RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Seed depredation negates the benefits of midstory hardwood removal on longleaf pine seedling establishment

John L. Willis[1,2], David K. Schnake[3], Branson Wetzstein[1], Justin Yow[1], Daniel Guinto[4], Stacy Ulrich[4], Christopher S. DePerno[4], Marcus A. Lashley[5]

Midstory hardwoods are traditionally removed to restore longleaf pine on fire-excluded savannas. However, recent evidence demonstrating midstory hardwood facilitation on longleaf pine seedling survival has brought this practice into question on xeric sites. Also, midstory hardwoods could facilitate longleaf pine seedling establishment, as hardwood litter may conceal seeds from seed predators or improve micro-environmental conditions for seedling establishment. However, little is known about these potential mechanisms. In this study, we tracked longleaf pine seed depredation and germination in artificially seeded plots (11 seeds/m^2) in a factorial design fully crossing hardwood retention or removal with vertebrate seed predator access or exclusion in the Sandhills Ecoregion of North Carolina, U.S.A. Seed depredation averaged 78% across treatments and was greatest in unexcluded plots. Hardwood retention did not affect seed depredation. Longleaf pine averaged 3.6 germinants/4 m^2 across treatments, and was six times more abundant where vertebrates had been excluded. Hardwood removal had a strong positive effect on seedling germination, likely due to the removal of litter, but only when vertebrates were excluded. Our results indicated midstory hardwoods are not facilitating longleaf pine seedling establishment. Nevertheless, our results indicated that hardwood removal may not increase longleaf pine seedling establishment, as seed depredation diminished the effectiveness of hardwood removal under mast seed availability. Collectively, these results demonstrate the underlying complexity of the longleaf pine ecosystem, and suggest that planting may need to be part of the restoration strategy on sites where seed depredation limits longleaf pine natural regeneration.

Key words: facilitation, germination, hardwoods, *Pinus palustris*, savanna restoration, seed predators

Implications for Practice

- Retaining midstory hardwoods will not improve longleaf pine seedling establishment.
- Restoration prescriptions that remove midstory hardwoods to improve longleaf pine seedling establishment on xeric sites may be offset by intense seed depredation during mast seed years.
- Planting longleaf pine seedlings may be required in stands with intense seed depredation.

Introduction

Restoring ecosystem function is often a goal in forest management (Stanturf et al. 2014). In the fire-dependent pine savannas of the southeastern United States, the occurrence of frequent, low-intensity fire is important for maintaining the open forest structure and biodiversity (Van Lear et al. 2005). Longleaf pine (*Pinus palustris* Mill.) is considered a foundational tree species for maintaining understory flammability, as its long, resinous needles and cones readily ignite and produce higher temperatures when ignited compared to several sympatric oak species (Fonda & Varner 2004; Mitchell et al. 2006; Dell et al. 2017). The open crown structure of longleaf pine also contributes to understory ignitability, as it allows for greater penetration of infrared radiation to the forest floor compared to other pine species (Sharma et al. 2012). In addition to its contribution to understory flammability, longleaf pine serves as the primary cavity tree for the endangered Red-cockaded Woodpecker (*Picoides borealis* Vieillot; Rudolph & Conner 1991). Consequently, regenerating longleaf pine is an important step in restoring the functional capacity of pine savannas.

Author contributions: JLW, DKS, BW, JY, DG, SU, CSD designed the research, analyzed the data, conducted the research, and wrote the manuscript; MAL designed the research and wrote the manuscript.

[1] Department of Forestry, Mississippi State University, 361 Thompson Hall, 775 Stone Blvd, Mississippi State, MS 39762, U.S.A.
[2] Address correspondence to J. L. Willis, email jw2905@msstate.edu
[3] Research Stations Division, North Carolina Department of Agriculture and Consumer Services, 2 West Edenton Street, Raleigh, NC 27606, U.S.A.
[4] Department of Forestry and Environmental Resources, Fisheries, Wildlife, and Conservation Biology, North Carolina State University, 110 Brooks Avenue, Raleigh, NC 27695, U.S.A.
[5] Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Thompson Hall, 775 Stone Blvd, Mississippi State, MS 39762, U.S.A.

doi: 10.1111/rec.12951
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.12951/suppinfo

Naturally regenerating longleaf pine is a complex process. Seed production requires almost 3 years and varies spatially and temporally in response to temperature and moisture (Guo et al. 2016; Chen et al. 2018). Seed availability often limits regeneration, as good seed years (2,500 cones/ha) occur once every 5–7 years (Wahlenberg 1946). Exceptionally large cone crops occur on 8–10-year intervals (Maki 1952). Longleaf pine has the largest seed among the southern pines, which is dispersed in the fall with the majority of seed falling near (20 m) the parent tree (Croker & Boyer 1975). Germination can occur within as little as one week provided optimal climatic conditions. Seedling establishment is maximized on mineral soil substrate, where germinants can easily access soil moisture (Boyer 1990). Once established, seedlings can remain in the grass stage, a morphological adaptation to fire where the only aboveground structures are long, grass-like needles, for 2–20 years before initiating height growth (Wahlenberg 1946; Croker & Boyer 1975; Brockway et al. 2006). Grass stage duration is influenced by competition and occurrence of fire, as longleaf pine is intolerant of shade and can quickly become overgrown without recurring fire.

Creating favorable conditions for longleaf pine regeneration may involve a series of silvicultural manipulations on fire-suppressed sites. Light availability is increased by reducing overstory density through a variety of harvesting techniques (Boyer & Farrar 1981; Mitchell et al. 2006; Brockway & Outcalt 2017). Additional mechanical, chemical, or prescribed fire treatments are often used to eliminate midstory hardwoods to restore the historical savanna structure and create a more flammable understory for the reintroduction of fire (Provencher et al. 2001; Kush et al. 2004; Jose et al. 2010). Prescribed fire is then applied prior to a mast seed year to expose mineral soil substrate for seedling establishment (Croker & Boyer 1975). Although this silvicultural prescription accounts for growth strategy and resource requirements of longleaf pine, regeneration responses to silvicultural manipulation vary across ecoregions (Brockway & Outcalt 2017), suggesting the traditional approach to regenerating longleaf pine may not be appropriate for all sites.

