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Research Article

Investigating the Role of Soil Legacy Effects in the Management of *Lespedeza cuneata*, an Invasive Legume

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ABSTRACT

Invasive plant species present a growing threat to biodiversity. Many invasive plants recruit microbial symbionts in their novel range that influence their growth and fitness. These soil legacy effects can linger after the removal of invasive species and impact restoration efforts. In a growth room experiment to investigate the legacy effects of an invasive legume and herbicide, *Lespedeza cuneata* was grown with either an intraspecific competitor or a native species in field-collected soils with and without history of an *L. cuneata* invasion and use of glyphosate herbicide. In separate experiments, we investigated seed germination and seed bank composition in these different soil types. Taken together, results suggest that legacy effects of *L. cuneata* invasion do not significantly contribute to its growth or spread in a floodplain in eastern North Carolina. The absence of soil legacy effects, which have been documented in other systems, may be attributed to frequent flooding observed in the system and the resulting homogenization of soil biota. Study findings also suggest that the application of glyphosate herbicide creates areas where *L. cuneata* can readily reinvade, as it significantly reduced the number and diversity of seedlings to emerge from the seed bank while increasing the aboveground biomass and nodule formation of *L. cuneata*. Concerning the restoration of native flora, data suggest that *Chasmanthium latifolium* may be negatively impacted by a *L. cuneata* invasion or glyphosate herbicide, while *Solidago altissima* and *Chamaecrista nictitans* could be successful in preventing areas from being reinvaded.

Index terms: glyphosate; invasive species; *Lespedeza cuneata*; plant ecology; restoration ecology; soil legacy effects

INTRODUCTION

Invasive species are an increasing threat to biodiversity across the globe (Wittenburg and Cock 2001). In addition to endangering aboveground flora and fauna (Butchart et al. 2010; Skórka et al. 2010), invasive plant species have also been found to alter the composition and diversity of the seed bank, with implications for the long-term composition of the plant community (Gioria and Osborne 2009a, 2009b). Invasive plant management is likely to benefit from efforts to restore populations of native plants. In addition to improving soil stability, native biodiversity, and ecosystem dynamics, increasing the abundance of native species also constrains and combats the spread of invasive species (Bakker and Wilson 2004).

The interactions between a plant and the soil it occupies can contribute to, or hinder, the success of an invasive species. When faced with barriers to establishment in their novel ranges nonnative plants must often rely on symbiotic relationships belowground to assist them in the formation of their invasive population (Richardson et al. 2000), both during seed germination (Meng et al. 2022) and later plant growth phases. In one such symbiosis, legumes form mutualistic relationships with rhizobia bacteria in the soil that convert atmospheric nitrogen into an organic form that plants can use (Sprent et al. 1987). Mutualistic soil symbionts are often generalists while soil pathogens tend to have more specialist relationships shaped by coevolution (Callaway and Aschehoug 2000). As a result, the predominantly positive plant–soil interactions can provide nonnative species a competitive advantage against native species. During the course of invasion, populations of exotic plants can accumulate soil mutualists, including soil rhizobia (Coykendall and Houseman 2014), but they might also be suppressed through time if the plant accumulates soil pathogens (Wolfe and Klironomos 2005). Negative feedbacks can also occur if the plant depletes the soil of required nutrients (Bennett and Klironomos 2018). Invasive plants may also influence the establishment of native plant species (Corbin and D'Antonio 2012) through soil legacy effects, such as accumulation of soil pathogens (Mangla and Callaway 2008), allelopathic compounds (Dommanget et al. 2014), or increased nitrogen levels (Nsikani et al. 2017). The consequences of such soil alterations are difficult to predict, as they are affected by the traits of specific species, community density, climate, and substrate conditions (Hess et al. 2019).

Beyond the soil legacy effects related to microbial communities, invasions can alter the composition of the community of dormant seeds in the soil, or seed bank (Gioria and Pyšek 2015). Clearly, the rate and spatial extent of invasion will depend upon seed production and dispersal mechanisms of the invading species (Landenberger et al. 2007) and the density of its seed bank. Furthermore, unassisted reestablishment of native plants after the removal of invasives will require the presence of their seeds, and the extent to which native seed banks persist after invasion might affect the potential for recovery of the plant community.

Protocols used to manage invasive plants, such as herbicide application, can also impact soils, with potentially lasting consequences. Glyphosate, a broad-spectrum, widely used herbicide, has been effective in managing invasive plants;

however, repeated use of this herbicide has caused some areas to experience a change in the composition of the herbaceous plant community (Duke and Powles 2008) as well as an increase in the number of species developing a resistance to the chemical (Benbrook 2016). In soils containing elevated concentrations of heavy metals, the application of glyphosate herbicide was found to significantly increase the leaching of copper, zinc, nickel, and other heavy metals present (Barrett and McBride 2006). In addition, soils treated with glyphosate herbicide have been found to have reduced soil microbial biomass and decreased dehydrogenase activity (Tejada 2009). Therefore, herbicide applications to invasive species may have complex effects on both native and nonnative plant species.

This study addressed soil legacy effects in an invasive legume, *Lespedeza cuneata* (Dum. Cours.) G. Don, and their potential consequences for management and restoration of a plant community in eastern North Carolina. Previous studies indicate that *L. cuneata* alters properties of the chemistry and the bacterial and fungal communities of the soil it occupies (Yannarell et al. 2011; Coykendall and Houseman 2014), with positive effects on the growth of *L. cuneata* (Coykendall and Houseman 2014) and its ability to outcompete native species (Crawford and Knight 2017). Much of the research on this invasive legume has focused on prairie habitats of the Midwest (Eddy et al. 2003; Silliman and Maccarone 2005; Coykendall and Houseman 2014; Quick et al. 2016; Crawford and Knight 2017), with a much smaller proportion taking place in the grasslands of eastern North America (Yannarell et al. 2011) where the invasion first began. Here, we investigate the soil legacy effects of *L. cuneata* in a different habitat where an invasion is ongoing—a floodplain in the coastal plain of eastern North Carolina. In a series of growth room experiments, we asked how a history of a *L. cuneata* invasion and the subsequent use of glyphosate herbicide may influence seed germination and plant growth of *L. cuneata* and three native plant species. We also asked how an *L. cuneata* invasion and use of herbicide impacts the naturally occurring seed bank. The objective of the study was to gain a better understanding of how the soil legacy effects, both from an invasive legume and the use of glyphosate herbicide, may influence the success of the invasive species and to help tailor efforts to restore native plant populations following the removal of *L. cuneata*.

