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Quantifying the Morphological and Physiological Effects of Drought, Grass Invasion, and Fire on Longleaf, Loblolly, and Slash Pine

Laura Young

QUANTIFYING THE MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF

DROUGHT, GRASS INVASION, AND FIRE ON LONGLEAF, LOBLOLLY, AND SLASH

PINE

by

LAURA L. YOUNG

(Under the Direction of Kerrie Sendall)

ABSTRACT

Climate change is altering ecosystems on a global scale, creating novel ecological scenarios with which plant species must cope. Factors such as altered precipitation and fire regimes and non-native plant invasion may negatively affect native plant species. The interactions between these stressors could also magnify their impacts. The complexity of multiple stressors and the effects they have on native pine seedlings are difficult to predict without evaluating their combinations in field experiments. In a multi-year study, I investigated the effects of drought and plant invasion on three southeastern pine species under pre and post-fire conditions. We planted longleaf (*Pinus palustris*), loblolly (*P. taeda*), and slash (*P. elliottii*) pine into a factorial common garden experiment with the following treatment combinations: (1) native vegetation, ambient precipitation; (2) grass invasion by cogongrass (*Imperata cylindrica*), ambient precipitation; (3) native vegetation, drought simulated by rain out shelters; (4) grass invasion, drought. I measured growth and physiological traits of the native pine seedlings to quantify differences between treatments and species as well as pre and post-fire differences. I found that under both pre and post-fire, drought alone was the most debilitating stressor physiologically for pine seedlings. Drought led to decreased growth and survival in comparison to seedlings affected by grass invasion and the combination of drought and grass invasion. Grass

invasion negatively affected seedling physiology and survival; however, in the pre-fire growing season the addition of the grass invader to drought treatments alleviated some of the physiological stress caused by unusually low seasonal rates of precipitation. Grass invasion also alleviated physiological drought stress of pine seedlings to some degree in the post-fire growing season, but I also found that the combination of grass invasion and drought resulted in significantly lower rates of seedling survival. Interactions between these stressors are thus very complex and may require unique management techniques under future climate conditions. This field experiment indicates that careful site selection will be necessary when establishing new pine plantations or restoring longleaf pine habitat, to avoid drought, plant invasion by cogongrass, and most importantly the combination of the two.

INDEX WORDS: Physiology, Multiple stressors, Drought stress, Cogongrass, Chlorophyll fluorescence, Seedling.

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GENERAL INTRODUCTION

Climate change has serious implications for the future of many species in the southeastern United States (Smith et al., 2015; IPCC, 2014). Many tree species are already experiencing the effects of invasive species introduction, increased drought, altered fire regimes, or a combination of these factors (NeSmith et al., 2018; Samuelson et al., 2012; Caldeira et al., 2015). Forests are equipped to deal with stressors, but the rate of change and quantity of new threats may prove to be too intense to allow native species to respond to effectively.

Plant invaders, drought, and altered fire regimes are currently affecting tree species in the southeastern United States (Barton, 1993; Caldeira et al., 2015; Samuelson et al., 2012). Invasive plant species can invade new areas that were previously unsuitable to them due to changes in climate. Temperature and precipitation changes can alter the borders of unsuitable habitat that used to constrain these invaders (Allen & Breshears, 1998). Invasive plants then compete with native vegetation, and if the invaders are successful it can cause a severe reduction in native plant diversity, in some cases transforming the landscape to a different habitat type (Brewer, 2008). Dominant tree species present in the eastern North American coastal plains are also severely affected by drought, indicated by the significant correlations between growth deficits and the Palmer Drought Index (Henderson et al., 2009; Parker et al., 2014). Longleaf pine (*Pinus palustris,* Mill.)*,* slash pine (*P. elliottii*, Engelm.)*,* and loblolly pine (*P. taeda,* L.) all show that trends in growth and physiology have a direct relationship to changes in precipitation, temperature, and drought (Samuelson et al., 2012). Changes in temperature and precipitation regimes have also altered historical fire behavior, causing larger and more frequent fires under drier conditions. Non-native species invasion adds to the fuel loading (biomass available to burn) by providing higher densities of biomass or changing the fuel continuity across the landscape,

both of which increase fire intensity (Hess & Tschinkel, 2017). Fire intensity and severity are also increased by reduced precipitation and warmer temperatures, leading to pre-drying of the increased fuels available. This creates a dangerous interaction between these three variables (Costanza et al., 2015; Stephens et al., 2018; Stocks et al., 1998). An analysis of the responses of dominant timber species in the southeastern region to these stressors is essential to make informed management decisions under future climate scenarios.

The rate at which drought, grass invasion, and fire regime changes associated with global climate change are occurring pose the largest hurdle for plants as they are exposed to new environments. Unlike animals, individual plants cannot migrate away from areas that are no longer suitable ecosystems for them. Instead they can move slowly over generations via seed dispersal, acclimate to the new factors present in the area, or extirpate and be replaced with another hardier species. Plants are already moving poleward and up in elevation, and those unable to migrate have begun to be replaced by more competitive species (Aitken et al., 2008; Hoffman & Sgro, 2011).

There are also many other barriers that prevent plants from migrating across a landscape to more suitable habitats in response to climate change. Large geographic barriers like mountain ranges, deserts, or oceans make it difficult for plants to colonize within a short time period. Many species also cannot cope with major variations in soil type, soil pH, precipitation regimes, elevation, periods of freezing, drought, and many other factors that already define the edges of most species ranges globally (Lafleur et al., 2010; Feeley et al., 2014). Therefore, acclimations of plant species within areas that they already inhabit is the most viable option for human assistance through management strategies to counter the effects of climate change.

Species-specific trait differences allow some species to better respond to stressors like drought, invasion, and fire. In general, gymnosperms are better at coping with drought stress due to superior resistance to embolism using adaptive anatomical structures and strategies such as exclusively using tracheids, as well as surfactant coating to avoid cavitation (Lens et al., 2013; Venturas et al., 2017). In addition, gymnosperms operate in a much safer hydraulic range, develop more advantageous root morphology, and generally possess leaf traits such as low surface area to volume ratios in comparison to angiosperms (Brodribb et al., 2005). They have also developed fire-resistance mechanisms and traits to deal with fire such as thick protective bark or substantial belowground biomass storage (Schubert et al., 2016). Identifying these traits and species, and testing their limits allows us to gather key information on how individual species will respond to changes in climate. This information then allows us to better compare native species resistance to stressors.

Longleaf pine, slash pine, and loblolly pine are three dominant pine species in the southeastern United States, making them excellent model species for climate change scenario research (Landers et al., 1995). Longleaf pine forests once dominated the landscape throughout the majority of the coastal plains, but these forests have been decimated by anthropogenic changes, and only a few million acres exist today (Landers et al., 1995). Longleaf pines offer many ecological benefits, sequestering carbon and creating important habitat with a unique herbaceous understory for hundreds of animal and plant species, many of which are now considered threatened or endangered (Jackson, 1989). Keystone species, such as the endangered gopher tortoise, that rely on longleaf pine habitat, also provide ecosystem services like burrows that are used by over 60 other animal species (Jackson, 1989). Longleaf pine is considered to be more drought tolerant than either slash or loblolly pine, quantified by higher water use

efficiency. However, the mechanisms driving this trait are still poorly understood (Table 1.1; Samuelson et al., 2012). Longleaf pine also produces a large taproot that allows it to seek resources lower in the water table when they become scarcer in surface soils. This may indicate that longleaf pine will outperform the others under drought conditions (Landers et al., 1995; Carey, 1992). This large taproot also stores large quantities of carbon belowground and allows juvenile longleaf pines to be more resistant to fire than younger slash and loblolly pines. The belowground storage potentially allows longleaf pine to resprout even following higher intensity burns that may become more common in the future (Carey, 1992; Abatzoglou et al., 2018). Taking future climate scenarios into account, longleaf pine may be a more viable option for current and future southern forestry practices, and a renewed interest has been placed on restoration efforts (South, 2006).

Although slash and loblolly pine do not provide the same level of ecological benefits as longleaf pine, they do represent an economic resource that supports the southeastern timber industry. Both pine species sequester carbon, although not as efficiently as longleaf pine, and they also provide habitat for some animal species. The closer planting spacing in slash and loblolly pine plantations shades the understory of these forest stands which results in a less complex ecosystem that cannot support the diversity found in longleaf pine habitat (South, 2006). Slash and loblolly pine, however, represented almost 95% of planted southern pines in the late 1990s due to their more lucrative return value and shorter management intervals (20-30 year vs. longleaf at 40-50 year) (Landers et al., 1995) and they still represent the majority of timber production in the southeast (Prestemon & Abt, 2002; Alavalapati & Carter, 2002). Although slash and loblolly pine have lower drought tolerance than longleaf pine, they do have a higher shade tolerance (Table 1.1). Lower shade tolerance in longleaf pine makes the grass invader

(discussed below) a potentially more debilitating stressor for this species in comparison to slash or loblolly pine. These species-specific differences in tolerance of environmental stressors may demonstrate advantages to species selection when specific stressors or combinations of stressors studied here are present in both plantations and natural ecosystems.

Imperata cylindrica (L.) P. Beauv., cogongrass, currently poses a serious threat to native vegetation in seven states in the southeastern United States (Holzmueller & Jose, 2011). The species was intentionally introduced from its native range in the eastern hemisphere to Florida for use as a foraging crop. Cogongrass was also accidently brought over through global shipping to invade additional locations the southeastern U.S. The invasive grass is able to grow readily in many different microclimates and is now considered to be one of the world's worst weeds (Bryson & Carter, 1993). The species is such a successful competitor that it almost exclusively exists in monotypic stands in its introduced and native ranges, making it difficult for other species to establish. Existing native vegetation is then required to compete for light, water, and other essential nutrients (Estrada & Flory, 2015; Toky & Ramakrishnan, 1983). Cogongrass also keeps the majority of its biomass belowground as it reproduces asexually via rhizomes that can puncture the roots of nearby vegetation and open them up to infection (Holly & Ervin, 2006). Cogongrass lowers the soil pH with the addition of organic matter and reduces the availability of nutrients to other types of vegetation by moving nutrients through rhizomes to daughter plants. It also interferes with mycorrhizal associations in pine seedling roots by releasing compounds that decimate fungal populations, which indirectly decreases fine root production of the pines (Trautwig et al., 2017; Holzmueller & Jose, 2011). In its native habitat, cogongrass typically exists in monotypic grasslands that regularly experience high temperatures and limited soil water availability. Cogongrass is also maintained by frequent fires, and can easily return immediately

post-burn. This suggests that cogongrass may be unaffected by any of the stressors present in the United States (Toky & Ramakrishnan, 1983).

Cogongrass invasion results in major ecosystem changes such as: (1) the shift in biodiversity from native vegetation to monotypic stands of cogongrass, (2) the altered fire regime that results in changes in fire transmission across the landscape and overall severity, and (3) the decreased growth in pine species for timber production resulting from resource competition (Brewer, 2008; Hess & Tschinkel, 2017). These alterations will result in different habitat types that are not complex enough to support the 900 endemic species that occur within longleaf pine habitat (Van Lear, 2005). Loss of wood production in the timber industry should stimulate interest in the treatment of this grass invader; however, the most viable current management strategy available for cogongrass is a combination of repeated mechanical treatments like discing the landscape (i.e. tearing up the land and the rhizomes of cogongrass with heavy machinery), followed by several chemical treatments with glyphosate to provide long term control (Dozier et al., 1998). Since cogongrass occupies such a large geographical area, these strategies may not prove to be economically efficient, so more research is required to determine how it may affect ecosystems in the future.

Droughts are already becoming more frequent, prolonged, and widespread and they are only predicted to worsen in the future (Dai, 2013; Smith, 2015). Water stress causes plants to close stomata and reduce rates of transpiration to conserve what little water is available. Decreased water movement leads to lower rates of photosynthesis and reduced growth rates, which negatively affect both timber production and natural ecosystem regeneration (Boyer, 1982). Seedlings are even more susceptible to drought, as they do not have the advanced root structure or carbon stores of older trees. This forces them to photosynthesize under stressful

conditions to maintain the biomass they have already accumulated (Samuelson et al., 2012).

Transpiration of water under drought scenarios severely increases the risk of cavitation, which is

caused by pulling an air bubble into the xylem instead of water. Air bubbles cause the water

column to break, resulting in the loss of functionality of that tracheid (Lens, 2013; Venturas et

al., 2017). Increased water stress on the seedling makes additional stresses like plant invasion

and secondary fire effects even more debilitating and pushing seedlings to operate under riskier

hydraulic margins to cope with these stresses can lead to mortality. Drought has already killed

millions of trees in the U.S. over the last two decades, so drought hardier species and careful site

selection may become more essential for long term forest rehabilitation and survival (Moore et

al., 2015; Guarin & Taylor, 2005).

Fire is becoming more frequent and intense in natural systems due to increased

temperatures and altered precipitation regimes. In managed areas, however, fire frequency is

often reduced because many slash and loblolly plantations no longer use prescribed fire for

management as these pine species are vulnerable to fire as seedlings. After fire exclusion persists

in these plantations for six to eight years, understory woody shrub encroachment begins,

resulting in a less complex ecosystem. The number of landscapes close to human development

have also increased dramatically in past decades, making it difficult to use fire to manage those

systems. Fire suppression has become increasingly common in the southeastern U.S. because

fires are costly to contain within human development and public opinion of fire as a management

strategy is not unanimously positive (Costanza et al., 2015; Van Lear, 2005). Fire behavior is

dependent upon a multitude of variables ranging from weather conditions like temperature,

relative humidity, and wind speed; to fuel characteristics like fuel loading and continuity (Bessie

& Johnson, 1995). Higher temperatures increase the pre-drying of fuels and reduce relative

humidity, making fires more likely to start and to keep burning. Lowered precipitation decreases fuel moisture which contributes to easier starts and more rapid and continuous burning, while increased precipitation results in more spring growth of vegetation that adds biomass to the landscape. More biomass increases fuel loading to create more intense fires (Stephens et al., 2018; Stocks et al., 1998).

Pine trees must deal with primary fire effects like removal of above ground vegetation by flames, or root death due to intense soil heating. Both are simple to quantify through post-burn survival surveys. However, trees must also deal with secondary fire effects that change the physical environment they occupy, which are much harder to quantify. Secondary fire effects consist of anything fire does to alter the physical environment. Flames heat the soil which stratifies compounds as they vaporize making it more hydrophobic (Certini, 2005). Fire can remove the vegetative layer, which exposes the ground to higher rates of soil moisture evaporation and creates a more difficult environment for seedlings to establish and survive in. Higher intensity burns reduce the soil organic matter, which removes soil nutrients that also aid in the water holding capacity of soils (Certini, 2005). Primary fire effects like tree mortality can also lead to secondary fire effects such as the loss of root structures that were preventing soil erosion for newly establishing trees. Species that are not equipped to deal with these effects or the magnitude and frequency of the climatically altered fire regimes may not be as successful as more fire tolerant species under future climate conditions.

To accurately measure the effects of the drought, grass invasion, and fire, I need to quantify the level of stress that each seedling experiences throughout the growing season. Photosynthetic rates and chlorophyll fluorescence emissions as well as other leaf level characteristics (respiration, transpiration, stomatal conductance, and water use efficiency) are

useful metrics for quantifying plant stress at any given time and have been proven to accurately represent stress in the field (Mommer et al., 2005). Leaf nitrogen is known to correspond with photosynthetic rates, and increased competition by the grass invader may lead to nitrogen limitations in native pines (Law & Waring, 2015). Chlorophyll fluorescence measures the electron transport rates and excitation of other electron sinks, CO_2 concentration in the chloroplasts, and the intercellular conductance of CO_2 in the mesophyll, something that has recently been shown to contribute to photosynthesis more than previously thought (Warren, 2008). Photosynthetically active radiation (PAR) and its associated energy absorbed by leaf tissues can follow one of three pathways: it can be used for photosynthetic processes, dissipated as heat, or emitted as fluorescence. Measuring the ratios of light used for photosynthetic processes versus heat loss and/or fluorescence can indicate the overall health and efficiency of the plant (Vogelman & Evans, 2002; Schreiber et al., 1995).

Growth is one of the most important metrics when predicting how plants will respond to different environments. Therefore characteristics like leaf cell elongation and biomass production are significantly affected when plants are exposed to stressors like nutrient competition, shade, and drought. A combination of height growth, diameter growth, and total biomass allow us to accurately represent the overall success of seedlings (Yano & Terashima, 2001; Sharp et al., 2004). Height and survival are good indicators of where and how well a plant is allocating the carbon it is fixing over time. More growth will either indicate more resources are available or higher resource usage efficiency in a species. Although there is the potential for etiolated growth in heavily shaded plots, pines typically gain height growth rapidly to compete for light with tight planting spacings. Southeastern pine species especially grow quickly in the early stages, gaining roughly two feet of growth per year, which should naturally surpass the

height of the grass invader (Carey, 1992). The combination of these morphological, physiological, and nutrient composition traits represents seedling stress and success across the treatments.

Several studies have been conducted to compare the relative tolerances of these three species to drought and/or fire (Barton, 1993; Samuelson et al., 2012; Hess & Tschinkel, 2017). Other studies have looked at the effects of either drought, invasive species encroachment, or fire on a single species (Henderson et al., 2009; Parker et al., 2014; Patterson et al., 2016). My experiment will examine drought, invasive species, fire, and most importantly the interaction between these different effects, something that is lacking in current research. Many climate models can predict how temperature changes will affect single factors, such as drought or fire intensity in the natural world. However, as one factor changes, it can modify other factors in the same ecosystem (Meng et al., 2014). For example, decreased precipitation may increase the prevalence and intensity of drought causing plants to transpire under riskier conditions or slow down photosynthesis and growth. Drought increases plant stress, which then increases the probability of encroachment by invasive species or mortality from fire. These feedbacks can amplify stress, and their complexity can be additive, synergistic, or offsetting. The magnitude of these feedbacks is difficult to predict without field studies that encompass the interactions of stressors to provide more information on the cascading effects of changes in climate (NeSmith et al., 2018; Figure 1.1). This novel field study will help to predict the individual and interactive effects of drought, grass invasion, and fire on growth, survival, physiology, and overall success of native pine species.

The following set of hypotheses deal with how I expect individual species to respond to drought, grass invasion, and the combination of stressors within this field experiment:

Ho All species will perform at the same physiological levels within each treatment. If the null hypothesis is supported, it will provide no reason for species selection due to possible dominant abilities to form climate tolerant stands.

HA Species that are more drought, shade, and fire tolerant will perform better in treatments with the respective stressor tolerance than those that do not possess these tolerances (Table 1.1). If supported, this may indicate potential benefits for species selection in both naturally restored climate tolerant stands and increased timber production under climatically altered stressors.

The following set of hypotheses cover how drought is going to affect seedlings of all species:

Ho Drought will have no physiological effect on seedlings. If supported, the null hypothesis will indicate that: all species have adapted mechanisms to cope with drought stress.

HA1 Drought stress will have a negative effect on pine seedlings, evidenced by lower rates of photosynthesis, growth, and survival. If supported, this will indicate that photosynthesizing under limited water conditions causes higher rates of water loss, which contribute to negative physiological effects on the seedlings.

HA2 Drought stress will have a positive physiological effect on pine seedlings, indicated by higher rates of photosynthesis, growth, and survival. If supported, this will indicate that a beneficial relationship between drought and the seedlings exists.

This set of hypotheses deals with how plant invasion is going to affect pine seedling species:

Ho Grass invasion will have no physiological effect on seedlings. If supported, the null hypothesis will indicate that: all species have adapted mechanisms to cope with stress from competition.

H$_{A1}$ Grass invasion will have a negative effect on pine seedlings, evidenced by lower rates of photosynthesis, growth, and survival from increased shading.

H$_{A2}$ Grass invasion will have a positive physiological effect on pine seedlings, indicated by higher rates of photosynthesis, growth, and survival. If supported, this will indicate that a beneficial relationship between cogongrass and the seedlings is present, possibly indicating cogongrass acts as a nurse plant.

The following set of hypotheses deals with how the combined stressors of drought and plant invasion will interact to affect the pine seedling species:

Ho Drought and grass invasion will have no interaction and no physiological effect on seedlings. If supported, the null hypothesis will indicate that: (1) all species have adapted mechanisms to cope with stress from both of these stressors, (2) there is no stress caused by drought or grass invasion, or (3) the effects of these stressors is exactly cancelled out by the two residing in the same area.

H$_{A1}$ Drought and grass invasion will have an additive negative effect on pine seedlings, showing an effect equal to the stress or drought and stress of grass invasion added together (Figure 1.2).

H$_{A2}$ Drought and grass invasion will have a negative, synergistic physiological effect on pine seedlings, demonstrating an effect magnitude that is more than the individual stressors of drought and grass invasion added together.

H$_{A3}$ Drought and grass invasion will have an offsetting effect on pine seedling physiology, resulting in less than the added effect of drought and grass invasion. If supported, this may indicate that either (1) some stressors do not have detrimental effects on seedlings (2) species have developed mechanisms to deal with some stressors better than others or (3) the stressors themselves affect each other to create negative feedbacks that lessen their effects.