Current regeneration approaches assume established vegetation inhibits the development of longleaf pine and seek to reduce overstory density and midstory hardwoods prior to longleaf pine seedling establishment (Menges & Gordon 2010). However, a growing body of evidence indicates that established vegetation may facilitate longleaf pine seedling survival on xeric sites, suggesting that current restoration efforts may inadvertently inhibit longleaf pine regeneration by removing midstory hardwoods (Wahlenberg 1946; Hiers et al. 2014; Loudermilk et al. 2016). Nevertheless, if midstory hardwoods are indeed facilitating longleaf pine regeneration, little is known about the mechanisms of facilitation, at what life history stage facilitation is occurring, and whether facilitation occurs across a gradient of overstory density.

Facilitation of longleaf pine regeneration by the hardwood midstory may occur through a variety of mechanisms acting on the early stages of seedling establishment. One potential mechanism is that hardwoods reduce seed predation pressure. Due to their relatively large size and carbohydrate content, longleaf pine seeds are often depredated by birds, small mammals, and insects (Boyer 1964; Croker & Boyer 1975; Gómez 2004). Maintaining a hardwood midstory may reduce predation intensity on longleaf pine seed by providing hardwood seed as an alternative food source for seed predators (Howe 1986; Butler et al. 2007). Also, midstory hardwoods may lengthen seed longevity on the forest floor by concealing seeds from seed predators through a combination of crown area and leaf litter. In addition, midstory hardwoods may increase longleaf pine germination by mitigating the risk of seed desiccation, which may be particularly influential on xeric sites with low overstory density.

Alternatively, some of the facilitative effects of maintaining a hardwood midstory for longleaf pine seedling establishment may be offset by a suite of regeneration inhibiting mechanisms. For example, maintaining a hardwood midstory could increase seed predator density, as southeastern fox squirrels (*Sciurus niger niger*) have been shown to select areas with greater oak (*Quercus* spp.) density (Prince et al. 2014). Predation pressure may also be increased by the presence of hardwood saplings, as vegetative cover has generally been shown to increase predation pressure (Royo & Carson 2008; Mattos & Orrock 2010). Seeds escaping depredation beneath a hardwood midstory may encounter a substrate limitation to seedling establishment where overstory density is high, as the combination of pine and hardwood litter may form an impenetrable barrier between the radicle of a new germinant and mineral soil. The combination of a hardwood midstory and a dense overstory may negatively impact the vigor of other understory species such as wiregrass (*Aristida stricta* Michx.; McGuire et al. 2001; Mulligan & Kirkman 2002), which have the potential to facilitate longleaf pine germination by concealing seeds, providing shade, or by providing germination space resulting from an overdispersed spatial patterning (Hovanes et al. 2018). Thus, maintaining a hardwood midstory could create a complicated set of regeneration trade-offs involving seed predators, microclimate, and substrate constraints that may vary across a gradient of overstory density and soils. The relative strength of these opposing effects could determine whether the overall impact of the hardwood midstory facilitates or inhibits longleaf pine seedling establishment spatially within and across stands.

To explore these potential relationships, we established a stand-scale, manipulative experiment in the Sandhills Ecoregion of North Carolina. We tracked depredation and germination of longleaf pine seeds in a factorial experiment where plots were uncaged or caged to exclude vertebrate seed predators (birds and small mammals), untreated or treated with herbicides to control midstory hardwoods, and located across a gradient of overstory basal area of all species. Specifically, we were interested in whether (1) seed depredation increased or decreased in the presence of midstory hardwoods; (2) germination increased with the exclusion of vertebrate seed predators; (3) the effect of midstory hardwoods/seed predators interacted with overstory density; and (4) whether the presence of certain substrate and plant cover types influenced longleaf seed depredation and germination. Our results will contribute to our understanding of the role midstory hardwoods play in pine savannas, and whether restoration treatments that eliminate the midstory are unintentionally impeding longleaf pine seedling establishment on xeric sites.

Methods

Site Characteristics

Our experiment was conducted on a mature longleaf pine stand in the Sandhills Ecoregion of North Carolina (35°3′ 34.6932″N, 79°22′ 22.0872″W). The average high temperature for the region ranged from 11.7°C in January to 32.4°C in July (Fayetteville, NC; NCEI, NOAA). Precipitation in the region fell mostly in the form of rain and averages 1,084 mm annually (Charlotte, NC; NCEI, NOAA). The soils at the site were variable, but all experimental blocks were established on Ultisols soils in the Candor soil series. Candor soils are sandy throughout the entire profile, but form a clay-to-loam bottom at the deepest depths (1.5–2.0 m).

The forest stands included in this study naturally initiated in the 1920s following clearcut harvesting. Since the early 1990s, these stands have been managed toward a savanna structure through mechanical removal of overstory hardwoods and the application of dormant season prescribed fire on a 3-year interval. Vegetation at the site was dominated by longleaf pine, which varied in basal area from 2 to 41 m^2/ha. Hardwood density in the midstory averages 6,228 stems/ha and was dominated by turkey oak (*Quercus laevis* Walter; 0.823) with minor components of black gum (*Nyssa sylvatica* Marsh.; 0.091) and sassafras (*Sassafras albidum* [Nutt.] Nees; 0.066). Midstory height ranged from 0.1 to 6.7 m, and averaged 1.2 m. Understory percent cover was dominated by wiregrass and a diversity of forbs.

Experimental Design

Our experiment was conducted as a randomized complete block. In the spring of 2017, we located 13 blocks (0.10 ha) across a gradient of basal area (6–25 m^2 all species considered). Within each block, we established eight measurement plots (2 × 2 m) similar in size to those used in Royo and Carson (2008). We randomly assigned each plot one of four treatments: control, midstory hardwood removal, vertebrate seed predator exclusion with midstory hardwood retention, and vertebrate seed predator exclusion with hardwood removal. Each treatment was replicated twice within each block ($N = 26$; Fig. 1).

