Investigating the role of soil legacy effects and community engagement in the management of *Lespedeza cuneata*, an invasive legume

by

Matthew Steven Hodges

December, 2020

Director of Thesis: Dr. Carol Goodwillie

Major Department: Biology

Invasive plant species present a growing threat to biodiversity. Many invasive plants are able to recruit microbial symbionts in their novel range and establish plant-soil feedbacks that influence growth and fitness. These alterations, referred to as soil legacy effects, can linger for decades after the removal of invasive species and impact efforts to restore native plant populations. The process of restoring organisms and their interactions with one another, referred to as ecological restoration, occurs by repairing these damages and alterations to ecosystem diversity and ecosystem dynamics.

In a series of growth room experiments, I analyzed the plant-soil feedback of an invasive legume, *Lespedeza cuneata*, and how soil legacy effects caused by invasion and use of glyphosate herbicide influence the growth and competitive interactions of three native plant species. In contrast to studies of *L. cuneata* in prairie ecosystems, my investigation suggests that positive plant-soil feedback does not significantly contribute to its growth or spread in the floodplains of eastern North Carolina, as a history of invasion did not significantly improve the seed germination, seedling survival, growth, or root nodule formation of the invasive legume. The absence of evidence for positive plant-soil feedback in my experiment might be attributed to frequent flooding observed in a floodplain system and the resulting homogenization of soil biota.

Findings from my study also suggest that the application of glyphosate herbicide alone 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 significantly increasing the aboveground biomass and nodule formation of *L. cuneata*. Concerning the restoration of native flora, my investigation suggests that *Chasmanthium latifolium*, as opposed to *Solidago altissima* or *Chamaecrista nictitans*, may be more susceptible to negative impacts caused by a *L. cuneata* invasion or glyphosate herbicide and therefore less suitable for initial efforts to restore populations of native flora. Results from my competition experiments also suggest that while *S. altissima* and *Cham. nictitans* may not be able to suppress populations of *L. cuneata*, the two native forbs would be successful in preventing areas from being reinvaded while areas occupied solely by *Chas. latifolium* may be at risk of reinvasion.

Control of invasive species requires active participation by conservation professionals and the public. Outreach events and citizen-science programs can provide members of the community of all ages and careers the opportunity to play an active role in conservation efforts through data collection, species monitoring, restoration, invasive species removal, or a wide variety of other necessary tasks. To assess undergraduate attitudes towards conservation and involve students in the management of an invasive plant, an engagement event was held on a local greenway with an ongoing invasion of *L. cuneata*. During the outreach event, participants manually removed invasive plants while engaging in discussions centered on invasive species, local flora, and conservation. Voluntary participant data surveys suggested that the event positively impacted participants' perception of the natural world and encouraged them to seek out similar opportunities in the future. Survey results also showed that opinions towards conservation were influenced by the undergraduate major of students.

Investigating the role of soil legacy effects and community engagement in the management of *Lespedeza cuneata*, an invasive legume

A Thesis

Presented To the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

by

Matthew Steven Hodges

December, 2020

Investigating the role of soil legacy effects and community engagement in the management of Lespedeza cuneata, an invasive legume

by

Matthew Steven Hodges

APPROVED BY:

DIRECTOR OF THESIS:

Dr. Carol Goodwillie, PhD

COMMITTEE MEMBER:

Dr. Claudia Jolls, PhD

COMMITTEE MEMBER:

Dr. Heather Vance-Chalcraft, PhD

COMMITTEE MEMBER:

Dr. David Chalcraft, PhD

COMMITTEE MEMBER:

COMMITTEE MEMBER:

CHAIR OF THE DEPARTMENT OF

Biology

Dr. David Chalcraft, PhD

DEAN OF THE GRADUATE SCHOOL:

Paul J. Gemperline, PhD

ACKNOWLEDGEMENTS

I would like to thank my adviser, mentor, and friend Dr. Carol Goodwillie for the immense support she has shown to not only my research, but also my professional development and career pursuits. I would like to also thank the members of my committee for their time and expertise over the course of this research. I would like to thank my parents, and fellow East Carolina University alumni, David and Robin Hodges for supporting my education in more ways than I could ever list here. I would like to thank Garett Thompson for repeatedly providing an extra set of hands in both the lab and in the field. Finally, I would like to thank Alisha Olney for loving me every step of the way, and providing me with more confidence than I could have ever imagined.

I would like to also thank the North Carolina Native Plant Society for supporting this research through the Tom and Bruce Shinn Grant, to The Garden Club of North Carolina for support through their scholarship program, to the East Carolina University Department of Biology for support through the Martha N. Jones Scholarship, and to Southwood Memorial Christian Church for support provided through their scholarship program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

I. Introduction

Ecological restoration is the process of restoring organisms and their interactions with one another by repairing the damages, typically caused by humans, to ecosystem diversity and ecosystem dynamics (Jackson, 1995). Initial efforts to repair these damages include identifying and reversing the causes of ecosystem degradation, including the removal of invasive species from the area (Hobbs and Norton, 1996). Invasive species are an increasing threat to biodiversity across the globe and can have negative impacts on agriculture, fisheries, forestry, enterprises, and human health (Wittenburg and Cock, 2001). In areas where invasive flora or fauna are present, reduced biodiversity (Butchart et al., 2010). In addition to their threat to aboveground flora, invasive plant species have also been found to alter the composition and diversity of the natural seed bank (Vilà and Gimeno, 2007; Gioria and Osborne 2009a; Gioria and Osborne 2009b). These alterations to the plant community and the seed bank cause potential impacts to the invaded area that are persistent and long lasting. Properties of the local ecosystem, including ecosystem production, are impacted by the reduction of biodiversity caused by invasive species (Vilà et al., 2015). Invasive plants cause negative effects that can extend to other trophic levels. In regions dominated by invasive plants, the reduction of biodiversity is observed in both plants and animals. For example, in areas of Europe dominated by *Solidago* spp., fewer bird species are observed because the resources originally produced by native plants have been reduced (Skórka et al., 2010). However, field surveys conducted at larger scales have suggested that a positive relationship exists between exotic species richness and native species richness (Londsdale, 1999; Sax, 2002). Previous research taking place on multiple continents has illustrated that at broader scales, the richness of exotic herbaceous plants was positively correlated to the richness of native herbaceous plants (Sax, 2002). This idea, often referred to as the “invasion paradox”, describes that while a loss of native biodiversity occurs at a local neighborhood scale, an increase in native species richness is observed at broader scales (Fridley et al., 2007).

Invasive species are introduced into their exotic ranges intentionally or unintentionally and are then able to establish large populations. The overwhelming majority of invasive woody plants were introduced to new environments after being intentionally used by nurseries, botanical gardens, or private landscaping, while the majority of invasive herbaceous species were introduced as contaminants in agricultural seed (Reichard and White, 2001). A potential explanation for the success of invasive species is a lack of natural predators in their introduced ranges, often referred to as the enemy release hypothesis (Williamson and Fitter, 1996). Two assumptions of this hypothesis are that specialist enemies of the invasive species will not be present in the introduced range and the specialist enemies of native species will not switch to the exotic species (Liu and Stiling, 2006). Evidence supporting the enemy release hypothesis illustrates that invasive plant species experience higher rates of herbivory in their native ranges in the presence of their natural predators compared to their introduced ranges where their natural predators are absent (Vilà et al., 2005). This absence of herbivory allows invasive plant populations to grow without means of natural control and results in higher population densities to exist in introduced ranges (Paynter et al., 2003).

Plants that successfully establish populations in introduced ranges often bear similar characteristics. Successful invasive plants typically have more vigorous spatial growth, higher fecundity, more efficient use of nutrients, longer flowering times, and a higher photosynthetic rate than that of the native species with which they compete (Moravcová, 2015). These plants also display higher seed production, lower mortality, longer survival time, and other characteristics associated with higher fitness (van Kleunen et al., 2010). Invasive plants that have been found to most drastically reduce species richness are those that are capable of clonal growth but lack mutualisms for nitrogen fixation (Vilà et al., 2015). Habitats most susceptible to invasions are those that have a history of land use, including areas surrounding large cities or along major roadways (Kuhman et al., 2015). Fragmented portions of land are also more vulnerable to invasive species establishing thriving populations (Vilà and Ibáñez, 2011).

Quick recognition and effective management strategies are necessary to prevent the establishment of large invasive plant populations and to minimize the disastrous effects they can have on an ecosystem (Simberloff, 2009). Simple manual removal is often an effective management strategy (Wittenburg and Cock, 2001), but it can require a large workforce. To satisfy this demand, several sources have been utilized to recruit manpower. Successful management efforts have used paid workers, community volunteers, and also convicts (Simberloff, 2009). Regardless of the source of the work force, training and supervision are still limiting factors in the success of management efforts. In some efforts at management, a non-native predator is intentionally introduced to control the target species. While claims exist suggesting this practice has been successful in some cases, the practice poses a high risk that the intentionally introduced species will have damaging effects to populations other than the target invasive species (Wittenburg and Cock, 2001; Havens et al., 2012; Havens et al., 2019). The use of herbicides can be successful in reducing invasive plant populations but can have adverse effects on the surrounding ecosystem (Wittenburg and Cock, 2001).

