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Ontogenetic responses of four plant species to additive and interactive effects of land-use history, canopy structure and herbivory

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Summary

1. The strength of interactions among species is often highly variable in space and time, and a major challenge in understanding context-dependent effects of herbivores lies in disentangling habitat-mediated from herbivore-mediated effects on plant performance.

2. We conducted a landscape-scale experiment that manipulated light availability in woodlands with either a history of agricultural use or no history of agricultural use and coupled this with performance measurements of three life stages on four perennial herbaceous species exposed to varying levels of herbivory.

3. We found that the context-dependent effects of herbivory on plant performance changed as plants grew: juvenile plant survival was reduced by herbivores in low-light habitats whereas biomass of adult plants was reduced by a more diverse insect fauna in high-light environments. A history of agricultural land use also had negative effects on seedling establishment and adult performance, independent of herbivory.

4. *Synthesis.* This work experimentally separates the habitat-mediated effects on plant performance from the herbivore-mediated effects on plant performance and highlights how context-dependent interactions depend on plant ontogenetic stages.

Key-words: context-dependent, grasshoppers, herbivory, light availability, old field, plant ontogeny, plant–herbivore interactions, tolerance

Introduction

Herbivores can exert strong negative effects on the performance and survival of individual plants, the dynamics of plant populations and the structure of plant communities (Maron & Crone 2006; Allan & Crawley 2011). Yet these effects are often highly variable in space and time (Maron, Baer & Angert 2014) and theoretical predictions for how environmental variation influences herbivore impacts often contradict (Hahn & Maron 2016). For example, two leading models make opposing predictions for how resource availability should influence herbivore impacts on plant performance in low versus high-resource environments. The compensatory continuum hypothesis predicts that the negative effect of herbivory should be damped in high-resource environments because plants can better tolerate herbivory (Maschinski & Whitham 1989). In contrast, the growth rate model predicts that plants are better able to compensate for herbivory in low-resource environments, because plant growth in low-resource environments is maintained below their maximum potential growth rate (Hilbert *et al.* 1981). Despite a number of studies evaluating the influence of resources on plant performance, few consistent patterns have emerged from these studies (Hawkes & Sullivan 2001), unless the limiting resource has been properly identified (Wise & Abrahamson 2007). However, a variety of ecologically relevant factors beyond resource availability, such as plant ontogenetic stage (Barton & Koricheva 2010; Massad 2013) or type or abundance of herbivores (Ali & Agrawal 2012; Louthan, Doak & Angert 2015), can also have important influences on a plant's response to herbivory. Explicitly incorporating multiple relevant factors into experimental designs may help to understand the variable outcome in field studies (Chamberlain, Bronstein & Rudgers 2014).

Disentangling how environmental or resource context may affect herbivory is becoming increasingly important when considering the myriad of human activities that create novel environmental conditions (Tylianakis *et al.* 2008). For instance, a large fraction of the terrestrial land surface is undergoing succession on abandoned agricultural lands, where

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the legacy of past agricultural land use can have persistent effects on ecosystems by altering soil properties and reducing reestablishment of certain plant species (Flinn & Vellend 2005; Cramer, Hobbs & Standish 2008). Agricultural legacies can subsequently affect plant performance through changes in soil properties, such as nutrients, structure or compaction (Fraterrigo, Turner & Pearson 2006; Baeten *et al.* 2011), or through increased herbivory rates on some palatable plants by altering plant quality or the composition of alternative vegetation available for consumption (Hahn & Orrock 2015a, 2016; de la Peña *et al.* 2016). Many post-agricultural systems are further modified by concurrent human activities, such as fire suppression and subsequent woody encroachment (Ratajczak, Nippert & Collins 2012). Canopy closure due to fire suppression results in reductions in light availability and increases in below-ground competition, which can also have substantial effects on plant performance (Harrington, Dagley & Edwards 2003). Light environment can be particularly important for affecting plant responses to herbivory by altering herbivore abundance and damage rates (Evans *et al.* 2012; Stoepler & Rehill 2012; Levey *et al.* 2016) or mediating the ability for plants to regrow after being damaged (Baraza, Zamora & Hódar 2010; Salgado-Luarte & Gianoli 2010). Despite what is individually known about land-use history and light availability, and the ubiquity of anthropogenic disturbances, the combined effects of multiple anthropogenic disturbances on biotic interactions such as herbivory are only beginning to be recognized and explored (Tylianakis *et al.* 2008; Thébault *et al.* 2014; Hahn & Orrock 2015a).