METHODS

Study Site

This study was focused on the South Tar Greenway in Pitt County, Greenville, North Carolina, a site of a *Lespedeza cuneata* invasion. The greenway runs through a cypress swamp in the flood plain of the Tar River. The plant community is dominated by bald cypress (*Taxodium distichum* (L.) Rich.), water swamp tupelo (*Nyssa biflora* Walter), and an understory of river oats (*Chasmanthium latifolium* (Michx.) Yates), and several species in the Asteraceae including tall goldenrod (*Solidago altissima* L.). While no congeners of *L. cuneata* are known to be present on the greenway, a few other native legumes are present including native panicled-leaf ticktrefoil (*Desmodium paniculatum* (L.) DC) and sensitive partridge pea (*Chamaecrista nictitans* (L.) Moench). The invasive legume kudzu (*Pueraria montana* (Lour.) Merr.) is present on the greenway, but not at sites within the range of this study. The trail was constructed in 2011, and by 2014 dense patches of *Lespedeza cuneata* were present. We suspect that the sand used to construct the trail likely contained seeds that were the source of the initial invasion, since the species is not common in surrounding areas. To manage the invasion, glyphosate herbicide has been applied once per year during July or early August at application rate of approximately 1 lb glyphosate acid per acre in highly localized areas that are especially dense.

Lespedeza cuneata

Lespedeza cuneata is a shrubby perennial with trifoliate leaves and small white flowers with purple markings. *Lespedeza cuneata* is abundant in disturbed areas with high light availability (Remaley 1998) and can establish massive seed banks that remain viable for several years (Barnewitz et al. 2009).

Native Species

Three native herbaceous plant species that are abundant at the study site were used in the study: *Chamaecrista nictitans* (Fabaceae), *Chasmanthium latifolium* (Poaceae), and *Solidago altissima* (Asteraceae). *Chasmanthium latifolium* (river oats) is a rhizomatous perennial grass growing up to 1.5 m tall. This species is popular in the native plant trade, as it is tolerant of shade and is a larval host plant for several native pollinators. *Solidago altissima* (tall goldenrod) is commonly found in disturbed areas where it spreads by both seed dispersal and rhizomatous growth. *Chamaecrista nictitans* (sensitive partridge pea), an annual nodule-forming legume, was included to allow us to compare the competitive ability of an invasive and native legume. All three species are contenders for native plant restoration following the removal of *L. cuneata* on the South Tar Greenway.

Seed Collection and Germination

Seeds of *L. cuneata*, *S. altissima*, *Cham. nictitans*, and *Chas. latifolium* were collected at several locations along the Greenville South Tar Greenway in fall 2018, bulked together, and stored at 4 °C until germination. Prior to germination all seeds were sterilized in a 5% bleach solution for 5 min, then rinsed thoroughly. Germination of *L. cuneata* was optimized by soaking seeds in sulfuric acid for 20 min and then rinsing thoroughly (Bentley 1933), as done in similar studies on the species (Coykendall and Houseman 2014). This scarification process is necessary to prevent the low germination success often observed in lab studies (Bentley 1933). Seeds of *Cham. nictitans* were nicked using a razor and then soaked in water for 24 hr (Carino and Daehler 2002). In both leguminous species, scarification procedures erode the outer husk that protects the seed, which would otherwise be removed through natural dispersal processes such as passage through an animal gut.

Growth Experiment

In May 2019, soils were collected from seven sites along the greenway. Sites were all located within a 0.10 km^2 area on the greenway, with the most distant sites being 0.74 km from one another. At each site, soil was collected from three areas within 8 m of one another for each of the three soil conditions used in this experiment. At each site soils were collected from areas that (1) had been invaded with *L. cuneata* (invaded), (2) were previously invaded with *L. cuneata* and sprayed with glyphosate herbicide the year prior to the experiment (sprayed), and (3) had no history of *L. cuneata* invasion or herbicide spraying within 4 m (uninvaded). Although the exact history of invasion at each site was unknown, patches were chosen that contained plants of sufficient size and stem girth to indicate several years of growth and *L. cuneata* stem densities of at least 10 plants per square meter. Soils were collected at a depth of 20 cm. Spades were wiped clean using ethanol between soil collections to minimize cross contamination of soil microbial communities. Soil samples were double bagged in plastic zip sealed bags and stored at room temperature.

The following day, debris was removed from field-collected soil. Plastic 10.16 × 10.16 cm pots were filled with a mixture of 25% field-collected soil and 75% Sungro (Sun Gro Horticultural, Agawam, Massachusetts) professional growing mix containing no added nutrients. For the remainder of this manuscript, we refer to soil treatments simply as invaded, sprayed, and uninvaded. However, the field-collected soil types account for only 25% of the soil by volume. This was done to introduce biotic components but minimize abiotic differences in the various field-collected soils that were not the focus of this study.

To test for the influence of soil legacy effects on *L. cuneata*, two individuals of the species were grown in each of the three soil types: invaded, sprayed, and uninvaded. Four replicate pots were used from each of the 21 soil collections, for a total of 84 pots (3 soil types × 7 sites × 4 replicates). To analyze the influence of *L. cuneata* soil legacy effects on growth and competition in native plants, individuals of the three native species were grown in the three soil types from each of the seven sites under two neighbor identities: (1) grown with another individual of the same native species (intraspecific), and (2) grown with an individual of *L. cuneata* (interspecific). Each soil × neighbor identity combination was replicated in four pots, resulting in a total of 504 pots (3 soil types × 7 sites × 2 neighbor identities × 3 native species × 4 replicates).

