



Experimental drought and plant invasion additively suppress primary pine species of southeastern US forests

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ABSTRACT

Climate change and non-native invasive species are two predominant drivers of global environmental change, yet little is known about how they might interact to affect native communities and ecosystems. Drought and plant invasions are intensifying in ecosystems worldwide, including ecologically and economically important pine forests of the southeastern United States. These stressors can alter resource availability and plant competition outcomes, and may together exert additive, synergistic, or offsetting effects on native species, but such outcomes are difficult to predict. We used a factorial common garden experiment to determine how simulated drought, invasion by *Imperata cylindrica* (cogongrass), and their interaction affected seedling survival and performance (relative growth rates of height and diameter, and biomass) of two native pine species, *Pinus elliottii* var. *densa* (South Florida slash pine) and *Pinus taeda* (loblolly pine). In general, loblolly pine outperformed slash pine over the course of the experiment, but the directions and magnitudes of each species' responses to the treatments were similar, with the two stressors often exhibiting additive negative effects on pine seedling performance. For both species, invasion significantly suppressed seedling survival, drought reduced relative growth rates in height, and drought and invasion had an additive negative effect on diameter compared to ambient conditions with resident plant communities. The suppressive effects of drought on these primary pine species suggests that increasing drought in the region could scale up to affect forest stand dynamics. Furthermore, the experimental demonstration of cogongrass impacts on pine seedling survival and performance should further motivate land owners and property managers to remove this noxious invasive species. To predict the long-term outcome of drought and invasion on forest stands, and more broadly on vegetation dynamics in ecosystems affected by these global change agents, additional evaluations of their separate and interactive effects are needed. Nonetheless, these results experimentally demonstrate that stress from experimental drought combined with competition from an aggressive grass invader can significantly suppress seedlings of primary pine species of southeastern US forests.

1. Introduction

Climatic change, including shifts in temperature and precipitation regimes, is creating novel abiotic conditions that can alter the structure and function of ecological communities. In particular, the extent and severity of drought is intensifying in many ecosystems worldwide due to climate change (Easterling et al., 2000; Hoerling and Kumar, 2004; IPCC, 2001), resulting in native species mortality (Breshears et al., 2005; Thomas et al., 2004; Vose et al., 2012) and displacement (Lenoir et al., 2008). Non-native invasive plant species also are threatening native ecosystem integrity (Vitousek et al., 1996) by competing with native plant species for limiting resources such as nutrients, light, and water (Wilcove et al., 1998), which can have cascading effects, for

example, by modifying wildlife habitat conditions (e.g., food sources and availability; Vose et al., 2012). Native and invasive species likely will interact in new ways as novel abiotic conditions caused by climate change, and drought specifically, shift species ranges (Hoffman and Parsons, 1997; Pounds et al., 1999; Woodward, 1987) and transform plant community dynamics by opening niches (Walther et al., 2002) and altering habitat suitability. Although drought and invasive species are primary abiotic and biotic stressors, respectively, little is known about how they might interact to affect native ecosystems.

There are three scenarios for how multiple global change stressors may interact to affect native ecosystems. Drought and invasion may exert negative effects that manifest additively such that the combination of stressors is equal to the sum of each acting independently

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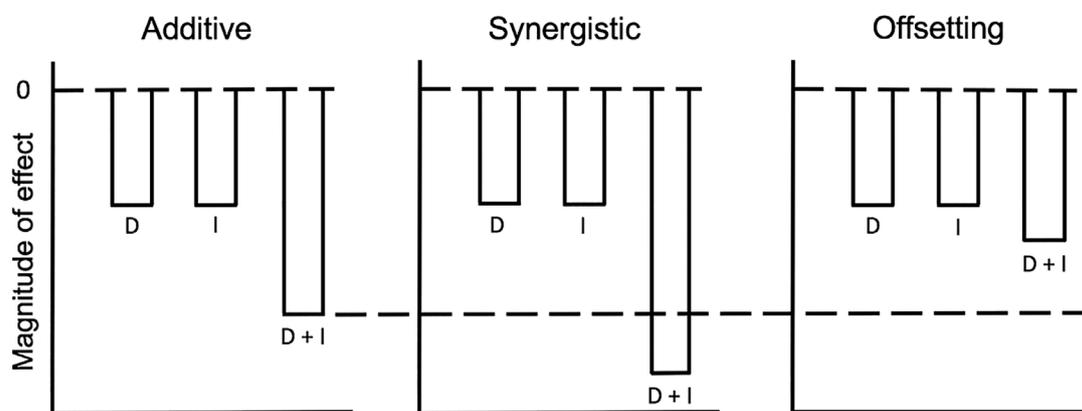


Fig. 1. Conceptual diagram illustrating three potential scenarios for the magnitude of independent (additive) or interactive (synergistic and offsetting) negative effects as a result of multiple stressors acting on ecological communities. Here, “D” represents drought and “I” represents invasion by a non-native species. The lower dashed line provides a reference point for the additive effects scenario.