In this study, we combine local herbivory experiments with landscape-scale habitat manipulations to disentangle the effect of habitat context and herbivores on the performance of multiple plant ontogenetic stages at large spatial scales relevant to conservation and management. We manipulated canopy structure in 64 1-ha longleaf pine woodland stands that were historically used for agriculture (> 60 years since abandonment) or that were never used for agriculture. Within these experimental landscapes, we measured herbivore damage and performance on transplanted seedlings exposed to or protected from grasshoppers, the dominant invertebrate herbivore in this system (Evans *et al.* 2012; Hahn & Orrock 2015a). Because context-dependent effects of herbivory on plant performance could differ among life stages (Boege & Marquis 2005; Massad 2013) or traits of different plant species (Maron & Kauffman 2006; Hahn & Orrock 2015a), we selected four species in the Asteraceae that vary in growth rate and measured the performance of three life stages that are known to be important for regulating the population dynamics of perennial herbs (Lauenroth & Adler 2008). We hypothesized that (i) the effects of herbivory on plant performance would be greatest on earlier life stages (i.e. seedling establishment and juvenile survival; Barton & Hanley 2013). We also hypothesized that, (ii) because plants in thinned patches grow faster compared to plants in unthinned patches (Harrington 2011), herbivory should have less of an effect on plant performance in thinned patches. Based on previous findings (Hahn & Orrock 2015a), we predicted that (iii) herbivory would have greater effects where alternative plant resources are low, particularly in post-agricultural sites. Collectively, we expected herbivory to have the greatest effect on juvenile plant survival in unthinned, post-agricultural habitats. By providing a comprehensive overview of the context-dependent effects of generalist herbivores on plant performance, our experiments reveal insight into the additive and interactive effects of abiotic and biotic factors on several critical life stages.

Materials and methods

STUDY SITE AND LANDSCAPE EXPERIMENT

We conducted this study at the Savannah River Site (SRS; Appendix S1 in Supporting Information), an 80 125 ha National Environmental Research Park near Aiken, South Carolina, USA, located within the historical range of the longleaf pine ecosystem. Much of SRS was converted to agriculture between 1865 and 1950. These farms were small and dispersed across the landscape, resulting in a many pockets of untilled woodlands (Kilgo & Blake 2005). All agricultural fields on SRS were abandoned in 1951 following government acquisition of SRS (Kilgo & Blake 2005). We determined historic land use by examining aerial photographs taken in 1951, just prior to governmental acquisition of SRS. As in Brudvig *et al.* (2013), cultivated fields in 1951 were classified as ‘post-agricultural woodlands’ and mature woodlands in 1951 were classified as ‘non-agricultural woodlands’. The post-agricultural woodlands were subsequently replanted with longleaf (*Pinus palustris*), loblolly (*P. taeda*), or slash (*P. elliottii*) pine trees in the 1950–1970s (Kilgo & Blake 2005). We selected 14 4-ha sites and one 8-ha site that encompassed a non-agricultural/post-agricultural boundary (Appendix S1). Blocking by sites that contained both types of land-use history reduces the potentially confounding issue of non-random selection of agricultural fields by statistically accounting for environmental variation of the site (Flinn & Vellend 2005). See Appendix S1 and Brudvig *et al.* (2013) for more detailed site descriptions.

Within this general design, we conducted a randomized, factorial manipulation of canopy closure (thinned or unthinned). Overstorey tree thinning occurred in early 2012, and 1-ha patches were thinned to 8–10 longleaf pine trees per hectare. The lower bound of the thinning treatment was set by the stand with the minimum number of pine trees so that we could thin to a consistent density across all of our sites. Control patches had an average tree density of 550–750 trees per hectare and the canopy in non-agricultural was slightly more dense due to hardwood encroachment compared to the post-agricultural pine plantations (approximately 77% and 66% canopy closure, respectively; Brudvig *et al.* 2013).

We have previously documented ecological effects of land-use history and overstorey tree thinning at our sites. The major ecological effects of agricultural land-use history are largely via changes in the composition of the understorey plant community and soil properties (Brudvig *et al.* 2013). Post-agricultural woodlands tend to have higher soil phosphorous, lower water holding capacity, lower organic matter and inorganic nitrogen, and more compacted soil, relative to non-agricultural woodlands (Brudvig *et al.* 2013). Overstorey thinning increased light levels, herbaceous plant cover, and grasshopper abundance, all by threefold or greater, relative to unthinned patches (Hahn & Orrock 2015b). However, differences in plant community composition between post-agricultural and non-agricultural sites persisted even after canopy thinning (Turley & Brudvig 2016).

LOCAL HERBIVORY EXPERIMENTS

We selected four plant species of Asteraceae: *Carphephorus bellidifolius* (Michx.) Torr. & A. Gray, *Sericocarpus tortifolius* (Michx.) Nees, *Solidago nemoralis* Aiton and *Solidago odora* Aiton. We selected Asteraceae species to control for phylogeny. The species we selected vary in over an order of magnitude in seed mass (Appendix S2), a trait correlated with growth rate and seed output (Westoby *et al.* 2002), allowing us to evaluate responses to herbivory along this axis of trait variation. These four species vary widely in their habitat occurrences, with *C. bellidifolius* and *Se. tortifolius* occurring more frequently in non-agricultural habitats, *So. nemoralis* occurring more frequently in post-agricultural habitats and *So. odora* occurring frequently in both habitat types, based on surveys conducted in the year prior to this experiment (Brudvig *et al.* 2013).

Grasshoppers are common invertebrate herbivores in our system (Evans *et al.* 2012; Hahn & Orrock 2015a) and include the most likely insect consumers of the focal plant species. The two most common species of grasshoppers at our site are *Melanoplus angustipennis* (subfamily: Melanoplinae) and *Schistocerca alutacea* (subfamily: Cyrtacanthacridinae; Hahn & Orrock 2015c), both species are mixed feeders that can eat a variety of grasses and forbs (Joern 1985). Grasshoppers in the subfamilies Gomphocerinae and Oedipodinae are also present at our site (Hahn & Orrock 2015c), although both of these subfamilies consume mainly grasses (Joern 1985) and thus were not likely to eat our experimental plants. We previously measured preference of the plant species to two of the dominant grasshopper species at our site using cafeteria-style (choice) feeding experiments (Hahn & Orrock 2015b). *Schistocerca* grasshoppers consumed similar amounts of *Se. tortifolius, So. nemoralis* and *So. odora*, but consumed lower amounts of *C. bellidifolius*. *Melanoplus* grasshoppers preferred *So. nemoralis* and to a lesser degree *So. odora* and consumed low amounts of *C. bellidifolius* and *Se. tortifolius*.