Following the preparation of soils, the appropriate seedlings were transferred into experimental pots from the trays of greenhouse soil where they had been germinated. Because seed germination was not completely synchronous, seedlings ranged somewhat in size and age. In each pot, care was taken to pair individuals of approximately the same size. Seedling transfers were conducted so that each soil type and site contained a range of seedling sizes and ages. For 2 wk following the initial transfer of seedlings into pots, dead seedlings were replaced to minimize losses to sample sizes.

Plants were raised in a growth room with mixed natural and artificial lighting. Artificial lighting was set to a 14 hr light period, followed by a 10 hr dark period. Temperatures within the room did not exceed 27 °C during the day and did not cool below 20 °C at night. All plants were watered from above as needed, the rate of which was influenced by room temperature and plant size. A layer of sterile sand was placed on top of the soil in each pot to minimize possible cross contamination of microbes from adjacent pots during watering (Crawford and Knight 2017). Pots were placed in plastic trays, which were rotated around the room twice weekly to reduce positional effects. Trays contained pots of the same soil type to limit the effects of contamination that might occur during watering. Within that constraint, the position of pots in trays was randomly assigned.

Plant size data were collected during the first weeks after potting and included in analyses as an explanatory variable. Methods for measuring initial size varied among species to accommodate their different morphologies. The number of leaves on *L. cuneata* and *Cham. nictitans*, the height of *Chas. latifolium*, and the width of the broadest leaf on *S. altissima* individuals were recorded. Mortality was recorded throughout the experiment. After 13 wk, all plants that had survived at least 10 wk were cut off at ground level, dried in an oven at 60.0 °C for 24 hr and weighed. For each pot containing two individuals of *L. cuneata*, soil was rinsed off and the root nodules were counted on both plants.

Germination Experiment

In September 2019, soils were collected again from the 21 locations on the South Tar Greenway using the methods described above. Seeds were germinated in 7.62 × 7.62 cm square pots consisting of four cells, with nine replicate pots for each of the 21 soils collected. The bottom half of each cell was filled with moist Sungro professional potting mix, the top half was then filled with field-collected soil. Each cell received seeds of a different species, prepared using the germination protocols described above: ten seeds of *L. cuneata*, ten seeds of *S. altissima*, five seeds of *Chas. latifolium*, or four seeds of *Cham. nictitans*. Seedlings of species not planted were removed as they emerged from the seed bank. Pots were placed in trays that were rotated about the room regularly in pairs to control for positional effects. Each pair of trays contained a replicate from each of the 21 soils collected, arranged randomly. Seed germination and mortality were recorded for 5 wk, after which no new germinant emerged.

Seed Bank Experiment

To see how the naturally occurring seed bank is impacted by a history of a *L. cuneata* invasion or the application of glyphosate herbicide, a separate study was conducted using field soil collected in September 2019. The soil was cold moist stratified at 4 °C for one month after field collection. The experiment was replicated on five 10.16 × 10.16 cm pots for each of the 21 soils collected. The bottom half of all pots were filled with moist Sungro professional potting mix. On top of the potting soil, 150 mL of field-collected soil was spread evenly. Pots were then placed in trays containing one replicate of each of the 21 soils and watered regularly for the duration of the experiment. Trays were rotated around the growth room every 3–4 d.

Seedlings were identified to species when possible and removed after they were identified and counted. Native vs. exotic status was recorded for each identified species based on USDA PLANTS Database (United States Department of Agriculture 2019). Graminoids could be identified only to family by the end of the experiment, although leaf morphological traits were used to attempt to sort seedlings into distinct unknown species. Recording of data ended 13 wk later, when the rate of seedling emergence became negligible.

Statistical Analysis

All data were analyzed using SAS 9.4 statistical software (SAS Institute Inc. 2013). To analyze aboveground biomass data collected from the growth room experiment, a series of mixed models were utilized that included soil type and neighbor identity as fixed independent variables and their interaction. The soil collection site was treated as a random effect. The initial size of individuals was included in all models as a continuous explanatory variable. For intraspecific competition treatments, the mean of the two individuals grown in the pot was used for all analyses. Effects of soil history and competition on aboveground biomass were investigated in *L. cuneata* with three levels of soil type (invaded, uninvaded, sprayed) and four neighbor identities (*L. cuneata*, *S. altissima*, *Cham. nictitans*, *Chas. latifolium*). Tukey's procedure was used to determine if pair-wise differences between soil types or neighbor identities were significant. For each of the three native species, separate models were run to assess the impact of soil type and neighbor identity (*L. cuneata* or another individual of the same native species) on aboveground biomass. Tukey's procedure was again used to determine if pair-wise differences between soil types were significant.

In the analysis of nodule formation of *L. cuneata* in the growth room experiment, the mean number of root nodules per pot served as the dependent variable. In this model, the neighbor factor was excluded because nodule data were collected only for individuals grown with another *L. cuneata* plant. As with the model described above, soil type remained as the independent variable, collection site was treated as a random effect, and initial size of individuals was included as a continuous explanatory variable. Tukey's procedure was used to determine if pair-wise differences between soil types were significant.

Mixed general linear models were used to test for soil legacy effects on seed germination and seedling survival of *L. cuneata* and native species. The models defined soil type as the fixed independent variable and site of soil origin as a random variable. For each cell, the total proportion of seeds to germinate over the course of the experiment and the proportion of the seedlings to survive to end of the experiment were determined. Tukey's procedure was used to determine if the proportion of seeds to germinate or the proportion of seedlings to survive was significantly different between the three soil types.

To test for an effect of *L. cuneata* invasion and herbicide on the seedlings emerging from the seed bank, mixed general linear models were used. For each pot the total number of seedlings, species richness, and proportion of all seedlings belonging to native species were calculated. Unknown species were deleted from the data set for calculations of proportion native seedlings. Analyses were run for each of the three dependent variables using soil type (invaded, sprayed, uninvaded) as the fixed independent variable and site where soil was collected as a random effect. Tukey's procedure was used to determine if the number of seedlings, species richness, or the proportion of seedlings belonging to a native species was significantly different between the three soil types. To assess if soil type significantly impacted the number of *L. cuneata* seedlings to emerge, the total number of *L. cuneata* seedlings in each of the three soil types was compared using a chi-square test.

