2009a). Cogongrass has significant impacts on phosphorous and potassium availability and alters pH of the soil (Brewer and Cralle 2003).

In Louisiana, cogongrass has been present since 1990, when a survey and eradication plan were enacted (Bryson and Carter 1993) and is primarily found in eastern Louisiana (Loewenstein and Miller 2007). Lee Memorial Forest is an LSU AgCenter property of more than 1200 acres in Washington Parish (30.874, -89.991). The area is managed for research as well as timber harvest. There are areas of hardwood bottomlands, longleaf pine restoration and timber plantations. A patch of cogongrass was first discovered in 2002 and the land manager has been monitoring and treating it biannually. Despite such intense management, new patches are discovered every year (Figure 1.1, J. Nehlig personal communication).

![Graph showing number of newly discovered cogongrass patches found annually in Lee Memorial Forest, Washington Parish, Louisiana. Data collected by Joe Nehlig, land manager of Lee Memorial Forest.](image)

Figure 1.1. Number of newly discovered cogongrass patches found annually in Lee Memorial Forest, Washington Parish, Louisiana. Data collected by Joe Nehlig, land manager of Lee Memorial Forest.
DISTURBANCE

As with many other invasive species, cogongrass is thought to be reliant on disturbance to be able to establish a population (Catford et al. 2009, Müller et al. 2016), though it is also found in less disturbed areas (Dozier et al. 1998). Müller et al. (2016) found that for both native and exotic plants, disturbance was more important for establishment success than pathogens and herbivores. Cogongrass spreads by seeds and by rhizome production. Anthropogenic dispersal from moving soil contaminated with rhizomes is thought to be a predominant source of spread (Dozier et al. 1998). A few studies have investigated disturbance and the spread of cogongrass. Holzmueller and Jose (2012) studied cogongrass in the Blackwater River State Forest in the Florida panhandle. They found that it was more than twice as likely to be in burned versus unburned areas. They also found a positive linear relationship between post-hurricane salvage biomass and cogongrass presence. Ervin and Holly (2011a) surveyed up to 90 meters from roadsides in DeSoto National Forest in Mississippi. They found that cogongrass was found in high proximity to roads, with few areas surveyed having cogongrass more than 30 meters from the roadside. They also found that human-caused disturbance had a much stronger relationship with the presence of cogongrass than did natural disturbance.

To investigate this at Lee Memorial Forest, I marked the location of 57 cogongrass patches using a Trimble GPS unit in December 2016 and January 2017. In addition to location data, annual maps of cogongrass patches and records of tree harvest and prescribed burning since 1998 were obtained from Lee Memorial Forest records. Management units at Lee Memorial Forest had not been fully delineated in a digital format. A partially delineated map from the Louisiana State University AgCenter was used along with paper maps to create a shapefile with all Lee Memorial Forest management units. Using ArcMap 10.4, information about
management activities was joined to the respective units and overlayed with the location of the
cogongrass patches. Association of new cogongrass patches with timing of disturbance was
determined by calculating the time between the last burn or harvest in a management unit and
when the cogongrass patch was first observed. In addition, a chi-square analysis was done to see
if management activities were associated with the presence of cogongrass patches in those units.
It is possible that patches could have arisen from rhizomes moved by mechanical equipment.
Especially areas that are harvested, since the companies hired do not wash off equipment.

Timber harvesting happens periodically at Lee Memorial Forest and sections of
and 2016. There were 35 cogongrass patches (61%) in management units that had a harvest
prior to the discovery of a patch in that unit (Figure 1.2). Of those patches 55% were found
within 2000 days (5.5 years). Timber harvesting activity was not associated with cogongrass
patch presence ($X^2=2.87$, $p=0.09$). This would seem to indicate that movement of rhizomes by
mechanical equipment is not driving cogongrass presence in Lee Memorial Forest.

Burning happens annually, though not in the same management units. There were 45
cogongrass patches (79%) found in units managed by prescribed burns. New cogongrass patches
were often found after burning, with 88% being found within 730 days (2 years) of the
prescribed burn (Figure 1.3). Prescribed burning is associated with cogongrass patch presence
($X^2=7.86$, $p=0.01$). The frequency of burning, and that it is a primary management tool in areas
with cogongrass may be a reason for this association. Most cogongrass patches (81%) are
located in areas that had some known management activity prior to discovery. However, 11
patches (19%) were in management units that had no prior management activities. Those few
could have germinated from windblown seed.
Figure 1.2. The timing of 35 cogongrass patch discoveries since the occupied management unit had timber harvested using heavy equipment. The mean number of days before discovery was 2032 (5.6 years).

Figure 1.3. The timing of 45 cogongrass patch discoveries since the occupied management unit had a prescribed burn. The mean number of days before discovery was 526 (1.4 years).
There are still unknowns such as the length of time a patch may be growing before it is large enough to be noticed. It remains to be investigated if management practices, especially prescribed burning, spread cogongrass, or if it is more likely to be noticed because of the management activity. The location of these patches does not necessarily indicate a causal relationship. In addition, there are also research activities that occur in Lee Memorial Forest in addition to management by harvest and prescribed burning that could potentially spread cogongrass.

ASSOCIATION WITH SOIL AND LAND COVER

The distribution of invasive species can be useful in predicting their continued spread by analyzing environmental variables associated with the presence of the species. Analysis of a distribution model for cogongrass found that in Alabama there was a strong correlation with soil variables, whereas in Mississippi, there was a stronger correlation with tree canopy cover (Ervin and Holly 2011b). A study using 3 soil types found in Mississippi (Mississippi Alluvial Plain, Blackland Prairie and Pontotoc Ridge) found that the soil types were significantly different when it came to cogongrass growth rate and distribution of biomass (Holly and Ervin 2007). The nutrient-rich Mississippi Alluvial Plain soil was more productive in all areas of plant growth measured, indicating that soils with higher nutrient contents are potentially more susceptible to invasion and establishment (Holly and Ervin 2007). A more comprehensive study using soil from 53 Mississippi counties covering a wide variety of soil characteristics showed that cogongrass was able to successfully establish in many different soil types (Bryson et al. 2010).

Using ArcGIS 10.4 soil type and land cover was overlayed with the location of the cogongrass patches. Soil type data with a 10-meter resolution were obtained from the United

There are 10 different cover types in Lee Memorial Forest, with cogongrass found in 7 of them (Figure 1.4). Over half (58%) of the cogongrass locations are found in the evergreen forest, and another 26% are found in shrub/scrub habitat (Table 1.1). A chi-square goodness of fit analysis of land cover confirmed that cogongrass is not evenly distributed across cover types, so there could be a cover type association ($X^2=91.09$, $p<0.001$). Though, this might be due to differences in use of areas in the forest, since the hardwood area is not actively managed.

![Figure 1.4. Land cover type and location of cogongrass patches on Lee Memorial Forest.](image-url)
Table 1.1. Cogongrass Patches on Lee Memorial Forest and associated cover type.

<table>
<thead>
<tr>
<th>Number of Cogongrass Patches</th>
<th>Vegetation Cover Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>Evergreen Forest</td>
</tr>
<tr>
<td>1</td>
<td>Deciduous Forest</td>
</tr>
<tr>
<td>3</td>
<td>Developed/Open Space</td>
</tr>
<tr>
<td>2</td>
<td>Woody Wetlands</td>
</tr>
<tr>
<td>2</td>
<td>Hay/Pasture</td>
</tr>
<tr>
<td>1</td>
<td>Herbaceous</td>
</tr>
<tr>
<td>15</td>
<td>Shrub/Scrub</td>
</tr>
</tbody>
</table>

There are two soil types found at Lee Memorial Forest (Figure 1.5). Ouachita-Jena-Bibb soil has 40% of the cogongrass patches. This is an acidic soil characterized by a loamy surface layer and subsoil, with a sandy substratum and is found on flood plains (Trahan et al 1997). Tangi-Savannah-Ruston soil, containing the other 60% of cogongrass patches is also acidic, but better drained and generally on higher slopes (Trahan et al 1997). A goodness of fit chi-square analysis indicates cogongrass is not evenly distributed between the two soil types ($X^2=48.57$, $p<0.001$). This could be due to flooding in the woody wetlands, which covered 35% of the area of Lee Memorial Forest. They are found predominantly on Ouachita-Jena-Bibb soil (94%). The soil type and the cover type combination may work together to explain the distribution of cogongrass in Lee Memorial Forest.
Figure 1.5. Soil type and location of cogongrass patches on Lee Memorial Forest.

