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Causes of a seedling recruitment advantage for an encroaching oak over a historically dominant oak in a fire-restored open oak woodland

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ABSTRACT

The lack of natural oak regeneration within forests is one of the most pressing conservation issues facing oak-dominated ecosystems in eastern North America. Although the restoration of suitable fire regimes appears to favor oaks over fire-sensitive mesophytes in many cases, differences in the effects of fire restoration on different species of oak have not received nearly as much attention. In particular, greater sapling recruitment of historically lowland *Quercus alba* compared to that of the historically upland and dominant *Quercus stellata* in response to fire restoration appears to be related to differences in seedling establishment, but the causes of these differences are not clear. In this study, we investigated species differences in acorn viability and rate of removal by predators, as well as differences in oak seedling emergence, survival, and vigor over two growing seasons (fall 2020 to fall 2022) in a restored oak woodland in northern Mississippi (USA). The proportion of viable acorns was greater for *Q. alba* than for *Q. stellata,* especially in 2020. Both species had similar acorn predation/removal rates in the winter of 2021. Seedling survival and vigor were greater in *Q. alba* than in *Q. stellata*, although these differences were more pronounced in patches dominated by the invasive *Microstegium vimineum*. Seedling emergence, survival, and vigor for both oak species were significantly lower in patches dominated by *M. vimineum* than in those dominated by native species. Neighboring vegetation and litter removal significantly increased seedling survival in both species. The positive effect of neighbor/litter removal on seedling survival was no greater in patches dominated by *M. vimineum* than in those dominated by native species, a result consistent with the existence of legacy effects of *M. vimineum*. Altogether, our findings suggest greater acorn viability combined with higher seedling survival and vigor in *Q. alba* than in *Q. stellata* may contribute to the lower regeneration rates of the historically dominant *Q. stellata* compared to encroaching *Q. alba* in fire-maintained woodlands. Negative effects of an invasive grass, *M. vimineum*, may further complicate restoration efforts.

1. Introduction

One of the most pressing issues facing oak-dominated ecosystems in eastern North America, behind outright habitat loss, is the lack of natural regeneration of oaks within forests. Despite increasing alarms being sounded since the 1950 s, the difficulty in maintaining oak (*Quercus* sp.) regeneration in eastern North American forests continues to be of paramount importance (Dey, 2014). However, this failure to regenerate is not limited to North America, as the replacement of oak by other species in old-growth and regenerating forests has been reported through the historic range in the northern hemisphere (Li and Ma, 2003; Götmark et al., 2005; Pulido and Díaz, 2005; Johnson et al., 2009; Spînu et al., 2020). These failures to regenerate include regional failures of oak species regeneration in all but the most xeric sites (Abrams, 1992).

The explanation for oak regeneration failure that has received the most attention is the so-called "fire-oak hypothesis." This hypothesis states that modern oak regeneration failure in large part is caused by canopy closure and increased competition from less fire-tolerant mesophytic trees as a result of prolonged fire exclusion (Abrams, 1992). In contrast to historic conditions, generally speaking, current oak-dominated forests have reduced fire frequency, less flammable understories, and denser canopies (Clebsch and Busing, 1989; Nowacki and

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Fig. 1. The Wildflower Loop 1 Restoration plot that has undergone oak restoration via canopy thinning and repeated prescribed fires since 2004. Restoration treatments have increased native plant species diversity and substantially increased oak sapling recruitment, but treatments have unintentionally increased the spread of *M. vimineum*.

Abrams, 2008). Most presettlement fire regimes in the eastern United States produced low- to mixed-severity surface burns which maintained oak and pine forests with open conditions, such as woodlands or savannas (Wright and Bailey, 1982; Frost, 1998). Although oaks remain dominant in the canopy, the regeneration potential of many stands is decreasing with time as mature, canopy trees age and understories become increasingly dominated by shade-tolerant species that recruit into the canopy (Dey, 2014).

The fire-oak hypothesis is based on the following assumptions: (1) Periodic fire has been an integral disturbance of forests in eastern North America for millennia; (2) oaks, specifically upland adapted oaks, have several physiological and physical characteristics that allow them to survive at higher rates than their mesophytic competitors in the presence of periodic fire regimes; (3) the decline and suppression of fire in the 20th century is the major reason for the chronic, widespread oak regeneration problem and; (4) reintroducing fire via repeated prescribed burning will promote oak regeneration (Brose et al., 2013).

Effective testing of the fire-oak hypothesis requires an adequate understanding of how fire regimes interact with demographic transitions at multiple stages of the oak life cycle to influence oak regeneration (Arthur et al., 2012). A significant bottleneck in oak regeneration appears to be the transition from the seedling to sapling size class in fire-excluded stands (Arthur et al., 2012; Brose et al., 2013). In general, when given ample sunlight for a period of time, oak saplings generally produce larger resprouting stems than do most mesophytes after a fire (Brose, 2011; Cannon and Brewer, 2013) and are better able to avoid topkill by subsequent fires than are mesophytes (Brewer, 2015). Hence, the combination of canopy openings and repeated fires shows considerable promise, provided the treatments are timed to coincide with adequate seed supply and abundant seedling recruits of oaks (Laatsch and Anderson, 2000; Iverson et al., 2008; Brose et al., 2013; Brewer, 2015; Waldrop et al., 2016). How the restoration of fire and canopy openness affects other life-cycle transitions in oaks (e.g., acorn supply and seedling emergence, growth, and survival) is not as well understood (Brose, 2011; Arthur et al., 2012; Funk et al., 2015).

Although the combination of repeated fire and canopy openings can promote regeneration of oaks as a group, how this combination differentially affects different species of oak is less clear. Fire-maintained woodlands of northern Mississippi (USA) were historically dominated by the slow-growing *Quercus stellata* Wangenh, but have shifted to a predominantly *Quercus alba* L.-dominated forest following modern fire exclusion (Surrette et al., 2008). This shift is likely related, at least in part, to greater shade tolerance in *Q. alba* than in *Q. stellata*, which could give *Q. alba* an advantage over *Q. stellata* with increasing canopy closure as a result of fire exclusion (Dey, 2014). Fires in northern Mississippi historically favored *Q. stellata* in the uplands and largely restricted *Q. alba* to floodplains, terraces, and the ecotone between uplands and terraces (Surrette et al., 2008). Comparison of land surveyor notes of witness tree sizes and locations in the 1830 s and 1840 s with tree species composition of mature upland forests in the early 2000 s in portions of Marshall and Lafayette Counties found substantial differences driven by the increase in *Q. alba* in the overstory and an increase in non-oak mesophytes in the midstory of upland areas (Brewer, 2001; Surrette et al., 2008).