We removed existing hardwoods through a chemical cut stump treatment with the goal of eliminating the midstory (Brushtox and methylated seed oil [61.6% Triclopyr] Ragan and Massey, Inc., Gig Harbor, WA, U.S.A.). In addition to chemically treating the stems, we raked all litter by hand from the hardwood control plots. Litter removal was added as an additional measure to remove physical legacy of the hardwood midstory and to simulate forest floor conditions following a prescribed burn. To reduce edge effects associated with hardwood removal, we surrounded each measurement plot on all sides by buffer plots (4.57 m).

Vertebrate seed predators (small mammals and birds) were controlled for by constructing exclosures (2 × 2 m). Each exclosure measured 4 m^2 and was constructed with hardware cloth (1.27 cm mesh size), similar to the material used by Royo and Carson (2008). To discourage burrowing, we fit flashing around the base of each exclosure and buried the flashing to a depth of 25 cm. In recognition of variation associated with fencing, we fenced plots not selected for seed predator exclusion with a single strand of twine, permitting seed predator access.

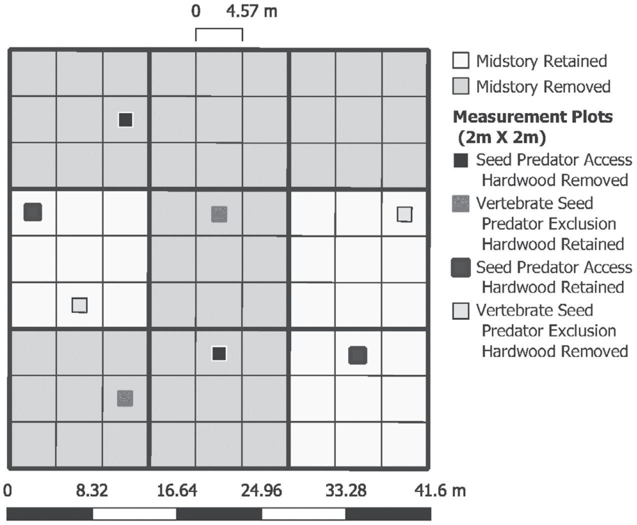

Figure 1. Example layout of an experimental block (0.10 ha). Each block consisted of nine sections (188 m^2), with each section containing nine plots (21 m^2). Each section was randomly selected for midstory hardwood removal (gray) or retention (white). Measurement plots (smaller squares; 4 m^2) were established within plots and randomly assigned one of four treatments: seed predator access with hardwood removal (solid black square with a white border); vertebrate seed predator exclusion with hardwood retention (moderately dark gray square); seed predator access (dark gray square), or vertebrate seed predator exclusion and hardwood removal (light gray square).

Each plot was artificially seeded with 11 longleaf pine seeds/1 m^2, which is approximately four times the recommended minimum rate for artificial seeding (3 seeds/1 m^2; Brockway et al. 2006), in late October, 2017. The seed originated from the North Carolina Forest Service's longleaf pine seedling orchard in Sampson County, NC, and included families from central and southeastern North Carolina as well as northeastern South Carolina. We chose this seed rate to increase the probability of attaining a germination response and to approximate seed rain conditions during a mast seed year (12 seeds/1 m^2; Boyer 1990). Further, artificial seeding was chosen because cone production at our site was low (<5 cones per tree; personal observations), and to account for potential differences in seed rain among blocks. Prior to seeding, we removed all established longleaf pine seedlings from the plots to prevent confusion in future germination surveys.

Field Measurements

To determine small mammal abundance, we placed 5 Sherman traps (H.B. Sherman Traps, Tallahassee, Florida, U.S.A.) diagonally across each of the 13 blocks with 10-m spacing between traps. Traps were baited with a mixture of oats and peanut butter and a cotton ball was placed in each trap for bedding material. All 13 trapping transects were sampled in October 2017 for five consecutive nights. Additionally, to determine a baseline estimate of seed predator impacts on longleaf pine seed availability independent of our treatments, we set three-seed plots diagonally across each of the 13 blocks with 20-m spacing between plots. Each seed plot contained 20 longleaf pine seeds. We considered a seed predated if it was missing, just the seed coat remained, or if it was being actively consumed. All 39 seed plots were sampled in October 2017 for five consecutive 24-hour periods.

We conducted all measurements of overstory basal area, vegetation cover, and substrate availability at the plot level. Prior to seeding, overstory basal area of all tree species was assessed from plot center with a prism (10 basal area factor). Existing conditions at the forest floor plot were assessed through ocular estimations of pine litter, hardwood litter, mineral soil grass, forbs, and hardwood stems cover to the nearest 5%. Estimates of substrate and vegetation were then summed to equal 100%, as we were interested only in the characteristics of the seedbed in the plots. We estimated the percentage of the plot shaded by the hardwood midstory to the nearest 5%. Seed germination was assessed monthly in all plots from November 2017–March 2018. Seeds were considered germinated if the cotyledons emerged from the seed coat. All seedlings were removed from the plots once recorded. During the final germination census, we measured seed depredation in a subset of randomly selected plots (40 plots) encompassing the entire range of treatments and overstory basal area. Seed depredation was measured on the first five longleaf pine seeds encountered in each plot. Seeds were considered predated if the seed coat was empty and showed evidence of seed predation (holes in the seed coat or ripping).

