

RESEARCH ARTICLE



Tree encroachment impacts on seed predator selection and seedling establishment in degraded pine woodlands

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Abstract

Questions: While much is known about the impact of tree encroachment on flammability in degraded pine woodlands, little is known about how encroachment is impacting other important ecosystem functions. We investigated how the availability of seed from four encroaching tree species and the presence of a midstorey and litter layer affect seed predator selection. Additionally, we investigated how seed predators, the midstorey, overstorey basal area, substrate availability, and vegetation cover affect germination for a foundational species (*Pinus palustris*) compared to an encroaching species (*Pinus taeda*). **Location:** Sandhills Ecoregion, NC, USA (35°3'34.6932" N, 79°22'22.0872" W).

Methods: We measured seed depredation of *Pinus palustris*, *Pinus taeda*, *Liquidambar styraciflua*, *Acer rubrum*, and *Quercus nigra* in cafeteria trials. Each trial was held within a 2 × 2 factorial involving vertebrate seed predator exclusion and midstorey and litter layer removal across a gradient of overstorey basal area (6–25 m²). Additionally, we measured *Pinus palustris* and *Pinus taeda* germination within each treatment and correlated germinant density to substrate and understorey vegetation cover.

Results: Granivory generally varied inversely with seed size, with small-seeded *Liquidambar styraciflua* experiencing the highest (27%) and large-seeded *Quercus nigra* (7%) and *Acer rubrum* (6%) the lowest depredation pressure. *Pinus palustris* and *Pinus taeda* germinant density was significantly highest where vertebrate seed predators were excluded and the midstorey and litter layer were removed. For both pine species, this result corresponded with a significant positive association with mineral soil and negative associations with hardwood and pine litter where vertebrate predators were excluded. Basal area did not affect granivory or germination for any species.

Conclusions: Our results demonstrate that granivores did not select *Pinus palustris*, and that large-seeded species encroachment was less inhibited by seed predators. *Pinus palustris* and *Pinus taeda* are depredated at comparable rates and germinate best under similar understorey conditions.

KEYWORDS

Aristida stricta, facilitation, germination, granivory, *Pinus palustris*, *Pinus taeda*, regeneration, seed diversity, substrate, woody encroachment

1 | INTRODUCTION

Frequent, low-intensity surface fire has sustained pine woodlands in the southeastern USA for centuries (Platt, 1999). The regular occurrence of fire helped maintain an open forest structure by limiting recruitment opportunities for trees and shrubs. Also, frequent burning influenced species composition, as tree species poorly adapted to survive fire were largely prevented from reaching reproductive maturity (Hoffmann, 2000; Gignoux et al., 2009). Thus, even though southeastern pine woodlands are considered among the most biodiverse ground layers in the world, the species and structural diversity of the tree community is often limited (Walker & Peet, 1984; Kirkman et al., 2001).

Pinus palustris is considered a foundational species in pine woodlands and is well adapted to surviving on sites with a frequent fire regime (Stambaugh et al., 2011). The regular occurrence of fire benefits *Pinus palustris* recruitment by exposing favorable mineral soil seedbeds. After establishment, *Pinus palustris* seedlings in the grass stage are generally resistant to fire (Wahlenberg, 1946; Knapp et al., 2018), as the apical bud is insulated by a tuft of needles, and can sprout from dormant axillary buds located in the root collar if the apical bud is killed (Farrar, 1975; Jin et al., 2019). *Pinus palustris* seedlings can remain in the grass stage for up to 15 years building carbohydrate reserves in its root system before initiating height growth (Wahlenberg, 1946). Upon exiting the grass stage, bolting seedlings are temporarily vulnerable to surface fire, but the risk of mortality diminishes once the apical bud grows beyond the average flaming range of surface fire (approximately 1 m) (Brockway et al., 2007). In addition, *Pinus palustris* rapidly develops thick bark, which helps insulate the cambium from surface fire (Hare, 1965; Jackson et al., 1999; Schafer et al., 2015). Species lacking a comparable suite of advantageous life history traits are often excluded from pine woodlands (Hoffman et al., 2012; Varner et al., 2016).

Decades of fire exclusion have altered the structure, function, and species composition of pine woodlands. For example, the absence of fire has enabled fire-resilient hardwood tree species to recruit into sub-canopy and canopy positions (Gilliam & Platt, 1999; Addington et al., 2015), where they reduce light availability, increase forest floor depth, and in some cases reduce litter layer flammability compared to *Pinus palustris* (Kane et al., 2008; Varner et al., 2016; Emery & Hart, 2020). Moreover, the absence of fire has allowed for the encroachment of fire-sensitive species, further augmenting reductions in resource availability and litter layer flammability (Hoffmann et al., 2005; Kreye et al., 2018). Collectively, these changes have affected understorey biodiversity and tree recruitment in degraded pine woodlands (Palik et al., 1997; Provencher et al., 2001; Hiers et al., 2007; Veldman et al., 2013).

Another functional change that has likely occurred in degraded woodlands is the contribution of seeds from encroaching tree species. Fire-sensitive tree species such as *Acer rubrum*, *Liquidambar styraciflua*, and *Quercus* spp., and other fire-tolerant pine species, including *Pinus taeda*, have steadily invaded southeastern pine woodlands and now influence annual seed rain (Hanberry et al., 2018).