(Breitburg and Riedel, 2005; Folt et al., 1999). Alternatively, two stressors may have synergistic interactive effects, whereby together they yield stronger negative effects than would be predicted based on each stressor acting in isolation. Finally, one stressor may act antagonistically with another and offset the effects of the other stressor, resulting in less negative impacts than would be expected compared to the additive scenario (Fig. 1). For example, a plant invader with a dense canopy may, under drought conditions, compete strongly against native species for both light and limited soil water, leading to synergistic negative effects. Alternatively, the dense invasion might offset drought stress by lowering ground-surface temperatures and air flow, thereby reducing evapotranspiration. Given the increasing severity and extent of both drought and plant invasions, the lack of quantitative studies investigating interactions between abiotic and biotic stressors (Todgham and Stillman, 2013) and the unpredictable nature of such interactions represent a critical knowledge gap.

Drought and plant invasions are intensifying in forests worldwide, including ecologically and economically important pine forests of the southeastern United States (Simberloff et al., 1997; Wang et al., 2010). Drought stress can degrade forest health and resistance to stressors such as fire, pathogens, insects, and plant invasions (Dale and Joyce, 2001; Vose et al., 2012), particularly in regions marked by high temperatures and long growing seasons (Aber et al., 2001). Both natural and planted coastal plain forests in the southeastern US are largely dominated by natural or improved varieties of *Pinus elliottii* (slash pine) and *Pinus taeda* (loblolly pine) forests. Because slash and loblolly pine forests have largely replaced *Pinus palustris* (longleaf pine) across its historic range, they represent important ecological refuges (Bremer and Farley, 2010; Gilman and Watson, 2006) and act as a major carbon sink in the US (Alavalapati et al., 2007; Turner et al., 1995). Furthermore, the economic importance of managed forests in the southeastern US is unrivaled as they supply 62% of the timber harvested in the U.S. (Smith et al., 2009) and 16% of global industrial wood, and the region produces more timber than any one country (Prestemon and Abt, 2002; Wear and Gries, 2002). Southeastern US forests are increasingly invaded by non-native plant species, including *Imperata cylindrica* (cogongrass), a perennial C4 grass native to Asia that can inhibit pine establishment (Daneshgar et al., 2008) and now covers hundreds of thousands of hectares across the region (Estrada and Flory, 2015; Schmitz and Brown, 1994). Cogongrass thrives in a wide range of soil conditions (MacDonald, 2004), is reportedly drought and fire tolerant (Bryson et al., 2010; Patterson, 1980), and strongly competes with native species for water and nutrients (Estrada and Flory, 2015; Kuusipalo et al., 1995; MacDonald, 2004). Thus, we hypothesized that drought, in combination with cogongrass invasion would have additive or synergistic negative effects on the survival and performance of pine seedlings.

We evaluated the independent and interactive effects of drought (simulated with rainout shelters) and invasion (by cogongrass) on slash and loblolly pine seedling survival and performance using a factorial common garden experiment. Across the drought and invasion treatments, we measured pine seedling survival, biomass, and relative growth rate, as well as abiotic conditions that might shape plant competitive outcomes, including soil moisture and light availability. Our results demonstrate that both drought and invasion significantly suppress the survival and performance of these ecologically and economically important pine species, and together these two stressors have the potential to dramatically alter southeastern US forests.

2. Materials and methods

2.1. Study species

Slash and loblolly pine occur naturally or are planted across tens of millions of hectares in the southeastern US and comprise more than half of the region’s standing pine volume (Baker and Langdon, 1990). They provide critical habitat for wildlife and generate billions of dollars in revenue for regional economies each year (Nowak, 2015). Slash pine is moderately to highly drought tolerant relative to other pine species (Burns and Honkala, 1990; Gilman and Watson, 2006) and can grow across a range of soil conditions from seasonally dry to wet soils near streams and swamps, and in hammocks and mesic flatwoods (Ewel and Myers, 1990). Loblolly pine has low to moderate drought tolerance (Burns and Honkala 1990; Gilman and Watson, 2006) and predominantly occurs in poorly drained soils in mesic forests, floodplains, and hydric hammocks (Ewel and Myers, 1990). Both species have low to moderate shade tolerance and exhibit poor establishment under competition (Burns and Honkala, 1990; Gilman and Watson, 2006). *Pinus elliottii* var. *elliottii* is the most common and widely distributed *P. elliottii* variety, occurring across the southeastern coastal plain from Louisiana to South Carolina, and south to Central Florida (USDA NRCS, 2016). South Florida slash pine (*Pinus elliottii* var. *densa*) is endemic to Central and South Florida, and unlike *P. elliottii* var. *elliottii*, has a distinct grass seedling stage (Fowells, 1965). The two varieties hybridize naturally where their ranges overlap and produce offspring that are indistinguishable from either variety (Lohrey and Kossuth, 1990).

