

THE EFFECTS OF PLANT INVASION AND DROUGHT ON PLANT-SOIL
INTERACTIONS

By

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To Iris and James, the next generation of environmentalists

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Plant-soil interactions are major drivers of plant community dynamics and are likely to be altered by anthropogenic global change with consequences for ecosystem structure and function. Interactions among global change factors have the potential to exacerbate ecological effects, but these interactions are notoriously difficult to predict. Plant invasions are accelerating worldwide with consequences for biodiversity, nutrient cycling, and disturbance regimes. Furthermore, plant invaders will experience shifts in abiotic conditions associated with climate change such as increased frequency and severity of drought. Here, I present research on the responses of plant and soil communities to interacting stressors and assess the potential consequences for ecosystem restoration. First, I assessed the response of longleaf pine forest plant communities to experimental invasion by *Imperata cylindrica* and experimental drought imposed with rainout shelters over four years. I found that invasion caused severe declines in diversity and shifts in composition of the native plant community, while drought had moderate effects on diversity and shifted the dominant functional groups. Soil moisture under drought conditions with the invader was higher than without the invader, and in combination the impacts of invasion and drought were lower than expected, indicating an ameliorating effect. Additionally, I evaluated the effects of invasion and drought and the consequent shifts in plant community on the soil microbial

communities. On the whole, drought was a stronger driver of bacterial communities than invasion, whereas fungal communities were interactively affected by the treatments. Functional groups of importance for plant communities including plant pathogens, mycorrhizal fungi, and nitrifiers were affected by both invasion and drought. Finally, I assessed the impacts of these shifts in soil microbial communities in response to invasion and drought on growth and competition of the dominant plant species in this ecosystem (longleaf pine and wiregrass). Interestingly, the effect of soil microbes on plant growth varied with competitive context. Additionally, soil legacy of invasion decreased the growth of wiregrass but not pine. Collectively, my research provides an evaluation of the role of plant invasion and drought on interactions in the plant-soil system and addresses the implications for native ecosystems under future biotic and abiotic conditions.

CHAPTER 1 INTRODUCTION

Framework: Interacting Stressors

Many biotic and abiotic stressors can influence plant communities and the literature on this topic is vast. Stress can be defined in different ways and the chosen definition can influence the conclusions drawn from multiple stressor research. One common definition is a condition that reduces performance or fitness of a species below optimal levels (Lichtenthaler 1996, Folt et al. 1999, Vinebrooke et al. 2004). This definition is useful when considering a single species but is difficult to apply at the community level because species have different optimal conditions (Thompson et al. 2018). For the current discussion, stress will include stimuli that cause a negative response at the level of organization assessed (i.e. species, population, or community level).

Abiotic stressors for plants include levels of environmental resources (e.g. water, light, nutrients) and conditions (e.g. temperature, soil pH), physical stressors associated with disturbance (e.g. fire, wind), and exposure to toxins (e.g. heavy metals, pollutants). Abiotic stressors can influence plant species composition, diversity, structure, and function in a community and influence soil ecosystem function in turn (Thuiller et al. 2005, Kardol et al. 2010). For abiotic stressors that are resource-based, plant communities are expected to be most strongly affected by reductions in the most limiting resource (van der Ploeg et al. 1999). The limiting resource will not necessarily be the same for all species within a community; therefore, different abiotic stressors can differentially affect species performance, and in some cases allow species to coexist if they are most limited by different resources or have access to different resource pools (Chesson 2000, Nippert and Knapp 2007). Fluctuating resource levels and

occasional stress from disturbance can also promote increased species diversity, as in the storage effect and intermediate disturbance hypothesis (Chesson 2000, Knapp et al. 2002).

For plants, biotic stressors primarily include herbivore or pathogen effects and plant-plant competitive interactions. Biotic stressors such as specialist pathogens can influence species diversity by creating density dependence in plant populations and allowing for species coexistence (Chesson 2000, Bever et al. 2015). Herbivory can increase plant diversity in communities where undefended plant species associate with highly defended species. The unpalatable species reduce herbivory on palatable species allowing them to persist (Rebollo et al. 2002, Rousset and Lepart 2002, Callaway et al. 2005). On the other hand, a generalist herbivore or pathogen can have the opposite effect on species coexistence, as the species with the greatest tolerance to herbivory/pathogens will dominate. Finally, competition with non-native invasive plants can act as biotic stress, especially if the invader has enhanced competitive ability compared to natives or novel weapons (Callaway and Ridenour 2004, Graebner et al. 2012).

Vegetation responses to stress take place on different time scales and levels of ecological organization. Individual plants respond most immediately to stress through various physiological mechanisms triggered by hormone signaling (Atkinson and Urwin 2012). Acclimation can only occur in response to stress that is within the environmental tolerance of an individual plant, and plants with higher phenotypic plasticity may show greater ability to acclimate to stressful conditions. Over longer time scales, plant populations can adapt to stressful conditions as conditions select for the most stress tolerant individuals. Changes in the abundance of different plant species depending on stress tolerance can lead to community reordering (Collins et al. 2012, Jones et al. 2016). Under severe or long-term stress, communities begin to lose the most susceptible species, which can alter ecosystem function as well as the trajectory of the

community recovery if the stressor is removed (Jones et al. 2016). The effects of stress on ecosystem function generally are less severe in communities with greater functional diversity (Fry et al. 2013).

Interacting stressors can have different effects on plant populations and communities than predicted from the individual stressors, i.e. synergistic or antagonistic responses (Figure 1-1). The terms for these responses have been used inconsistently, resulting in the over emphasis of synergy in ecological literature (Côté et al. 2016). Folt et al. (1999) proposed a framework with three potential null models for the interaction between two stressors. First, the comparative effect model (or dominance model) predicts that with two interacting stressors, the effect will be equal to that of the greater stressor alone. Deviation from this hypothesis results in increased or decreased effect in comparison to the effect of the strongest individual stressor. The comparative effect model should be applied when two stressors have similar mode of action and is often applied to limiting resources, as in the Law of the Minimum (van der Ploeg et al. 1999). Second, the multiplicative effect model predicts that the effects of the combined stressors will be the product of the effects of the individual stressors. Deviation from this hypothesis can be called “multiplicative synergism” or “multiplicative antagonism” (Folt et al. 1999). The multiplicative effect is expected when the response to stress is measured in terms of mortality (Côté et al. 2016). Finally, the additive effect model hypothesizes that the effect of two stressors will be equal to the sum of the effects of the individual stressors. Effects greater than and less than those predicted are considered synergistic and antagonistic, respectively. The additive effect is expected when the stressors have physiologically different modes of action, such as with interacting biotic and abiotic stressors, and this is the null model I will use throughout my work (Schäfer and Piggott 2018).

At the community level, stressors affect species differently: what is stressful for one species may not be stressful for another species. Thus, stress may alter competitive interactions and community composition (Schäfer and Piggott 2018). The alteration of biodiversity in response to multiple stressors depends on the type of stressors and how the tolerance of the species in that community to each of the stressors covaries (co-tolerance) (Vinebrooke et al. 2004). If species responses to each of the stressors tend to be positively correlated, then a dominance null model would be more appropriate, but if they are negatively correlated then an additive model would be more appropriate (Vinebrooke et al. 2004, Schäfer and Piggott 2018). Our ability to predict the effects of interacting stressors on plant communities depends on a better understanding of the applicability of the various null models under different circumstances and the standardization of selecting null models (Piggott et al. 2015, Thompson et al. 2018).

Abiotic stress and plant-plant competition are highly interconnected. Abiotic physical stress and the strength of competitive interactions tend to be negatively correlated (Grime 1977, Bertness and Callaway 1994). The stress gradient hypothesis predicts that positive interactions (i.e. facilitation) are more common under stressful environmental conditions, and is well supported in the literature (Bertness and Callaway 1994, Pugnaire and Luque 2001, Lortie and Callaway 2006, He and Bertness 2014), and these harsh conditions may reduce the success of introduced species. In contrast, low abiotic stress tends to favor strong competitive interactions, and disruption of these interactions, for example by disturbance, provides an opportunity for invasion by non-native species (Melgoza et al. 1990). Resource addition into a site with previously low resource availability or high variability in resource supply rate over time can also promote invasion because invaders are provided the opportunity to take advantage of excess resources (Huenneke et al. 1990, Alpert et al. 2000, Davis et al. 2000, Shea and Chesson 2002,

Koerner et al. 2015). Thus, abiotic stress can influence the success and impacts of biological invaders.

Invasion by non-native plants often reduces species diversity (Alvarez and Cushman 2002, Yurkonis et al. 2005, Adams and Engelhardt 2009, Gaertner et al. 2009, Gooden et al. 2009, Hejda et al. 2009, Vilà et al. 2011, Cook-Patton and Agrawal 2014); however, the mechanism of reduction of diversity is rarely addressed and may involve interactions with abiotic stress (Levine et al. 2003). Impacts may occur through direct competition, interference competition through production of allelopathic compounds (Hierro and Callaway 2003), or may be mediated by feedbacks with abiotic factors like nutrient availability or disturbance regimes (Mack and D'Antonio 1998, Ehrenfeld 2003).

Abiotic stress imposed by climate change is often expected to increase plant invasion and exacerbate invasion effects on native communities (Bradley et al. 2010b, Diez et al. 2012). Severe stress such as extreme drought can increase competitive effects of invaders on native species and prevent recovery after the stress event (Caldeira et al. 2015). The majority of research in this area has focused on how stressors such as climate change will impact the ability of invaders to colonize and spread, but relatively little is known about what the specific impacts of these combined stressors will be at the community level.

Current understanding of how stressors interact to influence plant communities is surprisingly limited, despite the fact that increased stress due to climate change is occurring on a global scale. More explicit identification of the expected outcomes of combined stressors is needed to identify general patterns and trends. Additionally, a better integration of organism responses to stressors across disciplines, particularly between agricultural and ecological studies as well as between terrestrial and aquatic systems, would be helpful to predict future responses to

global change. Finally, a systematic application of null models across studies would improve synthesis of data across studies.

Microbial Communities

The same stressors that influence plant communities can also impact soil biota, both directly and indirectly. Soil biota respond directly to abiotic conditions but also to changes in plant community structure or function that result from abiotic stress. Biotic and abiotic drivers of soil microbial communities are complex, interactive, and context dependent. The abiotic environment strongly affects soil microbes because of their close association with the soil matrix. Abiotic conditions such as pH, nutrients, temperature, soil moisture, and O₂ concentrations alter microbial communities, but consensus has yet to be reached on which of these is most important under what circumstances and for which aspects of the microbial community (Ramirez et al. 2010, Evans and Wallenstein 2012, Shen et al. 2015). Moreover, it is difficult to distinguish the direct effects of the abiotic environment on microbial communities from indirect effects resulting from vegetation responses to environmental factors. In a changing global environment these influences take on even greater complexity.

Most research about drivers of microbial communities is confined to very broad groupings; however, the ecological function of microbial taxa at the higher taxonomic levels is highly variable (Philippot et al. 2010). Therefore, assessing finer scale variation in microbial communities can provide a better understanding of the ecological niche of microbial taxa. At the most general level, global patterns in total microbial biomass in soils are driven primarily by soil moisture and nutrients (Serna-Chavez et al. 2013). While it is generally thought that bacteria are more sensitive to soil moisture conditions than fungi (Evans and Wallenstein 2012, Ochoa-Hueso et al. 2018), Blankinship et al. (2011) found that bacterial abundance was influenced by temperature, while fungal abundance was more sensitive to precipitation. Within bacteria,

nitrifiers and gram negative bacteria are generally more susceptible to water stress than gram positive bacteria, and Actinomycetes are among the most tolerant (Manzoni et al. 2011).

Additionally, bacterial communities appear to adapt to frequent dry-wet periods which would typically cause lysis in many soil microbes (Fierer et al. 2003). Warming alters soil microbial communities and can increase fungal:bacterial (F:B) ratios; however, it is difficult to distinguish independent warming effects from those driven by altered soil moisture conditions (Zhang et al. 2005).

Soil chemistry conditions also play a major role in shaping microbial communities. F:B ratios are dependent on soil C:N ratios, and nitrogen addition reduces fungal compared to bacterial activity (Frey et al. 2004, Fierer et al. 2009). Nitrogen addition has been shown to alter bacterial community composition (Coolon et al. 2013), and data suggest that changes in bacterial communities are due to direct effects of N availability rather than effects of N availability on pH or plant community shifts (Ramirez et al. 2010). Many studies have suggested that soil pH is a strong predictor of bacterial community composition across large spatial scales and pH can influence soil nutrient availability (Fierer et al. 2009, Lauber et al. 2009, Kaiser et al. 2016). Bacterial community shifts could be related to changes in the abundance of copiotrophs that thrive in high resource conditions (e.g., Actinobacteria, Bacteroidetes, and β -Proteobacteria) and oligotrophs that dominate in low resource condition (e.g., Acidobacteria and Verrucomicrobia) in response to variation in resource supply (Fierer et al. 2007, Ramirez et al. 2012). Only certain components of the microbial community are active at a particular time, while many microbes become dormant under stressful conditions. Total bacterial community composition varied most between locations associated with different soil types and less strongly with vegetation type, while only the active bacterial community, as measured with RNA sequencing, was altered by a

change in precipitation, possibly suggesting different dormancy responses of microbial groups (Felsmann et al. 2015).

Vegetation composition and productivity plays a key role in shaping soil microbial communities (Burns et al. 2015). Some microbial groups are intimately associated with plant hosts including mycorrhizal fungi, rhizobia, and plant pathogens, and these groups are strongly driven by plant abundance and species composition (Allen et al. 1995, Burrows and Pflieger 2002). Plant species also differ in the quality and quantity of resource inputs into the soil and many studies have shown increased activity of microbes in the rhizosphere compared with bulk soil (Van Der Krift et al. 2001, Fierer et al. 2007). Plant productivity and soil organic matter are major drivers of microbial biomass (Fierer et al. 2009). Plant traits can also influence some aspects of microbial communities; for example, slow growing conservative plant species have fungal dominated microbial associates (Orwin et al. 2010). Furthermore, some evidence suggests that microbial diversity increases with plant diversity (Felsmann et al. 2015).

Various lines of evidence suggest that biotic and abiotic drivers interact to shape microbial communities (de Vries et al. 2012). Abiotic conditions determine the suitability of a habitat for different plant species and productivity of a species at a site. These plant communities can then have both direct effects (e.g. plants supply resources) and indirect effects (e.g. plants alter the physical environment) on soil communities. This interdependence makes it difficult to distinguish the direct effects of plant communities on microbial communities (Wardle et al. 2004). Many abiotic factors such as warming, elevated CO₂, soil nutrients, pH, and precipitation have been shown to alter microbial communities; however, these effects may be mediated by changes in plant growth or physiology (Horner-Devine et al. 2003, Zhang et al. 2005, Lesaulnier et al. 2008, Thomson et al. 2010, Shen et al. 2015). de Vries et al. (2012) showed that various

abiotic factors influence microbial communities including precipitation, soil nutrients, and pH, but that plant functional traits, independent of the abiotic site characteristics, also contributed to microbial community structure. Therefore, both biotic and abiotic factors concurrently and possibly interactively influence microbial communities, but more research is needed to parse out the importance of each under different scenarios.

Soil microbes can play an important role in plant invasion and impacts of invasion on ecosystem processes. Invasive plants can alter microbial community composition directly or indirectly, either by changing the plant community composition or soil properties (Niu et al. 2007). Invaders can alter ecosystem process rates controlled by microbes, such as nutrient cycling and decomposition (Ehrenfeld et al. 2001, Kourtev et al. 2002a, Allison and Vitousek 2004). For example, *Microstegium vimineum* invasion changes N and P cycling, soil pH, base cations, and Al (Ehrenfeld et al. 2001), and these soil properties can then alter soil microbial communities (Kourtev et al. 2002b).

While many studies have shown changes in microbial communities with plant invasion (Broz et al. 2007), this trend is not universal (Carey et al. 2015), therefore we need a better understanding of how invaders modify microbial communities, and how these responses depend on the environmental context. Additionally, interactions of invaders with the microbial community may change over time. For example, local species may adapt through time since introduction of an invader, allowing native pathogens to infect the invader (Nijjer et al. 2007). The time scale over which this might occur is unknown and in some cases appears not to occur even over a century (Day et al. 2015).

Soil microbial communities can also influence invasion success. For example, escape from soil pathogens provides a possible mechanism for invasion in a new range (Mitchell and

Power 2003, Reinhart et al. 2003). Invasive plants can create positive plant-soil feedbacks favoring their own growth or they can increase negative feedbacks for native species (Niu et al. 2007, Xiao et al. 2014). Invasive plants can accumulate native pathogens that negatively affect the native plants more than themselves (Malmstrom et al. 2005, Eppinga et al. 2006, Mangla et al. 2008). Invaders can also alter soil mutualist abundance (Kourtev et al. 2002b, Hawkes et al. 2006). Invaders such as *Solidago canadensis* and *Centaurea maculosa* reduce mycorrhizal fungal abundance and diversity in soils (Mummey and Rillig 2006, Zhang et al. 2010). Invaders can also alter and disrupt mycorrhizal associations in more dependent native species (Mummey et al. 2005, Wolfe and Klironomos 2005, Vogelsang and Bever 2009, Hagan et al. 2013b). *C. maculosa* appears to be able to take advantage of mycorrhizal networks to enhance its own growth at the expense of native species (Callaway et al. 2004, Carey et al. 2004). *Alliaria petiolata* is a non-mycorrhizal invasive notorious for inhibiting AM colonization of native species with allelochemicals (Roberts and Anderson 2001), resulting in reduced native growth (Stinson et al. 2006). In contrast, one study showed no inhibitory effect of this plant on native plants or on AMF diversity (Koch et al. 2010), so even the effects of the most noxious invaders may be context dependent.

Plant-Soil Interactions

Plants have been shown to modify the soil environment through physical processes (e.g. alterations in soil temperature or pH (Raich and Tufekcioglu 2000, Hinsinger et al. 2003)), biogeochemical processes (e.g. nutrient cycling (Hinsinger 2001, Yelenik and Levine 2011)), as well as by modifying soil biotic communities (Ehrenfeld et al. 2005). These altered soil conditions created by the plant can result in differences in fitness of subsequent plants growing in that soil. Abiotic factors can also modify soil properties and biota to create soil legacies. Soil legacies can be either abiotic, such as changes in nutrient availability, or biotic, such as changes

in microbial community composition. Biotic soil legacies that are most influential for plant growth are typically associated with pathogens and microbial mutualists like rhizobia and mycorrhizal fungi (Klironomos 2002). Plants serve as the principal energy supply for these organisms, and plant fitness is influenced by their metabolic activities. Microbial communities can also indirectly affect plant fitness through regulation of biogeochemical processes. For example, nutrient availability for plants is strongly controlled by microbial decomposition and nutrient demand (Craine et al. 2007, Kuzyakov and Xu 2013).

Plant-soil feedback (PSF) experiments are a specific type of soil legacy experiment used to assess whether and how plant mediated changes in either soil abiotic conditions or soil microbial communities generate feedbacks to plant performance. In order to test for a PSF, both components (plant effects on soil and soil effects on plants) must be demonstrated. This is typically accomplished through a 2-stage experiment. The first stage, or priming stage, involves growing a single plant species in soil for a period of time to alter the soil characteristics. The second stage, or growth stage, involves growing conspecific plants in that soil, called “self-cultivated” or “home” soil, and comparing fitness of those plants to those grown under the same conditions but in soil not self-cultivated, referred to as “other” or “away” soil (Kulmatiski and Kardol 2008). To assess the reciprocal feedback of two or more plant species, the “other” soil would be soil primed by the other species. At the end of the experiment, plant traits are measured to estimate fitness; most often biomass, but growth rate, reproductive output, or survival can also be used. Reciprocal PSF experiments can demonstrate the theoretical potential for plant species coexistence based on differential response to soil microbial communities generated by different plant species (Bever 2003). However, there are a variety of issues that can reduce the direct applicability of these studies. They are typically conducted under highly controlled conditions

(Bever 1994, Mangan et al. 2010), which are unrealistic representations of processes that occur in nature, making conclusions drawn from them somewhat suspect. Additionally, different experimental approaches have been shown to yield different results, indicating that better methodological standardization is needed (Brinkman et al. 2010). Finally, in nature many plant root systems are often interacting in the soil, so one individual plant species is unlikely to be exclusively altering the soil environment. In contrast, using field experiments to provide more realistic scenarios during the soil priming stage can improve the relevance of these experiments to natural systems. Because realistic field experiments are unlikely to have only one species present, they are not directly comparable to PSF experiments and cannot accurately show the potential for species coexistence but can provide an understanding of the influence of soil biotic and abiotic legacies on the growth of different plant species.

Ideally, soil legacy experiments should be capable of distinguishing between biotic and abiotic changes in the soil. When trying to deconstruct the mechanisms behind plant-soil interactions, differences in plant performance are often compared between sterilized and unsterilized (live) soil. The difference in performance of plants grown in live versus sterile soil indicates the direct effect of microbes on the plants. Because soil sterilization can release nutrients in soil, this is often controlled for by adding a small amount of live soil inoculum to a sterile growth mixture and assuming that abiotic effects of sterilization are masked because of the small volume of added soil (Kulmatiski and Kardol 2008).

Few studies look at interspecific competition or community effects of soil legacies (Kulmatiski et al. 2008, Suding et al. 2013). The spatial scale of soil alteration by each individual plant is another consideration when attempting to infer or model community dynamics (Levine et al. 2006). Soil communities can be altered by environmental conditions making it difficult to

extrapolate experimental results to a broad range of field conditions, and therefore measurements must be taken under alternate scenarios (Kolb et al. 2002, Carvalho et al. 2010). This limitation is partly due to the lack of understanding of the biology of the soil microbial communities. Additionally, different plant-plant interactions may influence the outcome of legacy effects (Shannon et al. 2012), and therefore it is important to place the concept of soil legacies in the larger context of plant-plant interactions in order to evaluate its relative importance compared to direct competition, allelopathy, or other forms of indirect competition in driving plant community assembly (Bennett et al. 2011).

Studying the net effect of microbes on plant communities provides a means to “black box” the identity of the soil community while still revealing its effects on plant communities. As methods for characterizing the soil microbial community have become more tractable on large scales, the next step is to identify the major microbial players in plant-soil interactions so that we can extend our understanding of how they work to broader general patterns (Batten et al. 2008).

Based on this review, I identified a gap in our knowledge of how plant invasions and climate change interact to affect native ecosystems. Ecosystem responses will rely heavily on the responses of plant communities, soil communities, and their interactions as they drive many ecosystem functions. Specifically, I test the following questions:

1. How do plant invasion and drought individually and interactively affect native plant communities?
2. How do plant invasion and drought individually and interactively affect soil bacterial and fungal communities?
3. How do the legacies of changes in soil bacterial and fungal communities due to invasion and drought alter the performance of native plant species compared with the invader and competition between native and the invader?

To test these questions, I used longleaf pine forests of the southeastern US as a model system. This region is concurrently threatened by invasion by *Imperata cylindrica* (cogongrass) a

rhizomatous C₄ grass as well as increased frequency and severity of drought. I expected a negative effect of both invasion and drought on plant diversity and an additive effect of invasion and drought in combination. Furthermore, I expected that changes in microbial communities would mirror changes in the plant communities. I expected that the legacy effects of the invader on soil microbial communities would benefit the invader more than native species and the legacy of drought would favor native species over the invader. Finally, I address the implications of this research for restoration of native plant communities.

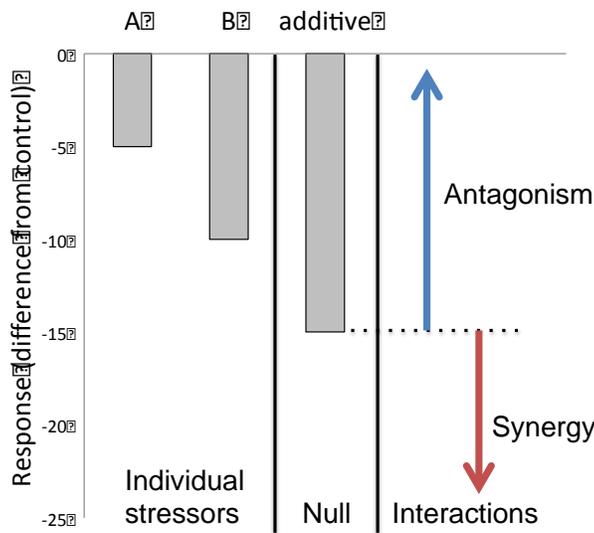


Figure 1-1. Model diagram of possible interactions between two stressors (A and B). A response equal to the sum of the individual stressors represents the null model. A more negative response designates a synergy between the two stressors while a less negative response indicates antagonism.

CHAPTER 2
GRASS INVASION AND DROUGHT INTERACT TO ALTER THE DIVERSITY AND
STRUCTURE OF NATIVE PLANT COMMUNITIES

Background

Plant community structure and function are determined by multiple biotic and abiotic drivers (Baruch and Jackson 2005, Gornish and Miller 2015), but anthropogenic environmental changes may alter these drivers and their effects on plant communities (Alvarez and Cushman 2002, Knapp et al. 2002). Many global environmental changes are occurring simultaneously, but effects of multiple stressors are difficult to predict based on evaluation of individual stressors because it is unknown if interactions between stressors will occur. In the absence of interactions, the effects will be additive such that the combined effect will be equal to the sum of effects from individual stressors (Zavaleta et al. 2003, Côté et al. 2016). Interactions between stressors can occur when the combined effects are greater than (synergistic) or less than (antagonistic) the predicted additive effect (Côté et al. 2016). Synergistic interactions among global change drivers have the potential to magnify effects on biodiversity and ecosystem function, while antagonistic interactions could partially ameliorate negative effects (Brook et al. 2008, Caldeira et al. 2015). Improved understanding of potentially complex interactions among stressors and their effects on native communities is necessary to predict long-term outcomes of global environmental change (Alpert et al. 2000, Bellard et al. 2013).