Despite greater historical dominance of fire-prone uplands by *Q. stellata*, a recent study of upland oak woodland restoration using thinning and prescribed burning revealed greater sapling recruitment by *Q. alba* than by *Q. stellata* (in terms of absolute numbers) in response to restoration (Brewer, unpublished). Thus, differences in shade and fire tolerance of *Q. alba* and *Q. stellata* alone may not be sufficient to explain interspecific differences in sapling recruitment responses to fire restoration. Greater sapling recruitment by *Q. alba* than by *Q. stellata* was in large part due to *Q. alba* having a larger seedling and sapling sprout bank than *Q. stellata* (Brewer, 2014). Such an observation suggests that factors operating at the seed and seedling stage interact with fire to influence which *Quercus* spp. recruit most successfully during ecological restoration of fire.

Numerous factors could cause differences in seedling establishment between *Q. alba* and *Q. stellata*, including differences in acorn supply and/or viability (Goodrum et al., 1971), acorn size (Goodrum et al., 1971; Guyette et al., 2004), predation (Pérez-Ramos and Marañón, 2008), competitive responses to native and/or invasive groundcover vegetation (Flory and Clay, 2010; Aronson and Handel, 2011; Brewer, 2011; Johnson et al., 2015; Morrison, 2017), and seedling herbivory (Knight et al., 2009; Morrison, 2017; Averill et al., 2018). Although all these factors are known to affect seedling establishment of oaks, differences between encroaching and historically dominant oak species have received little attention. An advantage of the encroaching *Q. alba* over the historically dominant *Q. stellata* in one or more of these acorn/seedling-related variables could negate any advantage *Q. stellata* has over *Q. alba* has in terms of fire tolerance of saplings and sprouts.

In this study, we investigated differences in acorn viability and the effects of several potentially interacting factors on acorn predation and/or removal, seedling emergence, seedling survival, and vigor of post oak *Q. stellata* and white oak *Q. alba* in a restored oak woodland at the Strawberry Plains Audubon Center. We tested the following non-mutually exclusive hypotheses to explain differences in seedling densities between *Q. alba* and *Q. stellata*: 1) Acorn viability - natural levels of acorn viability are higher for *Q. alba* than for *Q. stellata* in a fire-restored woodland; 2) Acorn predation/removal – acorn predation/removal is significant and is greater for *Q. stellata* than for *Q. alba*; 3) Seedling emergence and/or early seedling survival/vigor – seedling emergence and/or early seedling survival/vigor is greater in *Q. alba* than in *Q. stellata*, and; 4) Effect of invasives on acorn predation/removal, seedling emergence, and/or seedling survival/vigor – The seedling establishment advantage of *Q. alba* over *Q. stellata* is greater in patches dominated by invasives than in those dominated by native groundcover, due to differences in acorn predation/removal, seedling emergence, and/or early seedling survival/vigor. Results are relevant to regional upland oak restoration efforts and the methods may be applicable to other ecosystems where the historically dominant oak species is not responding to fire restoration as well as encroaching oak species.

2. Methods

2.1. Study Site

We conducted this study in an open woodland at the Strawberry Plains Audubon Center, a ~1000-ha wildlife sanctuary located in the loess plains of north-central Mississippi (USA) (34°50'00.7"N, 89°28'38.2"W). A long-term restoration ecological restoration experiment was established in 2004 with the goal of restoring open-canopy oak-hickory (*Quercus-Carya*) woodlands using repeated fires and canopy thinning. The experiment consisted of two pairs of adjacent 1-ha treated and control plots. One pair was established in 2004 and the other in 2017. The current seedling establishment experiment was conducted within the older and thus more restored of the two treated plots (treated plot at Wildflower Loop 1). We chose this plot because conditions were generally favorable for oak seedling establishment and recruitment into the sapling layer. Additionally, there were multiple patches of the invasive grass *Microstegium vimineum,* as well as large, mature *Q. alba* and *Q. stellata* trees as acorn sources (Fig. 1). Many native plant species have increased due to prescribed burning and restoration treatments, but so has *M. vimineum.*

2.2. Acorn collection and acorn viability tests

Prior to acorn collection, we identified and flagged eight mature trees of both *Q. alba* and *Q. stellata,* for a total of 16 trees, located in the two plots (restored and control) of Wildflower Loop 1 to expedite acorn collection and site selection. Each flagged tree was visited once during peak acorn drop to collect acorns from under the oak canopy. As such, each acorn census contained two or more unique oak trees that were visited during their peak acorn drop for the collections and not revisited after that census. We collected acorns off the woodland floor and immediately discarded acorns that were not potentially viable (i.e., acorns that were damaged, aborted, germinated, or rotten). We harvested every potentially viable acorn of *Q. alba* and *Q. stellata* acorn we could find under the flagged tree crowns during each acorn census. We collected acorns twice a week from the flagged, mature oaks in the Wildflower Loop 1 plots at the Strawberry Plains Audubon Center from 15 October to 8 November, 2020. Due to the periodic and variable nature of white oak group (subgenus *Leucobalanus)* acorn production (Johnson et al., 2009), we also collected acorns from 4 November to 6 November, 2022 to test for viability. Despite the prevalence of *Q. stellata* trees at these sites, we collected 601 *Q. alba* acorns compared to 283 *Q. stellata* acorns in 2020. In 2022, we collected 691 *Q. alba* acorns and 1500 *Q. stellata* acorns. We sorted the potentially viable acorns we collected in 2020 into bags according to their origin (i.e., restored vs. control plot). We used the acorns collected in 2020 for the field experiment.

Due to a low crop of viable *Q. stellata* acorns at Strawberry Plains (i.e., 73 viable acorns out of 283 collected acorns), we collected additional acorns of *Q. stellata* from Jeff Busby Park in Choctaw County, Mississippi, USA (33.4137° N, 89.2609° W) on 24 November 2020, to augment the supply needed for the seedling experiment.