Cogongrass is one of the most prolific and aggressive plant invaders in the southeastern United States. It spreads vegetatively throughout the region via rhizomes but purportedly produces viable seed primarily outside of Florida (MacDonald, 2004; MacDonald, Personal communication). Cogongrass establishment and spread are facilitated by natural and anthropogenic disturbances including fire, timber harvests, mowing, and tilling (Holzmueller and Jose, 2012; Lippincott, 2000). Cogongrass is threatening in part, because it is highly flammable and

facilitates longer duration, hotter fires that can damage or kill pine trees and other typically fire-tolerant species (Lippincott, 2000; Platt and Gottschalk, 2001). Furthermore, cogongrass routinely forms dense monocultures that can inhibit native plant species abundance, distribution, and diversity (Estrada and Flory, 2015), potentially leading to significant effects on ecosystem processes such as productivity, nutrient cycling, or decomposition. The species is difficult to manage due to rapid regeneration from dense rhizomatous networks belowground. Naturally seeded or planted pine forests are often invaded by cogongrass and trees can be exposed to invasion throughout all life stages.

2.2. Experimental design

To evaluate the effects of drought and cogongrass invasion on slash and loblolly pine, we conducted a common garden field experiment at the Bivens Arm Research Site (BARS) in Gainesville, FL (29.628489°N, -89.353370°W). Although drought and cogongrass may affect pine trees from germination through adulthood, here we focus specifically on the critical seedling life-history stage.

In May 2012 we established 40 4 m × 4 m plots and randomly assigned treatments to each of ten blocks. Treatments included (1) ambient precipitation, resident species only; (2) ambient precipitation, resident species plus cogongrass; (3) reduced precipitation via rainout shelters (hereafter referred to as “drought” plots), with resident species only; and (4) drought plots, with resident species plus cogongrass. We selected 12 native herbaceous understory species that occur in southeastern US pine forests and planted three individuals of each species into a 6 × 6 grid design. The native species consisted of seven grass and five forb species (Table 1). Plots were colonized by dozens of other native and naturalized species from the seed bank and surrounding area, such as *Bidens alba* and *Paspalum notatum*, thus we refer to plots without cogongrass as “resident” species plots. Cogongrass rhizomes were collected from an on-site population and grown for three months in a greenhouse. In June 2013 we planted nine cogongrass ramets in each “invaded” plot. All ramets survived transplantation. We have FDACS permit #2015-023 to conduct experiments with cogongrass at BARS. Soils at BARS are Portsmouth sandy loam (67% sand, 3% silt, and 30% clay) and comprised of Bivans sand (75%; 5–8% slope) and Blichton sand (25%; 2–5% slope; Natural Resources Conservation Service, Web Soil Survey).

In February 2013, we constructed wooden, lean-to style rainout shelters over drought-treated plots. We used corrugated polycarbonate roofing (89% roofing areal coverage and 89% light transmittance; Tufttex, Fredericksburg, VA) and aluminum gutters (Amorfill Aluminum, Gainesville, FL) to capture and direct precipitation offsite. We diverted surface and ground-water flow from drought plots by lining the perimeter of each with ground-level aluminum flashing (Amerimax aluminum flashing) and belowground (to one-meter depth)

plastic sheeting (20 mm thick; Global Plastic Sheeting Inc., Vista, CA). Control shelters were constructed over non-drought plots and topped with shade cloth (22% shade; International Greenhouse Company), which created comparable light levels in ambient and drought plots (mean ± SE percent light reduction ambient: 33.4 ± 1.01 and drought: 31.1 ± 1.2; $t_{(37)} = 1.5$, $P = 0.14$; see Alba et al., 2017 for additional shelter details). These light levels are within the range of light levels where slash and loblolly pine seedlings establish and cogongrass invades in southeastern US pine forests, which vary widely depending on management methods (Sharma et al., 2012).

In January 2015, we planted four bareroot seedlings each of slash and loblolly pine into all 40 plots at 0.5 m spacing in an alternating arrangement. For this experiment, we used *P. elliotii* var. *densa* (South Florida slash pine, hereafter slash pine) because seedlings were accessible when the experiment was initiated. Slash pine seeds were collected from a native stand in Avon Park, Florida and grown for one year at Andrews Nursery in north central Florida. Loblolly pine seeds were sourced from Livingston Parish, Louisiana and grown for one year at Dwight Stansel Farm in Wellborn, FL. Seedlings that did not survive after ten weeks were assumed to have died from transplant shock and were replaced.