Increased seed rain diversity has potential ramifications for future tree species composition, but little is known about the processes that may affect the fate of seeds from encroaching tree species. It is well known that granivores can limit seed availability for *Pinus palustris* (Boyer, 1964; Croker & Boyer, 1975). However, much of what is known about how seed depredation impacts tree recruitment in pine woodlands comes from studies that have isolated *Pinus palustris* from sympatric tree species (Boyer, 1964; Nolte & Barnett, 2000; Willis et al., 2019). Consequently, it is currently unknown how an influx of seeds from encroaching tree species will affect seed predator preference and how that may influence seedling layer species composition.

Interspecific patterns in seed depredation and germination may be influenced by the interaction between seed size and local variations in hardwood encroachment. In general, small mammals select larger seeds, whereas arthropods are more limited in the size of seeds that can be accessed (Hulme, 1998; Lundgren & Rosentrater, 2007; Mendoza & Dirzo, 2007; Galetti et al., 2015). Other research has shown that small-mammal granivory increases in areas with extensive vegetation cover (Ostfield et al., 1997; Manson & Stiles, 1998; Brown & Kottler, 2004; Orrock et al., 2004). Conversely, arthropods have been shown to be effective seed predators in frequently burned stands (Stuhler & Orrock, 2016) or in areas where midstorey vegetation has been removed (Willis et al., 2019). Thus, tree species seed availability may be indirectly affected by the density and size of the encroaching vegetation.

Encroachment may also create physical barriers that could have cascading effects on regeneration dynamics. In areas where encroachment has occurred, the existence of a dense forest floor could constrain the establishment of smaller-seeded species (Westoby et al., 2002; Varner et al., 2005). Increases in midstorey and canopy density could also exclude *Aristida stricta* from the understorey, which has been negatively associated with *Pinus taeda* invasion (Fill et al., 2017), but positively associated with *Pinus palustris* seedling establishment (Willis et al., 2019; Miller et al., 2019). Currently, it is unknown whether *Aristida stricta* inhibits *Pinus taeda* seedling establishment.

Fire exclusion has the potential to transform woodlands into structurally diverse, closed canopy forests (Bond et al., 2005). Myriad changes in fire-excluded southeastern pine woodlands have potential implications for future flammability, biodiversity, and future existence of this imperiled ecosystem. Here, we examined the impact of overstorey basal area, hardwood midstorey encroachment, substrate type and availability, and understorey vegetation type and density on seed depredation for *Pinus palustris* and four common encroaching tree species (*Pinus taeda*, *Liquidambar styraciflua*, *Acer rubrum*, and *Quercus nigra*) in degraded pine woodlands in the southeastern United States. In addition, we explored the effects of these same factors when vertebrate seed predators were excluded or unexcluded on *Pinus palustris* and *Pinus taeda* germination and correlated species-specific germination responses to the percent cover of *Aristida stricta*, herbaceous vegetation, pine litter, hardwood litter, and mineral



soil. Collectively, the information gained in this study will provide insight into the functional impact of tree encroachment on fire-maintained ecosystems and can help guide management efforts to restore degraded southeastern pine woodlands.

2 | METHODS

2.1 | Stand characteristics

The experiment was established in a mature *Pinus palustris* stand located in the Sandhills Ecoregion of North Carolina (35°3'34.6932" N, 79°22'22.0872" W). Average high temperatures in the region ranged from 11.1°C in January to 32.1°C in July (Arguez et al., 2010). Precipitation in the region occurs mostly in the form of rain and averaged 1 182 mm annually. Soils vary throughout the stand, but the experiment was conducted entirely on Candor sand (Soil Survey Staff Natural Resources Conservation Service, 2019), which features sand throughout the profile and a clay-to-loam bottom (1.5–2.0 m). The Candor sand soil series falls within the Sandy, kaolinitic, thermic Grossarenic Kandiodults family, and is moderately distributed throughout the Sandhills and upper Coastal Plain regions of North Carolina, South Carolina, and Georgia, USA.

The stand was naturally regenerated in the 1920s following extensive clearcutting. After establishment, the stand experienced approximately 70 years of fire exclusion. Efforts to restore the historical woodland structure began in the early 1990s and resulted in the mechanical removal of hardwood species from the midstorey and canopy and the reintroduction of fire through dormant season prescribed burning on approximately three-year intervals. Currently, *Pinus palustris* dominates the overstorey (>90%). The midstorey (1.2 m average height) is composed primarily of *Quercus laevis* (82%) with minor components of *Nyssa sylvatica* (9%) and *Sassafras albidum* (7%). *Aristida stricta* dominates the understorey and is complemented by a diversity of forbs and graminoids. The scientific nomenclature used in this manuscript was obtained from the United States Department of Agriculture Plants Database.