We prepared acorns for cold storage following the acorn storage methods proposed by the USDA NRCS (Connor, 2004). We used a float-sink method to estimate acorn viability. Acorns that sunk to the bottom of the bucket were labeled as viable and acorns that floated to the top of the bucket along the waterline were labeled as nonviable. We discarded all acorns that floated and stored viable acorns in breathable, wax paper bags at 3 °C until planting on 18 December 2020. We allowed all acorns to warm to air temperature one day prior to planting.

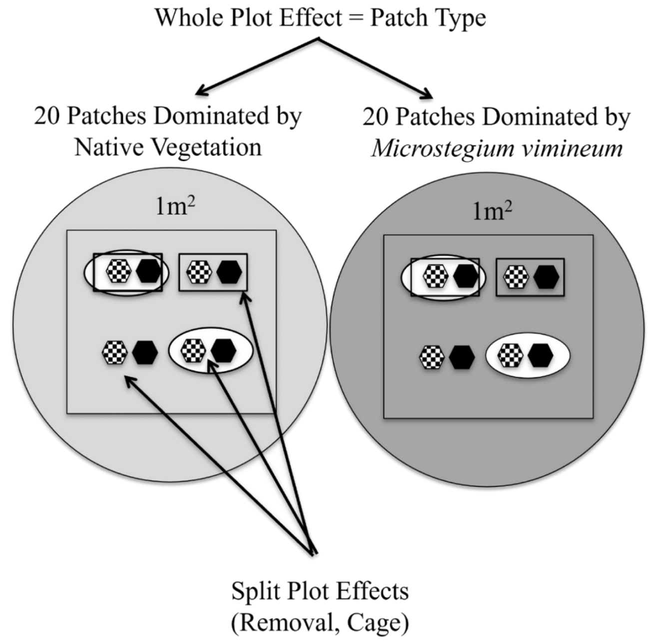

Fig. 2. The split-plot experimental design. *Quercus alba* acorns (checkered hexagon) and *Quercus stellata* acorns (black hexagon) are shown within plots where caged treatments are represented by a rectangle around the acorn pair and neighbor/litter removal treatments are represented by a white circle around the acorn pair. Within each of the 40 1-m^2 plots, there were four randomized subplots containing both acorn species, a neighbor/litter removal treatment, and a mammal exclusion cage treatment for a total of 160 subplots.

2.3. Acorn removal, seedling emergence, and seedling survival experiment

In mid-December, 2020, we established 40 1-m^2 plots where 20 1-m^2 plots were located within patches dominated by *M. vimineum* (with occasional *Lonicera japonica*) and 20 in areas lacking *M. vimineum* and

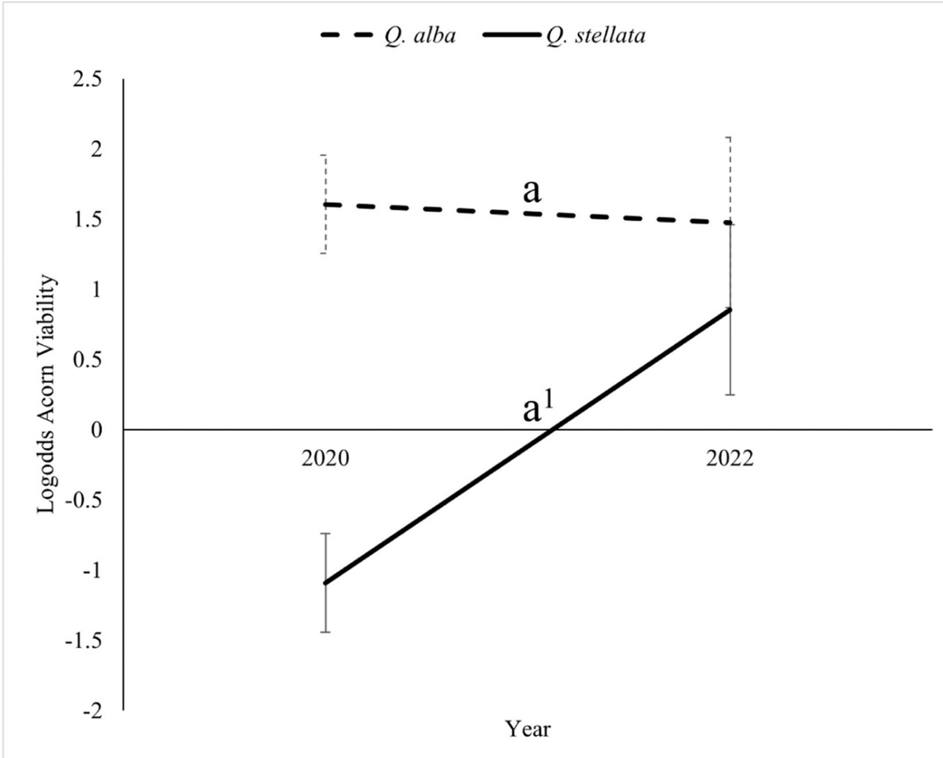

Fig. 3. Interaction plot of the difference in acorn viability of *Q. alba* and *Q. stellata* by year. Values are the logodds proportion of acorn viability for all acorns collected during a year. A logodds value of 0 is equivalent to 50% viability. Error bars are ± 1 standard error based on the mean square error for the analysis. The asterisk bar indicates statistical significance of $p < 0.01$ between treatments. The letters a and a^1 indicate that the slopes and thus the year x species interaction approached statistical ($p = 0.058$). The main effect of species on viability was highly statistically significant ($p < 0.001$).

dominated by native legumes, forbs, perennials, and/or bunchgrasses (hereafter referred to as *M. vimineum* patch and native vegetation patch, respectively) in the Wildflower Loop 1 restoration plot. In addition, we restricted the location of the plots to areas just past the dripline of mature oak trees within the restored woodland to mimic the natural seedfall location relative to mature oaks. To maximize environmental similarity between *M. vimineum* patches and native vegetation patches, to the extent possible, we equitably distributed plots of both patch types (patch type referring to the presence or absence of *M. vimineum*) at various levels of canopy cover and various positions along the east-facing slope of the restored woodland. We also avoided establishing plots in areas of high oak sapling density.