Statistical Methods

Objectives One to Three. Our primary objective was to evaluate the influence of vertebrate seed predators, midstory hardwoods, and overstory density on early natural regeneration dynamics of longleaf pine. We used generalized linear models to assess the main effects of treatment, overstory density, block, and the interaction between overstory density and treatment on seed depredation and germination. Higher-order interactions involving block were not explored, as block was not a factor of interest in this study. To account for zero-inflated count data, we selected a binary logistic distribution with logit link function for the model examining seed depredation and a Poisson distribution with a logarithmic link function for the model examining seed germination. We executed model selection through backwards selection. Model runs producing an interaction term exceeding the suggested threshold ($p > 0.25$; Bancroft 1964) were pooled and the model rerun with only main effects. Once a final model was determined, we examined the final model for overdispersion, model fit and the existence of outliers. A dispersion factor was calculated to assess the degree of overdispersion within the model ($\hat{c}$ = chi-square/degree of freedom). The final model for each response variable exceeded one and thus was considered overdispersed. As such, we used a *quasi-likelihood* approach, which inflated the standard errors of each factor by $\sqrt{c}$. We re-ran the adjusted final model and checked for significance. Model fit and potential outlier detection was accomplished by examining studentized deviance residual plots. Model fit was evaluated by visually inspecting how evenly the residuals were distributed around zero. Points on the plot exceeding 2 SDs of the mean were identified as potential outliers. The influence of such points was evaluated with Cook's distance statistic. Distance statistics exceeding one were identified as having potentially strong leverage on model fit. To evaluate leverage strength, we removed the potential outlier and re-ran the model. If all parameters in the model remained significant, we retained the potential outlier as part of the dataset. However, if changes in parameter significance occurred without the outlier, we removed the data point (Ramsey & Schafer 1997). Each factor or interaction in the final model was considered significant at $\alpha = 0.05$. We further explored significant main effects of treatment through post-hoc pairwise least significant difference tests. In addition to our analysis of seed depredation and germination, we tested for treatment effects on forest floor conditions including percent cover of wiregrass, herbaceous vegetation, oak litter, pine litter, and midstory hardwood canopy cover with analysis of variance. All tests were performed in SAS 9.4 (SAS Institute, Cary, NC, U.S.A.).

Objective Four. We assessed how the availability of different substrate and plant cover types influenced longleaf pine seed depredation and germination. We conducted a correlation analysis examining the relationship between percent cover of mineral soil, pine litter, oak litter, herbaceous vegetation, and wiregrass on the forest floor to observed rates of longleaf pine seed predation and germination within each plot. To remove the potentially confounding factor of excluding seed predators, we pooled plots together based on exclosure status. A lack of linearity between variables compelled us to use Spearman's correlation coefficients to determine the strength and direction of association of each relationship. We considered associations significant at $\alpha = 0.05$.

Results

Seed Depredation

We trapped for 311 trap nights with no small mammals captured. Of the 780 longleaf pine seeds placed to obtain a baseline estimate of seed predator impacts, 195 were consumed (0 on night 1, 10 on night 2, 23 on night 3, 36 on night 4, and 126 on night 5). Ants and millipedes were observed consuming seeds, and ant hills appeared near several of the seed plots. In all cases, ants and millipedes were believed to be the primary cause of seed depredation.

Longleaf pine seed depredation averaged 78% across all treatments. Seed depredation varied among treatments ($\chi^2 = 11.8$, $p = 0.008$; Table S1, Supporting Information). The highest rates of depredation occurred in plots with vertebrate predator access that lacked midstory hardwoods and litter, which differed from vertebrate predator-excluded plots with

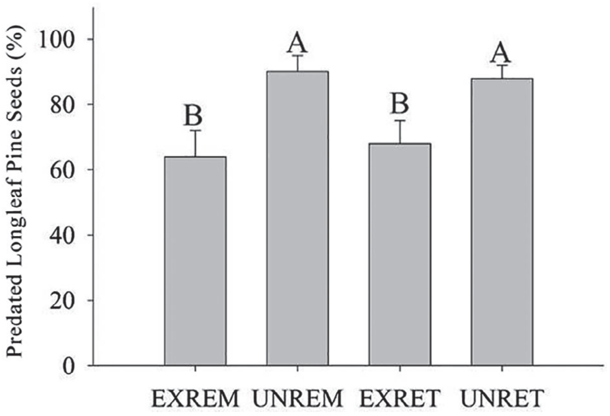

Figure 2. Average seed depredation percentage (+1 SE) treated for seed predator (birds and small mammals) exclusion and midstory hardwood removal (EXREM), midstory hardwood removal (UNREM), seed predator exclusion and midstory hardwood retention (EXRET), and control (UNRET) in the Sandhills Ecoregion, North Carolina, 2017–2018. Treatments with different letters were significantly different (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$).

($p = 0.021$) or without hardwood retention ($p = 0.005$; Fig. 2). Similarly, plots with vertebrate predator access and hardwood retention differed from vertebrate predator-excluded plots with ($p = 0.028$) or without hardwood retention ($p = 0.007$; Fig. 2). Hardwood retention did not significantly influence seed depredation within excluded plots ($p = 0.628$) or within plots with vertebrate predator access ($p = 0.834$), respectively (Fig. 1). Neither overstory basal area ($\chi^2 = 0.9, p = 0.349$; block $\chi^2 = 0.1, p = 0.879$), nor the interaction between overstory basal area and treatment ($\chi^2 = 5.1$, $p = 0.161$) influenced seed depredation (Table S1). No substrate or plant cover type was related to seed depredation in plots with or without vertebrate predator access (data not shown).

Germination

Longleaf pine seed germination averaged 3.6 germinants/4 m^2 across all factors. Seed germination was strongly influenced by treatment ($\chi^2 = 80.3$, $p < 0.001$; Table S2). The combined absence of midstory hardwoods and vertebrate predators significantly increased longleaf pine germination ($p < 0.001$), as germinants were at least four times more abundant in vertebrate predator-excluded plots lacking midstory hardwoods compared to any other treatment (Fig. 3). Also, germination was higher in vertebrate predator-excluded plots with hardwood retention compared to plots with predator access and midstory removal ($p = 0.006$; Fig. 3). Differences in overstory basal area did not influence germination ($\chi^2 = 1.3$, $p = 0.249$), nor did it interact with treatment ($\chi^2 = 2.5$, $p = 0.474$; Table S2). However, germination did vary among blocks ($\chi^2 = 7.6, p = 0.006$; Tables S2 & 1).