2.3. Data collection

To determine how drought and invasion treatments affected abiotic conditions in the plots we quantified soil percent volumetric water content (hereafter, soil moisture; HydroSense II; Campbell Scientific, Logan, UT) and photosynthetically active radiation (hereafter, light availability; ACCUPAR LP-80; Decagon Devices, Pullman, WA). We measured soil moisture at a depth of 0–12 cm ($n = 4$ subsamples per plot) monthly during the dry season (December to April) and bi-monthly during the wet season (May until December). We measured light availability at ground level, 0.5 m, and above the vegetation canopy (~1.5 m) in each plot ($n = 4$ subsamples per plot) monthly during the growing season (April through December). To characterize the density and extent of cogongrass invasion, we measured cogongrass cover in February, July, and October of 2015 by dividing each plot into a grid of quadrats and then averaging values among quadrats at the plot level for analysis.

To evaluate how slash and loblolly pine responded to drought and invasion, we quantified survival to harvest (one growing season), relative growth rates (RGR) in height and diameter, and biomass (clipped at the soil surface, dried to constant mass at 60 °C, and weighed) at final harvest. To quantify growth rates, we measured height to the apical meristem (mm) and root crown diameter (mm) two months after the trees were planted (March 2015) and again at final harvest (December 2015). We calculated RGR according to Hunt (1982) as $\ln(W_2) - \ln(W_1)/t_2 - t_1$, where W_2 and W_1 are the final and initial height and

Table 1
The scientific names and functional types of twelve native understory species planted in 2013 and the most common resident species in the plots in 2015 across all treatments.

Planted native species (2013)			Most common resident species (2015)		
Genus	Species	Functional group	Genus	Species	Functional group
<i>Andropogon</i>	<i>brachystachyus</i>	grass	<i>Ambrosia</i>	<i>artemisiifolia</i>	forb
<i>Andropogon</i>	<i>virginicus</i> var. <i>glaucus</i>	grass	<i>Aristida</i>	<i>stricta</i>	grass
<i>Aristida</i>	<i>stricta</i>	grass	<i>Baccharis</i>	<i>halimifolia</i>	shrub
<i>Eragrostis</i>	<i>elliotti</i>	grass	<i>Bidens</i>	<i>alba</i>	forb
<i>Eragrostis</i>	<i>spectabilis</i>	grass	<i>Bothriochloa</i>	<i>pertusa</i>	grass
<i>Muhlenbergia</i>	<i>capillaris</i>	grass	<i>Eragrostis</i>	<i>spectabilis</i>	grass
<i>Panicum</i>	<i>anceps</i>	grass	<i>Eupatorium</i>	<i>capillifolium</i>	forb
<i>Carophophorus</i>	<i>subtropicanus</i>	forb	<i>Muhlenbergia</i>	<i>capillaris</i>	grass
<i>Elephantopus</i>	<i>elatus</i>	forb	<i>Paspalum</i>	<i>notatum</i>	grass
<i>Liatrus</i>	<i>laevigata</i>	forb	<i>Pityopsis</i>	<i>graminifolia</i>	forb
<i>Pityopsis</i>	<i>graminifolia</i>	forb	<i>Solidago</i>	<i>fistulosa</i>	forb
<i>Solidago</i>	<i>fistulosa</i>	forb	<i>Urochloa</i>	<i>maxima</i>	grass

diameter, respectively, and t_2 and t_1 are the final and initial dates of measurement. We analyzed the log-transformed growth rates (Hunt, 1982) but present the untransformed data to facilitate ecological interpretation.

2.4. Statistical analysis

Cogongrass percent cover was analyzed with mixed model ANOVA using the nlme package in R version 3.2.3 (v.3.23, R Development Core Team). Fixed effects included drought, date, and a drought by date interaction, with a random effect of plot nested with block. Soil moisture, light availability, and pine performance (proportion of seedlings surviving per plot, RGR of height and diameter, and biomass) were analyzed using mixed model ANOVA, with soil moisture and light response models accounting for repeated measures. Response variables were transformed as necessary (square root of soil moisture, light availability and biomass, and log of RGR) to improve normality and homogeneity of variance based on inspection of residual-versus-predicted and residual-versus-quantile plots. The fixed effects for soil moisture and light availability were drought, invasion, date, and all interactions, with block as a random effect. For pine responses to the treatments, species were analyzed individually except for overall survival. Percent survival by plot was analyzed using proc mixed in SAS (v. 9.4, SAS Institute). Height and diameter RGR and biomass, which had unbalanced data sets due to unequal seedling survival across the treatments at final harvest, were analyzed using proc glimmix with a Gaussian distribution and logit link function. All post hoc models included Tukey’s adjustment for multiple comparisons.

3. Results

During the 2015 growing season, cogongrass percent cover was not affected by drought (mean ± SE drought: 54.7 ± 2.8; ambient: 57.7 ± 3.8; $F_{(1, 9)} = 3.0$; $p = 0.119$). Across the drought and ambient treatments in 2015, cogongrass percent cover increased from 53% ± 2.7 in February to 75% ± 2.4 in October. On average, soil moisture in drought conditions was 48% lower than under ambient conditions ($F = 145_{(1, 701)}$; $p < .0001$) and 37% higher in invaded plots relative to resident plots ($F = 7.3_{(1, 701)}$; $p = .007$) under drought conditions (Fig. 2a). Overall, light availability was 58% greater at ground level in resident plots compared to invaded plots (Fig. 2b).