The experiment was a split-plot design, in which the whole plot effect was patch type, with 20 pseudoreplicates of each patch type (40 total), and the split-plot treatments of cage and neighboring vegetation and litter removal were arranged as a 2 × 2 factorial and assigned randomly to four 0.25 × 0.25 m subplots within each whole plot (for 40 replicated factorial treatment arrangements or 160 total subplots; Fig. 2). The unmanipulated controls were no cage and neighbors/litter present, which mimicked the natural state of the woodland floor.

On 18 December, 2020, we planted a pair of *Q. alba* and *Q. stellata* acorns within each of the 160 subplots, for a total of 320 seedlings. We randomly selected acorns with respect to their origin and buried them just below the soil surface (1.5 to 3 cm deep) to minimize desiccation and roughly simulate the effects of scatter hoarders on burial (Perea et al., 2012). We planted each acorn at least 10 cm from the other acorn in the subplot and marked the location of each acorn with a small piece of metal wire and flagging tape. We implemented a mammal/bird exclusion cage treatment immediately after planting the acorns and maintained it biweekly throughout the experiment. We constructed mammal/bird exclusion cages from 19-Gauge hardware cloth (with 1.2 cm mesh) by cutting the hardware cloth into strips and shaping them into hollow cuboids (15 cm × 15 cm x 28 cm). The completed cages lacked a top or bottom. In total, we made 80 cages, 2 cages per plot. We secured the cages in the ground using metal wire and pinched the tops of the cages closed to prevent animals from entering and removing acorns. We replaced any cages that were knocked over or moved due to animals or fallen limbs and noted any mortality due to stochastic cage disturbances (e.g., a treefall and crushing a cage and seedling pair). We delayed the implementation of the neighbor/litter removal treatment until April of 2021, coincident with the beginning of the growing season. We implemented the neighbor/litter removal treatment by removing all leaf litter and surrounding vegetation within the subplot by hand.

2.4. Data collection

We recorded acorn predation/removal (i.e., missing acorn and evidence of digging) from January to March 2021 and then monitored seedling emergence, survival, and vigor biweekly from April 2021 to October 2022. We estimated acorn predation/removal by determining whether there were signs of predation and/or removal for each species. We quantified oak seedling emergence in April 2021 by assessing the presence of cotyledons derived from acorns that had not been removed by animals. We quantified oak seedling survivorship by identifying the last census in which green tissue was observed and then counting the number of censuses up to and including that census. We quantified oak seedling vigor, hereafter, greenness, by counting the number of censuses from April 2021 to October 2022 in which some green tissue was apparent (e.g., leaves, buds, new stem growth, and/or resprouting green stems from the roots). Seedling greenness counts and seedling survivorship counts were not always identical, because there were some cases in which censuses that revealed no green tissue were followed by censuses in which green tissue was apparent. Thus, we assumed reflushed oak seedlings during those censuses were dormant rather than dead.

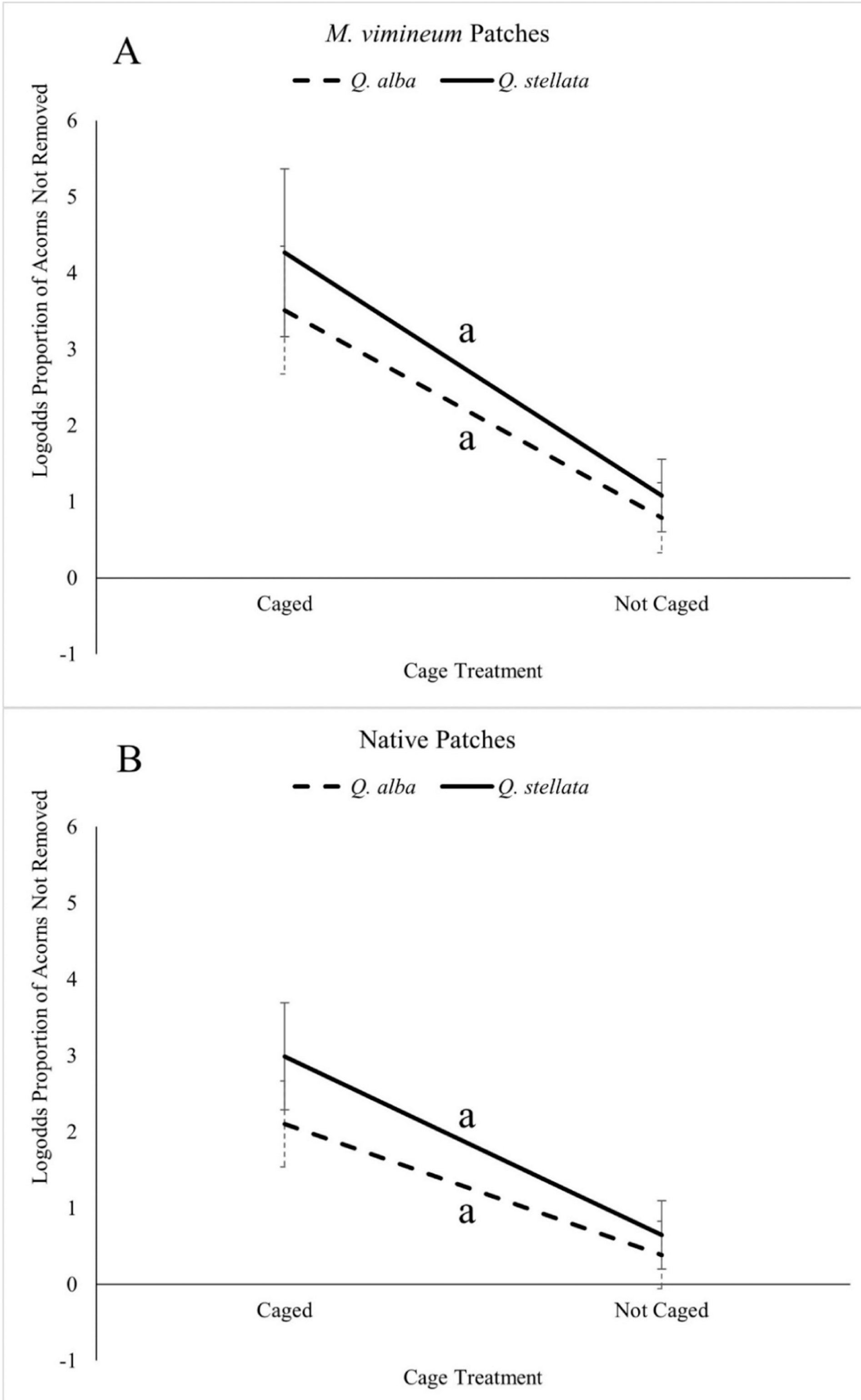

Fig. 4. Interaction plot of the differences in acorn predation/removal by species and cage treatments in patches dominated by A) *M. vimineum* and B) native vegetation in the winter of 2021. Values are the logodds proportions of acorns present (not removed). Error bars are ± 1 standard error based on the mean square error for the analysis. Equivalent lowercase letters indicate that the response slopes were the same and thus the species × cage interaction was not significant at $p = 0.05$.