We established study plots for a seed-addition experiment near the centre of each 1-ha experimental patch (combinations of land-use history and overstorey tree thinning). We selected areas with little or no vegetation cover and cleared vegetation by clipping stems at the soil surface, when necessary, to ensure there was no established vegetation inside the study plots, which was necessary in order to install the exclosures (see below). We added 100 seeds to 17.5 cm diameter subplots. Each subplot was seeded with only one species. Seeding densities ($\approx 4100\ \mathrm{m}^{-2}$) are similar to other seed-addition studies conducted in the longleaf pine ecosystem (e.g. Myers & Harms 2009). We surrounded the subplots with 4.5 cm tall plastic barriers, buried approximately 1 cm into the ground, to prevent seeds from being washed out. We then covered the groups of four barriers with a consumer exclosure, either a full exclosure to exclude consumers or a pseudo-exclosure. Exclosures were $0.125\ \mathrm{m}^2 \times 1$ m tall mesh (2.7 mm mesh) screen cages (pseudo-exclosures have holes to allow grasshoppers to enter) and have been previously used to reduce herbivory by grasshoppers (Families Acrididae and Tettigoniidae). Temperature and light availability do not differ between the two exclosure types (Hahn & Orrock 2015a). In order to install the exclosures and barriers, we removed the leaf litter and then replaced leaf litter (~1 cm) back into the barriers before we added the seeds, so the seeds were added on top of the leaf litter. The overstorey tree thinning treatment also reduced litter cover through the process of extracting trees, resulting in lower litter cover in these treatments. Plots were watered once immediately after adding seeds. We note that the installation process disturbed the top few cm of soil (~2–3 cm), but this effect was equal across all treatments. Seed additions occurred between 21 and 26 March 2013, which is near peak seedling establishment in the longleaf pine ecosystem (Glitzenstein *et al.* 2001). Although arthropods (beetles and ants) and small rodents are dominant seed predators in our system (Stuhler & Orrock 2016), the timing of our seed additions narrowed the window for granivores to consume the seeds. The seed-addition experiments described here use 32 one-ha experimental patches, with eight full replicates of land-use history (non-agriculture or post-agriculture), overstorey tree thinning (thinned or unthinned) and consumer exclosures (full exclosures or pseudo-exclosures).

We conducted a seedling transplant experiment in 2012–2013 in plots located within 2–3 m of the seed-addition experiment described above. Plants were obtained by germinating seeds collected on SRS and grown for approximately 60 days in a glasshouse at the Savannah River Ecology Laboratory. Seedlings were grown in a 1:1 mixture of potting soil and sand in mesh-bottom glasshouse trays (35 cm × 35 cm × 5 cm deep, 2 cm mesh). Plants of similar sizes were paired and haphazardly transplanted into each tray. Each tray received a total of eight plants, consisting of two individuals of each of the four study species. For this experiment, we transplanted a total of 1024 seedlings of the four species ($n = 264$ seedlings of each species) into the field between 2 and 26 July 2012. The trays were buried so that the top of the tray was flush with the soil surface. The open mesh bottom of the trays were directly exposed to the soil so that the plants could root into the native soil at each site allowing us to directly test whether plant performance differed between land-use histories. Observations upon extracting the plants at the end of the experiment confirmed that the plants rooted in the native soil in all treatment combinations (P.G. Hahn, personal observation). Grasshopper exclosures (described above) were installed immediately after the trays were transplanted into the field and were applied as split plots, with the one-hectare landscape manipulations (combinations of land-use history and canopy thinning) as the whole plots. We supplemented water during the first three weeks after transplanting into the field. The transplant experiments used 64 one-hectare experimental patches, with sixteen full replicates of land-use history (non-agriculture or post-agriculture), overstorey tree thinning (thinned or unthinned) and consumer exclosures (full exclosures or pseudo-exclosures).