Invasive plants can alter the structure and function of natural communities through changes in species interactions, biogeochemical cycling, and disturbance regimes (Brooks et al. 2004, Liao et al. 2008, Vilà et al. 2011). At the same time, climate change is expected to increase extreme weather events such as prolonged drought, which can cause stress to native plant communities and exacerbate impacts of invaders (Knapp et al. 2008, Bradley et al. 2010b, 2010a, Diez et al. 2012). Although prior studies have evaluated the effects of various climate change

factors on invasive plant establishment and performance (Dukes and Mooney 1999, Dukes et al. 2011, Eskelinen and Harrison 2014, Manea et al. 2016), it is unknown how global change stressors may influence an invader's effects on native species community dynamics.

The response of invaded communities to abiotic stress depends on the relative stress tolerance of native and invasive species and how stress influences species interactions such as competition and facilitation (Bertness and Callaway 1994, Tylianakis et al. 2008). For example, while invasive species are often expected to disproportionately invade high resource environments, some invaders have higher resource use efficiency than native species, allowing them to compete in low resource environments (Funk and Vitousek 2007). Invaders also may benefit when resources become available during extreme climate events or when competitive interactions are disrupted due to native species losses and reduced biotic resistance (Huenneke et al. 1990, Alpert et al. 2000, Davis et al. 2000, Diez et al. 2012). In such cases, climate change factors can interact synergistically with invaders to suppress native species (Caldeira et al. 2015). Conversely, climate change may inhibit invaders that are less tolerant of abiotic stress than native species (Bradley et al. 2009, Sorte et al. 2013, Liu et al. 2017), thereby reducing the effects of invasion. Moreover, invaders may mitigate climate change effects on native communities if they moderate stressful abiotic conditions (Rodriguez 2006). The nature of these interactions, whether additive, synergistic, or antagonistic, may change in magnitude and even direction through time as either the invader becomes increasingly dominant, climate stress is increasingly severe, or thresholds in the ability of native and invasive plants to persist are exceeded due to restricted resource access or extreme physical stress. Thus, experimental studies that assess how interactions between stressors change over time are needed to forecast their net effects on community diversity and structure.

C₄ grasses are problematic invaders across the globe (D'Antonio et al. 2001, Milton 2004, Flory and Clay 2010, Hager et al. 2016). They typically have high drought tolerance and therefore could become more problematic as frequency or severity of drought increases; however, they also have high water use efficiency and may not draw down water resources as much as C₃ competitors (Sage and Monson 1999, Ward et al. 1999). Therefore, it is difficult to predict how native plant communities will respond to C₄ grass invasion under predicted future changes in precipitation. To evaluate the individual and interactive effects of plant invasion and climate change on plant communities and how they change over time, we established a factorial field experiment with invasion by *Imperata cylindrica* (cogongrass), a rhizomatous C₄ grass native to Southeast Asia (Estrada and Flory 2015), and chronic drought (simulated with rainout shelters, Alba et al. 2017). *Imperata cylindrica* is a globally problematic invader of warm temperate to tropical systems and a Federal Noxious Weed in the US with severe impacts on threatened longleaf pine ecosystems (Brewer 2008). Climate change predictions forecast more frequent and prolonged droughts in many regions including the southeastern US (Wang et al. 2010, Singh et al. 2013). *Imperata cylindrica* is predicted to increase in range and impacts in response to climate change (Bradley et al. 2010b). Our specific objectives were to 1) quantify the effects of drought on the invader and resident plant species; 2) compare the independent and interactive effects of invasion and drought on plant species richness, diversity, evenness, community structure, and dynamics; and 3) assess whether the relative effects of individual and interacting stressors on plant communities change over time.

Methods

Experimental Design

To determine the effects of *I. cylindrica* invasion and drought on native plant communities, we established a common garden field experiment at the University of Florida

Bivens Arm Research Site (BARS) in Gainesville, FL (29° 37' N, 82° 21' W; MAP 1300 mm, MAT 20.5°C). Soils are primarily Bivans sand (75%; 5%–8% slope) and Blichton sand (25%; 2%–5% slope; Natural Resources Conservation Service, Web Soil Survey). To prepare the site, the area was mowed and tilled. Then, in May 2012, we established native plant communities in each of 40 4 m x 4 m plots spaced 2.5 m apart with 20 bare root longleaf pine (*Pinus palustris*) seedlings (Florida Forest Service, Chiefland, FL) and 36 native perennial grass and forb seedlings (12 spp. x 3 individuals; The Natives Inc., Davenport, FL). By establishing replicate plant communities, we controlled for initial plant community composition. Herbaceous seedlings were grown in growth chambers and then in a greenhouse for a total of four months prior to transplanting. Species were selected based on their occurrence in longleaf pine forests and suitability for the site (See Table A-1 for species list). Plots were not weeded to maintain composition and numerous other species recruited from the seed bank and surrounding environment during the study.

A blocked factorial combination of *I. cylindrica* invasion and precipitation reduction (hereafter referred to as “drought”) was applied in spring 2013 (10 replicates per treatment combination), one year after the native plant communities were established (Figure A-1). For the invasion treatment, we planted nine *I. cylindrica* seedlings per “invaded” plot. Rhizomes were collected from an on-site population and plants were grown from rhizomes in a greenhouse for six weeks before being transplanted into the experimental plots. The simulated drought treatment consisted of rainout shelters with 89% areal coverage of polycarbonate roofing with 89% light penetration (TUFTEX PolyCarb, Fredericksburg, VA), gutters connected to pipes to move precipitation off site, root-impenetrable belowground plastic barriers to 1 m depth to prevent subsurface flow of water into the plots, and aluminum flashing buried to 5 cm depth and

extending 10 cm above the soil surface to divert overland water flow (Alba et al. 2017). Based on a systematic study of effectiveness of rainout shelter design (Yahdjian and Sala 2002), we anticipated that high roof cover would be required to achieve a moderate level of reduction in soil moisture. As expected, the average reduction in soil moisture in our experiment ranged from 30-50% in the drought-treated plots. We constructed structures over no-drought control plots (hereafter “ambient”) with 22% white shade cloth to account for shading by the rainout shelters.

Vegetation Surveys

To assess the effects of invasion and drought on plant communities, percent areal cover of all woody and herbaceous plant species in the plots was quantified beginning one year after the initiation of the treatments. Cover was recorded in July, October, and February from July 2014 to February 2018. July and October surveys represented mid and late growing season (rainy season), respectively, and February represented the winter dormant season (dry season). During peak growing season in July, we were particularly interested in the species richness and community composition patterns, and therefore percent cover of all species was recorded. In October and February, we were interested in changes in the dominant species and therefore only species with 5% or greater cover per subplot were recorded. Cover was evaluated within six 0.75 x 0.75 m sub-plots in each plot by the same person (C. Fahey) over time for consistency. Because it was common for canopies of species to overlap, total vegetation cover often exceeded 100%. The USDA Plants Database (plants.usda.gov) was used to determine species functional group and life history strategy (annual/biennial or perennial).

Abiotic Measurements

Soil moisture was recorded every 2-4 weeks (61 total time points) using a HydroSense II soil water sensor paired with CS659 12 cm soil water probe (Campbell Scientific Inc., Logan, UT, USA). Measurements were taken in each of four quadrants per plot and averaged for each

plot. Because many plant species can access deeper water reserves, soil moisture data also were collected at six depths (10, 20, 30, 40, 60, and 100 cm) in one location in each plot using a PR2 profiler probe connected to an HH2 data logger (Dynamax, Houston, TX, USA) at 14 time points. Photosynthetically active radiation (PAR) was measured every four weeks beginning in April 2015 using an ACCUPAR LP-80 ceptometer (Decagon Devices, Pullman, WA). PAR was measured in each of the four cardinal directions facing the center of the plot at 0.5 m and at ground level (Alba et al. 2017). Average percent light availability per plot was used for statistical analyses.

Statistical Analysis

To test if the drought treatment influenced *I. cylindrica* cover over time, we used a mixed effects model with drought treatment and date as fixed effects and plot nested within block as a random effect. To test for effects of invasion and drought treatment combinations on the dependent variables we performed mixed effects models with invasion, drought, and date as fixed effects and plot within block as a random effect. Dependent variables included soil moisture, light availability, species richness, diversity, evenness, and percent cover (total, resident, perennial grasses, annual forbs, and perennial forbs). Plots with only one species present were excluded from evenness calculations. Diversity was calculated as the exponent of the Shannon diversity index (H') (Jost 2006). For analyses including all time points (i.e., all percent cover analyses, diversity, and evenness), the July time points were subset to species with 5% cover or greater per subplot. Analyses of richness, colonization, extinction, and community composition included all species. Soil profiler probe data was averaged across the 14 time points and then differences between treatments were tested at each depth with ANOVAs. Mixed effects models were performed using the 'lme' function in the nlme package in R (Pinheiro et al. 2016).

Plant community composition was analyzed for July of each year (with *I. cylindrica* excluded) using ordination by nonmetric multidimensional scaling (NMDS) of the Bray-Curtis dissimilarity matrix by plot. We tested for differences in community composition with invasion, drought, date, and their interactions with a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and permutations constrained within each block. Community data were standardized relative to total plot cover (%) prior to calculating dissimilarity using the ‘decostand’ function of the vegan package (Oksanen et al. 2016). We tested for homogeneity of group dispersions with the multivariate analogue of Levene's test (Anderson and Walsh 2013). NMDS scores were calculated with the ‘metaMDS’ function of the vegan package. PERMANOVA and homogeneity of group dispersions were calculated with the ‘adonis’ and ‘betadisper’ functions in vegan. All statistical analyses were performed in R version 3.3.1 (R Development Core Team 2016).

Results

Percent Cover

Total live vegetation cover was similar among plots in July and October, but plots with *I. cylindrica* had nearly twice as much live vegetation cover than uninvaded plots in February (February mean \pm SE; uninvaded = 29.4% \pm 2.0, invaded = 55.3% \pm 2.2; Figure 2-1 a). Total cover of species other than *I. cylindrica* was strongly influenced by invasion (mean \pm SE; uninvaded: 60.5% \pm 1.8; invaded: 18.5% \pm 1.4), an effect that was stronger in July and October than in February (date x invasion; $F_{1,436}=10.5$, $P=0.001$; Figure 2-1 b). In the uninvaded plots, total cover of species other than *I. cylindrica* was not affected by the drought treatment in the first year but was lower in drought plots throughout the following two years (invasion x drought; $F_{1,27}=5.2$, $P=0.03$). *Imperata cylindrica* cover increased in the first year and a half of sampling, peaked at 76.3% cover in October 2015, and then declined. *Imperata cylindrica* cover declined

sharply in February 2018 following a particularly severe cold weather event (date; $F_{1,218}=3.5$, $P=0.06$; Figure 2-1 c). *Imperata cylindrica* cover was similar in ambient and drought plots throughout the first two years of sampling, after which *I. cylindrica* cover was slightly lower in drought plots than ambient plots (drought; $F_{1,9}=4.4$, $P=0.07$).

Functional Groups

We grouped herbaceous species other than *I. cylindrica* into the three most abundant functional groups: perennial grasses, annual forbs, and perennial forbs. Percent cover of all three groups was significantly lower in invaded plots regardless of drought treatment (mean cover: perennial grass: uninvaded 15.7%, invaded 3.6%; annual forbs: uninvaded 19.3%, invaded 3.7%; perennial forbs: uninvaded 7.5%, invaded 2.4%) however, functional groups responded differently to drought in the uninvaded plots. Perennial grass abundance did not differ significantly between drought and ambient plots (date x drought; $F_{1,436}=3.1$, $P=0.08$). Annual forbs were more abundant in drought than ambient plots (mean cover; ambient: 15.5%, drought: 23.2%; drought; $F_{1,27}=6.7$, $P=0.02$) and perennial forbs were less abundant in the drought than ambient plots (mean cover; ambient: 10.8%, drought: 4.3%; drought; $F_{1,27}=9.0$, $P=0.01$; Figure A-5 a-c). Woody species other than longleaf pine made up less than 3% cover in the plots on average. Longleaf pine cover was significantly lower in uninvaded drought, invaded ambient, and invaded drought plots compared to uninvaded ambient plots and this difference increased over time (date x invasion x drought; $F_{1,436}=18.7$, $P<0.001$).

Species Richness and Turnover

In July 2014, one year after initiation of treatments, species richness was similar across all treatments (mean \pm SE; 15.3 ± 0.4 ; Figure 2-2 a); however, across the subsequent three years, richness was on average 58% lower in invaded plots than uninvaded plots (mean \pm SE; uninvaded: 15.7 ± 0.6 , invaded: 6.6 ± 0.4). Species richness in the invaded plots declined by 41%

from 2014 to 2015 (mean \pm SE; 15.6 ± 0.4 to 9.2 ± 0.8). Richness continued to decline in invaded plots from 2015 to 2016 (mean \pm SE; 9.2 ± 0.8 to 4.5 ± 0.4) but increased slightly in 2017 (mean \pm SE; 6.1 ± 0.4 ; date x invasion; $F_{1,116}=62.1$, $P<0.001$; Figure 2-2 a). In uninvaded plots, species richness was significantly lower under drought (mean \pm SE; ambient: 18.1 ± 0.6 , drought: 13.0 ± 0.5), and this effect increased over time as richness was 20% lower in 2015, 35% lower in 2016, and 38% lower in 2017 (date x drought; $F_{1,116}=2.9$, $P=0.09$); however, there was no difference in richness with drought in the invaded plots (invasion x drought; $F_{1,27}=38.1$, $P<0.001$; Figure 2-2 a). Total number of species present across all plots of each treatment in 2017 was 65% lower in invaded plots than uninvaded plots (uninvaded: 49 species, invaded: 17 species), 22% lower in drought plots (38 species), and 51% lower in invaded drought plots (24 species).

The number of colonizing species was consistently higher in uninvaded plots compared to invaded plots (mean \pm SE; 5.1 ± 0.4 vs. 1.9 ± 0.2 ; invasion; $F_{1,36}=55.2$, $P<0.001$; Figure 2-2 b), but drought treatment only affected colonization events from July 2016 to July 2017 in uninvaded plots (mean; ambient = 7.3, drought = 4.7; invasion x drought; $F_{1,36}=4.6$, $P=0.04$). Number of extinction events was unaffected by drought treatment, but the effect of invasion decreased over time. From 2014 to 2015, regardless of drought treatment, the number of plot level extinctions was nearly twice as high in invaded plots as in uninvaded plots (mean; invaded: 7.9 vs. uninvaded: 4.0). By 2016, cumulative number of plot level extinctions was 32% higher in invaded plots (mean; invaded = 13.5, uninvaded = 10.2). By 2017, cumulative plot level extinctions were similar in uninvaded plots and invaded plots (mean; uninvaded: 13.6 vs. invaded: 14.7, date x invasion; $F_{1,76}=7.4$, $P=0.008$; Figure 2-2 c).

Diversity

In July 2014 plant species diversity (e^H) was similar among all plots. From July 2014 to February 2015, diversity declined more rapidly in invaded plots than uninvaded plots and remained less than 2.1 for the duration of the study (date x invasion; $F_{1,436}=6.6$, $P=0.01$; Figure 2-3 a). Diversity was lower in the uninvaded drought than uninvaded ambient plots but no difference was observed between invaded drought and invaded ambient plots (invasion x drought; $F_{1,27}=6.1$, $P=0.02$; Figure 2-3 a). Species evenness also decreased from 2014 to 2015 in the invaded plots but not in the uninvaded plots (invasion; $F_{1,27}=40.3$, $P<0.001$, date; $F_{1,408}=34.4$, $P<0.001$); however, evenness was not significantly affected by drought (invasion x drought; $F_{1,408}=0.5$, $P=0.50$ Figure 2-3 b).

Community Composition

Invaded and uninvaded plant communities became more dissimilar over time (PERMANOVA date x invasion; Pseudo- $F_{1,159}=12.7$, $P<0.001$; Table A-2) and a significant interaction between invasion and drought was observed (invasion x drought; Pseudo- $F_{1,159}=7.7$, $P<0.001$; Figure 2-4). Despite the significant interaction, the R^2 values show that the effects of invasion on community composition were much stronger than the invasion x drought effect (invasion: $R^2=0.41$; invasion x drought: $R^2=0.02$; Table A-2). To better understand the treatment effects, we analyzed plant communities for each time point separately. In July 2014, invasion and drought effects were each significant but there was no interaction (invasion: Pseudo- $F_{1,39}=2.9$, $P<0.01$; drought: Pseudo- $F_{1,39}=2.3$, $P=0.01$; Figure 2-4). During July 2015, there was a significant interaction between invasion and drought, where uninvaded ambient plots were more similar to invaded plots than uninvaded drought plots (invasion x drought: Pseudo- $F_{1,39}=2.0$, $P=0.04$). During July 2016 and 2017, only invasion had a significant effect on community composition (2016: Pseudo- $F_{1,39}=12.3$, $P<0.01$; 2017: Pseudo- $F_{1,39}=9.3$, $P<0.01$). Overall, there

was also greater group dispersion in the invaded than uninvaded plots (Pseudo- $F_{1,158}=16.9$, $P<0.001$; Figure 2-4), which was significant for 2015-2017 (2014: Pseudo- $F_{1,38}=1.8$, $P=0.2$; 2015: Pseudo- $F_{1,38}=10.2$, $P=0.003$; 2016: Pseudo- $F_{1,38}=13.9$, $P=0.002$; 2017: Pseudo- $F_{1,38}=14.9$, $P<0.001$; Table A-3). This effect indicates that the invaded communities (with *I. cylindrica* excluded from analysis) became more dissimilar to each other than the uninvaded communities. PERMANOVA results are insensitive to heterogeneity in dispersion for balanced designs such as ours (Anderson and Walsh 2013), so differences between treatments in the PERMANOVA can be confidently attributed to differences in group centroids rather than spread.

Soil Moisture and PAR

On average, soil moisture in the top 12 cm was 41% lower in drought plots than ambient plots (mean \pm SE; $11.5\% \pm 0.2$ vs. $19.5\% \pm 0.2$). Soil moisture was similar in the ambient plots with and without the invader (mean \pm SE; $20.1\% \pm 0.3$ vs. $19.0\% \pm 0.3$); however, the invaded drought plots had higher soil moisture compared to the uninvaded drought plots (mean \pm SE; $13.4\% \pm 0.3$ vs. $9.6\% \pm 0.2$). This interaction varied significantly by date (date x invasion x drought; $F_{1,2595}=9.5$, $P=0.002$), where the effect of the drought treatment increased with the duration of the experiment and did not appear to vary strongly with large natural variation in precipitation (Figure A-2; Hoover *et al.*, 2018).

Soil moisture differed between ambient and drought treatments up to 40 cm depth but not at 60 or 100 cm depth (depth 10 - 60 cm: $F_{1,27} > 5.2$, $P<0.006$; Figure A-3). Light availability at the ground was significantly lower in the invaded plots, especially in the winter and early in the growing season but was not affected by drought (date x invasion: $F_{18,646}=16.7$, $P<0.001$; Figure A-4). Light availability at 0.5 m was lower in invaded plots and was also lower in drought vs. ambient uninvaded plots depending on date (date x invasion x drought: $F_{18,646}=1.7$, $P=0.04$).

Discussion

Our results show that the biotic stress from an invasive grass dramatically altered native plant communities, while the abiotic stress from chronic drought had moderate effects that were partially ameliorated by the invader. Invasion by *I. cylindrica* had major effects on resident species cover, diversity, evenness, and community composition, while drought had more moderate effects on plant communities, including alteration of dominant functional groups and a modest reduction in diversity. Together, the effects of invasion and drought combined were similar to invasion alone and lower than would be expected in an additive model (Côté et al. 2016). Thus, we found an antagonistic interaction between invasion and drought, and we were uniquely able to identify the likely cause of the antagonism because we documented the ameliorating effect of invasion on soil moisture. These findings show that in uninvaded communities, drought had significant effects on plant community structure but when invasion and drought were combined, invasion was the primary driver of community structure regardless of drought.

Invasive plants generally have higher water use than natives species and have been shown in some cases to reduce water availability to native species (Levine et al. 2003, Cavaleri and Sack 2010, Caldeira et al. 2015); however C₄ species typically have higher water use efficiency (Sage and Monson 1999). We observed higher soil moisture in invaded drought plots compared to uninvaded drought plots and, interestingly, this modulation of soil moisture by the invader only occurred under drought conditions. This effect was likely due to a combination of higher water use efficiency of *I. cylindrica* and reduced soil surface temperature and increased humidity (Alba et al. 2017), potentially reducing transpiration and evaporative soil water loss. These data indicate that higher soil moisture in invaded drought plots is a mechanism whereby invasion can moderate drought effects on community structure and suggest that *I. cylindrica* does

not dominate via water competition. Instead, this invader likely outcompetes native species for light by maintaining a dense live canopy and thick layer of thatch throughout much of the year. Therefore, despite the potentially lower water consumption of C₄ invaders, they are likely to continue to be problematic under increased drought due to high drought tolerance and competitive ability.

Previous studies have suggested that some plant invaders alter community dynamics by suppressing colonization of native species, and that drought reduces diversity mainly through local extinction of rare species (Tilman and El Haddi 1992, Yurkonis et al. 2005). Our results showed that *I. cylindrica* both prevented species from colonizing and promoted the loss of resident species from the invaded plots, but over time lower colonization became more important in driving differences in richness. Additionally, drought reduced species richness by 38%, which was almost exclusively due to lower colonization in drought-treated plots, particularly in the fourth year of the experiment. These findings suggest that the balance of colonization and extinction changes over time and that it is critical to identify and remove invaders before species losses and recruitment limitation occur, particularly in threatened longleaf pine forests where endemic habitat specialists are strongly affected by *I. cylindrica* invasion (Brewer 2008). Losses of native species in invaded areas may not only lead to less biotic resistance (Fargione and Tilman 2005), but also loss of ecosystem function and lower habitat quality (Vilà et al. 2011).

Researchers have hypothesized that climate change will promote plant invasions because many invasive species have high tolerance for environmental stress (Dukes and Mooney 1999, Theoharides and Dukes 2007) and high phenotypic plasticity (Davidson et al. 2011). Additionally, climate stress may lower native community resistance to invasion (Dukes and Mooney 1999, Diez et al. 2012); however, there is no consensus on whether empirical evidence

supports this hypothesis (Bellard et al. 2013, Sorte et al. 2013). The native communities in our study showed very low biotic resistance to invasion regardless of drought treatments.

Additionally, drought had a minor effect on the invader despite the relatively severe and long-term drought we imposed. Thus, invaders that are highly resistant to climate stress should be the target of management and research regardless of their ameliorating effects on abiotic stress.

Additionally, *I. cylindrica* is known to alter fire severity in longleaf pine forests (Brooks et al. 2004), so other interactions in this system, such as between drought and fire, must be considered.

Native ecosystems around the globe are concurrently threatened by plant invaders and increasingly severe effects of climate change (Baruch and Jackson 2005, Going et al. 2009), but little is known about how these factors interact at the community scale. Interactions between biotic and abiotic stressors can be complex, and our results show that plant invasion and drought can interact in unexpected ways with profound consequences for resident plant communities. We demonstrated that *I. cylindrica* invasion and drought do not act synergistically; that is, drought did not promote invasion or exacerbate invasion impacts (Hamilton et al. 1999). Instead, the invader ameliorated effects of the drought by maintaining soil moisture. Regardless, high losses of diversity, likely due in part to intense light competition with the invader, indicate that even invaders with high water use efficiency will be problematic under future drought scenarios. Moreover, even in the absence of synergistic or additive interactions, combinations of multiple global change drivers can reduce biodiversity and alter community structure in threatened ecosystems. Therefore, although drought may not enhance susceptibility of this ecosystem to invasion, low biotic resistance indicates that invasions are a major threat to their persistence under current and future precipitation regimes. Removal of such high-impact invaders should be a top priority of natural areas management.