2.5. Data analysis

We conducted all analyses using R 4.1.0 (R Core Team, 2020). To test the acorn viability hypothesis, we quantified acorn viability as a nominal response variable (viable vs. not viable) and fit it to a generalized linear model (function glm) containing the predictors species and year, assuming a binomial response distribution (family = binomial). To test all remaining hypotheses, we fit acorn predation/removal rates,

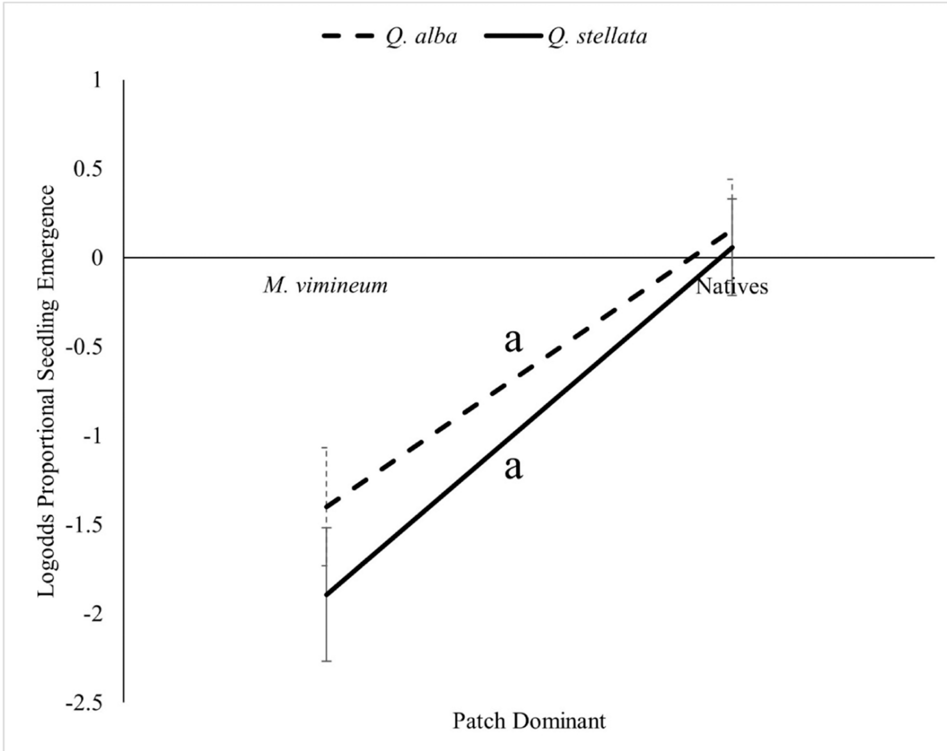

Fig. 5. Interaction plot showing the difference in oak seedling emergence by patch treatments and species in the winter of 2021. Values are the logodds proportion of oak seedlings present. A logodds value of 0 is equivalent to 50% emergence. Error bars are ± 1 standard error based on the mean square error for the analysis. Equivalent letters indicate that the slopes were the same and thus the interaction was not significant at $p = 0.05$.

seedling emergence rates, survivorship, and greenness to predictors in generalized linear mixed models using the lmer function (package *lmerTest*) (Kuznetsova et al., 2017). To test the acorn predation/removal and effect of invasives on acorn predation/removal hypotheses, we fit the nominal response variable of acorn predation/removal (removed vs. not removed) to a generalized linear mixed model containing the fixed effects of species (*Q. alba* vs. *Q. stellata*), cage (cage vs. no cage), and patch dominants (invasives vs. natives) and the random effects of patch and subplot. We assumed a binomial response distribution. The cage treatment was nested within patch, and the species effect was nested within subplot. To test the seedling emergence/early survival hypothesis, we fit the nominal response variable of emergence (green tissue present in April 2021 vs. not present in April 2021) to a generalized linear mixed model containing the fixed effects of species and patch dominants and the random effect of patch. Only those observations that were not removed by animals during the winter of 2021 were included in the analysis. Because predation/removal by animals resulted in cases in which only one species was present, we were not able include subplot as a random effect in this analysis. Hence, we nested species within the random effect of patch. We assumed a binomial response distribution. Because missing observations resulted in a lack of independence between main effects and interaction, we used type 2 sums of squares using the Anova function in R (package *car*), which estimated main effects before estimating interactions. To test the hypotheses related to seedling survival/vigor and the effect of invasives on seedling survival/vigor over time, we fit the count variable (number of censuses alive (or green) from April 2021 to October 2022) to a generalized linear mixed model containing the fixed effects of species, patch dominants (invasives vs. natives), neighbor/litter removal, and their interactions and the random effect of patch. Species and neighbor/litter removal were nested within the random effect. We added 1 to all counts and assumed a Poisson response distribution (family = poisson). We calculated expected marginal means for all analyses using the emmeans function (package *emmeans* (Lenth, 2023)), which reported the mean counts on a natural log scale for a Poisson response distribution and the log odds scale for a binomial response distribution. We reported standard error bars derived from the mean squared error for the test of a given effect or interaction.

3. Results

3.1. Acorn Viability

When averaged over both years, the proportion of viable acorns was significantly greater in *Q. alba* than in *Q. stellata* (species $F_{(1,12)} = 25.761$, $p < 0.001$; Fig. 3). Viability increased for *Q. stellata* between 2020 and 2022 but not for *Q. alba*, resulting in a year × species interaction that approached statistical significance (year × species $F_{(1,12)} = 4.378$, $p = 0.058$; Fig. 3). The main effect of year was not statistically significant (year $F_{(1,12)} = 3.359$, $p = 0.092$).