DATA COLLECTION

We counted the cumulative number of seedlings that established in the seed-addition plots during two surveys periods: early June and late August 2013. On the transplanted seedlings, we measured herbivore damage and plant performance at three time points: September 2012, June 2013 and September 2013. In September 2012 and June 2013, we estimated plant-level herbivory as the proportion of leaves with > 5% leaf tissue removed by chewing insects. This is a non-destructive sampling technique that estimates the relative, not absolute, damage to plants (Root 1996). We recorded plant survival in June 2013, approximately 1 year after planting. To reduce shading from overcrowding, we thinned each plot to one randomly selected individual plant per species in June 2013. In September 2013, we recorded whether or not a plant flowered and then destructively sampled the plants by collecting 3–6 leaves at regular intervals along the tallest stem and scanned these leaves using a portable scanner (VuPoint Solutions, City of Industry, CA, USA). We then calculated the area removed by leaf chewing insects using IMAGEJ (National Institutes of Health, Bethesda, MD, USA) and converted this to the proportion of leaf tissue removed. We did not differentiate chewing damage caused by different insect guilds (e.g. orthopertans, coleopterans, lepidopterans). During this last survey, many of the plants became prohibitively large to count all the leaves. We harvested all above-ground plant material in September 2013 and measured dried above-ground biomass production. All four of the species are able to reproduce clonally and all of our measures were at the genet level. In sum, we quantified plant performance of four ontogenetic stages over the two-year study: seedling establishment, first-year survival, flowering in year two and above-ground biomass production after the second year. We also surveyed grasshopper abundance along three 15 × 1 m transects in each patch during September of 2012 and 2013. We used a stick (approximately 1 m long) to perturb the vegetation as we walked along the transects and counted grasshoppers as they flushed (Hahn & Orrock 2015b). We recorded the cover of herbaceous vegetation in four 1-m^2 quadrats along each transect.

Herbivore exclosure cages effectively reduced the proportion of leaves damaged in September 2012, but not at subsequent surveys (Appendix S3). Most of the chewing damage in the June 2013 survey appeared to be cause by leaf beetles (Family Chrysomelidae) prior to our survey (P.G. Hahn, personal observation). We also observed a more diverse assemblage of insects on the experimental plants during both surveys in 2013 and in both exclosures types, including aphids (n = 21 total plants with aphids), stem galls (n = 11 on *Solidago odora* and n = 1 on *S. nemoralis*) and lepidopteran larvae (n = 7; P.G. Hahn, personal observation). However, we did not intensively search the plants for the presence or abundance of insects during our surveys, because our focus was on the response of plant growth between the exclosure types.

STATISTICAL ANALYSES

We used linear mixed models to separately analyse each of the four measures of plant performance for each of the four species. We focused our analyses on performance metrics, rather than explicitly taking a demographic modelling approach, because our data only encompassed the first 2 years, which may not appropriately capture longer-term dynamics for long-lived perennial herbs (Lauenroth & Adler 2008). In our models, the response variable was the metric of performance for the four ontogenetic stages (i.e. number of seedlings established, survival after 1 year, flowering in year two and above-ground biomass production in year two). Land-use history, overstorey tree thinning, herbivory (full exclosure or pseudo-exclosure) and all interactions were fixed effects. We included site as a random (blocking) effect and site × land-use history × overstorey tree thinning as a random effect to account for the grasshopper exclosures being applied as a split-plot design.

To evaluate the effects of herbivore damage by chewing insects in the second year of the study (i.e. when the plants were also attacked by non-orthopteran insects) on final plant biomass and whether this relationship varied among habitat types, we constructed linear mixed models with the proportion of leaves damaged in June 2013, land-use history, overstorey tree thinning and all interactions as fixed-effect predictor variables. The response variable was the final dried above-ground biomass (g). We pooled plants from both exclosure treatments for this analysis, because there was no difference in damage between the exclosure types in 2013 (Appendix S3). Site was included as a random effect. We conducted similar analyses using the proportion of leaf tissue removed measured in September 2013. However, this measure of herbivore damage was not related to final plant biomass (data not shown). Finally, we evaluated grasshopper abundance in each plot using linear mixed models. We included herbaceous plant cover (continuous variable), land-use history and thinning as fixed effects. Site was included as a random effect. The response variable, grasshopper counts, was averaged across the three transects per patch and across the 2 years.

We natural log-transformed the number of seedlings established (+1) and above-ground biomass (+0 or +1) for all species to meet the assumptions of normality and homogeneous variance, but did not transform any of the other response variables. We averaged survival of the two plants within each exclosure after 1 year. We examined the residuals of all models to ensure they did not violate the assumptions of normality and homogeneity of variance. All statistical analyses were conducted using the mixed procedure in SAS version 9.4 (SAS Institute., Cary, NC, USA).

Results

In the seed-addition experiment, establishment of three species significantly increased inside the grasshopper exclosures, averaged across all other treatments (Fig. 1, Table S1). For *S. odora*, the exclosure treatment was only evident in the unthinned non-agricultural habitats (exclosure × land-use × thinning interaction: $F_{1,28}$ = 5.5, P = 0.026). There was also some evidence for greater establishment in non-agricultural or thinned patches. *Solidago nemoralis* had marginally significantly greater establishment in non-agricultural compared to post-agricultural sites ($F_{1,21}$ = 3.4, P = 0.078). The land-use history by thinning interactions was significant or marginally significant for *Se. tortifolius* ($F_{1,21}$ = 3.7, P = 0.069) and *So. odora* ($F_{1,21}$ = 4.5, P = 0.046). Both species had higher establishment in unthinned post-agricultural patches and thinned non-agricultural patches compared to thinned post-agricultural patches and unthinned non-agricultural patches, averaged across the exclosure treatments (Fig. 1b,d).

Grasshopper exclosures affected juvenile survival of all four plant species and also interacted with other factors (Table S1). *Carphephorus bellidifolius* was affected by the three-way interaction between exclosures, land-use history and overstorey thinning ($F_{1,56}$ = 4.2, P = 0.046), with the greatest reductions in survival due to grasshopper herbivory in the thinned, post-agricultural treatment and the unthinned non-agricultural treatment (Fig. 2a). *Sericocarpus tortifolius* ($F_{1,56}$ = 5.9, P = 0.019), *Solidago nemoralis* ($F_{1,56}$ = 8.9, P = 0.004) and *So. odora* were all affected by the interaction between grasshopper exclosures and thinning, although the effect was only marginally significant for *So. odora* ($F_{1,56}$ = 3.0, P = 0.091). For all three of these species, grasshopper herbivory reduced survival in unthinned patches, but not in thinned patches (Fig. 2b–d).