Glyphosate is a broad-spectrum herbicide commonly used in the management of invasive plants, in addition to its applications in the agricultural industry. Currently the most applied herbicide globally, glyphosate is produced in higher volumes than any other herbicide

(Benbrook, 2016). While susceptibility varies with species, glyphosate is taken up by plant tissues and then is transported to the plant's meristems, roots, and leaves. Once there, the chemical inhibits a necessary enzyme of the shikimate pathway (Duke and Powles, 2008). The repeated use of this herbicide for nearly fifty years has caused some areas to observe 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 humans and other fauna, long term exposure to this herbicide has been shown to have negative consequences for health. Long-term glyphosate exposure in humans has been linked to the development of non-Hodgkin lymphoma (Guyton et al., 2015). Fish exposed to the herbicide were found to have adverse alterations to tissue and biochemistry (Jiraungkorrskul et al., 2003). The length of time glyphosate herbicide persists in the soil following application is dependent on the texture of the soil (Tejada, 2009). 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). Even though the popularity of glyphosate herbicide continues to grow, largely because of its affordability and effectiveness (Benbrook, 2016), there is growing evidence of its potential for negative consequences to human health and the environment.

Long-term invasive plant management is likely to also benefit from efforts restoring populations of native plants. In addition to improving soil stability, native biodiversity, and ecosystem dynamics, increasing the number and diversity of native species also constrains and combats ongoing spread of invasive species (Bakker and Wilson, 2004). Without restoration of native plant species, the invasive species that was the target of removal can reinvade the area if proper precautions are not taken; other exotic species also can move into the disturbed area (Harms and Hiebert, 2006). Restoration efforts that restore populations of native species following an invasion must be tailored to the specific environment and invasive species being removed. For example, in a study focusing on the restoration of native flora following the removal of invasive *Microstegium vimineum* (Trin.) A. Camus, it was found that the use of herbicide had a significant negative impact on the biomass and species richness of native graminoids used in restoration efforts; in contrast, herbicide use had a positive impact on the same characteristics of forbs. The same study found that the effect of specific site where restoration took place significantly influenced the performance of native graminoids, forbs, ferns, and woody species (Flory and Clay, 2009). Similarly, in lab experiments, native legumes were found to have a higher biomass in soil that was previously invaded by an exotic legume than when they were grown in soils with no history of invasion (Komatsu and Simms, 2019). However, these results from lab experiments do not necessarily carry over into real world field conditions (Yelenik and Levine, 2011).

The association between an invasive plant and the soil it occupies can contribute to, or hinder, the success of an invasion as well as the outcome of native plant restoration after its removal. Plant-soil feedback describes the process where a plant alters the physical, chemical, or biological properties of the soil it occupies to influence its own growth and fitness in either a positive or negative manner (Bever et al., 2010). Previous research suggests that these alterations by the plant to the soil provide mechanisms for plant invasion (Kulmatiski et al., 2008).

Positive plant-soil feedback can occur when the presence of the microbial community recruited by the plant encourages subsequent growth and fitness (Klironomos, 2002). This may involve the establishment of mycorrhizal fungi or nitrogen-fixing or free-living bacteria in the soil, which improve the fitness of the host plant (Reinhart and Callaway, 2006). Plants can also alter chemical properties of the soil, either directly or through their association with microbial communities. Positive feedbacks can occur when efficient nutrient cycling of high-quality litter adds nutrients to the soil (Bennett and Klironomos, 2018).

When faced with barriers to establishment in their novel ranges non-native plants, including *L. cuneata*, must often rely on positive plant-soil feedback through symbiotic relationships belowground to assist them in the formation of their invasive population (Richardson et al., 2000). In one such symbiosis, legumes form mutualistic relationships with bacteria in the soil. Soil rhizobia residing in root nodules on legumes convert atmospheric nitrogen into an organic form that plants can use. The rhizobia are protected by the plant from desiccation and provided nutrients from the plant's root system (Sprent et al., 1987).

Negative feedbacks through microbial associations can occur when soil pathogens that associate with the plant after colonization hinder plant growth and reduce fitness (Coykendall and Houseman, 2014). In addition, plant growth can be negatively affected by herbivores or parasites that accumulate in the soil. Negative feedbacks are also possible if the plant depletes the soil of nutrients (Bennett and Klironomos, 2018). Furthermore, some plants can introduce harmful chemicals into the environment through allelopathy, reducing the performance of plants with which they could compete (Ooka and Owens, 2018). Negative feedback, for example through allelopathy or soil pathogens, can create higher levels of plant diversity, as the negative feedback prevents one species from creating a monoculture in the area (Kulmatiski et al., 2008).

Plant soil feedbacks can influence the growth and performance of invasive plants. Just as the absence of natural aboveground herbivores can increase the prevalence of invasive plants, so can the absence of belowground pathogens (Reinhart and Callaway, 2006). Mutualistic soil symbionts tend to be generalists while soil pathogens are the result of coevolution and tend to be more specialist (Callaway and Aschehoug, 2000). The abundant relationships with soil mutualists and the lack of specialized soil pathogens can provide nonnative species a competitive advantage against native species. The dynamic of this plant-soil interaction is likely to change over the course of the invasion, however, as the invasive plant accumulates pathogens over time leading to negative feedback (Wolfe and Klironomos, 2005). For example, in a study involving an invasive legume, individuals grown in recently invaded soil were found to have a higher biomass than those grown in soils with a longer history of invasion (Lau and Suwa, 2016).

Following the removal of an invasive plant population, changes to the physical, chemical, or biotic properties of the soil can persist, typically referred to as soil legacy effects (Corbin and D'Antonio, 2012). These modifications to the soil may hinder the establishment of native plant species being reintroduced through restoration efforts. Therefore, careful consideration must be taken when determining what species to reintroduce through restoration. In the case of some invasive plants, it has been found that the accumulation of soil pathogens by the invader has negative consequences for native plants (Mangla and Callaway, 2008). Native plants introduced through restoration efforts can also be hindered through allelopathic compounds released by some invasive plant species (Dommanget et al., 2014). These chemicals released by the invasive plant can have varying effects on the native plant population, and the most effective restorations utilize native plants that are the least susceptible to the compounds (Hess et al., 2019). While allelopathic compounds might linger in the soil for only a few days, other alterations such as increased nitrogen levels can persist for decades (Nsikani et al., 2017). The effects of such soil alterations are difficult to predict, as they are influenced by the exact species, community density, climate, and substrate conditions (Hess et al., 2019). Soil legacy effects can create numerous obstacles for restoring native plant populations and must be considered when determining restoration strategies following the manual removal of nonnative individuals (Nsikani et al., 2018). Efforts such as topsoil removal, topsoil amendments, or introduction of tolerant native species may be necessary to mitigate these lingering effects and re-establish a thriving native population of plants (Hess et al., 2019).

My study addressed the plant-soil interactions in an invasive legume, *Lespedeza cuneata* (Dum. Cours.) G. Don, and their potential consequences for management and restoration of an invasion in a greenway in eastern North Carolina. The species was first introduced to North Carolina from China in 1896 as a means of erosion control and forage (Ohlenbusch and Bidwell., 2007). *L. cuneata* has established invasive populations in the grasslands of midwestern and eastern United States and is labeled as a noxious weed by the USDA in nearly all states. It produces five times as many seeds per plant as native congeners with which it co-occurs in prairie habitats (Woods et al., 2009), posing a challenge to control efforts. Previous research has indicated that individuals of *L. cuneata* out-shade the native species around it, causing a significant decrease in native plant cover (Brandon et al., 2004). The most effective means of management for *L. cuneata* combines herbicide treatment and mowing, while prescribed burning has been shown to be ineffective (Stevens, 2002) and mowing alone may actually benefit this species (Brandon et al., 2004). Previous studies indicate that *L. cuneata* alters properties of the chemistry, bacterial communities, and fungal communities in the soil it occupies (Coykendall and Houseman 2014; Yannarell et al., 2011) and that these changes in soil properties increase the growth of *L. cuneata* (Coykendall and Houseman 2014) and its ability to outcompete native species (Crawford and Knight, 2017). Differences in *L. cuneata* performance in previously invaded soil versus soil that has never been invaded are most evident in live soil and are absent in soils that have been sterilized through autoclaving or lack of live inoculation (Coykendall and Houseman, 2014). This illustrates the role the microbial community plays in the positive plant-soil feedback observed (Crawford and Knight, 2017; Coykendall and Houseman, 2014).

All studies to date concerning the soil legacy effects and plant soil feedback of *L. cuneata* have concerned populations in midwestern prairie habitats. This study was the first to analyze the plant-soil feedback of *L. cuneata* in the eastern US, the site of first introduction. Growth room experiments were used to investigate how a history of *L. cuneata* invasion may influence seed germination and plant growth of *L. cuneata* and three species of the native flora. The study also addressed the effects of an *L. cuneata* invasion on the naturally occurring seed bank. The objective of the study was to gain a better understanding of how the soil legacy effects of an invasive legume may influence its success as an invasive species and to help tailor efforts to restore native plant populations following the removal of *L. cuneata*.