3.2. Acorn Predation/Removal

Exclusion cages reduced acorn predation/removal of both oak species in both patch types prior to oak seedling emergence in April 2020 (cage $X^2_{(\mathrm{df}=1;\ 320\ \mathrm{obs})} = 31.307$, $p < 0.001$; Fig. 4). The cages were equally effective at reducing predation/removal of both oak species, as indicated by a species × cage interaction that was not statistically significant (species × cage $X^2_{(\mathrm{df}=1;\ 320\ \mathrm{obs})} = 0.548$, $p = 0.459$; Fig. 4). Although acorn predation/removal was slightly lower in the *M. vimineum* patches (Fig. 4A) than in the native vegetation plots (Fig. 4B), the main effect of patch type was not statistically significant (patch type $X^2_{(\mathrm{df}=1;\ 320\ \mathrm{obs})} = 1.494$, $p = 0.222$). Although predation/removal of *Q. stellata* acorns appeared to be slightly lower than that of *Q. alba acorns* (Fig. 4), the main effect of species was not significant (species $X^2_{(\mathrm{df}=1;\ 320\ \mathrm{obs})} = 1.590$, $p = 0.207$).

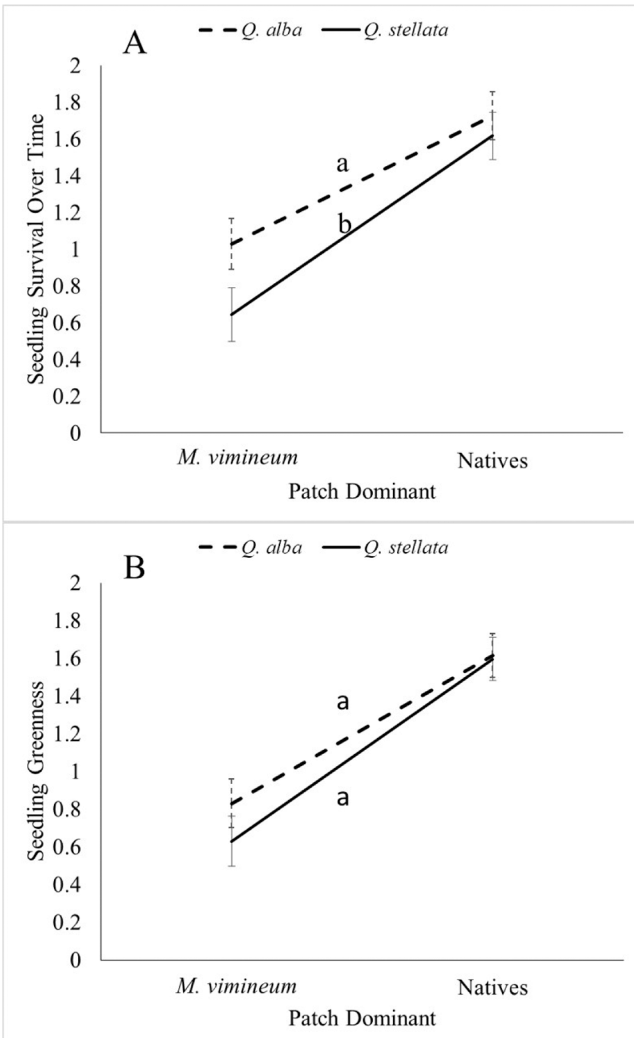

Fig. 6. Interaction plot showing difference in A) survival and B) greenness of oak seedlings over the course of two growing seasons (Seedling Survival Over Time) of *Q. alba* and *Q. stellata* by patch type. Values are the log-transformed mean of census counts in which the seedling was alive or green (maximum number of counts = 12; log maximum = 2.48). Error bars are ± 1 standard error based on the mean square error for the analysis. Different letters in 6 A indicate that the slopes were different and thus the species × patch type interaction was significant at $p = 0.05$. Seedling survival over time was significantly higher in *Q. alba* than in *Q. stellata* ($p = 0.001$). Equivalent letters in 6B indicate that the slopes were not significantly different and thus the species × patch type interaction was not significant at $p = 0.05$. Seedling greenness did not differ significantly between *Q. alba* and *Q. stellata* ($p = 0.187$).

3.3. Seedling Emergence in Native and Invasive Patches

Oak seedling emergence in April 2020 was significantly higher in native vegetation patches than in *M. vimineum* patches (patch type $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 28.439$, $p < 0.001$). Both oak species had similar rates of seedling emergence (species $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 0.706$, $p = 0.401$), and the species × patch type interaction was not statistically significant (species × patch type $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 0.425$, $p = 0.515$; Fig. 5).

3.4. Survival and Greenness of Seedlings over the First Two Years

Oak seedlings, regardless of species, showed significantly higher survival over time (inclusive of initial emergence) in native vegetation patches than in *M. vimineum* patches (patch type $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 20.294$, $p < 0.001$; Fig. 6A). *Q. alba* seedlings showed significantly higher survival than *Q. stellata* seedlings (species $X^2_{(\text{df}=1:\ 250\ \text{obs})} = 10.570$, $p = 0.001$), and this trend was especially pronounced within *M. vimineum* patches (species × patch type $X^2_{(\text{df}=1:\ 250\ \text{obs})} = 4.318$, $p = 0.038$; Fig. 6A). As with survival over time, the number of censuses in which oak seedlings were green was greater in patches of native vegetation than in patches dominated by *M. vimineum* (patch type $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 28.997$, $p < 0.001$; Fig. 6B). Unlike with survival over time, oak seedling greenness did not differ significantly between the two species (species $X^2_{(\text{df}=1:\ 250\ \text{obs})} = 1.498$, $p = 0.221$), nor was the species × patch type significant (species × patch type $X^2_{(\text{df}=1:\ 250\ \text{obs})} = 1.742$, $p = 0.187$; Fig. 6B).