Biomass production varied widely among the four species, with maximum above-ground biomass production of the largest species *So. nemoralis* being over 10-fold greater than the smallest species *C. bellidifolius*. *Sericocarpus tortifolius* produced the second highest levels of above-ground biomass and *So. odora* produced the third highest values (Fig. 3). Biomass production of all four species was at least five times greater in thinned patches compared to unthinned patches (Fig. 3; Table S1). For *Se. tortifolius* ($F_{1,40.4}$ = 4.3, P = 0.046) and *So. nemoralis* ($F_{1,53.7}$ = 4.4, P = 0.042), the thinning effect was significantly greater in non-agricultural sites compared to

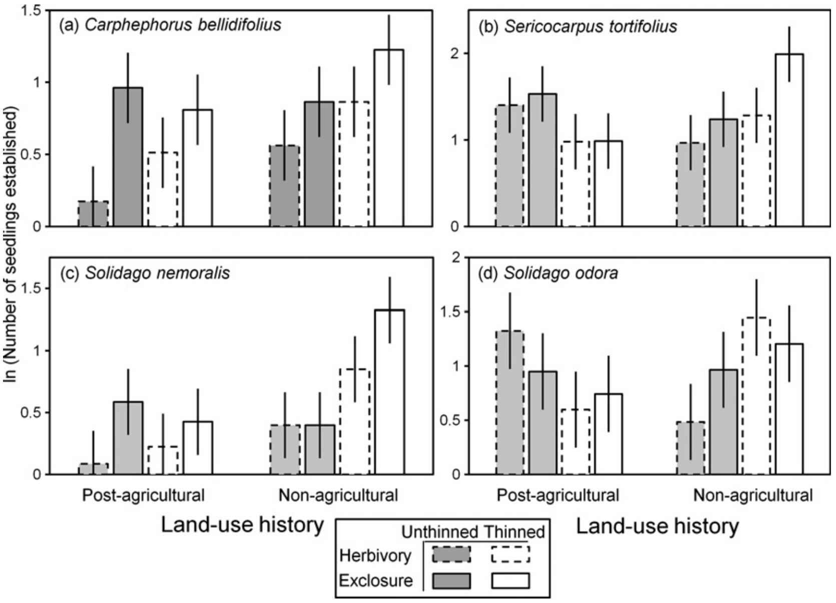

Fig. 1. Seedling establishment after one growing season for four species of perennial herbs (a–d). The experiment, conducted at the Savannah River Site, South Carolina USA, manipulated overstorey tree canopy (unthinned or thinned) and access to grasshopper herbivores (open or exclosures) in post-agricultural (> 60 years since abandonment) and non-agricultural (no history of agriculture) habitats. Note that the *y*-axis changes among species. Error bars are 1 SE.

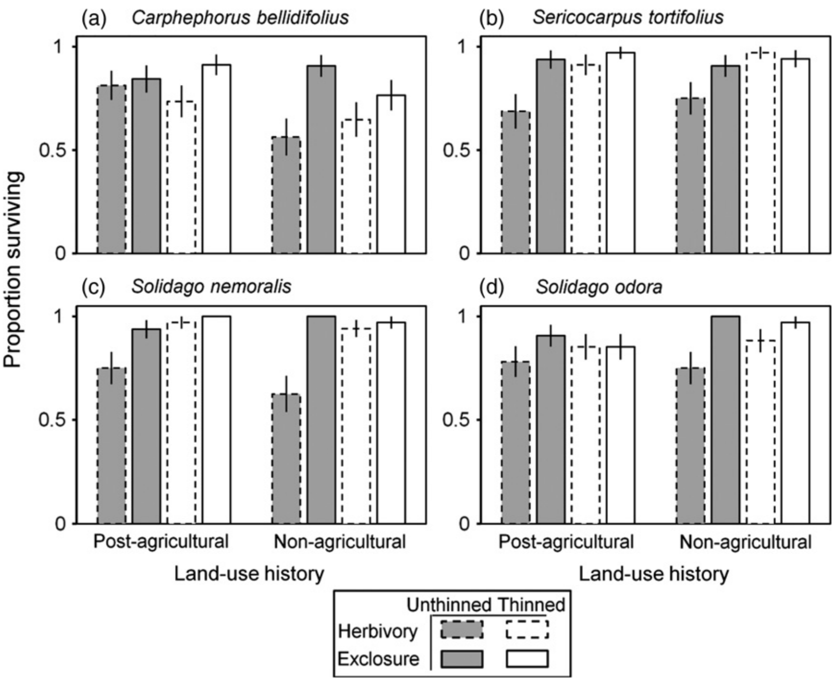

Fig. 2. Proportion of juvenile plants surviving after 1 year for four species of perennial herbs (a–d). The experiment, conducted at the Savannah River Site, South Carolina USA, manipulated overstorey tree canopy (unthinned or thinned) and access to grasshopper herbivores (open or exclosures) in post-agricultural (> 60 years since abandonment) and non-agricultural (no history of agriculture) habitats. Error bars are 1 SE.

post-agricultural sites (i.e. land-use history × thinning interaction). The magnitude of the exclosure effect also differed between the thinning treatments for *So. nemoralis* ($F_{1,70.2} = 4.5$, $P = 0.038$), although the exclosure treatments was not significant within either of the thinning treatments (linear contrast for the exclosure effect within unthinned patches: $P = 0.72$; exclosure effect within thinned patches: $P = 0.78$). The general patterns for plants flowering in the second year were qualitatively similar to the results of the biomass production analysis (Table S1, Fig. S1).