Treatment affected the abundance of wiregrass ($F = 4.756$, $p < 0.001$), oak litter ($F = 30.627$, $p < 0.001$), pine litter ($F = 35.053$, $p < 0.001$), and hardwood shading ($F = 60.970$, $p < 0.001$). On average, plots subjected to midstory hardwood removal had higher percent cover of wiregrass ($p = 0.021$) and lower percent cover of oak litter ($p < 0.001$), pine litter ($p < 0.001$), and midstory shading ($p < 0.001$; Table 2). Within vertebrate predator-excluded plots, longleaf pine germination was positively associated with the availability of mineral soil ($p < 0.001$, $\rho = 0.555$; Fig. 4). Although weaker, a significant positive relationship was detected between seed germination and wiregrass cover ($p = 0.038$, $\rho = 0.287$; Fig. 4). Conversely, moderately strong negative associations were detected between seed germination and percent cover of pine ($p < 0.001$, $\rho = -0.578$) and oak litter ($p < 0.001$, $\rho = -0.632$; Fig. 4). No significant relationships were detected between longleaf pine germination and any substrate type or vegetation cover in plots with vertebrate predator access (Fig. 4).

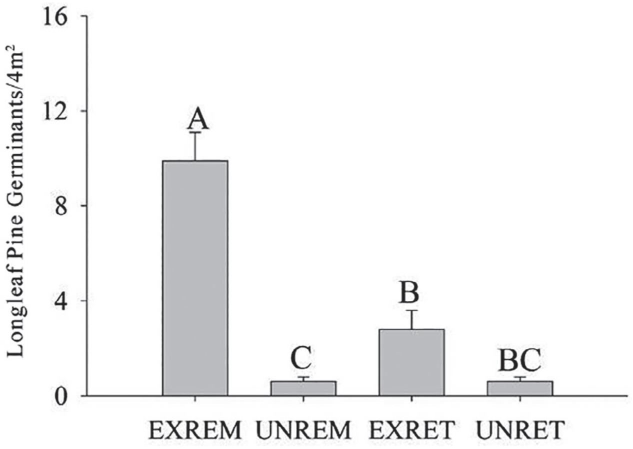

Figure 3. Average longleaf pine seed germination (+1 SE) in plots treated for seed predator exclusion (birds and small mammals) and midstory hardwood removal (EXREM), midstory hardwood removal (UNREM), seed predator exclusion and midstory hardwood retention (EXRET), and control (UNRET) in the Sandhills Ecoregion, North Carolina, 2017–2018. Treatments with different letters were significantly different (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$).

Table 1. Average, overstory basal area (±1 SE), and germinants per block in the Sandhills Ecoregion, North Carolina, 2017–2018.

Block	*Basal Area (m^2/ha)*	*Germinants*
3	9.9 ± 1.1	1.5 ± 0.7
4	5.3 ± 0.7	2.7 ± 1.3
5	6.4 ± 0.7	6.0 ± 2.4
6	6.7 ± 1.4	2.5 ± 1.1
7	10.1 ± 1.8	2.1 ± 1.7
8	24.3 ± 3.2	3.4 ± 1.7
9	12.2 ± 1.6	3.0 ± 2.1
10	20.4 ± 2.3	4.3 ± 2.2
11	15.8 ± 1.8	3.9 ± 2.1
12	18.1 ± 3.9	2.6 ± 1.2
13	17.0 ± 3.2	7.1 ± 2.6
14	13.8 ± 3.0	3.5 ± 2.3
15	16.1 ± 3.0	6.4 ± 3.0

Table 2. Average percent cover (±1 SE) of wiregrass, herbaceous vegetation, oak litter, pine litter, mineral soil, oak stems, and midstory canopy cover in plots treated by excluding seed predators and removing the hardwood midstory, excluding seed predators and retaining the hardwood midstory, removing the hardwood midstory, and control in the Sandhills Ecoregion, North Carolina, 2017–2018. Treatments with different letters were considered significantly different for each response variable (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$).

Treatment	*Wiregrass (%)*	*Herbaceous (%)*	*Oak Litter (%)*	*Pine Litter (%)*	*Mineral Soil (%)*	*Midstory Canopy Cover (%)*
Excluded removed	24 ± 3^{ab}	4 ± 1^{a}	0 ± 0^{b}	0 ± 0^{b}	70 ± 3^{a}	0 ± 0^{b}
Excluded retained	16 ± 3^{b}	2 ± 1^{b}	25 ± 4^{a}	45 ± 6^{a}	11 ± 3^{b}	35 ± 3^{a}
Predator access removed	31 ± 4^{a}	3 ± 1^{ab}	0 ± 0^{b}	0 ± 0^{b}	66 ± 5^{a}	0 ± 0^{b}
Predator access retained	17 ± 3^{b}	3 ± 1^{ab}	33 ± 5^{a}	40 ± 6^{a}	2 ± 2^{b}	55 ± 6^{a}