Across all four treatments, slash pine survival (23% ± 3.8) was lower than that of loblolly pine (62% ± 3.8; $F_{(1, 63)} = 53.9$; $p < .0001$). Slash pine survival was 58% lower under drought (13.8 ± 5.2% survival) than ambient (32.5 ± 7.5) conditions and 72% lower with the invader than with resident species (Fig. 3; Table 2). Invasion offset any effects of drought on slash pine survival (Fig. 3a; Table 2, significant drought × invasion interaction). Loblolly pine survival was 38% lower under drought (47.5 ± 8.7) than ambient (76.3 ± 7.2) conditions and 26% lower when growing with the invader (52.5 ± 9.8) than with resident species only (71.3 ± 6.1; Fig. 3b; Table 2). In contrast to slash survival, invasion did not significantly offset the effect of drought on loblolly survival (Table 2, no drought × invasion interaction).

Drought, but not invasion, was associated with lower relative growth rates in height of both pine species (Fig. 4; Table 2; Table 3). Height RGR of slash pine was 47% slower under drought (0.0379 ± 0.010 mm mm⁻¹ day⁻¹) than ambient (0.0720 ± 0.019 mm mm⁻¹ day⁻¹) conditions, while loblolly was 42% slower under drought (0.0854 ± 0.0064 mm mm⁻¹ day⁻¹) than ambient (0.147 ± 0.0069 mm mm⁻¹ day⁻¹) conditions. In terms of stem diameter, slash pine only grew larger under baseline conditions of ambient precipitation with resident species, while remaining stagnant when exposed to drought or invasion independently or in concert (Fig. 5a, Table 2; Table 3). Growth in loblolly diameter was 43% slower under drought (0.00666 ± 0.00077 mm mm⁻¹ day⁻¹) than

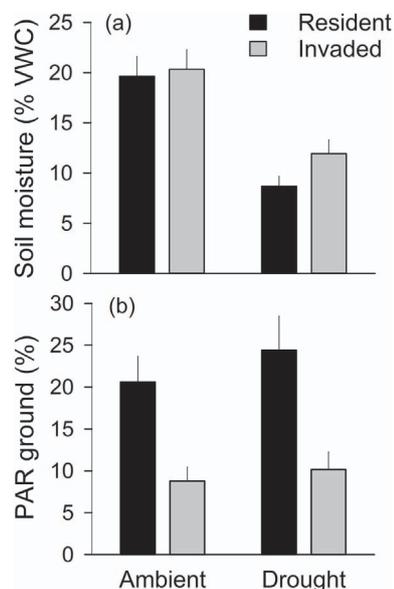


Fig. 2. Mean ± SE of soil moisture (percent volumetric water content) averaged over 2015 (a) and percent light availability at the ground level (PAR) from April through December 2015 (b), in plots exposed to ambient or drought conditions and with resident species only or resident species invaded by *Imperata cylindrica* (cogongrass).

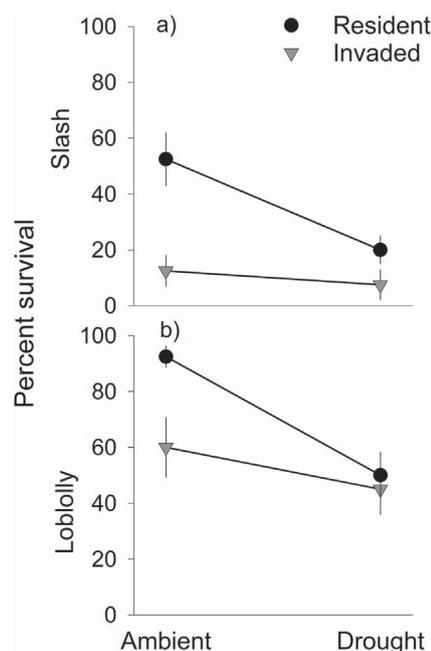


Fig. 3. Mean ± SE percent survival of slash (a) and loblolly (b) pine seedlings exposed to drought and invasion treatments.

ambient (0.0177 ± 0.00082 mm mm⁻¹ day⁻¹) precipitation and 39% slower when growing with the invader (0.00693 ± 0.00074 mm mm⁻¹ day⁻¹) compared to resident (0.0114 ± 0.00085 mm mm⁻¹ day⁻¹; Table 2; Table 3) species. For both species, drought and invasion had an additive negative effect on diameter (Fig. 5), with growth in slash and loblolly reduced by 241% and 71%, respectively, under both stressors relative to baseline conditions (Fig. 5, Table 2; Table 3).