2.2 | Experimental design

Our experiment used a 2 × 2 factorial randomized complete-block design. Thirteen experimental blocks (0.10 ha) were established across the stand in areas with low (6–10 m²/ha), medium (11–20 m²/ha), and high residual basal area (21–25 m²/ha). Each block contained eight measurement plots (2 m × 2 m) surrounded by a 4.57 m buffer. Measurement plots were randomly assigned one of four treatments: vertebrate seed predator exclusion with hardwood midstorey removal, vertebrate seed predator exclusion with hardwood midstorey retention, no seed predator exclusion with hardwood midstorey removal, and no seed predator exclusion with hardwood midstorey retention (Figure 1 and Appendix S1). Each block contained two replicates of each treatment (Figure 1).

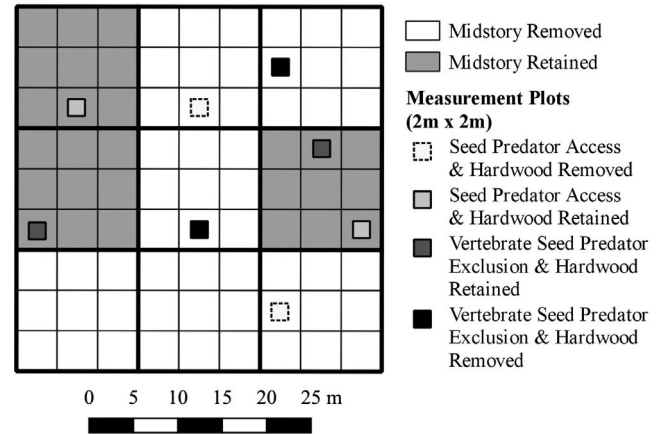


FIGURE 1 Example layout of an experimental block (0.10 ha). Each block consisted of nine sections (188 m²), with each section containing nine plots (21 m²). Each section was randomly selected for midstorey hardwood removal or retention. Measurement plots (4 m²) were established within plots and randomly assigned one of four treatments: seed predator access with hardwood removal; seed predator access with hardwood retention, vertebrate seed predator exclusion with hardwood retention; or vertebrate seed predator exclusion and hardwood removal

Midstorey removal was accomplished with a cut stump treatment (Brushtox and methylated seed oil [61.6% Triclopyr] Ragan and Massey, Inc., Gig Harbor, WA, USA) conducted in the spring of 2017. The buffer surrounding measurement plots assigned midstorey removal were also treated to reduce edge effects (Figure 1). In addition, all litter was raked by hand from the measurement plots to remove the legacy of the midstorey. Also, this procedure removed pine litter associated with the overstorey, as it was operationally infeasible to separate litter types at the plot.

Vertebrate seed predators (small mammals and birds) were controlled for by installing hardware cloth enclosures (1.27 cm mesh size; Appendix S1). Flashing was fit around the exterior of each enclosure to a depth of 25 cm to discourage burrowing. To account for any potential bias associated with fencing, we fenced plots not selected for seed predator exclusion with one strand of twine (Appendix S1).

To examine seed predator selection, we conducted two cafeteria trials (feeding trials where foragers are simultaneously offered a variety of palatable items to determine preference) in October and November of 2018. In each trial, 10 seeds of *Pinus palustris*, *Pinus taeda*, *Liquidambar styraciflua*, *Acer rubrum*, and *Quercus nigra* were each placed in a single Petri dish lid located on the forest floor in the center of each measurement plot. The Petri dish lid was placed on top of the litter layer in measurement plots with midstorey retention (Appendix S2). Each trial lasted for 72 hr. A seed was considered predated if it was either missing or partially damaged. Three lids were removed from the trials for either not being recovered or tipped over when located.

For germination, we artificially seeded *Pinus palustris* and *Pinus taeda* in each plot at rate of 12 seeds/m² in early November 2018. *Pinus taeda* was selected for this trial because it has been extensively planted as a commercial species within the historical

range of *Pinus palustris*, regularly produces good seed crops, and generally outcompetes *Pinus palustris* when fire is excluded as a result of its greater initial height growth (Baker & Langdon, 1990; Boyer, 1990). The seeding rate used in this study was four times the recommended minimum rate for direct seeding *Pinus palustris* (Brockway et al., 2007) and equal to the seed rain conditions during a mast year for *Pinus palustris* (Boyer, 1990). Germination tests conducted prior to dispersal revealed germination rates exceeding 85% for both species. *Pinus palustris* and *Pinus taeda* seed was obtained from the North Carolina Forest Service's Claridge Nursery in Goldsboro, NC, USA.

2.3 | Field measurements

All measurements of basal area, forb and graminoid cover, and substrate availability were collected at the measurement plot level. Basal area was quantified through point sampling with a 2.296-factor metric prism swung in the center of each plot. All tree species counted in the variable radius plot were recorded, but species other than *Pinus palustris* were exceedingly rare. To quantify conditions at the forest floor, we conducted ocular estimates of percent cover of *Aristida stricta*, forbs, mineral soil, pine litter, and hardwood litter cover to the nearest 5% immediately prior to dispersing seed. Germination was assessed monthly from December 2018 to April 2019. A seed was considered germinated once the cotyledon extended beyond the seed coat.