SPATIAL DISTRIBUTION

I performed a hotspot analysis using a spatial weights matrix calculated using Delaunay triangulation to visualize infestation intensity (Getis and Ord 1992, De Smith et al. 2018). To examine the spatial distribution of cogongrass, data points were examined using Ripley’s K to find any evidence of clustering. The spatial weights matrix was not used in the Ripley’s K analysis. The 57 patches of cogongrass on Lee Memorial Forest could be divided into five clusters. The areas to the North are a hotspot, where the cluster of cogongrass patches were all
near each other. There is an aggregation toward the South as well, where there is a coldspot
cluster, indicating that the patches are farther away from each other, but still have a clustered
distribution (Figure 1.6). The average number of neighbors used for the Delaunay triangulation
was 5.54 indicating that each patch was connected to an average of 9.73% of the total patches for
the cluster analysis. The Ripley’s K function showed slight but significant clustering when the
nearest neighbor was within 640 meters, but beyond that the distribution is not statistically
different from random. (Figure 1.7). This indicates that either wind is not dispersing seeds far, or
multiple rhizomes are transported to a localized area.
Figure 1.6. Distribution of cogongrass patches on Lee Memorial Forest. A higher GiZScore indicates other patches in close proximity. Higher values on the heat map indicate clustering of patches.
Figure 1.7. Results of Ripley's K function of the cogongrass distribution in Lee Memorial Forest. The vertical axis \( L(d) \) is the difference between the pattern observed and a randomly distributed pattern and the horizontal axis is the distance from one cogongrass plant to the next. The dashed lines represent a 95% confidence interval. The red line represents the results and the blue line represents the idealized situation. Clustering occurs when patches are located near each other, but when the distance to the nearest patch extends beyond 640 meters there is a random pattern.

CONCLUSION

Cogongrass at Lee Memorial Forest reflects previous findings from other areas. The cover types of evergreen forest and shrub/scrub appear to be a more important factor in where new patches are discovered than soil type. This could be a reflection of searching intensity, since there are no management actions in the woody wetlands, though they are a large portion of the landcover. Management may be a potential source of spread, but new patches are discovered within a few years of any activity. Monitoring of areas with management actions should be more thorough when it is within that timeframe. There also appears to be some periodicity in the
discovery of new patches, with peaks approximately every 5 years (Figure 1.1). It is likely there is some germination by seed occurring because there are patches that are in units that have not had any active management. Research activities also occur in Lee Memorial Forest, which also have the potential to transport rhizomes and should also be monitored. Patches appear to be clustered, especially in the north, indicating that areas within 640 meters of a known patch should be more intensely monitored because of increased risk of invasion.

REFERENCES


CHAPTER 2. BIOGEOGRAPHIC EFFECTS OF SOIL MICROBES ON GROWTH OF COGONGRASS

INTRODUCTION

Invasive species disrupt ecosystem processes, reduce biodiversity and land quality (Vitousek and Walker 1989, Vitousek et al. 1996, Levine et al. 2003, Brewer 2008, Davies 2011) and cost billions of dollars in direct and indirect impacts on natural and agricultural systems (Daneshgar and Jose 2009, Pimentel et al. 2011, Divate et al. 2017). Thus, there is an urgent need to understand the mechanisms by which plants invade new ecosystems. Research on mechanisms of invasion has largely focused on the traits of plants (van Kleunen et al. 2010), but successful plant invasions may also be the result of changes in trophic interactions (Catford et al. 2009). For example, the loss of predators, consumers, or pathogens as species move from their native range to introduced ranges may result in unchecked population growth (Keane and Crawley, 2002, Agrawal et al. 2005, Heger and Jeschke 2018). This release from natural enemies (Keane and Crawley 2002) has mostly been studied in the context of plant-herbivore interactions, however, there are a myriad of macro-and micro-invertebrates, bacteria, and fungi that have negative effects on plants, thereby acting as enemies (Mitchell and Power 2003).

Soil microbes can have powerful impacts, both positive and negative, on individual plant growth and fitness (van der Heijden et al. 2008). Microbes increase production of aboveground biomass, number of leaves and their chlorophyll content (Lau and Lennon 2011) and alter the timing of flowering (Wagner et al. 2014). Additionally, they increase tolerance to abiotic conditions (Rodriguez et al. 2008), control gene expression (Yang et al. 2009) and increase invasiveness (Aschehoug et al. 2012, Aschehoug et al. 2014). The clonal plant Stachys sylvatica produced more stolons and fewer flowers when exposed to the soil mycorrhizal community from a hedgerow as compared to one from the forest interior (de la Peña 2011). There can also be
differences in microbial community effects depending on plant genotype. Western genotypes of *Boechera stricta* are more sensitive to microbial inoculum than an eastern genotype (Wagner et al. 2014). Relationships between nitrogen-fixing bacteria and mycorrhizal fungi and plants provide increased access to nutrients (Delavaux et al. 2017). Microbes can also provide protection by consuming pathogens in the rhizosphere (van der Heijden et al. 2008, de Deyn 2017). Microbial pathogens and consumers reduce plant growth or cause death (Reinhart and Callaway 2006). Soil microbial communities also contribute to the community composition of plants by influencing interspecific interactions between plants (Klironomos 2002, Bever et al. 2010). A study of two dune grasses found that nematodes preferentially reduce root mass of one of the species, allowing the other to have a competitive advantage in the intake of nutrients (van der Putten and Peters 1997). An invasive aster was found to cultivate a generalist fungus, which negatively impacted growth of two native species, with limited effect on itself (Mangla and Callaway 2008). Klironomos (2002) found that plants that accumulated more pathogens were less abundant in an old field.

Plants, by way of chemical signals and carbon allocation, can also affect the composition and relative abundance of microbes in the rhizosphere (Garbeva et al. 2004, Berg and Smalla 2009). Kourtev et al. (2002) found the structure and function of soil communities differed depending on plant species and whether or not they were invasive. The invasive grass *Bromus inermis* increased the abundance of rare species of soil bacteria, which in turn appeared to increase the abundance of *B. inermis*, suggesting that some relationships between plants and soil microbes are highly mutualistic (Piper et al. 2015). However, the effect of plants on soil microbial communities are not always consistent within a species. A study of four genotypes of *Populus augustifolia* showed that 70% of the variation in microbial community composition was
due to genotype (Schweizer et al. 2008). Microbial communities in the rhizosphere of three lineages of clonal *Phragmites australis* populations were more similar within lineages, even when growing in sympatry (Bowen et al. 2017). These results suggest that the effects of plants on soil microbial communities are complex and context specific.

The positive and negative soil feedbacks generated by individual plant species then can influence plant competition (Bever 2003) which can result in cascading effects across plant and soil communities in space and time (Bever 2003, Belnap et al. 2005, van der Heijden et al. 2008, Bauer et al. 2017). Consequently, the complex relationships between plant and microbial communities are likely to be disrupted when plant species or genotypes are introduced to new ranges. For example, two invasive lineages of *Phragmites australis* reduced biomass production of the native *Spartina alterniflora* by 7% when grown concurrently in *Phragmites* cultivated live soil, compared to sterile soil. Whereas, a native lineage of *P. australis* increased biomass production by 6% (Allen et al. 2018).

A crucial step toward understanding plant-microbe interactions is assessing the way in which soil microbial communities change over time in response to plants, and how those changes affect plant performance (Bever 2003, Garbeva et al. 2004, Inderjit and van der Putten 2010, Maron et al. 2014, Perkins et al. 2015, Bauer et al. 2017). To this end, plant-soil feedback experiments are commonly used to test the effects of whole soil microbial communities on plant performance (Klironomos 2002, Beckstead and Parker 2003, Kulmatiski et al. 2008, te Beest et al. 2009, Bever et al. 2010, Brinkman et al. 2010). Soil microbial communities may shift toward higher abundances of pathogenic microbes over time resulting in a negative feedback, or plants may cultivate specific beneficial microbes via chemical signaling or carbon sharing resulting in a

Cogongrass (*Imperata cylindrica* (L.) Beauv.) is an invasive species found throughout the Southeastern US (Dozier et al. 1998, Lucardi et al. 2014, Burrell et al. 2015). To better understand if release from pathogenic microbes plays a role in its success, I explored the effects of soil microbial communities on cogongrass growth. I conducted two parallel plant-soil feedback experiments utilizing live and sterilized soils from both the native range and the invaded range of cogongrass. The use of a biogeographic experimental design allows us to assess whether changes in trophic level interactions contribute to cogongrass invasion success. I focused on the following questions: 1) Does release from soil microbes in the native range increase performance upon arrival in a novel environment? 2) Does cogongrass alter the soil microbial community to affect plant performance and do they differ between native and non-native ranges? 3) Do separate populations of cogongrass in the invaded range have similar performance reactions to the microbial community?

MATERIALS AND METHODS

**Focal Species**

Cogongrass is a clonal C4 perennial grass species native to Southeast Asia that has invaded across the Southeast United States (Dozier et al. 1998, Burrell et al. 2015). There are three known clonal lineages in the United States (Burrell et al. 2015). Cogongrass has strong negative effects worldwide including accelerated decomposition rates (Holly et al. 2009), faster nitrogen uptake than pine seedlings (Daneshgar and Jose 2009), decreasing species richness (Brewer 2008) and increasing landscape flammability (Lippincott 2000, Estrada and Flory 2015) and as an agricultural weed (Chikoye et al. 2005). It grows in a wide variety of soil types, from
heavy clay to sandy soils and in a wide range of nutrient conditions (Jose et al. 2002, Bryson et al. 2010).

In September 2017, rhizomes of cogongrass were collected from two unmanaged populations in Washington Parish, Louisiana, USA: Bogue Chitto State Park (BC) (30.779, -90.143) and Lee Memorial Forest (LMF) (30.874, -89.991). A permit was obtained from the Louisiana Office of State Parks and permission granted from the LMF land manager. Both sites were mixed pine-hardwood forests. Rhizomes were rinsed in tap water to remove adhering soil particles, then placed into a 5% bleach solution for 5 minutes for surface sterilization (Maron et al. 2014). Each rhizome was cut into segments containing at least 0.5 grams of plant material and 5 nodes. Mass, number of nodes and location of collection were recorded for each segment prior to planting.