This set of hypotheses covers how fire will affect the landscape, which indirectly influences the success of the pine seedlings:

H$_O$ Fire will have no effect on drought and/or plant invasion or their combination as evidenced by treatment differences between the pre-fire and post-fire seasons. If supported, this will indicate (1) native pine species are equipped to deal with secondary fire effects or (2) prescribed burning does not amplify the stressors of plant invasion or drought.

H$_{A1}$ Fire will increase the magnitude of drought. If supported this will indicate (1) species in this experiment are not resistant to the secondary effects of fire or (2) there are significant interactions between drought and fire that will negatively affect ecosystems.

H$_{A2}$ Fire will increase the magnitude of plant invasion. If supported this will indicate the invading species is more fire tolerant or more effective at taking over an early successional landscape than the native pines

H$_{A3}$ Fire will decrease the magnitude of drought stress on the pine seedlings. If

supported, this will indicate that fire reset the successional stages of the plots allowing

fast growing pioneer species to form a denser herbaceous layer reducing soil moisture

evaporation.

H$_{A4}$ Fire will decrease the magnitude of grass invasion. If supported, this will indicate

that grass invader is less fire tolerant or less capable of establishing in early successional

plant stages than the native pines

I expect physiology and growth to correlate well as photosynthetic processes lead to

carbon assimilation that results in growth, well documented in the literature. This allows us to

use short term physiological trends to predict trends in longer term growth effects.

Plants have an immediate physiological response to changes and stresses in their

environment, which eventually translates to long term growth effects (Shinovara & Leskovar,

2014). I used these well-supported responses that plants exhibit to different factors to help

determine the level of stress each seedling is experiencing. When under stress, plants typically

close stomata and reduce rates of transpiration and photosynthesis to retain water and increase

water use efficiency. Slowed physiological responses under stress, then negatively affect growth

and survival rates (Tatrai et al., 2016; Samuelson et al., 2012). Throughout this experiment I aim

to quantify differences in stress levels of seedlings in treatments representing all pertinent

stressors as well as potential species-specific responses. The proposed project is divided into two

chapters consisting of (1) the initial gas exchange and growth measurements of all three species

under drought and invasion treatments pre-fire and (2) chlorophyll fluorescence and growth

measurements of all three species after fire is introduced to the landscape and seedlings are exposed to all three stressors.

Table 1.1: Relative drought, shade, and fire tolerances of longleaf pine (*Pinus palustris* Mill.), slash pine (*P. elliottii* Engelm.), and loblolly pine (*P. taeda L.*) in the North American southeastern coastal plains ecosystem.

Plant trait	Longleaf pine	Slash pine	Loblolly pine
Drought tolerance	High	Moderate	Moderate
Shade tolerance	Low	Low	Moderate
Fire tolerance	High	Low	Moderate
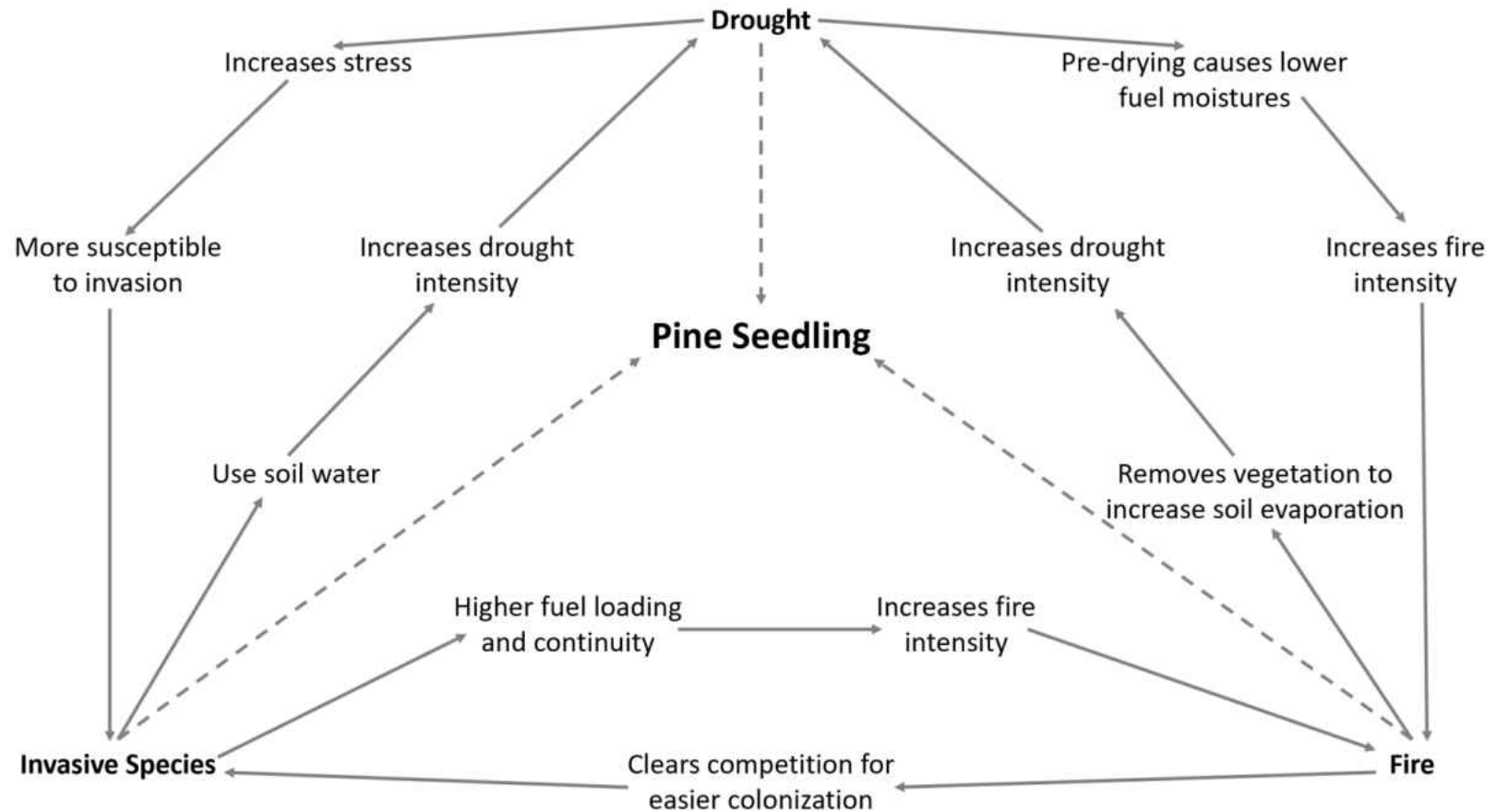
Figure 1.1: Representation of the three negative stressors: drought, invasive species, and fire on the pine seedling and how these stressors may be amplified by their interactions. Solid lines indicate positive feedbacks, or an increase in the magnitude of the affected stressor. Dashed lines indicate negative feedbacks, or a decrease in the magnitude of the affected stressor.
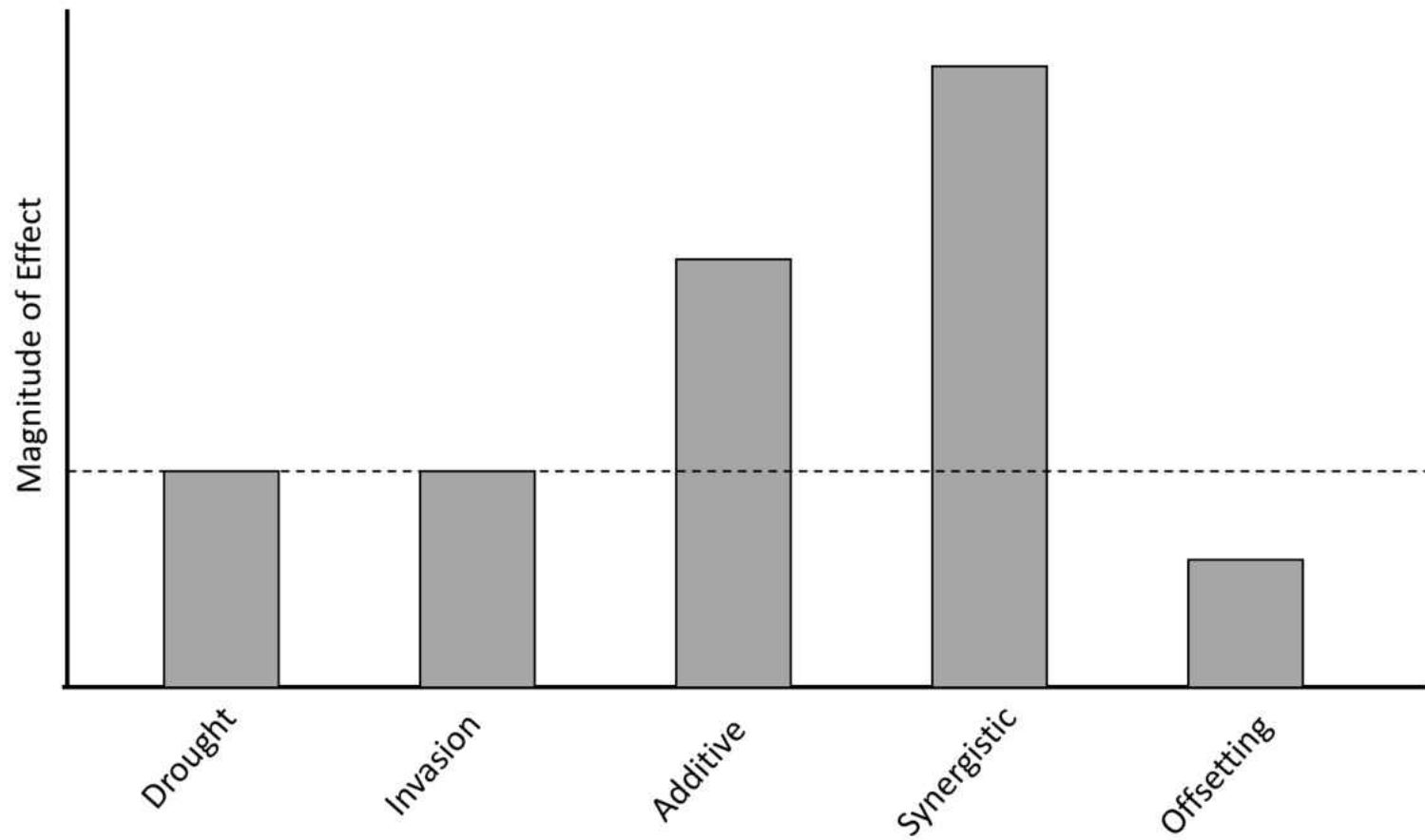
Figure 1.2: Representation of hypothesized outcomes of singular and interacting stressors and the magnitude of their effects.

CHAPTER 1

GRASS INVASION HAS OFFSETTING EFFECTS ON PROLONGED DROUGHT

AFFECTING SOUTHEASTERN PINE SEEDLINGS

ABSTRACT

Understanding how interactive effects of abiotic and biotic stressors will affect seedling growth and survival is critical for predicting how forests will respond to climate change. In a factorial field experiment, I investigated the effects of drought and an aggressive, non-native grass species, *Imperata cylindrica* (cogongrass), on the growth and physiological responses of three species of pine seedlings (*Pinus palustris* Mill., *P. taeda* L., and *P. elliottii* Engelm.). Seedlings were planted into each of four treatments: (1) native plant dominated, ambient precipitation; (2) invaded, ambient precipitation; (3) native plant dominated, drought; (4) invaded, drought. Competition by cogongrass for light, water, and soil nutrients resulted in a decrease in rates of photosynthesis and growth; however, drought in the native dominated plots had the strongest negative effect on the pine species leading to a 27% reduction in the rate of photosynthesis and a 36% decrease in overall growth rates. Interestingly, drought stress was less extreme for pines growing in invaded plots compared to uninvaded plots due to higher levels of soil moisture, indicating that cogongrass alleviated water stress of the pine species. However, more long-term effects of cogongrass invasion such as reduced light availability and increased belowground competition may prove to have serious implications for forested ecosystems in the southeastern United States.

INTRODUCTION

Climate change is influencing ecosystem functionality on a global scale by altering abiotic factors like precipitation and temperature, which then allow for changes in vegetative ranges. Different geographic habitable ranges can aid in the spread of invasive species by moving the borders of temperature and precipitation that originally contained them, stressing the native vegetation with additional non-native competition (Allen & Breshears, 1998; Henderson & Grissino-Mayer, 2009; Holzmueller & Shibu, 2011; Weltzin et al., 2003; Vitousek, 1994). More intense, widespread droughts lasting for prolonged time periods are becoming increasingly common in the southeastern United States (Dai, 2013; IPCC, 2014; Smith et al., 2015). Increased temperatures and decreased soil water availability associated with climate change altered drought may have devastating effects on terrestrial ecosystems, potentially leading to high rates of plant mortality. Plant die back and mortality removes the insulating ground cover causing even higher rates of soil water evaporation as well as loss of diverse habitat (Allen & Breshears, 1998; Caldeira et al., 2015).

Cogongrass (*Imperata cylindrica* (L.) P. Beauv.; hereafter cogongrass) is an invasive species native to Asia that currently poses a significant threat to native vegetation in the southeastern United States (Holzmueller & Jose, 2011). Cogongrass can grow readily in many different microclimates forming monotypic stands (i.e. areas having only one species represented) that make it difficult for other species to establish. Native vegetation must compete with the fast-growing grass invader for light, water, and space in areas where cogongrass is prevalent (Estrada & Flory, 2015). Cogongrass can alter the environment it invades by lowering the soil pH with the addition of organic compounds. It can also reduce the availability of soil nutrients to native vegetation by redistributing nutrients from the soil further away from native

plants and closer to cogongrass daughter plants via rhizomes (Trautwig et al., 2017; Holzmueller & Jose, 2011). This species is a current threat to native species in seven states in the U.S., specifically in the southeast, and control options for the grass invader are costly and infeasible for larger areas. We will be dealing with cogongrass across plantations and native ecosystems, making it imperative to understand the larger ecosystem effects.

The effects of these stressors on juvenile pines are important to understand, as the seedling life stage is typically more vulnerable to biotic and abiotic stressors like drought and competition compared to older saplings and mature trees. For example, drought can reduce available water stores to deeper regions of the soil that cannot be accessed by the limited root structures of seedlings. These individuals also lack the underground storage structures of older trees to draw upon as an alternative to photosynthesizing under limiting water conditions. Seedlings then operate under a riskier hydraulic strategy that can lead to mortality (Boyer, 1982; Samuelson & Stokes, 2012). Seedling mortality represents a significant economic loss if replanting is necessary. Mortality in natural systems attempting to regenerate also represents a significant loss as cones are not produced yearly. There is a three to four year waiting period for another mass year (i.e. year of significant cone production), if a cone crop and drought happen concurrently and result in unsuccessful regeneration. This can ultimately lead to large spans of time without any successful regeneration, slowing down natural regeneration (Alavalapati & Carter., 2002; Carey, 1992). Both economic and ecological systems are affected by altered precipitation regimes and plant invasion, making these individual and interactive effects on valuable species important to understand.

Longleaf, slash, and loblolly pine are dominant pines of the southeastern U.S. that are important both ecologically and economically. Longleaf pine is native to the southeast and the

habitat it fosters has ecological benefits. The diverse herbaceous understory resulting from wider planting spacing provides habitat for hundreds of native species, many of which are threatened or endangered (Carey, 1992; Jackson, 1989; South, 2006). Longleaf pine dominated the landscape in past decades (covering over 3.2 million acres); however, due to anthropogenic development an industry, it now occupies less than 3% of its native domain. The main cause of this decline was an increase in intensively managed slash and loblolly plantations, which can be grown in shorter time intervals (20-30 years versus longleaf at 40-50 years) and an increase in fire exclusion policies. Longleaf pine habitat relies on fire to maintain its diverse understory layer; keeping it in a younger successional stage dominated by bunchgrasses, so a reduction in natural fire contributes to its shrinking population (Landers et al., 1995; Van Lear, 2005). Loblolly and slash pine are important in the southeast today and represent the largest portion of timber exported from the U.S., accounting for 16% of the global timber industry (Prestemon & Abt, 2002; Alavalapati & Carter, 2002). Research indicates, however, that these two common plantation species may not respond well to climate change, while longleaf pine has been shown to be more drought resistant (Carey, 1992; Landers et al., 1995).

Research on seedling responses to drought, grass invasion, and their interaction is limited by a lack of manipulative field experiments that allow us to measure direct and indirect physical effects. In particular, few field experiments exist in the coastal plain region. The studies that do examine the interacting effects of drought and plant invasion represent predominately semi-arid climates (Caldeira et al., 2015; English et al., 2005). While the studies within coastal plain ecosystems focus on overall changes to native ecosystems monitoring cover, vegetation type, and abiotic factors that leave us to estimate the results of these changes on an individual study species (Alba et al., 2017; Fahey et al., 2018). It is essential to examine in a field experiment

specifically how individual species with economic and ecological benefits to the ecosystem will be directly affected by these stressors.

The goal of this study was to evaluate the individual and interactive effects of drought and grass invasion on the seedling stage of three southeastern pine species. We planted longleaf, slash, and loblolly pine seedlings into a factorial common garden experiment and measured growth and physiological traits to quantify differences in success and establishment. Measurements included physiological traits such as light saturated photosynthesis (A_{sat}) and transpiration rates (E), and morphological traits like height growth and biomass production. We also measured abiotic factors in each research plot, including light availability and soil moisture, to determine their effect on the relative success of individuals in each treatment. I hypothesized that drought and competition from a grass invader would negatively affect physiological traits and growth of the pine seedlings, but the combined effects of drought and increased competition would be additive, causing the most significant negative effects on seedlings (Figure 1.2). I also hypothesized that species responses to the experimental treatments would differ depending on their relative drought and shade tolerances (Table 1.1). For example, longleaf pine is the most drought tolerant of the study species and should therefore be less susceptible to the drought treatment. The most shade tolerant species loblolly pine, should be more successful in heavily shaded plots.

MATERIALS AND METHODS

Experimental site and species

The study site is located on the University of Florida's campus in Gainesville, Florida (29°37'42.53"N 82°21'12.28"W, elevation 22m). The climate is humid subtropical, and the area

experienced an average temperature of 20.4 °C. Gainesville received 1203 mm of precipitation in

2017, which fell primarily during summer and fall (US Climate Data, 2017). The temperature

and total precipitation observed during the span of this study were within the normal historical

range, but the distribution of precipitation resulted in an uncharacteristically dry spring followed

by a wetter than average late summer and fall. Soils consisted of well drained, easily permeable,

loamy sand in the typic quartzipsamments series common for forested land in the southeastern

coastal plain region (National cooperative soil survey, 2018). The site is surrounded by forest,

protecting it from the nearby urban environment, however no trees shade the experimental

research plots.

The site was established in 2013 with a randomized block design consisting of four

treatments replicated ten times for a total of 40 4m x 4m plots (Figure 2.1) (Alba et al., 2017).

The four treatments were: (1) native vegetation, ambient precipitation (NA) (2) invaded by

cogongrass, ambient precipitation (IA) (3) native vegetation, reduced precipitation (ND) and (4)

invaded, reduced precipitation (ID) (Figure 2.2). Drought was simulated through rain-out shelters

constructed above each plot using 4×4 inch wooden posts and Plexiglass roofing. The rain runs

down this "roof" into a gutter and diverts roughly 60% of the precipitation to the outside of the

plot. Aluminum flashing was buried around the edges of each plot to prevent the reentry of any

runoff water from the gutters or adjacent plots. Plant invasion was simulated by cogongrass,

which is currently threatening ecosystems in many states in the southeast (Bryson & Carter,

1993; Dozier et al., 1998; Estrada & Flory, 2015). Native and invasive species plugs were

planted after the establishment of the plots. For detailed schematics of the experimental design

for this project see Alba et al. (2017). At the start of the current study in 2017, cogongrass had

established nearly 100% cover in the 20 grass invasion plots, outcompeting almost all natives in

the four years since planting. The drought plus grass invasion plots included both rain out shelters and cogongrass introduction. The area between plots was mowed regularly and the outer edges of each plot were sprayed with herbicide to remove all competition between plots and to contain the invasive species.

Longleaf, slash, and loblolly pine differ in growth form, drought tolerance, and shade tolerance (Table 1.1). Loblolly and slash pine both grow rapidly in height, allocating the majority of carbon assimilated to primary height growth in the shoot apical meristem. Longleaf pine on the other hand, stores most of its carbon belowground developing a large taproot, which causes it to stay in a grass-like stage for 0-20 years (Carey, 1992; Landers et al., 1995). These species have different drought tolerances, characterized by increased rates of water use efficiency during drier months. This results from either decreased transpiration, photosynthesis, or both. Species-specific differences in shade tolerance are also present within these three pines (Table 1.1).