II. Methods

II-A: Study Site

This study was focused on the South Tar Greenway in Pitt Co., Greenville, NC, a site of an ongoing *Lespedeza cuneata* invasion. The greenway runs through a cypress swamp in the flood plain of the Tar River. Prior to being converted into a public greenway, a portion of the area was originally the site of a landfill. The USDA PLANTS database was used to determine species nomenclature. The native tree community is dominated by bald cypress (*Taxodium distichum* (L.) Rich.), water swamp tupelo (*Nyssa biflora* Walter), and American sycamore (*Platanus occidentalis* L.). The herbaceous plant community at the site includes river oats (*Chasmanthium latifolium* (Michx.) Yates) and several species in the Asteraceae including late goldenrod

(*Solidago altissima* L.), blue mist flower (*Conoclinium coelestinum* (L.) DC.), and white aster (*Symphyotrichum ericoides* (L.) G.L. Nesom). Invasions of kudzu (*Pueraria montana* (Lour.) Merr.) and Japanese privet (*Ligustrum japonicum* Thunb.) are also present in the area. The trail was constructed in 2011 by Greenville City Recreation and Parks Department. By 2014, dense patches of *Lespedeza cuneata* were present. The sand used to construct the trail likely contained seeds of the invasive legume and was the source of the initial invasion (Goodwillie and Jolls, 2018).

II-B: Lespedeza cuneata

Standing to 2 m tall, *L. cuneata* is a flowering perennial that is abundant in disturbed areas. *Lespedeza cuneata* is distinguished by its trifoliate leaves with wedge-shaped leaf bases and small white flowers with purple markings. The species produces both chasmogamous (open) and cleistogamous (closed, obligately self-fertilizing) flowers, and single-seeded indehiscent fruits (Gucker, 2010). The species can establish massive seed banks within the soil, as seeds may remain viable for several years (Stevens, 2002). *Lespedeza cuneata* occurs more often in areas of direct sunlight and performs poorly under shade conditions (Remaley, 1998). On the study site, dense patches of *L. cuneata* are present. To manage the invasion, undergraduate students annually remove individuals of *L. cuneata* manually as part of a service-learning course. In addition, Greenville City Recreation and Parks department has applied glyphosate herbicide in select areas that are especially dense.

II-C: Native Species

Three herbaceous perennial plant species native to the study site were used in a growth experiment and a seed germination experiment: *Chamaecrista nictitans* (L.) Moench (Fabaceae), *Chasmanthium latifolium* (Poaceae), and *Solidago altissima* (Asteraceae). Native species used in this investigation were restricted to those whose seeds could be collected in necessary quantities and could successfully germinate and grow in growth room conditions. *Chasmanthium latifolium*, commonly referred to as river oats, is a cool season perennial grass growing up to five feet tall. This native grass species is popular in the native plant trade, as it is tolerant of shade and is a host plant for several native pollinators. *Solidago altissima*, tall goldenrod, is a wildflower commonly found in disturbed areas using underground rhizomes to spread. This wildflower is found in abundance throughout the study site. *Chamaecrista nictitans,* an annual nodule forming legume, was included to allow us to compare the competitive ability of an invasive and native legume. While only *Chas. latifolium* is popular in the native plant trade, all three species are contenders for native plant restoration following the removal of *L. cuneata* on the South Tar Greenway.

II-D: Seed Collection and Germination

Seeds of *L. cuneata, S. altissima, Cham. nictitans,* and *Chas. latifolium* were collected along the Greenville South Tar Greenway in fall of 2018. Collections were made for each species at various locations along the study site and then bulked together. Seeds were refrigerated for eight to twelve months until germinated.

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 (Coykendall and Houseman, 2014). To germinate seeds of *Cham. nictitans*, a corner was nicked using a razor and seeds were then soaked in water for 24 h (Carino and Daehler, 2002). In a preliminary test, cold moist stratification was not found to increase germination rates substantially in *Chas. latifolium* and *S. altissima*, so seeds were planted directly into soil. All seeds were planted in trays of standard potting medium prior to being transplanted into experimental pots.

II-E: Growth Experiment

On May 19, 2019, soils were collected from areas that 1) had been invaded with *L. cuneata* for at least 4 yr (invaded), 2) were previously invaded with *L. cuneata* invasion and sprayed with glyphosate herbicide the year prior to the experiment (sprayed), and 3) had no history of *L. cuneata* invasion or herbicide spraying (uninvaded). At seven sites along the greenway, soils of each type (invaded, sprayed, and uninvaded) were collected within 8 m of each other. Spades were used to collect soil samples at a depth of 20 cm. Tools were wiped clean using ethanol between soil collections to minimize cross contamination of soil microbial communities. Soil samples from invaded sites were collected from the middle of *L. cuneata* patches, soil samples from sprayed sites were collected from the center of sprayed areas to minimize perimeter effects, and uninvaded sites were checked for individuals of *L. cuneata* prior to soil collection. No individuals of *L. cuneata* were found within 4 m of uninvaded collection sites. Soil samples were double bagged in plastic zip sealed bags and stored at room temperature in a cooler without ice.

The following day, major debris was removed from field collected soil. Experimental 10.16 × 10.16 cm pots were filled with a mixture of 25% field collected soil and 75% Sungro (Sun Gro Horticultural, Agawam MA) professional growing mix. The growing mix used did not contain any additional fertilizer. Containers and tools used to mix soil and fill experimental pots were sterilized with ethanol between soil samples.

To test for plant-soil feedback effects in *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 (4 replicates × 3 soil types × 7 sites).

To analyze the influence of *L. cuneata* soil legacy effects on growth and competition in native plants, individuals of the three selected native species were grown in two neighbor treatments in each of the three soil types (invaded, sprayed, and uninvaded) from each site. One neighbor treatment consisted of the native individual grown with another individual of the same species, while the other treatment consisted of the individual of the native species grown with an individual of *L. cuneata.* This design resulted in 18 soil type × neighbor treatment combinations (3 soil types × 3 native species × 2 neighbor treatments). Each soil type × neighbor treatment combination was replicated in four pots for each of the seven sites, resulting in a total of 504 pots (18 combinations × 4 replicates × 7 sites).

Following the preparation of soil in pots, the appropriate seedlings were transferred into experimental pots from the trays of greenhouse soil where they had been germinated. Seedlings were transferred between May 21 and May 23. At the time of transfer, seedlings of *L. cuneata* were 2-4 weeks old, *S. altissima* were 3-5 weeks old, *Cham. nictitans* were 2-4 weeks old, and *Chas. latifolium* were 3-6 weeks old. In each pot, care was taken to pair two individuals of approximately the same size. Seedling transfers were planned and conducted so that each soil type and site contained a range of seedling sizes and ages. Tools used for transplanting seedlings were sterilized with ethanol between soil collection sites to minimize cross contamination of the soil microbial community. Following the initial transfer of seedlings into pots, deceased seedlings were replaced for 2 wk until June 6.

Plants were raised in a growth room at East Carolina University with mixed natural and artificial lighting. Pots were watered from above. Watering was performed as needed to keep soils moist and increased in frequency as plants grew. A layer of sterile sand was placed on top of the soil on each pot and maintained for the duration of the experiment to minimize possible cross contamination between soil microbial communities from adjacent pots during watering (Crawford and Knight, 2017) and inhibit fungus gnat infestation. Experimental pots were placed in plastic trays and rotated around the room twice weekly to minimize positional effects. Trays contained experimental pots of the same soil type to minimize the effects of contamination that might occur during watering. The position of pots within trays was randomly assigned. To manage fungus gnat and thrips outbreaks, pirate bugs (*Orius insidiosus*) were released twice into the room. This means of pest control does not influence the soil microbial communities. Stakes were used to keep *Cham. nictitans* and *S. altissima* individuals from leaning on and shading other pots.

Measurements of plant size were collected from all plants at the start of the experiment to factor out the effects of initial size on aboveground biomass after 13 weeks. To gauge initial size, the number of leaves on *L. cuneata*, the number of leaves on *Cham. nictitans,* the height of *Chas. latifolium,* and the width of the broadest leaf on *S. altissima* individuals were recorded. Over the course of the entire experiment, the date of first flower was recorded for individuals of *Cham. nictitans,* the only species to flower during the course of the experiment. After the first 2 wk of replacing dead seedlings, mortality was recorded for the remainder of the experiment. At the conclusion of the experiment 13 wk later, remaining plants were cut off at ground level and plant tissue was dried in an oven at 60.0 ° C for 24 h. Specimens were weighed immediately after being removed from the oven to determine dry aboveground biomass. For intraspecific competition treatments, the mean biomass of the two individuals was used. For each pot containing two individuals of *L. cuneata*, the total number of root nodules was determined. To remove soil and untangle the roots from one another, roots were rinsed above a sieve. Debris collected in the sieve was also examined for nodules that were removed from the roots during rinsing.

All data collected were analyzed using SAS statistical software. To analyze the effects of competition, plant-soil feedback, and *L. cuneata* legacy effects on aboveground biomass, nodule formation, and time to flowering a series of mixed models were utilized. Individuals of all species that survived to at least week 10 of the experiment were included in statistical analysis. All mixed models included soil type and neighbor identity as fixed independent variables and their interaction. The site where soil was collected 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 models. A total of five separate mixed models were used to analyze data concerning dry aboveground biomass of all four species, and nodule formation of *L. cuneata*. For the two models analyzing aboveground biomass and nodule formation of *L. cuneata,* three soil types (invaded, uninvaded, sprayed) and four neighbor identities (*L. cuneata, S. altissia, Cham. nictitans, Cham. latifolium)* were included. For each of the three native species, separate models were run assessing the impact of soil type and neighbor identity on the aboveground biomass. For each of these three models, three soil types (invaded, uninvaded, sprayed) and two neighbor identities (*L. cuneata,* same native species) were present. Tukey's procedure was used to determine if the difference of response variables between soil types or neighbor identities was significant for all ten mixed models.