Biomass production was affected by the interaction between the proportion of leaves damaged in June 2013 and the thinning treatment for two plant species: *Se. tortifolius* and *So. nemoralis* (Table S1). For both species, the relationship between biomass and herbivore damage was negative in thinned patches and flat (*Se. tortifolius*) or positive (*So. nemoralis*) in unthinned patches (Fig. 4b,c). Biomass production of *C. bellidifolius* and *So. odora* was not related to the proportion of damaged leaves in June 2013 (Table S1; Fig. 4a,d). Land-use history did not affect the relationship between leaf damage and biomass for any species (Table S1).

Grasshopper abundance was greater in thinned patches compared to unthinned patches and was positively related to herbaceous plant cover in thinned patches (Appendix S4). Land-use history did not affect grasshopper abundance (Appendix S4).

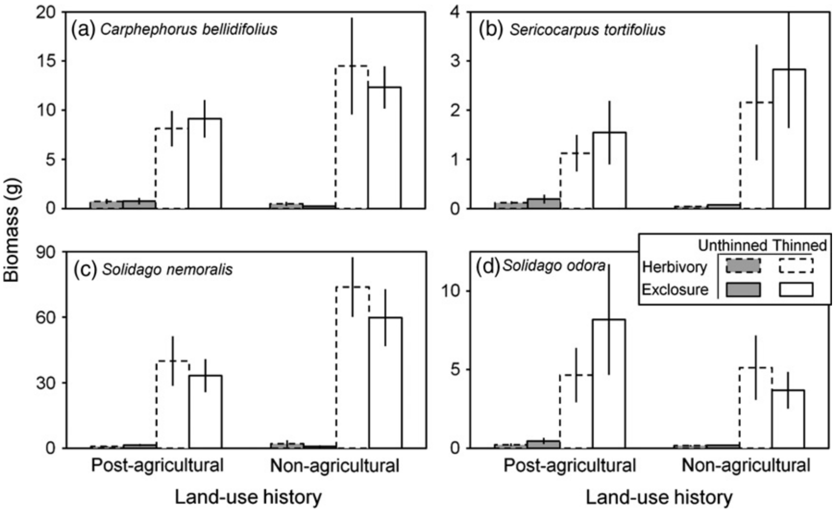

Fig. 3. Biomass (g) of four species of perennial herbs (a–d) that survived 2 years. The experiment, conducted at the Savannah River Site, South Carolina USA, manipulated overstorey tree canopy (unthinned or thinned) and access to grasshopper herbivores (open or exclosures) in post-agricultural (> 60 years since abandonment) and non-agricultural (no history of agriculture) habitats. Note that the *y*-axis changes among species. Error bars are 1 SE.

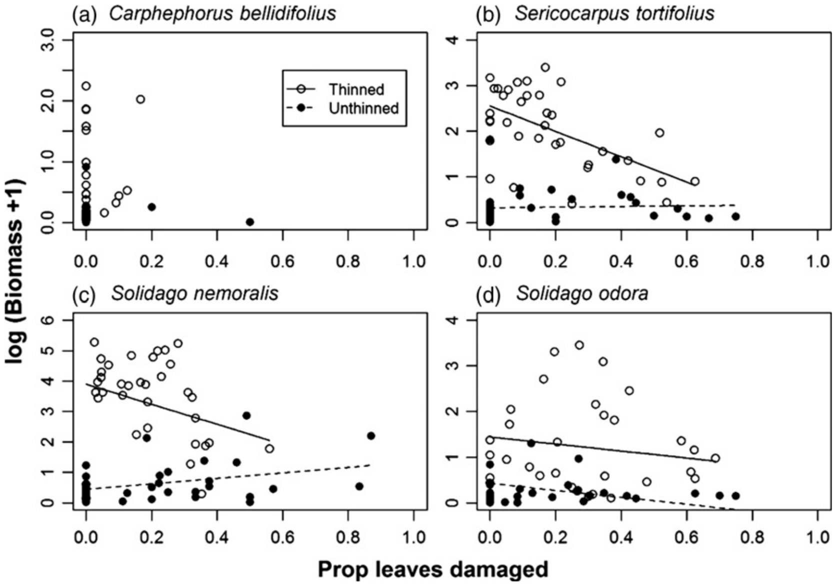

Fig. 4. Relationship between the proportion of leaves damaged by chewing insects in June 2013 and final above-ground biomass of two-year-old plants harvested in September 2013 from thinned (open dots) or unthinned (closed dots) longleaf pine woodlands. These relationships did not differ among land-use histories (Table S1), so for clarity only the relationships within the overstorey thinning treatments are shown. Note that the *y*-axis changes among species.