A total of 120 seedlings of each pine species (n = 3 seedlings per species per plot, n = 360 total individuals) were planted into the research plots in January 2017. Each plot was divided into four quadrants and each seedling was randomly assigned a position within quadrants one through three with roughly one meter spacing between each seedling. Quadrant four was not used (Figure 2.1; n = 3 individuals per species per plot). Seedlings were sourced from Andrew's Nursery (Chiefland, FL) and were not modified for superior drought tolerance. Pines were one year old at the time of planting and measured on average 2cm, 20cm, and 30cm for longleaf, slash, and loblolly pine respectively. The cogongrass was 1-1.5 m tall when the seedlings were planted in. Spring of 2017 was unseasonably dry, so I watered the seedlings in all treatments occasionally, and allowed them six months to establish before I began physiological measurements.

Weather data were collected regularly throughout 2017 using a remote automatic weather station (RAWS) installed on site (but outside the treatment plots). Light data were taken between the hours of 1100 and 1400 using a ceptometer in the center of the four sides of every plot, 0.5 m from the plot edge (and then averaged for a plot total) at three different heights: ground level, 0.5 m above ground level, and 1.5 m above ground level. Ground level measurements were used to estimate seedling light availability as seedlings never grew taller than 0.5m (Figure 2.3). Within the treatment plots, soil moisture measurements were taken monthly in four locations (at least 20 cm from either side of the plot edge), and plot averages were calculated from those data (Figure 2.4).

Plant growth and physiology measurements

All seedlings were monitored from June to September for both morphological and physiological traits. Height growth was recorded monthly for each surviving seedling, measured from the ground to the apical meristem. Diameter of each seedling was recorded at ground level for slash and loblolly pine. Longleaf pine diameter proved difficult to measure in an accurate and repeatable manner due to its grass-like growth form, so I do not report diameter measurements for this species here.

Due to the small size of pine seedlings, destructive harvesting of plant tissues was minimized during the growing season. Thus, leaf tissues were collected in small quantities (one to two needles) in July for nutrient analyses. Blades of cogongrass were also collected from 15-20 plants per treatment. Samples were dried at 60 °C for at least 72 hours and then ground in a ball mill (SPEX Sample Prep LCC 8000 Mixer/Mill, Metuchme, NJ, USA) and packaged in tins (6µg) to prepare for elemental analyses. They were then processed using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) to measure

carbon and nitrogen content of each tissue sample. At the end of the growing season, biomass was sampled through destructive harvesting of the seedlings. Roots were washed and above ground (AG) and belowground (BG) biomass tissues were weighed separately after being dried at 60 °C for at least 72 hours.

Gas exchange rates were measured biweekly from June to September using a Li-Cor 6400XT (LICOR 6400, Li-Cor Inc., Lincoln, NE, USA) with the 2×3 cm LED lighted chamber. A subsample of eight seedlings per species per treatment were randomly selected at each measurement period to collect rates of light-saturated photosynthesis, dark respiration, stomatal conductance, and water use efficiency (light saturated photosynthesis/transpiration, A_{sat}/E) between the hours of 0800 and 1500. For each seedling measured, five needles were placed lengthwise (3cm side) inside the chamber with care taken to ensure that there was no overlapping or self-shading of needles. I exposed each seedling to photosynthetically active radiation (PAR) of 1500 µmol m^{-2} s^{-1}, humidity of 55-65%, CO_2 levels of 400 µmol mol^{-1}, and ambient temperature conditions. Measurements were recorded after the leaf tissues reached a steady state inside the chamber under these conditions (usually two to eight minutes). I also measured light saturated photosynthesis, stomatal conductance, and water use efficiency of the cogongrass present within the plots, clamping blades into the chamber lengthwise (3cm) to quantify its response to drought conditions.

The 2×3 LED chamber for the Li-Cor 6400XT assumes a leaf surface area of 6cm^2. Since I was measuring needles instead of broadleaved species, they filled only a portion of the chamber. Ordinarily, needles would be collected for each gas-exchange measurement to obtain an exact needle area for each. However, the seedlings had limited needles due to their small size, and destructive harvesting of needles for every measurement could have proven debilitating for

the plants. Therefore, using ImageJ software, I developed a species-specific standard estimate for needle surface area using average needle width. For each species, forty needles from ten different plants were collected, scanned, and average width was determined. Total leaf area in the chamber was calculated by taking the average width of each species multiplied by five (the number of needles placed in the chamber for each measurement) and then three (length of the chamber in cm). Surface area in the chamber for the cogongrass was calculated in the same way as the pine seedlings (n = 20 blades) to find average width, which was then multiplied by three (length of the chamber). These values were used to recompute all Li-Cor output to be representative of the actual leaf area in the 2 × 3 chamber.

Statistical analyses

Independent variables were transformed as necessary (square root of photosynthesis, log of height growth and biomass) to achieve normality and equal variance. Different transformations were used on different datasets as normality was not improved across all datasets with the same transformations. Data were analyzed for light and soil moisture levels to examine changes in abiotic factors between treatments using a mixed-model ANOVA that incorporated treatment, time period, and the interaction of these two variables as fixed effects and plot as a random effect. Light and soil moisture levels were not considered explanatory variables in this model as they have been accounted for in the treatment effects. Significant values were determined at P = 0.05. Data were analyzed for photosynthesis, conductance, respiration, and WUE using a mixed model ANOVA that incorporated treatment, species, time period, and the interaction between treatment and species as fixed effects and plot as a random effect. I chose not to look at the interaction of time period with any other variable because all months were statistically similar with the exception of September, which represented a slowdown in

physiology and growth in all treatments and all species, characteristic of plants at the end of a growing period. Biomass, height growth, and leaf nutrient data were analyzed using a mixed model ANOVA that included treatment, species, and the interaction between these two as fixed effects and plot as a random effect. Above and below ground biomass were analyzed separately for trends. All post hoc tests used Tukey's comparison for multiple pairs.

RESULTS

Plot level differences in light and soil moisture

Seedling light availability was significantly affected by plant invasion, and it impacted the growth and success of seedlings. Photosynthetically active radiation (PAR) measured at the soil surface was significantly different with plots containing grass invasion (IA and ID) receiving 70% less light than those without cogongrass (NA, ND; $P < 0.0001$, n =1600; Figure 2.3). Light availability also differed significantly over the course of the year with more light reaching seedlings during the summer months (May-July) and less light during the winter months (October–December; $P < 0.0001$, n = 1600).

Soil moisture was monitored to estimate the severity of drought and its possible interaction with other abiotic and biotic stressors. Soil moisture availability differed between all treatments, indicating the success of the rain-out shelters and the manipulation of soil water availability by cogongrass (Figure 2.4; $P < 0.0001$, n = 2184). Annual average soil moisture was 20% higher in the IA plots compared to the NA plots, while drought reduced soil moisture by 47%. The effect of drought in plots with the grass invader (ID) were minimized, with soil moisture being reduced only 22% compared to the native ambient plots. Soil moisture also varied over the course of the experiment with the lowest water availability during spring and the

highest during summer and fall, which was consistent with the trends in annual precipitation (P < 0.0001, n = 2184; Figure 2.4). All treatments followed similar seasonal trends across the year of measurements in both soil moisture and light availability (Figures 2.3 & 2.4).

Seedling responses to drought

Drought had the most debilitating effects on pine seedlings, all of which experienced significantly reduced rates of growth and photosynthesis. Seedlings subjected to drought (ND) showed a 29% decrease in rates of A_{sat} (P < 0.0001, n = 294; Figure 2.5 & 2.6) resulting in a 36% decrease in growth rates compared to seedlings in the native ambient plots (NA; P = 0.0002, n = 164; Figure 2.7). Rates of photosynthesis correlated well with growth measurements taken over the same period (R^2= 0.74; Figure 2.9). Total biomass and AG biomass of all three species, as well as BG biomass of longleaf and loblolly pine, showed no significant differences between treatments. BG biomass in slash pine, on the other hand, was increased by 58% in the ND plots in comparison to the other two species, representing a possible drought mitigation strategy (P = 0.0146, n = 115). All pine species in this treatment showed drought resilience evidenced by lower stomatal conductance (ND = 0.15 ± 0.012, NA = 0.27 ± 0.014) and a 27% increase in water use efficiency (WUE) for all species in comparison to individuals in the native ambient plots (P < 0.0001, n = 294; Figures 2.5 & 2.6).

Seedling responses to grass invasion

Grass invasion had a significant impact on seedlings, causing increased AG growth to escape competition for light and decreased rates of photosynthesis due to competition with the grass invader for light and belowground resources. Rates of A_{sat} were 21% lower than those in the NA plots (P < 0.0001, n = 264) and stomatal conductance rates were also lowered by 15% in comparison to seedlings in the NA plots (P < 0.0001, n = 291). In contrast, significantly higher

rates of height growth (18.4cm ± 1.41) were observed in individuals in the IA plots in comparison those in the NA plots (16.18cm ± 1.12) (P < 0.0001, n = 164). Slash pine seedlings had lower accumulations of BG biomass specifically in the IA treatment (1.03 ± 0.18) than the other two species (2.61 ± 0.84, 2.5 ± 1.3; P = 0.0146, n = 127; Figure 2.7) indicating below ground competition with the grass invader, documented in the literature, may have affected growth and success in this treatment. Interestingly, pines in this treatment had higher levels of nitrogen than those in the NA treatments, indicating the pines were potentially passively benefitting from the concentration of nutrients caused by the cogongrass (P = 0.0757, n = 231; Figure 2.8).

Seedling responses to the combined stressors of drought and grass invasion

Pine seedlings subjected to the combination of drought and grass invasion were less physiologically impacted than those experiencing only drought, however individuals were unable to attain the rapid rates of height growth that were observed in seedlings in the IA treatment. Cogongrass alleviated water stress by increasing soil moisture availability causing individuals to do comparatively better than those in the drought treatments indicated by both higher rates of growth and photosynthesis. Seedlings in the ID had a 17% decrease in rates of A_{sat} which was less impactful than the 29% decrease observed in seedlings in the ND plots in comparison to individuals within the NA plots (P < 0.0001, n = 294). Photosynthetic trends were mirrored by height growth (14.77 ± 1.52) and stomatal conductance (0.20 ± 0.013) which were both lower than individuals in the NA but higher than those in the ND plots (P = 0.0002, n = 164; P < 0.0001, n = 291; Figures 2.5 & 2.6). Height growth for seedlings in this treatment was only 8% lower than height growth measured in the NA plots, which was less drastic than the 36% decrease in growth observed in the ND plots. Seedlings did however have significantly lower

rates of growth (14.77 ± 1.52) in comparison to the IA plots (18.4 ± 1.41) requiring seedlings in the ID treatment to compete more intensely with the grass invader for light (P = 0.0002, n = 164). Since multiple stressors are present, the offsetting effects on drought from the grass invader lessening the physiological stress on the seedlings alone, may not be enough to significantly increase long term survival and success.

Cogongrass responses to drought

Cogongrass was virtually unaffected by drought as it showed no difference in photosynthesis, conductance, or water use efficiency between treatments with and without reduced precipitation (P = 0.5709, 0.7216, 0.3477, n = 42) (Figure 2.5). Growth of the cogongrass was not measured as it covered nearly 100% of the cover area of invaded plots. It was also approximately the same height and vigor in both treatments prior to the establishment of the native pines and showed no changes throughout the season (personal observation).

Species specific effects across all treatments

Overall, longleaf pine represented a more conservative physiological strategy across all treatments in comparison to the other two species. Longleaf pine operated at lower rates of photosynthesis (8.42 ± 0.40) than slash pine (11.61 ± 0.54) or loblolly pine (12.30 ± 0.61) (P < 0.0001, n = 294) which may have contributed to the decreased biomass production (1.88 ± 1.0), in comparison to slash and loblolly pine respectively (3.13 ± 0.28, 3.42 ± 0.25) (P < 0.0001, n = 116). Despite this, longleaf pine did demonstrate higher rates of growth (25.02 ± 1.30) in comparison to both slash pine (13.75 ± 0.94) and loblolly pine (10.54 ± 0.92), however this disparity was most likely due to differences in seedling growth forms where I measured needle elongation in longleaf pines and apical meristem elongation in slash and loblolly pines (Figures 2.5, 2.6, & 2.7; P < 0.0001, n = 161).

Loblolly and slash pine possessed similar rates of height and diameter growth and operated with somewhat similar physiological strategies. Slash pine differed from loblolly pine in below ground biomass accumulation with lower rates in the IA plots and higher rates in the ND plots, but still maintained similar rates of height growth. Loblolly pine showed a statistically higher rate of respiration than the other two species ($P < 0.0001$, n = 294) (data not shown) as well as increased conductance in comparison to both slash and longleaf pine ($P < 0.0001$, n = 291; Figure 2.5). Loblolly pine also contained higher levels of carbon than found in longleaf pine, most likely resulting from the increased physiological rates ($P < 0.0001$, n = 224; Figure 2.8).

Seasonal trends in physiology observed for all species indicated that month of measurement was a significant fixed effect ($P < 0.0001$, n = 29). The lowest rates of photosynthesis were in September (9.22 ± 0.64) with June (10.65 ± 0.49), July (11.58 ± 0.58), and August (11.40 ± 0.74) representing significantly higher rates. Pine seedlings also showed a seasonal slow-down in conductance rates with lower rates of stomatal conductance in September in comparison to the other three months ($P < 0.0001$, n = 291). The differences in physiological traits between months was most likely due to plants naturally slowing down photochemical processes at the end of the growing season.

DISCUSSION

Climate change alters the intensity of drought and plant invasion stress, leading to significant negative changes in ecosystems (Barton, 1993; Caldeira et al., 2015; NeSmith et al., 2018). The complexity of combined stressors taking their interactions into consideration makes it difficult to predict the overall effects on a pine seedling without an interactive field experiment. Here using physiological and growth measurements, I demonstrate that although singly both

stressors debilitate the physiology and success of all three pine species, drought and plant invasion can interact to alleviate some of the physiological stress on pine seedlings.

My treatments were successful in manipulating soil water content and light availability for seedlings, which allowed me to create water and light limited scenarios in this field experiment. These abiotic measurements also showed us the feedbacks drought and plant invasion had on the ecosystem, by examining how relative water and light availability were affected between the treatments. I was able to confirm and expand upon earlier studies showing that cogongrass invasion limits light availability by as much as 70% to native species, reducing their growth and survival (Brewer, 2008; Dozier et al., 1998; Estrada & Flory, 2015). I also found that while cogongrass limited light available to seedlings, it simultaneously impacted drought intensity by reducing soil water evaporation, increasing soil water availability (NeSmith et al., 2018). Due to the abnormally dry spring accentuating the drought treatments, there were physiological benefits to pine seedlings in the invaded drought plots.

Both drought and plant invasion negatively affected seedling physiology and growth, however there were some interactions between the stressors that resulted in unpredicted responses. Drought was the most debilitating stressor for juvenile pines, indicated by the decreased rates of photosynthesis and conductance leading to less growth. My initial hypothesis, predicting that the two stressors would have an additive negative effect was not supported when grass invasion was examined in addition to drought. I observed higher rates of photosynthesis and conductance in seedlings in these treatments caused by increased soil water availability. As found in other studies, I believe that cogongrass may have alleviated some water stress by decreasing soil moisture evaporation, as VWC was significantly higher in this treatment in comparison to drought plots that did not contain the invader (Espeleta et al., 2004; Estrada &

Flory, 2015; NeSmith et al., 2018; Figure 3.10). However, long-term survival may still be negatively impacted by the combination of stressors if height growth to escape the shade caused by the invader is limited by competition and drought. Individuals in the ID treatment showed no increase in height growth in response to competition for light in comparison to seedlings in uninvaded plots. Whereas pine seedlings in the IA were able to grow taller than every other treatment to compete more effectively for light, increasing chances of survival.

My data support the hypothesis that species differences in shade, drought, and fire tolerance contributed to their relative success across treatments. Loblolly and slash pine were affected similarly, responding to drought and plant invasion with comparative physiological and growth responses. Their increased physiological rates in comparison to longleaf pine aided in the competition for light and survival under physiologically demanding situations across all treatments, demonstrating their moderate tolerances to a variety of stresses.

In contrast, I observed significantly reduced rates of photosynthesis and stomatal conductance in longleaf pine in comparison to the other two species. Although other studies have found superior drought tolerance in longleaf pine indicated by higher WUE (Foster & Brooks, 2001; Henderson & Grissino-Mayer, 2009; Patterson et al., 2016), this experiment did not support longleaf pine possessing this ability, which is consistent with the results of Samuelson et al. (2012). I believe this is most likely due to studying these species in the seedling stage. Longleaf pine uses a large taproot to secure water under drought conditions, and this is not possible for a seedling to produce in a single growing season. I demonstrate here a seedling phenomenon and would not expect these species to respond the same at a sapling, or mature tree life stage. Examining the seedling stage, however, is still representative of an important initial step that must be successful in order for individuals to continue on to more mature life stages.

The lowered shade tolerance of longleaf pine in combination with the surprising lack of drought resistance, resulted in this species experienced the most debilitating effects across all treatments, signifying that seedlings of longleaf pine may not be as equipped to deal with climate change as slash and loblolly pine.

Although cogongrass offset drought stress for the native pines, invasion has many other negative ecological consequences. Cogongrass was unaffected by drought, growing rapidly to outcompete all native vegetation within the invaded plots. Replacement of the understory habitat in longleaf pine ecosystems by monotypic stands of cogongrass would decimate the biodiversity that occurs exclusively in the rich herbaceous understory (Brewer, 2008; Estrada & Flory, 2015). As climate change continues to alter the borders of habitable ranges for invaders, it will become more important to protect areas of longleaf pine habitat from cogongrass invasion to protect biodiversity, especially considering how vulnerable the species appears to be. It is also financially validated to consider management techniques in timber production, since drought and plant invasion had negative effects on physiology and growth, which would have long term negative effects on wood production. Initial treatments for cogongrass or drought mitigation should be considered, even though costly, as their resulting effects may be large enough in scale to make treatments worthwhile.

This novel experiment demonstrates the physiological and growth responses in native pine species to the combined influences of reduced precipitation and cogongrass invasion. I found that overall, drought is a debilitating stressor for all pine species and would recommend that local precipitation regimes and soil water holding capacity be considered when selecting sites for plantations or restoration efforts. Using improved drought tolerant varieties of timber is another possible solution to combatting this stressor. I observed that the combined effects of

drought and plant invasion under abnormally dry conditions were offsetting physiologically which corroborates the results found by Fahey et al. (2018), however this contrasts the additive effects found by NeSmith et al. (2018) and the synergistic effects observed by Caldeira et al. (2015). These varied outcomes from the same combination of drought and plant invasion further demonstrates the complexity of interacting stressors and their feedbacks, making it apparent that we need more field experiments allowing the direct observation of the interaction of these factors.
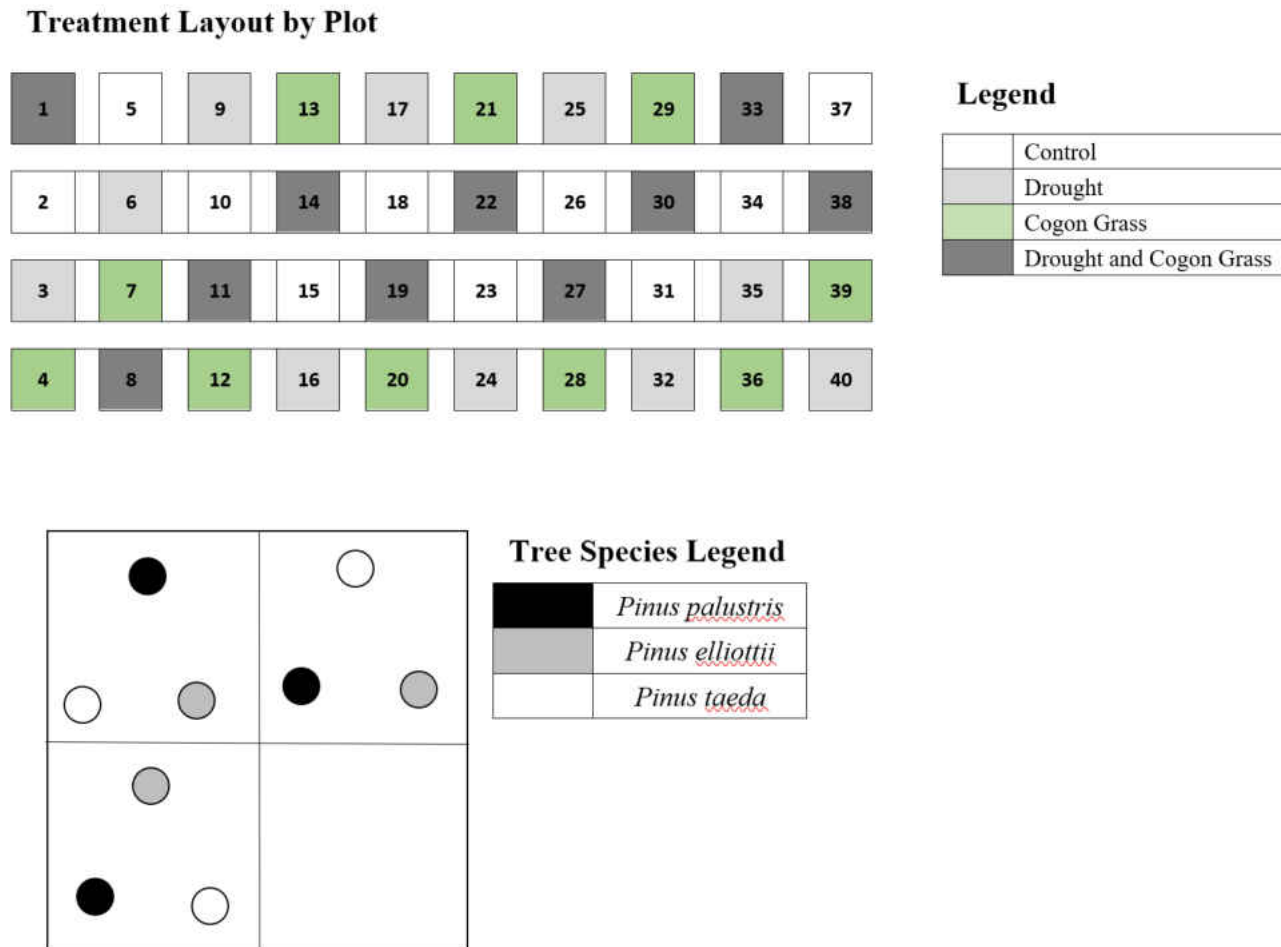
Figure 2.1: Factorial common garden block design layout of the four treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought (ten replicates) at the field site (top). Planting design for each 4m x 4m plot with three individuals per species for a total of n = 9 individuals per plot (bottom). Seedlings were planted roughly 1 m apart from seedlings in all directions. The fourth quadrant was left unused as it contained soil data collection equipment for an ongoing experiment.