Table 1.—Results from the mixed general linear model for the effects of soil type (invaded, uninvaded, or sprayed), neighbor species (*Lespedeza cuneata*, *Chamaecrista nictitans*, *Chasmanthium latifolium*, or *Solidago altissima*), and their interaction on aboveground dry biomass of *Lespedeza cuneata*. Significant *P*-values (<0.05) indicated with bold.

Source	df	*F*	*P*
Soil Type	2, 284	4.25	**0.015**
Neighbor Species	3, 284	4.53	**0.004**
Neighbor Species × Soil Type	6, 284	0.50	0.812
Site × Soil Type	18, 284	1.51	0.084

RESULTS

Growth Experiment Results

Data from the growth experiment were analyzed to determine the effects of soil history, including both *Lespedeza cuneata* invasion and herbicide application, on growth and competition. Twenty individuals of *L. cuneata* that died within the first 10 wk of the experiment were eliminated from the data set. Mortality of *L. cuneata* did not differ among the three soil types ($\chi^2 = 0.4$, df $= 2$, $P = 0.819$). Dry aboveground biomass varied significantly by soil type (Table 1, Figure 1). In pairwise comparisons, mean aboveground biomass of *L. cuneata* individuals grown in sprayed soil was 22.2% greater than those grown in uninvaded soil ($t_{296} = 2.33$, $P = 0.02$) and 24.5% greater than those grown in invaded soil ($t_{296} = 2.61$, $P = 0.0.01$), while the biomass of *L. cuneata* grown in uninvaded soil and invaded soil was similar ($t_{296} = 0.28$, $P = 0.782$) (Table 2, Figure 1).

The aboveground biomass of *L. cuneata* (Table 1, Figure 1) varied significantly with neighbor identity. Individuals of *L. cuneata* grown with *Chasmanthium latifolium* had the greatest biomass, which was 29.6% greater than the biomass of those grown with another *L. cuneata*, a difference that was found to be statistically significant ($t_{296} = 2.77$, $P = 0.006$) (Table 2, Figure 1). The mean biomass of *L. cuneata* was similar when grown with another individual of the same species, *Chamaecrista nictitans* ($t_{296} = 0.53$, $P = 0.598$), or *Solidago altissima* ($t_{296} = 0.54$, $P = 0.589$) (Table 2, Figure 1). The interaction between neighbor identity and soil type was not significant in determining the biomass of *L. cuneata* (Table 1).

The number of root nodules per *L. cuneata* individual varied significantly among soil types. As with biomass, individuals grown in sprayed soils had the highest mean number of root nodules. The number of nodules formed per individual was significantly greater in sprayed soil than in invaded soil ($t_{61} = 2.73$, $P = 0.008$), which was the only pair-wise comparison determined to be statistically significant (Figure 2). Spraying was found to increase the mean number of nodules by 77.1% compared to invaded soil.

Data were also analyzed to investigate if legacy effects, caused by an *L. cuneata* invasion or by glyphosate herbicide, impacted the growth and competition of native species. One individual of *Chas. latifolium* and 19 of *Cham. nictitans* died during the first

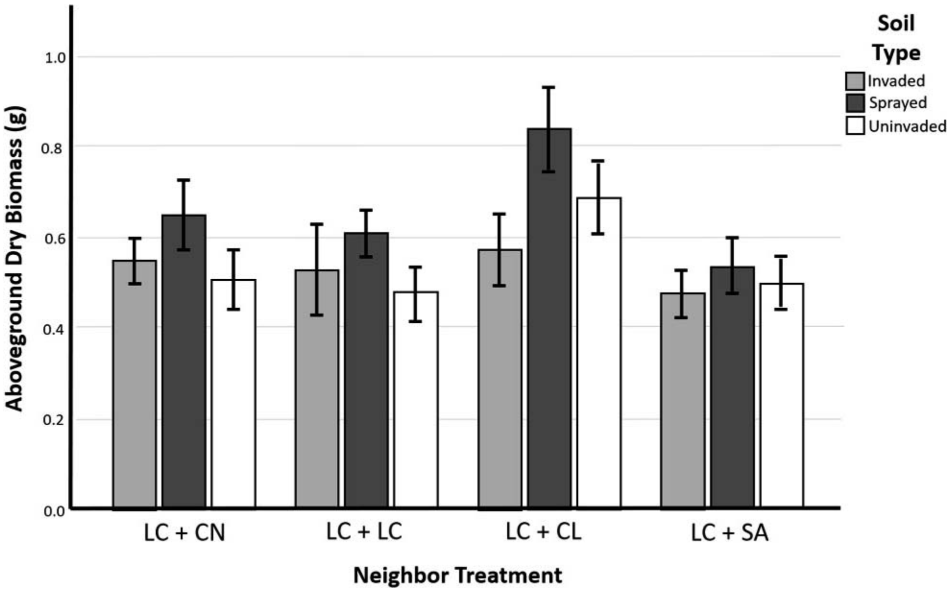

Figure 1.—Mean aboveground dry biomass (± 1 SE) of *Lespedeza cuneata* (LC) when grown with another individual of *Lespedeza cuneata*, *Chamaecrista nictitans* (CN), *Chasmanthium latifolium* (CL), and *Solidago altissima* (SA) in uninvaded (U, white), invaded (I, light gray), or sprayed (S, dark gray) soil. Aboveground dry biomass varied significantly among soil type ($F_{2,284} = 4.25$, $P = 0.015$) and neighbor identity ($F_{2,284} = 4.53$, $P = 0.004$). The interaction of neighbor × soil was not significant ($F_{6,284} = 0.50$, $P = 0.812$).

10 wk of the experiment and were therefore excluded from the biomass data set. No association was found between soil type and the mortality of *Cham. nictitans* ($\chi^2 = 2.0$, df = 2, $P = 0.368$).