Soils

Soils from the native range of cogongrass were collected from two sites in Okinawa, Japan (26.363, 127.827) in mid-June and early July 2017. Japanese soils were chosen because the earliest introduction of cogongrass to the United States was in packing material of a shipment of satsuma plants from Japan in 1912 (Tabor 1952). All soils were air-dried and had large root material removed prior to shipping. Soils were imported under USDA permit P330-16-00306 and arrived within 10 days of shipping. Invaded-range soils were collected from two different locations in Lee Memorial Forest (30.876, -89.99 and 30.873, -89.996), Louisiana, USA on June 13, 2017. All soils were collected from areas known to be free of cogongrass to mimic unoccupied areas in both native and invaded ranges.

In the laboratory, all soils were processed to remove remaining plant matter and reduce the size of aggregated soil clumps. The two soil sources from each geographic region were
thoroughly homogenized prior to use in experiments. Soil samples were sent to the Soil Testing and Plant Analysis Lab at Louisiana State University (www.lsuagcenter.com/portals/our_offices/departments/spess/servicelabs/soil_testing_lab) for analysis of mineral and organic content. Results of those tests are reported in Table 2.1.

Table 2.1. Comparison of initial soil composition before mixing with pasteurized sand.

<table>
<thead>
<tr>
<th></th>
<th>Louisiana Soil</th>
<th></th>
<th>Japanese Soil</th>
<th></th>
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</thead>
<tbody>
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<td></td>
<td>Live</td>
<td>Sterile</td>
<td>Live</td>
<td>Sterile</td>
</tr>
<tr>
<td>% Organic Matter</td>
<td>2.57</td>
<td>2.24</td>
<td>6.13</td>
<td>5.49</td>
</tr>
<tr>
<td>Calcium, ppm</td>
<td>316.42</td>
<td>198.77</td>
<td>3009.12</td>
<td>3265.45</td>
</tr>
<tr>
<td>Copper, ppm</td>
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<td>0.18</td>
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<td>1.22</td>
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<td>Magnesium, ppm</td>
<td>47.09</td>
<td>37.47</td>
<td>313.67</td>
<td>227.31</td>
</tr>
<tr>
<td>pH (1:1 Water)</td>
<td>5.92</td>
<td>5.16</td>
<td>7.48</td>
<td>6.67</td>
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<td>Potassium, ppm</td>
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<td>16.88</td>
<td>27.05</td>
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<td>Zinc, ppm</td>
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<td>0.63</td>
<td>7.31</td>
<td>8.18</td>
</tr>
<tr>
<td>Carbon %</td>
<td>1.56</td>
<td>1.46</td>
<td>3.96</td>
<td>2.99</td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>0.07</td>
<td>0.07</td>
<td>0.34</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Effects of naïve soil microbial communities on cogongrass**

In the first phase of the plant-soil feedback experiment, I grew cogongrass in either live soils or sterilized soils to assess the effects of naïve soil microbial communities on the establishment and growth of cogongrass. Naïve soil microbial communities are defined as soil communities that have not previously had cogongrass growing in them. In July 2018, all soils were mixed with pasteurized sand (heated to 180°C) 1:1 by volume. Then, 1500 mL of the soil-sand mixture was placed in a 1.8 L round nursery pot. Fifty-four pots were filled with Japanese soil and 54 pots were filled with Louisiana soil. To establish a treatment consisting of microbes
present or absent, half of the pots of each soil type were sterilized via autoclaving. The efficacy of sterilization was confirmed using sterilization indicator strips placed in the middle of the pot. All pots, sterilized or not, were covered with aluminum foil to prevent contamination prior to planting.

On September 16, 2017, rhizomes were randomly selected and planted into the prepared pots. Thirteen pots were prepared for each soil source-rhizome source-sterilization treatment combination for a total of 104 pots. Emergence, maximum height (measured from the soil to the tip of the longest green leaf in cm) and number of leaves in each pot, were monitored weekly. Any pots that did not have emergent vegetation were replanted on September 30, 2018 with previously collected but unused rhizomes from the same location as those that did not emerge. After 14 weeks, all plants were harvested, separating aboveground and belowground parts. All biomass was dried at 70° C for 48 hours and weighed (Figure 2.1).

Figure 2.1. Diagram of test of naïve soil microbial communities. Japanese and Louisiana soil were kept separate but went through the same process of sterilization and planting.
Effects of cultivated soil microbe communities on cogongrass

To test the effects of cogongrass soil microbe community cultivation on future growth, on January 12, 2018 new rhizomes were planted into either “experienced soils” or sterilized soils. Experienced soils were unsterilized, live soils from the previous experiment in which cogongrass grew for 14 weeks. Live soils from the first experiment were mixed with pasteurized sand (heated to 180º C) 1:1 by volume. To test for differences associated with rhizome origin, soils were constrained to re-use within previous rhizome planting type. Soils with the same soil origin/rhizome combination were homogenized, mixed with pasteurized sand as described previously, and 1500 mL were placed into 1.8 L round pots. Twelve pots of each soil/rhizome combination were sterilized via autoclaving and twelve pots had the soil community left intact.

Due to the short natural photoperiod during the time of the experiment, supplemental lighting was used to extend the photoperiod to 12 hours. At weekly intervals, emergence, maximum height and number of stems in each pot were measured. After 14 weeks, all plants were harvested, and aboveground and belowground biomass was separated. All biomass was dried at 70º C for 48 hours and weighed (Figure 2.2).

Figure 2.2. Diagram of test of cultivated soil microbial communities. Soils were kept separate from each other and by previous rhizome source exposure and by soil collection location. All went through the same process of sterilization and planting.
Statistical Analysis

Japanese and Louisiana soil varied greatly from each other in mineral content (Table 2.1). Japanese soil had a close to neutral pH, where Louisiana soil is acidic. Japanese soil also had over twice as much % carbon, four times as much % nitrogen and micronutrients ranged from 1.9 to 8.5 times higher than in Louisiana soil. Due to these soil composition differences and to more clearly assess the impact of both microbes and rhizome source, the data were analyzed separately for Japanese and Louisiana soil. In addition, response variables were checked for correlation via linear regression using JMP Pro 14.

A two-way ANCOVA was performed using SAS 9.4 with rhizome source and microbial presence/absence as fixed factors for each of the response variables. In addition, initial rhizome weight was included in the model as a covariate, since there were a range of values (0.44 g – 1.86 g in the initial phase and 0.75g – 1.14 g in the feedback phase). Post-hoc Tukey tests were run to determine pairwise significance (P<0.05) of the least-square means for each combination of microbe/rhizome effect. Response variables included aboveground biomass, height at 12 weeks, and number of leaves at 12 weeks. Before analysis, all response variables were natural log transformed due to non-normal distribution and heteroscedasticity.

The influence of microbial presence and rhizome source on emergence of the rhizomes was analyzed using logistic regression in SAS 9.4. Slope parameters were analyzed by maximum likelihood to determine if they were significantly different from zero, then plots of predicted probability were generated for each predictive variable.

To investigate microbial influence and compare results from initial and feedback phases in both locations, a relative interaction (RI) index (Armas et al. 2004) was calculated as
RI Index = \frac{B_w - B_o}{B_w + B_o} \quad \text{with variance} = n \frac{C^2_w + C^2_o}{m} \left(1 + \frac{(B_w - B_o)^2}{(B_w + B_o)^2} - 2 \cdot \rho \cdot \frac{(B_w - B_o)}{B_w + B_o} \right)

where \ \rho = \frac{\sigma^2_w - \sigma^2_o}{\left(\frac{n}{\sigma^2_w} + \frac{m}{\sigma^2_o}\right)} \quad \text{and} \quad B_w \text{ is the mean measured effect in live soil, } B_o \text{ is the mean measured effect in sterile soil, } \sigma^2_w \text{ is the variance in the live soil, } \sigma^2_o \text{ is the variance in the sterile soil, } n \text{ is the sample size of live soil and } m \text{ is the sample size of sterile soil. Responses range from } -1 \text{ to } 1, \text{ with negative values indicating the measured effect was greater in sterile soil and positive values indicating the measured effect was greater in live soil. The index is symmetric around zero, which means the RI index can be used to compare the response of cogongrass to soil microbes across soil and treatment types. Data used to calculate RI index was not transformed and values were compared using two-sample t-tests assuming unequal variances to test for differences both within and between treatments.}

RESULTS

Correlation of response variables

Aboveground biomass was not correlated with height of the plants in either Japanese (F_{1,36}=1.87, p=0.18, r^2=0.05) or Louisiana soil (F_{1,43}=2.28, p=0.14, r^2=0.05). There was a correlation between aboveground biomass and number of leaves in both Japanese (F_{1,36}=14.70, p<0.001, r^2=0.29, Y=-1.41+0.35X) and Louisiana soil (F_{1,43}=40.29, p<0.001, r^2=0.48, Y=-2.67+0.64X). Number of leaves and height were chosen as response variables because they appeared to represent differences between the two populations. Height and leaf number were correlated in Japanese soil when all samples were combined (F_{1,36}=28.12, p<0.001, r^2=0.44, Y=6.51-1.05X) but when rhizome sources were considered separately, no correlation was evident (LMF:..
$F_{1,16}=2.88, p=0.11, r^2=0.15$, BC: $F_{1,18}=0.002, p=0.97, r^2<0.001$). Similarly, in Louisiana soil there was a correlation when all samples were included ($F_{1,43}=9.90, p=0.003, r^2=0.19, Y=4.79-0.72X$), but the LMF rhizome had a positive correlation ($F_{1,19}=8.17, p=0.01, r^2=0.30, Y=-0.42+0.82X$) and the BC rhizome had a negative relationship ($F_{1,22}=5.45, p=0.029, r^2=0.16, Y=5.33-0.84X$). Because of these differences, all response variables were included in subsequent analysis.