Removing neighboring vegetation and litter increased oak seedling survival (neighbor/litter removal $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 5.585$, $p = 0.018$; Fig. 7A). The positive effect of neighbor/litter removal on oak seedling survival was no greater in patches dominated by *M. vimineum* than in those dominated by native species, as indicated by a neighbor/litter removal × species interaction for oak seedling survival that was not significant ($X^2_{(\text{df}=1;\ 250\ \text{obs})} = 1.929$, $p = 0.165$; Fig. 7A). As with oak seedling survival over time, oak seedling greenness was greater when neighbors/litter were removed than when neighbors/litter were left intact (neighbor/litter removal $X^2_{(\text{df}=1;\ 250\ \text{obs})} = 7.291$, $p = 0.007$; Fig. 7B). Unlike with oak seedling survival, the positive effect of neighbor/litter removal on oak seedling greenness was significantly greater in *M. vimineum* patches than in native patches (neighbor/litter removal × patch type $X^2_{(\text{df}=1;\ 250)} = 4.389$, $p = 0.036$; Fig. 7B).

4. Discussion

The lack of natural regeneration of oaks within forests is an important issue facing oak-dominated ecosystems in eastern North America. The explanation that has received the most attention, the fire-oak hypothesis (Clebsch and Busing, 1989; Nowacki and Abrams, 2008), could be extended to predict that fire-tolerant oaks that historically upland sites should benefit more from fire restoration than less fire-tolerant oaks encroaching from lowland sites (i.e., *Q. stellata* should benefit more than *Q. alba*). Support for this hypothesis, however, depends on whether there are other overriding factors such as greater rates of seedling establishment in the encroaching oak species.

Our results support the hypotheses that higher seedling densities of *Q. alba* than in *Q. stellata* in upland areas result in part from acorn viability being greater in *Q. alba* than in *Q. stellata*, although viability did increase for *Q. stellata* in 2022. We cannot explain this difference, but contributing factors could include differences in oak male reproductive strategies and/or increased outcrossing in *Q. alba* compared to *Q. stellata*, potentially due to differences in interspecific sexual barriers (e.g., a stronger mating incompatibility in *Q. stellata* than in *Q. alba*; Lagache et al., 2014). Regardless of the causes, we suggest that lower viability of *Q. stellata* compared to *Q. alba* could be a significant limiting factor in the regeneration of *Q. stellata* and part of the reason why *Q. alba* dominated the seedling and sapling layers of the fire-restored oak woodland studied here (Surrette and Brewer, 2008; Brewer, 2014).

Acorn removal/predation rates were substantial (~24 to 27%) for both species, but we found no differences between the two species or between patches dominated by invasive *M. vimineum* and those dominated by natives. The lack of differences between the two species suggests that potential dispersers and/or predators did not respond to differences in acorn size (larger for *Q. alba*). The lack of differences between patch types is consistent with a previous finding that showed that the survival of seeds of larger seeded tree species, particularly oak species, was unaffected by the presence of the invader (Flory and Clay, 2010). While Flory and Clay (2010) suggest that dense patches of *M. vimineum* may provide better habitat for small herbivores and seed predators than native patches, they found no effect of direct evidence of this.