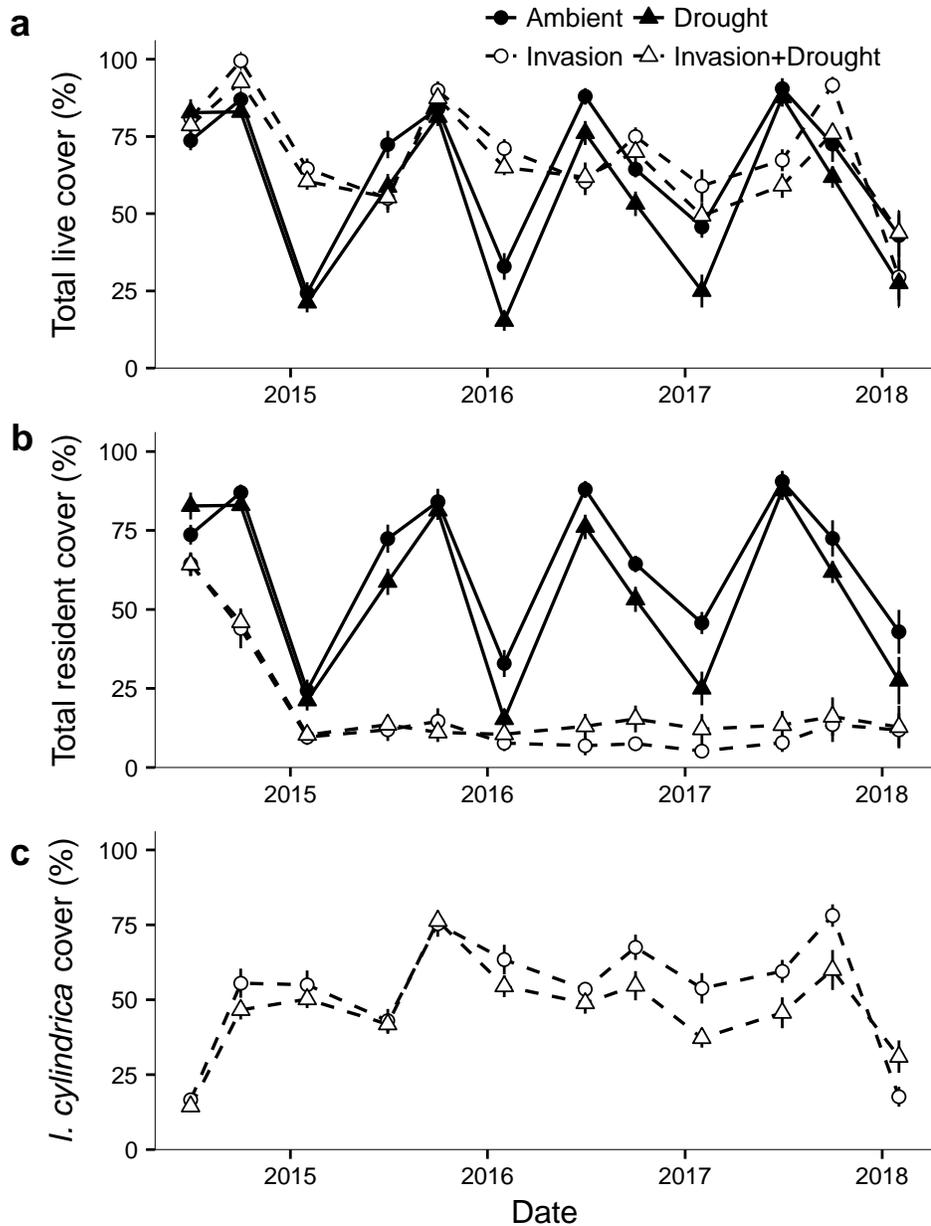


Figure 2-1. Seasonal changes in vegetation cover over 4 years. Percent cover of (a) total live vegetation, (b) resident vegetation (i.e., species other than *Imperata cylindrica*), and (c) *Imperata cylindrica* (Mean \pm SE; N=10). Ambient = Uninvaded plots with ambient precipitation, Drought = Uninvaded plots with experimental drought, Invasion = Plots invaded with *I. cylindrica* and ambient precipitation, Invasion+Drought = Plots invaded with *I. cylindrica* and experimental drought.

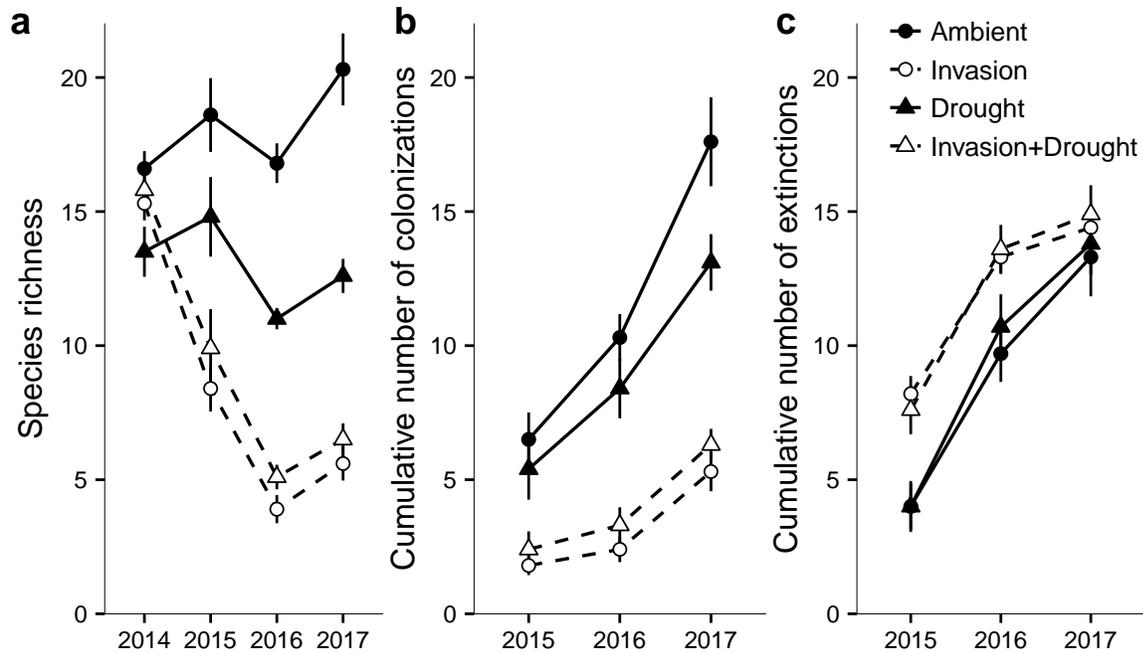


Figure 2-2. Plant community dynamics in response to the individual and interactive effects of invasion and drought. (a) Plant species richness; (b) cumulative number of plot level colonization and (c) extinction events measured in the summer of each year (Mean \pm SE; N=10). For (b) and (c), year indicates the end of the time period over which colonization and extinction were measured.

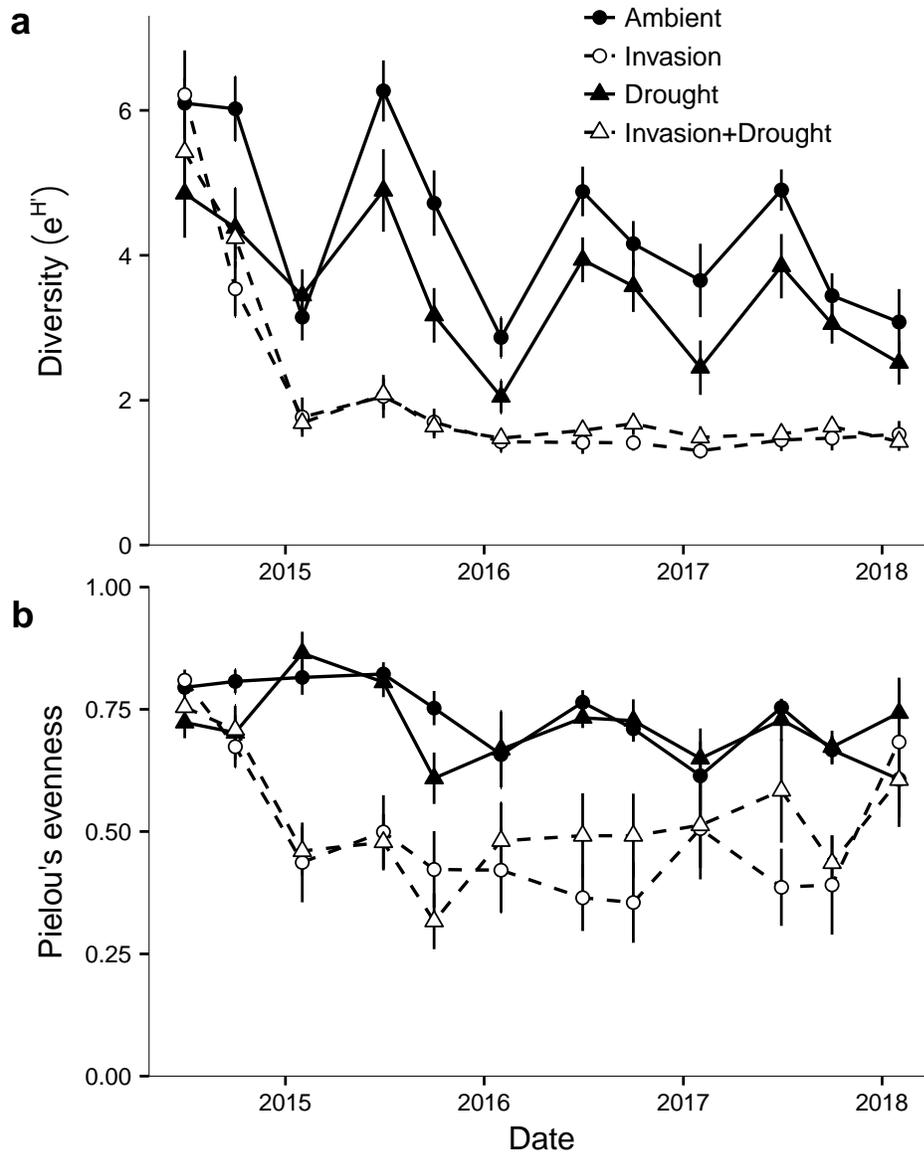


Figure 2-3. Seasonal changes in diversity metrics in response to the individual and interactive effects of invasion and drought. (a) Diversity ($e^{H'}$) and (b) Pielou's evenness per plot over time (Mean \pm SE; N=10).

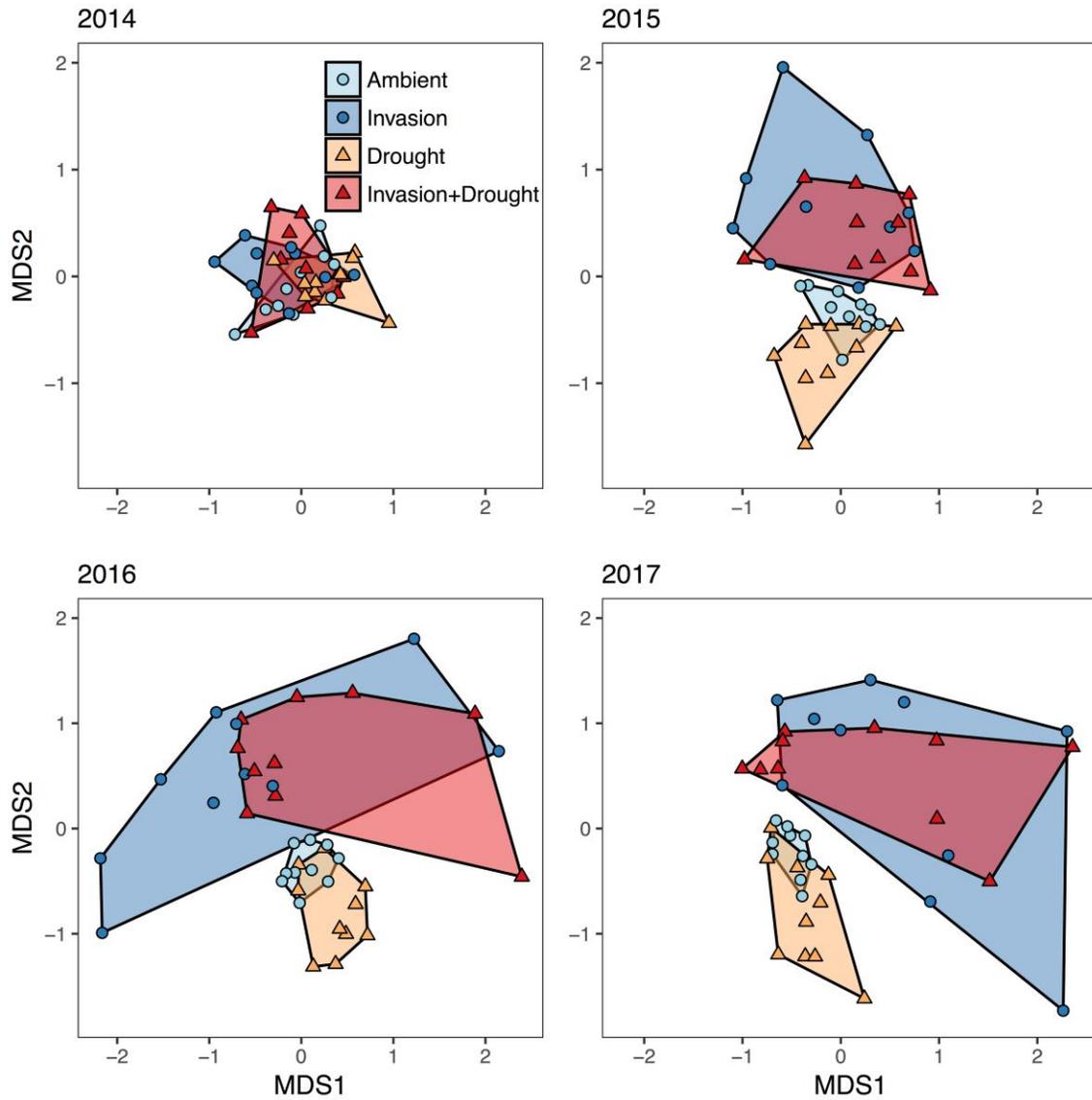


Figure 2-4. Non-metric multidimensional scaling (NMDS) ordination plots of summer plant community composition 2014 – 2017. Ordination based on Bray-Curtis dissimilarity among plots with *I. cylindrica* excluded from the analysis. Two-dimensional NMDS stress: 2014 = 0.187, 2015 = 0.204, 2016 = 0.164, 2017 = 0.170.

CHAPTER 3
PLANT INVASION AND DROUGHT INTERACTIVELY STRUCTURE SOIL MICROBIAL
COMMUNITIES

Background

Soil provides essential ecosystem services such as nutrient cycling and water storage, and soil microbial communities can moderate provisioning of these services. However, responses of soil microbes to interacting global change factors, such as changes in precipitation, nitrogen deposition, or introduction of invasive species, remain difficult to predict. Some studies have found significant changes in microbial community composition in response to multiple global change factors (Castro et al. 2010), whereas others have found no changes in fungal and bacterial community composition (Carey et al. 2015) or that global change impacts are overshadowed by seasonal variation (Matulich et al. 2015). Additionally, microbial communities may respond directly to abiotic changes such as increased temperature or drought (Sheik et al. 2011), but indirect effects via changes in plant communities also may occur (Zak et al. 2003, Berg and Smalla 2009, Lange et al. 2014). Plant invasion can cause profound shifts in plant species composition, driving associated changes in soil microbial communities (Kourtev et al. 2002b). Decreased precipitation has also been shown to alter the soil microbiome (Ochoa-Hueso et al. 2018); however, it is presently unknown how these factors interact to influence soil microbial communities. For example, invasive plants could alter the response of soil moisture to drought either through physiological (e.g., water-use efficiency) or biophysical (e.g., shading) effects. Conversely, drought could influence the survival or competitive success of invasive plants. Therefore, additional research is needed to tease apart responses of soil microbes to interacting biotic and abiotic global change drivers.

Plant communities play a major role in shaping soil microbial communities through effects of root structure and root exudation as well as litter inputs and microclimate control (Zak

et al. 2003, Burns et al. 2015). Plant species can be particularly strong drivers of certain microbial groups such as plant pathogens and mutualists, which often have specialist plant hosts or variable host responses (Bever 2002, Augspurger and Wilkinson 2007, Wang et al. 2012). Therefore, marked changes in plant community composition such as those that occur during plant invasion are likely to have major impacts on microbial communities and these effects may differ across microbial functional groups, such as mycorrhizal fungi or plant pathogens; however, few studies have assessed the response of different microbial taxa to invasion. Some invasive plants modify the soil microbiome in ways that have negative impacts on native plant species, including reductions in mutualists that benefit native species and accumulation of pathogens that disproportionately harm native species (Eppinga et al. 2006, Stinson et al. 2006, Batten et al. 2008, Barto et al. 2011). Thus, plant invaders have the potential to modify soil microbiomes in ways that benefit invader performance, but not all invaders display this ability (Del Fabbro and Prati 2015) and a better understanding of which microbial groups could drive these responses is needed.

Climate change is likely to increase the global frequency and severity of droughts (Burke et al. 2006, Singh et al. 2013), with major consequences for microbial communities. Drought can cause rapid declines in microbial activity as well as shifts in abundance of microbial taxa such as Actinobacteria, Chloroflexi, and Glomeromycota, with consequences for ecosystem function and plant-microbe interactions (Castro et al. 2010, Manzoni et al. 2011, Ochoa-Hueso et al. 2018). However, plants can ameliorate the impacts of drought on microbes through changes in microclimate and plant-water relations (Alba et al. 2017). Furthermore, certain microbial groups have been shown to mediate plant response to drought (Augé 2001, Yang et al. 2009). Therefore,

plant-microbe interactions are likely to play an important role in ecosystem response to climate change induced drought.

Microbial responses to environmental factors have historically focused on total microbial biomass, fungal:bacterial ratios, or individual microbial groups (Allen et al. 1995, Blankinship et al. 2011, Bragazza et al. 2015); however, treating microbial communities as a “black box” can limit our ability to understand the ecological interactions occurring belowground. High throughput sequencing methods allow for a more detailed picture of changes in microbial communities at different taxonomic levels as well as for the organization of microbes into general functional groups (Nguyen et al. 2016). These methods have yet to be used to evaluate the response of soil microbial communities to the interaction of plant invasion and climate change. In addition to assessing microbial diversity and overall community composition, the response of particular microbial taxa and functional groups to changes in the abiotic and biotic environment now can be assessed (Matulich et al. 2015, Anthony et al. 2017), thereby facilitating evaluation of how microbial responses to global change shape whole ecosystem function (Cline et al. 2018b).

To evaluate the potential interactive effects of plant invasion and drought on soil microbial communities, we sampled soil from a long-term, fully crossed invasion x drought field experiment and sequenced bacterial and fungal communities (the dominant components of the soil microbiome). Our overarching question was: How do plant invasion and drought independently and interactively affect bacterial and fungal diversity and microbial community composition? In Chapter 2, analysis of the effects of invasion and drought on resident plant communities indicated 58% decrease in richness in response to plant invasion compared with a 28% decrease in response to drought. Because plant communities influence microbial diversity,

we hypothesized that microbial communities would respond to plant invasion and drought, including a more dramatic response to invasion and less extreme response to drought (Thakur et al. 2015). In particular, we expected this pattern to hold true for plant-dependent species, such as plant pathogens and mycorrhizal fungi. However, because bacteria are typically more vulnerable to drought than fungi, and certain bacterial and fungal groups are known to respond differently to wet versus dry conditions (Castro et al. 2010, Maestre et al. 2015, Zhang et al. 2016, Meisner et al. 2018), we hypothesized that drought would have a larger effect on these sensitive taxa. The interaction between invasion and drought is more difficult to predict because the invader in our system (*Imperata cylindrica*) is relatively resistant to drought and also moderated drought effects on soil moisture (Alba et al. 2017; Chapter 2). Thus, we hypothesized that microbial community responses to invasion might be similar in the ambient and drought plots because the invasion may moderate the response of microbial communities to drought.

Methods

Study System

Longleaf pine (*Pinus palustris*) dominated forests historically covered ~30 million hectares of the southeastern US, making up more than 50% of upland areas, with an additional 33% of upland areas in mixed stands including longleaf pine. Longleaf pine forests cover less than 3% of their original extent and major efforts are focused on restoring these ecosystems (Frost 2007). Longleaf pine forests have an open canopy and diverse understory plant communities maintained by fire, but invasive species such as *Imperata cylindrica* (cogongrass) can greatly reduce diversity and threaten rare native plant species (Hardin and White 1989, Walker and Silletti 2007, Brewer 2008).

Imperata cylindrica is a globally distributed invasive grass infesting over 500 million ha in tropical and subtropical regions worldwide. It was introduced to the United States from Asia

and has invaded the southeastern US from Florida to Virginia and westward to Texas (Estrada and Flory 2015). Climate models suggest that *I. cylindrica* invasion will accelerate under future climate change (Bradley et al. 2010b), but experimental evidence of cogongrass response and impacts under climate change are lacking. The southeastern US is expected to experience increased frequency and duration of drought over the next several decades (Singh et al. 2013), and therefore it is of critical importance that the impacts of *I. cylindrica* invasion and drought on native ecosystems be evaluated.

Experimental Design

The study site was located at the University of Florida Bivens Arm Research Site (BARS) in Gainesville, Florida, USA (29°37'42"W, 82°21'14.4"W). Mean annual temperature and precipitation are 20.5°C and 1300 mm, respectively. Soils are primarily Portsmouth sandy loam (78% sand, 19% silt, and 3% clay) composed of Blichton sand (25%; 2%–5% slope) and Bivans sand (75%; 5%–8% slope; Natural Resources Conservation Service, Web Soil Survey). The study area was mowed and tilled to prepare the site. In May 2012, we established plant communities in 40, 4 m x 4 m plots with 36 native perennial grass and forb seedlings (12 spp. x 3 individuals; The Natives Inc., Davenport, FL) and 20 longleaf pine seedlings per plot (Florida Forest Service, Chiefland, FL).

In spring 2013, a fully crossed combination of *I. cylindrica* invasion and precipitation reduction (hereafter referred to as “drought”) was applied (10 replicates per treatment combination). We planted nine *I. cylindrica* seedlings into each invasion plot. The drought treatment consisted of rainout shelters with 89% polycarbonate roofing cover (TUFTEX PolyCarb, Fredericksburg, VA) and gutters connected to pipes to move the water off site. Around each plot belowground plastic barriers were inserted to 1 m depth (to prevent subsurface

flow of water), and aluminum flashing extended 10 cm above the soil surface (to reduce overland flow). We constructed structures over no-drought control plots (hereafter “ambient”) with 22% white shade cloth to mimic shading by the rainout shelters. The experimental setup is described in detail in Alba *et al.* (2017).

Soil samples were collected in May 2016 from the experimental plots using a 5 cm diameter hammer corer. Surface plant litter was removed, and three cores were extracted per plot to a depth of 15 cm. All roots were hand sorted from the soil and separated into fine (<1 mm diameter) and coarse (>1 mm) fractions, washed, dried at 60 °C for 48 hours, and weighed. The 5-15 cm fraction of each of the three cores per plot was homogenized through a 2 mm sieve and composited. Soils were then frozen at –10 °C until DNA extraction. A 5 g subsample of fresh soil was dried at 105 °C for 72 hours and weighed to calculate gravimetric soil moisture.

DNA Extraction, PCR, and Illumina Sequencing

Genomic DNA was extracted from each soil sample (0.25 g) using MoBio PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The 16S rRNA and fungal ITS genes were amplified for each sample using primer sets of F515/R806 (Bates et al. 2011) and 5.8S-FUN/ITS-FUN (Taylor et al. 2016), respectively. The primers were modified for the Illumina platform by fusing CS1 and CS2 linker primers for forward and reverse primers, respectively.

Polymerase chain reactions were conducted with 50 µL assays. For the 16S amplification, 25 µL of GoTaq® Colorless Master Mix (Promega, Madison, Wisconsin, USA), 5 µL of BSA (5 ng µL⁻¹), 1 µL of each primer (10 µM), 15 µL of PCR-grade water, and 3 µL of a genomic DNA template (5 ng µL⁻¹) were mixed in a 200-µL PCR tube for each sample. The following thermal profile was used for PCR: an initial denaturation and enzyme activation step

of 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 sec, 50 °C for 60 sec, and 72 °C for 90 sec, with a final extension of 72 °C for 10 min. For the fungal ITS amplification, 25 µL of GoTaq® Colorless Master Mix, 5 µL of BSA (5 ng µL⁻¹), 0.8 µL of each primer (10 µM), 18.4 µL of PCR-grade water, and 18.4 µL of a genomic DNA template (5 ng µL⁻¹) were mixed in a 200-µL PCR tube for each sample. The following thermal profile was used for the fungal PCR: an initial denaturation and enzyme activation step of 96 °C for 2 min, followed by 30 cycles of 94 °C for 30 sec, 58 °C for 40 sec, and 72 °C for 120 sec, with a final extension of 72 °C for 10 min. Qualities of PCR products were evaluated by agarose gel electrophoresis. Additional rounds of PCR were performed to fuse CS1/CS2 linker primers to the indices and adapters before Illumina MiSeq sequencing at Génome Québec (Montréal, Québec, Canada).

Sequence Data Processing

QIIME 1.9.0 toolkit (Caporaso et al. 2010) was used to process the Illumina sequences. Chimeric 16S and ITS sequences were identified using reference-based (May 2013 version of Greengenes database, (McDonald et al. 2012)) and abundance-based methods, respectively, via USEARCH (Edgar 2010), and removed for downstream analyses. Operational taxonomic units (OTUs) were determined at the $\geq 97\%$ similarity level of the nucleotide sequences (Stackebrandt and Goebel 1994) using the open-reference OTU picking option. Taxonomy was assigned to each OTU via Greengenes (McDonald et al. 2012) and UNITE (Kõljalg et al. 2013) databases for 16S and ITS sequences, respectively.

For the 16S sequences, *de novo* sequences, which accounted 90.1% of OTUs but only 18.6% of the total sequences, were removed for downstream analyses. After non-bacterial sequences and singletons were removed, remaining 16S sequences were aligned using PyNAST (Caporaso et al. 2010) to build a phylogenetic tree using FastTree (Price et al. 2009). The

bacterial sequences were rarefied at 71,166 sequences per sample, and analyzed via Phylocom (Webb et al. 2008). The package “vegan” (Oksanen et al. 2016) in R 3.4.1 (R Development Core Team 2016) was used for dbRDA.