**Effects of naïve soil microbial communities on cogongrass**

There were no significant effects of microbe presence/absence on cogongrass emergence, regardless of rhizome or soil source (Tables 2.2 & 2.3). In sterile Japanese soil, 46% of the Lee Memorial Forest (LMF) rhizomes and 62% of the Bogue Chitto State Park (BC) rhizomes emerged, while in sterile Louisiana soil both rhizomes had 85% emergence. Emergence in Japanese live soil was 86% for both rhizomes. In Louisiana soil it was 71% for the LMF rhizome and 93% for the BC rhizome.

In Japanese soil, rhizome source, presence/absence of microbes or their interaction had no significant effect on plant aboveground biomass (Table 2.4). However, the mean height of the LMF plants was 106% greater than the mean height of the BC plants (Table 2.5). Rhizome source also affected the number of leaves with individual BC plants producing 325% more leaves than LMF plants (Tables 2.4 & 2.5). There was also an interaction between rhizome and the presence/absence of microbes. In sterile soil, LMF plants produced 104% more leaves, but microbes had no influence on the number of leaves produced by BC plants (Table 2.5).
Table 2.2. Maximum likelihood estimates of emergence with multiple predictor variables from logistic regression model for cogongrass exposure to naïve soil, N= 54 for each soil source. The dependent variable was categorized as 0=no emergence and 1=emergence. Emergence was any observable aboveground growth even if the plant later died.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Factor</th>
<th>Estimate</th>
<th>SE</th>
<th>Wald</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Microbe Presence</td>
<td>1.36</td>
<td>1.23</td>
<td>1.23</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td>Rhizome Source</td>
<td>-0.393</td>
<td>0.891</td>
<td>0.195</td>
<td>0.659</td>
</tr>
<tr>
<td></td>
<td>Microbe*Rhizome</td>
<td>-0.380</td>
<td>1.567</td>
<td>0.059</td>
<td>0.808</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Microbe Presence</td>
<td>0.080</td>
<td>1.470</td>
<td>0.003</td>
<td>0.957</td>
</tr>
<tr>
<td></td>
<td>Rhizome Source</td>
<td>-0.780</td>
<td>1.294</td>
<td>0.364</td>
<td>0.547</td>
</tr>
<tr>
<td></td>
<td>Microbe*Rhizome</td>
<td>-0.869</td>
<td>1.761</td>
<td>0.243</td>
<td>0.622</td>
</tr>
</tbody>
</table>

Table 2.3. Influence of factors on the probability of rhizome survival from the logistic regression model. Results for naïve soil exposure and after cogongrass cultivation of microbial communities are included.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Factor</th>
<th>Probability Range Naïve Soil</th>
<th>Probability Range After Microbe Cultivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>LMF Rhizome</td>
<td>0.70 – 0.86</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>BC Rhizome</td>
<td>0.77 – 0.93</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Microbes Present</td>
<td>0.85 – 0.93</td>
<td>0.63 – 0.96</td>
</tr>
<tr>
<td></td>
<td>Microbes Absent</td>
<td>0.69 – 0.77</td>
<td>0.63 – 0.96</td>
</tr>
<tr>
<td>Louisiana</td>
<td>LMF Rhizome</td>
<td>0.72 – 0.85</td>
<td>0.34 – 0.79</td>
</tr>
<tr>
<td></td>
<td>BC Rhizome</td>
<td>0.93</td>
<td>0.33 – 0.79</td>
</tr>
<tr>
<td></td>
<td>Microbes Present</td>
<td>0.72 – 0.93</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Microbes Absent</td>
<td>0.85 – 0.93</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Table 2.4. Results of the ANCOVAs for cogongrass growth in naïve soil. Soil sources and response variables were analyzed separately.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Response Variable</th>
<th>Factor</th>
<th>DF</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Aboveground Biomass</td>
<td>Microbe Presence</td>
<td>1, 33</td>
<td>2.93</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 33</td>
<td>0.48</td>
<td>0.496</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 33</td>
<td>1.81</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td>Height at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 33</td>
<td>0.65</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 33</td>
<td>59.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 33</td>
<td>0.77</td>
<td>0.385</td>
</tr>
<tr>
<td></td>
<td>Leaves at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 33</td>
<td>3.77</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 33</td>
<td>19.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 33</td>
<td>8.08</td>
<td>0.008</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Aboveground Biomass</td>
<td>Microbe Presence</td>
<td>1, 40</td>
<td>8.18</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 40</td>
<td>7.05</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 40</td>
<td>1.65</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>Height at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 40</td>
<td>0.00</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 40</td>
<td>32.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 40</td>
<td>6.15</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Leaves at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 40</td>
<td>17.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 40</td>
<td>41.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 40</td>
<td>0.02</td>
<td>0.8849</td>
</tr>
</tbody>
</table>

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Table 2.5. Means and standard errors of response variables for cogongrass exposure to naïve soils. Means subdivided by rhizome and soil treatment are provided only if it was a statistically significant effect.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Response</th>
<th>Treatment</th>
<th>Mean</th>
<th>St. Error</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Aboveground Biomass (g)</td>
<td>Live Soil</td>
<td>0.65</td>
<td>0.04</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>0.92</td>
<td>0.11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>0.71</td>
<td>0.07</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>0.78</td>
<td>0.07</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Live Soil</td>
<td>32.24</td>
<td>2.83</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>32.29</td>
<td>3.64</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>44.27</td>
<td>1.96</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>21.46</td>
<td>1.38</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Leaf Number</td>
<td>Live Soil</td>
<td>22.33</td>
<td>3.26</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>26.64</td>
<td>3.54</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>7.96</td>
<td>1.88</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>33.85</td>
<td>2.84</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Live, LMF</td>
<td>9.58</td>
<td>1.26</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile, LMF</td>
<td>19.50</td>
<td>3.97</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Live, BC</td>
<td>35.08</td>
<td>3.64</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile, BC</td>
<td>32.00</td>
<td>4.77</td>
<td>8</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Aboveground Biomass (g)</td>
<td>Live Soil</td>
<td>0.48</td>
<td>0.03</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>0.35</td>
<td>0.04</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>0.34</td>
<td>0.04</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>0.48</td>
<td>0.03</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Live Soil</td>
<td>21.99</td>
<td>1.86</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>22.41</td>
<td>1.99</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>28.05</td>
<td>1.99</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>17.08</td>
<td>1.01</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Live, LMF</td>
<td>30.72</td>
<td>1.82</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Live, BC</td>
<td>15.28</td>
<td>0.79</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile, LMF</td>
<td>25.63</td>
<td>3.35</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile, BC</td>
<td>19.20</td>
<td>1.84</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Leaf Number</td>
<td>Live Soil</td>
<td>19.74</td>
<td>1.87</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>12.14</td>
<td>1.67</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>9.48</td>
<td>0.90</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>21.75</td>
<td>1.74</td>
<td>24</td>
</tr>
</tbody>
</table>
In Louisiana soils, live soil resulted in a 37% increase in mean aboveground biomass compared to sterilized soil (Table 2.5). The BC plants had a 41% increase in mean aboveground biomass compared to the LMF plants (Table 2.5). Rhizome source and the interaction between rhizome and microbe presence affected height (Table 2.4). The mean height of LMF plants was 64% taller than the mean height of BC plants (Table 2.5). This was more pronounced in live soil, where the mean height of the LMF plants was 101% taller than the mean height of BC plants (Table 2.5). In sterile soil, LMF plants were 33% taller than BC plants. Live soil resulted in a 63% increase in the number of leaves produced overall and the BC plants produced 129% more leaves than the LMF plants (Table 2.5).