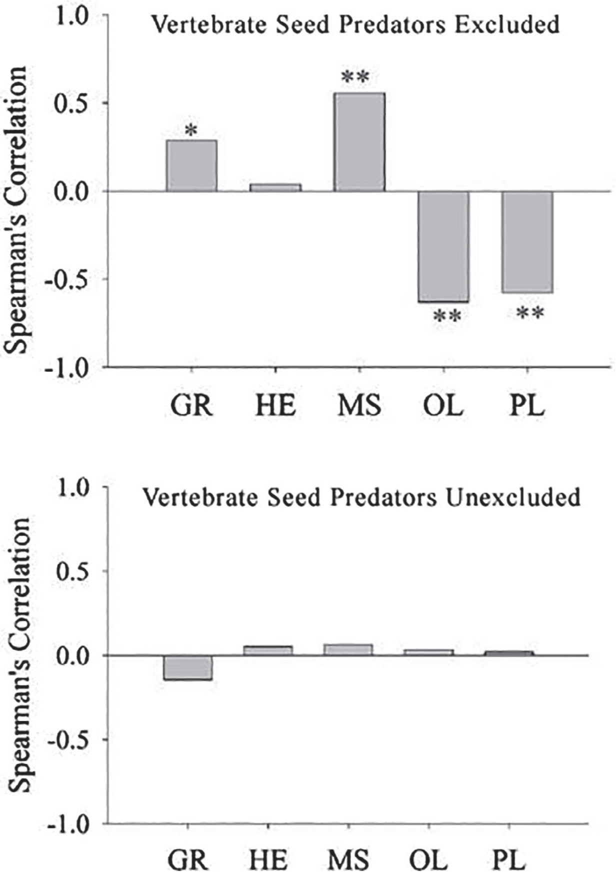

Figure 4. The strength and direction of association between longleaf seed germination and percent cover of wiregrass (GR), herbaceous vegetation (HE), mineral soil (MS), oak litter (OL), and pine litter (PL), in plots that seed predators (birds and small mammals) were excluded or not in the Sandhills Ecoregion, North Carolina, 2017–2018. Association strength and direction was assessed through Spearman's rank correlation coefficients. Bars with * represent significant relationships at $\alpha = 0.05$ and ** at $\alpha = 0.01$.

Discussion

Seed Depredation

It has long been known that longleaf pine seed provides an important food resource for insects and wildlife (Boyer 1964; Croker & Boyer 1975; Gómez 2004). However, the relative importance of seed depredation as an inhibiting factor to longleaf pine natural regeneration has not been studied as intensively as competition for resources (Pecot et al. 2007). Our results demonstrate that seed depredation had a strong negative influence on longleaf pine regeneration. Moreover, due to its early occurrence in the regeneration process, seed depredation has the potential to offset restoration treatments aimed at providing favorable conditions for longleaf pine seedling establishment.

The strong negative effect of seed depredation was surprising considering that each plot was seeded at approximately a mast rate (Boyer 1990). Mast seeding is a perennial plant strategy that is thought to be at least partially driven by seed predators, as irregular peaks in seed production maximize the odds of seed escape by overwhelming seed predator populations (Janzen 1971; Silvertown 1980). Our singular seeding rate prevented us from examining whether seed predator satiation had occurred; however, results of approximately 90% seed depredation across unexcluded plots exceeded values reported in previous studies of seed depredation in mast years (Xiao et al. 2005; Linhart et al. 2014; Soler et al. 2017), suggesting that seed predator satiation was not achieved in our study. We acknowledge, however, that mast seed availability was limited to our experimental plots, which may have contributed to the lack of seed predator satiation.

Another unexpected result was the intensive seed depredation that occurred in the excluded plots. The purpose of our experimental design was to exclude vertebrate seed predators, which we suspected would be the primary seed predator (Croker & Boyer 1975). No evidence was discovered that would lead us to believe that vertebrate seed predators breached the exclosures. Further, we did not capture any small mammals, a result that is consistent with Sasmal et al. (2017), who determined that small mammal abundance was extremely low in frequently burned longleaf pine communities. This result, combined with our observations of ants and millipedes consuming seeds, leads us to believe that invertebrate seed predators were the primary seed predator in excluded plots and likely contributed to seed depredation in all plots. If indeed invertebrates were the primary seed predator, the intensity of seed depredation is surprising considering that longleaf pine seeds lack an elaiosome, which has previously been shown to be important for invertebrate seed selection (Stuble et al. 2010; Cumberland & Kirkman

2013). Also, theory and empirical evidence suggest that invertebrate seed predators should become satiated during a mast year due to their seed specialization and relative immobility compared to vertebrate seed predators (Holling 1959; Holling 1965; Soler et al. 2017). Nevertheless, recent evidence demonstrates that some invertebrate seed predators are capable of overcoming satiation during mast years through rapid reproduction and aggregative responses to seed availability (Bogdziewicz et al. 2018), suggesting that strong invertebrate seed depredation is possible.

Seed Germination

The traditional paradigm of regenerating longleaf pine on fire-excluded sites considers competing vegetation and the organic layer as inhibiting factors to seedling establishment (Croker & Boyer 1975; Boyer 1990; Mitchell et al. 2006). However, evidence from xeric sites suggests that midstory hardwoods can facilitate longleaf pine regeneration (Wahlenberg 1946; Loudermilk et al. 2016). Our results indicate that midstory hardwood retention had no effect on longleaf pine seedling germination, as germinant density was equivalent with and without midstory retention in plots with seed predator access. Moreover, consistent with traditional restoration practices, germinant density was maximized in excluded plots where mineral soil was exposed and the midstory was removed.

One explanation for the lack of midstory hardwood facilitation was the litter layer composition of our site. Given the original moisture-holding capacity of hardwood litter (Kreye et al. 2013; Kreye et al. 2018), we thought hardwood litter may be less inhibiting to longleaf pine seedling establishment than pine litter. Our results indicated that hardwood litter is just as inhibiting to longleaf pine germination as pine needles; which may have been driven by the fact that turkey oak was the dominant midstory species at our site. Compared to more mesophytic hardwood species, turkey oak litter has been shown to absorb less moisture and dry faster (Kreye et al. 2013). Therefore, the microenvironment of the seedbed may not have been as conducive to seedling establishment as it may have been if the midstory was dominated by mesophytic species with greater litter moisture-holding capacity.

Another potential benefit to retaining the hardwood midstory was the shade produced on the forest floor. We cannot say for certain whether the shade created by hardwood retention positively or negatively impacted seedling germination, as our experimental design did not allow us to separate the effects of shade from substrate availability. However, it is clear from our results that any potential shading benefits were offset by the strong negative effects created by the litter layer. Thus, we believe that midstory hardwood facilitation is either not occurring at our site, or is acting on post-germination life history stage.

Implications for Longleaf Pine Restoration on Xeric Sites

The longleaf pine ecosystem is regarded as one of the most biodiverse, fire-maintained ecosystems in North America (Noss et al. 1995; Means 2006). Longleaf pine plays an important role in maintaining biodiversity through increasing understory flammability, helping to promote the frequent ignitions that maintain the open savanna structure (Fonda & Varner 2004; Dell et al. 2017). Thus, regenerating longleaf pine is critical to the overall maintenance of the longleaf pine ecosystem.