For the fungal ITS sequences, *de novo* sequences, which accounted for 45.2% of OTUs but only 0.7% of the total sequences, were removed for downstream analyses. The fungal data were rarefied at 25,650 sequences per sample. Analyses were conducted for total fungal sequences and for arbuscular mycorrhizal (AM) fungi, a monophyletic group comprising the phylum Glomeromycota, as a subset of total fungi. FUNGuild was employed to categorize the OTUs into functional groups, including pathogens, saprotrophs, and mutualists (Nguyen et al. 2016). We used only taxa for which the confidence ranking for guild assignment was “probable” or “highly probable” and a unique role was identified in FUNGuild.

Statistical Analyses

Statistical analyses were conducted in R 3.4.1 (R Development Core Team 2016). Mixed-effect ANOVAs using the *nlme* package (Pinheiro et al. 2016) were conducted with the invasion and drought treatments as fixed effects and block as a random effect. We used OTU richness, Shannon diversity index, Pielou’s evenness, and Chao1 index (Chao 1984) as diversity indicators for bacterial and fungal communities. Microbial community composition was analyzed separately for bacteria, whole fungal community, and AM fungal community using ordination by nonmetric multidimensional scaling (NMDS). For the bacterial community we used the weighted and unweighted UniFrac distance matrix to account for phylogenetic distance, and for the fungal community we used the Bray-Curtis dissimilarity matrix because UniFrac distance is not valid for the ITS region. We tested for differences in community composition with invasion, drought, and their interactions with a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and permutations constrained within each block. NMDS scores were

calculated with the ‘metaMDS’ function of the vegan package (Oksanen et al. 2016). PERMANOVA was calculated with the ‘adonis’ function in vegan. We used the ‘envfit’ function in vegan to determine correlations between environmental characteristics and community composition. We used Mantel tests to determine the correlations between plant community distance, bacterial community distance, and fungal community distance using the ‘mantel’ function in vegan.

Results

Bacterial Community

The invasion and drought treatments significantly affected bacterial diversity (Figure 3-1 a). The drought treatment effects were evident for all measures of diversity, including decreased Shannon diversity index, evenness, OTU richness, and Chao 1 index (Table B-1). Invasion by *I. cylindrica* significantly increased Shannon diversity index and evenness of soil bacteria but had no effect on richness or Chao 1 index (Table B-1).

Soil bacterial community structure was also significantly altered by *I. cylindrica* invasion and drought. PERMANOVA of unweighted UniFrac distance showed a significant invasion x drought interaction, where drought had the largest effect and invasion had a smaller effect and invasion+drought had an intermediate effect (Table B-5). Only drought was a significant predictor of bacterial community structure based upon weighted UniFrac distance (Figure 3-2 a-b; Table B-4).

Bacterial Taxa

Relative abundance of bacterial taxa was fairly consistent across plots and treatments with Acidobacteria, Proteobacteria, and Verrucomicrobia as the dominant phyla (Figure 3-3), but individual phyla were affected by the treatments. Thus, we analyzed the effect of invasion and drought on the most abundant phyla and families (>1% relative abundance). Actinobacteria were

20% more abundant in drought than ambient plots while Bacteroidetes, Nitrospirae, and Planctomycetes were 11-32% less abundant (Drought (D): $F_{1,27} > 5.4$, $P < 0.03$).

Verrucomicrobia were 15% more abundant in drought than ambient plots but 16% less abundant in invaded plots than uninvaded plots (Invasion (I): $F_{1,27} = 17.2$, $P = 0.0003$; D: $F_{1,27} = 10.1$, $P = 0.004$). Acidobacteria, Chloroflexi, and Firmicutes were not significantly affected by the treatments (Figure 3-5).

Four bacterial families had higher relative abundance under drought (Acidobacteriaceae, Bradyrhizobiaceae, Gaiellaceae, and Koribacteraceae), while three families had lower relative abundance under drought (Chitinophagaceae, Ellin515, and Syntrophobacteraceae) (D: $F_{1,27} > 5.2$, $P < 0.03$). Chthoniobacteraceae relative abundance decreased by 21% in response to invasion and increased by 29% in response to drought (I: $F_{1,27} = 19.6$, $P = 0.0001$; D: $F_{1,27} = 23.7$, $P < 0.0001$), while Sinobacteraceae increased by 21% in response to invasion (I: $F_{1,27} = 5.3$, $P = 0.03$). Solibacteraceae were 44% more abundant in uninvaded drought plots than uninvaded ambient plots but no different in the invaded plots (I x D: $F_{1,27} = 7.2$, $P = 0.01$). Five families showed no significant response to the treatments (Bacillaceae, Burkholderiaceae, Gemmataceae, Hyphomicrobiaceae, Rhodospirillaceae).

Relative abundance of bacteria associated with nitrification, including *Nitrospira* (primarily a nitrite oxidizer) and *Nitrosovibrio* (an ammonium oxidizer), was interactively affected by the invasion and drought treatments (I x D: $F_{1,27} = 9.0$, $P = 0.006$). Specifically, nitrifiers were similarly abundant in the ambient and drought uninvaded plots and the invaded drought plots, but more abundant in the invaded ambient plots. The relative abundance of nitrogen fixing bacteria including *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Frankia*,

Methylobacterium, *Azospirillum*, was 52% higher in invaded plots than uninvaded plots (I: $F_{1,27} = 8.3$, $P = 0.008$).

Fungal Community

Fungal diversity was not significantly affected by the invasion or drought treatments (Figure 3-1 b). While fungal OTU richness, diversity, and evenness were slightly higher in the invaded drought treatment than the other three treatments, differences were not statistically significant (Table B-2). Invasion and drought interactively affected community composition of fungi (Table B-6; Figure 3-2 c), where the invaded ambient plots and uninvaded drought plots were different from the uninvaded ambient plots, but the invaded drought plots were similar to the uninvaded ambient plots.

Fungal Taxa

Relative abundance of fungal phyla was highly variable across plots and treatments (Figure 3-4). We analyzed the effects of the treatments on the most abundant fungal phyla and families (>1% relative abundance). The only fungal phylum affected by the treatments was Glomeromycota (AM fungi), which were 40% less abundant under drought (D: $F_{1,27} = 5.06$, $P = 0.03$). Among the fungal families, Atheliaceae were nearly 10 times more abundant in invaded ambient plots than uninvaded ambient plots, whereas they were only twice as abundant in invaded drought plots as uninvaded drought plots (I x D: $F_{1,27} = 4.7$, $P = 0.04$). Chaetomiaceae, Hypocreaceae, Nectriaceae, and Trichocomaceae were more abundant under drought in uninvaded plots but unaffected by drought in invaded plots (I x D: $F_{1,27} > 5.5$, $P < 0.03$). Thelephoraceae were 90% less abundant in invaded plots but unaffected by drought (I: $F_{1,27} = 4.37$, $P = 0.046$). Chaetosphaeriaceae, Cortinariaceae, Lipomycetaceae, Rhizophydiaceae were not significantly affected by the treatments (Figure 3-6).

There was a significant interaction between invasion and drought on AM fungal diversity, where diversity was 6% lower under drought in uninvaded plots but 10% higher under drought in invaded plots (I x D: $F_{1,27} = 6.3$, $P = 0.02$; Figure 3-1). There were no significant effects of invasion or drought on AM fungal richness or evenness (Table B-3), but community composition of AM fungi was significantly affected by both invasion and drought (I: Pseudo-F = 1.6, $P = 0.04$, D: Pseudo-F = 2.6, $P < 0.001$; Figure 3-2).

Fungal Guilds

We classified fungal OTUs into trophic modes and guilds based on the FUNGuild database. In particular, we were interested in mycorrhizal fungi and plant pathogens as these groups are likely to play a role in plant community dynamics. In the fungal dataset, 39.5% of OTUs were identified as having known guilds. Fungal guilds were interactively influenced by the invasion x drought treatments (I x D x Guild; $F_{3,135} = 6.2$, $P < 0.001$). Pathotrophs, which include plant and animal pathogens as well as fungal parasites, were significantly more abundant in invaded ambient than invaded drought plots but not different in uninvaded ambient and drought plots (I x D; $F_{1,27} = 5.9$, $P = 0.02$; Figure 3-7 a). These differences were driven mainly by plant pathogens (I x D; $F_{1,27} = 4.5$, $P = 0.04$; Figure 3-8 c). Saprotrophs were more abundant in uninvaded drought plots than uninvaded ambient plots, but not influenced by drought in invaded plots (I x D; $F_{1,27} = 8.9$, $P = 0.006$; Figure 3-7 b). Symbionts as a whole were not significantly affected by the treatments (Figure 3-7 c) and neither were ectomycorrhizal fungi (Figure 3-8 b).

Drivers of Microbial Community Structure

Although the bacterial and fungal communities were significantly correlated ($r = 0.19$, $P = 0.002$), neither bacterial nor fungal communities were significantly related to the plant community composition based on a Mantel test of the Bray-Curtis dissimilarity matrices (bacterial: $r = -0.06$, $P = 0.93$; fungal: $r = -0.04$, $P = 0.84$). We assessed the role of several biotic

and abiotic environmental factors, including total plant cover, plant species richness, fine root biomass, coarse root biomass, bulk density, and mean percentage of sand, silt, and clay in the soil, in structuring microbial communities. Fine root biomass was twice as high in invaded versus uninvaded plots and 25% lower in drought than ambient plots (I: $F_{1,27} = 19.8$, $P = 0.0001$; D: $F_{1,27} = 3.9$, $P = 0.06$). Coarse root and rhizome biomass was interactively affected by invasion and drought where drought decreased coarse root biomass by 71% compared to ambient plots in uninvaded treatment, while drought decreased coarse root biomass by only 43% compared to ambient in invaded plots (I x D: $F_{1,27} = 7.4$, $P = 0.01$; Figure B-2). On average, coarse root biomass was over six times higher in invaded than uninvaded plots. Fine root biomass and total plant cover were significant predictors of bacterial community composition (fine root biomass: $R^2 = 0.22$, $P = 0.03$; plant cover: $R^2 = 0.16$, $P = 0.01$), while none of the environmental factors assessed predicted fungal community composition.

Discussion

Our results demonstrate that soil bacterial communities in the longleaf pine ecosystem are primarily driven by drought with more moderate responses to invasion by *I. cylindrica*. Fungal diversity was unaffected by the invasion and drought treatments but fungal community composition and some particular fungal taxa and functional groups responded interactively to invasion and drought.

The greater response to drought of bacterial diversity than fungal diversity fits with our hypothesis and is consistent with previous studies that documented greater sensitivity of bacteria to soil moisture conditions in a variety of systems (Gordon et al. 2008, Clark et al. 2009, Cregger et al. 2012, Barnard et al. 2013). The lack of fungal diversity response to invasion did not fit our expectation, which was based on the idea that higher plant diversity would create greater fungal niche space (Cline et al. 2018a). However, fungal community composition changed in response

to invasion and drought, which suggests that while the total number and relative abundance of OTUs remains relatively consistent, fungal community reordering occurs to favor different OTUs in response to the treatments (Lankau and Lankau 2014, Roy-Bolduc et al. 2016).

Invasion had dramatic effects on the plant community and total root biomass, while drought had more moderate effects (Chapter 2; Figure B-2); hence, we expected that bacterial and fungal communities would be strongly influenced by invasion and only moderately by drought.

Relative abundance of bacterial phyla responded strongly to drought. In particular, we found that Actinobacteria were favored under drought, while Bacteroidetes, Nitrospirae, Planctomyces, and Proteobacteria were less abundant in drought plots. Previous studies have shown responses of bacterial taxa to drought that are not fully consistent across studies, but Actinobacteria appear to consistently be associated with drought or aridity (Battistuzzi and Hedges 2009, Acosta-Martínez et al. 2014, Evans et al. 2014, Maestre et al. 2015, Ochoa-Hueso et al. 2018). Furthermore, most previous studies on the impacts of drought have been conducted in dryland systems (Clark et al. 2009, Cregger et al. 2012, Maestre et al. 2015, Ochoa-Hueso et al. 2018); therefore more studies are needed to identify patterns of bacterial responses to drought within and across systems. In our study, the only phylum that exhibited a response to both drought and invasion was Verrucomicrobia, which was more abundant in drought plots and less abundant in invasion plots. From a functional standpoint, bacterial genera of particular significance for the nitrogen cycle responded strongly to invasion (Sy et al. 2001, Lee et al. 2012, Bahulikar et al. 2014) so that interactions with N-cycling and *I. cylindrica* invasion are likely.

Within phyla, bacteria are highly diverse in function and metabolism but studies have shown ecological coherence at the level of order and below (Philippot et al. 2010). Bacterial families were most often affected by drought with seven of the 15 most abundant families

responding only to drought, however these families showed diverse ecological functions including aerobes and anaerobes, freeliving heterotrophs and endosymbionts, and some families for which little is known. One family responded only to invasion, one family to invasion and drought independently, and one family to the interaction of invasion and drought. These results suggest that at multiple taxonomic levels, bacteria are more sensitive to soil moisture conditions than to plant invasion and the concurrent alteration of plant communities.

While overall diversity of fungi was unaffected by the invasion and drought treatments, community composition and lower taxonomic levels responded to the treatments. Community composition of fungi in the uninvaded ambient plots was similar to the invaded drought plots, but dissimilar to the invaded ambient plots and uninvaded drought plots (Figure 3-2 d). This result suggests that invasion and drought may counteract each other in combination to alleviate their effects. At the level of phylum, only relative abundance of Glomeromycota (AM fungi) was affected by the treatments. AM fungal relative abundance decreased in response to drought alone (Ochoa-Hueso et al. 2018), while community composition responded to both invasion and drought. AM fungal diversity displayed an interactive response to drought and invasion where, in uninvaded plots, drought lowered AM fungal diversity but in invaded plots drought drove greater AM fungal diversity (Figure 3-1 c). Results from previous studies on AM fungal response to invasion have been mixed, with some studies showing decreased AM fungal inoculum potential and diversity, as well as shifts in community composition, and others showing no response to invasion even in studies of the same invader (Roberts and Anderson 2001, Hawkes et al. 2006, Mummey and Rillig 2006, Stinson et al. 2006, Burke 2008, Barto et al. 2011, Koch et al. 2011). Furthermore, many of these studies have focused on a single non-mycorrhizal invader, *Alliaria petiolata*, and mycorrhizal invaders may have different effects on AM fungal communities.

Imperata cylindrica associates with AM fungi, so the change in community composition but similarity in overall AM fungal abundance in invaded plots may indicate that the invader maintains mycorrhizal symbiosis while altering the AMF species composition to benefit its own growth.

Multiple fungal families composed mainly of saprotrophs and pathogens showed the same interactive response to the treatments where their abundance was higher in uninvaded drought plots than uninvaded ambient plots, but no different across the invaded plots (Chaetomiaceae, Hypocreaceae, Nectriaceae, and Trichocomaceae). This pattern may indicate that these taxa are drought tolerant and that the greater soil moisture associated with the invader in drought plots was sufficient to reduce the abundance of drought-tolerant taxa (Chapter 2). Additionally, total saprotroph abundance was higher in drought than ambient plots in the uninvaded treatment (Figure 3-7). This response may reflect increased detritus for decomposition under drought in uninvaded plots (Treseder et al. 2016). The lack of difference in the abundance of saprotrophs between the ambient and drought treatment in the invaded plots could be due to the low-quality litter of *I. cylindrica* (Hagan et al. 2013a) or that the invader was more resistant to the drought treatment as seen in Chapter 2. Pathotrophs, and specifically plant pathogens, were most abundant in invaded ambient plots (Figure 3-7 & Figure 3-8) indicating that the invader can accumulate pathogens, but it is unknown whether these pathogens have differential effects on native plant species as has been seen for other invaders (Mangla et al. 2008). In the invaded drought plots, however, the abundance of plant pathogens was lower, indicating that the pathogens associated with invasion may be susceptible to drought. Symbiotrophs as a whole and ectomycorrhizal fungi were slightly less abundant in invaded plots but neither trend was significant. On the other hand, certain ectomycorrhizal families did show significant responses.

For example, Thelephoraceae were less abundant in the invaded plots. Conversely, Atheliaceae, which contains some ectomycorrhizal fungi but also saprotrophs, were more abundant in invaded ambient plots, but showed low abundance in all other treatment combinations. Therefore, even within functional groups, fungi still show taxa-specific responses.

The drivers of soil microbiome structure are mixed and taxon specific. While bacterial and fungal communities were strongly correlated, neither was significantly related to patterns in the plant community. This result differs from previous findings from California grasslands suggesting both bacterial and fungal communities are correlated with plant communities (Matulich et al. 2015). Additionally, plant invasion was a weaker driver of bacterial communities than drought. Drought reduced all measures of bacterial diversity as well as bacterial community composition, while invasion increased diversity and evenness but not richness and Chao1 index. The majority of abundant bacterial phyla and families responded to the drought treatment, though different taxa either increased or decreased under drought. These observations indicate that abiotic conditions are stronger drivers of bacterial communities than invasion and consequent changes in plant communities. However, bacteria associated with the nitrogen cycle were more abundant with invasion. Fungal diversity was comparatively resilient to both invasion and drought; however, community composition and specific fungal taxa responded to both drought and invasion. Additionally, AM fungi were less abundant under drought, but plant pathogens were more abundant with invasion. Our results provide a better understanding of the drivers of soil microbiomes, and their potential role in ecosystem function and plant community dynamics.

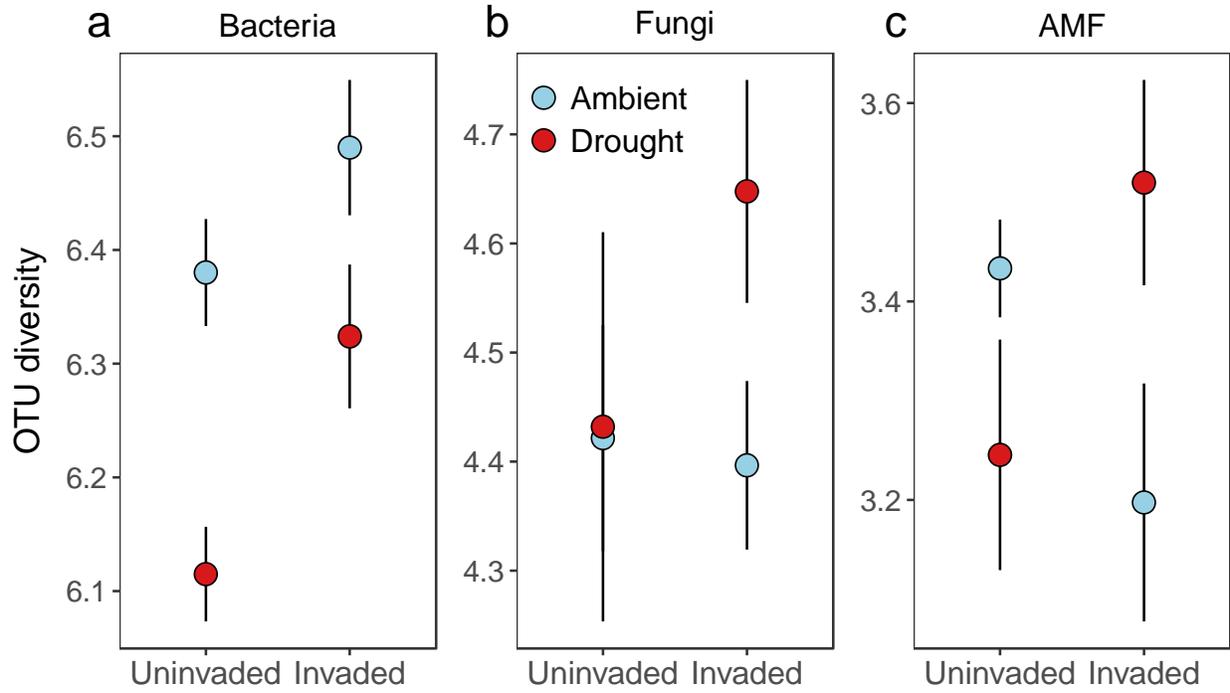


Figure 3-1. Shannon diversity index of a) bacterial, b) all fungal, and c) arbuscular mycorrhizal fungal OTUs.

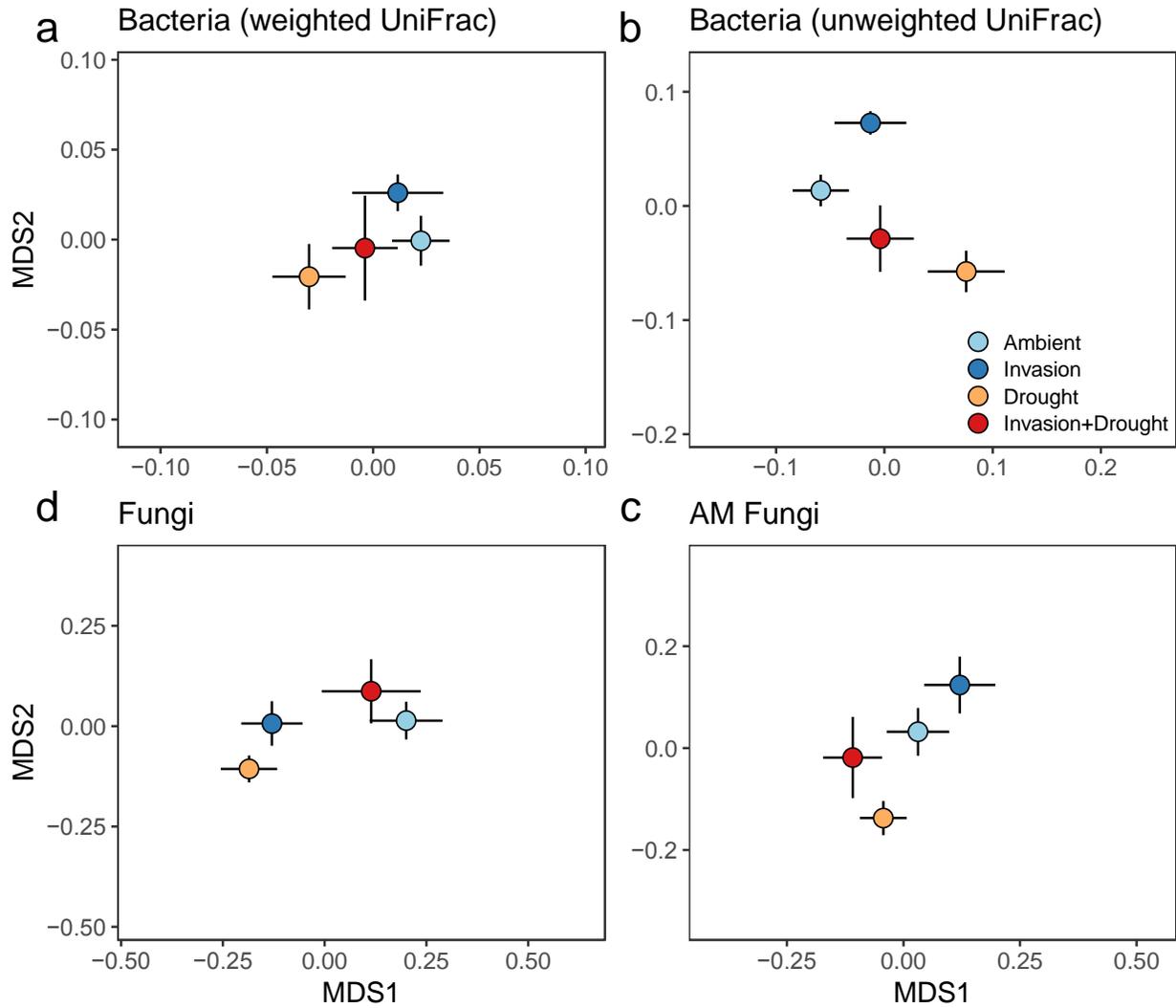


Figure 3-2. Nonmetric multidimensional scaling ordination of a) weighted UniFrac distance of the bacterial community (considers relative abundance), b) unweighted UniFrac distance of the bacterial community (considers presence-absence), c) Bray-Curtis dissimilarity of the whole fungal community, d) Bray-Curtis dissimilarity of the arbuscular mycorrhizal fungal community. Larger points with error bars indicate the mean \pm SE of the 10 plots per treatment and smaller background points show each plot NMDS values.