Microbes influenced cogongrass aboveground growth and leaf production in opposite ways depending on the source of the soil. The RI index for aboveground biomass and number of leaves were negative in Japanese soil, indicating a negative effect of microbes on these two traits. In contrast, there was a positive effect of Louisiana soil on the same traits (biomass: $t_{78.1}=-25.29$, $p<0.001$, leaves: $t_{70.7}=-16.52$, $p<0.001$) (Figures 2.3 & 2.5). Microbial presence/absence did not appear to influence height (Figure 2.4), until separated by rhizome source. Microbes positively influenced height for LMF plants in both Japanese and Louisiana soil, but negatively influenced BC plant heights in both soil types (Japan: $t_{36}=-5.64$, $p<0.001$, Louisiana: $t_{37}=-10.73$, $p<0.001$) (Figure 2.6). Microbes negatively influenced the number of leaves for LMF plants in Japanese soil, but positively influenced it in Louisiana soil. In Louisiana soil, the rhizome source did not differ in leaf production ($t_{41.7}=-0.95$, $p=0.35$) (Figure 2.7). Microbes affected both rhizomes similarly in aboveground biomass production, but LMF plants were more strongly affected (Japan: $t_{35.1}=3.67$, $p<0.001$, Louisiana: $t_{32.7}=-4.12$, $p<0.001$) (Figure 2.8).
Figure 2.3. Means (+/- standard error) of RI Index for aboveground biomass in soil containing microbes compared to sterile soil. An asterisk indicates there was a significant difference (p<0.05) between the RI Indices of naïve and cogongrass cultivated microbes.
Figure 2.4. Means (+/- standard error) of RI Index for height at 12 weeks in soil containing microbes compared to sterile soil. An asterisk indicates there was a significant difference (p<0.05) between the RI Indices of naïve and cogongrass cultivated microbes. Japan and Louisiana values for naïve soil microbes were not significantly different from zero.
Figure 2.5. Means (+/- standard error) of RI Index for number of leaves at 12 weeks in soil containing microbes compared to sterile soil for rhizomes. An asterisk indicates there was a significant difference (p<0.05) between the RI Indices of naïve and cogongrass cultivated microbes. An asterisk indicates there was a significant difference (p<0.05) between the phases.
Figure 2.6. Means (+/- standard error) of RI Index for height at 12 weeks in soil containing microbes compared to sterile soil divided by rhizomes from Lee Memorial Forest (LMF) and Bogue Chitto State Park (BC). An asterisk indicates there was a significant difference (p<0.05) between the RI Indices of plants grown from the two rhizome sources. The index value for BC rhizomes grown in Louisiana soil with microbes cultivated by cogongrass is not statistically different from zero.
Figure 2.7. Means (± standard error) of RI Index for number of leaves at 12 weeks in soil containing microbes compared to sterile soil for rhizomes divided by rhizomes from Lee Memorial Forest (LMF) and Bogue Chitto State Park (BC). An asterisk indicates there was a significant difference (p<0.05) between the RI Indices of plants grown from the two rhizome sources. The index value for BC rhizomes grown in Louisiana soil with microbes cultivated by cogongrass is not statistically different from zero.
Effects of cultivated soil microbe communities on cogongrass

Rhizome source predicted emergence in Japanese soil that had previously been conditioned by growing cogongrass in those soils (Wald=5.64, P=0.018) with the BC rhizome having a predicted 63% chance of emergence and the LMF rhizome having a 96% chance of emergence. In Louisiana soil microbial presence (Wald=9.35, P=0.002) had a large influence on emergence with a predicted 78% probability of emergence in the presence of microbes and only a 33% chance in sterile soil (Tables 2.3 & 2.6).
Table 2.6. Maximum likelihood estimates of emergence with multiple predictor variables in feedback with cogongrass cultivated soil microbial communities, N= 48 for each soil source. The dependent variable was categorized as 0=no emergence and 1=emergence. Emergence was any observable aboveground growth even if the plant later died.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Effect</th>
<th>Estimate</th>
<th>SE</th>
<th>Wald</th>
<th>P-value</th>
<th>Estimate</th>
<th>SE</th>
<th>Wald</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Microbe Presence</td>
<td>1.099</td>
<td>0.882</td>
<td>1.552</td>
<td>0.213</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhizome Source</td>
<td>2.398</td>
<td>1.193</td>
<td>4.037</td>
<td>0.045</td>
<td>2.625</td>
<td>1.105</td>
<td>5.641</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Microbe*Rhizome</td>
<td>9.216</td>
<td>166.3</td>
<td>0.003</td>
<td>0.956</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louisiana</td>
<td>Microbe Presence</td>
<td>4.007</td>
<td>1.300</td>
<td>9.497</td>
<td>0.002</td>
<td>2.028</td>
<td>0.663</td>
<td>9.346</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Rhizome Source</td>
<td>1.609</td>
<td>0.966</td>
<td>2.775</td>
<td>0.096</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microbe*Rhizome</td>
<td>-3.314</td>
<td>1.549</td>
<td>4.578</td>
<td>0.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Japanese soil, rhizome source, presence/absence of microbes or their interaction had no statistically significant effect on plant aboveground biomass or 12-week leaf count. Rhizome source influenced height, with a 65% increase in mean height of LMF plants compared to BC plants (Tables 2.7 & 2.8).

In Louisiana soil, microbe presence affected aboveground biomass produced, with a 117% increase in mean aboveground biomass in sterile soil compared to live soil. Neither rhizome source nor presence/absence of microbes influenced the height or number of leaves (Tables 2.7 & 2.8).
Table 2.7. Means and standard deviations of response variables after exposure to a microbial community cultivated by cogongrass.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Response</th>
<th>Treatment</th>
<th>Mean</th>
<th>St. Error</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Aboveground Biomass (g)</td>
<td>Live Soil</td>
<td>1.34</td>
<td>0.10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>1.67</td>
<td>0.15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>1.65</td>
<td>0.13</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>1.20</td>
<td>0.06</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Live Soil</td>
<td>43.63</td>
<td>3.11</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>49.14</td>
<td>3.13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>54.09</td>
<td>2.12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>32.76</td>
<td>1.60</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Leaf Number</td>
<td>Live Soil</td>
<td>17.52</td>
<td>1.66</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>18.56</td>
<td>1.56</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>16.78</td>
<td>1.49</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>19.93</td>
<td>1.73</td>
<td>14</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Aboveground Biomass (g)</td>
<td>Live Soil</td>
<td>0.06</td>
<td>0.01</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>0.13</td>
<td>0.05</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>0.08</td>
<td>0.03</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>0.08</td>
<td>0.02</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Live Soil</td>
<td>8.16</td>
<td>1.24</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>13.30</td>
<td>2.79</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>12.65</td>
<td>1.86</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>6.01</td>
<td>0.93</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Leaf Number</td>
<td>Live Soil</td>
<td>3.31</td>
<td>0.64</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterile Soil</td>
<td>4.38</td>
<td>1.29</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMF Rhizome</td>
<td>3.31</td>
<td>0.84</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC Rhizome</td>
<td>4.38</td>
<td>0.92</td>
<td>8</td>
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</tbody>
</table>
Table 2.8. Results of the ANCOVAs of the Feedback Growth Phase. Soil Sources and Response variables were analyzed separately.

<table>
<thead>
<tr>
<th>Soil Source</th>
<th>Response Variable</th>
<th>Factor</th>
<th>DF</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Aboveground Biomass</td>
<td>Microbe Presence</td>
<td>1, 32</td>
<td>1.96</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 32</td>
<td>2.93</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 32</td>
<td>0.01</td>
<td>0.928</td>
</tr>
<tr>
<td></td>
<td>Height at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 32</td>
<td>2.12</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 32</td>
<td>42.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 32</td>
<td>0.86</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Leaves at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 32</td>
<td>0.13</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 32</td>
<td>1.71</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 32</td>
<td>2.18</td>
<td>0.150</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Aboveground Biomass</td>
<td>Microbe Presence</td>
<td>1, 22</td>
<td>9.29</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 22</td>
<td>2.65</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 22</td>
<td>0.21</td>
<td>0.653</td>
</tr>
<tr>
<td></td>
<td>Height at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 16</td>
<td>1.48</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 16</td>
<td>3.60</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 16</td>
<td>0.01</td>
<td>0.908</td>
</tr>
<tr>
<td></td>
<td>Leaves at 12 Weeks</td>
<td>Microbe Presence</td>
<td>1, 16</td>
<td>0.78</td>
<td>0.389</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizome Source</td>
<td>1, 16</td>
<td>1.14</td>
<td>0.303</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbe*Rhizome</td>
<td>1, 16</td>
<td>0.48</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Microbial impact was negative in all cases for aboveground biomass production (Figure 2.3), height (Figure 2.4) and for number of leaves (Figure 2.5). Although the effect was much greater in Louisiana for all plant traits (biomass: $t_{30.4}=8.90, p<0.001$, height: $t_{23.5}=6.48, p<0.001$, leaves: $t_{23}=2.8, p=0.01$). When divided by rhizome source, microbial presence negatively affected the height and aboveground biomass production in all soils and rhizomes, except height in Louisiana soil, which was not significantly different from zero (Figures 2.8 & 2.6). There was a stronger microbial effect on LMF rhizomes in Louisiana soil for aboveground biomass production ($t_{23}=2.63, p=0.015$) and a stronger effect in Japanese soil on the BC rhizome for height ($t_{26.3}=-4.03, p<0.001$). Microbes had a negative effect in both soils on leaf production for LMF rhizomes, and a neutral to positive effect for the BC rhizomes (Figure 2.7).

**Comparing Naïve Soil Effects to Cogongrass Cultivated Soil Microbial Effects**

Soil microbes had neutral or negative effects on cogongrass growth in Japanese soil for all response variables in the initial and feedback phases. After microbial communities were cultivated, the strength of the reaction weakened for aboveground biomass production and number of leaves (biomass: $t_{73}=-4.69, p<0.001$, leaves: $t_{55.2}=-3.12, p=0.003$). The response became less negative after conditioning to microbes for 14 weeks (Figures 2.3 & 2.4). Microbial cultivation had the opposite effect for height and moved in a negative direction ($t_{55.5}=4.19, p<0.001$) (Figure 2.4). In Louisiana soil after cultivation, soil microbes caused all response variables to move from neutral or positive values to negative (biomass: $t_{29.8}=16.84, p<0.001$, height: $t_{53.3}=8.16, p<0.001$, leaves: $t_{51.8}=9.51, p<0.001$) (Figures 2.3-2.5).