2.4 | Statistical methods

We used generalized linear mixed models to identify factors influencing seed depredation and germination. In both models, we used a negative binomial distribution and a log link to account for overdispersion. Gauss-Hermite quadrature was used to obtain parameter estimates. Denominator degrees of freedom were determined with the containment method, which assigns degrees of freedom to fixed effects based on the smallest rank contribution from the G-side random-effects list (SAS Institute, 2015). Initial analysis indicated significant differences among species, prompting the use of species-specific models for seed depredation and germination. Each model consisted of the main effects of block, treatment, and continuous basal area. Also, the interaction between treatment and basal area was included in the model. Treatment was considered a categorical fixed effect, while basal area was considered a continuous fixed effect. Block was considered a categorical random effect. Although 13 blocks were initially established, given the physical proximity of some of the blocks within the stand, measurement plots were consolidated into five blocks. Factors were considered significant at $\alpha = 0.05$. All models were checked for overdispersion. Normality of the residual errors was confirmed with quantile-quantile plots. In addition to seed depredation and germination, we examined the effect of treatment on the percent cover of *Aristida*

stricta, mineral soil, herbaceous vegetation, pine litter, and hardwood litter with ANOVA. The above analyses were conducted using the GLIMMIX and GLM procedures in SAS 9.4 software (SAS Institute, Cary, North Carolina, USA).

To explore the influence of substrate availability and ground layer vegetation on seed depredation and germination, we conducted species-specific multivariate correlation analyses. Separate analyses were completed for measurement plots with different levels of vertebrate seed predator exclusion. The analyses used Spearman's correlation coefficients to determine the strength and direction of the associations between hardwood litter, pine litter, mineral soil, forbs, and *Aristida stricta* percent cover on seed depredation and germination. The analysis of seed depredation was conducted only in plots without seed predator exclusion, while our analysis of germination was limited to plots where vertebrate seed predators were excluded. Associations were considered significant at $\alpha = 0.05$. The multivariate correlation analyses were conducted using the CORR procedure in SAS.

3 | RESULTS

3.1 | Seed depredation

Seeds from the five tree species were depredated at different rates ($F = 81.28, p < 0.0001$). Granivory generally corresponded inversely with seed size (Figure 4). *Liquidambar styraciflua*, the smallest-seeded species, was depredated at a higher rate than any other species (Figure 2). *Pinus taeda* and *Pinus palustris*, the species with the next smallest seeds, experienced higher granivory than *Acer rubrum* and *Quercus nigra*, the two largest-seeded species, but did not differ from one another (Figure 2). Treatment influenced the depredation of *Pinus taeda* and *Pinus palustris* seeds (Table 1; Figure 3). For both species, granivory was significantly lower where vertebrate seed predators were excluded and midstorey hardwoods were removed compared to other treatments (Figure 3). Midstorey retention did not significantly influence seed depredation for any species (Figure 3).

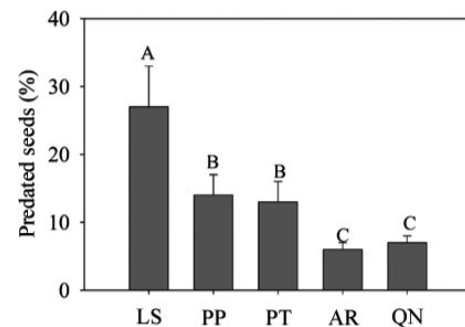


FIGURE 2 Estimated mean depredation percentage (± 1 SE) averaged across all treatments for *Liquidambar styraciflua* (LS), *Pinus palustris* (PP), *Pinus taeda* (PT), *Acer rubrum* (AR), and *Quercus nigra* (QN) in the Sandhills Ecoregion, North Carolina, 2018–2019. Treatments with different letters were significantly different (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$)



Neither basal area nor its interaction with treatment significantly influenced granivory for any species (Table 1). No examined factors significantly influenced seed depredation for *Liquidambar styraciflua*,

Quercus nigra, or *Acer rubrum* (Table 1). Percent cover of understorey vegetation and substrate was not significantly related to granivory for any species (data not shown).

TABLE 1 Results of a generalized linear mixed model examining the influence of basal area, treatment, and the interaction between basal and block on seed depredation in the Sandhills Ecoregion, North Carolina, USA, 2018–2019

Species	Factor	DF	DDF	F ratio	p value
<i>Pinus palustris</i>	Basal area	1	88	1.90	0.1716
	Treatment	3	88	6.39	0.0006
	Basal area × treatment	3	88	2.67	0.0526
<i>Pinus taeda</i>	Basal area	1	88	3.41	0.0682
	Treatment	3	88	2.88	0.0400
	Basal area × treatment	3	88	1.87	0.1490
<i>Liquidambar styraciflua</i>	Basal area	1	88	3.40	0.0684
	Treatment	3	88	1.42	0.2420
	Basal area × treatment	3	88	1.15	0.3349
<i>Acer rubrum</i>	Basal area	1	88	0.55	0.4611
	Treatment	3	88	0.66	0.6429
	Basal area × treatment	3	88	0.10	0.9581
<i>Quercus nigra</i>	Basal area	3	88	0.22	0.6434
	Treatment	3	88	1.85	0.1436
	Basal area × treatment	3	88	0.75	0.5235

Note: Effects with $p < 0.05$ were considered significant. Bold terms are statistically significant at 0.05.