Slash pine biomass was 45% lower under drought than ambient conditions when growing with resident species, but there was no effect of drought under the invasion treatment. In parallel, there was only an effect of invasion on biomass under ambient precipitation, where presence of invasion resulted in 53% lower slash biomass compared to

Table 2

Results of mixed model ANOVAs testing the fixed effects of drought, invasion, and their interaction on slash and loblolly pine survival, relative growth rate of height (RGR height) and diameter (RGR diameter), and aboveground biomass. P-values less than or equal to .05 indicate significant differences ($\alpha = 0.05$).

Fixed effects	Source of variation	Survival (%)			RGR height(mm/day)			RGR diameter (mm/day)			Biomass (g)		
		d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Slash pine	Drought (D)	1, 27	8.89	0.0060	1, 25	3.91	0.0593	1, 25	4.11	0.0535	1, 10	1.64	0.2287
	Invasion (I)	1, 27	17.43	0.0003	1, 25	0.78	0.3857	1, 25	6.53	0.0171	1, 10	0.80	0.3907
	D × I	1, 27	4.78	0.0376	1, 25	0.66	0.6896	1, 25	0.51	0.4828	1, 10	1.02	0.3360
Loblolly pine	Drought (D)	1, 27	11.87	0.0019	1, 84	17.5	< 0.0001	1, 84	12.4	0.0007	1, 25	14.26	0.0009
	Invasion (I)	1, 27	5.05	0.0330	1, 84	3.04	0.0849	1, 84	11.3	0.0012	1, 25	5.89	0.0228
	D × I	1, 27	2.72	0.1110	1, 84	0.01	0.9407	1, 84	1.0	0.3199	1, 25	0.19	0.6627

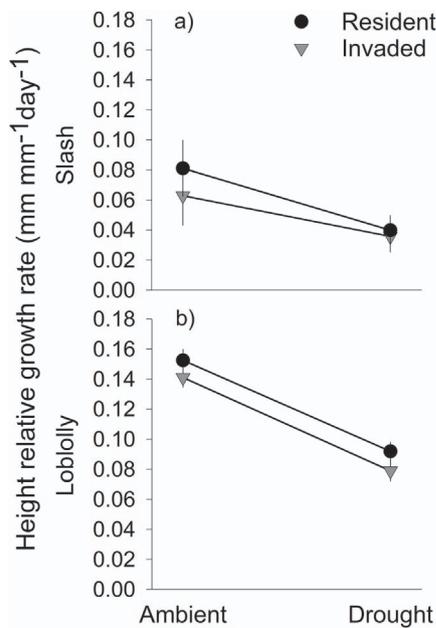


Fig. 4. Mean \pm SE of relative growth rates of height of slash (a) and loblolly (b) pine seedlings exposed to drought and invasion treatments.

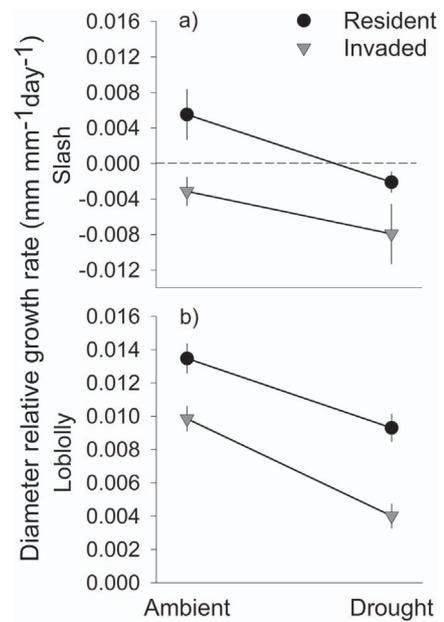


Fig. 5. Mean \pm SE of relative growth rates of diameter of slash (a) and loblolly (b) pine seedlings under drought and invasion treatments.

resident vegetation (Fig. 6a). Despite the tendency for the invader to offset the drought effect, the drought by invasion interaction was not statistically significant, possibly due to low power associated with low slash pine seedling numbers at harvest. In contrast to the slash pine results, both drought and invasion significantly affected loblolly pine biomass (Fig. 6b; Table 2). Average loblolly seedling biomass was only half as much under drought (8.6 ± 2.1 g) compared to ambient (16.3 ± 2.4 g) conditions (Table 2). Separately, invasion resulted in 36% less loblolly biomass (9.7 ± 2.1 g) compared to seedling performance in resident species plots (15.2 ± 2.4 g). Thus, there was an additive negative effect of drought and invasion on loblolly seedlings,

resulting in 70% lower biomass under the combined treatments relative to baseline ambient/resident conditions (Fig. 6b).

4. Discussion

Climate change and plant invasions are two predominant drivers of global environmental change, yet it is difficult to predict how these abiotic and biotic stressors will affect native species. Here we demonstrate that experimental drought and invasion by an aggressive non-native grass, both individually and in concert, significantly suppressed slash and loblolly pine seedlings. In general, loblolly pine outperformed

Table 3

Mean \pm SE of final height, diameter, biomass, and survival of slash and loblolly pine seedlings under drought and invasion treatments.