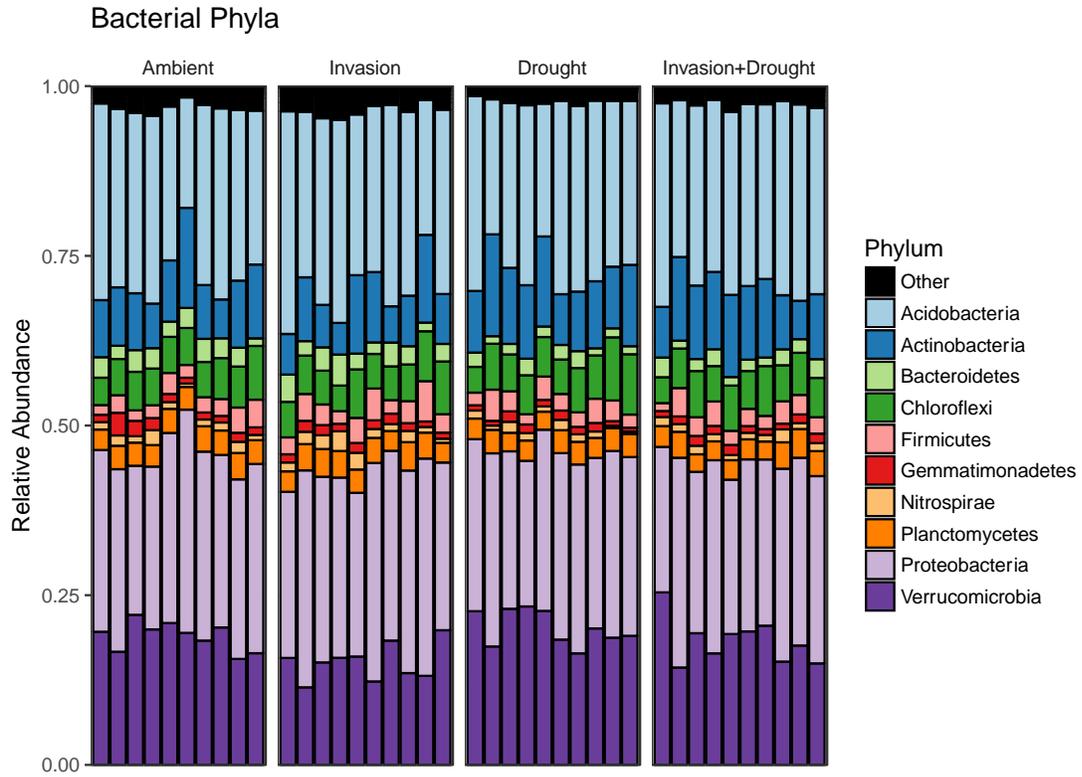


Figure 3-3. Relative abundance of bacterial phyla by plot in each invasion x drought treatment. Each bar represents one plot.

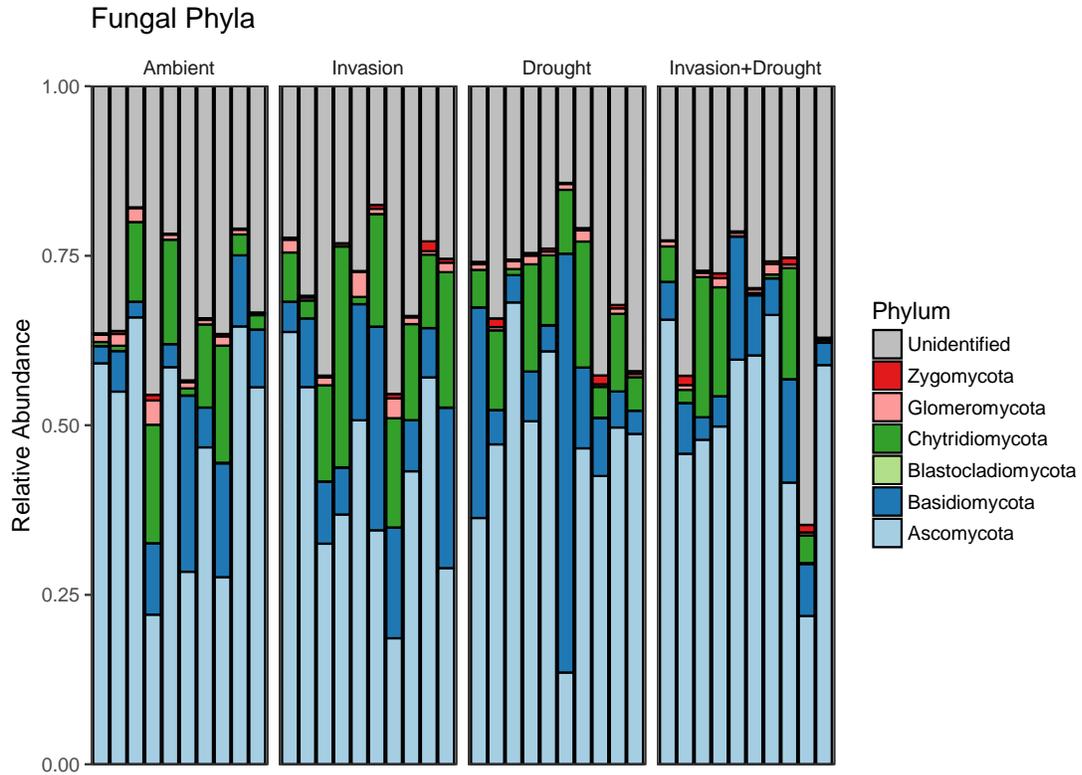


Figure 3-4. Relative abundance of fungal phyla by plot in each invasion x drought treatment. Each bar represents one plot.

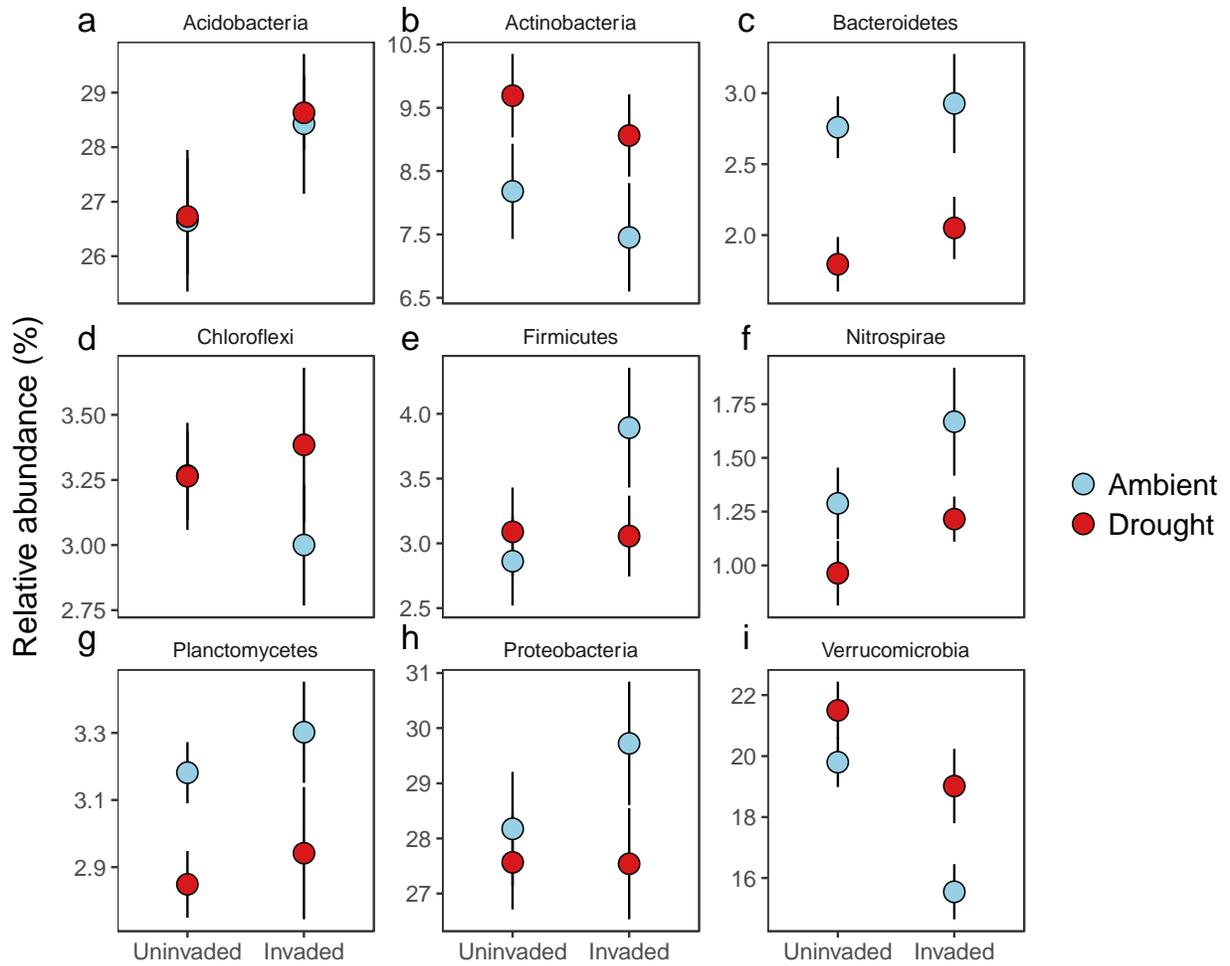


Figure 3-5. Relative abundance of the most abundant bacterial phyla (>1% mean relative abundance) in response to invasion and drought treatments (mean \pm standard error): a) Acidobacteria, b) Actinobacteria, c) Bacteroidetes, d) Chloroflexi, e) Firmicutes, f) Nitrospirae, g) Planctomycetes, h) Proteobacteria, i) Verrucomicrobia.

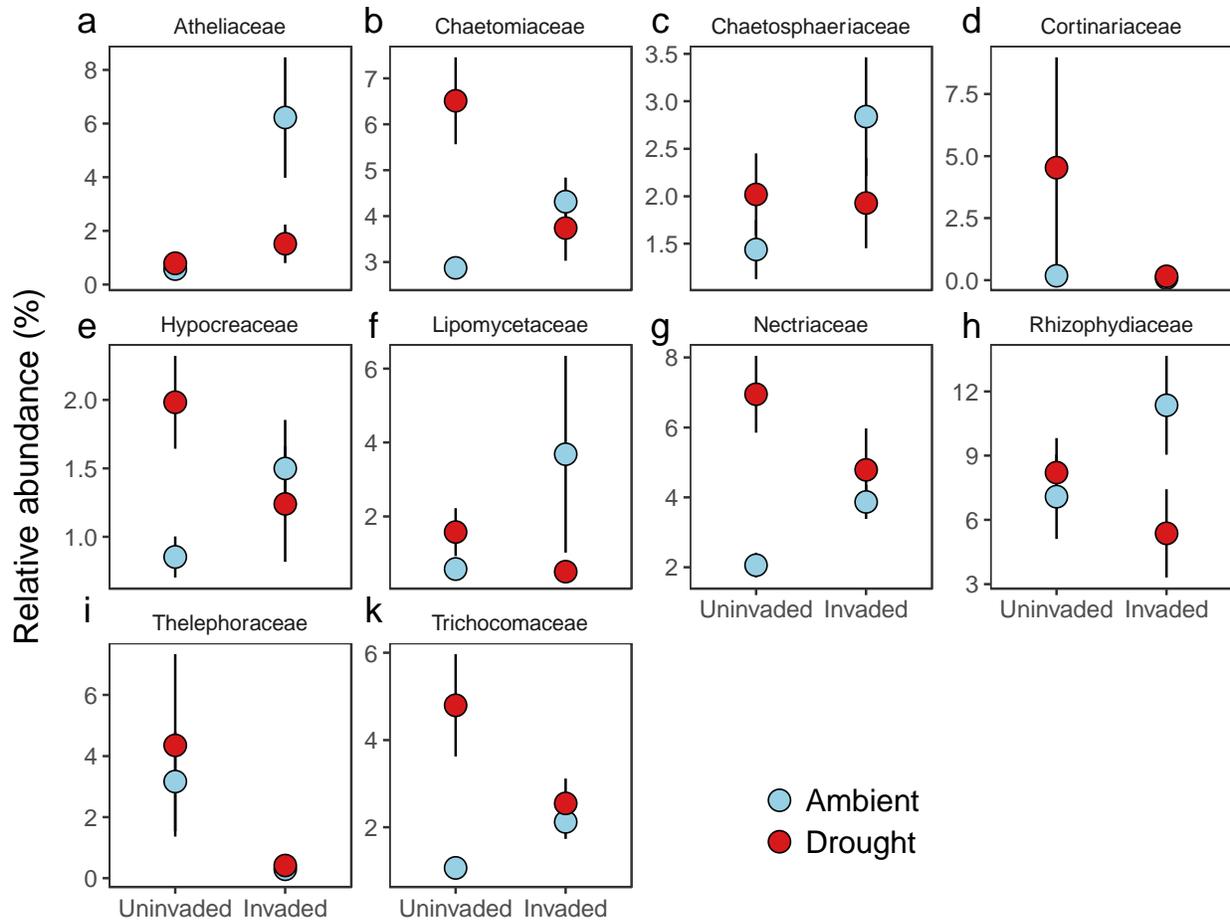


Figure 3-6. Relative abundance of the most abundant fungal families (>1% mean relative abundance) in response to invasion and drought treatments (mean \pm standard error): a) Atheliaceae, b) Chaetomiaceae, c) Chaetosphaeriaceae, d) Cortinariaceae, e) Hypocreaceae, f) Lipomycetaceae, g) Nectriaceae, h) Rhizophydiaceae, i) Thelephoraceae, and k) Trichocomaceae.

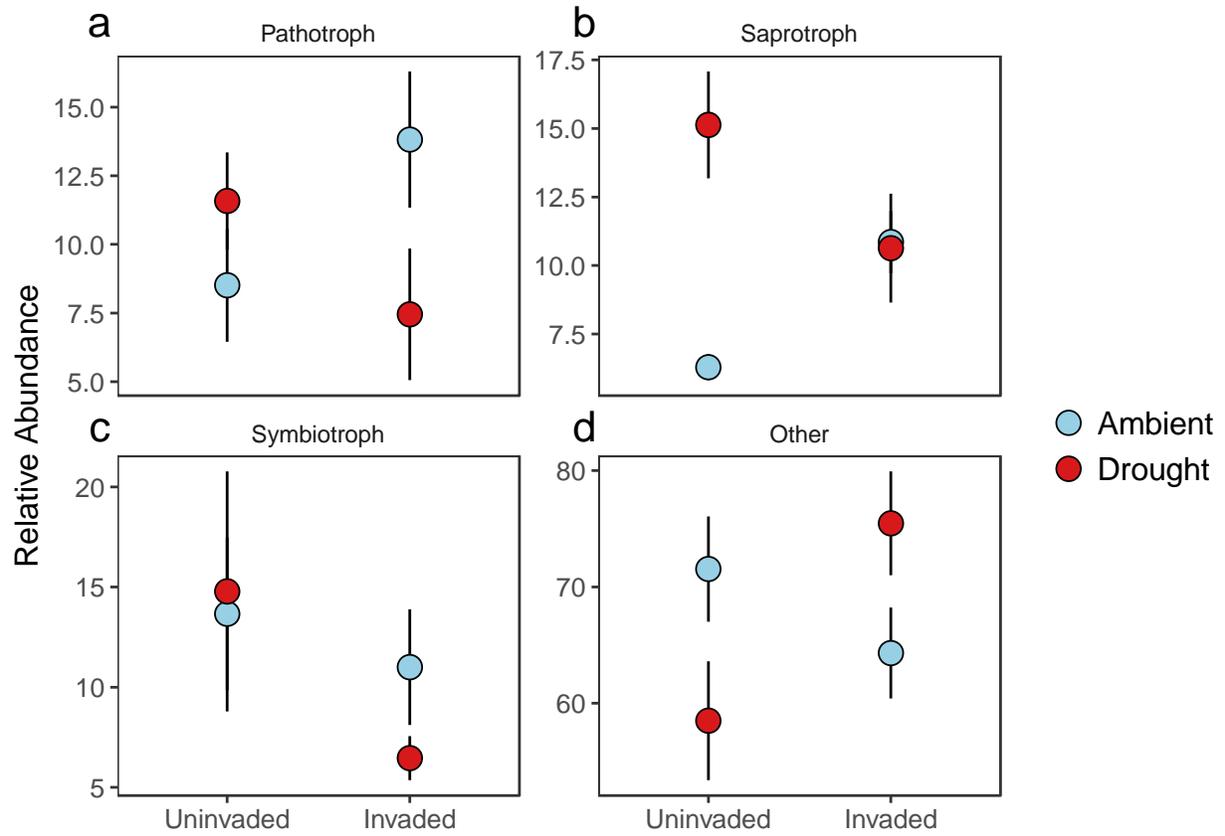


Figure 3-7. Relative abundance of fungi by trophic mode: a) pathotrophs, b) saprotrophs, c) symbiotrophs, and d) other, in response to invasion and drought treatments (mean \pm standard error). Trophic mode was assigned according to FUNGuild.

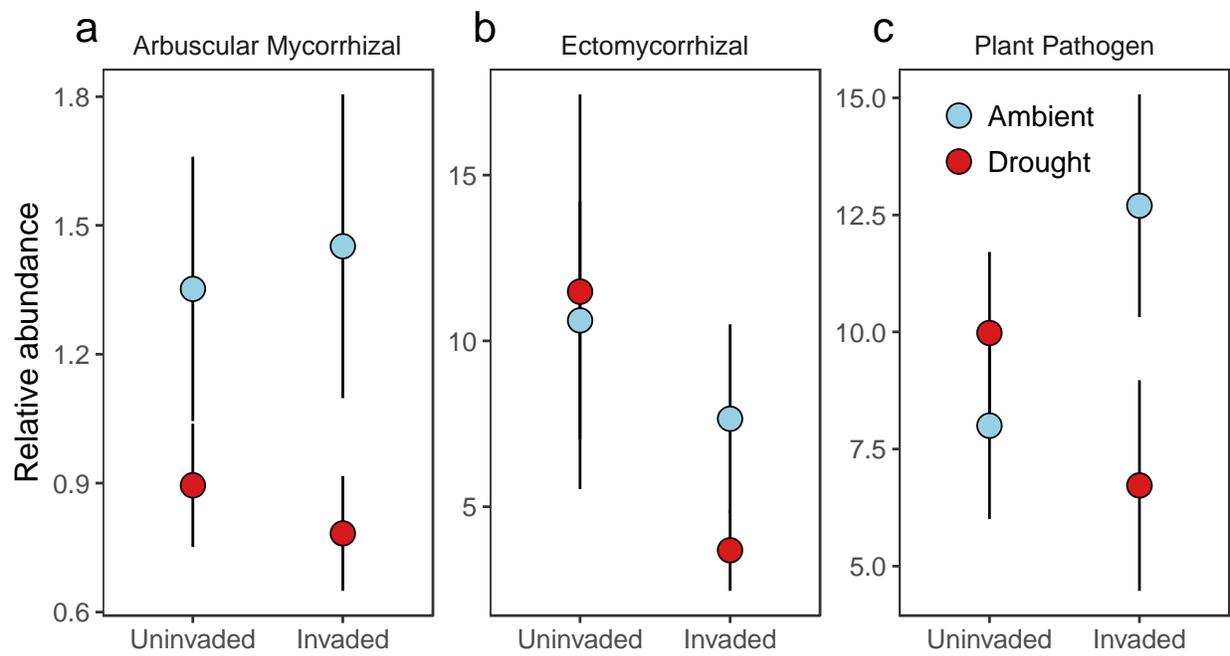


Figure 3-8. Relative abundance of selected fungal guilds (mean \pm standard error): a) arbuscular mycorrhizal fungi, b) ectomycorrhizal fungi, c) plant pathogens. Guilds were assigned according to FUNGuild.

CHAPTER 4 COMPETITION AND SOIL LEGACIES ALTER THE ROLE OF SOIL MICROBES IN PLANT INVASION

Background

Invasive plants are problematic because they can alter natural communities and ecosystems and are costly to land managers in time and resources (Mack et al. 2000, Flory and Clay 2010, Simao et al. 2010). When nonnative plants invade a community, they are subjected to novel interactions with species not present in their native range. These interactions can include interspecific competition, herbivory, pathogens, and mutualisms. The success of a plant species in a new range is partially dependent on the relative strengths of these interactions. Soil biota can play a critical role in mediating competition between native and invasive plants, for example if invaders accumulate pathogens that inhibit native species or degrade mutualistic networks (Wolfe and Klironomos 2005, Stinson et al. 2006, Mangla et al. 2008). These interactions may differ among species with traits similar to the invader and those with different traits (Bever et al. 2010). For example, species that share common mycorrhizal types (i.e., arbuscular mycorrhizae (AM) or ectomycorrhizae (ECM)) have the potential to share resources through common mycorrhizal networks (Robinson and Fitter 1999). Additionally, more closely related species are more likely to have common pathogens (Gilbert and Webb 2007); hence species that share traits with an invader could experience more negative interactions associated with soil microbes.

Soil microbes can influence invasive plants and interactions with native plants both directly and indirectly (Wolfe and Klironomos 2005, Inderjit and van der Putten 2010). Direct plant-plant competition is related to the availability of resources and resource use by each competing plant species (Tilman 1982, Maron and Marler 2008; Figure 4-1 a-c), and soil microbes can influence competition by altering availability of soil resources to plants (Ehrenfeld et al. 2001). Specifically, saprotrophs drive ecosystem processes, including decomposition and

nutrient cycling, which affect soil nutrient availability to plants; however, invaders can disrupt these processes, for example via altered plant litter quality and quantity (Gordon 1998, Holly et al. 2009, Lee et al. 2012; Figure 4-1 g). Additionally, soil mutualists such as mycorrhizal fungi can increase access to soil resources for associated plant species and can be involved in direct transfer of nutrients between plants (Robinson and Fitter 1999, He et al. 2006). Invaders may take advantage of or degrade mutualistic networks thus indirectly altering soil resource availability to native species (Marler et al. 1999, Mummey and Rillig 2006). Plants can also interact directly through interference competition via production of phytochemicals (Bennett et al. 2011; Figure 4-1 j-l). Some microbes can breakdown phytochemicals thus reducing their effects on competing plant species (Li et al. 2015). On the other hand, some invaders cause degradation of mycorrhizal networks by production of phytochemicals which indirectly affect native species (Roberts and Anderson 2001, Stinson et al. 2006, Cipollini et al. 2012). Invaders and native species may respond differently to soil pathogens if invaders are released from natural enemies in the introduced range (Mitchell and Power 2003). Furthermore, some invaders have been shown to accumulate pathogens that disproportionately inhibit native species (Mangla et al. 2008; Figure 4-1 f). The complexity of these interactions has made it difficult to parse out the role of microbes in plant competition and invasion and an improved understanding of these interactions could improve outcomes for restoration of invaded ecosystems.

Plant-soil interactions are likely to change under altered environmental conditions associated with climate change. For example, drought is one of the strongest abiotic drivers of change in microbial community structure and function (Manzoni et al. 2011), and the effects of soil moisture on microbial communities can in turn influence plant performance and competition (Kaisermann et al. 2017, Fry et al. 2018). Drought may have multiple impacts on plant-soil

interactions, including effects on the availability of and plant access to soil resources (Figure 4-1 a-c), the abundance and composition of soil biota (Evans and Wallenstein 2012; Figure 4-1 d-i), and the production and movement of allelochemicals in the soil (Figure 4-1 j-m). All of these effects may directly or indirectly affect plant performance, but it is unknown how they will interact to influence native versus invasive plants.

The aim of this study was to evaluate the potential effects of soil legacy of invasion and drought on plant performance. The invader, cogongrass (*Imperata cylindrica*), is a warm season, rhizomatous, perennial grass native to Southeast Asia that has invaded 500,000 ha in Florida and over 500 million ha worldwide (MacDonald 2004, Estrada and Flory 2015). It is listed as a Federal Noxious Weed partly due to its impacts on threatened longleaf pine ecosystems in the southeastern US (Brewer 2008). Longleaf pine (*Pinus palustris*) and wiregrass (*Aristida stricta*) are foundation species in longleaf pine forests (Noss 1989). These species differ in their mycorrhizal associations; cogongrass and wiregrass both associate with arbuscular mycorrhizal fungi, while pine is ectomycorrhizal. This difference in mycorrhizal associations is likely to influence how soil biota affect the interactions among these plants (Toju et al. 2014).

The specific objectives of this study were to; 1) Determine the strength and direction of the impacts of microbial communities on performance of cogongrass and native species, 2) Evaluate how legacy effects of invasion and drought on soil microbial communities influence cogongrass and native species performance, 3) Assess the role of soil microbes and legacy effects on competitive interactions between cogongrass and native species, and 4) Quantify the effect of soil microbes on net productivity of cogongrass and natives in competition. We expected that microbial effects on native species would be negative due to the effects of plant pathogens but neutral/positive for the invader because of enemy release. In Chapter 2 and

Chapter 3, we showed that invasion and drought both influence plant and soil microbial community composition; therefore, we expected substantial soil legacy effects of both invasion and drought on native species. Because of the drastic competitive effects of cogongrass invasion on native plants, we hypothesized that microbes promote cogongrass' competitive ability compared to native plants. Finally, we hypothesized that overall microbes would limit plant productivity in competition due to pathogen effects, and that soil legacy effects of invasion and drought would limit productivity due to differences in pathogen and mutualist abundance observed in Chapter 3.

Methods

Field Experiment

We conducted this study at a long-term factorial invasion x drought field experiment at the University of Florida Bivens Arm Research Site. Detailed methods for this experimental setup have been previously described (Alba et al. 2017). Briefly, we established 40 plots, each 4 m x 4 m, in spring 2012. In each plot, we planted 20 longleaf pine seedlings and 36 native herbaceous seedlings (12 spp. x 3 individuals). In spring 2013, a crossed combination of drought treatment and cogongrass invasion was applied. The drought treatment was implemented by constructing rainout shelters with 89% roof cover, which reduced soil moisture by 40% on average over 5 years. Similar shelters were constructed over ambient rainfall plots with shade cloth instead of roofing to create similar light levels across the treatments (Chapter 2). The invasion treatment was applied by planting nine seedlings of cogongrass into the invasion plots, which resulted in 50% invader cover on average. The invasion and drought treatments served as the soil legacy conditioning for this greenhouse experiment.