Figure 2.2: Photographic representation of the differences in cover between plots with native vegetation (left) and plots invaded by cogongrass (right).
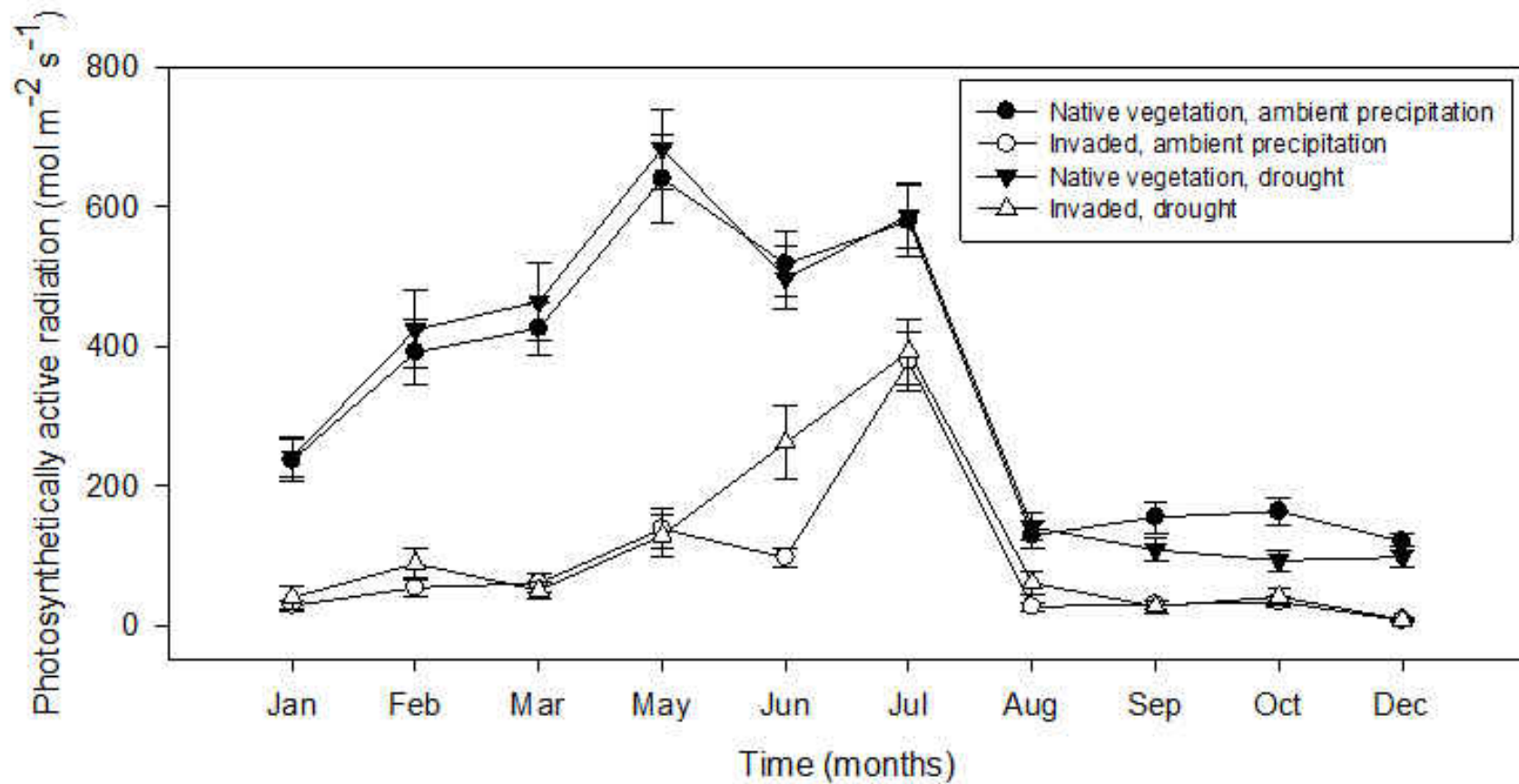
Figure 2.3: 2017 Monthly average (± standard error) light measurements of photosynthetically active radiation (PAR) at ground level (10 replication plots per treatment, 4 measurements per plot, n = 40 per month) reported across the four treatments: control, grass invader (cogongrass, *Imperata cylindrica*), drought (rain out shelters), and drought plus grass invader (rainout shelters and cogongrass) (P < 0.0001, F = 387.53, n = 2184). Data was not collected in April due to inclement weather.
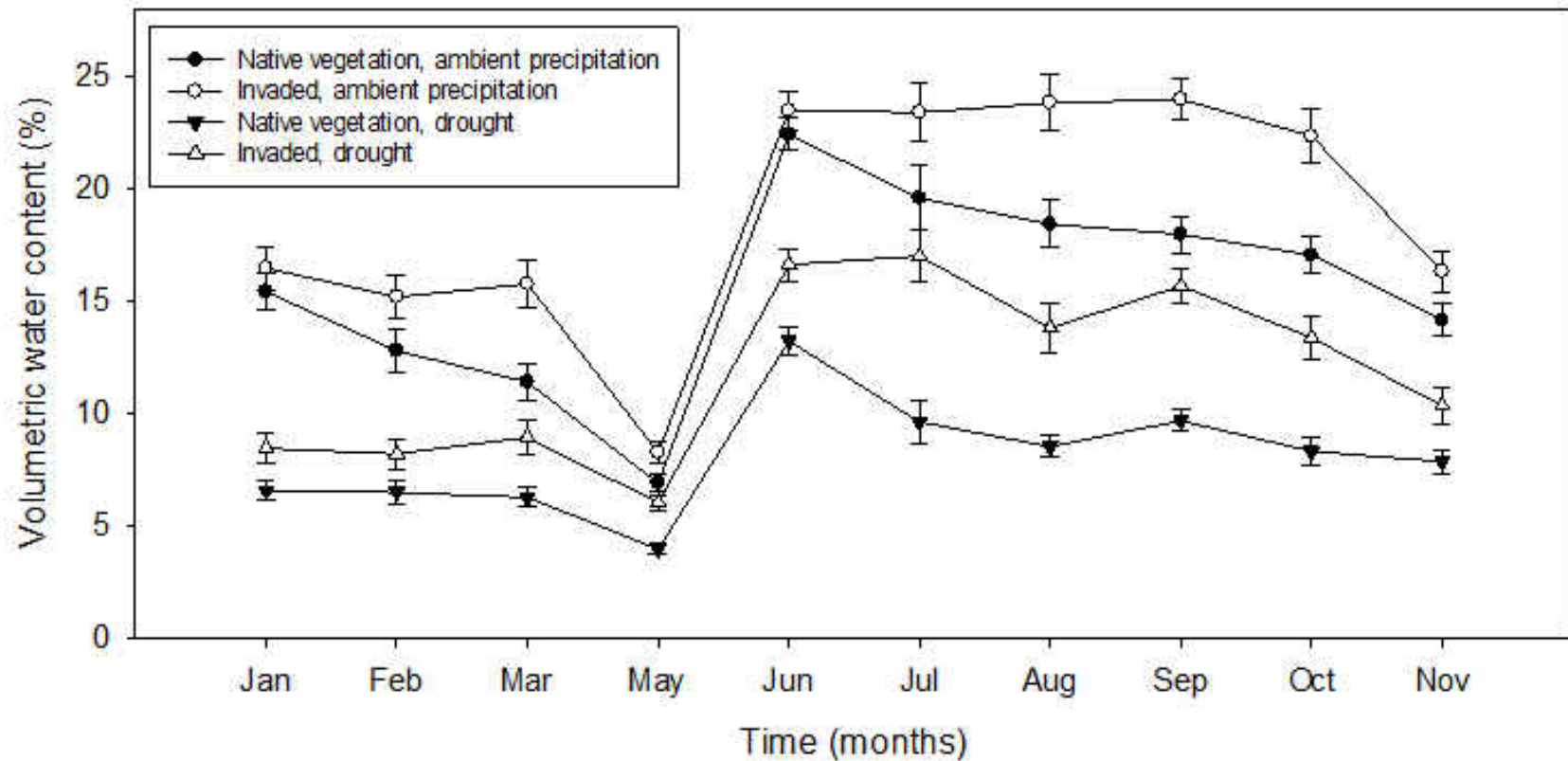
Figure 2.4: 2017 monthly average (± standard error) volumetric water content taken at 12 cm belowground across the four treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought. Measurements were averaged for ten replication plots per treatment with four measurements per plot (n=40, per treatment per month) (P < 0.0001, F = 186.60, n = 1560). Data was not collected in April due to inclement weather.
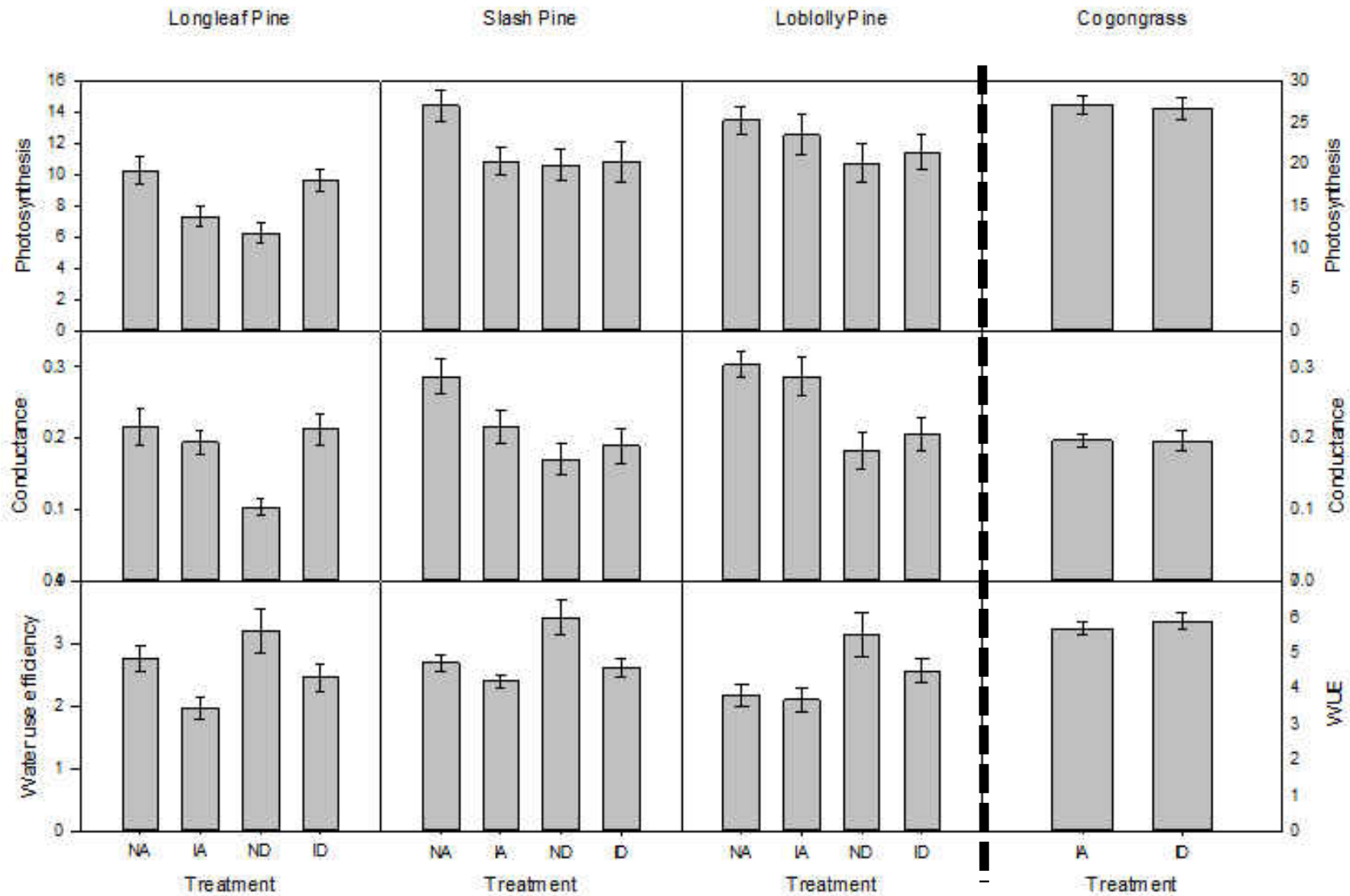
Figure 2.5: Average light saturated photosynthesis (± standard error; top), average stomatal conductance during light measurements (± standard error; middle), and average water use efficiency (± standard error; bottom) for all three species pooled (longleaf, loblolly and slash pine) and the grass invader across the four treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought. Cogongrass uses the righthand scale.
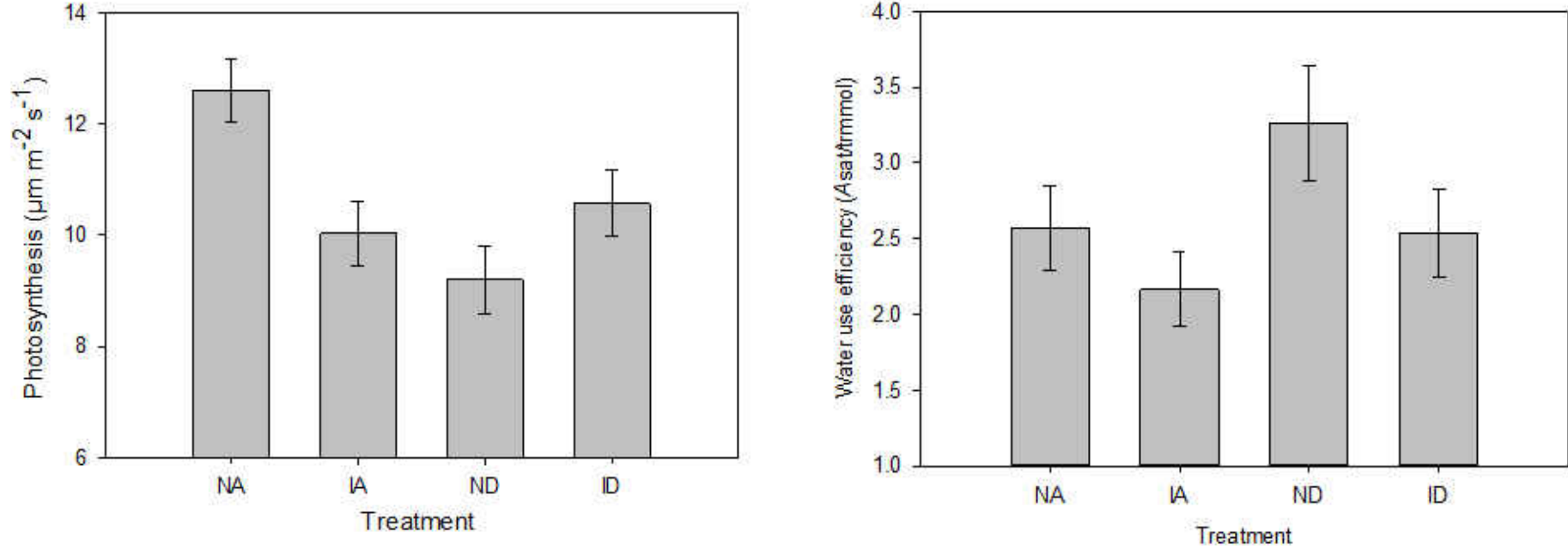
Figure 2.6: Average rates (± standard error) of photosynthesis (left) and water use efficiency (right) pooled for all species for the entire growing season.
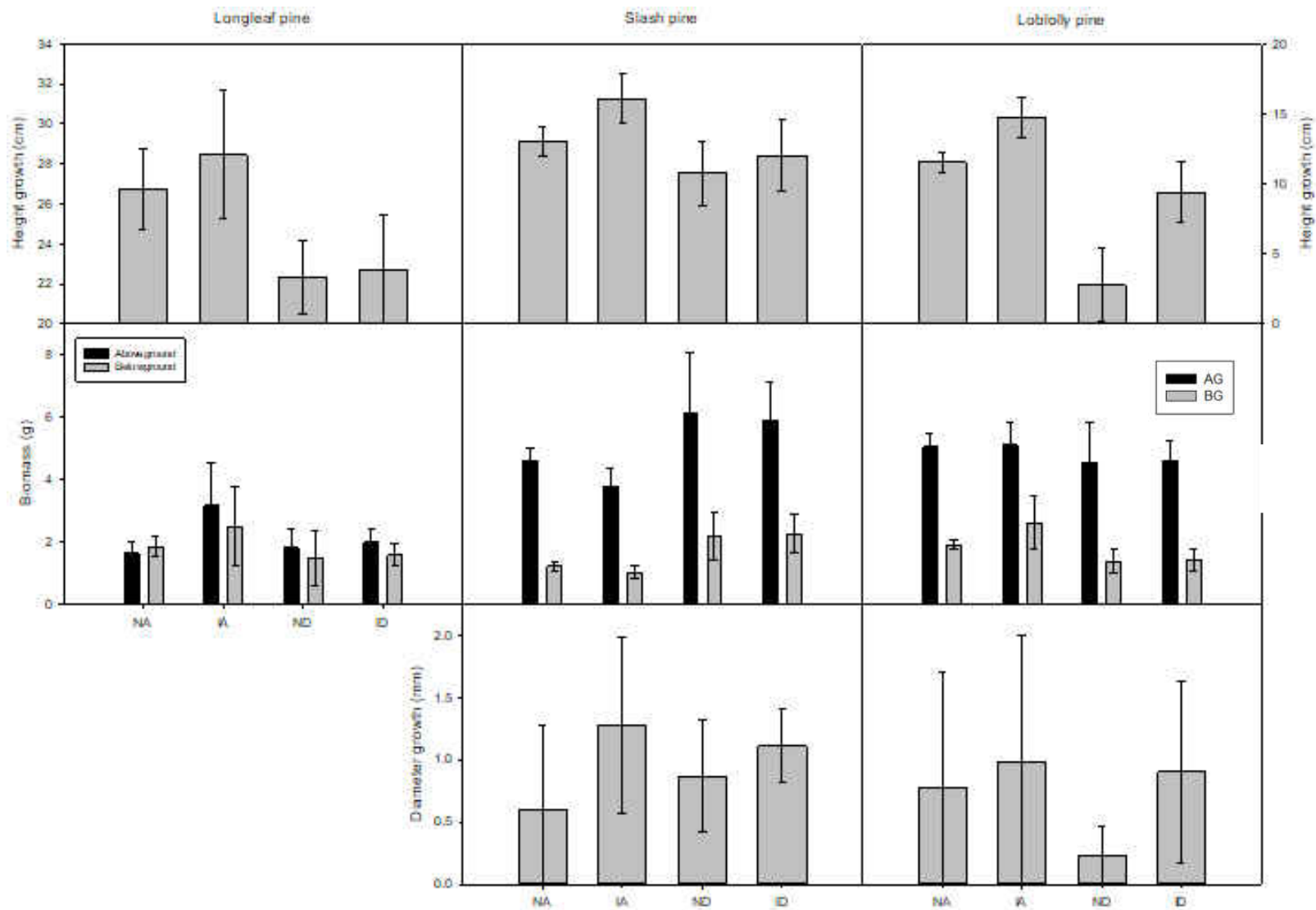
Figure 2.7: Average height growth from February to September (± standard error; top), average above and below ground biomass (± standard error; middle), and average diameter growth from February to September (± standard error; bottom) for all three species (longleaf, loblolly and slash pine) across the four treatments. Slash and loblolly pine use the right-hand height growth scale.
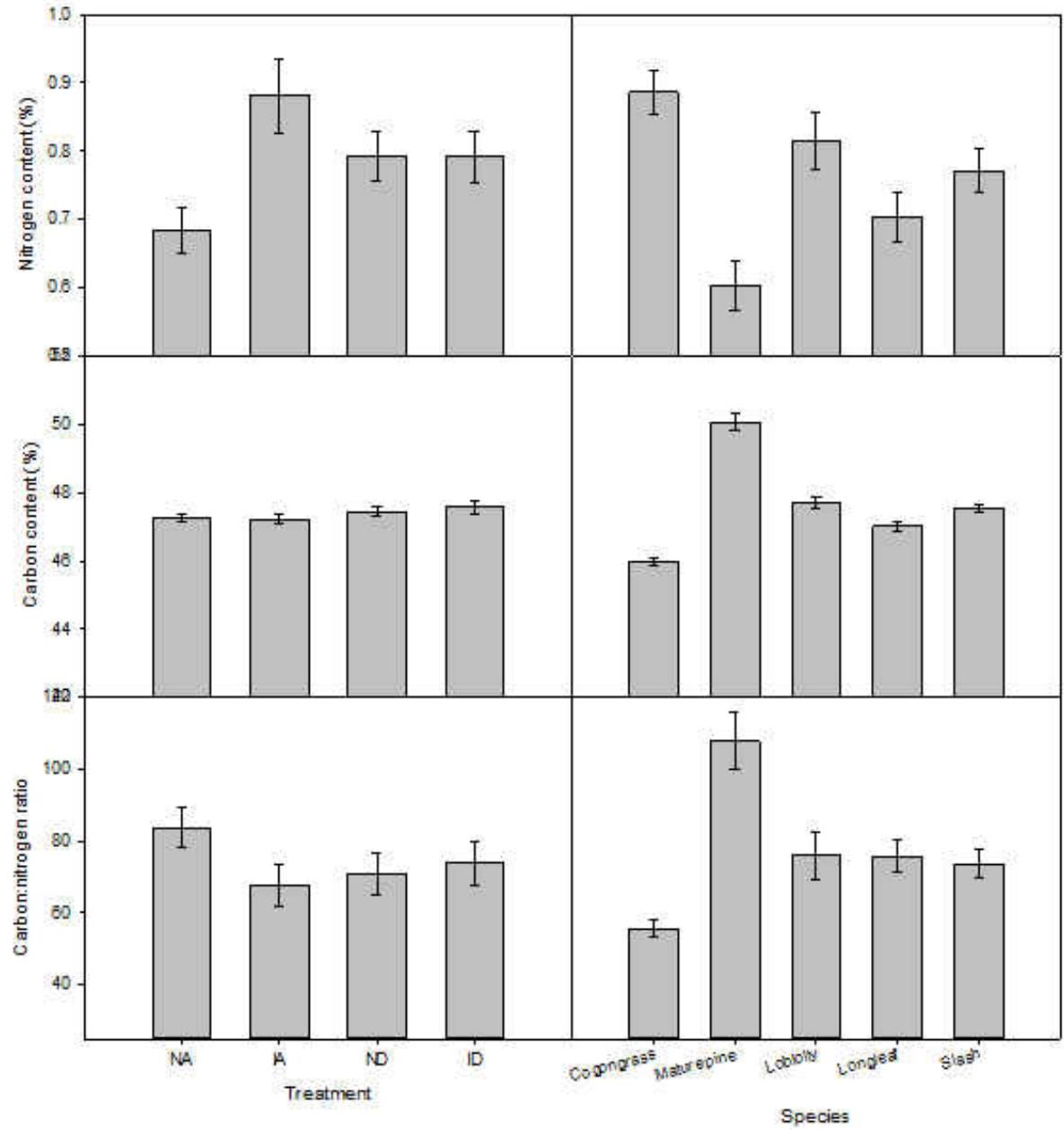
Figure 2.8: Average percentage (± standard error) of nitrogen content in the native pines across the four treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought (top left). Average percentage (± standard error) of nitrogen content for each species sampled: longleaf, slash, loblolly, large longleaf pine, and cogongrass pooled for all treatments (top right). Average percentage (± standard error) carbon content by treatment and species (middle) and average (± standard error) carbon:nitrogen ratio by treatment and species (bottom).
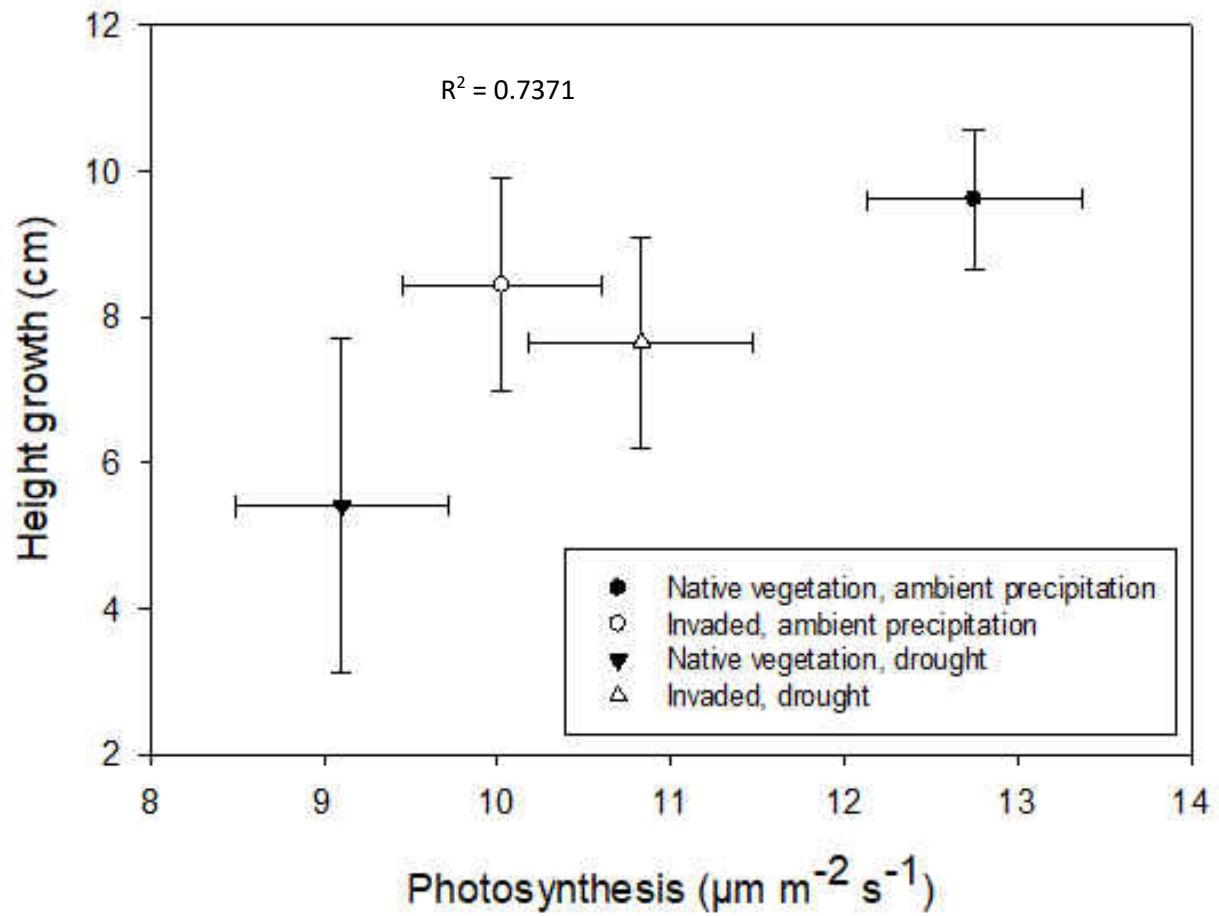
Figure 2.9: Average rates of photosynthesis (± standard error) for all species versus average