While the final aboveground biomass of *S. altissima* was found to be similar across all three soil types, that of *Cham. nictitans* and *Chas. latifolium* varied significantly among soil types (Table 3, Figure 3). As with *L. cuneata,* the mean biomass of *Cham. nictitans* grown in sprayed soils was significantly greater than those grown in uninvaded ($t_{136} = 2.96$, $P = 0.004$) and invaded soils ($t_{136} = 2.10$, $P = 0.038$), which were not significantly different from each other ($t_{136} = 0.22$, $P = 0.829$) (Table 2, Figure 3A). Compared to uninvaded soil, individuals of *Cham. nictitans* grown in sprayed soil reached 32.5% greater aboveground biomass, and 36.8% greater than those grown in invaded soil. In contrast, the aboveground biomass of *Chas. latifolium* individuals grown in uninvaded soil was 22.3% greater than those grown in invaded soil ($t_{153} = 1.98$, $P = 0.050$) and 28.6% greater than sprayed soil ($t_{153} = 2.44$, $P = 0.016$) (Table 2, Figure 3B).

The identity of the neighbor had a significant effect on aboveground biomass of *S. altissima* and *Cham. nictitans* (Figure 3), but not *Chas. latifolium* (Tables 2 and 3, Figure 3). *Chamaecrista nictitans* grown with another individual of the same species had an average aboveground biomass that was 32.6% lower than those grown with *L. cuneata* ($t_{136} = 2.96$, $P = 0.004$) (Table 2, Figure 3A). Similarly, *S. altissima* grown with an individual of *L. cuneata* reached an aboveground biomass 60.8% greater than those grown with another individual of *S. altissima* ($t_{156} = 9.05$, $P < 0.001$) (Table 2, Figure 3C). The interaction between neighbor identity and soil did not significantly affect the aboveground biomass of any of the native species (Table 3).

Seed Germination Experiment Results

Of the four species included in the study, seed germination was found to vary significantly with soil type only in *S. altissima* (Table 4). Average germination was 19.4% lower in sprayed soil than compared to uninvaded ($t_{167} = 2.51$, $P = 0.013$) or invaded soil ($t_{167} = 2.52$, $P = 0.013$), which were not different from each other. Soil type did not impact the survivorship of seedlings for *L. cuneata* or any of the three native species (Table 4).

Seed Bank Experiment Results

A total of 1430 seedlings of 86 species and 34 plant families emerged from the seed bank in soil samples. Dogfennel (*Eupatorium capillifolium* (Lam.) Small) was the most abundant seedling to emerge during the 14 wk experiment, with 333 seedlings emerging in total. With 475 seedlings and 11 species,

Table 2.—Mean aboveground dry biomass (± 1 SE) of *Lespedeza cuneata*, *Chamaecrista nictitans*, *Chasmanthium latifolium*, and *Solidago altissima* for each of the three soil types (uninvaded, invaded, sprayed; averaged across neighbor identity and sites) and for neighbor identity (averaged across soil types and sites).

	Soil			Neighbor			
Species	Uninvaded	Invaded	Sprayed	*L. cuneata*	*Cham. nictitans*	*Chas. latifolium*	*S. altissima*
L. cuneata	0.54 g ± 0.04	0.53 g ± 0.04	0.66 g ± 0.04	0.54 g ± 0.05	0.57 g ± 0.05	0.70 g ± 0.05	0.50 g ± 0.05
Cham. nictitans	1.17 g ± 0.22	1.12 g ± 0.23	1.55 g ± 0.22	1.53 g ± 0.21	1.03 g ± 0.21		
Chas. latifolium	1.26 g ± 0.10	1.03 g ± 0.09	0.98 g ± 0.10	1.02 g ± 0.66		1.16 g ± 0.66	
S. altissima	5.15 g ± 0.29	4.79 g ± 0.26	4.85 g ± 0.26	6.08 g ± 0.18			3.78 g ± 0.18

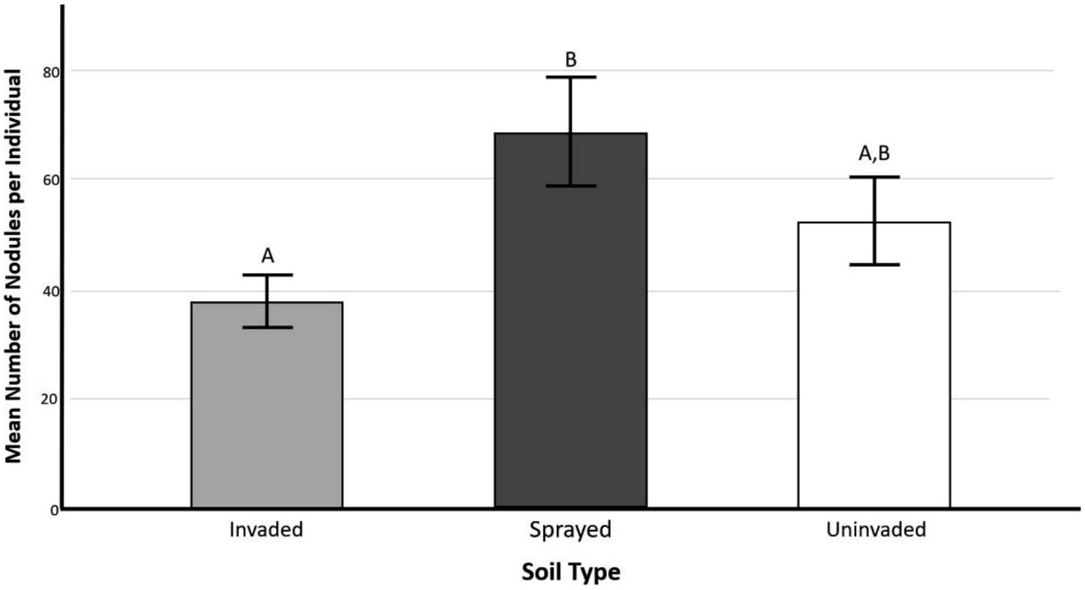

Figure 2.—Mean (± 1 SE) number of root nodules per individual of *Lespedeza cuneata* grown in invaded soils, sprayed soil, and uninvaded soil. Nodule number varied significantly among soil types ($F_{2,61} = 3.72$, $P = 0.0300$). Significant pairwise comparisons, as determined by Tukey's post hoc procedure ($P < 0.05$), are depicted using different letters.