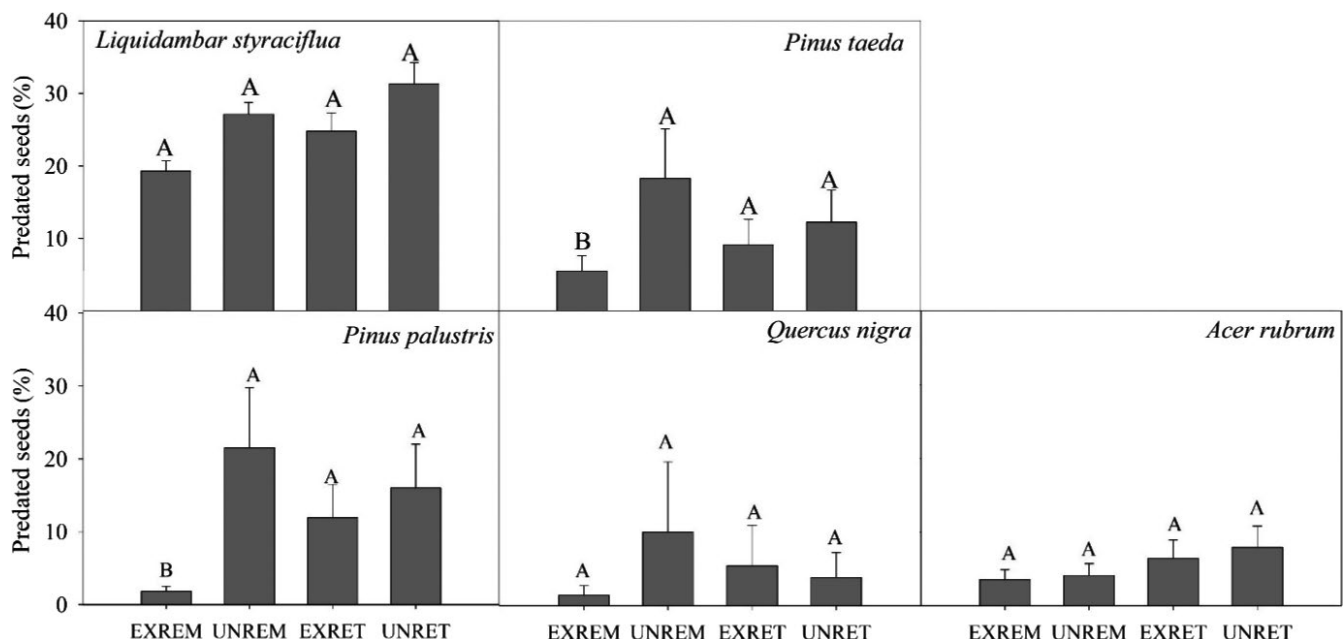


FIGURE 3 Estimated average seed depredation percentage (± 1 SE) of *Pinus palustris*, *Pinus taeda*, *Liquidambar styraciflua*, *Acer rubrum*, and *Quercus nigra* in plots with vertebrate seed predator exclusion and the hardwood midstorey removal (EXREM), no seed predator exclusion and hardwood midstorey removal (UNREM), vertebrate seed predator exclusion and hardwood midstorey retention (EXRET), and no seed predator exclusion and hardwood midstorey retention (UNRET) in the Sandhills Ecoregion, North Carolina, USA, 2018–2019. Treatments with different letters were significantly different (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$).

Species	Factor	DF	DDF	F ratio	p value
<i>Pinus palustris</i>	Basal area	1	92	1.08	0.3024
	Treatment	3	92	8.16	<0.0001
	Basal area × treatment	3	92	1.40	0.2473
<i>Pinus taeda</i>	Basal area	1	92	1.76	0.1884
	Treatment	3	92	10.14	<0.0001
	Basal area × treatment	3	92	1.82	0.1482

Note: Effects with $p < 0.05$ were considered significant. Bold terms are statistically significant at 0.05.

TABLE 2 Results of a generalized linear mixed model examining the influence of basal area, treatment, and the interaction between basal and block on germination in the Sandhills Ecoregion, North Carolina, USA, 2018–2019

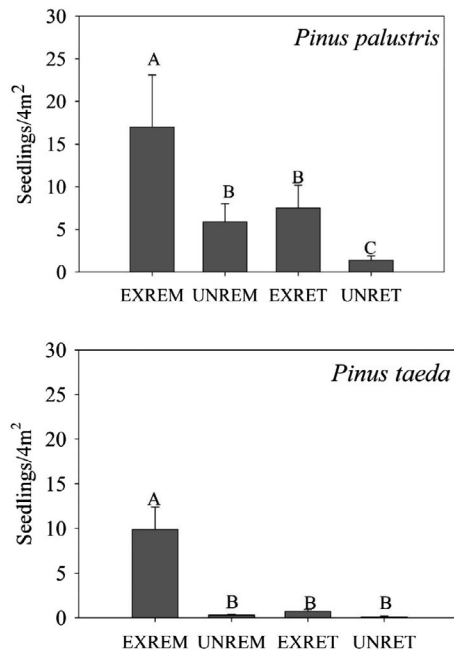


FIGURE 4 Estimated average germination (± 1 SE) of *Pinus palustris* and *Pinus taeda* in plots with vertebrate seed predator exclusion and hardwood midstorey removal (EXREM), no seed predator exclusion and hardwood midstorey removal (UNREM), vertebrate seed predator exclusion and hardwood midstorey retention (EXRET), and no seed predator exclusion and hardwood midstorey retention (UNRET) in the Sandhills Ecoregion, North Carolina, USA, 2018–2019. Treatments with different letters were significantly different (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$)