Our results support the hypothesis that *Q. alba* seedlings showed

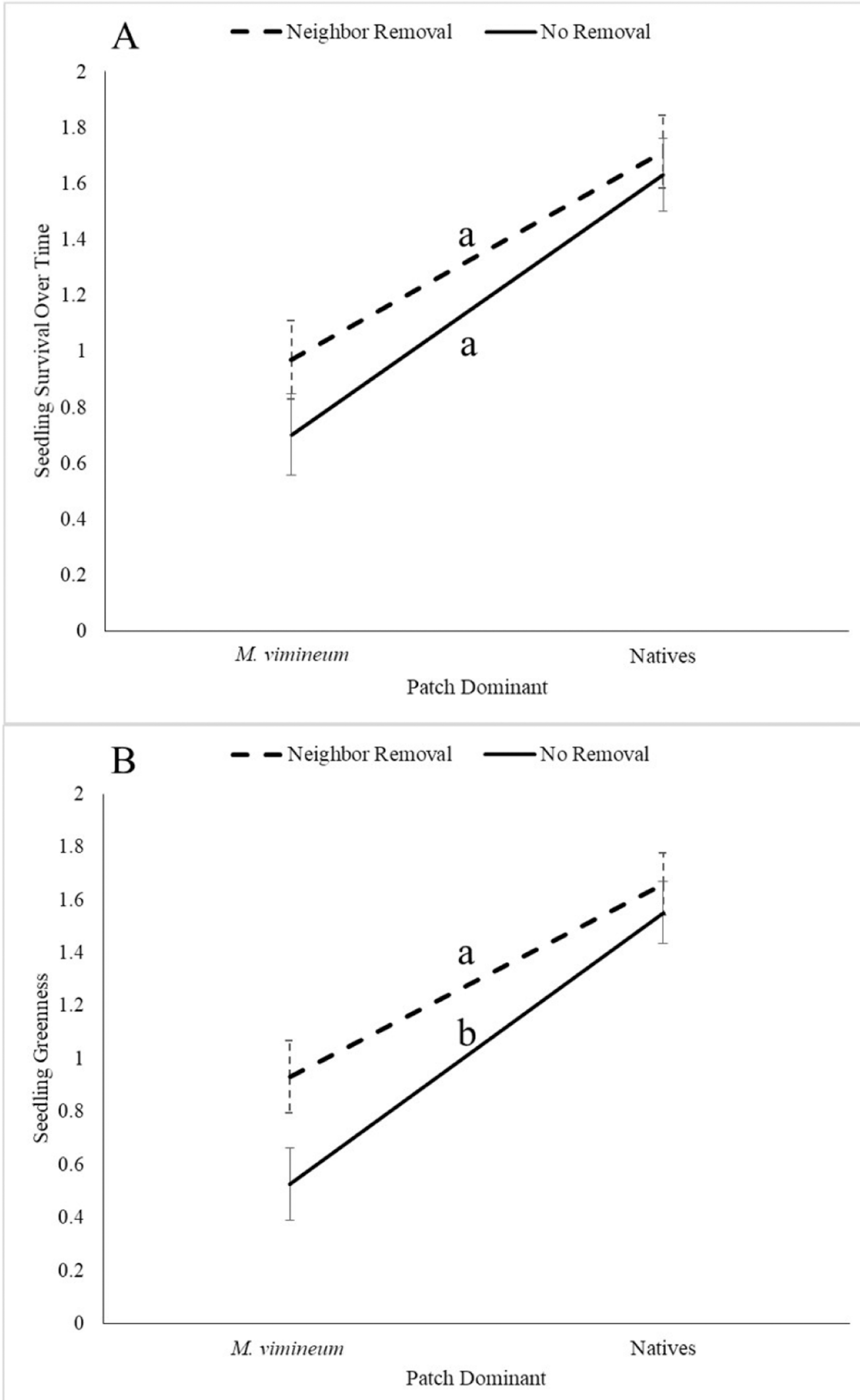

Fig. 7. Interaction plot showing the difference in A) seedling survival and B) greenness over time as a function of neighbor/litter removal in either *M. vimineum* or native vegetation patches. Values are the log-transformed mean number of census counts in which the transplant was alive or green (maximum number of counts = 12; log maximum = 2.48). Error bars are ± 1 standard error based on the mean square error for the analysis. For survival, equivalent letters indicate that the slopes were not significantly different and thus the neighbor/litter removal × patch type interaction was not significant at $p = 0.05$. Oak seedling survival over time was significantly higher in neighbor/litter removal treatments than in removal controls ($p = 0.018$). For greenness, different letters indicate that the slopes were significantly different and thus the neighbor/litter removal × patch type interaction was significant at $p = 0.05$. Oak seedling greenness was significantly higher in neighbor/litter removal treatments than in controls ($p = 0.002$) and significantly higher in native patches than in *M. vimineum* patches ($p < 0.001$).

~10% higher survival than *Q. stellata* seedlings in the first two years following germination. The reasons for this difference remain unclear but are likely related to physiological and life history differences between the species, including the larger acorn size of *Q. alba*. In addition, the same physiological factors responsible for lower acorn viability in *Q. stellata* could also have affected seedling viability and vigor. Whatever the reason, our results suggest that lower seedling survival in *Q. stellata* compared to *Q. alba* could be a significant limiting factor in the regeneration of *Q. stellata* and another reason why *Q. alba* dominated the seedling and sapling layers of the fire-restored oak woodland studied here.

In our study, we found evidence of approximately 60 to 70% lower seedling emergence, survival, and vigor of both species of oaks in patches dominated by an invasive grass than in patches dominated by native species (Figs. 5–7). The positive effect of removing neighbors and litter on seedling greenness was greater in *M. vimineum* patches than in native patches (Fig. 7B), suggesting a competitive effect of *M. vimineum*. The negative effect of being in a *M. vimineum* patch on seedling survival appeared to be slightly but significantly greater for *Q. stellata* than for *Q. alba*. *M. vimineum* can inhibit the survival or growth of hardwood species including *Q. alba* and *Q. stellata* at the seedling level, but the impact of invasive species on oak growth starting from the seed stage to seedling stage was not well understood prior to our study. These results agree with those of previous studies that showed that oak seedling survival can be reduced by *M. vimineum* (Flory and Clay, 2010; Aronson and Handel, 2011; Brewer, 2011; Johnson et al., 2015).

We found evidence consistent with the hypothesis that *M. vimineum* exerted a negative legacy effect on oak seedling survival. Although seedling survival was lower in patches dominated by *M. vimineum* than native vegetation, we found no evidence that the positive effect of neighbor/litter removal on survival was greater in *M. vimineum* patches than in native patches (Fig. 7A). This result suggests that some of the negative effects of *M. vimineum* on seedling survival persisted after *M. vimineum* removal (i.e., legacy effects). *M. vimineum* was not tall enough to shade acorns during spring emergence in April 2021, indicating that legacy effects of *M. vimineum* in the soil likely reduced oak seedling emergence. We therefore suggest that an inhibitory soil-mediated legacy effect was partially responsible for the reduced emergence and survival of oaks in patches of *M. vimineum*. It is possible that *M. vimineum* disrupted arbuscular mycorrhizal connections of the oak seedlings, thus reducing their ability to successfully emerge from seed and survive the initial two growing seasons. Alternatively, we cannot rule out the possibility that other environmental differences between *M. vimineum* and native patches were responsible for differences in oak seedling survival. The environmental conditions that favored the establishment of *M. vimineum* might have negatively affected oak seedling survival in our study. Further study specifically examining the inhibitory effect of *M. vimineum* on oak seedling emergence and survival is warranted.

We found no evidence of a “lighthouse” effect; i.e., the preferential targeting of oak seedlings in *M. vimineum* patches by grazing herbivores. This finding contrasts with those of published studies on native perennial herbaceous species and tree species, in which deer preferentially consumed native plants in patches of invasive species (Averill et al., 2018; Knight et al., 2009; Morrison, 2017). In contrast to studies that found substantial herbivory by white-tailed deer (*Odocoileus virginianus*) on oak seedlings (Aronson and Handel, 2011), we saw very little herbivory of oak seedlings in our study. There are many possible reasons for such regional differences in herbivory in the eastern United States. One possibility is there are regional differences in the concentration of deer in oak ecosystems. Another possibility, given that white-tailed deer were present at our site, is that the restoration of productive and species-rich herbaceous groundcover vegetation has provided numerous alternatives for deer to browse. Whatever the reason for the differences observed among studies, we suggest that the high levels of herbivory commonly reported in oak forests in the northeastern and midwestern USA do not necessarily apply to the southern USA and/or to certain stand conditions (e.g., restored open oak woodlands with productive groundcover vegetation).

5. Conclusions

Although considerable attention has focused on the role that fire plays in natural oak regeneration, our results suggest that factors other than fire that limit acorn and seedling viability may be as important for successful restoration as factors preventing the transition from the seedling to the sapling stage. We have demonstrated that acorn and seedling viability can be lower for *Q. stellata*, the more fire-tolerant but poorer viable acorn-producer, than for *Q. alba*, the slightly less fire-tolerant but more prolific viable acorn producer. If restoration of the historically dominant species of oak is a desired management goal, then acorn and seedling viability (along with acorn crop size) must be considered alongside the use of fire and canopy manipulation to alleviate the seedling to sapling bottleneck in fire-excluded stands. That said, fire is crucial for successful natural regeneration of *Q. alba*, and this species is important for wildlife no matter where it happens to be successful. Negative effects of an invasive grass, *M. vimineum*, which responds positively to fire restoration, may further complicate restoration efforts, as it appears to negatively affect both species, especially the more fire-tolerant *Q. stellata*.

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CRediT authorship contribution statement

G. L. Williams: Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing– Review & Editing, Visualization. **J. Stephen Brewer:** Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing – Review & Editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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