When separated by rhizome source, microbes had no real effect on the BC rhizome for any of the response variables, with the exception of aboveground biomass production in Louisiana soil, which went from positive to negative ($t_{13}=6.91, p<0.001$) (Figure 2.4). The LMF
rhizome was different in every response variable after microbe cultivation in all soil types. In Japanese soil the response moved in a positive direction, though still staying negative, for aboveground biomass production ($t_{33}=-4.9$, $p<0.001$) and height ($t_{28.4}=2.13$, $p=0.042$). While the movement was from positive to negative for number of leaves ($t_{32}=-6.79$, $p<0.001$) (Tables 2.4-2.6). In Louisiana all response variables moved from positive to negative after cogongrass cultivated soil microbes (biomass: $t_{18.1}=14.26$, $p<0.001$, height: $t_{16.3}=6.90$, $p<0.001$, leaves: $t_{15}=10.42$, $p<0.001$) (Figures 2.4-2.6).

**DISCUSSION**

There was a strong positive effect of naïve soil microbe communities on the growth of cogongrass in the invaded range. This pattern of facilitation during colonization may improve cogongrass establishment and drive patterns of invasiveness in new ranges (Inderjit and van der Putten 2010, Maron et al. 2014, Reinhart and Callaway 2006). This contrasts with the native range where naïve soil microbial communities had a negative effect on cogongrass growth. Soil microbial communities in the native range may act as a top-down control on cogongrass abundance and distribution (Klironomos et al. 2002, Bever 2003, Bever et al. 2010, Inderjit and van der Putten 2010) and by moving to new ranges, cogongrass may be released from natural enemies (Keane and Crawley 2002).

Although there are positive effects of naïve soil microbial communities on cogongrass growth, cultivated soil microbial communities had strong negative effects on cogongrass growth in both the invaded and native range. The reversal of soil microbial community effects in the introduced range is likely the result of the rapid accumulation and cultivation of soil pathogens. Negative soil feedbacks typically result in low growth and reduced population size within communities (Klironomos et al. 2002). However, field observations show persistent robust
populations of cogongrass that expand rapidly after colonization (Wilcut et al. 1988) This disconnect between negative soil feedback and rapid expansion and growth may be due to changes in plant behavior in response to soil microbial community changes over time. For example, invasive species with negative soil feedbacks may escape negative soil conditions through higher investment in root growth (Cortois et al. 2016). Additionally, invasive species exhibit more intensive root foraging behavior than non-invasive introduced species (Keser et al. 2014) and negative soil conditions may further promote root elongation in an attempt to escape soil pathogens.

Grasses, such as cogongrass, are more likely to have negative soil feedbacks due to the large amount of biomass devoted to underground growth (Kulmatiski et al. 2008, Cortois et al. 2016). For example, two invasive grasses *Ammophila arenaria* and *Bromus inermis* had lower biomass in field-collected, conspecific cultivated live soils when compared to sterilized soils (Beckstead and Parker 2003, Otfinowski et al. 2016). *Bromus inermis* experienced strong negative feedbacks in both native and invaded range soils, but the effect was much stronger in the invaded range. Despite strong negative feedbacks, *B. inermis* is an aggressive invaded or the northern prairies of North America (Otfinowski et al. 2016).

An estimated 70% of monocot families are rhizomatous (Pan and Clay 2002) and perennial, such as cogongrass, are likely to be successful invaders since invasive plants have high frequencies of clonal growth (Thompson et al. 1995, Lake and Leishman 2004, Lloret et al. 2005, Pyšek and Richardson 2008). Clonal plants are physiologically integrated so that risk is spread out among many ramets (Caraco and Kelly 1991, Fischer and van Kleunen 2001, Pan and Price 2002). In this way, the plant can minimize the impact of parasitic or pathogenic organisms with an increase in the number of ramets, resulting in a decreased chance of mortality for the
genet as a whole (Pan and Price 2002). Ramets are preferentially placed in areas with the best soil conditions (de Kroon and Hutchings 1995, Fischer and van Kleunen 2001) and can potentially grow away from negative conditions. For example, in a sedge species, Carex arenaria, the soil condition of the parent plant was a larger determinant of growth than the ramet soil condition (D’Hertefeldt and van der Putten 1998). The aster Lactuca sibirica was infected with a systemic rust fungus and ramets were uninfected if more than 45 cm away from parent plants, even when still physiologically connected (Wennstrom and Ericson 1992). Similarly, cogongrass could increase growth in underground structures in invaded areas to grow to uncolonized areas to take advantage of a naïve microbial community.

Cogongrass is thought to have been introduced to the United States from two locations, first accidentally from Japan and a second time from the Philippines for research purposes (Patterson et al. 1980, Estrada et al. 2017). The second time, samples were brought to Florida, where they thrived, Mississippi, where they were killed in a hard frost within a few years and Texas, where they survived less than a year (Hubbard et al. 1944). A few previous studies have tried to differentiate plants from two locations under the assumption that Mississippi accessions were descended from the Philippine plants. Plants from Mississippi were found to have a consistently shorter height and less biomass production than plants from Alabama near the Japanese introduction (Patterson et al. 1980). In this study there was no observable difference between plants at either location in the field, however in the greenhouse difference in growth form were evident and consistent. In the greenhouse, the LMF plants grew taller and the BC plants sent up many more stems and leaves. Later genetic studies showed that the clonal lineage with the widest geographical variation is most similar to Japanese accessions and is predominant in the northern Gulf Coast region (Burrell et al. 2015). Burrell et al. (2015) found that there was
equal or greater genetic variation within lineages than between lineages. There is enough variation that samples collected from the northern Gulf Coast can be divided into Mississippi and Alabama subtypes (Lucardi et al. 2014). There was no evidence of hybridization between clonal lineages (Burrell et al. 2015), so it is possible that the growth differences observed are due to phenotypic plasticity rather than genetic contribution from different lineages. While the overall trend of microbes on aboveground biomass was the same for both rhizome sources, the BC source population was less affected by the presence of microbes. Without genetic analysis it is not possible to tell if the different rhizome sources represent the same clonal lineage and the same introduction source or if the phenotypic differences are due to genetic differences.

The phenotypic expression of plants from the BC population mirrored populations described in Patterson et al. (1980), with the BC plants consistently short with a large number of leaves. The two populations responded differently to microbial assemblage modification, with the BC plants not altering aboveground biomass allocation between leaves and height. The LMF plants, however, exhibited large changes in plant traits between the microbe presence-absence treatments. While it had increased leaf production initially, after microbial cultivation there was a decrease in leaf production due to microbes. The same pattern held for height. LMF plants may be less suited to persist in shaded areas or may expand quickly to find less shaded areas. However, the BC plants could be more likely to persist in shaded areas because increased leaf production enables a plant to capture more light and produce more photosynthate (Chapin et al. 1987). The decreased height of the BC plants in response to microbes may be of little concern when growing in an area shaded by trees. The BC plants, therefore, may be well suited to invade shaded areas. Patterson (1980) and Holly and Ervin (2007) found cogongrass allocated more biomass to aboveground structures when shaded, and Estrada et al. (2017) found that Florida
populations grown in shade did not persist into a second year. The source populations for my study were in shaded areas and had been persisting for years (J. Nehlig personal communication). Further investigations could explore context dependence in invasive ability depending on habitat and population source.

Invasive capacities and strategies may vary by phenotype (te Beest et al. 2009, Andonian et al. 2012). *Centaurea solstitialis* seeds from Argentina, an invaded region where there is rapid spread, germinated later than other seeds, but had similar biomass at the end of the study (Andonian et al. 2012). Though not directly measured, this would indicate that these plants were growing much faster and had a higher relative growth rate than those from other regions, potentially contributing to the rapid spread. In Argentina, there was a greater production of shoots when grown in live soil compared to sterilized soil, differing from other regions tested, including California, which also has a rapidly spreading population (Andonian et al. 2012). These might represent phenotypically adaptive strategies facilitated by the microbial community.

The genetic component of both plant and microbial communities has the potential to reveal which plant populations are more likely to become invasive and what causes an introduced plant to become invasive. Increased sampling of plants from native areas combined with genetic sequencing could uncover variation in invasive potential. Genomic areas that differ between populations can be evaluated for interactions with soil microbes. While many studies have looked at soil microbial community responses to plant chemical compounds (Lorenzo et al. 2013, Zhu et al. 2017) and changes along invasion gradients (Sun et al. 2013, Piper et al. 2015, Collins et al. 2016), a literature search did not reveal any studies assessing before and after microbial assemblages in the same location. This could be done in both invaded and native ranges, to see if soil microbial changes are consistent.
The enemy release hypothesis is conceptually straightforward, but simply escaping old enemies does not mean a species will become invasive. It cannot be directly determined if release from soilborne enemies through rapid expansion or positive feedbacks is a determining factor in invasive ability. The effect of changes in the microbial community on native plants and the community needs to be explored as well (Keane and Crawley 2002, Aschehoug et al. 2014, Heger and Jeschke 2014, Bauer et al. 2017). Differences between populations could indicate underlying reasons for invasion success and could depend on biogeographic area, even within the same species. Part of this could be the way the phenotype/genotype, soil microbial community and nutrient content of the soil interact. Teasing apart these interactions has the potential to give more insight into differences in degree of invasiveness. In addition, it is possible there is not a single causal mechanism for invasion success (Catford et al. 2009). It is possible that after a certain time lag where plants with negative feedback rapidly expand their range, soil microbiota will no longer be naïve. It will be only marginally beneficial to grown into new areas and rapid expansion will cease. At that point, negative feedback will serve as a mechanism for coexistence as may be the case in the current home range.