height growth (± standard error) for all species over the entire growing season, with each point

representing one of the four treatments.
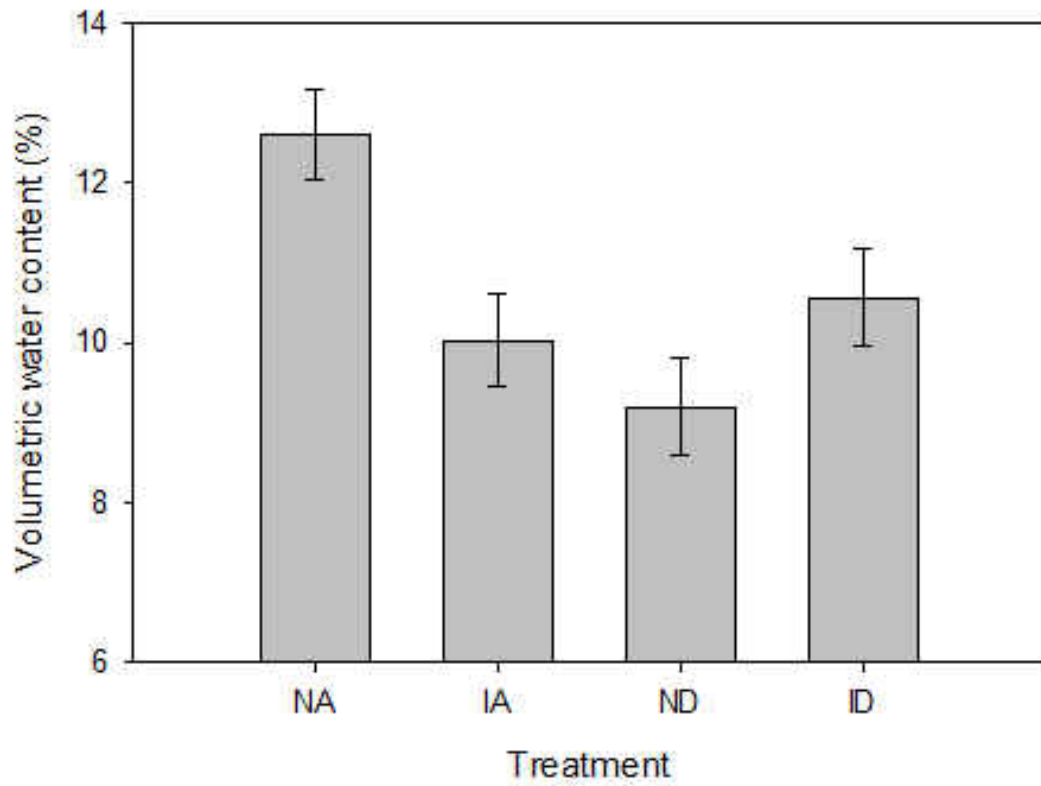
Figure 2.10: 2017 yearly average (± standard error) volumetric water content between treatments measured at 12 cm below the surface.

CHAPTER 2

HOW ARE TIMBER SPECIES OF THE SOUTHEASTERN COASTAL PLAINS AFFECTED

BY DROUGHT AND PLANT INVASION IN A POST-BURN ENVIRONMENT?

ABSTRACT

Changes in natural systems such as altered precipitation or fire regimes often have both direct and indirect effects on organisms. These factors can also interact to amplify each other and understanding the combination of these effects is essential for predicting how climate change will affect forested ecosystems in the future. In a factorial field experiment, I examined the physiological and growth responses of three southeastern pine seedling species under four treatments: (1) native vegetation, ambient precipitation (NA), (2) grass invasion, ambient precipitation (IA), (3) native vegetation, reduced precipitation (ND), and (4) grass invasion, reduced precipitation (ID). I exposed my research plots to a prescribed burn before planting in seedlings in order to look at post-fire effects on *Pinus palustris, P. taeda,* and *P. elliottii* in combination with the treatment effects. My results show that pine seedlings in the IA and ID treatment showed minimal signs of physiological stress. This was most likely due to the grass invader alleviating drought stress by reducing soil water evaporation. In spite of this, pine seedlings experienced significantly more mortality in ID plots than individuals in the NA plots. Pine seedlings in the ND and the IA plots did show physiological stress over the season, with rates of chlorophyll fluorescence (a proxy used to quantify plant stress) lower than seedlings in the NA plots. However, seedlings in these treatments had higher rates of survival than those in the plots with the combined stressors, which may indicate ecosystems dealing with both stressors will have significant impacts on native pine species of the southeast.

INTRODUCTION

Climate change can directly affect ecosystems by altering precipitation and temperature regimes that contribute to the success and establishment of species in their native ranges (Smith et al., 2015; Allen & Breshears, 1998; Weltzin et al., 2003). Climate change also has many indirect effects on ecosystems, which are more difficult to quantify. Indirect effects can include influencing fire frequencies and intensities, which can have significant synergistic (more than the added effect of the two stressors) effects on natural systems by amplifying stressors like drought and non-native species invasion (Figure 1.2). Drought has become more frequent and intense across the United States in recent years and is predicted to increase in magnitude (Dai, 2013; IPCC, 2014; Smith et al., 2015). In response to high levels of water stress, plants often close their stomata and reduce transpiration rates, limiting their ability to photosynthesize and grow. These physiological responses can result in a significant reduction in productivity and survival of tree species, in both plantation and natural forested systems (Singh & Reddy, 2011). Aggressive invaders like cogongrass (*Imperata cylindrica,* described in detail in Chapter 1) are virtually unaffected by drought and can move into forested areas and outcompete native vegetation that is not as well-equipped for these new conditions (Estrada & Flory 2015; Dozier et al., 1998; Brewer, 2008). Drought and plant invasions often occur simultaneously, and their interactive effects on native vegetation have complex effects that may be additive, synergistic, or offsetting (Figure 1.2). Long term field studies incorporating multiple climate stressors are important for predicting these highly variable outcomes (NeSmith et al., 2018, Caldeira et al., 2015).

Fire is an important component of native southeastern forests, especially longleaf pine stands, as an ecological resource. Natural fire supports the diverse herbaceous understory layer, keeping the vegetation in an early successional stage dominated by native bunchgrasses that

hinders woody shrub encroachment (Landers et al., 1995, Hess & Tschinkel, 2017). Fire behavior has been significantly altered over the last few decades with natural systems experiencing shorter return intervals or more frequent fires, larger burn area, and higher fire severity (Costanza et al., 2015; Stocks et al., 1998). Simultaneously, fire exclusion has become more common due to the increase in the number of lucrative slash and loblolly pine plantations as these species are more vulnerable to fire. Ecological and economic benefits are gained from the correct timing and number of prescribed burns, a common land management technique. However, if prescribed burning is used in areas that have been invaded by non-native species, then we may be assisting in the colonization of this area by removing native competition for the invader (Balch et al., 2012). In addition, if drought is concurrent with prescribed burning, fire can modify soil components to make it more hydrophobic after being exposed to heat. Fire can also remove vegetation and soil organic matter that served as a fertilizer as well as a barrier to soil moisture evaporation. Both of these fire effects can lead to interactions of factors that can amplify drought, and create novel scenarios for seedlings (Certini, 2005).

Longleaf (*Pinus palustris* Mill.), slash (*P. elliottii* Engelm.), and loblolly (*P. taeda* L.) pine are important timber species of the southeastern U. S., especially in fire prone landscapes. Loblolly and slash pine are planted commonly as fast-growing plantation species that account for roughly 16% of the global timber industry, representing significant economic income to stakeholders in the southeastern U. S. (Prestemon & Abt, 2002). These plantation species, however, do not support the herbaceous understory layer commonly found in longleaf habitat because plantation pines are planted closer together. Tighter planting spacing shades the soil surface, making it difficult for diverse vegetation to establish (South, 2006). The fire exclusion that occurs within and around areas surrounding plantations and human development leads to

woody shrub encroachment, which creates a less complex ecosystem that cannot support the biodiversity of longleaf pine habitat. Longleaf pine is native to the coastal plains and sequesters carbon at a faster rate than loblolly and slash pine. It is also a less intensively managed carbon sink because of the longer return intervals (40 years versus 20-30 years) (Alavalapati & Carter, 2002). Longleaf pine habitat is characterized by a bunchgrass understory that supports over 900 endemic species, some of which are threatened or endangered (Jackson, 1989; Van Lear, 2005). Bunchgrass represents a patchy fuel type that allows for the low intensity transmission of fire across the landscape. Low intensity burns have enough residence time to burn off the above ground vegetation keeping the landscape in a younger successional state, but a low enough intensity to not heat the soil enough to kill below ground root stores (Hess & Tschinkel, 2017).

I aimed to quantify the individual and interacting stresses of drought and a grass invader (cogongrass) using physiological and growth traits of three native pine species in a post-fire landscape. I planted one-year-old longleaf, loblolly, and slash pine seedlings into a factorial common garden experiment one week after a prescribed burn. Following a two month acclimation period, I measured physiological and morphological traits of seedlings across the growing season. I measured the photosynthetic efficiency of seedlings using chlorophyll fluorescence, which compares the proportion of leaf absorbed light energy transferred to photochemistry versus heat and fluorescent emissions. Higher chlorophyll fluoresce values imply that a larger proportion of incoming radiation is being used for photosynthetic processes, which is indicative of lower stress and higher light-use efficiency within the leaf. Lower values indicate that less energy is transferred to photosynthetic machinery and a larger proportion is fluoresced or lost as heat. Reductions in the proportion of light energy used in photosynthetic processes can be due to limitations in water, nutrients, or light all of which reduce the maximum rates of

possible photosynthesis. A healthy seedling will have a maximum dark-adapted quantum yield of 0.8 (F_v/F_m). Seedling height and survival was monitored in July, September, and December. I also measured abiotic factors like soil moisture and light availability within the treatment plots to evaluate how these factors affected the relative success of the native pines. I hypothesized that drought would be amplified by the secondary fire effects and therefore be the most detrimental stressor for the seedlings. However, grass invasion and the combination of invasion and drought would still negatively affect seedlings in comparison to the native, ambient plots. I also hypothesized that species would respond differently to these stressors based on relative fire, drought, and shade tolerances. For example, longleaf pine is drought and fire tolerant, so I predicted it would be the least affected by water stress. Loblolly pine is shade tolerant and, thus would outperform slash and longleaf pine in plots shaded by the grass invader (Table 1.1).

MATERIALS AND METHODS

Experimental site and species

For this experiment, I used the same research site in Gainesville, Florida that was established in 2013 (Alba et al., 2017) and is described in Chapter 1 (see Chapter 1 for full site and plot description; 29°37'42.53"N 82°21'12.28"W, elevation 22m). In 2018 the site experienced an average temperature of 21.6 °C and 1531mm of precipitation, which fell predominantly in the spring and winter and was wetter than average for the area (US Climate Data, 2018). In April 2018 all plots underwent a prescribed burn, then seedlings of three pine species (*Pinus palustris, P. elliottii,* and *P. taeda*) were planted in the plots as shown in Figure 3.1 (n = 5 per species per plot). Seedlings were 1.5 years old, purchased from Anderson's Nursery (Chiefland, FL) and had an average height of 12 cm, 36 cm, and 41 cm for longleaf, loblolly, and slash pine respectively at the time of planting. Seedlings were given eight weeks to

establish after planting, after which I monitored morphological and physiological traits from July through December. I did not water any seedlings during the establishment phase due to the increased precipitation. Cogongrass reproduces asexually via rhizomes and can easily resprout after mowing, fire, or mechanical removal. Thus, there was no replanting of the grass invader into the plots post burn as belowground rhizomes of the cogongrass were still intact following the fire. I did not estimate cogongrass growth post-burn as it occupied nearly 100% of cover available three weeks after the burn.

Site and plot-level abiotic data were collected regularly throughout 2018 using a remote automatic weather station (RAWS) installed on site (but outside the treatment plots). We measured soil moisture data monthly, using the average of four measurements per plot taken at least 20 cm from the edges (n=40 per treatment; Figure 3.2). Plot-level light data were averaged from ceptometer measurements taken in the center of the four sides of the plots (0.5 m from the edge). We measured light throughout the canopy in the plots (at the soil surface, 0.5 m, and 1.5 m above the soil surface), but I only report data recorded at the soil surface because seedlings never grew taller than 0.5 m in height (Figure 3.3). The prescribed burn in April required us to tear down/rebuild the rainout structures, so soil moisture and light data were not collected in the months surrounding the burn.

Longleaf, slash, and loblolly pine differ in juvenile growth form as well as fire tolerance. Longleaf pine is characterized as a fire tolerant species, even in the seedling stage, as it can survive prescribe fire by using large below ground stores of biomass to regrow needles lost to fire. This species remains in a grass-like stage for 0-20 years, contrasting with the growth forms of slash and loblolly pine which primarily allocate carbon to aboveground tissues (Carey, 1992; Landers et al., 1995). Once longleaf pine exits the grass stage it grows rapidly over a short

period of time to elevate its apical meristem above the level of potentially damaging fire. This height increase allows longleaf pine to withstand low intensity burns without having to replace its above ground biomass. While juvenile loblolly and slash pine are vulnerable to fire, more mature pines (6+ years) develop a thicker outer bark layer that helps protect the vascular cambium from damaging heat (Schubert, 2016).