Discussion

By linking local herbivore exclosures with experimental manipulations of large-scale habitat factors, our work provides several insights regarding the role of herbivores and environmental context in shaping performance of multiple ontogenetic stages on four plant species. First, as expected, we found that grasshopper herbivory caused the greatest reduction in plant performance on earlier ontogenetic stages, particularly seedling establishment, and in most cases, this was independent of habitat (Fig. 1). We also found support for our second hypothesis that herbivory has the greatest effects on limiting plant performance in low-light conditions: in most instances, juvenile survival was reduced by grasshoppers under canopy where light was limited (Fig. 2). Grasshopper herbivory did not affect adult performance of any of the four plant species in the second year (Fig. 3). However, counter to our expectations, damage by all insects reduced biomass production of adult plants for two plant species and this effect was only evident in thinned patches where ground layer productivity, grasshopper abundance (Fig. 4, Appendix S4) and likely other insect taxa (Joern & Laws 2013) are higher. We found partial support for our third hypothesis that herbivory would have stronger effects on plant performance in post-agricultural habitats (Hahn & Orrock 2015a; de la Peña *et al.* 2016), although the effects of thinning were so large that they may have damped differences between post-agricultural patches (Hahn & Orrock 2015b). Collectively, our work has several key implications. First, it suggests that environmental context is clearly important for understanding plant–herbivore interactions and it demonstrates that the effect of context differs among ontogenetic stages. Secondly, our results illustrate that past land-use history can have long-lasting effects on plant performance that are independent of herbivory.

CONTEXT-DEPENDENT EFFECTS OF HERBIVORY ON PLANT PERFORMANCE

Mortality of seedlings or juvenile plants due to invertebrate herbivores can exert strong pressures on patterns of plant recruitment and community composition (Allan & Crawley 2011; Barton & Hanley 2013). We found strong negative effects of grasshopper herbivory on seedling establishment and juvenile plant survival of all four plant species. For three species herbivory only reduced juvenile survival in habitats with intact canopies (Fig. 2). Thus, our results suggest that herbivory can reduce the performance of early life stages when growth is limited by low-resource conditions, such as competition for light or soil resources (i.e. in the unthinned patches in our experiment). Our findings of greater effects of herbivory on plant performance in low-resource environments have been partially supported by other studies (Maschinski & Whitham 1989; Hawkes & Sullivan 2001; Wise & Abrahamson 2007). However, the combined effects of herbivory and resource limitation were most apparent for plants in earlier life stages, as we found the strongest negative effects of herbivory on seedling establishment and juvenile survival.

In the second year of the transplant experiment, we found no effect of grasshopper exclosures on flowering, biomass production or chewing damage. However, we did find that the proportion of leaves damaged in early in the season reduced plant biomass at the end of the growing season for two of the four species (*Se. tortifolius* and *So. nemoralis*) in thinned patches (i.e. high-resource environments) but not in unthinned patches (Fig. 4). These patterns are potentially explained by two non-mutually exclusive mechanisms. First is the switch in the dominant herbivore taxa that we observed between the first- and second-year plants. Much of the early season chewing damage on second-year plants appeared to be caused by leaf beetles (Family Chrysomelidae) prior to our first survey in June (P.G. Hahn, personal observation). We also observed higher frequencies of lepidopterans, coleopterans and hemipterans particularly in thinned patches (P.G. Hahn, personal observation). These taxa were rarely observed on first-year plants, either the transplanted seedlings in 2012 or the seedlings established from the seed-addition experiment in 2013. All of these insect taxa can be more specialized than orthopterans (Forister *et al.* 2015), which are often generalist herbivores (Joern 1985). Furthermore, because larvae of these specialist insect taxa often arrive on a host plant via oviposition by an adult, specialists may be less likely to colonize seedlings and juvenile plants (Price & Wilson 1979; Castagneyrol *et al.* 2013). This point is bolstered by our finding that the grasshopper exclosures were effective at increasing seedling establishment as part of the seed-addition experiment, but did not reduce herbivory on the adult plants in the same year.

The second potential explanation for why herbivory reduced performance of adult plants in the second year only in thinned patches is that herbivory in high-resource environments may reduce a plant's ability to acquire carbon via photosynthesis, whereas plants in low-resource environments are maintained below their maximum potential growth rate and herbivory would not affect their ability to accumulate additional carbon, as postulated by the limiting resource model (Wise & Abrahamson 2007). As such, plants in low-resource conditions may be better able to compensate for herbivory (Hilbert *et al.* 1981; Hakes & Cronin 2012; Burghardt 2016). Interestingly, we found the opposite patterns on these plants during the first year of growth, where plants exposed to grasshopper herbivory were significantly smaller compared to plants in grasshopper exclosures, most often in the unthinned treatments (Hahn & Orrock 2015b) and these plants subsequently experienced greater mortality (Fig. 2). Compensatory growth after herbivory can be related to physiological plant traits, such as carbon storage in roots or shoots (Hochwender *et al.* 2012; Barton 2013). Thus, first-year plants may not have accumulated enough below-ground carbon storage to be able to compensate for herbivore damage, resulting in reduced growth (Hahn & Orrock 2015b) and survival (Salgado-Luarte & Gianoli 2010; Fig. 2) in low-resource environments. Finally, the two fastest growing species, *Se. tortifolius* and *So. nemoralis*, were better able to compensate for herbivore damage in shade than the two slower-growing species (Fig. 4), which is potentially related to faster-growing species' ability to more rapidly accumulate carbon after tissue removal (Atkinson *et al.* 2014).