REFERENCES


CHAPTER 3. COGONGRASS SOIL LEGACY EFFECTS ON TWO NATIVE PLANTS

INTRODUCTION

A major cause of anthropogenic global change is the accidental and purposeful introduction of species into new ranges (Crosby 1986, Kowarik and von der Lippe 2008). Invasive species have strong negative effects on ecosystem function (Tilman 1999, Levine et al. 2003), population persistence (Brewer 2008, Davies 2011), and can lead to the extinction of rare species (Baider and Florens 2011, Akasaka et al. 2017). For example, invasive species may have cascading effects across multiple trophic levels resulting in potentially irreversible changes to food web dynamics (Belnap et al. 2005, Perkins and Nowak 2012). These problems have led ecologists to seek ways to restore ecosystems via the removal of non-native species. However, success of these actions has been limited, potentially because of the legacy effects that species may exert on systems long after their removal (Maron and Jeffries 2001, Cuddington 2011, Elgersma et al. 2011, Corbin and D’Antonio 2012).

The term ‘legacy effects’ refers to the changes to abiotic or biotic system properties of soil that persist after a plant species has been removed from the soil (Corbin and D’Antonio 2012). Abiotic changes can include persistent chemical exudates from microbes or roots in the soil (Kuusipalo et al. 1995, Hierro and Callaway 2003, Hagan et al. 2013), degradation of leaf litter (Liao et al. 2008, Holly et al. 2009), alteration of nutrient loads and cycling (Liao et al. 2008, Daneshgar and Jose 2009) and changes to soil pH (Corbin and D’Antonio 2012). Biotic changes occur via alterations in microbial community assemblages, including fungal and bacterial species, nematodes, and soil microinvertebrate abundance and composition (Kourtev et al. 2002, Mangla and Callaway 2008).
These abiotic and biotic changes are not necessarily independent but may be the result of interactions among factors (Reinhart and Callaway 2006, Perkins and Nowak 2012, van der Putten et al. 2013,). As such, the resulting legacy effects can be highly variable, temporal and context dependent (Klironomos 2002, Stinson et al. 2006, Kardol et al. 2007, Meisner et al. 2014). Some recent examples include staged removal of invasive *Cytisus scoparius*, which showed an initial increase of nitrogen one month after removal, but a decrease of 70% within 10 months with no further decrease over time (Grove et al. 2015). Additionally, Douglas-fir planted in removal areas were 37% smaller 22-months post removal of *C. scoparius* and there was an increase in other exotics the longer the time since removal (Grove et al. 2015). Another study involving restoration of pine areas in Hungary showed no legacy of the pine, but when these areas had invasive *Asclepias syriaca* there were transient negative effects on grass, but positive effects on the species richness of other native species (Szitár et al. 2016). Following invasive *Rhododendron ponticum* removal, the total cover of grass and forbs did not change over the ensuing 20-years (Maclean et al. 2018b). There was a significant increase in bryophyte cover, returning to a near uninvaded level causing a different community composition than in uninvaded areas with no change in nutrients or pH, though microbial changes were not examined. A last example with invasive *Phalaris aquatica* showed that after 11 years of growth, a microbial legacy was created following removal of this weed. However, the legacy affected only 1 of 3 native plants tested (Pickett et al. 2019). Hence, legacy effects are likely to be species specific and studies should be targeted to understand specific problematic species effect on predominant native species when undertaking restoration in an area previously dominated by an invasive species. Nurturing survival and expansion of rare species in such areas may be more successful as well with an understanding of legacy effects.
Cogongrass (*Imperata cylindrica* (L.) Beauv.) is an invasive species in the southern United States that has strong negative effects on both natural and managed pinelands. Cogongrass reduces pine seedling survival (Daneshgar et al. 2008, Daneshgar and Jose 2009), increases the intensity of prescribed burns (Lippincott 2000), and is a low-quality forage for grazers (Hubbard et al. 1944, Dozier et al. 1998). It also has negative effects on native plant growth via chemically mediated interactions (Koger and Bryson 2004, Xuan et al. 2009, Hagan et al. 2013, Javaid et al. 2015) and can cause changes to soil microbial communities (see previous chapter). The varied mechanisms by which cogongrass affects communities of native plants may also lead to persistent legacy effects after management actions, but the only studies investigating these have used manufactured leachate, which may not accurately reflect conditions in the field (Maclean et al. 2018a). Here, I evaluate the legacy effects of cogongrass on native species establishment and growth in the Southeastern United States.

**METHODS**

Two native species commonly found in Louisiana pinelands were used to estimate the legacy effects of cogongrass. *Arnoglossum ovatum* (Walt) H.E. Robins, is an aster commonly found in the southeastern United States in a wide variety of habitats (Smith 1996, Anderson 1998). Little bluestem, *Schizachyrium scoparium* (Michx) Nash, is a dominant grass throughout North America (Gaines et al. 1954, Platt 1999, USDA 2019) and is often used in restoration of prairies and pinelands (Aschenbach 2010, Tober and Jensen 2013). Seed of both species were purchased from The Ecology Center at University of Louisiana-Lafayette (ecology.louisiana.edu) in February 2018.

To mimic management actions on cogongrass, in March 2018, I first established cogongrass “populations” in the greenhouse. Three rhizome segments weighing 1 gram each
were planted in ten 3L round pots containing Sta-Green Moisture Max potting mix plus fertilizer (Pursell Industries, Sylacauga, Alabama) in a greenhouse at Louisiana State University and watered as needed. To establish a source of control soil that did not have previous cogongrass growth, on June 2018, I filled ten additional 3L pots with potting soil and watered at the same frequency and kept in the same conditions as pots containing cogongrass.

In July 2018, cogongrass was harvested and all potting soil from pots containing cogongrass was combined. Cogongrass-exposed soil was mixed in a 1:5 ratio by volume with the same, but unused, potting soil. Though this may have diluted the effects, it was done to ensure a sufficient number of replicates for the experiment described below. After mixing, 1200 mL of the substrate was placed in a 1.5 mL pot. A total of 36 pots were prepared with this cogongrass-exposed soil. The same procedure was followed to make 36 pots of cogon-free soil using the control potting soil from the greenhouse. Pots were randomly placed in three incubators set for 14 hrs light (28°C)/10 hrs dark (25°C) and watered as needed.

Two flats containing the two native plant species were started, one with the unmixed cogongrass-exposed soil and the other with unmixed cogongrass-free soil. The potting soil used to start the seeds was not mixed with any other material. Seeds of both A. ovatum and S. scoparium were direct seeded onto the soil and placed in incubators at the same settings mentioned previously. In September 2018, one-month-old seedlings were transplanted into pots, 18 of each species were planted in cogongrass-exposed soil and 18 were planted in cogongrass-free soil for a total of 72 pots. After growing for 11 weeks they were harvested. Aboveground and belowground mass was separated and weighed after drying for 48 hours at 70°C.

To determine if there were any legacy effects in the soil after cogongrass growth total biomass was analyzed using the Friedman’s Test in SAS 9.4. This test was chosen because the
distribution of the data was not normal, even if natural log transformed, and blocking was required due to the use of three incubators. Each species was analyzed separately using untransformed values. Survival data were analyzed via a chi-square test. In addition, data were standardized using the relative interaction (RI) index (Armas et al. 2004).

\[
\text{RI Index} = \frac{B_w - B_o}{B_w + B_o}
\]

with variance:

\[
\text{variance} = \frac{\sigma_w^2 + \sigma_o^2}{n + m} \left(1 + \frac{(B_w - B_o)^2}{(B_w + B_o)^2} \cdot \frac{2 \cdot \rho(B_w - B_o)}{B_w + B_o}\right),
\]

where

\[
\rho = \frac{n \sigma_w^2 - m \sigma_o^2}{n \sigma_w^2 + m \sigma_o^2}
\]

and \(B_w\) is the mean measured effect in cogongrass-exposed soil, \(B_o\) is the mean measured effect in cogongrass-free soil, \(\sigma_w^2\) is the variance in the cogongrass-exposed soil, \(\sigma_o^2\) is the variance in the cogongrass-free soil, \(n\) is the sample size of cogongrass-exposed soil and \(m\) is the sample size of cogongrass-free soil. Responses range from -1 to 1, with negative values indicating the measured effect was greater in cogongrass-free soil and positive values indicating the measured effect was greater in cogongrass-exposed soil. The index is symmetric around zero.