Varying shade and drought tolerances may also contribute to the relative success of seedlings in each treatment. Drought tolerance, defined by increased water use efficiency under drought conditions in comparison to other species, may help species like longleaf pine fare better in the plots with reduced precipitation. However, loblolly pine is more shade tolerant and is generally more successful in establishing under stressful conditions in comparison to the other two species (NeSmith et al., 2018; Shiver et al., 2000). This may enable its success in plots heavily shaded by the grass invader (Table 1.1).

Growth and physiological trait measurements

I monitored morphological traits including height growth and survival to quantify the magnitude of the treatment effects post-fire. Height and survival of planted seedlings were monitored in July, September, and December. I chose these months to represent the entire growing period, with three trips to the field site. This was the highest feasible number of trips for one person to accomplish in a season. Seedling survival was calculated as the percentage of the starting population that remained alive at each measurement period. A seedling was considered deceased after no green foliage was left on the individual. Height of slash and loblolly pines was measured on surviving individuals as the distance from ground level to the apical meristem. Growth was then calculated as the difference in height from the previous period subtracted from the height of the current period. Longleaf pine seedlings remained in their grass stage throughout

this experiment, so height measurements were not meaningful for this species. In a previous growing season, I compared needle elongation of longleaf pine to apical meristem elongation of slash and loblolly pine. However, the two traits were not well-correlated and did not represent an accurate picture of juvenile carbon storage for the longleaf pine species (Carey, 1992; Landers et al., 1995), so I do not report height growth for this species here.

Chlorophyll fluorescence was measured in July, September, and December on the three pine species. At each measurement period, eight to ten seedlings per species per treatment were randomly selected from a pool of unmeasured individuals, so no seedling was measured multiple times (i.e., so no pseudo replication occurred). Measurements were taken at night between 2200 and 0400 to ensure that all plants were dark adapted prior to measurements. The first plot measured each night was randomly chosen so the same plots were not being repeatedly measured at the same time of night.

Maximum dark-adapted chlorophyll fluorescence (F_v/F_m) and quantum yield of photosystem II (ϕ_{PSII}) was measured using the FluorPen (FluorPen FP 110, Photon Systems Instruments, Drasov, Czech Republic). The FluorPen is made for use with broadleaved species, so I spread several needles over the surface of the display to ensure no gaps occurred between needles (Needles tended to lay relatively straight across the display, but if overlapping occurred, it should have minimal effect since only the outer cell layers that are directly exposed to the light pulses undergo chlorophyll fluorescence). Minimum fluorescence (F_0) was measured by exposing the plant to modulated actinic light pulses to close all reaction centers in photosystem II. The seedling was then exposed to a dark pulse followed by a single saturating light pulse ($3000 \, \mu mol/m^{-2}s^{-1}$) to record maximum fluorescence (F_m) (and heat dissipation). Maximum dark-

adapted quantum yield of PSII was calculated using the formula F_v/F_m, where variable

fluorescence $(F_v) = F_m-F_0$ (Bolhar-Nordenkampf & Oquist, 1993; Schreiber et al., 1995).

Also using the FluorPen, light response curves of PSII quantum yield were measured on

light-adapted needles between the hours of 1000 and 1500. Measurements were taken during this

timeframe because it represented times of day when seedlings experience the highest temperature

and lowest soil water availability and are thus representative of times when the seedlings are

under the most physiological stress. I measured light response curves by exposing the leaf to five

levels of increasingly intense actinic light (100, 200, 300, 500, 1000 μmol m^{-2} s^{-1}). After a stable

state of fluorescence was achieved at each light level, the FluorPen exposed the tissue with a

saturating light pulse to record maximum fluorescence. Curves were measured on roughly three

seedlings per species per treatment in September and December. For simplicity, I report only the

plant response at the first light step (100 μmol m^{-2} s^{-1}), and light-adapted maximum quantum

yield from this data set as they represented species stress levels under the most natural

conditions.

Statistical analyses

Data were transformed to achieve normality and equal variance as necessary (mortality

statistics were run on ranked data). Light and soil moisture data were evaluated to examine the

differences between these abiotic factors and their interactions with a mixed model ANOVA that

incorporated treatment, time period, and the interaction between treatment and time period as

fixed effects and plot as a random effect. Significant values were determined at $P = 0.05$.

Quantum yield, light response curve (ϕPSII), survival, and height growth data were evaluated

using a mixed model ANOVA with treatment, species, time period, and the interaction between

treatment and species as fixed effects and plot as a random effect. I chose not to look at the

interaction of time period and any other variable as the time period differences represented natural seasonal changes that would be expected without treatment effects present (US Climate Data, 2018). All post hoc tests were conducted with Tukey's all pairs for multiple comparisons and t-tests for those with only two variables.

<div align="center">RESULTS</div>

Plot level differences in light and soil moisture

We measured light availability to the seedlings and soil water content to quantify how reduced precipitation and grass invasion affected the research plots post-fire in order to better explain seedling physiology and growth. There were significant differences in light availability between treatments caused by shading from the grass invader ($P < 0.0001$, $n = 1440$). Seedlings in NA plots received 50% more light than individuals in both invaded treatments (IA &ID). The prescribed burn removed all surface vegetation resetting the successional stages of uninvaded plots which repopulated with fast growing pioneer species that blocked more light from the seedlings in comparison to the preexisting native vegetation from the first year of the experiment. However, cogongrass still covered roughly 100% of the invaded plots and represented a denser cover type, evidenced by the 42% reduction in light available to seedlings in the invaded plots in comparison those in uninvaded plots ($P < 0.0001$, $n = 1280$) (Figure 3.3). There was also a difference in light availability between time periods with more light available during summer months and less during late fall and winter ($P < 0.0001$, $n = 1440$). All treatments followed the same seasonal trends across the months of measurement oscillating with natural patterns of precipitation (Figures 3.2 & 3.3).

There were also significant differences between treatments in volumetric water content (VWC) with the highest VWC in the NA and IA plots (15.20 ± 0.89, 14.88 ± 0.88 respectively)

and the lowest in the ND plots (4.95 ± 0.58; P < 0.0001, n = 2495; Figure 3.2). Seedlings in the

ND treatments experienced a 67% reduction in water availability and the ID received 21% less

water than the NA plots. IA plots, however, only experienced a 2% decrease in soil water

availability compared to the NA plots. There was a significant difference in time period (P <

0.0001, n = 2495) with higher soil water availability in the spring and early summer and less in

late summer and fall, correlating directly with monthly precipitation (Figure 3.2).

Seasonal trends in height growth

Total height growth pooled for both slash and loblolly pine from April to December was

similar across all treatments, most likely due to the short measurement period coupled with

higher than average rainfall. I would expect to see more significant results that mirrored the

physiological results with multiple growing seasons of data collection, as photosynthesis and

growth correlate well. However, loblolly pine grew taller than slash pine in all treatments (P <

0.0001, n = 275; Figure 3.4).

Seasonal trends in dark and light-adapted quantum yield

Seasonal trends in all physiological measurements were observed throughout the

experiment. Higher rates of light and dark-adapted quantum yield and maximum fluorescence

occurred in summer and winter correlating well with the increased precipitation, and lower rates

were observed in fall, which was comparatively drier. There was a significant difference in light-

adapted quantum yield and maximum fluorescence between September and December, with

significantly lower values in September (P < 0.0001, n = 284; P = 0.0068, n = 90; data not

shown). These seasonal trends were mirrored by dark-adapted quantum yield measurements that

were higher in the wetter months of July and December and lower in September (P < 0.0001, n =

284; data not shown).

Seedling responses to drought

Pine seedlings subjected to drought experienced significant physiological stress, as evidenced by their lower rates of dark-adapted quantum yield in comparison to individuals in the NA treatment (P = 0.0036, n = 284; Figure 3.5). There were no differences in light-adapted quantum yield between species or treatments (Figures 3.6 & 3.7). However, these plots still maintained a survival rate comparable to rates observed in the NA plots (Figures 3.8 & 3.9). Cogongrass was unaffected by the drought treatment; it showed no differences in success of re-establishment (it covered approximately 100% of plots with grass invasion 3 weeks post burn) or quantum yield (light- or dark-adapted) in plots with and without precipitation removal (data not shown).

There were also significant differences between species in dark-adapted quantum yield and survival. Longleaf pine had significantly lower rates of dark-adapted quantum yield and survival in comparison to slash and loblolly pine, consistent across all treatments. Slash pine performed comparatively better than longleaf pine, however, loblolly pine represented the most successful species throughout all treatments with the highest rates of dark-adapted quantum yield, growth, and overall survival respectively (P < 0.0001, n = 284; P < 0.0001, n = 275; P < 0.0001, n = 36; Figures 3.4, 3.5, & 3.8).

Seedling responses to grass invasion

Grass invasion as a single stressor significantly impacted survival rates and had a lesser effect on physiological stress. Individuals in the IA plots experienced lower rates of dark-adapted quantum yield in comparison to those in the NA plots, most likely due to light limitation and competition with the invader for soil water and nutrients (P = 0.0036, n = 284; Figure 3.5). Survival rates were more significantly reduced in these plots compared to rates in the NA plots,

indicating the potential of either interacting secondary fire effects or the sheer magnitude of cogongrass competition on native vegetation (P = 0.0072, n = 36; Figures 3.8 & 3.9). Seedlings of all three species in this treatment experienced similar growth rates and light adapted quantum yield measurements in comparison to seedlings in all other treatments (Figure 3.6 &3.7).

Seedling responses to the combined stresses of drought and grass invasion

Grass invasion significantly decreased rates of survival, which interestingly was not predicted by the physiological measurements. Individuals in this treatment experienced similar rates of dark-adapted quantum yield measurements as individuals in the NA plots, signifying a lower level of water stress on seedlings caused by reduced soil moisture evaporation as a result of the dense grass invader (Figure 3.10). However, this treatment also had the highest rates of mortality, significantly higher than rates observed in the NA plots (p = 0.0036, n = 284; Figure 3.8). Since photosynthetic processes and growth are comparable between seedlings in the NA plots and those in the ID plots, the increased mortality is likely due to variables not measured directly, such as belowground competition or fire modified soil characteristics in combination with drought and plant invasion.

Physiologically seedlings in both invaded treatments did better than those subjected to reduced precipitation, indicated by higher rates of dark-adapted quantum yield in seedlings in invaded plots. However, seedlings experiencing competition with cogongrass (IA & ID) experienced lower rates of survival in both treatments when compared to rates in the NA plots. This indicates stress on the seedlings was present in these plots that was not explained by physiological measurements.

DISCUSSION

Drought, plant invasion, and fire influenced by climate change can create unfamiliar ecosystems by affecting physical environmental factors like light or soil components. These factors can also interact with each other causing unique combinations of novel abiotic and biotic changes in forested ecosystems of the southeast like intensified or decreased drought and changes in vegetative cover (Costanza et al., 2015; Barton, 1993; Stephens et al., 2018; Caldeira et al., 2015). I examined physiological and growth responses of slash, loblolly, and longleaf pine to drought and plant invasion under post-fire conditions. I found that stressors have interactions that contribute to transitions in vegetation type to faster-growing species, intensifying drought, and increased fire behavior characteristics that do not exist in single variable scenarios. Seedlings were physiologically stressed by reduced precipitation, indicated by lower levels of chlorophyll fluorescence. The combination of the grass invader and reduced precipitation resulted in alleviation of the physiological stress by increasing soil moisture. However, measured rates of physiological stress in seedlings were not well-correlated with seedling mortality. For example, the highest rates of mortality were observed in the ID plots, but those seedlings did not exhibit a stress response that differed from the NA plots, indicating that there are potentially confounding factors that require further research to understand.

Unseasonably high rainfall in combination with the timing of the prescribed fire allowed for rapid recolonization of both native and non-native species into the plots post fire (Barton, 1993; Weltzin et al., 2003; Van Lear, 2005). Cogongrass commonly deals with grass-fire cycles in its native range. Fire removes other vegetation allowing cogongrass to quickly repopulate due to underground rhizome storage causing it to reestablish to nearly100% cover in the invaded plots three weeks after the burn (Balch et al., 2012; Toky & Ramakrishnan, 1983). The

prescribed fire changed the successional stage of the native plots replacing the patchy distribution of woody species and bunch grasses with fast-growing herbaceous pioneer species. New denser plant cover decreased seedling light availability in the native plots relative to pre-burn conditions (Capitanio & Carcaillet, 2008). The total native cover was therefore similar to the total plant cover in the cogongrass plots, which caused soil moisture conditions in the NA and IA, as well as ND and ID, plots to converge (compare Figures 2.4, 2.10, & 3.2, 3.10). This convergence was due to reduced soil water evaporation in all plots (as opposed to only the invaded plots prior to the burn), which led to similar physiological results in the invaded and uninvaded plots (Estrada & Flory, 2015; NeSmith et al., 2018; Figure 3.10).

Although individuals in the ND plots exhibited the most physiological stress, they also had the highest rates of survival in comparison to both invaded treatments. This could indicate that drought is a familiar stressor to seedlings that causes them to decrease transpiration rates, allowing for photosynthetic functionality in a safer margin as temperature and precipitation vary throughout the year (Shinohara & Leskovar, 2014; Singh & Reddy, 2011). Whereas seedlings in plots with the novel combination of drought and plant invasion (ID) continue functioning physiologically as if there are no water limitations present due to increased soil moisture, ultimately resulting in mortality. Based on the literature, it is also likely that variables I did not measure, such as below ground competition could have had significant negative effects on individuals within cogongrass plots that could have contributed to the higher mortality rates (Holly & Ervin, 2006; Holzmueller & Jose, 2011; Trautwig et al., 2017). Even if seedlings can withstand severe drought and shade from competition, the lowered rates of photosynthesis will not allow for optimal growth conditions which will negatively impact both timber production and natural ecosystem regeneration.

Changes in ecosystems resulting from decreased precipitation, plant invasion, or fire effects create unfamiliar challenges and systems for native species (Caldeira et al., 2015; Barton, 1993; Stephens et al., 2018). These three variables not only affected success of the seedlings in my field experiment, but also affected each other by changing the magnitude of the treatment effects (Figure 1.1). Fire changed predominant native vegetation types, which in turn increased soil moisture and decreased light availability to the seedlings. Pine seedlings in the ND treatments experienced significant physiological stress in comparison to seedlings in the NA plots even with during periods of seasonally high precipitation, indicating either (1) drought is debilitating to seedlings whether minor or severe or (2) fire changed soil properties and/or vegetation in a way that accentuated the intensity of drought. It is difficult to quantify whether results can be attributed to fire effects since we burned all plots in the experiment and were unable to monitor metrics like soil porosity and soil organic matter between burned and unburned plots. However, it is well documented in the literature that fire does modify many soil characteristics like porosity, hydrophobicity, and water holding capacity that likely would amplify drought stress (Certini, 2005; Van Lear, 2005; Capitanio & Carcaillet, 2008).

When examining responses between my three study species, I found that loblolly pine outperformed slash and longleaf pine, which is consistent with findings in other seedling studies (Nesmith et al., 2018; Shiver et al., 2000). Drought and grass invasion affected loblolly pine to a lesser degree. This species had higher rates of fluorescence, which is indicative of lower plant stress, most likely leading to increased rates of photosynthesis. Loblolly pine also had higher growth and survival in comparison to other species in all treatments. More photosynthesis and growth may be beneficial during seedling establishment as it provides a larger carbon budget for initial growth. However, higher rates of photosynthesis that result in more growth early on forces

loblolly pine to operate under a riskier hydraulic margin. Higher photosynthetic rates must be maintained to support the increased biomass, requiring more stomatal opening, leading to more water loss and the distinct possibility of mortality if droughts become significantly more intense in short time periods (Lens et al., 2013; Venturas et al., 2017; Dai, 2013). However, with careful site selection to maximize soil water holding capacities and optimal vegetative cover, I would recommend loblolly pine as the plantation species of choice as it had higher rates of growth and photosynthesis in comparison to slash pine under all conditions, resulting in more wood production.

The extent of longleaf pine habitat has already significantly declined due to land use changes to more intensively managed plantations or urban environments (Landers et al., 1995; Van Lear, 2005). The 3% of the original native range still occupied by longleaf pine may be significantly threatened by factors such as increased drought, fire severity, and plant invasion. I hypothesized that longleaf pine would have higher rates of physiological processes under stressful conditions due to its shade/fire/drought tolerance. My findings refute that hypothesis as longleaf pine had significantly lower efficiency in photosynthetic processes in response to all treatment effects, indicated by lower rates of dark-adapted quantum yield in comparison to slash and loblolly pine. Lower physiological rates led to significantly higher rates of mortality than the other two pine species, indicating that longleaf pine may be more significantly impacted by climate change. Longleaf pine was most negatively affected by the shade caused by grass invasion than any other stressor or stressor combinations, which is consistent with its relatively low shade tolerance and wide planting spacings and naturally widely spaced regeneration (Carey, 1992; South, 2006). A species that exists in a grass-like stage for years is unlikely to survive underneath a meter of cogongrass blades that in some cases block light completely from the soil

surface. Longleaf pine seedlings cannot coexist with invasive cogongrass without experiencing large decreases in seedling survival, leading us to consider the serious implications of cogongrass invasion into longleaf pine habitat.

Cogongrass invasion in an ecosystem provides competition for light, space, and nutrients but also alters the fire behavior typical in southeastern pine forests. Higher fire severity associated with the increased fuel loading and continuity from cogongrass results in mortality of native pines during prescribed burns or natural fires (Balch et al., 2012; Toky & Ramakrishnan, 1983; Hess & Tschinkel 2017; Figure 3.11). These habitat types are adapted to low intensity surface fires, where thick bark protects young trees from mortality (Landers et al., 1995; Schubert et al., 2016). When burning my 4m × 4m plots of cogongrass, I saw minimum flame lengths of 12-15 feet, which would immediately crown into the canopy killing even mature pines, which could result in the necessity of fire exclusion in cogongrass invaded forests. Fire exclusion and invasion by cogongrass will significantly impact biodiversity of the southeastern U.S. by continuing to reduce the already limited habitat available. These factors in combination with drought will also impact the overall success of timber species for wood production and native habitat reforestation.

Treatment Layout by Plot
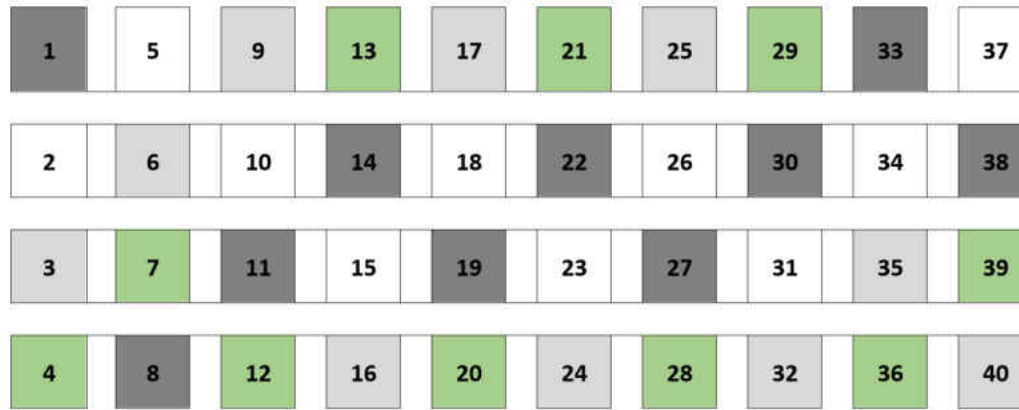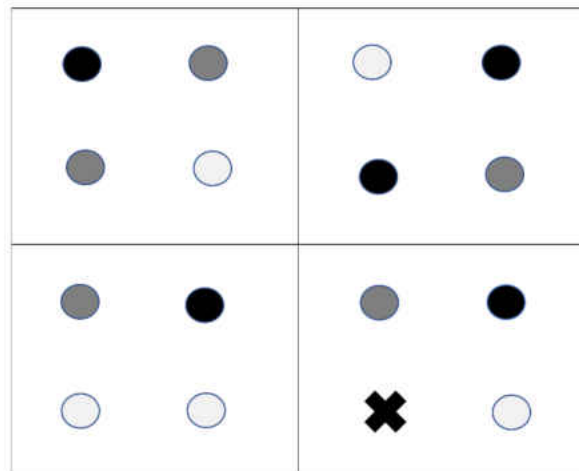
Figure 3.1: The overall plot layout with the locations of the different treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought (top). Example of the planting layout for each treatment plot, updated to add more trees from the previous season's planting layout (bottom). Each plot was divided into four quadrants and four individuals were planted into each quadrant with the exception of one randomly chosen blank spot (n = 5 per species per plot, for a total of 15 individuals).
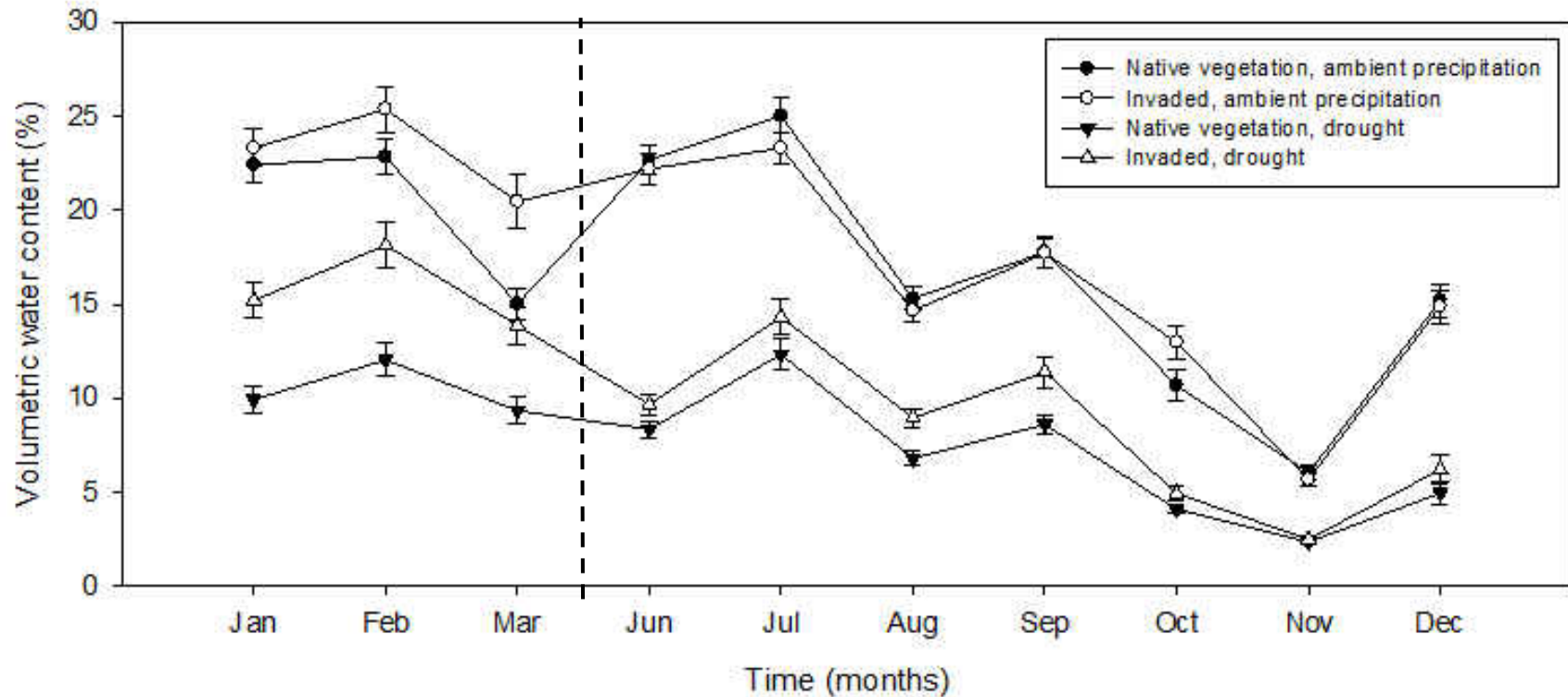
Figure 3.2: 2018 monthly average (± standard error) volumetric water content measured across the four treatments (10 replication