To determine the relationship between the aboveground biomass and the number of nodules formed on *L. cuneata* in each of the three soil types, three separate linear regression models were used. The total number of nodules and the mean aboveground biomass was used for each intraspecific competition treatment of *L. cuneata*. In each of the three models, the number of nodules was the explanatory variable and the aboveground biomass was treated as the response variable.

II-F: Germination Experiment

On September 4, 2019, field soil was collected again to perform a seed germination assay. Soils were collected from the same 21 locations at seven sites on the South Tar Greenway using the same collection and decontamination procedures as the initial collection. Soils were stored at 4°C for four days before being placed in experimental containers. Seeds were germinated in 7.62 × 7.62 cm pots consisting of four 3.81 × 3.81 cm cells. A total of 189 pots were used, nine 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. All cells within a pot contained field soil from the same location. In each pot, ten seeds of *L. cuneata* were planted in one cell, ten seeds of *S. altissima* in another cell, five *Chas. latifolium* in the third cell, and four seeds of *Cham. nictitans* in the remaining cell. All seeds were planted over the course of four days, with a different species planted each day. Seeds were sterilized and prepared for planting as described above. Seedlings of species not planted were removed as they emerged from the seed bank. Seedlings of the four study species that emerged in cells other than the ones they were intentionally planted in were also removed, with the exception of *S. altissima* whose small buoyant seeds tended to float from cell to cell as watering occurred. Watering occurred regularly to keep field soil moist. Experimental pots were placed in trays that were rotated about the room regularly in pairs to account for environment differences within the growth room. Each pair of trays contained a replicate from each of the 21 soils collected, arranged randomly. I recorded seed germination and mortality two weeks after the first seeds were planted. Subsequent data collection occurred regularly to track further germination and seedling mortality.

A mixed general linear model was used to test for soil legacy effects on the seed germination and seedling survival of *L. cuneata* and native species. The model 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 was determined. Two separate models were used, one assessing the effect of soil type on seed germination and another assessing the effect of soil type on seedling survival. 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.

II-G: Seed Bank Experiment

To see if 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 until October 21, 2019. A total of 105 10.16 × 10.16 cm pots were used, five 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 such that each had a replicate of each of the 21 soils, Watering occurred regularly to keep soil moist for the duration of the experiment. Trays were rotated regularly to minimize positional effects.

As seedlings emerged, physical characteristics including leaf arrangement, shape, margins, color, and presence of hairs were used to recognize different species. To prevent overcrowding in pots, seedlings of abundant species were removed after they were counted so that only approximately 10 individuals of each remained. These 10 individuals were allowed to grow and, in some cases, produce flowers to be used to accurately identify each species. Radford, Ahles,and Bell (1968), Weakley(2010), and online resources were used to identify species. Plant profiles from the USDA PLANTS Database (United States Department of Agriculture, 2019) were used to determine native vs. exotic status for each identified species. Following identification, all remaining individuals of a species were counted and removed from the pots for the duration of the experiment. Graminoids (Poaceae, Cyperaceae, and Juncaceae) could be identified only to family, although leaf morphological traits were used to attempt to sort seedlings into distinct unknown species. Recording of data ended on January 21, 2020. The few seedlings that emerged after this point were removed as they appeared and not included in the data set. Individuals that emerged prior to this date but had yet to be identified were allowed to grow so that identification could be made.

A mixed general linear model was used to test for an effect of *Lespedeza cuneata* invasion and herbicide on the abundance, diversity, and composition of the seedlings emerging from the seed bank. For each pot the total number of seedlings, species richness, Shannon-Wiener diversity index, 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 and species. A mixed general linear model was run for each of the six 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, Shannon-Wiener diversity index, 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.

III. Results

III-A: Growth Experiment Results

Data gathered from the growth experiment were analyzed to determine the effects of the plant-soil feedback of *Lespedeza cuneata* and the impact of herbicide on growth and competition. Twenty individuals of *L. cuneata* that died within the first 10-weeks of the experiment were eliminated from the data set, leaving 415 to be included. Mortality of *L. cuneata* did not differ among the three soil types ($x^2 = 0.4$, df = 2, p = 0.8187). Soil type had a significant impact on the dry above ground biomass ($F_{2,296} = 4.11$, p = 0.0174, Table 1.1; Fig. 1.1) and the number of root nodules per individual of *L. cuneata* ($F_{2,61} = 3.72$, p = 0.0300; Fig. 1.2). Means reported ± 1SE throughout. Overall, the biomass of *L. cuneata* grown in uninvaded soil (0.54 g ± 0.04) and invaded soil (0.53 g ± 0.04) was similar ($t_{296} = 0.28$, p = 0.7818). Individuals of *L. cuneata* grown in sprayed soil had a significantly greater aboveground biomass (0.66 g ± 0.04) than those grown in uninvaded soil ($t_{296} = 2.33$, p = 0.0202) or invaded soil ($t_{296} = 2.61$, p = 0.0095) (Fig. 1.2). Individuals grown in sprayed soils also had the highest mean number of root nodules per individual (68.87 ± 7.93), while those grown in invaded soils had the lowest (38.92 ± 7.60) (Fig. 1.2). The number of nodules formed per individual was significantly greater in sprayed soil than in invaded soil ($t_{61} = 2.73$, p = 0.0083), which was the only pairwise comparison determined to be statistically significant (Fig. 1.2). The relationship between the number of nodules per individual and aboveground biomass was positive and significant for those grown in uninvaded soil ($t_1 = 2.52$, $p = 0.0203$, $r^2 = 0.2411$, Fig. 1.3A) and sprayed soil ($t_1 = 5.16$, $p = 0.0023$, $r^2 = 0.3645$, Fig. 1.3C). While the relationship was found to be statistically significant, the amount of variation explained by the model was found to be minor. No significant relationship was detected for those grown in invaded soils ($t_1 = 1.28$, $p = 0.2149$, $r^2 = 0.0661$, Fig. 1.3B).

The identity of the neighbor significantly impacted the aboveground biomass of *L. cuneata* ($F_{2,296} = 4.48$, $p = 0.0043$, Table 1.1; Fig. 1.1). The biomass of *L. cuneata* when grown with another individual of the same species (0.5362 g ± 0.05) was similar when grown with *Chamaecrista nictitans* (0.57 g ± 0.05; $t_{296} = 0.53$, $p = 0.5980$) or *Solidago altissima* (0.50 g ± 0.05; $t_{296} = 0.54$, $p = 0.5894$) (Fig. 1.1). Individuals of *L. cuneata* grown with *Chasmanthium latifolium* had the greatest biomass (0.70 g ± 0.05), a size that was significantly greater than the biomass of those grown with another individual of *L. cuneata* ($t_{296} = 2.77$, $p = 0.0059$) (Fig. 1.1). The interaction between neighbor treatment and soil type was not significant in determining the biomass of *L. cuneata* ($F_{6,296} = 0.55$, $p = 0.7689$, Table 1.1).

Data were also analyzed to determine the impact of soil legacy effects, caused by an invasion of *L. cuneata* and the use of herbicide, on the growth and competition of native species. One individual of *Chas. latifolium* did not survive to the 10-week threshold and was therefore not included in the data set. The remaining 251 individuals of *Chas. latifolium* survived the entire duration of the experiment. All individuals of *S. altissima* survived for the entire experiment; all 252 individuals to be included in the data set. Nineteen individuals of *Cham. nictitans* were excluded from the data set due to mortality in the first 10-weeks of the experiment, leaving a sample size of 233. No association could be identified between soil type and the mortality of *Cham. nictitans* ($x^2 = 2.0$, df = 2, p = 0.3679).

While the final aboveground biomass of *S. altissima* was found to be similar across all three soil types ($F_{2,156} = 0.80$, p = 0.4521, Table 1.2), the aboveground biomass of *Cham. nictitans* ($F_{2,136} = 2.72$, p = 0.0696, Table 1.2) and *Chas. latifolium* ($F_{2,153} = 3.36$, p = 0.0373, Table 1.2) varied significantly between soil types (Fig. 1.4). Similar to the trend observed in *L. cuneata*, the mean biomass of *Cham nictitans* was similar grown in uninvaded vs. invaded soil (1.17 g ± 0.22 vs. 1.12g ± 0.23, respectively) was similar ($t_{136} = 0.22$, p = 0.8294), but individuals grown in sprayed soils (1.55 g ± 0.22) were significantly larger than those grown in uninvaded soil ($t_{136} = 2.96$, p = 0.0037) or invaded soil ($t_{136} = 2.10$, p = 0.0378) (Fig. 1.4A). In contrast, the aboveground biomass of *Chas. latifolium* was greatest when grown in uninvaded soils (1.26 g ± 0.10) and the lowest when grown in sprayed soil (0.98 g ± 0.10). The biomass of *Chas. latifolium* grown in uninvaded soil was significantly greater than those grown in invaded soil (1.03 g ± 0.09) ($t_{153} = 1.98$, p = 0.0499) or sprayed soil ($t_{15\,3}$= 2.44, p = 0.0158) (Fig. 1.4B).