Asteraceae was the most common family to occur. The most abundant exotic species to emerge was Brazilian vervain (*Verbena brasiliensis* Vell.) with 196 seedlings. Of the species that could be fully identified, 61.5% were native to the area while 38.5% were exotic.

The total number of seedlings that emerged from the seed bank varied significantly among soil types ($F_{2,96} = 9.96$, $P = 0.001$). A similar number of seedlings per pot emerged from invaded soils (15.83 ± 3.83, mean ± 1 SE throughout) and uninvaded soils (16.26 ± 3.83) ($t_{96} = 0.22$, $P = 0.828$). Significantly fewer seedlings emerged from sprayed soils (8.43 ± 3.83) than uninvaded soils ($t_{96} = 3.75$, $P < 0.001$) and invaded soils ($t_{96} = 3.97$, $P < 0.001$). Counter to expectations, *L. cuneata* seedlings were not more frequent in soils of invaded than uninvaded or sprayed sites ($\chi^2 = 4.59$, df = 2, $P = 0.101$). Species richness of seedlings per pot also varied with soil type ($F_{2,96} = 6.33$, $P = 0.0026$). Uninvaded soils (6.34 ± 1.12) and invaded soils (5.91 ± 1.12) had similar numbers of species per pot ($t_{96} = 0.80$, $P = 0.427$), while sprayed soil had significantly lower species richness (4.51 ± 1.12) than uninvaded soils ($t_{96} = 0.3.40$, $P = 0.0010$) and invaded soils ($t_{96} = 2.61$, $P = 0.011$). Across all replicates a total of 52 species occurred in uninvaded soils, 44 species in invaded soils, and 42 species in sprayed soils. Soil type did not significantly influence the proportion of seedlings belonging to native species ($F_{2,96} = 1.75$, $P = 0.180$).

Table 3.—Results from the mixed general linear model for the effect of soil type (invaded, uninvaded, or sprayed), neighbor type (intraspecific or *Lespedeza cuneata*), and their interaction on the aboveground dry biomass of *Chamaecrista nictitans*, *Chasmanthium latifolium*, and *Solidago altissima*. Significant *P*-values (<0.05) indicated with bold.

Species	Source	df	F	P
Cham. nictitans	Soil Type	2, 124	3.29	**0.04**
	Neighbor	1, 124	10.62	**0.001**
	Neighbor × Soil	2, 124	0.23	0.795
	Site × Soil	18, 124	2.47	**0.002**
Chas. latifolium	Soil Type	2, 141	3.41	**0.036**
	Neighbor	1, 141	2.39	0.124
	Neighbor × Soil	2, 141	0.29	0.752
	Site × Soil	18, 141	1.42	0.131
S. altissima	Soil Type	2, 144	0.84	0.434
	Neighbor	1, 144	86.30	**<0.001**
	Neighbor × Soil	2, 144	0.54	0.583
	Site × Soil	18, 144	1.48	0.105

DISCUSSION

Results from this investigation provide no evidence that legacy effects caused by an ongoing invasion contribute significantly to the growth or spread of *Lespedeza cuneata* in the floodplains of eastern North Carolina. Individuals grown in previously invaded soil were similar in size to those grown in uninvaded soil. Furthermore, a history of *L. cuneata* invasion did not increase the emergence of *L. cuneata* seeds from the seed bank, the germination success of *L. cuneata* seeds, or the survival of *L. cuneata* seedlings. In studies conducted in grassland prairie systems, however, individuals of *L. cuneata* grown in previously invaded soil grew to a significantly larger size (Coykendall and Houseman 2014; Crawford and Knight 2017) than those grown in uninvaded soil. Similar positive feedback trends have been observed in other invasive legumes, including winter vetch (*Vicia villosa* Roth; Lau and Suwa 2016). Several factors might account for the differences between this investigation and previous studies.

One factor possibly contributing to the absence of evidence in this experiment for legacy effects caused by a history of *L. cuneata* invasion may be the homogenization of soil bacterial