3.2 | Germination

Overall, *Pinus palustris* ($15,025$ germinants $\text{ha}^{-1} \pm 3,525$) averaged more germinants than *Pinus taeda* ($2,350$ germinants $\text{ha}^{-1} \pm 600$) ($F = 72.39$, $p < 0.0001$). Treatment influenced germination for both pine species (*Pinus palustris*: $F = 8.16$, $p < 0.0001$; *Pinus taeda*: $F = 10.14$, $p < 0.0001$; Table 2). Germination of both *Pinus palustris* and *Pinus taeda* was higher where vertebrate seed predators were excluded and the midstorey was removed compared to any other treatment (Figure 4). Germination of *Pinus palustris* was also lower where the midstorey was retained and seed predators were not excluded compared to other treatments (Figure 4). In contrast,

no further differences in germination were detected among treatments for *Pinus taeda* (Figure 4). Differences in basal area did not significantly influence germination for either pine species (data not shown) (*Pinus palustris*: $F = 1.08$, $p = 0.3024$, $R^2 = 0.03$; *Pinus taeda*: $F = 1.76$, $p = 0.1884$, $R^2 = 0.04$; Table 2). Similarly, *Pinus palustris* and *Pinus taeda* germination was not affected by the interaction of treatment and basal area (*Pinus palustris*: $F = 1.40$, $p = 0.2473$; *Pinus taeda*: $F = 1.82$, $p = 0.1482$; Table 2).

Percent cover of *Aristida stricta* ($F = 5.2$, $p = 0.0023$), hardwood litter ($F = 32.1$, $p < 0.0001$), pine litter ($F = 43.2$, $p < 0.0001$), and mineral soil ($F = 207.2$, $p < 0.0001$) were significantly affected by treatment. Treatments with midstorey removal averaged significantly less hardwood and pine litter and more mineral soil than treatments with hardwood retention (Table 3). *Aristida stricta* percent cover was highest where vertebrate seed predators were excluded and the midstorey was retained compared to where seed predators had access regardless of midstorey retention (Table 3). Treatment did not impact herbaceous percent cover ($F = 2.30$, $p = 0.0774$; Table 3). Regardless of seed predator exclusion, mineral soil percent cover had a positive association with *Pinus palustris* germination (excluded: $p = 0.0046$, $\rho = +0.3869$; unexcluded: $p = 0.0003$, $\rho = +0.4862$), while pine (excluded: $p = 0.0258$, $\rho = -0.3091$; unexcluded: $p = 0.0363$, $\rho = -0.2911$) and hardwood litter (excluded: $p < 0.0001$, $\rho = -0.5572$; unexcluded: $p = 0.0003$, $\rho = -0.4821$) had a negative association with germination (Figure 5). The same general relationships of substrate and vegetation associations with *Pinus palustris* were found with *Pinus taeda* when vertebrate seed predators were excluded (Figure 5). However, no substrate and vegetation associations were associated with *Pinus taeda* germination without vertebrate seed predator exclusion (Figure 5).

4 | DISCUSSION

4.1 | Seed depredation

Selective post-dispersal granivory has the potential to modify the species composition and structure of ecosystems (Costa et al., 2017; Larios et al., 2017). While limited to five tree species and one stand, our results indicate that seed size influenced granivory, as smaller-seeded species experienced greater seed depredation than

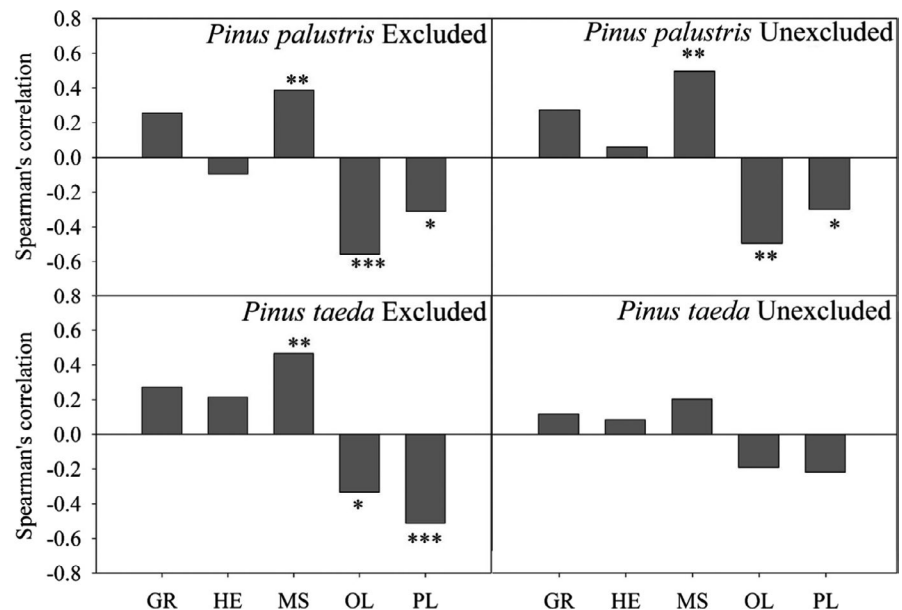


TABLE 3 Estimated average percent cover (± 1 SE) of mineral soil, wiregrass, herbaceous vegetation, pine litter, and hardwood litter in plots with vertebrate seed predator exclusion and midstorey hardwood removal (EXREM), vertebrate seed predator exclusion and hardwood midstorey retention (EXRET), no seed predator exclusion and midstorey hardwood removal (UNREM), and no seed predator exclusion and midstorey hardwood retention (UNRET), in the Sandhills Ecoregion, North Carolina, USA, 2018–2019