The identity of the neighbor, either another individual of the same native species or an individual of *L. cuneata*, significantly impacted the aboveground biomass of *S. altissima* ($F_{2,156}$ = 81.93, $p < 0.0001$, Table 1.2) and *Cham. nictitans* ($F_{2,136} = 8.75$, p = 0.0037, Table 1.2) (Fig. 1.4). The identity of the neighbor did not affect the biomass of *Chas. latifolium* ($F_{2,153} = 2.42$, p = 0.1219, Table 1.2). Individuals of *Cham. nictitans* grown with another individual of the same species had a biomass (1.03 g ± 0.21) that was significantly lower than those grown with *L. cuneata* (1.53 g ± 0.21, t_{136}=2.96, p =0.0037) (Fig. 1.4A). In a similar trend, individuals of *S. altissima* that were grown with another individual of *S. altissima* (3.78 g ± 0.18) reached a biomass that was significantly lower than those grown with *L. cuneata* (6.08 g ± 0.18, $t_{156} = 9.05$,

$p < 0.0001$) (Fig. 1.4C). The interaction between neighbor treatment and soil did not significantly impact the one-month size or aboveground biomass of *Cham. nictitans*, *Chas. latifolium*, or *S. altissima* (Table 1.2).

No overall trend was observed regarding response to the seven different sites where soil was collected (Fig. 1.5). The four species displayed different responses to soils from the seven collection sites. While *Cham. nictitans* grew the largest in soils from site 7, *S. altissima* grown in soils from site 7 had the lowest aboveground biomass. Soil originating from site 3 produced the smallest individuals of *L.* cuneata, but also produced some of the largest individuals of *Chas. latifolium.*

III-B: Germination Experiment Results

Soil type (uninvaded, invaded, sprayed) did not significantly influence the germination success of *L. cuneata* seeds ($F_{2,180} = 1.70$, $p = 0.1861$, Table 1.3; Fig. 1.6). Soil type did not impact the survivorship of *L. cuneata* seedlings over the seven weeks ($F_{2,180} = 1.75$, $p = 0.1785$, Table 1.3)

The germination success of *Cham. nictitans* ($F_{2,180} = 0.55$, $p = 0.5781$) and *Chas. latifolium* ($F_{2,180} = 0.16$, $p = 0.8492$) was similar across all three soil types (Table 1.3; Fig. 1.6). However, the germination success of *S. altissima*, however, was found to significantly differ between soil types ($F_{2,180} = 7.16$, $p = 0.0010$, Table 1; Fig. 1.6). Germination of *S. altissima* was similar between invaded soil (0.57 ± 0.05) and uninvaded soil (0.57 ± 0.05) ($t_{167} = 2.52$, $p = 0.9527$) but significantly lower in sprayed soil (0.4597 ± 0.05) than uninvaded ($t_{167} = 2.51$, $p = 0.0129$) or invaded soil ($t_{167} =2.52$, $p = 0.0128$) (Fig. 1.6). Soil type did not impact the survivorship of seedlings for any of the three native species (Table 1.3).

III-C: Seed Bank Experiment Results

A total of 1,430 individuals of 86 different species and 34 different families emerged from the seed bank in soil samples over the course of this experiment (Table 1.4). Dogfennel (*Eupatorium capillifolium* (Lam.) Small) was the most abundant seedling to emerge during the 14 wk experiment, with 333 seedlings emerging across all samples (Table 1.4). With 475 seedlings and 11 different species, Asteraceae was the most abundant family to emerge from the seed bank (Table 1.4). The most abundant exotic species to emerge was Brazilian vervain (*Verbena brasiliensis* Vell.) with 196 seedlings. Of the species whose native status could be determined, 61.5% belonged to a species native to the area while 38.5% belonged to a known exotic species. Overall, *L. cuneata* was not more frequent in invaded than uninvaded or sprayed sites ($x^2 = 4.59$, df = 2, p = 0.1009). Soil type significantly influenced the total number of seedlings that emerged from the seed bank ($F_{2,96} = 9.96$, p = 0.0001; Fig. 1.7A). A similar number of seedlings emerged from invaded soils (15.83 ± 3.83) and invaded soils (16.26 ± 3.83) ($t_{96} = 0.22$, p = 0.8284). Significantly fewer seedlings emerged from sprayed soils (8.43 ± 3.83) than uninvaded soils ($t_{96} = 3.75$, p = 0.0003) and invaded soils ($t_{96} = 3.97$, p = 0.0001) (Fig. 1.7A). Soil type also significantly impacted the species richness of seedlings that emerged ($F_{2,96} = 6.33$, p = 0.0026; Fig. 1.7B). Uninvaded soils (6.34 ± 1.12) and invaded soils (5.91 ± 1.12) had a similar species richness ($t_{96} = 0.80$, p = 0.4271), 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 = 0.0106)(Fig. 1.7B). Soil type significantly impacted the diversity of seedlings that emerged, as measured by the Shannon Wiener Index ($F_{2,96} = 4.70$, p = 0.0113; Fig 1.7C). Similar to the total number and the species richness of the seedlings to emerge, seedlings that emerged from uninvaded soils (1.40 ± 0.21) and invaded soils (1.34 ± 0.21) had a similar Shannon Wiener Index ($t_{96} = 0.49$, p = 0.8785) (Fig. 1.7C). However, seedlings that emerged from sprayed soils

(1.05 ± 0.21) had a significantly lower Shannon Wiener Index than uninvaded soils ($t_{96} = 2.38$, $p = 0.0141$) or invaded soils ($t_{96} = 2.87$, $p = 0.0501$) (Fig. 1.7C) Soil type did not significantly influence the proportion of seedlings belonging to a native species ($F_{2,96} = 1.75$, $p = 0.1796$).

IV. Discussion

Results from my investigation suggest that positive plant-soil feedback does not significantly contribute to the growth or spread of *Lespedeza cuneata* in the floodplains of eastern North Carolina. Individuals grown in previously invaded soil grew to a similar size as those grown in uninvaded soil (Fig. 1.1). 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 (Table 1.3), or the survival of *L. cuneata* seedlings (Table 1.3). 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) and formed a significantly higher number of root nodules (Coykendall and Houseman, 2014) than those grown in uninvaded soil. Similar 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 my investigation and previous studies.

The absence of evidence for positive plant-soil feedback in my experiment might be attributed to frequent flooding observed in a floodplain system and the subsequent homogenization of soil biota as a result. In river floodplain ecosystems, flooding and hydrology are the most influential factors explaining biodiversity patterns and ecosystem dynamics (Wolfgang et al., 1989). Increases in surface connectivity, as well as flood pulses, have been shown to have a homogenizing effect on the composition of the bacterial community within the soil (Mayr et al., 2020). Floods have been described as “rubber erasers” (Bozelli et al., 2015), removing the environmental heterogeneity created and beta diversity maintained when these areas are isolated during low water periods (Thomaz et al., 2007). Flooding events influence the structural and potential diversity of soil bacterial communities (Furtak et al., 2020), and can decrease microbial biomass within the soil (Unger et al., 2009). Previous research suggests that a relationship exists between the composition of the soil microbial community and an area's hydrological connectivity and flooding frequency (Argiroff et al., 2017). Similar trends of homogenization due to flooding have been documented in flora and fauna. The beta diversity of zooplankton (Bozelli et al., 2015), understory vegetation (Johnson et al., 2014), and grassland bird species (Żmihorski et al., 2016) have all been found to be reduced by flooding events. In the floodplain system where my study is focused, it is likely that populations of *L. cuneata* do not have time to recruit and accumulate microbe symbionts in soil biota prior to the landscape being homogenized through flooding events.

Differences in my results and previous research may also be due to differences in methodology. Because of logistical restraints, this research was conducted using soil collected from pre-existing populations of *L. cuneata*. While it is known that these areas had been invaded for at least four years, the exact age of invasions is unknown and likely to vary among sites. In contrast, previous studies (Coykendall and Houseman 2014; Crawford and Knight, 2017) used soil collected from experimentally invaded plots and were therefore able to control the invasion, including age and density, and other environmental factors. A research design utilizing experimentally invaded sites, as opposed to using pre-existing invasions and herbicide applications, minimizes the overall variation present in the data, and thus is more likely to detect an effect of soil legacy. To minimize variation other than the presence of *L. cuneata* and the application of glyphosate herbicide in this study, uninvaded, invaded, and sprayed soils were collected within a short distance of each other at multiple sites. This design, however, assumes that the soil legacy effects of a *L. cuneata* invasion or glyphosate herbicide are restricted to a relatively small spatial scale. It is possible that experiments utilizing a larger spatial scale between treatments would yield data better illustrating the impact of a *L. cuneata* invasion or herbicide use.

In addition to reaching a similar aboveground biomass, individuals of *L. cuneata* grown in invaded and in uninvaded soils formed a similar number of root nodules (Fig. 1.2). Many non-native 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. A positive relationship between the number of nodules and biomass was significant for those individuals grown in uninvaded soil (Fig. 1.3C) and sprayed soils (Fig. 1.3B). The results from my study cannot determine if a greater biomass was achieved because a greater number of nodules was formed, or if a greater number of nodules was formed as a result of a greater overall biomass. However, these data do certainly illustrate a relationship between plant size and root nodules. The lack of a significant relationship between nodule formation and aboveground biomass in invaded soils suggests that nodule formation is most beneficial in the earliest stages of an invasion, rather than in an ongoing invasion. Similar results have been found in other invasive legumes. Individuals of *V. villosa* grown in recently invaded or uninvaded soil had a significantly stronger relationship between size and the number of nodules formed compared to areas with older invasion histories (Lau and Suwa, 2015).