Species	Treatment		Height (mm)		Diameter (mm)		Biomass (g)		Survival	
	Drought	Invaded	mean	SE	mean	SE	mean	SE	mean	SE
Slash pine	Ambient	Resident	32.9	4.57	7.4	0.70	11.6	2.80	52.5	8.98
	Ambient	Invaded	31.0	5.07	5.6	0.28	5.4	0.98	15.0	5.24
	Drought	Resident	22.1	2.22	5.9	0.40	4.6	0.72	20.0	4.74
	Drought	Invaded	17.7	2.28	6.9	0.07	5.3	1.01	7.5	5.06
Loblolly pine	Ambient	Resident	69.3	3.80	7.8	0.55	18.9	2.93	92.5	3.62
	Ambient	Invaded	66.7	3.08	6.7	0.43	13.7	1.85	57.5	9.39
	Drought	Resident	54.2	6.22	6.2	0.59	11.4	2.49	50.0	7.91
	Drought	Invaded	47.9	3.72	4.6	0.32	5.7	1.12	45.0	8.51

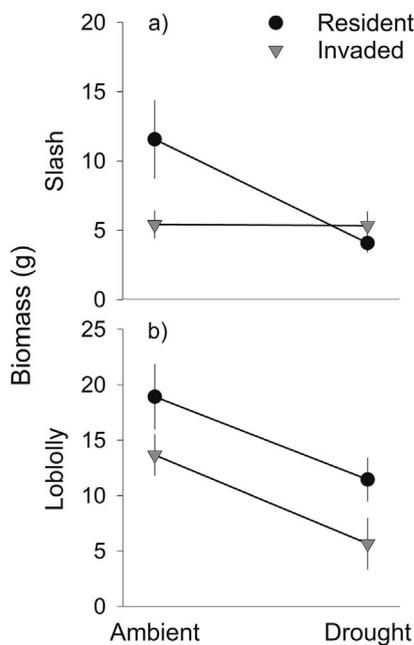


Fig. 6. Mean \pm SE biomass of slash (a) and loblolly (b) pine seedlings grown under drought and invasion treatments.

slash pine over the course of the experiment, consistent with the findings of Shiver et al. (2000) who showed that loblolly had higher survival and growth than slash across several sites in Georgia and northern Florida. However, we found that despite higher performance by loblolly overall, the magnitudes of both species' responses to the treatments were similar. For both species, each stressor alone inhibited seedling survival, while the combination of drought and invasion resulted in 86% and 51% fewer surviving slash and loblolly seedlings, respectively, when compared with pine survival under ambient conditions with resident species. In addition, seedling performance metrics tied closely to juvenile and adult tree performance (McGrath and Duryea, 1994), including RGR of stem diameter in both species and first-year biomass in loblolly, exhibited additive negative responses under both stressors that led to critical reductions in growth relative to baseline conditions. We found that interactive effects, where the two stressors in combination have greater (synergistic) or lesser (offsetting) effects than expected, were uncommon, suggesting that the effect of each stressor acting in isolation is to some degree predictive of their effect in the presence of the other stressor. Overall, our results indicate that the combination of abiotic stress from drought and biotic stress from a plant invader can severely affect slash and loblolly pine, two of the most ecologically and economically important forest species in the southeastern US.

The majority of experiments that have investigated how low soil moisture affect slash and loblolly pine have demonstrated strong detrimental effects on tree performance. Our findings not only support but greatly expand the inference space of these previous studies, which were largely conducted from a timber production or maximum-yield perspective (Bongarten and Teskey, 1987; Clark and Saucier, 1989, 1991; VanderSchaaf and South, 2003), were implemented in field settings where site location was considered a proxy for drought (Shoulders, 1977), or were conducted in a less realistic greenhouse setting where low soil moisture was imposed by limiting irrigation (Bongarten and Teskey, 1987). In contrast, we experimentally isolated the effect of drought on pines in an ecologically relevant setting using diverse plant communities growing and competing under field conditions. In this novel experimental context, we found that for both pine species, drought but not invasion, drove a reduction in seedling height growth, possibly because seedlings growing under lower light conditions in the dense invader canopy were cued to prioritize growth in height. In contrast, the relative growth rates in stem diameter of both

pinus were strongly inhibited by both drought and invasion, which could have important implications for stand dynamics given that this trait is strongly linked to water-stress acclimation and pine tree survival (McGrath and Duryea, 1994). Biomass was the only performance metric for which the species responded in a different way: slash pine biomass tended (although not statistically significant) to be lower due to the drought treatment only when growing with resident species, while loblolly pine biomass was reduced by drought regardless of the invader.