Silvicultural efforts to naturally establish longleaf pine seedlings on fire-excluded sites have traditionally applied prescribed fire to remove midstory hardwoods and expose mineral soil. Our results support this restoration strategy on sites where advance longleaf pine seedlings are lacking, as seedling germination was not improved by retaining midstory hardwoods regardless of vertebrate seed predator exclusion. Burning in the growing season prior to seed-fall may benefit wiregrass expansion through improving seed production (Outcalt 1994). Expanding wiregrass cover may, in turn, improve conditions for longleaf pine seedling establishment, as seed germination was positively associated with wiregrass in the absence of vertebrate seed predators. Once a new cohort of longleaf pine seedlings are established, it may be prudent to lengthen the burning rotation to allow the sprouting midstory to facilitate longleaf pine seedling survival (Wahlenberg 1946; Loudermilk et al. 2016).

Another important lesson from this study is that seed depredation can be a stronger barrier to longleaf pine seedling establishment than mineral soil availability and midstory hardwood presence under mast seed conditions. This result suggests that seed predators may override restoration treatments that would otherwise increase longleaf pine seedling establishment even in years when seed is not limiting. In stands where severe seed depredation is occurring, planting longleaf pine seedlings is likely the only viable management alternative for obtaining regeneration.

Acknowledgments

The authors would like to thank the undergraduate research programs at Mississippi State University and North Carolina State University (NCSU) for providing financial support for this project. Also, we thank the North Carolina Department of Agriculture Consumer Services Research Stations Division, and the Fisheries, Wildlife, and Conservation Biology Program at NCSU for their operational support of the project. Additionally, the authors thank A. Royo for sharing his experiences in conducting seed predator studies. Finally, thanks to Sasha for bringing this issue to light.

LITERATURE CITED

Bancroft TA (1964) Analysis and inference for incompletely specified models involving the use of preliminary test(s) of significance. Biometrics 20:427–442

Bogdziewicz M, Marino S, Bonal R, Zwolak R, Steele MA (2018) Rapid aggregative and reproductive responses of weevils to masting of North American oaks counteract predator satiation. Ecology 99:2575–2582

Boyer WD (1964) Longleaf seed losses to animals on burned seedbeds. Restoration Note SO-6. US Department of Agriculture Forest Service, Southern Forest Experiment Station, New Orleans, Louisiana

Boyer WD (1990) *Pinus palustris* Mill. Pages 405–412. In: Burns RM, Honkala BH (eds) Silvics of North America. Conifers. Vol 1. Forest Service, United States Department of Agriculture, Washington D.C.

Boyer WD, Farrar RM (1981) Thirty years of management on a small longleaf pine forest. Southern Journal of Applied Forestry 5:73–77

Brockway DG, Outcalt KW (2017) Influence of reproduction cutting methods on structure, growth and regeneration of longleaf pine forests in flatwoods and uplands. Forest Ecology and Management 389:249–259

Brockway DG, Outcalt KW, Boyer WD (2006) Longleaf pine regeneration ecology and methods. Pages 95–133. In: Shibu J, Jokela EJ, Miller DL (eds) The longleaf pine ecosystem: ecology, silviculture, and restoration. Springer, New York

Butler DW, Green RJ, Lamb D, McDonald WJF, Forster PI (2007) Biogeography of seed-dispersal syndromes, life-forms and seed sizes among woody rain-forest plants in Australia's subtropics. Journal of Biogeography 34:1736–1750

Chen X, Brockway DG, Guo Q (2018) Characterizing the dynamics of cone production for longleaf pine forests in the southeastern United States. Forest Ecology and Management 429:1–6

Croker TC, Boyer WD (1975) Regenerating longleaf pine naturally. Research Paper SO-105. US Department of Agriculture Forest Service, Southern Forest Experiment Station, New Orleans, Louisiana

Cumberland MS, Kirkman LK (2013) The effects of the red imported fire ant on seed fate in the longleaf pine ecosystem. Plant Ecology 214:717–724

Dell JE, Richards LA, O'Brien JJ, Loudermilk EL, Hudak AT, Pokswinski SM, Dyer LA (2017) Overstory-derived surface fuels mediate plant species diversity in frequently burned longleaf pine forests. Ecosphere 8:1–15

Fonda RW, Varner JM (2004) Burning characteristics of cones from eight pine species. Northwest Science 78:322–333

Gómez JM (2004) Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. Evolution 58:71–80

Guo Q, Zarnoch SJ, Chen X, Brockway DG (2016) Life cycle and masting of a recovering keystone indicator species under climate fluctuation. Ecosystem Health and Sustainability. 2:1–11

Hiers JK, Walters JR, Mitchell RJ, Varner JM, Conner LM, Blanc LA, Stowe J (2014) Ecological value of retaining pyrophytic oaks in longleaf pine ecosystems. The Journal of Wildlife Management 78:383–393

Holling CS (1959) The components of predation as revealed by a study of small mammal predation of the European pine sawfly. Canadian Entomologist 91:293–320

Holling CS (1965) The functional response of predators to prey density and its role in mimicry and population regulation. Memoirs of the Entomological Society of Canada 48:3–86

Hovanes KA, Harms KE, Gagnon PR, Myers JA, Elderd BD (2018) Overdispersed spatial patterning of dominant bunchgrasses in southeastern pine savannas. The American Naturalist 191:658–667

Howe HF (1986) Seed dispersal by fruit-eating birds and mammals. Pages 123–189. In: Murray D (ed) Seed dispersal. Academic Press, New York

Janzen DH (1971) Seed predation by animals. Annual Review of Ecology and Systematics 2:465–492

Jose S, Ranasinghe S, Ramsey CL (2010) Longleaf pine (*Pinus palustris* P. Mill.) restoration using herbicides: overstory and understory vegetation responses on a coastal plain flatwoods site in Florida, U.S.A. Restoration Ecology 18:244–251

Kreye JK, Varner JM, Hiers JK, Mola J (2013) Toward a mechanism for eastern North American forest mesophication: differential litter drying across 17 species. Ecological Applications 23:1976–1986