Treatment	N	Mineral soil (% cover)	<i>Aristida stricta</i> (% cover)	Herbaceous (% cover)	Pine litter (% cover)	Hardwood litter (% cover)
EXREM	26	67.5 (2.6) ^a	29.3 (2.8) ^a	1.9 (0.6) ^a	1.7 (4.1) ^b	<1 (2.8) ^c
EXRET	26	5.8 (2.6) ^b	18.5 (2.8) ^b	<1 (0.6) ^a	54.2 (4.1) ^a	16.6 (2.8) ^b
UNREM	26	71.3 (2.6) ^a	19.8 (2.8) ^{ab}	2.5 (0.6) ^a	6.5 (4.1) ^b	<1 (2.8) ^c
UNRET	26	2.3 (2.6) ^b	14.0 (2.8) ^b	<1 (0.6) ^a	46.9 (4.1) ^a	33.3 (2.8) ^a

Note: Treatments with different letters were considered significantly different within each response variable (Tukey's honest significance test post-hoc comparisons $\alpha = 0.05$).

FIGURE 5 The direction and strength of the association between *Aristida stricta* (GR), herbaceous vegetation (HE), mineral soil (MS), oak litter (OL), and pine litter (PL) and *Pinus palustris* and *Pinus taeda* germination in plots with vertebrate seed predator exclusion or no seed predator exclusion in the Sandhills Ecoregion, North Carolina, USA, 2018–2019. The direction and strength of the associations were evaluated with Spearman's rank correlation coefficients. Asterisks indicate significance at: *, $\alpha = 0.05$; **, $\alpha = 0.01$; and ***, $\alpha = 0.001$



larger-seeded species. This result contradicts a previous report examining granivory of tree species from a similar seed size range in a northern hardwood forest (Royo & Carson, 2008), but is consistent with the results of a study examining granivory on exotic species in a nearby pine savanna (Krall et al., 2014) and a broad study examining pine invasiveness in Mediterranean shrublands (Carrillo-Gavilán et al., 2010). We suspect the selection for smaller-seeded species was likely influenced by the local seed predator population. Although seed predator identity was not recorded in this study, small-mammal abundance has been shown to be extremely low in frequently burned pine woodlands (Sasmal et al., 2017), and circumstantial evidence suggests that invertebrates are acting as the primary seed predators at our stand (Willis et al., 2019). Based on this assumption, the low occurrence of *Quercus nigra* granivory may be explained by the principles of optimal foraging theory, as foraging invertebrates may have avoided acorns due to their thick seed coat or size (Rey et al., 2002; Lichti et al., 2017). However, this logic does not correspond with the apparent selection for *Liquidambar styraciflua* over *Acer rubrum* or either pine species. Neither seed size nor seed coat toughness should have discouraged the depredation of *Acer rubrum*, *Pinus palustris*, or

Pinus taeda. Also, we are unaware of any secondary compounds or digestive inhibitors that may have made *Acer rubrum* or either pine species undesirable. While the underlying mechanism remains unknown, our results demonstrate that granivores may constrain the encroachment of small-seeded species in degraded pine woodlands more strongly than large-seeded species and that *Pinus palustris* is not necessarily the preferred tree seed for granivores.

One caveat to our interpretation of granivory is the assumption that seed predators are not benefiting tree recruitment through secondary dispersal (Vander Wall et al., 2005). To our knowledge, no species examined in this study has been shown to benefit from secondary seed dispersal and seed depredation has been shown to reduce colonization of *Pinus nigra* following wildfire (Ordóñez & Retina, 2004). Thus, seeds selected by seed predators were considered functionally absent from the seedbank.

Microhabitat structure is another factor known to influence seed predator behavior (Orrock et al., 2004). In general, microhabitats with vegetation cover are thought to provide favorable environments for seed predators at risk of predation from larger predators (Matos & Orrock, 2010; Greenler et al., 2019). Also, variability in timing and

frequency of prescribed fire affects the distribution of plant types and seed production (Lashley et al., 2014) and the phenology of fruiting (Lashley et al., 2015) which may contribute to seed depredation risks by influencing seed availability. However, our results indicate that midstorey retention did not affect seed depredation for any species in plots without seed predator exclusion, demonstrating that the midstorey is not facilitating regeneration by increasing seed availability (Louise Loudermilk et al., 2016). Moreover, seed depredation was not associated with *Aristida stricta* or herbaceous vegetation cover for any species. These results could be related to the seed predator population at our stand, as the impact of vegetation cover has been primarily linked with small-mammal behavior (e.g., *Peromyscus* spp; Zwolak, 2009). Also, it is possible that the relatively open crown structure of *Quercus laevis*, the dominant species in the midstorey, on xeric sites (Hiers et al. 2007) and the relatively discontinuous distribution of *Aristida stricta* on the forest floor may not have provided enough cover to encourage foraging. Regardless, our results indicate the encroachment of hardwood species into the midstorey has not altered relationships in seed depredation at our stand.