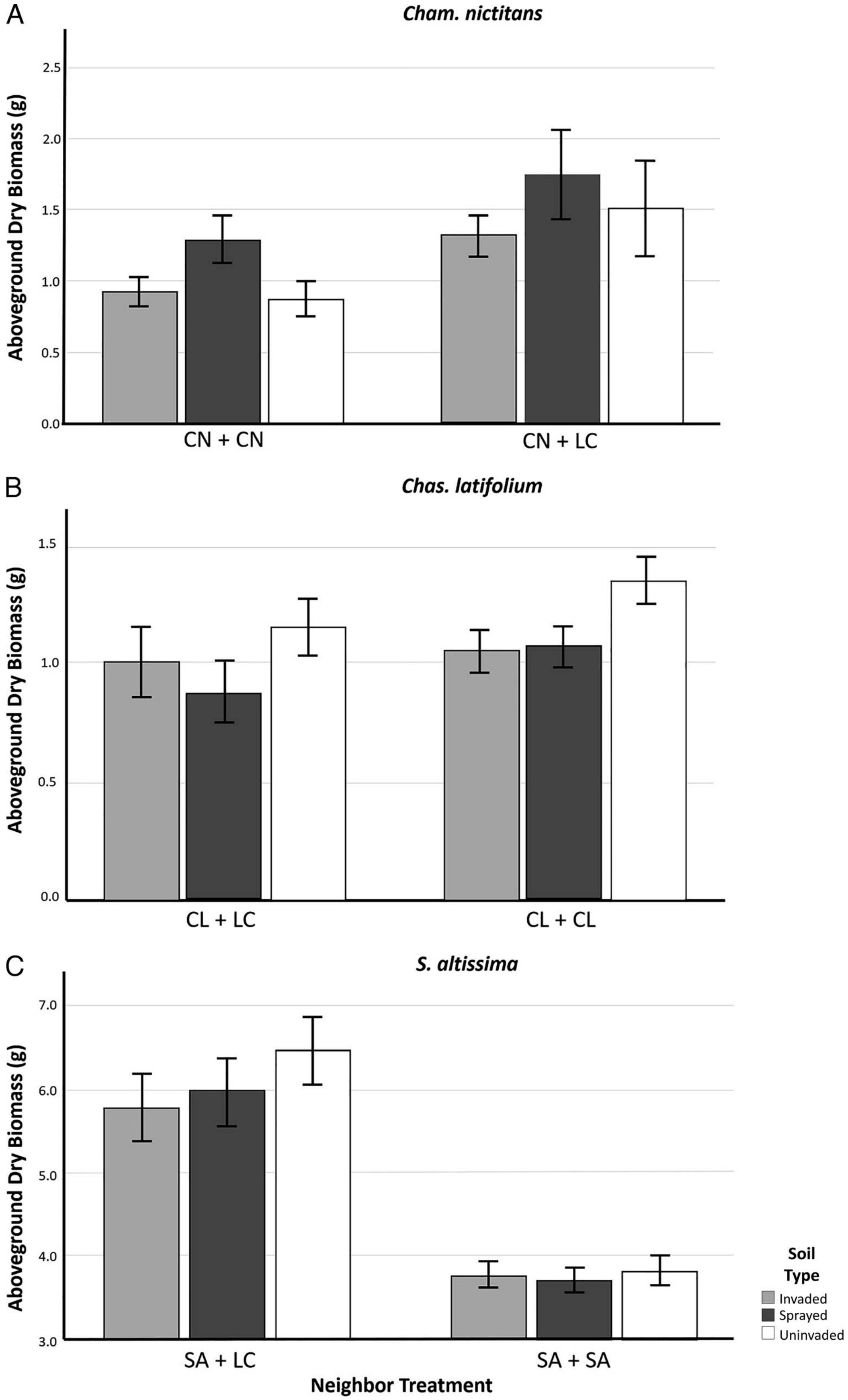

Figure 3.—Mean (± 1 SE) aboveground dry biomass of (A) *Chamaecrista nictitans* (CN), (B) *Chasmanthium latifolium* (CL), and (C) *Solidago altissima* (SA) when grown in uninvaded (U, white), invaded (I, light gray), or sprayed (S, dark gray) soil with either another individual of the same species or with an individual of *Lespedeza cuneata* (LC). The aboveground dry biomass of *Cham. nictitans* ($F_{2,124} = 3.29$, $P = 0.04$) and *Chas. latifolium* ($F_{2,141} = 3.41$, $P = 0.036$) varied significantly among soil types, but soil type did not contribute significantly to the final biomass of *S. altissima* ($F_{2,144} = 0.84$, $P = 0.434$). Neighbor type was a significant source of variation of the final biomass of *Cham. nictitans* ($F_{2,124} = 10.62$, $P = 0.001$) and *S. altissima* ($F_{2,144} = 86.30$, $P < 0.0001$) but not *Chas. latifolium* ($F_{2,141} = 2.39$, $P = 0.124$). The interaction of neighbor type × soil type was not found to be a significant source of variation for any of the three species.

Table 4.—Results from the mixed general linear model for the effect of soil type (uninvaded, invaded, sprayed) on germination success and seedling survival for *Lespedeza cuneata*, *Chamaecrista nictitans*, *Chasmanthium latifolium*, and *Solidago altissima*. Significant *P*-values (<0.05) indicated with bold.

	Germination			Seedling survival		
Species	df	*F*	*P*	df	*F*	*P*
L. cuneata	2, 180	1.70	0.186	2, 107	1.75	0.179
Cham. nictitans	2, 180	0.55	0.578	2, 148	0.30	0.744
Chas. latifolium	2, 180	0.16	0.849	2, 72	1.19	0.309
S. altissima	2, 180	7.16	**0.001**	2, 167	1.99	0.14

communities caused by frequent flooding, as has been shown in other floodplain systems (Mayr et al. 2020). In river floodplain ecosystems, flooding and hydrology are the most influential factors explaining biodiversity patterns and ecosystem dynamics (Wolfgang et al. 1989). Previous research has found a relationship between flooding frequency and a reduction of beta diversity in zooplankton (Bozelli et al. 2015), understory vegetation (Johnson et al. 2014), and grassland bird species (Żmihorski et al. 2016). Although flooding effects on soil microbial community structure are less well understood, hydrology may be a contributing factor in the lack of *L. cuneata* legacy effects observed in our study.

Methodological factors may have also contributed to the differences observed between these results and previous research. Because of logistical constraints, our study used field-sampled soils from areas with and without preexisting invasions to test for soil legacy effects. In contrast, previous studies of *L. cuneata* (Coykendall and Houseman 2014; Crawford and Knight 2017) used a two-phase approach where soils were first conditioned in experimentally invaded and control plots and then used to test for feedback in plant growth assays. The field-sampling approach we used has the benefit of inoculating with soil communities that have developed for longer periods of time under natural conditions; however, variation associated with abiotic factors or other vegetation can confound the impact of soil history (Pernilla Brinkman et al. 2010) and may have constrained our ability to detect it. We attempted to minimize variation due to abiotic factors by limiting the field-sampled soil to 25% of the growth medium. In addition, we controlled for other factors by collecting uninvaded, invaded, and sprayed soils within a short distance of each other at each site. In doing so, however, we assumed that the soil legacy effects of a *L. cuneata* invasion or glyphosate herbicide were restricted to a relatively small spatial scale. A mismatch between the spatial scale of soil community effects and our sampling design might also explain the absence of clear feedback effects in our results.