Individuals of *L. cuneata* grown in invaded soils grew the fewest number of root nodules, significantly lower than individuals grown in sprayed soil (Fig. 1.2). This result might be attributed to the location of rhizobia during the soil collection process. In my study, invasive soils were obtained by collecting soil directly around invasive individuals, often requiring *L. cuneata* individuals to be manually removed with their roots. Rhizobia in these locations are likely to be present in the root nodules of living legumes, rather than free-living in the soil being collected. For sprayed treatments, soil was collected from areas where individuals of *L. cuneata* had been eradicated the previous year using glyphosate herbicide. Following the death of the legume, either native or non-native, the nodules found on the plant roots deteriorate allowing the rhizobial contents to return to the soil (Grains Research and Development Corporation, 2013). While the presence of an invasive legume can increase the density of rhizobia communities when compared to uninvaded areas, the means of invasive individual removal can preserve or diminish this accumulation of symbionts. Mowing preserves the rhizobia community more than the application of herbicide or pulling (Komatsu and Simms, 2019). In a 2014 study performed by Coykendall and Houseman, individuals of *L. cuneata* grown in previously invaded soil produced a greater number of root nodules than those grown in uninvaded soils. However, researchers in that study used herbicide and burning as a means of management prior to soil collection for their invaded treatment. Their invaded treatment, like the sprayed treatment utilized in my study, produced a significantly higher number of root nodules following some form of herbicide management.

The results of my study suggest that the application of glyphosate herbicide alone creates areas where *L. cuneata* can readily reinvade. While *L. cuneata* did not germinate more successfully (Table 1.3) or produce seedlings with a greater survival in the sprayed sites (Table 1.3), individuals grown in sprayed sites achieved a significantly greater biomass than those grown in either uninvaded soil or invaded soil (Fig. 1.1). In addition, individuals of *L. cuneata* grown in sprayed soil produced a significantly greater number of root nodules than those grown in currently invaded soil (Fig. 1.2). These alterations to the soil, coupled with other environmental (Jiraungkorrskul et al., 2003; Glusczak et. al, 2007) and health risks (Guyton et al., 2015) makes the use of glyphosate herbicide alone an ineffective long-term management strategy and will likely lead to reinvasion by *L. cuneata* and other invasive flora.

My research supports previous work showing that native grasses may be more susceptible to the negative soil legacy effects caused by a *L. cuneata* invasion than native forbs. Neither of the two forbs utilized in this study, *Solidago altissima* (Fig. 1.4C) or *Chamaecrista nictitans* (Fig. 1.4A), was impacted by the soil legacy effects of a *L. cuneata* invasion. Individuals of *S. altissima* and *Cham. nictitans* grown in invaded soil showed similar germination rates (Fig. 1.6), seedling survival, and biomass (Fig. 1.4) as those grown in uninvaded soils. However, results from my study and previous research (Kalburtji and Mosjidis, 1993) indicate that a history of a *L. cuneata* invasion negatively impacts the growth of native grasses. While the germination success (Fig. 1.6) and seedling survival of *Chasmanthium latifolium* was similar in invaded soil and uninvaded soil, individuals grew to a significantly smaller biomass when grown in invaded soil as opposed to uninvaded soil (Fig. 1.4B). Similar results were found in a 1993 study concluding that root exudates from *L. cuneata* significantly reduced the biomass, radicle length, and coleoptile length of tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) and bahia grass (*Paspaalum notatum* Flueggé). The same study showed that root exudates decreased the germination, radicle length, and coleoptile length of bermudagrass (*Cynodon dactlyon* (L.) Pers.) (Kalburtji and Mosjidis, 1993).

The use of glyphosate herbicide did not negatively impact either of the two native forbs used in this research. Aboveground biomass of *S. altissima* was similar across in all soil types (Fig. 1.4C). *Cham. nictitans* followed a trend similar to *L. cuneata*, in that its aboveground biomass was significantly greater in the sprayed soil than in uninvaded soil or invaded soil (Fig. 1.4A). In a contrasting trend, but similar to previous research (Flory and Clay, 2009), individuals of *Chas. latifolium* grown in sprayed soil had the lowest biomass, significantly lower than those grown in uninvaded sites (Fig. 1.4B). This continues to support the notion that native grasses may be more susceptible to chemical changes in the soil and may be less appropriate for the initial phase of native plant restoration.

Results from my investigation suggest that individuals of *L. cuneata* grown with either of the two forb species experience interspecific competition that is not significantly more intense than the intraspecific competition within a monoculture. Individuals of *L. cuneata* grown with either of the two native forbs used in this study, *S. altissima* and *Cham. nictitans*, were similar in size to those grown with *L. cuneata* (Fig. 1.1). However, *S. atissima* (Fig. 1.4C) and *Cham. nictitans* (Fig. 1.4A) reached a significantly higher biomass when grown with an individual of *L. cuneata* than when grown with another individual of the same native species. These results suggest that while *S. altissima* and *Cham. nictitans* may not be able to drive out populations of *L. cuneata* through rigorous competition, the two native forbs would be successful in preventing areas from being reinvaded. Soil type was not found to influence the effect of competition for either of the two native forb species, suggesting that *L. cuneata* does not compete more aggressively in previously invaded soils than it does in uninvaded soils.

When grown with the native grass, however, individuals of *L. cuneata* reached a significantly larger aboveground biomass (Fig. 1.1) than when grown with another *L. cuneata*. Meanwhile, interspecific vs intraspecific competition was not found to significantly impact the aboveground biomass of *Chas. latifolium* (Fig. 1.4B). Results from this investigation suggest that the use of *Chas. latifolium* in initial efforts restoring native flora may be unsuccessful, as *L. cuneata* will likely reinvade areas occupied solely by the native grass species as populations of the invasive legume expand. As with forb species, soil type was not found to influence the effect of competition for *Chas. latifolium*.

My results concerning competition are inconsistent with evidence illustrating *L cuneata* as an aggressive invader. While pots were regularly watered, it is likely competition for water and nutrients occurred between the two plants within each pot. As plants reached considerable heights, particularly *S. altissima* and *Cham. nictitans,* competition for light likely occurred throughout the growth room. Over the 11-week growth period, individuals of *S. altissima* (4.93 g ± 0.15) reached a significantly larger overall size than *L. cuneata* (0.58 g ± 0.02), *Chas. latifolium* (1.09 g ± 0.05), or *Cham. nictitans* (1.29 g ± 0.09). As a result, individuals of *S. altissima* grown with another of the same species may have experienced more intense competition for water, nutrients, and light than any other pair of species, and to a greater degree than they would experience in natural field conditions. During the final weeks of the experiment, pots containing at least one individual of *S. altissima* required much more frequent watering. In contrast, *L. cuneata* may not have reached a large enough size to experience competition to the extent observed in natural field conditions.

While emergence of seeds from the seed bank was not impacted by a *L. cuneata* invasion, it was significantly impacted by the application of glyphosate herbicide. A history of an *L. cuneata* invasion did not significantly increase the seedling abundance of *L. cuneata*, the overall abundance of seedlings (Fig. 1.7A), impact species richness (Fig. 1.7B), or the diversity of seedlings (Fig. C). It is possible that the seed bank has been homogenized across the landscape through regularly occurring flooding events, as proposed for soil microbiota. Previous research conducted in floodplain systems has found that seed bank density varies greatly, from 260 to 11, 260 seeds/m^2 (Greulich et al., 2019). Flooding events are likely to also contribute to the long-distance dispersal of *L. cuneata* to new, uninvaded areas. Therefore, the impacts of a *L. cuneata* invasion on the seed bank would not be restricted to solely the soil being actively invaded. However, the use of glyphosate herbicide significantly reduced the total number (Fig. 1.7A), species richness (Fig. 1.7B), and diversity (Fig. 1.7C) of the seedlings that emerged from the seed bank. The use of glyphosate herbicide as currently administered leaves patches of area barren of flora and a seed bank that cannot naturally replenish the area with native flora. These results 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 plant-soil feedback and legacy effects (Coykendall and Houseman 2014; Yannarell et al. 2011), the results may not translate to field conditions and active restoration projects (Reichenborn et al., 2020). In my study, limitation of resources including nutrients, water, and light within my growth room experiment likely differed from their availability in field conditions. Furthermore, this experiment was conducted over 13-weeks of growth, rather than a long-term restoration project. Therefore, results concerning the effects of plant-soil feedback and *L. cuneata* legacy effects reflect only impacts during early growth. There is a need for long-term, field experiments to yield applicable information concerning the role of plant-soil feedback in *L. cuneata* invasions and how legacy effects influence efforts to restore native plant communities.

The native species included in this research were restricted to species whose seeds could be collected in the necessary quantity and germinated in a growth room setting. Many of the candidates initially considered for the study had to be excluded due to these two criteria. This research could be expanded by utilizing a more diverse pool of candidate species for native flora restoration. While results from this research indicate that a native grass, as opposed to native forbs, responds differently to the legacy effects and competition of a *L. cuneata* invasion, research including a wider array of native grasses is necessary to test whether this trend is general. Likewise, the inclusion of more native forbs would allow for more species to be identified that could successfully repopulate areas once invaded with *L. cuneata*.