4.2 | Filters on germination

Multiple factors have the potential to influence the transition from seed-to-germinant. Our results indicate that *Pinus palustris*, a foundational species in pine woodlands in the southeastern United States, and *Pinus taeda*, a common invader of pine woodlands, germinant density responded similarly to our treatments and abiotic factors. Germinant density of both species was most abundant when the midstorey and litter layers were removed, and vertebrate seed predators were excluded. These results are not particularly surprising, as it is well known that mineral soil provides a favorable seedbed for *Pinus* and that seed depredation can limit seed availability (Crocker & Boyer, 1975). Also, our results indicate that any potential facilitative effects on seedling establishment created by midstorey retention are not affecting germinant density for either species (Wahlenberg, 1946; Louise Loudermilk et al., 2016; Prévosto et al., 2020). Similarly, neither species' germinant density was statistically improved by proximity to *Aristida stricta* cover, as has been noted in previous studies (Miller et al., 2019; Willis et al., 2019). However, *Aristida stricta* cover had a biologically relevant positive effect on germination for both species, indicating that *Aristida stricta* was not impeding germination. Nevertheless, *Aristida stricta* may impede the future survival of established seedlings through intense below-ground competition for resources or by increasing fuel load density which can increase fire intensity (Bond, 2008; Fill et al., 2017).

Despite their general similarities, patterns of germinant density of *Pinus palustris* and *Pinus taeda* were not identical. For example, *Pinus palustris* germinants were more abundant than those of *Pinus taeda* in plots treated with the singular application of vertebrate seed predator exclusion or midstorey removal. Our results demonstrate that *Pinus palustris* retained statistical associations with mineral soil

and litter cover in plots without seed predator exclusion, while the substrate associations with *Pinus taeda* became statistically indistinguishable. Several factors make it difficult to reconcile these results. First, the results from our cafeteria trials demonstrate nearly identical patterns of granivory for both species across treatments. Thus, differences in seed depredation pressure are likely not the cause of lower *Pinus taeda* germinant density in plots without vertebrate seed predator exclusion. Second, differences in seed size are unlikely to explain the differing patterns of germinant density across substrates in plots with vertebrate seed predator exclusion, as germination of *Pinus palustris*, the larger-seeded species, was also limited by forest floor retention. Future studies that manipulate the organic layer and midstorey independently will be better able to provide insight on these confounding results.

5 | CONCLUSIONS

Decades of fire exclusion have enabled tree species formerly limited or excluded by frequent burning to invade southeastern pine woodlands. Changes in tree species composition have the potential to negatively affect biodiversity in the understorey by reducing flammability and resource availability. Moreover, the presence of encroaching tree species may create a series of ecological feedbacks that limit the recruitment of fire-promoting species such as *Pinus palustris*, which could threaten the long-term sustainability of the ecosystem. Our results indicate that in the presence of seeds from four common encroaching tree species, *Pinus palustris* is not preferred by seed predators and that granivory varied inversely with seed size. Collectively, this suggests that encroaching species with larger seeds may have an advantage invading or expanding within degraded pine woodlands compared to smaller-seeded species. It also indicates that granivory may situationally benefit *Pinus palustris* recruitment by reducing competition from smaller-seeded species. Our results also demonstrate that midstorey and litter layer retention did not reduce granivory or facilitate germination for either *Pinus* species at our stand. Hence, any potential nurse effects mediated by midstorey retention on xeric sites appears to be limited to later life history stages. Finally, our results demonstrate that *Pinus palustris* and *Pinus taeda* had similar rates of seed depredation, substrate associations, and had seedling densities that were biologically improved by proximity to *Aristida stricta* cover. These results indicate that management efforts seeking to promote *Pinus palustris* under the context of restoring degraded pine woodlands to historical reference conditions are just as likely to recruit *Pinus taeda*. Thus, efforts should be made to remove nearby *Pinus taeda* seed sources at the beginning stages of restoration.

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AUTHOR CONTRIBUTIONS

JW conceived the research idea, obtained funding, collected data, performed statistical analyses and wrote the manuscript; DS and CD collected data and wrote the manuscript; ML wrote the manuscript; BW and JY collected data.

DATA AVAILABILITY STATEMENT

The data associated with this project are publicly available on the internal server of the USDA Forest Service Research. Data Archive <https://doi.org/10.2737/RDS-2021-0008>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. Example images of the treatments used in this study:

- (a) no seed predator exclusion with hardwood midstorey retention;
- (b) no seed predator exclusion with hardwood midstorey removal;

- (c) vertebrate seed predator exclusion with hardwood midstorey retention; and (d) vertebrate seed predator exclusion with hardwood midstorey removal.

Appendix S2. An example of a loaded Petri dish lid used in the cafeteria trials.

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