Greenhouse Experiment

In a greenhouse at the University of Florida, we established an experiment to test the performance of three species; cogongrass, longleaf pine, and wiregrass, in response to soil microbial communities from the invasion x drought field experiment. In May 2017, soil samples were collected to provide inoculum by taking four soil cores in each plot with a 5 cm diameter x 15 cm deep hammer corer. The soil corer was sterilized with 80% ethanol between each plot. The four cores per plot were composited and sieved through a 2 mm sieve. Roots were removed to reduce phytotoxicity effects.

Background soil medium consisted of a 1:1 mix of sand and local topsoil collected near the field experiment. Topsoil was sieved through a 6 mm screen to remove rocks and large roots. Sand and topsoil were mixed in a cement mixer for ~2 minutes. The soil mixture was autoclaved for one hour on three successive days and then stored in a cold room until planting. Half of each soil sample from the field plots was sterilized by autoclaving in the same way and the other half was kept as live inoculum. One-gallon pots were filled $\frac{3}{4}$ with the sterile soil medium then covered with a thin layer (125 g) of live or sterile inoculum (~5% of soil volume). This small volume of soil inoculum was used to reduce the effects of nutrient release from sterilization and abiotic effects of the treatments (Kulmatiski and Kardol 2008). Finally, 5 cm of sterile soil was added to cap the inoculum.

Longleaf pine seeds (Sheffield's Seed Company, Locke, NY) were surface sterilized twice in 10% bleach for 15 minutes and rinsed. They were then soaked for 24 hours at room temperature in DI water. Excess water was drained and then seeds were stored for 7 days at 4 °C before planting into sterilized sand for germination. Wiregrass seeds (The Natives Inc., Davenport, FL) were surface sterilized for 5 minutes, rinsed with DI water, and planted into

sterilized sand for germination. Cogongrass rhizomes were collected from a population near the field experiment. Sheaths and fine roots were removed and then rhizomes were cut into three node segments and surface sterilized for two minutes. They were then rinsed and planted into sterilized sand.

Seedlings of pine and wiregrass were transplanted into the pots in June 2018. These species were allowed to establish for one month and then cogongrass seedlings were transplanted into the pots. In total, we had four species combinations (each of the three species alone and all three species together in competition), two inoculum treatments (live/sterile), and four soil-conditioning treatments (ambient uninvaded, ambient invaded, drought uninvaded, drought invaded), with 10 independent replicates (corresponding with the 10 replicates in the field experiment) for a total of 320 pots. The pots were grouped into 10 blocks in the greenhouse matching the blocking structure of the field experiment.

Seedlings that died within two weeks after transplanting were replaced with sterile seedlings of the same age. Seedlings that died after the first two weeks were also replaced to maintain competition effects but were excluded from the statistical analyses. Seven pine seedlings died after the first two weeks, all of which were in the sterile inoculum treatment. Two wiregrass seedlings died and no cogongrass seedlings died (Table C-4). In late-November, above- and belowground biomass was harvested. Roots were washed free of soil, and for competition pots separated by species, and then above- and belowground biomass was dried at 60 °C for at least 72 hours before being weighed.

Statistical Analysis

To test for effects of live versus sterile soil inoculum, invasion and drought soil legacy combinations, and competition, we used mixed effects models with inoculum, invasion, drought,

competition, and all possible interactions as fixed effects, and field plot nested within block as a random effect. Response variables included total plant biomass for each species, root: shoot ratio, and relative competition intensity. We calculated the relative competition intensity (RCI) index as the difference between the biomass when grown alone and biomass when grown in competition divided by the biomass when grown alone (Weigelt and Jolliffe 2003). We also assessed the total biomass of all three species in the competition treatment in response to soil inoculum (live vs. sterile) and soil legacy (invasion x drought) with a mixed model including inoculum, invasion, and drought as fixed effects and plot nested within block as a random effect. In order to determine the relative importance of direct competitive effects on the focal species of the biomass of each of the other species in the competition treatment, we ran a mixed model for each species in the competition treatment including soil inoculum and the biomass of each of the other species in the pot as fixed effects, and plot and block as random effects.

A significant statistical interaction between the soil inoculum and soil legacy of invasion or drought indicates that the response to the soil legacy differed in the live versus the sterile soil and therefore is referred to as a biotic soil legacy (i.e. resulting from soil microbial communities). A significant individual effect of soil legacy with no significant interaction term indicates that live and sterile soil inoculum treatments responded similarly and cannot be attributed solely to microbes and therefore is referred to as an abiotic soil legacy (i.e. not resulting from soil microbial communities). We expected abiotic effects to be minimized because we added inoculum that made up only 5% of total soil volume.

While raw data were used for analyses, for visualization we also calculated the relative difference in total biomass and root:shoot ratio between the live and sterile treatment as (live-sterile)/sterile. Because the inoculum soils were kept separate by plot and not pooled by

treatment, the plants formed natural pairs grown in live and sterile soil and replication was maintained when calculating these indices (Rinella and Reinhart 2018). Additionally, as detailed below there were no biotic effects of drought; thus, the ambient and drought data have been pooled in the figures. Figures with raw data are presented in Appendix C (Figure C-1 to Figure C-3).

Results

For cogongrass grown alone, plants grown in live soil had 12% greater total biomass than those in sterile soil, regardless of the soil legacy of invasion or drought. However, for cogongrass in competition, plants grown in live soil had 38% lower biomass than in sterile soil (inoculum x competition: $F_{1,108} = 25.7$, $P < 0.001$; Figure 4-2). There was a legacy effect of drought on cogongrass performance such that cogongrass grown in soil with a history of drought did better in both live and sterile soils, indicating an abiotic legacy effect (drought: $F_{1,27} = 6.2$, $P = 0.02$); however, the magnitude of this effect was relatively small (8% increase; Table C-1).

Pine seedlings exhibited responses that were the opposite of those just reported for cogongrass. When pine seedlings were grown alone, they had 25% lower biomass in live soil than in sterile soil regardless of soil legacy (Figure 4-2). When grown in competition, however, pine seedling biomass was 17% higher when grown in live than sterilized soil (inoculum x competition: $F_{1,101} = 49.1$, $P < 0.001$; Table C-2).

Whether alone or in competition, wiregrass biomass was lower in live than sterilized soil (27% decrease in biomass) (Figure 4-2). Across live and sterile soil, there was a negative effect of soil legacy of invasion on wiregrass (10% decrease). More specifically, wiregrass biomass was 15% lower when grown alone in live soils with a history of invasion than in live uninvaded soils (invasion x inoculum x competition: $F_{1,106} = 4.0$, $P = 0.047$), indicating both abiotic and biotic effects of invasion, but only when this native grass was grown alone (Table C-3).

Treatments also modulated plant allocation to roots versus shoots – i.e. root:shoot ratio (RSR) – but the direction of treatment effects differed by plant species. For cogongrass, RSR was higher in live soil than sterile soil (inoculum: $F_{1,108} = 14.1$, $P = 0.0003$). For pine, RSR was higher in sterile soil, an effect that was larger when the pine seedlings were grown alone (inoculum x competition: $F_{1,101} = 12.5$, $P = 0.0006$). For wiregrass, RSR was higher in sterile than live soil and higher in soils with a legacy of drought (inoculum: $F_{1,106} = 4.3$, $P = 0.04$; drought: $F_{1,27} = 4.7$, $P = 0.04$; Figure 4-3) but inoculum x drought was not significant for wiregrass, suggesting an abiotic legacy of drought.

The relative competition intensity (RCI), indicating the relative difference in total biomass when grown alone compared to competition, was higher for cogongrass in live soil (inoculum: $F_{1,36} = 25.8$, $P < 0.001$), but higher for pine in sterile soil (inoculum: $F_{1,30} = 23.8$, $P < 0.001$) and unaffected by soil inoculum for wiregrass (inoculum: $F_{1,33} = 0.05$, $P = 0.83$; Figure 4-4). Soil legacy had no effects on RCI. When the biomass of the other species in the competition treatment pots was taken into account using linear mixed effects models, cogongrass was negatively affected by live soil and by pine biomass but not wiregrass biomass (pine biomass: $F_{1,29} = 18.2$, $P = 0.0002$; inoculum: $F_{1,29} = 19.6$, $P = 0.0001$). However, pine was negatively affected by both cogongrass and wiregrass biomass, and competition with cogongrass had a larger effect on pine biomass in the live soil inoculum (wiregrass biomass: $F_{1,29} = 45.4$, $P < 0.0001$; cogongrass biomass x inoculum: $F_{1,29} = 6.6$, $P < 0.02$). Wiregrass was only negatively affected by pine biomass and inoculum but not cogongrass biomass (pine biomass: $F_{1,29} = 49.8$, $P < 0.0001$; inoculum: $F_{1,29} = 21.8$, $P = 0.0001$).

We assessed total plant biomass (sum of the three species) in the competition treatment to determine if there was an effect of soil microbes or soil legacy on total community production.

Biomass was 15% lower in live soil (inoculum: $F_{1,32} = 12.4$, $P = 0.001$) and was a 10% lower in soils with a legacy of invasion, when averaged across both live and sterile soil pots (invasion: $F_{1,27} = 8.4$, $P = 0.007$; Figure 4-5).

Discussion

Soil microbial communities play an important role, both directly and indirectly, in plant community dynamics and the maintenance of diversity (Van Der Heijden et al. 2008, Bever et al. 2010). In the context of our conceptual diagram (Figure 4-1), we found evidence for pathways of interaction among native and invasive plants that were mediated by microbial communities and the biotic legacies of plant invasion but not drought. We discovered that soil microbes stimulated the invader's growth but depressed both native species when grown alone. This pattern is consistent with the hypothesis that invaders escape from belowground enemies in the introduced range (Mitchell and Power 2003, Reinhart et al. 2003). Surprisingly, in our study, the effect of soil microbes switched from positive to negative for the invader when grown in competition with native species and switched from negative to positive for longleaf pine when grown in competition (Figure 4-2). Moreover, although the effect of soil microbes on wiregrass remained negative in competition, the magnitude of the effect was dampened. Therefore, our results indicate that competition between plant species can modify the interaction between plants and microbes and even change the direction of these effects (Abbott et al. 2015). Many plant-soil interaction studies have assessed plant responses to soil legacies of different plant species individually, but plant species in isolation are likely to have different interactions with soil microbial communities than plants in competition, especially when invasive plants are present (Shannon et al. 2012, Crawford and Knight 2017). Furthermore, measured changes in the root:shoot ratio of these plants indicated that these complex effects are likely associated with differing responses of root allocation to inoculation between invasive cogongrass and the native

pine, whereby cogongrass allocates more to roots in live soil and pine allocates less to roots in live soil (Figure 4-3).

Addition of live inoculum to sterilized soil could influence plant interactions with microbial communities in three major ways: introduction of pathogens that harm plants, promotion of mycorrhizal associations that usually benefits plants, and introduction of saprotrophic microbes. Plant species often show host specific responses to pathogens that can drive differences in plant fitness and may differentially affect native and non-native species (Mitchell and Power 2003, Reynolds et al. 2003, Mangla et al. 2008). The effects of mycorrhizae on plant-plant competition could be complex. Mycorrhizae can be involved in transfer of resources between plant species, resulting in altered competitive dynamics (Robinson and Fitter 1999); they can increase access to soil nutrients, but can also act parasitically under certain conditions (Johnson et al. 1997); and the benefits provided by arbuscular mycorrhizae versus ectomycorrhizae differ (Smith and Read 2008). Saprophytic microbes can compete with plants for soil nutrients (immobilization) or they can convert nutrients to available forms (mineralization) depending on resource ratios (Hodge et al. 2000).

With this background, we can speculate on the potential causes of the switch in effect of microbes observed for the invader (cogongrass) and the native pine when grown alone compared with in competition (Figure 4-2). Soil microbes caused greater relative allocation to roots of the invader, but lower root allocation on average for both pine and wiregrass (Figure 4-3). Higher root:shoot ratios are often indicative of lower belowground resource availability or more intense belowground competition (Poorter et al. 2011), suggesting that cogongrass was more limited by nutrients in live soil, while pine was more limited in sterile soil. Based on preliminary observation, we observed abundant mycorrhizal colonization of pine roots in the inoculation

treatment and minimal contamination with mycorrhizal fungi in the sterile treatment (data not shown). We hypothesize that the ectomycorrhizal mutualism in pine in the inoculated soils allowed the species to compete effectively for nutrients with saprotrophic microbes and other plants (Figure 4-1 g-i). In general, cogongrass is notorious for its high belowground allocation to rhizomes and strong competitive ability belowground, and our results indicate that soil microbes can further intensify this competitive imbalance, but may not benefit cogongrass in all situations. Previous studies have shown changes in allocation patterns in response to soil microbes (D'Hertefeldt and Van Der Putten 1998) and Te Beest et. al. (2009) suggest that changes in allocation for an invader may relate to the evolution of increased competitive ability.

Another possible contributing mechanism to the difference in microbial effect on root:shoot ratio for the invader versus native species is limitation of root biomass by pathogen accumulation (de Kroon et al. 2012). Lower root allocation in live soil for either or both of the native species could potentially be caused by pathogen effects. However, this mechanism seems unlikely for pine in competition where the effect of microbes on total biomass was positive. Alternatively, pathogens associated with longleaf pine might spillover to cogongrass when grown in competition, thereby creating a more negative effect on cogongrass and reduced competition with pine (Mordecai 2011).

In addition to demonstrating a change in plant response to microbes under competition, we also showed that microbial communities can modify competitive interactions between plant species. Competition intensity (RCI) was higher for cogongrass in live soil but higher for pine in sterile soil, while inoculum did not affect competition intensity for wiregrass (Figure 4-4). Soil microbes have been shown to alter plant competitive interactions in some cases (Hodge and Fitter 2013, Abbott et al. 2015, Hortal et al. 2017), but the depth of understanding of these

interactions is limited. Furthermore, we found that pine responded to the biomass of the competing species and this response was modulated by soil microbes, while cogongrass and wiregrass responded directly to the inoculum treatment and to pine biomass but not to one another. Pine appeared to respond more to the changes in biomass of the other species in the competition treatment but not to the inoculum directly. Therefore, as cogongrass and wiregrass were inhibited by the live soil treatment, pine experienced less competition and higher production. The presence of soil microbes appears to be essential to the competitive ability of pine and the presence of a robust microbial community may prove to be important to the restoration of longleaf pine forests.

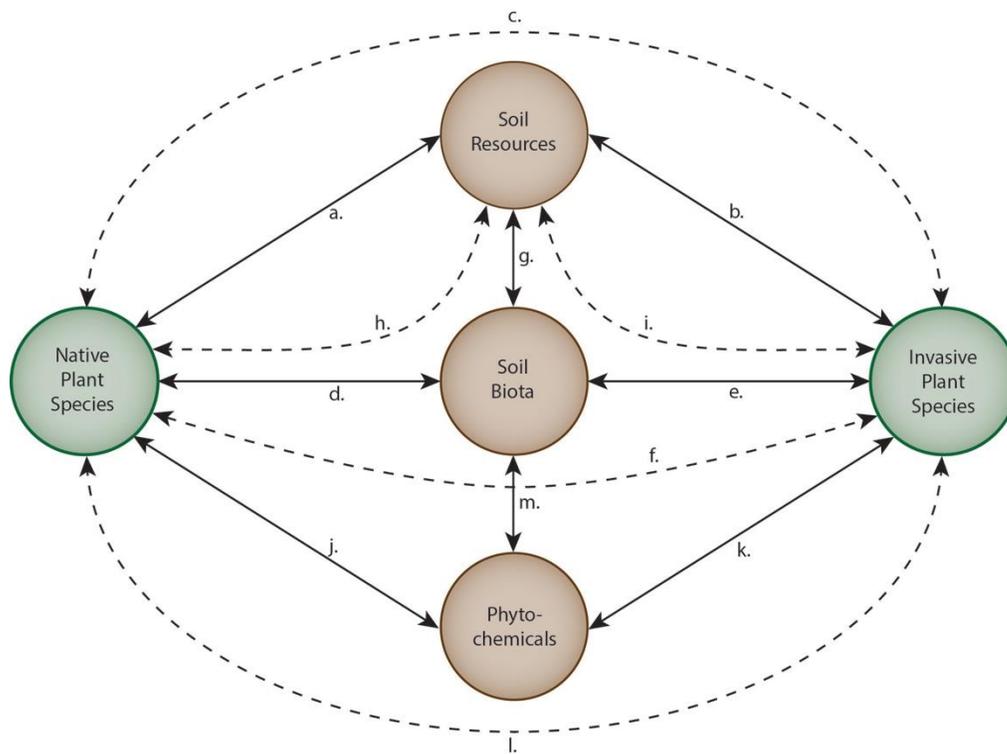
Soil legacy of invasion and drought had surprisingly limited effects overall; however, there were a few significant effects worth noting. In the one instance of a significant biotic effect of soil legacy, wiregrass was negatively affected by soil microbes from the invaded soils when grown alone, indicating that cogongrass may accumulate pathogens that have a negative effect on some native species (Mangla et al. 2008, Kelly et al. 2009, Flory and Clay 2013). This hypothesis is corroborated by the results in Chapter 3, where we found that invaded plots had higher relative abundance of fungal plant pathogens. As C₄ grasses, wiregrass and cogongrass are functionally more similar to each other than to pine, so cogongrass may accumulate pathogens that have more pronounced effects on functionally similar species (Gilbert and Webb 2007). In Chapter 3, we also observed that invasion changed the community composition of AM fungi; therefore, it is possible that cogongrass legacy altered the AM fungal community causing a decrease in the benefit received by wiregrass and therefore a more negative net effect of microbes (Figure 4-1 e-f). While previous studies have observed legacy effects of drought on plant-soil feedbacks (Kaisermann et al. 2017, Fry et al. 2018), we observed no significant biotic

effects of drought on plant performance despite significant changes in the microbial community in response to drought in the field. In our system, microbial communities may recover rapidly upon rewetting in the greenhouse, or the taxa that changed in abundance in the drought treatment may have minimal influence on the performance of these three plant species. Additionally, it should be noted that these are highly conservative results because we used a small volume of inoculum and also more realistic than many studies because the initial soil legacy phase was conducted in the field (Kulmatiski and Kardol 2008, Kulmatiski et al. 2008, Heinze et al. 2016).

We expected that using a small volume of soil inoculum would minimize abiotic effects of the soil legacy treatments; however, we observed some instances of significant abiotic soil legacy effects. Cogongrass growth was improved by soil legacy of drought and wiregrass root:shoot ratio was greater with soil legacy of drought. Additionally, we showed that microbial communities and invasion legacy can control plant productivity (Van Der Heijden et al. 2008), but the effect of invasion legacy appears to be at least partly abiotic as it occurred in both live and sterile soils. While the mechanism behind these abiotic effects is unclear, it is possible that allelochemicals in the invaded soil could reduce net biomass (Figure 4-1 j-1). Previous research has suggested that cogongrass may release allelochemicals that reduce growth of wiregrass and pine species and inhibit colonization by mycorrhizal fungi for both species (Hagan et al. 2013b).

Many conservation efforts are focused on habitat restoration, including the removal of invasive species and establishment of native communities; therefore, studies on the legacy effects of problematic invaders under different abiotic conditions are needed to inform management decisions for restoration (Smith-Ramesh and Reynolds 2017). Our results show that soil microbes can fundamentally alter the competition between native and invasive plants. Therefore, it is critical that competitive interactions among plants be taken into account in

studies of microbial effects on plant performance (Shannon et al. 2012, Crawford and Knight 2017). Soil biotic and abiotic legacies had more minor, although in some cases significant, effects on relative performance of different plant species and total plant productivity. Hence, the microbial legacy of invasion may not be a substantial barrier to restoration of sites invaded by cogongrass; however, degraded soil communities from management practices could inhibit restoration of longleaf pine (D'Antonio and Meyerson 2002). More broadly, understanding the role of soil microbial communities in mediating plant competition could improve the success of restoration efforts for native ecosystems.



- a. Direct effect of the native species on soil resources and the effect of soil resource availability on the native species
- b. Direct effect of the invader on soil resources and effect of soil resource availability on the invader
- c. Indirect effect of the native and invasive species on each other via competition for soil resources
- d. Direct effect of the native species on soil biota and the effect of soil biota on the native species
- e. Direct effect of the invader on soil biota and the effect of soil biota on the invader
- f. Indirect effect of native and invasive species on each other via soil biota (e.g. pathogen accumulation)
- g. Direct effect of soil biota on soil resource availability via competition and nutrient cycling
- h. Indirect effect of the native species on soil resources via soil biota (e.g. associations with mutualists)
- i. Indirect effect of the invader on soil resources via soil biota (e.g. associations with mutualists)
- j. Release of phytochemicals by the native species
- k. Release of phytochemicals by the invader
- l. Indirect effect of the invader and the native species on each other via phytochemicals (i.e. interference competition)
- m. Direct effect of phytochemicals on soil biota (e.g. degraded mutualisms) and direct effect of soil biota on phytochemicals (e.g. chemical degradation)

Figure 4-1. Conceptual diagram of possible belowground interactions between an invader and native species. Green circles represent competing plant species and brown circles represent aspects of the soil ecosystem that may mediate plant competition. Solid lines indicate direct effects and dashed lines indicate indirect effects.

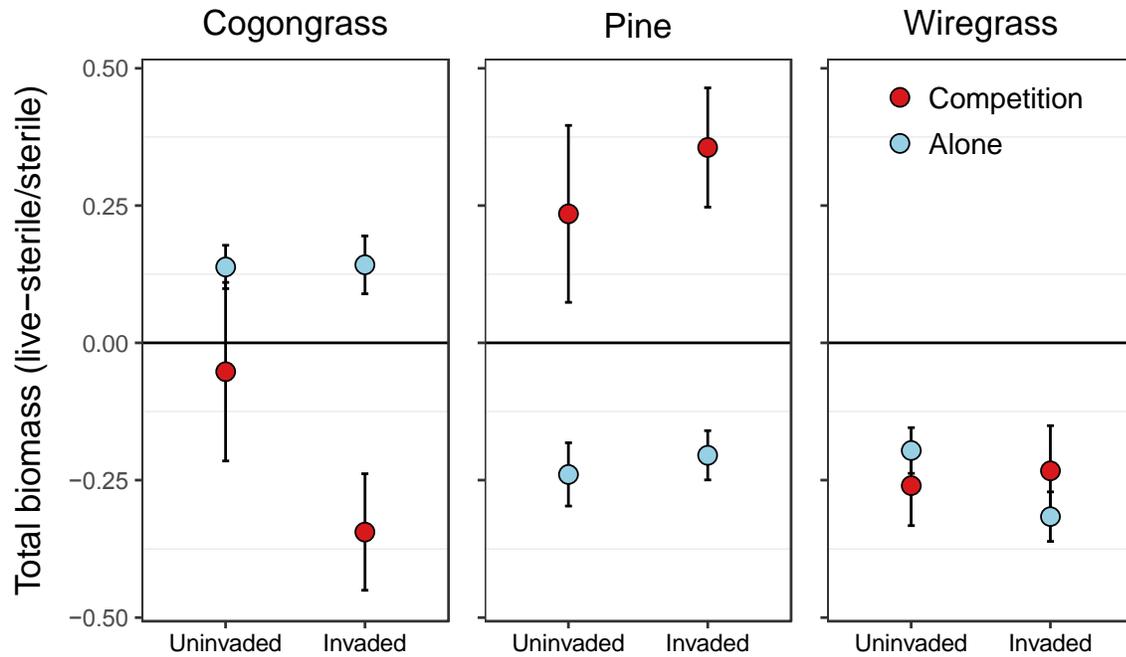


Figure 4-2. Relative difference in total biomass between live and sterile soil inoculum in soil with a history of native species or invasion for three species either alone or in competition (mean \pm standard error). Points above the zero line indicate a positive effect of live inoculum compared to sterile inoculum and points below the zero line indicate a negative effect of live inoculum. Soil legacies of ambient precipitation versus drought had no significant effects and are therefore combined in the figure. Biotic legacy of invasion was only significant for wiregrass when grown alone. Competition and soil inoculum had a significant interactive effect on all species.

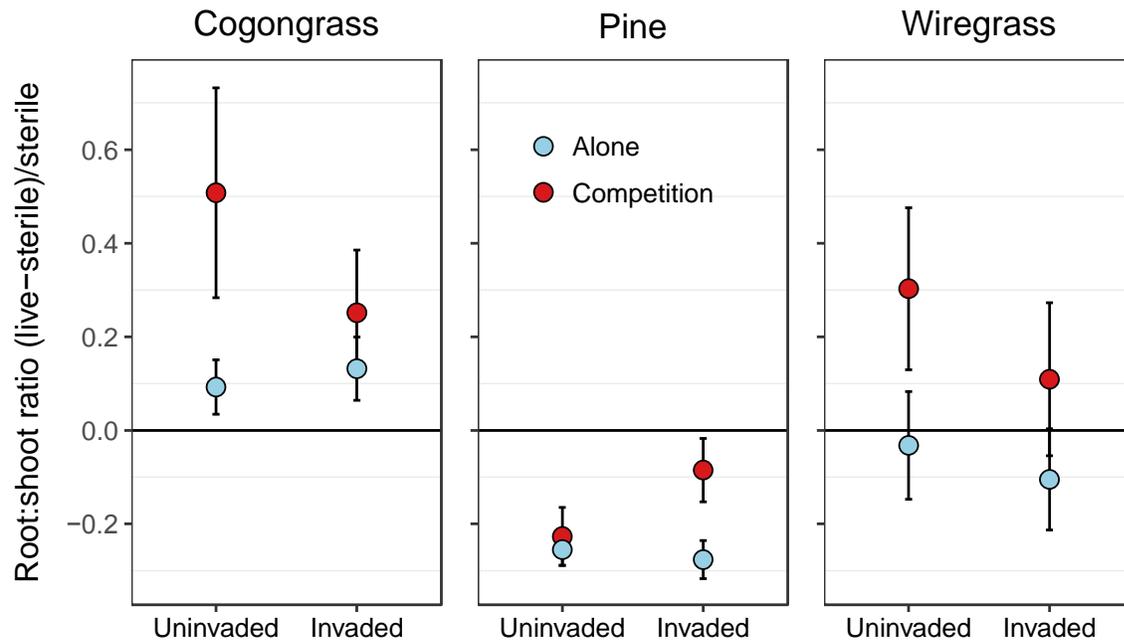


Figure 4-3. Relative difference in root:shoot ratio between live and sterile soil inoculum treatments (mean \pm standard error). Points above the zero line indicate a positive effect of live inoculum compared to sterile inoculum and points below the zero line indicate a negative effect of live inoculum. Drought had no significant effect on the difference between live and sterile soil and is combined in the figure. Cogongrass showed a significant response to inoculum. Pine showed a significant inoculum x competition interaction. Wiregrass showed significant inoculum and drought (not shown) effects.