Kreye JK, Varner JM, Hamby GW, Kane JM (2018) Mesophytic litter dampens flammability in fire-excluded pyrophytic oak–hickory woodlands. Ecosphere 9:1–10

Kush JS, Meldahl RS, Avery C (2004) A restoration success: longleaf pine seedlings established in a fire-suppressed, old-growth stand. Ecological Restoration 22:6–10

Linhart YB, Moreira X, Snyder MA, Mooney KA (2014) Variability in seed cone production and functional response of seed predators to seed cone availability: support for the predator satiation hypothesis. Journal of Ecology 102:576–583

Loudermilk EL, Hiers JK, Pokswinski S, O'Brien JJ, Barnett A, Mitchell RJ (2016) The path back: oaks (*Quercus* spp.) facilitate longleaf pine (*Pinus palustris*) seedling establishment in xeric sites. Ecosphere 7:1–14

Maki TE (1952) Local longleaf seed years. Journal of Forestry 50:321–322

Mattos KJ, Orrock JL (2010) Behavioral consequences of plant invasion: an invasive plant alters rodent antipredator behavior. Behavioral Ecology 21:556–561

McGuire JP, Mitchell RJ, Moser EB, Pecot SD, Gjerstad DH, Hedman CW (2001) Gaps in a gappy forest: plant resources, longleaf pine regeneration, and understory response to tree removal in longleaf pine savannas. Canadian Journal of Forest Research 31:765–778

Means DB (2006) Vertebrate faunal diversity of longleaf pine ecosystems. Pages 157–213. In: Jose S, Jokela EJ, Miller DL (eds) The longleaf pine ecosystem: ecology, silviculture, and restoration. Springer, New York

Menges ES, Gordon DR (2010) Should mechanical treatments and herbicides be used as fire surrogates to manage Florida's uplands? A review. Florida Scientist 73:147–174

Mitchell RJ, Hiers JK, O'Brien JJ, Jack SB, Engstrom RT (2006) Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. Canadian Journal of Forest Research 36:2724–2736

Mulligan MK, Kirkman LK (2002) Competition effects on wiregrass (*Aristida beyrichana*) growth and survival. Plant Ecology 163:9–50

Noss RF, LaRoe ETIII, Scott JM (1995) Pages 1–58. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. Vol 28. U.S. Department of the Interior, National Biological Service, Washington D.C.

Outcalt KW (1994) Seed production of wiregrass in central Florida following growing-season prescribed burns. International Journal of Wildland Fire 4:123–125

Pecot SD, Mitchell RJ, Palik BJ, Moser EB, Hiers JK (2007) Competitive responses of seedlings and understory plants in longleaf pine woodlands: separating canopy influences above and below ground. Canadian Journal of Forest Research 37:634–648

Prince A, DePerno CS, Gardner B, Moorman CE (2014) Survival and home-range size of southeastern fox squirrels in North Carolina. Southeastern Naturalist 13:456–462

Provencher L, Herring BJ, Gordon DR, Rodgers HL, Galley KE, Tanner GW, Hardesty JL, Brennan LA (2001) Effects of hardwood reduction techniques on longleaf pine sandhill vegetation in northwest Florida. Restoration Ecology 9:13–27

Ramsey FL, Schafer DW (1997) The statistical sleuth: a course in methods of data analysis. Duxbury Press, Belmont, California

Royo AA, Carson WP (2008) Direct and indirect effects of a dense understory on tree seedling recruitment in temperate forests: habitat-mediated predation versus competition. Canadian Journal of Forest Research 38:1634–1645

Rudolph DC, Conner RN (1991) Cavity tree selection by red-cockaded woodpeckers in relation to tree age. The Wilson Bulletin 103:458–467

Sasmal I, DePerno CS, Swingen MB, Moorman CE (2017) Influence of vegetation type and prescribed fire on *Peromyscus* abundance in a longleaf pine ecosystem. Wildlife Society Bulletin 41:49–54

Sharma A, Jose S, Bohn KK, Andreu MG (2012) Effects of reproduction methods and overstory species composition on understory light availability in longleaf pine–slash pine ecosystems. Forest Ecology and Management 284:23–33

Silvertown JW (1980) The evolutionary ecology of mast seeding in trees. Biological Journal of the Linnean Society 14:235–250

Soler R, Espelta JM, Lencinas MV, Peri PL, Pastur GM (2017) Masting has different effects on seed predation by insects and birds in antarctic beech forests with no influence of forest management. Forest Ecology and Management 400:173–180

Stanturf JA, Palik BJ, Dumroese RK (2014) Contemporary forest restoration: a review emphasizing function. Forest Ecology and Management 331:292–323

Stuble KL, Kirkman LK, Carroll CR (2010) Are red imported fire ants facilitators of native seed dispersal? Biological Invasions 12:1661–1669

Van Lear DH, Carroll WD, Kapeluck PR, Johnson R (2005) History and restoration of the longleaf pine-grassland ecosystem: implications for species at risk. Forest Ecology and Management 211:150–165

Wahlenberg WG (1946) Longleaf pine: its use, ecology, regeneration, protection, growth, and management. Charles Lathrop Pack Forestry and Forest Service, Washington D.C.

Xiao Z, Zhang Z, Wang Y (2005) The effects of seed abundance on seed predation and dispersal by rodents in *Castanopsis fargesii* (Fagaceae). Plant Ecology 177:249–257

Coordinating Editor: Valter Amaral

Supporting Information

The following information may be found in the online version of this article:

Table S1. Results from a generalized linear model examining the effects of treatment, block, overstory density, and the interaction between treatment and overstory density on longleaf seed depredation in the Sandhills Ecoregion, North Carolina, 2017–2018.

Table S2. Results from a generalized linear model examining the effects of treatment, block, and overstory density on longleaf germination in the Sandhills Ecoregion, North Carolina, 2017–2018.

Received: 10 September, 2018; First decision: 13 November, 2018; Revised: 21 March, 2019; Accepted: 21 March, 2019; First published online: 16 April, 2019