Typically, invasive grasses inhibit tree seedling establishment, growth, and survival due to lower light, soil moisture, or soil nutrient conditions (D'Antonio and Vitousek, 1992; Flory and Clay, 2010). Despite the apparent effects of cogongrass on native species and ecosystem functions, relatively few other studies have quantified impacts of invasion on native plant communities (Brewer, 2008; Daneshgar and Jose, 2009). We show here that invasion by cogongrass occurs rapidly in terms of increased cover over a growing season, and that invader stands can greatly reduce light availability. Surprisingly, however, there was some indication that cogongrass maintained higher levels of soil moisture in the drought treatment, suggesting its potential to offset drought stress to pines. While there was evidence of an offsetting effect of the two treatments on slash pine survival and biomass, cogongrass generally limited other resources (e.g., light as we have shown, or possibly nutrient availability) such that pine seedlings did not benefit from the slightly higher soil moisture observed in invaded plots. These findings of cogongrass' strong competitive ability mirror those of Daneshgar et al. (2008), who conducted an observational study in plots with cogongrass, native species, or no vegetation and measured survival, height, root collar diameter, and biomass of planted loblolly pine seedlings. They found that cogongrass inhibited seedling survival and suppressed seedlings for all growth responses compared to native vegetation or no vegetation treatments. Our expanded comparison with two species under experimental conditions shows that for survival, invasion more strongly inhibited slash than loblolly pine, while for biomass, it more strongly affected loblolly than slash. Regardless, it is clear that cogongrass invasions have significant implications for slash and loblolly pine seedling establishment and performance.

While some studies have evaluated how drought and competition individually affect pine seedling performance, little is known about the combined effects of these stressors. We are aware of only one study that has tested the combination of plant competition and relatively lower soil moisture on slash and loblolly pine seedlings. Stransky and Wilson (1966) planted seedlings into plots with and without turfgrass competition and after four months, erected rainout shelters to simulate drought. They found little effect of lower soil moisture without competition but the combination of reduced rainfall and competition with turfgrass resulted in 80% lower slash and loblolly seedling survival. In contrast to our results, where we found slightly higher soil moisture in invaded plots, Stransky and Wilson (1966) reported lower soil moisture in plots with plant competition. However, they compared bare ground to plots with plant competition whereas our comparison was between resident species only and invaded plant communities. Regardless, the difference in results between our study and Stransky and Wilson (1966) indicates plant responses to multiple stressors may be context and system specific. More recently, Dávalos et al. (2014) evaluated the effects of multiple stressors, including non-native plant invasion, on the survival and growth of four rare plant species in the US, and concluded that interactions among stressors were present yet unpredictable and require multifactor approaches to elucidate. Given the predicted increased prevalence of drought and other climate change factors, and the spread of plant invaders (Van Kleunen et al., 2015), natural and managed ecosystems are increasingly likely to be subjected to multiple stressors operating outside of historic norms in terms of timing or severity.

In this experiment we evaluated South Florida slash pine (*Pinus elliottii* var. *densa*), which is less widely distributed and less often planted than *Pinus elliottii* var. *elliottii*. The ranges of the two varieties extend

over mostly separate geographic regions, although they co-occur in Central Florida. More importantly, they have distinct life histories where var. *densa* has a 'grass' seedling stage and var. *elliottii* does not. Thus, although the responses to drought and invasion we observed are congruent with previous findings for other varieties, we urge caution in extrapolating our results for var. *densa* to var. *elliottii*, or to other coastal plain pine species or varieties. In addition, we focused on first-year seedling performance, which is known to be particularly influential for the long-term growth patterns of slash and loblolly pine trees (Bongarten and Teskey, 1987; Clark and Saucier, 1989, 1991; Stransky and Wilson, 1966), but studies that focus on earlier (seed) and later (juvenile and adult) pine life history stages are needed. Furthermore, in our experiment there was 48% less soil moisture in drought-treated versus to ambient plots, which we consider to be a moderate drought compared to historical drought in the region. However, "drought" has a wide range of definitions depending on temporal and spatial scales, soil type, and plant communities, as well as human perspectives, needs, and approaches (Florida Climate Center, 2017). Finally, studies that evaluate pine responses to multiple stressors across variable field sites would provide more robust measures to predict the outcome of drought and invasion effects on forest stand dynamics. Despite these important caveats, we found some generality in how loblolly and South Florida slash pine respond to abiotic and biotic stressors.

Our drought by invasion factorial experiment is the first to demonstrate both the independent and combined effects of multiple stressors on slash and loblolly pine seedling survival and performance. The effect of drought on seedlings of both species was significant, suggesting that land managers should carefully select field sites for plantations, and may benefit from considering different pine varieties or those with improved drought tolerance in the face of climate change. In addition, our results demonstrate experimentally the dramatic effects of cogongrass invasion on pine seedlings, which should further motivate land owners and property managers to remove this noxious invasive species. Additional work is needed to determine the longer-term effects of drought and invasions on pine forests, but clearly both of these stressors, and in particular their combination, may have profound consequences for southeastern US pine forests.

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