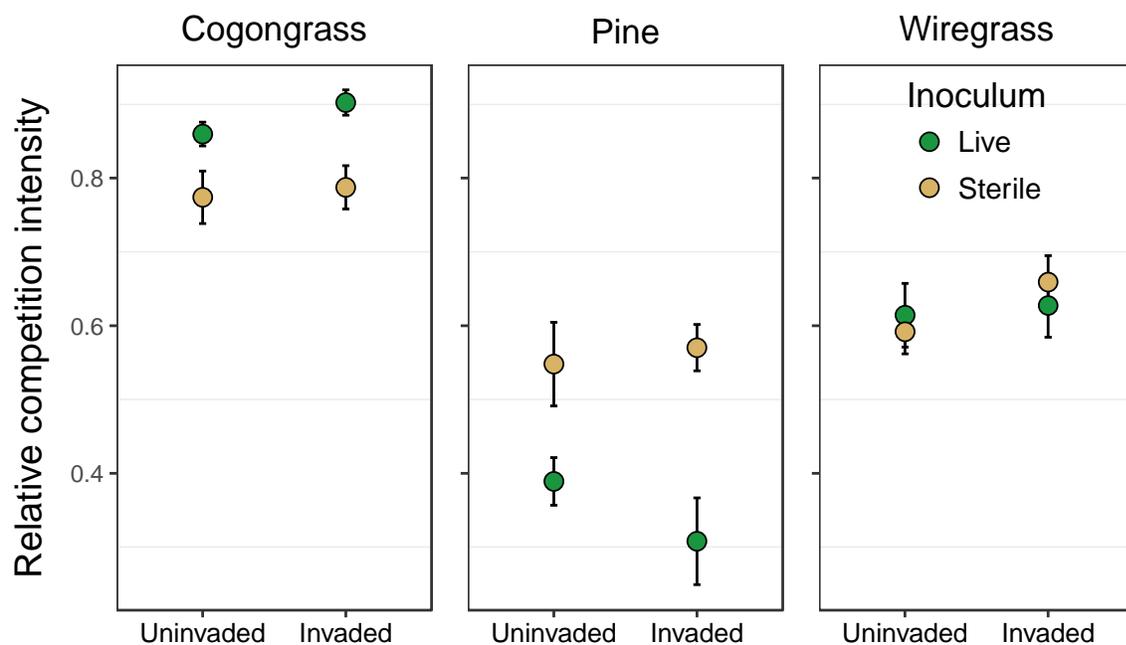


Figure 4-4. Relative competition intensity (RCI) for each species under live or sterile soil inoculum with a history of invasion or no invasion (mean \pm standard error). RCI is calculated as the difference between the biomass when grown alone and biomass when grown in competition divided by the biomass when grown alone, so higher values indicate a greater effect of competition on plant biomass. Soil legacy of drought had no significant effect and is combined in the figure. The effect of live versus sterile inoculum was significant for cogongrass and pine but not wiregrass.

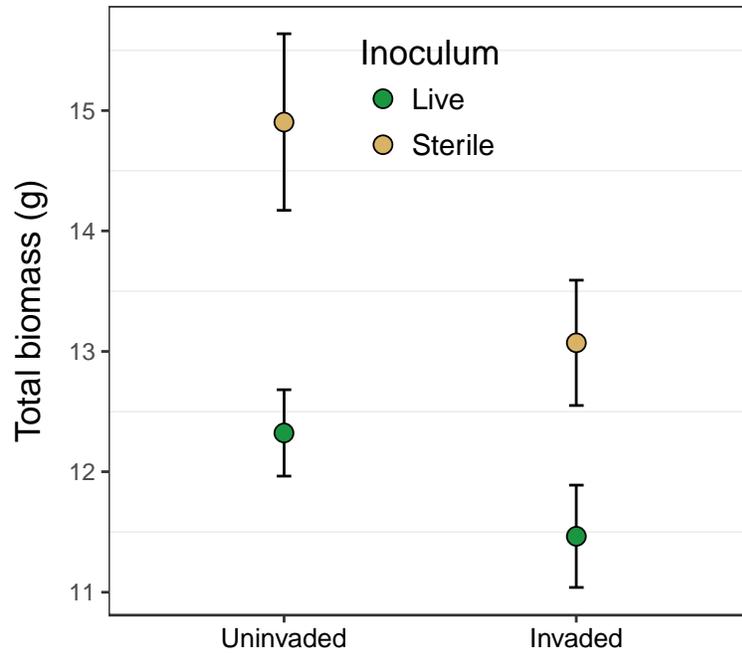


Figure 4-5. Effect of soil legacy of invasion and drought and live or sterile soil inoculum on total biomass production per plot in the competition treatment (mean \pm standard error). Total biomass includes above- and belowground biomass of three species (cogongrass, pine, and wiregrass). There was a significant effect of live versus sterile inoculum and of legacy of invasion.

CHAPTER 5 CONCLUSIONS

The number of studies addressing plant-soil interactions has increased exponentially in recent years as the important role of soil biota in all aspects of ecosystem function and community structure has become more apparent (Kulmatiski and Kardol 2008, van der Putten et al. 2013). Additionally, the role of soil microbiomes in plant invasion has gained interest; however, studies of these interactions have focused on a few notable species that are known to have major impacts (Roberts and Anderson 2001). Furthermore, most studies of invader-soil biota interactions assess plant performance in isolation under ideal conditions and do not consider the context dependency of these interactions.

As a model system, I used an ecologically understudied, but highly problematic invasive species, *Imperata cylindrica*, to make a comprehensive assessment of the interactive effects of invasion and drought on plant-soil interactions (Estrada and Flory 2015). In Chapter 2, I found dramatic effects of invasion by *Imperata cylindrica* on plant diversity and community composition (Figure 5-1 a). Invasion reduced species richness by nearly 60% after just two years and caused the site level extinction of more than half the plant species. Drought caused more moderate effects on plant diversity including a 30% reduction in richness and reordering of the dominant functional groups (Figure 5-1 b). *Imperata cylindrica* cover was not significantly affected by drought (Figure 5-1 c). Soil moisture was 30% higher in *I. cylindrica* invaded plots with the drought treatment compared to uninvaded drought plots thereby moderating the effects of drought on the plant community (Figure 5-1 d). This effect created an antagonistic interaction based on an additive model of interacting stressors (Figure 1-1). However, the major effects of invasion persisted, indicating that regardless of future drought conditions, *I. cylindrica* will continue to be a problematic invader.

In contrast to the responses of the plant community, in Chapter 3, I found that soil microbial communities overall showed greater response to drought than to invasion (Figure 5-1 e,f). Bacterial diversity and community composition responded strongly to drought, and most bacterial taxa responded to drought independently of the presence of the invader. Invasion had moderate positive effects on bacterial diversity compared to the native dominated plant community (Figure 5-1 f,g). Fungal diversity was more resilient to both treatments, but community composition responded interactively to invasion and drought. Furthermore, multiple fungal taxa and functional groups responded interactively to invasion and drought including groups with prime significance for plants, such as AM fungi and plant pathogens.

Finally, in Chapter 4, I found that the direct effect of soil microbes on performance of the invader was positive (Figure 5-1 h), while the effect on native species was negative when grown alone (Figure 5-1 i). The striking result from this study was the switch in the effect of microbes on performance of the invader and the native pine when grown in competition. Microbes had a positive effect on *I. cylindrica* biomass when grown alone but a negative effect when grown in competition with pine and wiregrass. Pine showed the reverse effect where microbes had a negative effect when grown alone but a positive effect when grown in competition. Thus, microbes altered competitive interactions between the invader and native plants (Figure 5-1 j,k). Surprisingly, the legacy effects of these changes in microbial communities resulted in only minor responses in plant performance. I showed that the indirect effect of soil biotic legacy of drought on the invader and the natives was not significant (Figure 5-1 l,m), but that the biotic legacy of invasion had a small negative effect on biomass of wiregrass but not pine or the invader cogongrass (Figure 5-1 n,o).

In summary, I assessed the ecological impacts of plant invasion and chronic drought on plant communities, soil microbial communities, and how these effects feedback to performance of native versus invasive plants. Overall, my results show that both invasion and drought have major consequences for native ecosystems, but they may act on different levels of the ecosystem. Additionally, while indirect effects of soil legacies of invasion and drought may be minor, microbes play a major role in competition between native and invasive plants and should be considered in management of invasive species (Eviner and Hawkes 2008, Harris 2009, Elgersma et al. 2011).

Recent studies have suggested that reestablishing soil communities can increase the success of ecosystem restoration in highly degraded systems; however, previous studies have found no benefit of soil community amendment (Harris 2009, Kardol et al. 2009, van der Bij et al. 2018). Furthermore, the role of plant-soil interactions in restoring invaded systems is highly context dependent and may be influenced by future climate change (Wolfe and Klironomos 2005, Harris et al. 2006, Bradley et al. 2009, Elgersma et al. 2011). Therefore, it may be unrealistic and potentially counterproductive to attempt to create a one-size-fits-all recommendation for land managers across different settings (Eviner and Hawkes 2008). Here, I provide a complete picture of the effects of a widespread invasive plant under current and possible future climate conditions on plant-soil interactions that can inform managers dealing with restoration of *I. cylindrica* invaded pine forests. Together, my results suggest that the direct competition from cogon grass will continue to be problematic to native communities under future precipitation regimes and the legacy of cogon grass invasion may have negative effects on restoration of wiregrass, but potentially more importantly, pine requires a robust microbial community regardless of soil legacy to compete effectively. This result suggests that

management such as top-soil removal could inhibit longleaf pine restoration as mycorrhizal propagules decrease exponentially with depth (Genney et al. 2006). Furthermore, the comprehensive methodology used here could be adapted and applied to other plant invaders and alternative abiotic conditions.

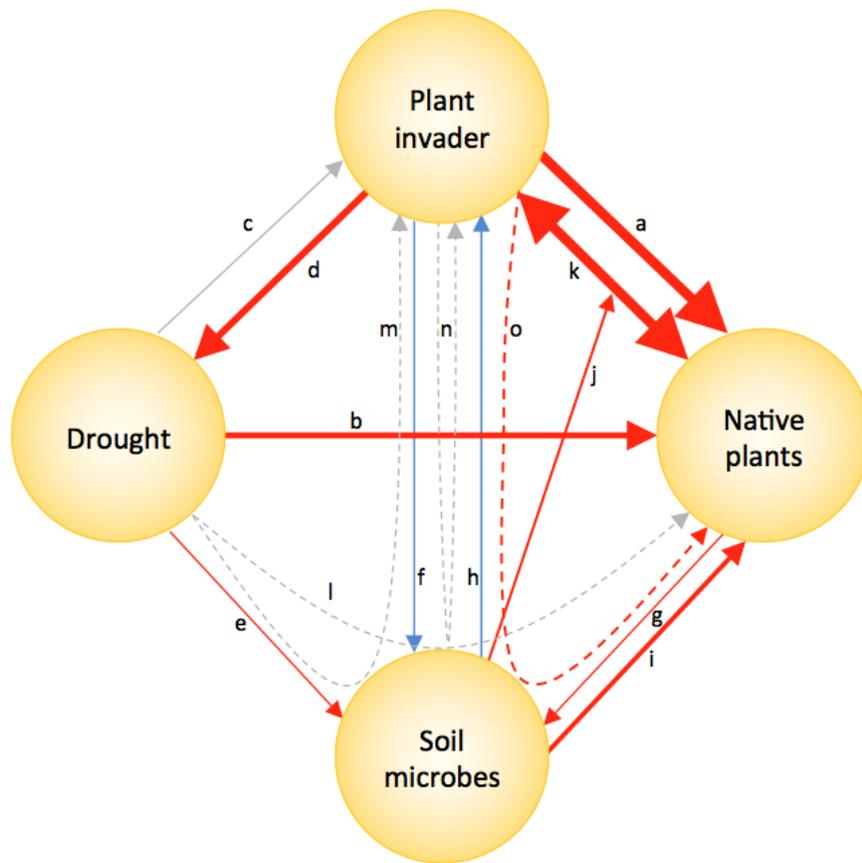


Figure 5-1. Synthesis diagram of the combined results of the experiments in this dissertation. Solid arrows indicate direct effects while dashed arrows indicate indirect effects. Arrow widths are proportional to the size of the effect indicated. Red arrows indicate a negative effect, blue arrows indicate a positive effect, and gray arrows indicate hypothesized interactions with no significant effect. The direct effects of invasion and drought on native plants and soil microbes specifically refer to the effects on Shannon diversity index. The direct effect of drought on the invader (specifically cogongrass) indicates change in percent cover. The direct and indirect effects of soil microbes and soil legacies on native plants and the invader refer to total biomass of longleaf pine and wiregrass, and cogongrass grown alone.

APPENDIX A
CHAPTER 2 SUPPLEMENTAL INFORMATION

Experimental Plots

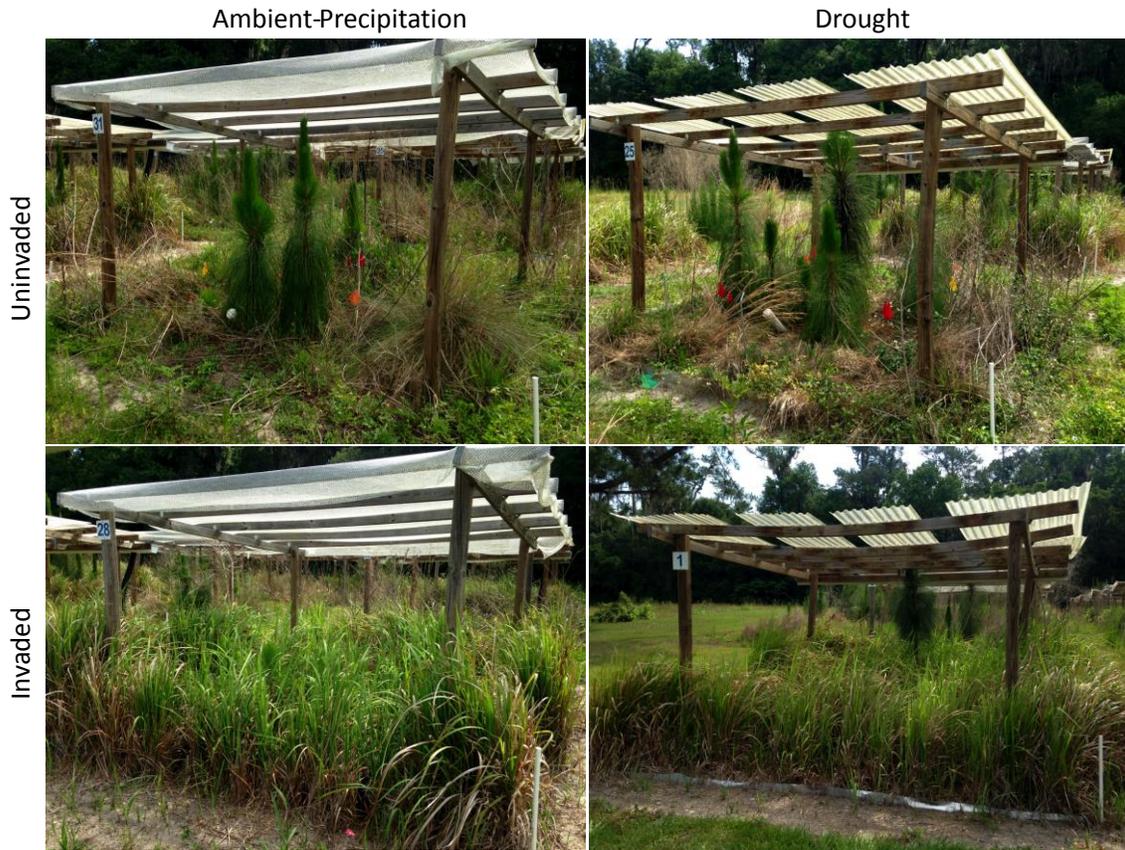


Figure A-1. Experimental plots at the Bivens Arm Research Site, Gainesville, Florida in May 2016, showing factorial combination of treatments. Top left: uninvaded plot with ambient precipitation. Top right: uninvaded plot with drought treatment. Bottom left: plot invaded with *Imperata cylindrica* and ambient precipitation. Bottom right: plot with invasion and drought treatments.

Abiotic Data

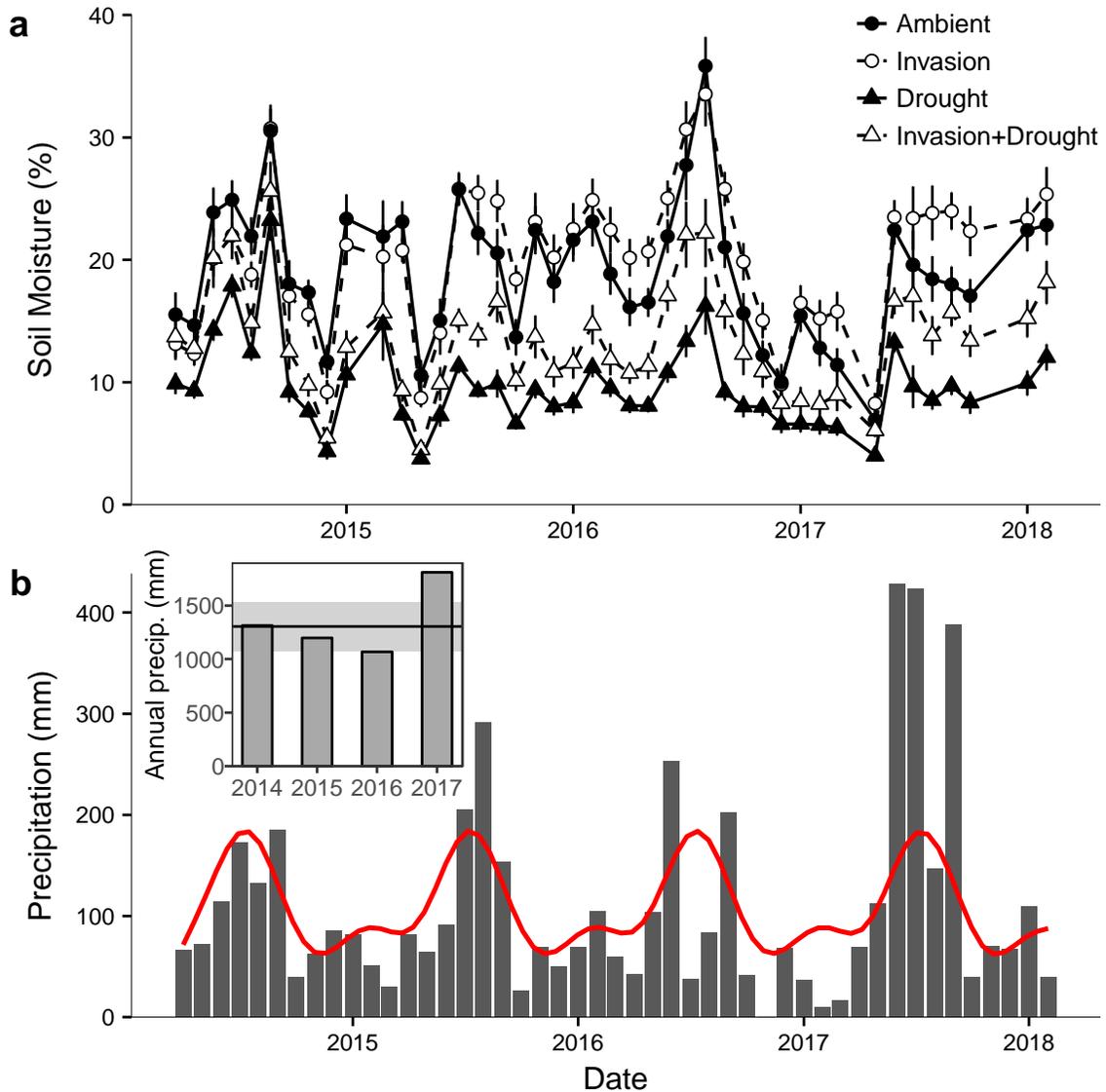


Figure A-2. Soil moisture and precipitation over the duration of the experiment. A) Monthly percent soil moisture to 12 cm depth (Mean \pm SE; N=10); B) sum of monthly precipitation measured at Gainesville Regional Airport, FL. Red line indicates 100-year average precipitation by month. Inset shows total annual precipitation by year. Black line indicates 100-year average of total annual precipitation and light gray shading indicates \pm 1 S.D. from 100-year average.

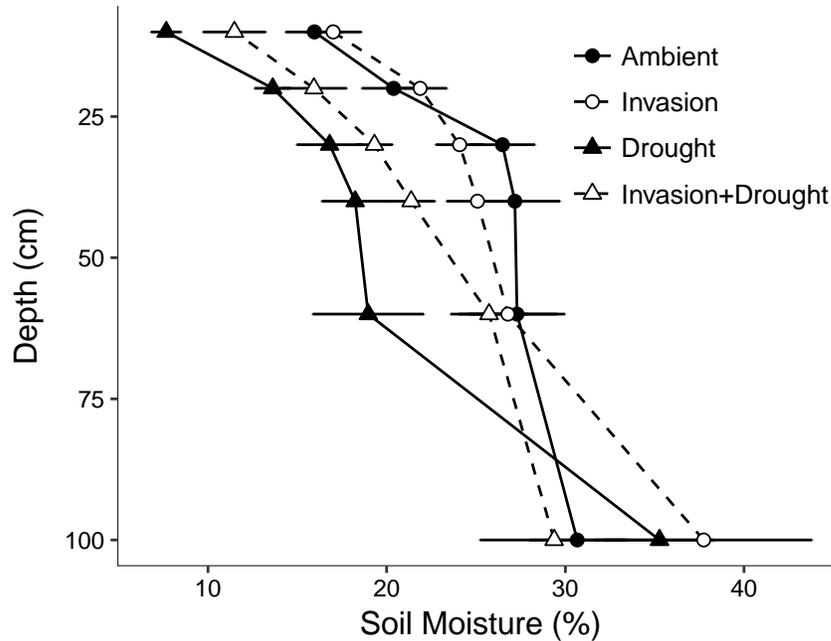


Figure A-3. Percent soil moisture by depth (Mean \pm SE). Points indicate averages across 10 replicates per treatment and across 14 sampling dates (9-26-14, 11-11-14, 11-24-14, 12-20-14, 1-21-15, 6-8-16, 6-22-16, 7-21-16, 8-22-16, 9-14-16, 9-30-16, 10-14-16, 1-6-17, 4-7-17). Soil moisture differed significantly between ambient and drought treatments to 40 cm depth but not at 60 or 100 cm depth (invasion \times drought \times depth; $F_{1,306}=5.0$, $P=0.03$).

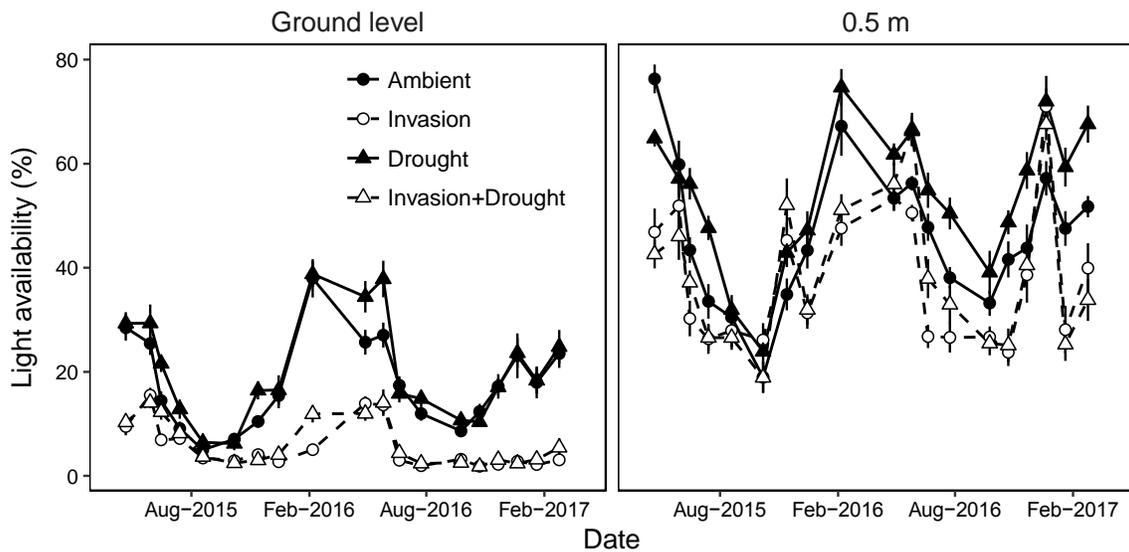


Figure A-4. Percent availability of photosynthetically active radiation at ground level and 0.5 m height above the soil surface (Mean \pm SE; $N=10$). Percent light availability was calculated as (ambient light – light below canopy)/ambient light $\times 100$.