RESULTS

Overall, both species were able to survive in both soil types. The survival rate was the same for both species with 94% surviving in the cogon-free soil and 83% surviving in the cogon-exposed soil. The soil legacy of cogongrass did not affect the ability of \(S.\ scoparium\) \((X^2=1.13, p=0.29)\) or \(A.\ ovatum\) \((X^2=0.07, p=0.29)\) to emerge and persist for the 3 month duration of the experiment.

The cogon-exposed soil negatively impacted total biomass production of \(S.\ scoparium\) \((Q=7.22, p=0.007)\). Total biomass was reduced by 65% for plants that grew in cogon-exposed
potting soil. Cogon-exposed soil elicited less of a response for *A. ovatum*. Biomass was reduced by 26% (Table 3.1), however the Friedman’s test showed that there was no significant differences (Q=0.68, p=0.41) when accounting for the incubator used.

Table 3.1. Results of 11 weeks of growth of two native plant species. Potting soil either had cogongrass growing in it for 3 months (cogon-exposed) or did not (cogon-free).

<table>
<thead>
<tr>
<th>Species</th>
<th>Response Variable</th>
<th>Soil Type</th>
<th>Mean (g)</th>
<th>Standard Error</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. scoparium</em></td>
<td>Total Biomass</td>
<td>Cogon-free</td>
<td>0.961</td>
<td>0.24</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cogon-exposed</td>
<td>0.336</td>
<td>0.10</td>
<td>15</td>
</tr>
<tr>
<td><em>A. ovatum</em></td>
<td>Total Biomass</td>
<td>Cogon-free</td>
<td>0.700</td>
<td>0.14</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cogon-exposed</td>
<td>0.520</td>
<td>0.09</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 3.1. Index value (+/- standard error) showing effect of cogongrass precultivating soil on total biomass production of two native species. Negative values indicate that the growth response variable was lower in cogon-exposed potting soil. Both values are significantly different from zero.

DISCUSSION

These results show that legacy effects are a potential barrier to restoration of cogongrass infested areas. After 11 weeks growing in cogon-exposed soil both *S. scoparium* and *A. ovatum*
had reduced biomass with a stronger effect on the grass species (Figure 3.1). Cogongrass alters the soil microbial community (see previous chapter). Potting soil has an unknown microbial composition, potentially containing a variety of bacteria and fungi via the manufacturing, packaging and transportation processes. In addition, microbes may be added through watering and air exposure. A variety of fungi and algae were observed to be growing in the potting soil (L. Radunzel-Davis, personal observation) while in the incubators. Previous research on S. scoparium showed that when exposed to fungal spores from multiple prairie soils, growth was not affected (Ji et al. 2010) and that growth responses in live soil was positive compared to sterile soil (Bauer et al. 2017). But, this contrasts to an earlier study showing that S. scoparium had reduced growth in live soil compared to sterilized soil, indicating a sensitivity to soil microbial communities (Anderson and Roberts 1993). There may be specific microbes that S. scoparium is sensitive to that are not found in all areas. Specific impacts of microbial changes on native plants due to cogongrass should be investigated in future experiments due to strong linkages between microbial community and plant species and community (Belnap et al. 2005, Mangla and Callaway 2008, de Kroon et al. 2012, Fitzpatrick et al. 2017, Reese et al. 2018).

Cogongrass has already been shown to produce allelopathic compounds (Koger and Bryson 2004, Xuan et al. 2009, Hagan et al. 2013, Javaid et al. 2015). In vitro studies have shown that cogongrass root extract decreases germination (Koger and Bryson 2004) and acts as a fungicide (Javaid et al. 2015). A study using cogongrass soil leachate applied to native plants in pots found only one plant, the grass Aristida stricta, to be negatively affected (Hagan et al. 2013). They theorized that bluestem grasses, such as S. scoparium may also be resistant, however S. scoparium was shown previously to have reduced seedling growth when exposed to root extracts of Andropogon virginicus, another native grass (Rice 1972). Another study
exposed *S. scoparium* to hydrocinnamic acid, a known allelochemical, and found it caused reduced shoot and root biomass (Williamson et al. 1992). Hydrocinnamic acid was one of 36 compounds identified in rhizome and root extracts of cogongrass (Xuan et al. 2009). This compound should be investigated further to explore if it is common and if it is a main causative agent of reduced biomass. Interestingly, another study found that *A. virginicus* performed better than other natives when in competition with cogongrass (Daneshgar and Jose 2009b). This could be due to similar root chemical exudates, which would make *A. virginicus* a good native candidate to use in comparison studies with cogongrass since often plants have evolved tolerance to root exudate chemicals in the home range (Callaway and Aschehoug 2000).

It has already been documented that cogongrass alters the communities it invades, decreasing plant species richness and increasing ground shading (Brewer 2008). And areas have been difficult to rehabilitate (Kuusipalo et al. 1995). A meta-analysis of plant-soil feedback studies showed that, overall, native species were not inhibited by soil legacies of exotic species and that native grasses in general had positive feedbacks in soil conditioned by exotics (Meisner et al. 2014). These conflicting results show the importance of exploring specific interactions, especially in cases of a dominant exotic species on a dominant restoration species. The success of *S. scoparium* in a restoration effort may be important, since it allows other species to grow and produce more biomass than other grasses (Wilsey 2010). However, in areas dominated by cogongrass there may be a tradeoff between successful establishment and restoration diversity.

One unanswered question is how long soil legacies persist. A study of multiple iterations of growth showed that there is the potential for some species to have a longer lasting legacy, even when a second species conditions the soil after the first is removed (Wubs and Bezemer 2017), but this did not hold true for all species, and there may be only transient effects (Corbin
and D’Antonio 2012). Use of a native cover crop that modifies the soil in a way that will benefit native plants could enhance restoration success (Krueger-Mangold et al. 2006, Sheley et al. 2006, Perry et al. 2009). The use of activated carbon to counteract the effect of allelochemicals during restoration has also been explored (Callaway and Aschehoug 2000, Kulmatiski 2011). A study of activated carbon had mixed results, with more efficacy against the legacy of some plants than others (Kulmatiski 2011). It was only effective in increasing native plant abundance when used along with native seed addition. It also had the effect of decreasing soil microbial lipids, so the benefits may be not only neutralizing chemicals, but also biotic alterations (Kulmatiski 2011).

REFERENCES


CHAPTER 4. CONCLUSION

Cogongrass has shown that its reputation as one of the world’s worst weeds is well deserved. It has been able to persist and expand into new patches despite continuous monitoring and chemical control at Lee Memorial Forest. Patches there are clustered and occur in areas that are less managed. A greater number of patches are found every five years, indicating some unexplored potential cyclical pattern. Monitoring intensity should be concentrated near known clusters and after prescribed burning and timber harvesting activities.

Cogongrass modifies the soil that it occupies. In Louisiana, it initially benefits from growth in soil with a naïve microbial community. These microbes act on the plant to greatly increase aboveground biomass production. In native Japanese soil, the soil microbes decrease aboveground biomass production. Sampling other invaded, as well as other native areas would show if this trend can be generalized and if there are some interaction effects with nutrient quality or other conditions. The two populations of cogongrass used in this study did not respond in the same way. Some populations of cogongrass may have more invasive potential in some areas than others. This could be explored further by growing rhizomes from populations with different phenotypic growth forms in the greenhouse in a range of shaded conditions to see if some are more likely to grow and persist. In some cases, there may be a low probability of cogongrass expanding into an area if it is less likely to become abundant.

The changes in microbial composition are another area that can be explored in greater depth. Genetic sequencing of the microbes in the soil can reveal more details on the microbial changes that occur in the soil and give insight into what is being favored and what is being disfavored by cogongrass. Microbial composition can be compared before and after cogongrass growth and with different soil types and locations to determine if a pattern can be established.
The microbial and chemical alterations in soil by cogongrass have the potential to influence growth by subsequent occupiers. This should be more fully explored for areas that are going to be restored. The use of seed addition, activated carbon or even a temporary cover crop all may have an impact on the success of restoration. Legacy effects are not the same for all species. Effects on the most abundant native plants or desired rare native species should be determined to increase chances of restoration success. If there are effects on either these, then extra steps can be taken to minimize the soil legacy.

Cogongrass remains problematic throughout the Gulf Coast states. Increased study of soil modifications that lead to its success have already given some insights into what makes it invasive. The naïve soil microbial community enhances biomass production, and the alterations to that community by cogongrass encourage rapid lateral growth. These alterations to either the soil microbial community and/or the chemicals released into the soil by cogongrass can have negative consequences for native plant biomass production. Continuing these lines of research have the potential to yield information that can be used to stop the spread of cogongrass and restore high priority areas.
VITA

Lori considers herself from Wisconsin but spent more than half her life far from the Midwest, including 2 years in the Philippines as a Peace Corps Volunteer. She earned her bachelor’s degree in wildlife ecology with a focus on international agriculture and natural resources and an Environmental Studies Certificate from the University of Wisconsin-Madison. She developed an interest in invasive species after working for the Departments of Natural Resources in Wisconsin and Minnesota, where her primary objective was to eliminate invasive plants from State Natural Areas. She temporarily changed careers, becoming a Certified Veterinary Technician and worked in small animal hospitals as well as a wildlife rehabilitation center. Earning this degree will represent a return to her original career path.