My investigation was the first to investigate the plant-soil feedback of *L. cuneata* in a floodplain ecosystem in eastern North Carolina. The results from this study illustrate that the positive plant-soil feedback and legacy effects of *L. cuneata* documented in other systems do not directly translate to all systems. 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. There is also a need for field-based experiments to determine the influence of plant-soil feedback and the impact of *L. cuneata* legacy effects in the larger picture of plant communities and ecosystems. Research involving a variety of management strategies including glyphosate herbicide, organic herbicide, manual removal, and burning may be beneficial in determine their effect on the restoration of native flora populations and the soil legacy effects these management strategies may leave behind.

My findings illustrate that in a floodplain system, the use of glyphosate herbicide causes stronger soil legacy effects than the manual removal of *L. cuneata*. Following the manual removal of *L. cuneata*, the soil left behind does not appear to be impacted by strong legacy effects that will significantly impede the establishment of native forb populations (Fig. 1.4A,C). It also appears that within a floodplain system, populations of *L. cuneata* do not load the soil with a significantly greater quantity of seeds in the seed bank that emerge and reinvade the area. However, the process to manually remove invasive flora requires a great deal of manpower and time, and even more to restore populations of native plants. If the use of glyphosate herbicide is continued, effort should be taken to replant native forbs, including *S. altissima* and *Cham. nictitans*, in their place to prevent the possibility of reinvasion. It is possible that the use of annual forbs, such as *Cham. nictitans,* for initial restoration of native flora would be less successful than perennial forbs, as their success relies on their reseeding of the next generation. A restored community of native flora is the end goal of successful invasive plant management.

Table 1.1: Results from the mixed general linear model for the effects of soil type, neighbor species, and their interaction the aboveground dry biomass of *Lespedeza cuneata*. Significant *P*-values (< 0.05) indicated with bold.

	df	*F*	*P*
Soil Type	2, 296	4.11	**0.0174**
Neighbor	3, 296	4.48	**0.0043**
Neighbor × Soil Type	6, 296	0.55	0.7689

Table 1.2: Results from the mixed general linear model for the effect of soil type, neighbor type (intraspecific or *L. 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	Treatment	df	*F*	*P*
Cham. nictitans	Soil Type	2,136	2.72	**0.0696**
	Neighbor	1,136	8.75	**0.0037**
	Neighbor x Soil	2,136	0.26	0.7738
Chas. latifolium	Soil Type	2,153	3.36	**0.0373**
	Neighbor	1,153	2.42	0.1219
	Neighbor x Soil	2,153	0.29	0.7501
S. altissima		2,156	0.80	0.4521
	Neighbor	1,156	81.93	**<0.0001**
	Neighbor x Soil	2,156	0.51	0.5992

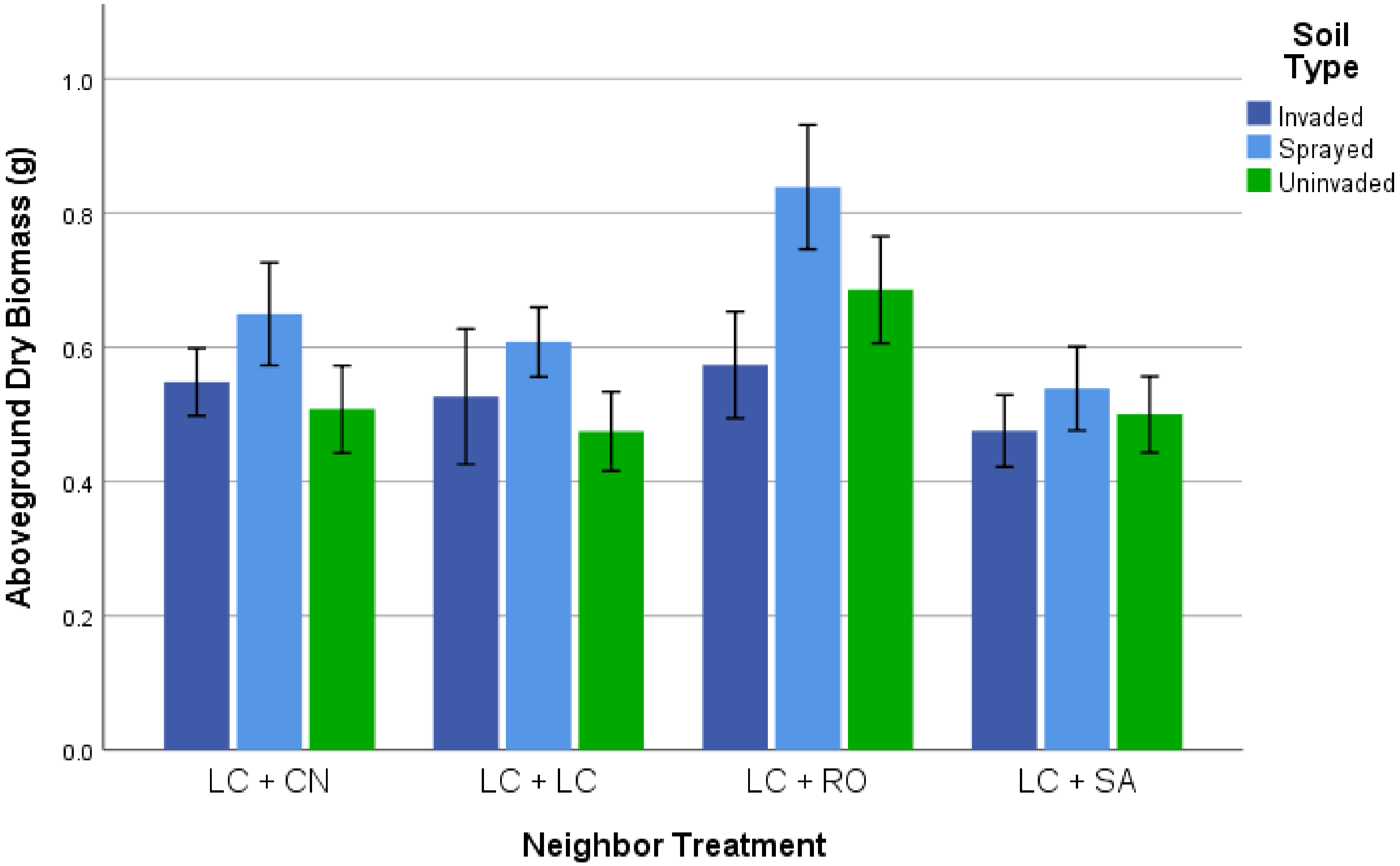


Figure 1.1: Mean aboveground dry biomass (± 1SE) of *Lespedeza cuneata* (LC) when grown with another individual of *Lespedeza cuneata*, *Chamaecrista nictitans* (CN), *Chasmanthium latifolium* (RO), and *Solidago altissima* (SA) in uninvaded (U, green), invaded (I, dark blue), or sprayed (S, light blue) soil. Soil type ($F_{2,296}$=4.11, *P*=0.0174) and neighbor ($F_{2,296}$=4.48, *P* =0.0043) were both found to significantly impact aboveground dry biomass. The interaction of neighbor × soil was not significant ($F_{6,296}$=0.55, *P* =0.7689).