plots per treatment, 4 measurements per plot, n = 40 per treatment, per month). Demonstrating the unseasonably wet winter and spring

in contrast to the drier fall period (P < 0.0001, F = 505.94, n = 2496). Dashed line indicates where the burn took place in late April,

data was not collected in April and May due to the tear down/rebuild of the rainout shelters for the burn.
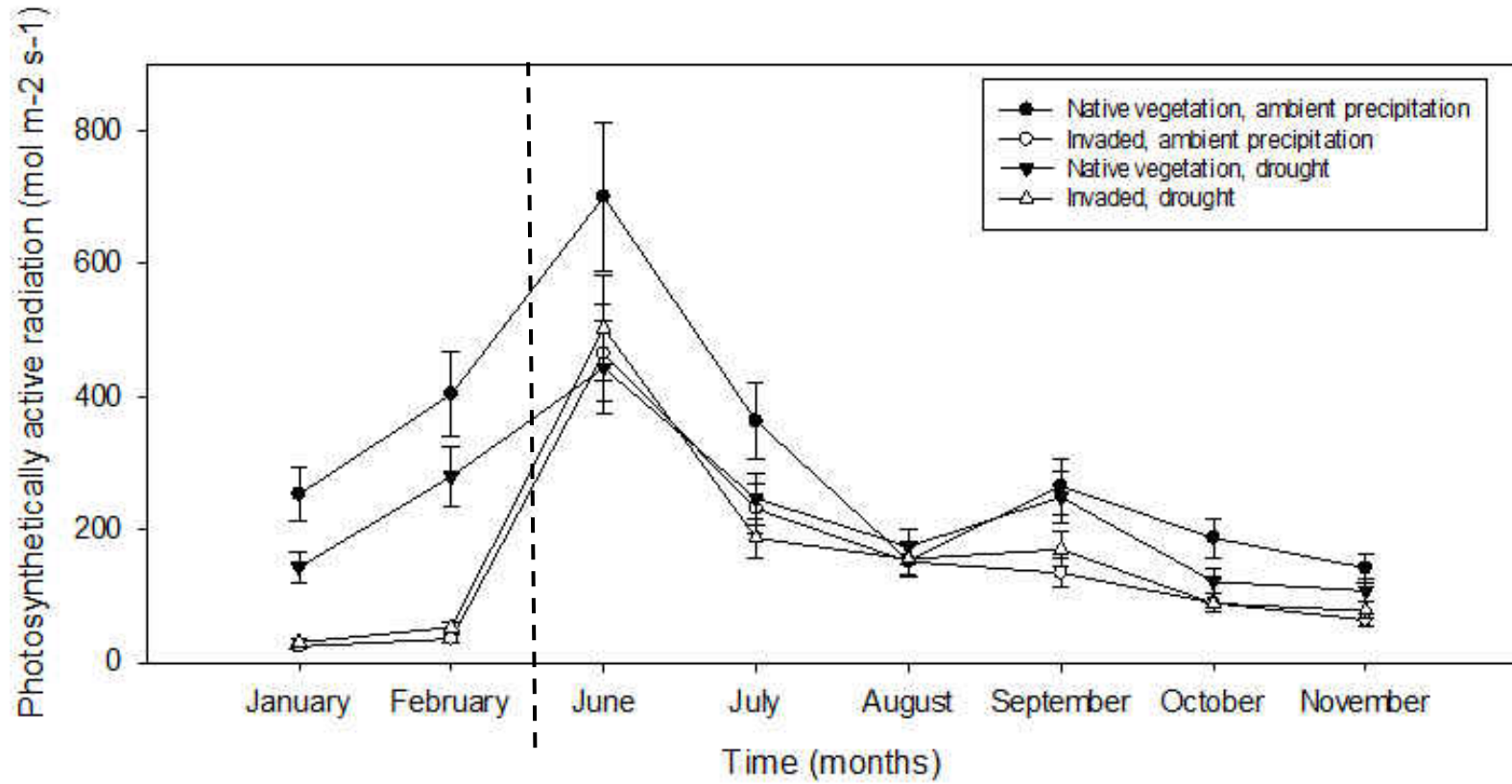
Figure 3.3: 2018 monthly average (± standard error) light measurements of photosynthetically active radiation (PAR) at ground level (10 replication plots per treatment, 4 measurements per plot, n = 40 per treatment, per month) reported across the four treatments: control, grass invader, drought, and drought plus grass invasion (P < 0.0001, F = 35.92, n = 1440). Dashed line indicates where the burn took place in late April, data was not collected in March, April, and May due to the tear down/rebuild of the rainout shelters for the burn.
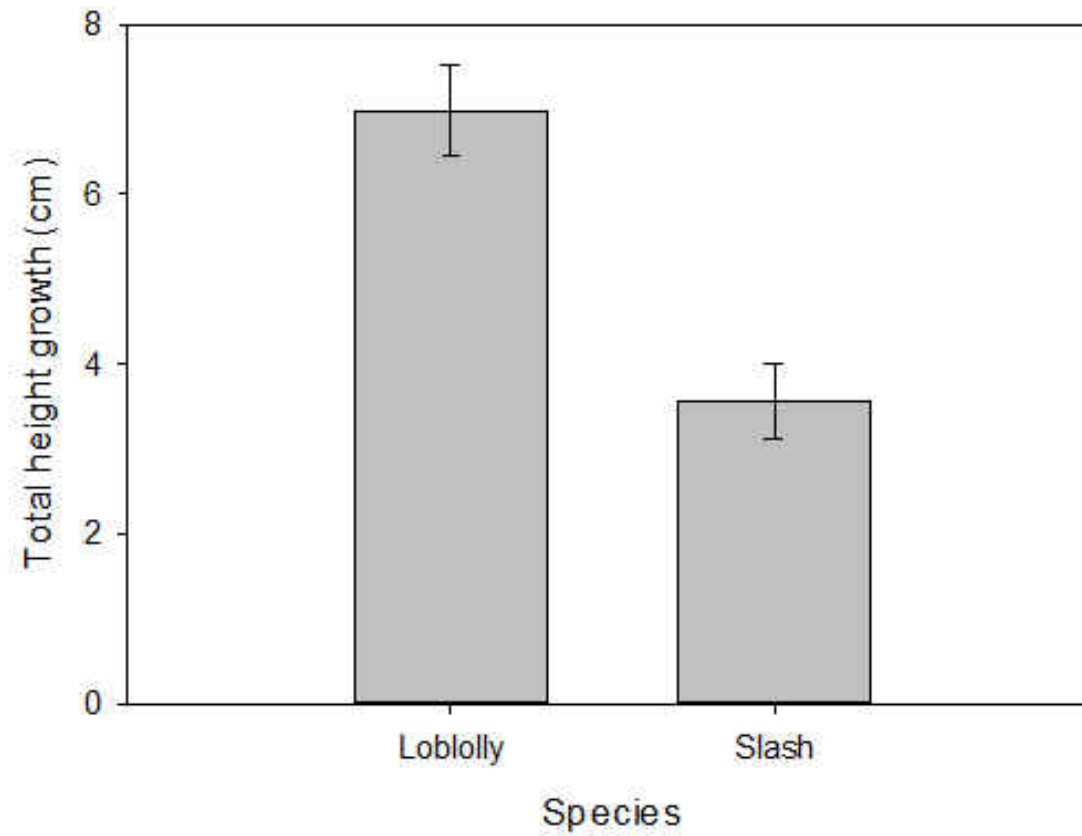
Figure 3.4: Average (± standard error) total height growth for slash and loblolly pine pooled for all treatments over the 2018 growing season.
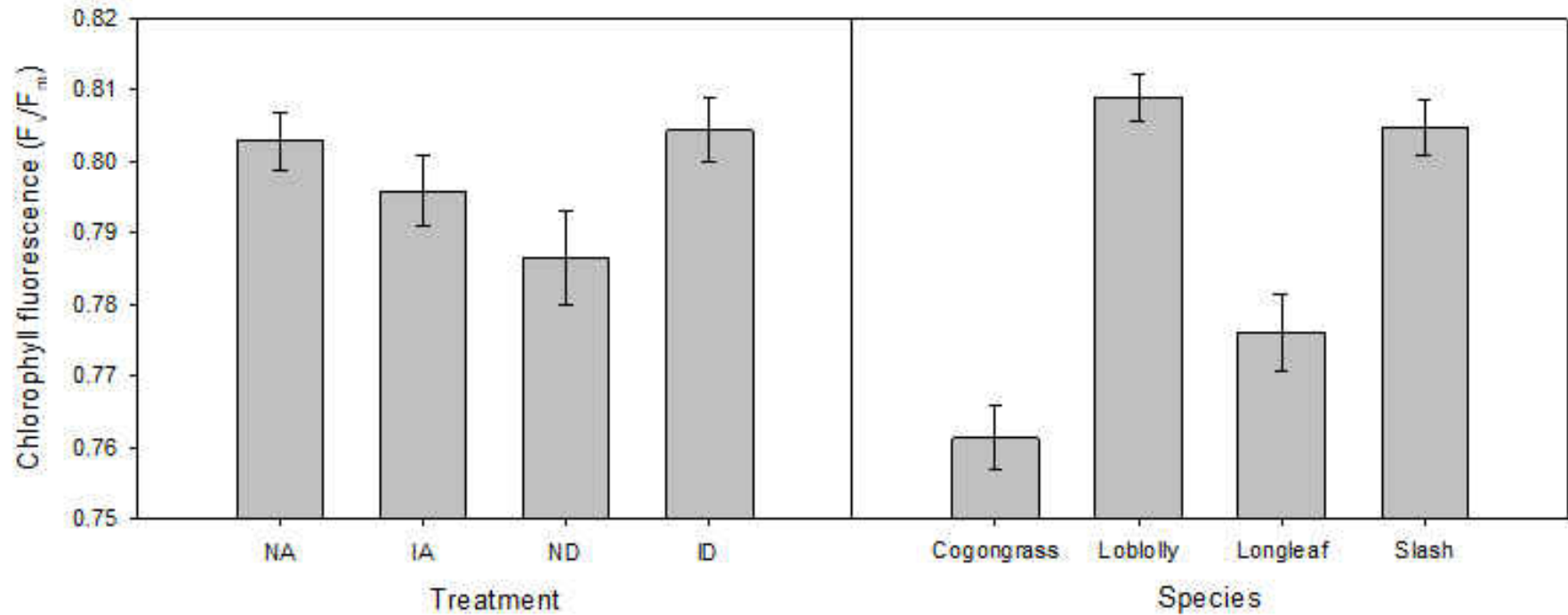
Figure 3.5: Average (± standard error) chlorophyll fluorescence (dark-adapted quantum yield, F_v/F_m) for each treatment, pooled for all species (left). Average (± standard error) chlorophyll fluorescence for each species, pooled for all treatments (right).
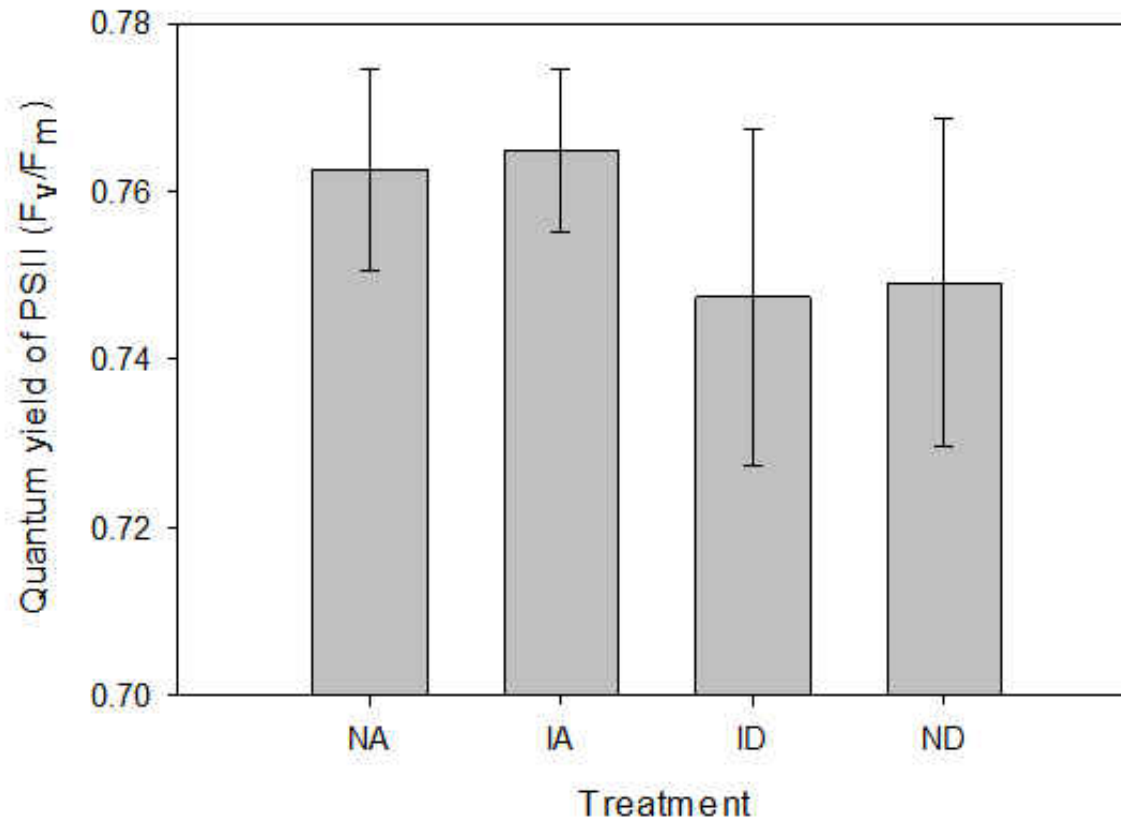
Figure 3.6: Average (± standard error) light-adapted quantum yield pooled for the native pine seedlings between the four treatments:

native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought.
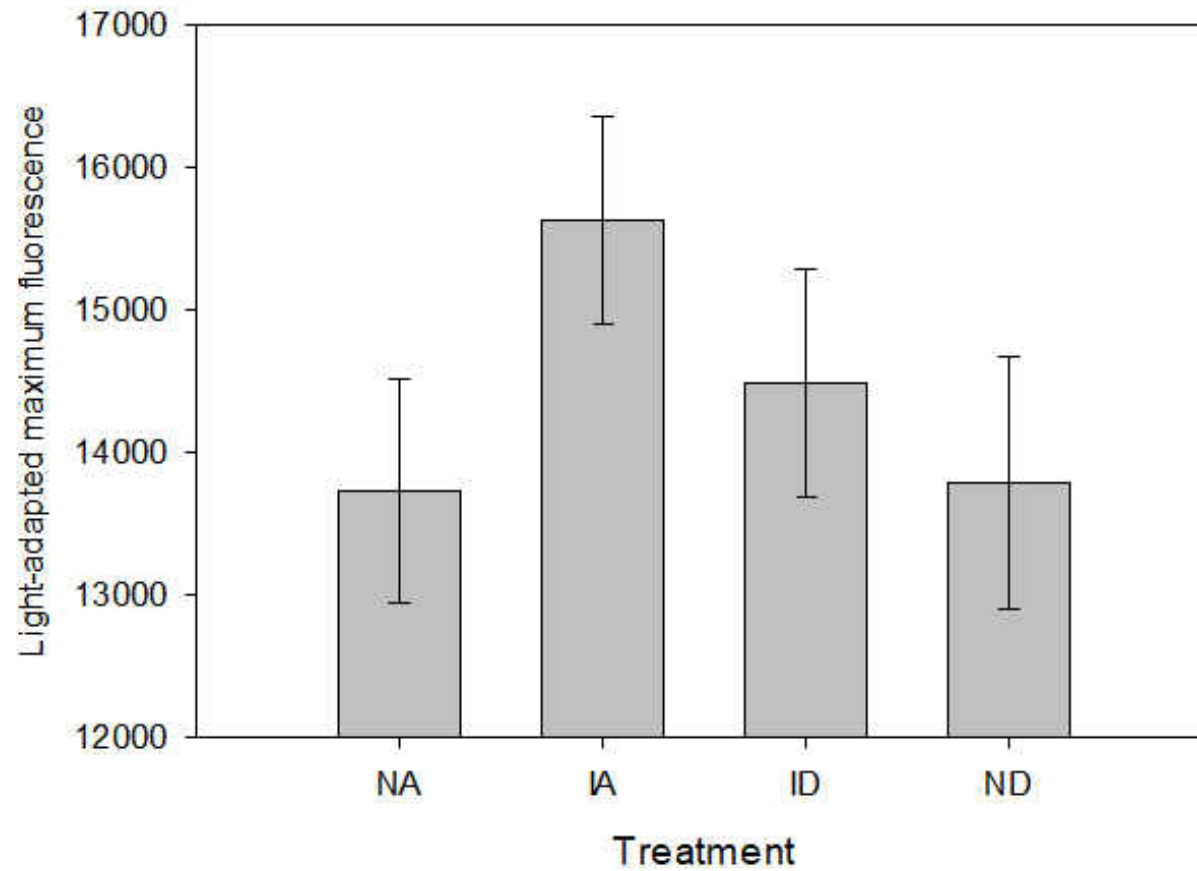
Figure 3.7: Average (± standard error) light-adapted maximum fluorescence pooled for all species, measured at the first light step (100 µm m^{-2} s^{-1}). The four treatment averages represent: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought.
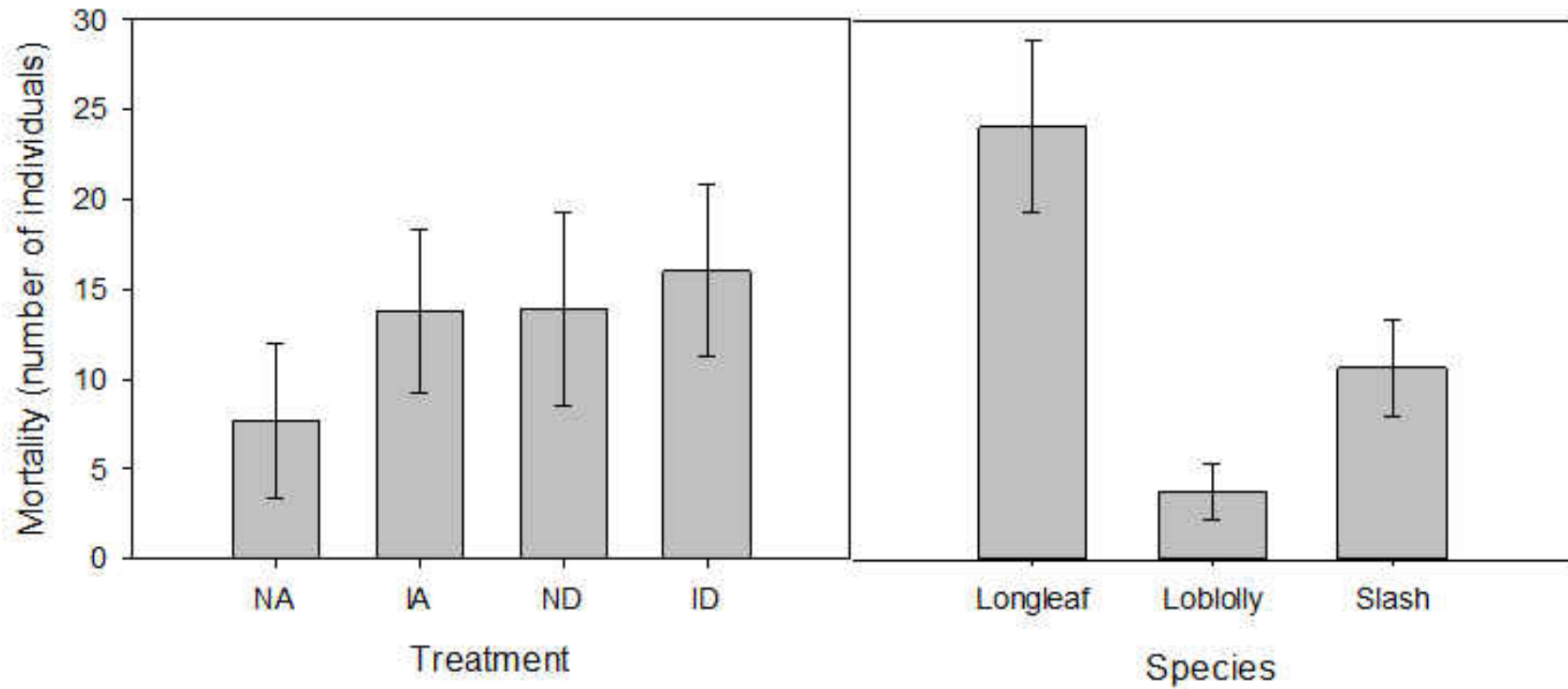
Figure 3.8: End of season mortality averages (± standard error) pooled for all species across the four treatments: native vegetation, ambient precipitation; native vegetation, drought; invaded, ambient precipitation; invaded, drought (left). Average (± standard error) mortality pooled for all treatments between the species (right).
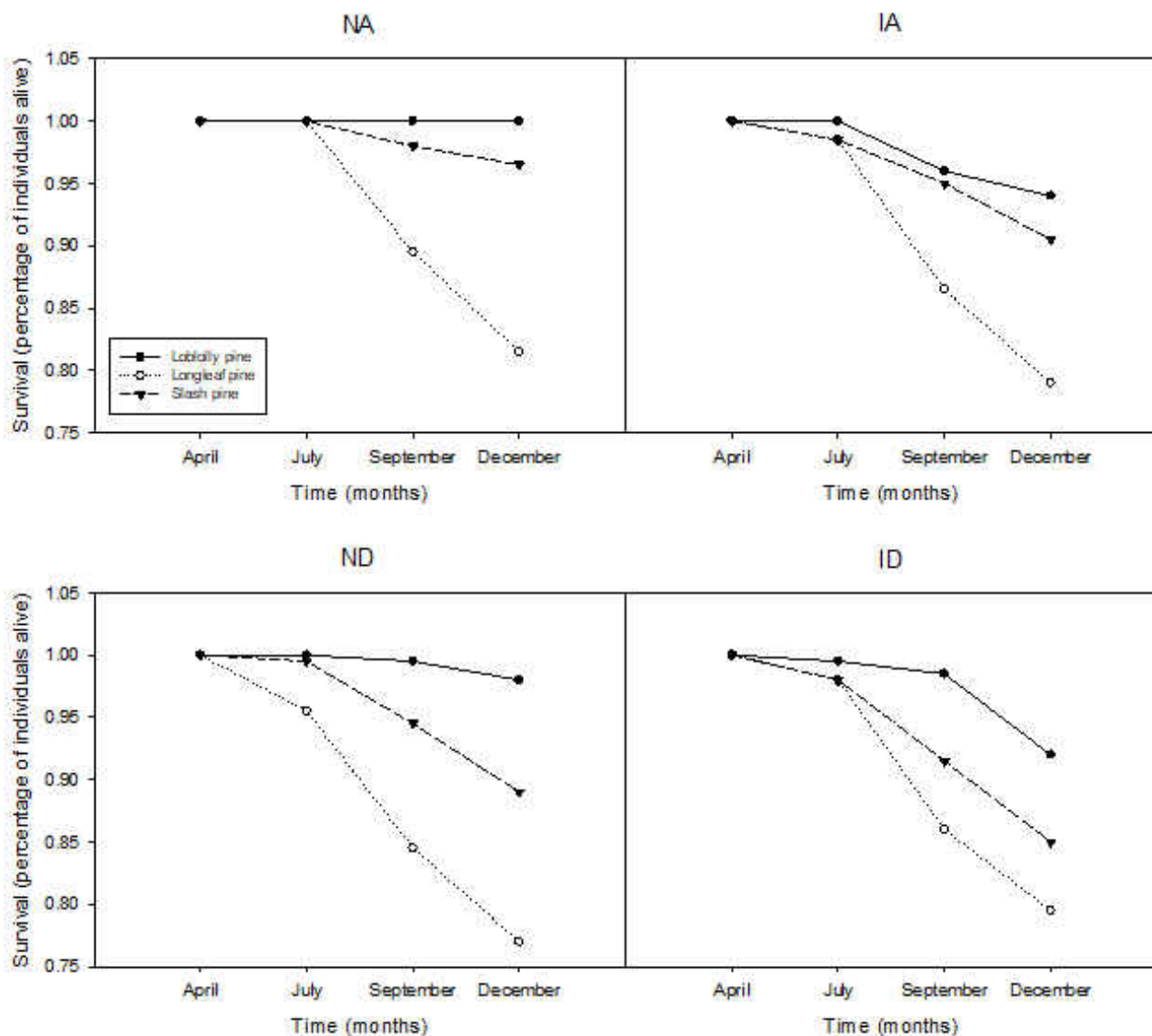
Figure 3.9: Mortality trends over time shown as survivorship curves where each species

(loblolly, longleaf, and slash pine) are shown on each graph representing one of the four

treatments: native vegetation, ambient precipitation (top left); native vegetation, drought (bottom

left); invaded, ambient precipitation (top right); invaded, drought (bottom right).
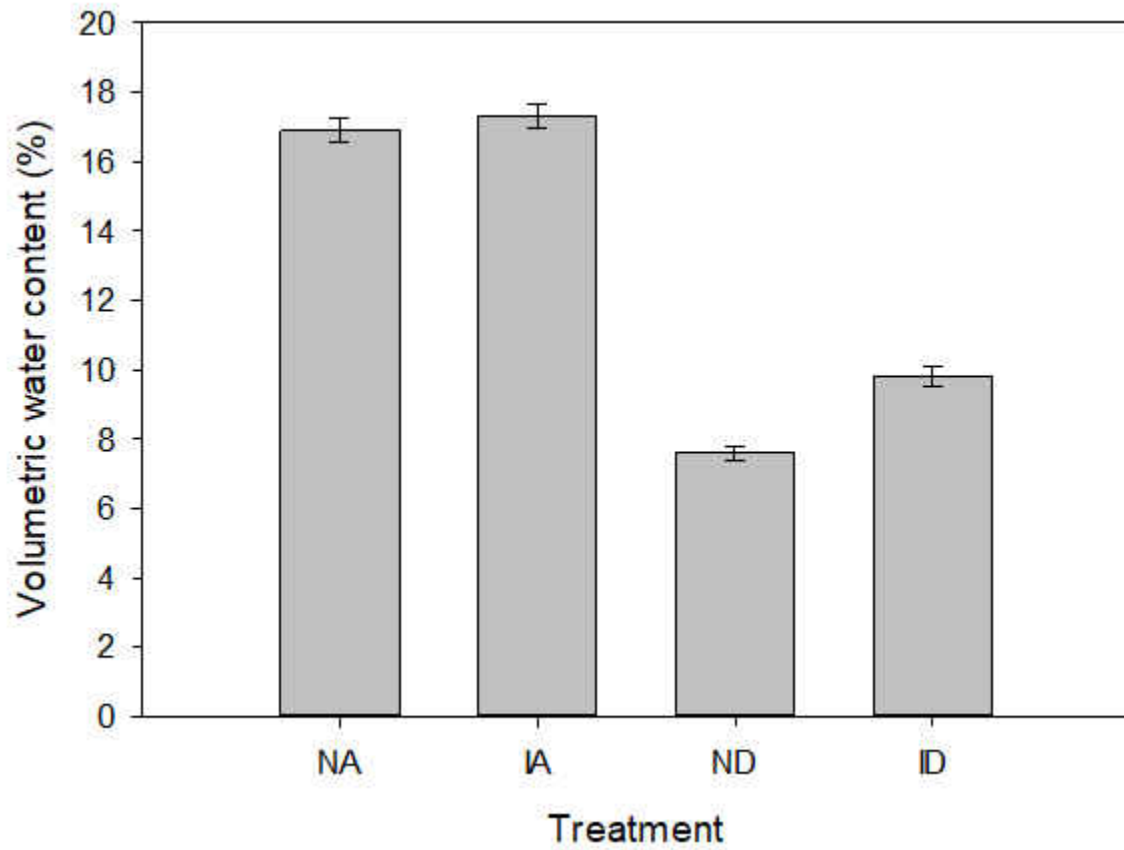
Figure 3.10: Average (± standard error) yearly volumetric water content measured at 12 cm

below the surface across the four treatments: native vegetation, ambient precipitation; invaded,

ambient precipitation; native vegetation, drought; invaded, drought.

Figure 3.11: Contrasting fire behavior observed in native vegetation plots (left) and plots invaded by non-native cogongrass (right).

SIGNIFICANCE

Environmental alterations associated with climate change are affecting many different aspects of functional terrestrial ecosystems. Moisture levels, fire regimes, and extent of plant invasions are changing, and this alters plant species composition within these ecosystems (Brewer, 2008; Hess & Tschinkel, 2017). Many studies have already indicated that North American plants are migrating northward and higher in elevation at alarming rates in response to their native ranges becoming uninhabitable due to stressors like drought and increased temperatures (Lustenhouwer et al., 2018; IPCC, 2014). Additionally, invasive species are moving more aggressively into new areas that were previously unsuitable before changes, such as increased drought and fire frequency occurred. Drought and increased fire severity stress the existing native vegetation allowing invasive cogongrass, which is well adapted to fire and drought in its native range, to outcompete the natives (Toky & Ramakrishnan, 1983). Current research indicates that fire intensities have been modified by changes in precipitation, fuel loading and continuity, and temperature (Stephens et al., 2018; Barton, 1993; Abatzoglou et al., 2018; Syphard et al., 2018). I witnessed these effects in my field experiment during the prescribed burn, where fire behavior was significantly more intense in invaded plots in comparison to plots with native vegetation (Figure 3.11). Fire behavior of this intensity causes concern for the survival of fire reliant forested ecosystems of the southeast.

I quantified the response of pine seedlings to interactive effects of drought and plant invasion in a coastal plain ecosystem throughout two growing seasons. Returning to my hypotheses, I found that pre-fire the combination of drought and plant invasion had offsetting effects, instead of additive like I expected. This was most likely due to the grass invader alleviating water stress for the native pines by lowering rates of soil moisture evaporation. In the

post-fire season, I observed almost no physiological stress on pines in the drought plus grass invasion treatments, however this was contrasted by the highest rates of mortality in comparison to all other treatments. The combination of drought and grass invasion appeared to be more detrimental on pine seedling establishment post burn. Unfortunately, it is difficult to objectively compare the physiological results of the two seasons of this experiment to identify pre and post-fire effects, because of the dramatic difference in precipitation across years. The site experienced lower than average spring rainfall the first season, followed by higher than average spring rainfall in the second. Abiotic factors like light levels and soil moisture were also significantly changed between the two seasons caused by the fast-growing pioneer species in the native plots resulting from the burn. These differences led to less drastic treatment differences post-burn since the pioneer species more heavily shaded the plots and prevented soil water evaporation, similar to the effects of the invasive cogongrass.

I estimated the interactions of drought, plant invasion, and fire by looking at differences in the combined treatment plots in comparison to plots with single variables, as well as differences between pre-fire and post-fire years. I found that as I predicted at the start of this experiment, the three stressors interacted to influence the relative effect of each factor on the success of native pine seedlings (Figure 4.1). For example, fire changed the vegetation type present in the native plots, resetting them to a younger successional stage common directly following a disturbance. The new vegetation regime caused changes in soil moisture availability, which was higher in native plots in the post-fire season in comparison to the native plots in the pre-fire season. Light availability was also much lower in the native plots post-fire as the fast-growing pioneer species were denser and more herbaceous than the sparsely populated woodier vegetation and grasses that existed before the burn. In the post-fire season, drought plots received

more than four times the average rate of precipitation in the winter and spring, which I would

predict to have nullified or at least significantly decreased drought effects on the seedlings (US

Climate Data, 2018; Boyer, 1982; Singh, 2011). However, drought still had the most debilitating

physiological effect on seedlings across all treatments, which may indicate that fire altered soil

characteristics (well documented in the literature) to decrease the absorption and water holding

capacity of soils and accentuate drought effects (Certini, 2005; Capitanio & Carcaillet, 2008;

Syphard et al., 2018). These interactions should be studied in more detail to isolate the specific

mechanisms by which these stressors are affected. Studies should include more intense cover

type monitoring, sampling of all aspects of soil characteristics from porosity to soil organic

matter content, and should observe longer-term effects by examining these treatments for

multiple growing seasons under the same conditions.

I hypothesized that the relative success of species within the treatments would depend on

their tolerance to drought, shade, and fire. Loblolly pine was more successful in all experimental

treatments across both seasons compared to the other two species, advocating for its use as a

more climate change-tolerant plantation species. However, drought was a debilitating stressor on

all three species under decreased and increased rainfall, and with and without prescribed burning.

The timber industry will be significantly impacted by decreased wood production, even in

loblolly plantations, if droughts are not mitigated by advantageous site selection, improved

timber varieties, or irrigation techniques. Longleaf pine was the most negatively affected species

in terms of physiology, growth, and survival under all conditions. My results therefore did not

support its alleged superior drought tolerance (Carey, 1992; Landers et al., 1995). What I found

is most likely a seedling phenomenon, and more mature longleaf pine may demonstrate much

higher drought tolerance after developing a tap root. The seedling stage however, is still very

important in both plantations and natural systems and data on the effects of drought and grass

invasion on this life stage should prove useful for management decisions. Significant impacts to

longleaf ecosystems may occur from the debilitating effects of drought and plant invasion

observed in this field experiment which indicate more intense protection efforts may be

necessary (Van Lear, 2005; Landers et al., 1995; Brewer, 2008).

  Longleaf pine ecosystems have already been decimated to less than 3% of their native

range and do not appear to respond well to drought, plant invasion, and fire, indicating even

more habitat could be lost. The combination of fire exclusion and transformation to monotypic

stands of cogongrass across the landscape will threaten biodiversity in the southeastern U.S.

where many species are already threatened or endangered (Brewer, 2008; Jackson, 1989; Van

Lear, 2005). We must be incredibly vigilant of the remaining longleaf pine ecosystems and take

precautions to prevent cogongrass introduction and spread. However, such an aggressive species

with no feasible management solutions for the size of the geographic area it infests will most

likely continue to invade its entire habitable range, potentially eliminating even more longleaf

pine habitat (Dozier et al., 1998; Estrada & Flory, 2015; Bryson & Carter, 1993). The impending

loss of this habitat should motivate restoration efforts for longleaf pine to use unique solutions

for reestablishing this diverse ecosystem. For example, freezing temperatures currently seem to

be the most limiting factor that defines the borders of cogongrass infestation. Therefore,

managers may need to consider moving longleaf pine restoration efforts further north. Longleaf

pine can withstand cooler average temperatures as its native range extends far past the habitable

range of cogongrass. However, habitable ranges of the many endemic species occurring within

longleaf pine ecosystems must also be considered. I urge that research into possible solutions for

the conservation of longleaf pine communities continue, either in conservation of existing areas

or the establishment of new habitat. Endangered species like the gopher tortoise could then be relocated to these more stable areas, preserving this species before extinction occurs from complete loss of habitat, as it seems likely these areas will become even more threatened by climate change in the future.
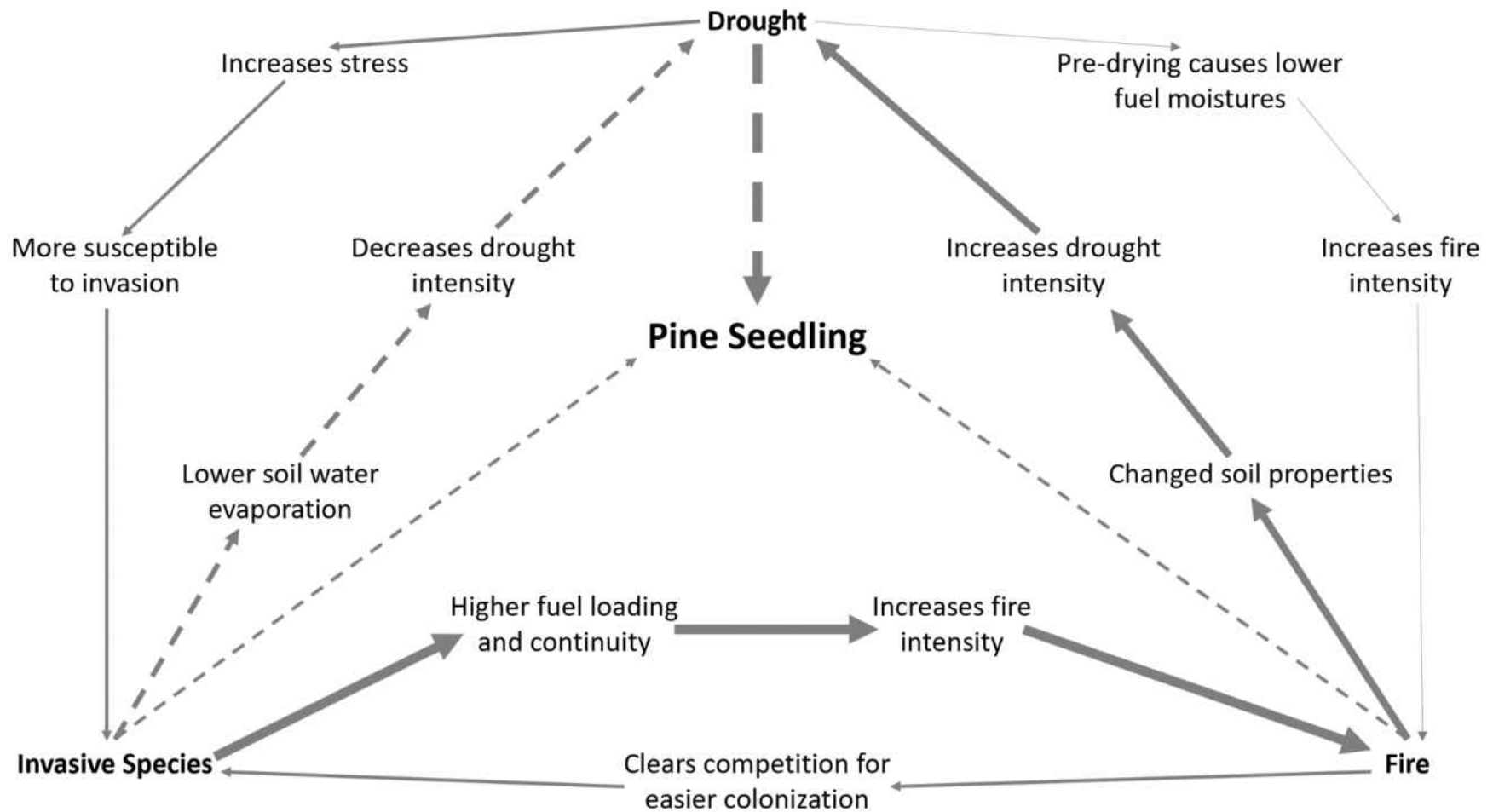
Figure 4.1: Negative effects of drought, invasive species, and fire and their interactions on the relative success of a pine seedling. Solid lines represent positive feedbacks that amplify the magnitude of effects, dashed lines represent negative feedbacks that offset the magnitude of effects. Thicker arrows represent more significant effects and skinnier arrows represent less important effects. This figure is modified from my original hypotheses to better represent the actual results of this study.

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