Plant Functional Groups

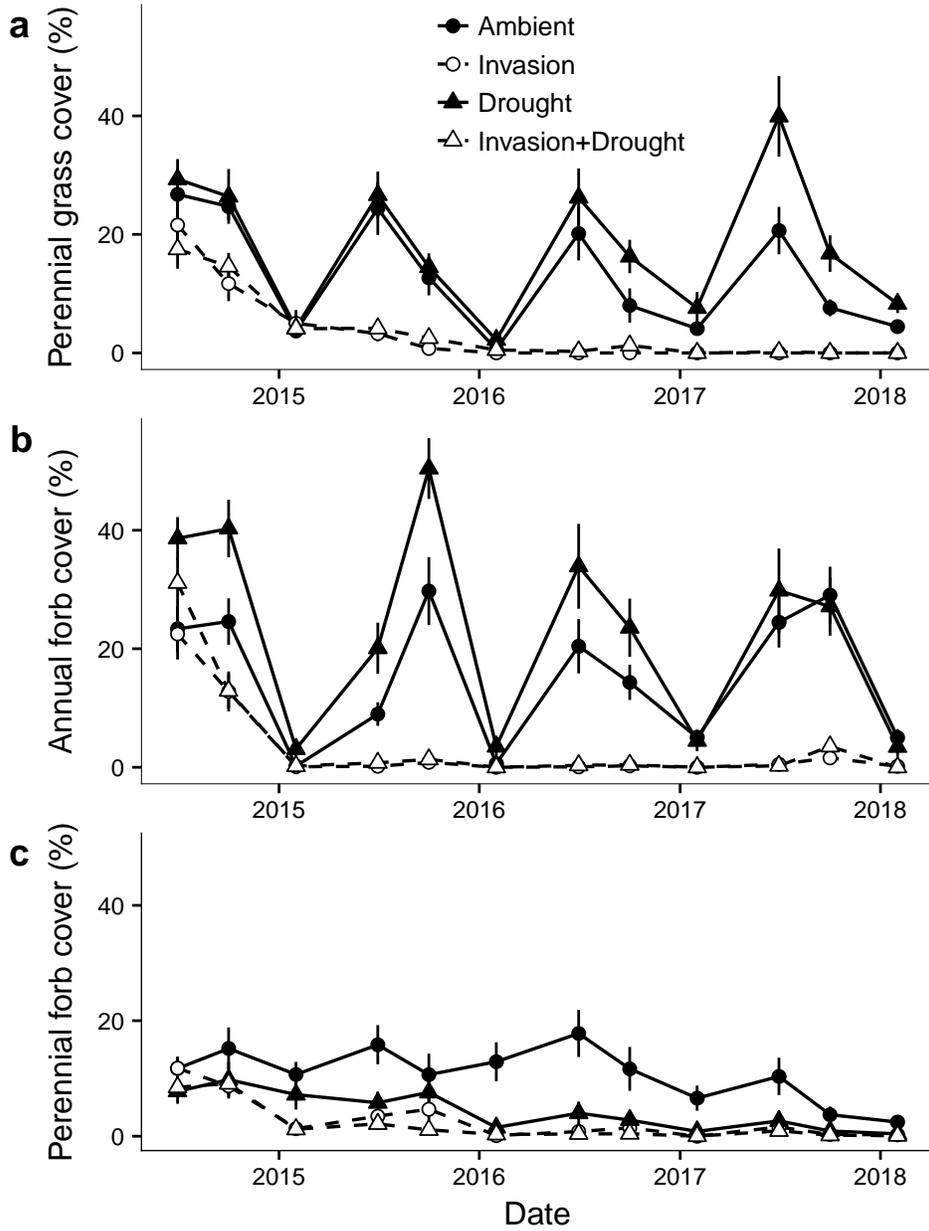


Figure A-5. Effects of invasion and drought on plant functional groups. Percent cover of A) perennial grasses (excluding *I. cylindrica*), B) annual forbs, and C) perennial forbs (Mean \pm SE; N=10).

Tables

Table A-1. List of herbaceous species planted into the plots.

Species	Functional group
<i>Andropogon brachystachyus</i>	grass
<i>Andropogon virginicus glaucus</i>	grass
<i>Aristida stricta</i>	grass
<i>Eragrostis ellioti</i>	grass
<i>Eragrostis spectabilis</i>	grass
<i>Muhlenbergia capillaris</i>	grass
<i>Panicum anceps</i>	grass
<i>Carophephorus subtropicanus</i>	forb
<i>Elephantopus elatus</i>	forb
<i>Liatrus laevigata</i>	forb
<i>Pityopsis graminifolia</i>	forb
<i>Solidago fistulosa</i>	forb

Table A-2. Results of the PERMANOVA of the main and interactive effects of invasion, drought, and date on plant community composition.

	Df	SumOfSqs	Pseudo F	R2	P
Date	1	3.86	31.58	0.09	<0.01
Invasion	1	17.74	145.24	0.41	<0.01
Drought	1	0.59	4.85	0.01	<0.01
Date:invasion	1	1.55	12.69	0.04	<0.01
Date:drought	1	0.09	0.70	0.01	0.57
Invasion:drought	1	0.93	7.65	0.02	<0.01
Date:invasion:drought	1	0.14	1.13	0.01	0.29
Residual	152	18.57		0.43	

Table A-3. Results of the PERMANOVA of the main and interactive effects of invasion and drought on plant community composition by year. F-values presented are pseudo-F values.

	2014					2015			
	Df	SumOfSqs	F*	P	R2	SumOfSqs	F*	P	R2
Invasion	1	0.36	2.87	<0.01	0.07	2.12	8.70	<0.01	0.18
Drought	1	0.28	2.25	0.01	0.05	0.39	1.61	0.11	0.03
Invasion:drought	1	0.11	0.85	0.49	0.02	0.49	2.00	0.04	0.04
Residual	36	4.46				8.79			
	2016					2017			
	Df	SumOfSqs	F*	P	R2	SumOfSqs	F*	P	R2
Invasion	1	3.09	12.27	<0.01	0.24	2.63	9.25	<0.01	0.19
Drought	1	0.31	1.24	0.31	0.02	0.20	0.71	0.76	0.01
Invasion:drought	1	0.54	2.14	0.06	0.04	0.57	2.00	0.07	0.04
Residual	36	9.07				10.22			

APPENDIX B
CHAPTER 3 SUPPLEMENTAL INFORMATION

Figures

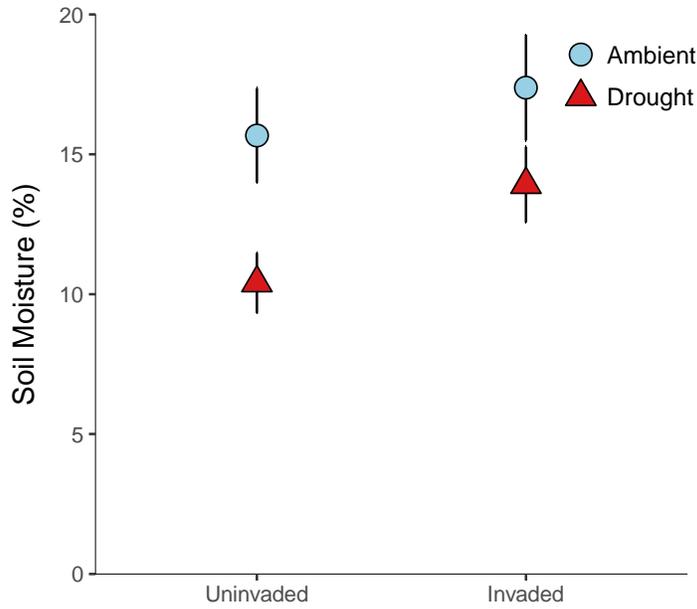


Figure B-1. Gravimetric soil moisture in ambient and drought plots either invaded by *Imperata cylindrica* or uninvaded.

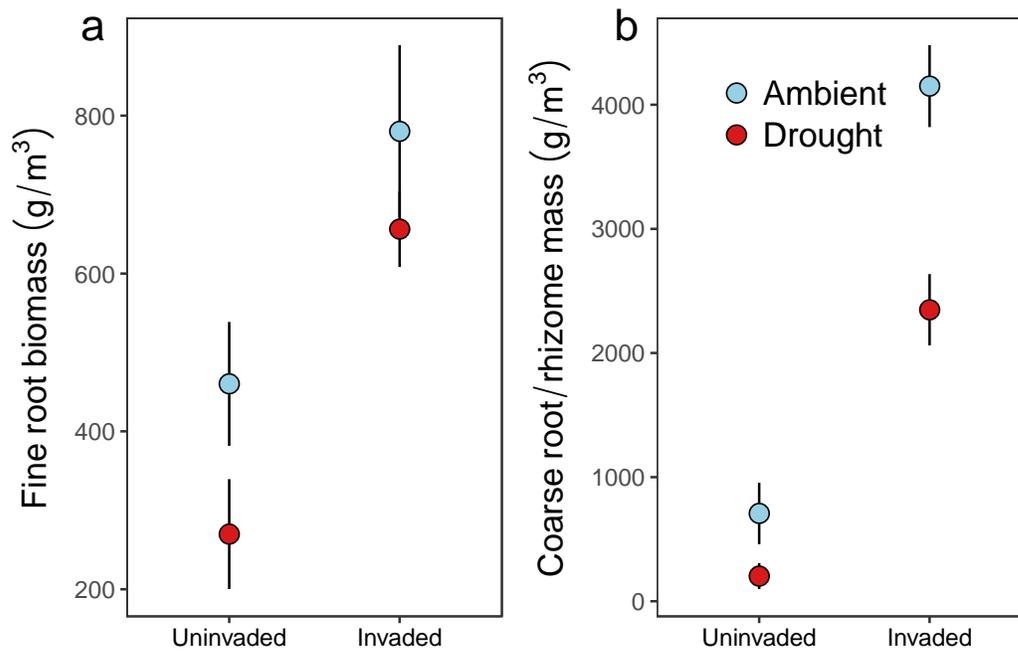


Figure B-2. Root biomass in invaded and uninvaded plots with ambient precipitation or drought at the 5-15 cm depth (mean \pm SE). a) Fine root biomass (< 1 mm diameter), b) Coarse root and rhizome biomass (>1 mm diameter).

Tables

Table B-1. Results of mixed effects model on bacterial richness, Shannon diversity index, and evenness.

Treatment	numDF	denDF	richness		shannon		evenness	
			F-value	p-value	F-value	p-value	F-value	p-value
Invasion	1	36	2.68	0.11	8.85	0.01	10.09	<0.001
Drought	1	36	5.98	0.02	16.19	<0.001	17.65	<0.001
Invasion:drought	1	36	2.15	0.15	0.86	0.36	0.34	0.56

Table B-2. Results of mixed effects model on fungal richness, Shannon diversity index, and evenness.

Treatment	numDF	denDF	richness		shannon		evenness	
			F-value	p-value	F-value	p-value	F-value	p-value
Invasion	1	36	0.06	0.81	0.62	0.44	0.75	0.39
Drought	1	36	0.42	0.52	1.16	0.29	1.15	0.29
Invasion:drought	1	36	3.09	0.09	0.98	0.33	0.58	0.45

Table B-3. Results of mixed effects model on arbuscular mycorrhizal fungal richness, Shannon diversity index, and evenness.

Treatment	numDF	denDF	richness		shannon		evenness	
			F-value	p-value	F-value	p-value	F-value	p-value
Invasion	1	36	0.11	0.75	0.04	0.85	0.36	0.55
Drought	1	36	0.21	0.65	0.44	0.51	1.42	0.24
Invasion:drought	1	36	2.57	0.12	6.35	0.02	2.77	0.11

Table B-4. Results of PERMANOVA on weighted UNIFRAC distance of the bacterial community.

Treatment	Df	Sum of sqs	Pseudo-F	P	R ²
Drought	1	0.032	4.251	0.002	0.10
Invasion	1	0.013	1.708	0.088	0.04
Invasion:drought	1	0.009	1.246	0.261	0.03
Residual	36	0.270			

Table B-5. Results of PERMANOVA on unweighted UNIFRAC distance of the bacterial community.

Treatment	Df	Sum of sqs	Pseudo-F	P	R ²
Drought	1	0.124	2.396	0.001	0.06
Invasion	1	0.063	1.206	0.122	0.03
Invasion:drought	1	0.069	1.326	0.037	0.03
Residual	36	1.869			

Table B-6. Results of PERMANOVA on Bray-Curtis dissimilarity matrix of the fungal community.

Treatment	Df	Sum of sqs	Pseudo-F	P	R ²
Drought	1	0.364	1.605	0.068	0.04
Invasion	1	0.278	1.225	0.189	0.03
Invasion:drought	1	0.820	3.612	0.001	0.09
Residual	36	8.168			

Table B-7. Results of PERMANOVA on Bray-Curtis dissimilarity matrix of the arbuscular mycorrhizal fungal community.

Treatment	Df	Sum of sqs	Pseudo-F	P	R ²
Drought	1	0.751	2.639	0.001	0.06
Invasion	1	0.453	1.592	0.043	0.04
Invasion:drought	1	0.416	1.461	0.085	0.04
Residual	36	10.242			

APPENDIX C
CHAPTER 4 SUPPLEMENTAL INFORMATION

Figures

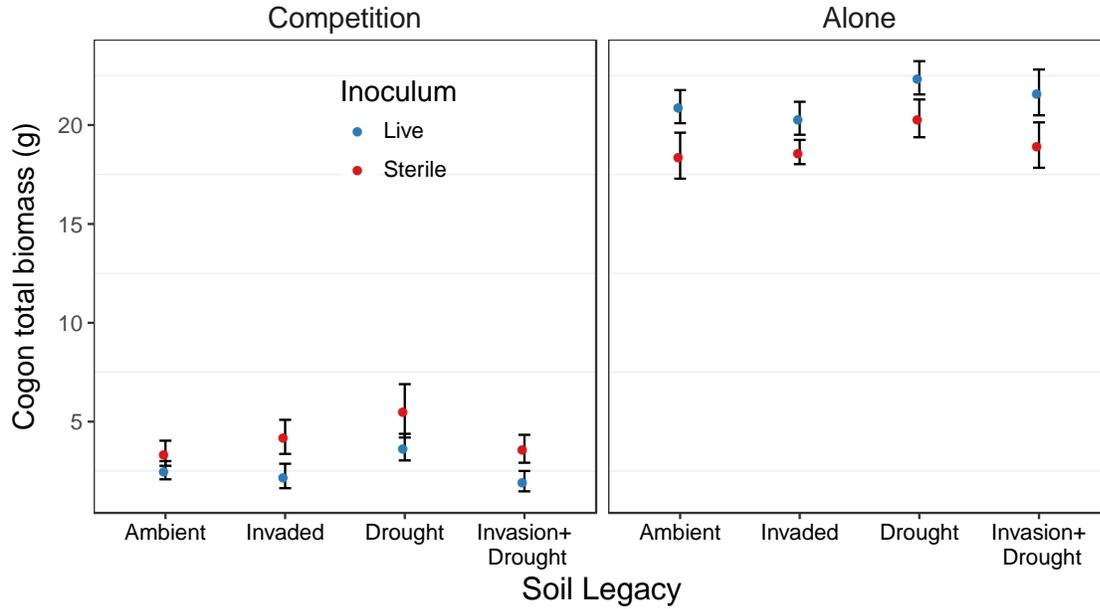


Figure C-1. Change in total biomass of cogongrass in live or sterile soil with soil legacies of invasion x drought grown alone or in competition with pine and wiregrass.

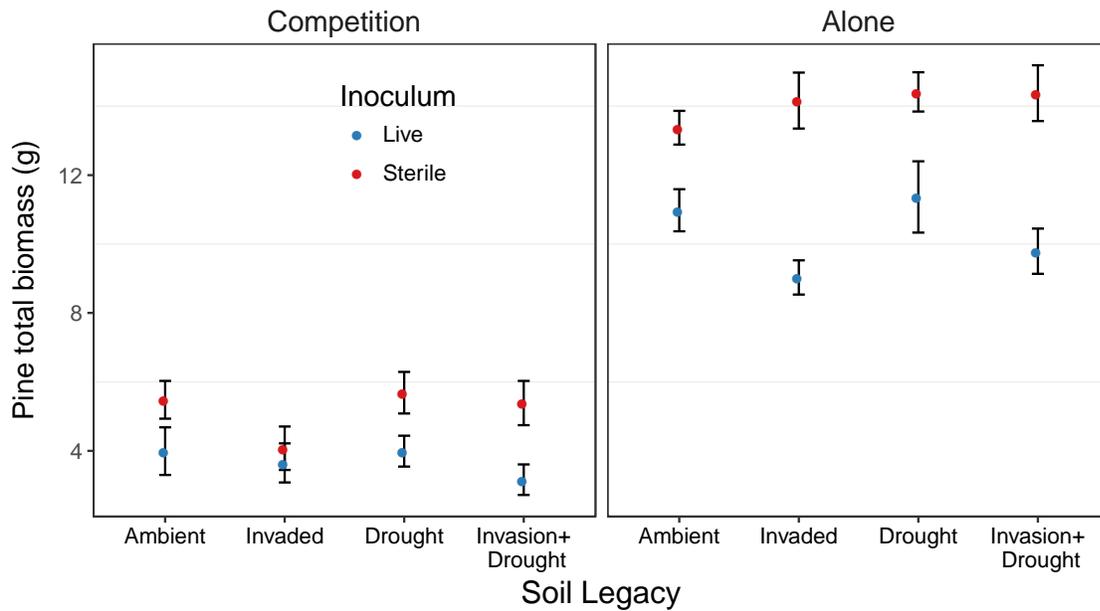


Figure C-2. Change in total biomass of longleaf pine in live or sterile soil with soil legacies of invasion x drought grown alone or in competition with wiregrass and cogongrass.

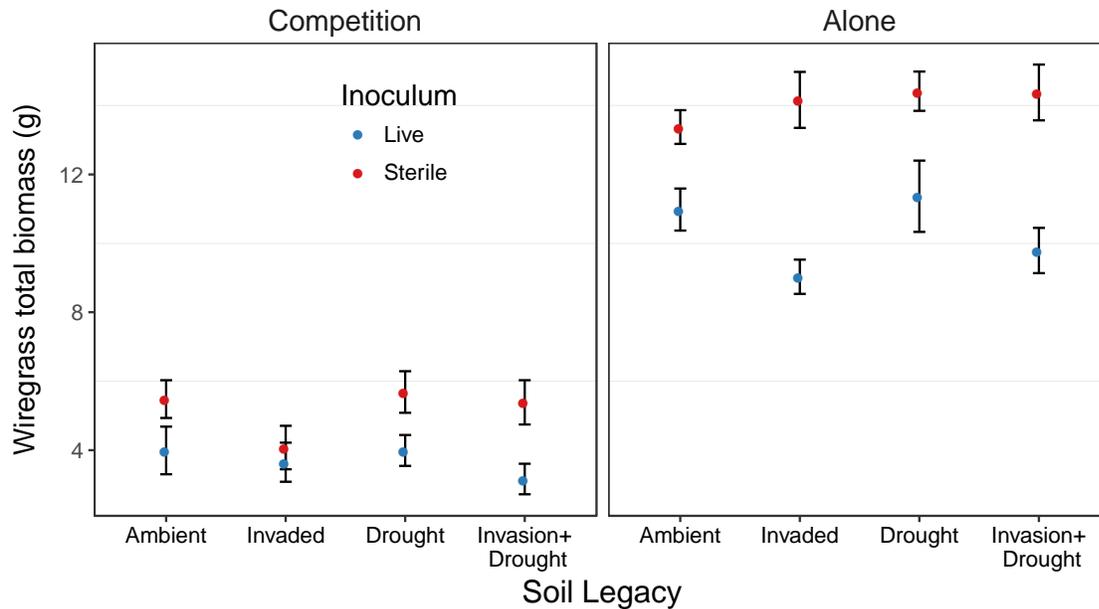


Figure C-3. Change in total biomass of wiregrass in live or sterile soil with soil legacies of invasion x drought grown alone or in competition with pine and cogongrass.

Tables

Table C-1. Results of mixed effects of soil legacy of invasion and drought, live or sterile inoculum, alone or in competition, and their interactions on total cogongrass biomass.

	numDF	denDF	F-value	P-value
(Intercept)	1	108	597.2597	<0.0001
Invasion	1	27	3.4757	0.0732
Drought	1	27	6.1531	0.0196
Inoc	1	108	0.7529	0.3875
Comp	1	108	2006.0846	<0.0001
Invasion:drought	1	27	3.8374	0.0605
Invasion:inoc	1	108	0.1311	0.718
Drought:inoc	1	108	0.0015	0.9695
Invasion:comp	1	108	0.0433	0.8355
Drought:comp	1	108	0.7209	0.3977
Inoc:comp	1	108	25.6847	<0.0001
Invasion:drought:inoc	1	108	0.8142	0.3689
Invasion:drought:comp	1	108	0.6874	0.4089
Invasion:inoc:comp	1	108	0.0653	0.7988
Drought:inoc:comp	1	108	0.1521	0.6973
Invasion:drought:inoc:comp	1	108	0.0004	0.9833

Table C-2. Results of mixed effects of soil legacy of invasion and drought, live or sterile inoculum, alone or in competition, and their interactions on total pine biomass.

	numDF	denDF	F-value	P-value
(Intercept)	1	101	849.4689	<.0001
Invasion	1	27	1.7429	0.1979
Drought	1	27	1.4933	0.2323
Inoc	1	101	13.9675	0.0003
Comp	1	101	343.2132	<.0001
Invasion:drought	1	27	0.9757	0.332
Invasion:inoc	1	101	3.5228	0.0634
Drought:inoc	1	101	2.2234	0.139
Invasion:comp	1	101	1.4311	0.2344
Drought:comp	1	101	1.6539	0.2014
Inoc:comp	1	101	49.1315	<.0001
Invasion:drought:inoc	1	101	0.0292	0.8648
Invasion:drought:comp	1	101	0.0004	0.9841
Invasion:inoc:comp	1	101	0.0635	0.8016
drought:inoc:sp	1	101	0.5093	0.4771
invasion:drought:inoc:sp	1	101	3.1502	0.0789

Table C-3. Results of mixed effects of soil legacy of invasion and drought, live or sterile inoculum, alone or in competition, and their interactions on total wiregrass biomass.

	numDf	denDf	F-value	P-value
(Intercept)	1	106	734.5888	<0.0001
Invasion	1	27	8.1932	0.008
Drought	1	27	2.2282	0.1471
Inoc	1	106	72.3774	<0.0001
Comp	1	106	683.5666	<0.0001
Invasion:drought	1	27	0.0302	0.8633
Invasion:inoc	1	106	2.1672	0.1439
Drought:inoc	1	106	1.0251	0.3136
Invasion:comp	1	106	0.0002	0.9883
Drought:comp	1	106	0.39	0.5336
Inoc:comp	1	106	15.7356	0.0001
Invasion:drought:inoc	1	106	0.0208	0.8855
Invasion:drought:comp	1	106	0.1546	0.695
Invasion:inoc:comp	1	106	3.7407	0.0558
Drought:inoc:comp	1	106	0.5738	0.4505
Invasion:drought:inoc:comp	1	106	1.3981	0.2397

Table C-4. List of seedlings that died by treatment and were excluded from analysis.

species	competition	inoculum	invasion	drought	dead count
pine	alone	sterile	uninvaded	ambient	1
pine	alone	sterile	invaded	ambient	1
pine	competition	sterile	uninvaded	drought	2
pine	competition	sterile	invaded	drought	1
pine	alone	sterile	invaded	drought	2
wiregrass	competition	live	uninvaded	ambient	1
wiregrass	alone	sterile	invaded	ambient	1

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BIOGRAPHICAL SKETCH

Catherine Fahey grew up in Ithaca, New York. After graduating high school in 2006, she went on to study environmental science and international development at Cornell University and studied abroad in New Zealand and Chiapas, Mexico. She took part in research internships at Hubbard Brook Experimental Forest in New Hampshire, Arnot Forest in New York, Amando Bermudez National Park in the Dominican Republic, and Blodgett Forest and Sequoia National Park in California where she did her honors thesis research with Dr. Robert York and Dr. Teresa Pawlowska. After earning a Bachelor of Science degree in 2010, she spent a year as a laboratory manager for Dr. Teresa Pawlowska in the Department of Plant Pathology at Cornell working on evolution of mycorrhizal fungi. She joined the Department of Biology at University of Florida with Dr. Kaoru Kitajima in 2011, working in the diverse tropical forests of Panama and received her master's degree in 2014. She then joined Dr. S. Luke Flory's lab in the Agronomy Department at the University of Florida to work on plant invasions in the southeast US. In August 2018, she received her Ph.D. in Interdisciplinary Ecology.