We also found that land-use history can alter the effect that herbivores have on the performance of some plant species (i.e. the most palatable or small, vulnerable plant species). Seedling establishment of *Solidago odora* was only enhanced by herbivore exclosures in the unthinned non-agricultural patches (Fig. 1). Juvenile survival of the smallest plant species *Carphephorus bellidifolius* was reduced by grasshopper herbivory in unthinned non-agricultural patches and thinned post-agricultural patches (Fig. 2). A potential explanation for this pattern is that altered soil conditions in post-agricultural sites (Brudvig *et al.* 2013) may have impeded the ability of the slow-growing *C. bellidifolius* to capitalize on the increased light availability in thinned patches. Differences in soil properties can also affect herbivory rates by changing plant tissue quality (de la Peña *et al.* 2016). We did find differences in herbivory rates on these plants in the first year of growth in some post-agricultural habitats (Hahn & Orrock 2015b), but not on established, second-year plants in this study (Table S1). Land-use history can also alter alternative food sources available to herbivores by changing the abundance and composition of the neighbouring plant community, increasing damage to some palatable species in post-agricultural sites compared to non-agricultural sites (Hahn & Orrock 2015a, 2016). The dominant grasshopper species also differ between land-use histories (Hahn & Orrock 2015c), and these species may have slightly different dietary preferences (Hahn & Orrock 2015b), but this does not appear to drive differential herbivory between land-use histories (Hahn & Orrock 2015a). Collectively, these results demonstrate that habitat-specific effects of herbivory on juvenile plant survival may negatively affect recruitment under certain environmental conditions, specifically low-light environments and for certain plant species, most likely smaller species. The effects of land-use history on herbivory, however, seem more nuanced likely because of the numerous ecological effects of historic agriculture on contemporary ecological processes (Cramer, Hobbs & Standish 2008).

HABITAT EFFECTS ON PLANT PERFORMANCE

The interaction between land-use history and canopy structure affected adult performance of two plant species, and these effects were largely independent of herbivory. As expected, overstorey tree thinning substantially increased flowering probability and adult size for all four species (Figs 3 and S1). Land-use history can also affect plant growth and allocation (Fraterrigo, Turner & Pearson 2006; Baeten *et al.* 2011). Our study found that the effect of overstorey tree thinning on adult size was greater in non-agricultural habitats for *Se. tortifolius* and *So. nemoralis*. Similarly, nearly twice as many *C. bellidifolius* flowered in thinned non-agricultural habitats compared to thinned post-agricultural habitats, although this effect was not significant ($P = 0.11$). Interestingly, there were no differences in light conditions in these plots measured as part of a parallel experiment (Hahn & Orrock 2015b; see also Methods: Site Description). However, soil properties, such as organic matter, nutrients, do differ among land-use histories in our plots (Brudvig *et al.* 2013; see also Methods: Site Description) and microbial communities have been shown to be affected by land-use history in other grassland systems (Fierer *et al.* 2013). Viewed in the light of these previous studies, our findings suggest that altered soil conditions in post-agricultural sites may constrain the ability of plants to capitalize on the increased light availability or reduced competition with overstorey plants created by the overstorey tree thinning treatment. Reduced performance of plants that are typical of longleaf pine habitat but absent from post-agricultural sites (e.g. *C. bellidifolius* and *Se. tortifolius*) helps to explain why land-use legacies persist in plant communities even after restoration efforts are implemented (Turley & Brudvig 2016).

IMPLICATIONS FOR UNDERSTANDING CONTEXT-DEPENDENT INTERACTIONS

Understanding context-dependent plant–herbivore interactions is critical for predicting plant abundance, distributions, and population dynamics across broad spatial scales (Maron, Baer & Angert 2014). Our large-scale experimental study highlights contingencies in how plant–herbivore interactions influence the performance of multiple life stages that are important for regulating long-term population dynamics of perennial herbs (Lauenroth & Adler 2008). Our most important findings in regard to understanding context-dependent plant–herbivore interactions are that: (i) generalist herbivores (grasshoppers) have strong negative effects on early life stages only when resources are limited, (ii) a more diverse insect fauna are important at later life stages and can affect plant performance mainly in high-resource environments where insects are more abundant, and (iii) habitat context also affects plant performance independent of herbivory, which may make context-dependent herbivory difficult to detect without factorial experiments. We acknowledge that because plants often invest heavily in defence against herbivory during the most important life stages for population growth rates (Ehrlén 2003), the longer-term impacts of herbivory on population growth rates under varying environmental conditions will require continued investigation. Nevertheless, by characterizing how life stages that are important to longer-term population dynamics are affected by habitat characteristics and herbivores, our findings may help to inform future demographic models and understanding of long-term population dynamics.

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.340p0 (Hahn & Orrock 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. ANOVA table from main analyses.

Fig. S1. Proportion of experimental plants flowerings in year two.

Appendix S1. Site description and map of sites.

Appendix S2. Supplemental information on plant species and seed collection.

Appendix S3. Effectiveness of the herbivore exclosures.

Appendix S4. Grasshopper abundance in the experimental patches.