In addition to reaching a similar aboveground biomass, individuals of *L. cuneata* grown in invaded and uninvaded soils formed a similar number of root nodules. Many nonnative legumes, including *L. cuneata*, produce nodules in their novel habitats, indicating that either the particular rhizobia species necessary for their nodule formation are distributed across continents or that they can form associations with a wide array of rhizobia species (Richardson et al. 2000). It is possible that the similar nodule formation in uninvaded and invaded soils is due to rhizobia being abundant in the soil prior to the invasion by *L. cuneata*. These results might also reflect the homogenizing effects of frequent flooding on rhizobia distribution.

An alternative explanation for the trends observed in nodule formation is that greater root mass and perhaps plant size in general created an opportunity for more nodules. While we cannot determine the causality driving the trend observed, a significant positive correlation was found to exist between the number of nodules and aboveground biomass of individuals in both uninvaded and sprayed soils (Supplemental Table S1). Further research is necessary to determine if the growth of more nodules causes a greater aboveground biomass or if larger plants are able to produce more nodules.

Our inability to detect legacy effects of invasion on root nodule production might reflect, in part, the location of rhizobia when soil was collected. Individuals of *L. cuneata* grown in sprayed soils with a history of invasion had the most root nodules, and significantly more than those grown in currently invaded soil. When living legumes are present, rhizobia are likely to be found in the root nodules, rather than free-living in the soil collected from around them. Following the death of legumes, as occurred in the glyphosate spraying treatment, root nodules deteriorate, and live rhizobia in the nodules return to the soil (Grains Research and Development Corporation 2013). Congruent with this idea, a study focusing on another invasive leguminous shrub (*Genista monspessulana* (L.) L.A.S. Johnson) found that hand-pulling reduced the abundance of rhizobial symbionts to preinvasion levels whereas herbicide treatment caused a significantly higher density of soil-dwelling rhizobia to nodulate (Komatsu and Simms 2019). Our results suggesting positive soil legacy effects, coupled with other environmental (Jiraungkorrskul et al. 2003; Glusczak et al. 2007) and health risks (Guyton et al. 2015), highlights the need for careful planning to prevent undesirable outcomes when using glyphosate herbicide as a tool for invasive plant management.

Although greenhouse experiments may not accurately predict outcomes in the field, our results provide some evidence regarding potential competitive interactions between *L. cuneata* and native plants. Across all species and analyses, soil type was not found to influence the effect of competition, although neighbor identity did affect the outcome. This suggests that size and growth rate of competitors is more influential than soil legacy effects in determining results. Likewise, data indicate that the native species used in this study do not suffer negatively from the legacy effects caused by an invasion of *L. cuneata* or the use of glyphosate herbicide. Independent of soil type, results do suggest that either of the native forbs, *Cham. nictitans* or *S. altissima*, would likely be more capable of preventing reinvasion than *Chas. latifolium*.

Legacy effects caused by populations of invasive plants not only have the potential to impact the abiotic and microbial properties of the soil, but also the bank of seeds the soil contains. The number of *L. cuneata* seedlings to emerge from the seed bank was similar in uninvaded ($n = 10$), invaded ($n = 22$), and sprayed ($n = 19$) soil types. Although the seed dispersal mechanism contributing most to the spread of *L. cuneata* remains unclear, previous research suggests that animals (Eddy et al. 2003; Silliman and Maccarone 2005; Quick et al. 2016), air

(Quick et al. 2016), and water (Silliman and Maccarone 2005) all serve as vectors to some degree. It is possible that in the floodplain system where our study focuses, *L. cuneata* is spread during flooding and high-flow events, allowing for long-distance dispersal across the landscape. Therefore, the impacts of a *L. cuneata* invasion on the seed bank would not be restricted to the soil being actively invaded.

The seed bank within the soil holds potential for restoration following invasive plant management; however, it is impacted by the legacy effects of the exotic species and management practices. Data from our study suggest that soil legacy effects caused by an invasion of *L. cuneata* did not significantly impact the overall abundance or species richness of seedlings to emerge from the seed bank. However, similar to findings of previous research, the use of glyphosate herbicide significantly reduced the total number and species richness of the seedlings that emerged from the seed bank. The magnitude of reduction has been found to be comparable to that of overgrazing, deforestation, or tillage (Rodriguez and Jacobo 2013). The use of glyphosate herbicide as currently administered leaves areas barren of flora and a seed bank that cannot naturally replenish the area with native flora. Our results, along with others, suggest that while a history of *L. cuneata* invasion does not significantly alter soil properties in a floodplain ecosystem, the use of glyphosate herbicide does create areas susceptible to reinvasion by *L. cuneata* or another exotic species.

While greenhouse experiments provide valuable information concerning isolated impacts of soil legacy effects (Yannarell et al. 2011; Coykendall and Houseman 2014), the results may not translate to field conditions and active restoration projects (Reichenborn et al. 2020). In our study, availability of resources including nutrients, water, and light within the growth room experiment likely differed from that of field conditions. Short-term studies of soil legacy effects only reflect impacts during early growth. Furthermore, our research was limited to a small pool of candidate species for native flora restoration. While results from this research found that a native grass, as opposed to native forbs, responded differently to the legacy effects and competition of a *L. cuneata* invasion, research including a wider array of native plant species is necessary to test whether this trend is general and to identify more potential native species for restoration of invaded areas.

CONCLUSIONS

The results from this study of *Lespedeza cuneata* in a floodplain plant community illustrate that the positive benefit of soil legacy effects documented in prairie and grassland habitats do not directly translate across all systems. In contrast to previous studies, individuals grown in uninvaded and invaded soils reached similar aboveground biomasses and formed similar number of root nodules. In addition, a history of invasion did not significantly increase the quantity of germinable seeds in the seed bank, germination success of *L. cuneata*, or seedling survival of individuals after germination. Information concerning the effects of ecosystem characteristics, primarily hydrology, is necessary to better manage this invasive plant in areas outside of the midwestern prairies where most of current literature is focused. Moreover, there is a need for long-term field experiments to understand how legacy effects influence *L. cuneata* invasions and efforts to restore native plant communities. As glyphosate herbicide is continued to be used as a tool for invasive plant management, effort should be taken to replant native forbs in their place to prevent the possibility of reinvasion. A restored community of native flora is the end goal of successful invasive plant management.

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