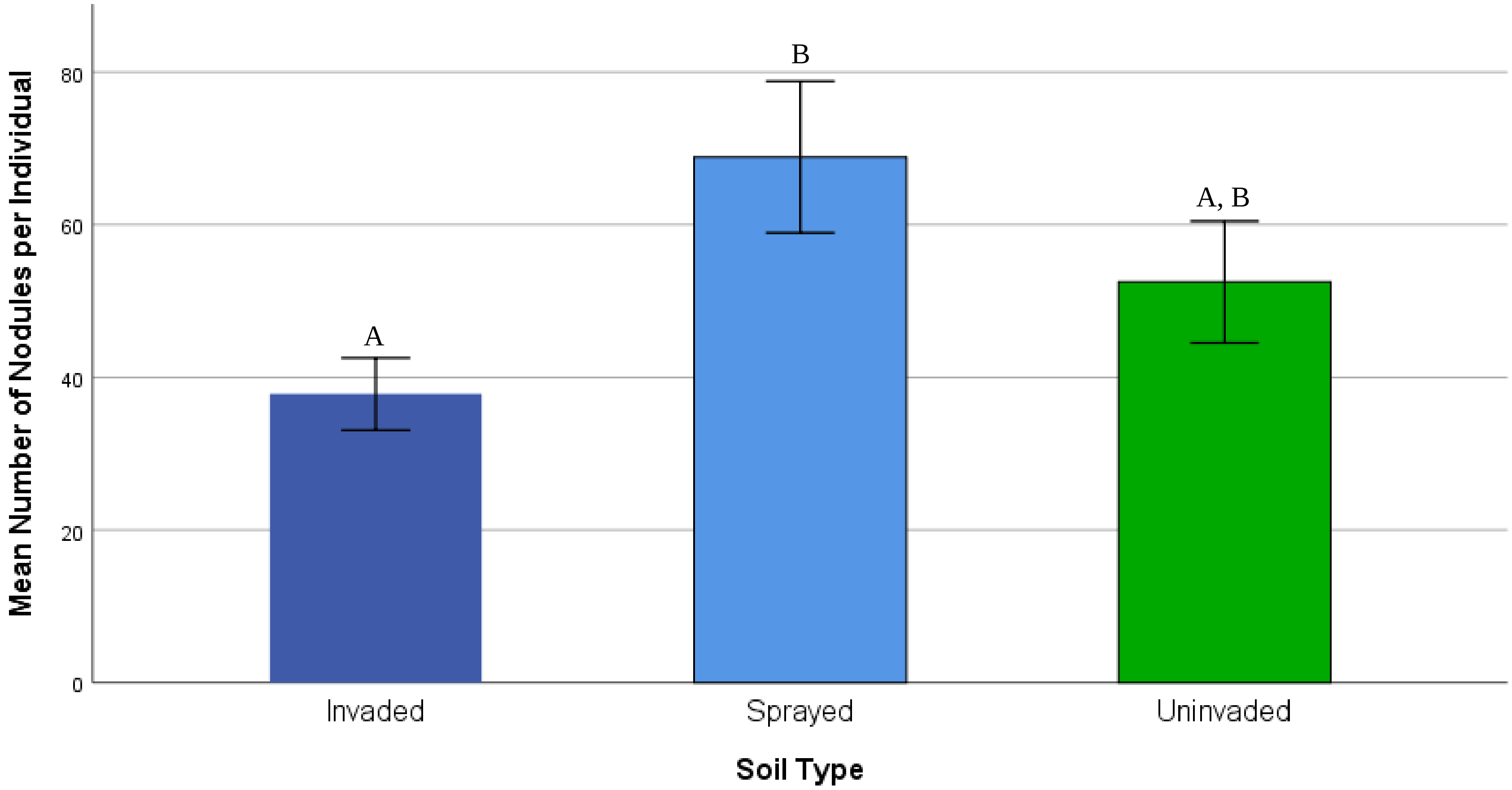
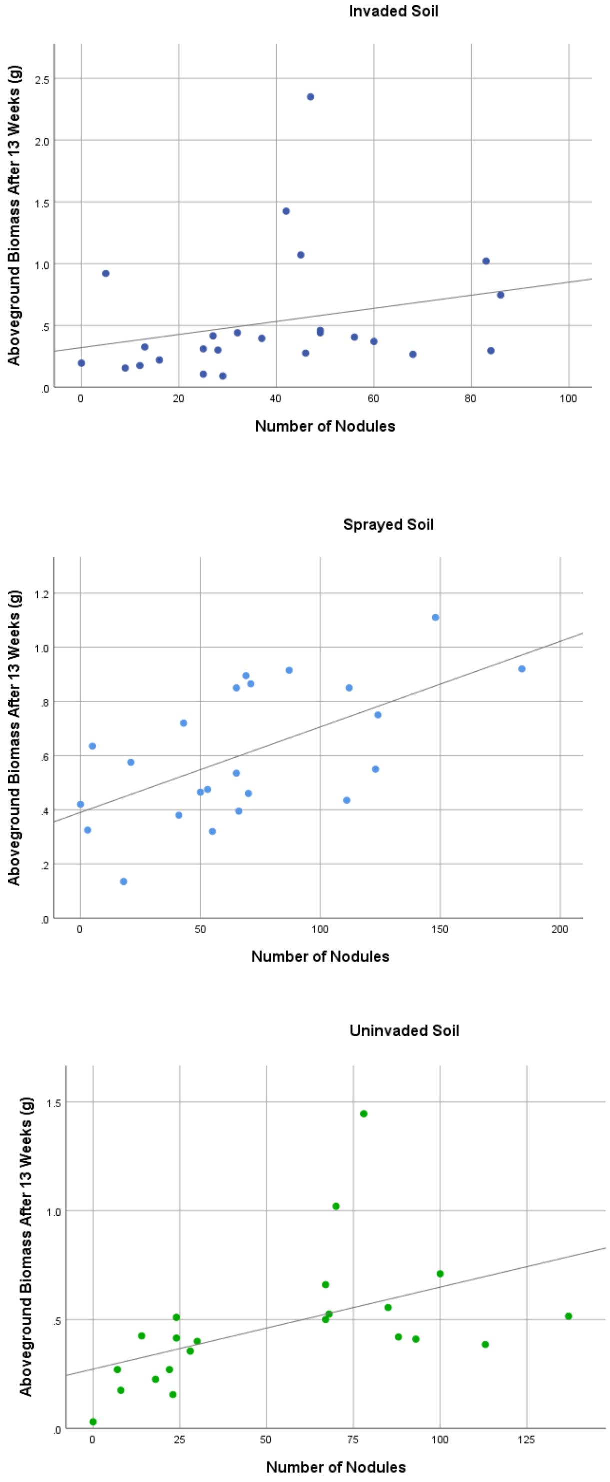


Figure 1.2: Mean (± 1SE) number of root nodules per individual of *Lespedeza cuneata* grown in invaded soils, sprayed soil, and uninvaded soil. Soil type significantly impacted the number of nodules per individual ($F_{2,61}$=3.72, P =0.0300). Significant differences between soil type, as determined by Tukey's post-hoc procedure ($P < 0.05$), are depicted using different letters.



A)

P = 0.0203

$r^2 = 0.2411$

B)

P = 0.2149

$r^2 = 0.0661$

C)

P = 0.0023

$r^2 = 0.3645$

Figure 1.3: Relationship between the number of root nodules per individual and aboveground final biomass of *Lespedeza cuneata* after 13weeks when grown in A) uninvaded soil (t_1=2.52, *P* =0.0203, r^2=0.2411), B) invaded soil (t_1=1.28, *P* =0.2149, r^2=0.0661), and C) sprayed soil (t_1=5.16, *P* =0.0023, r^2=0.3645).

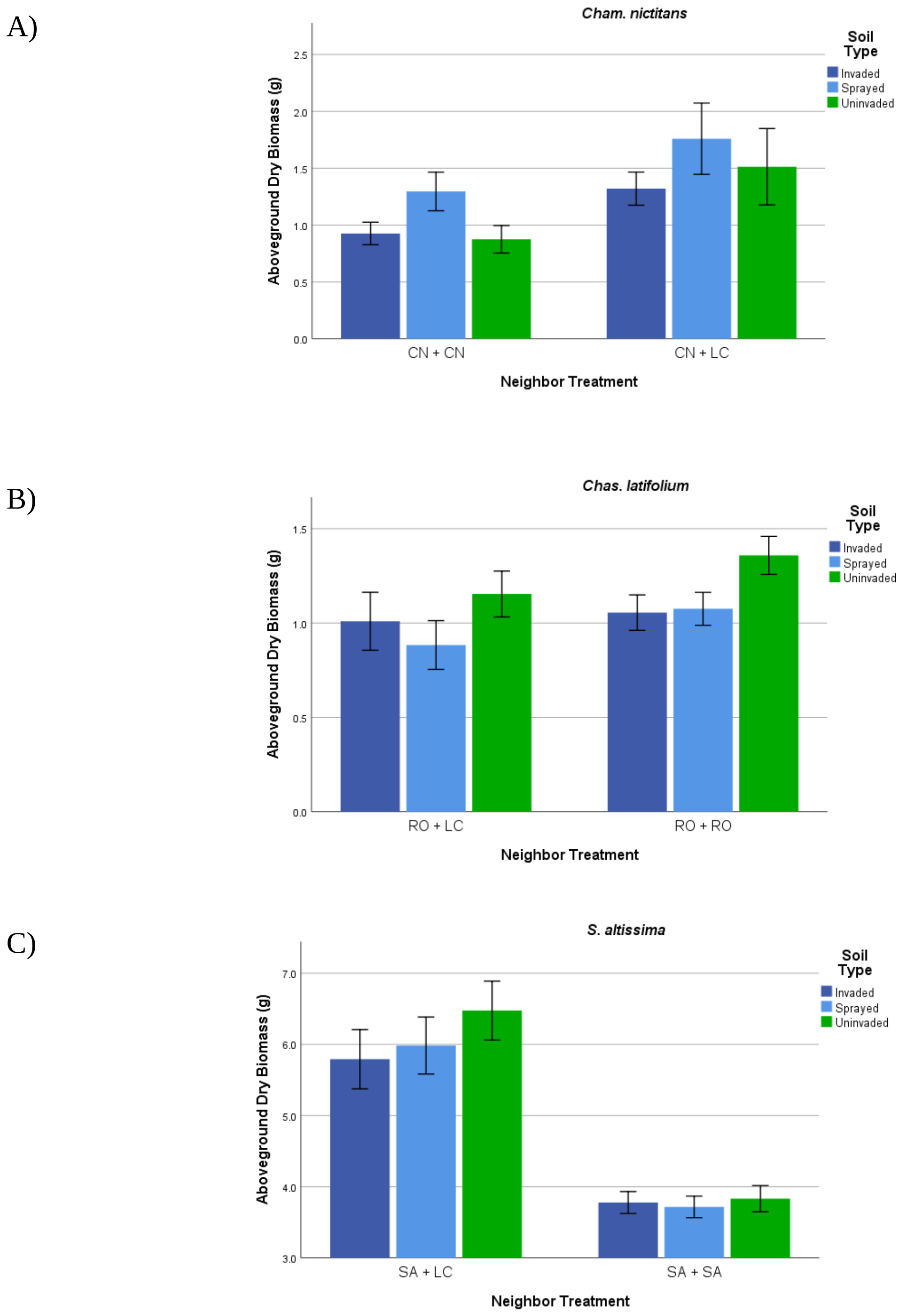


Figure 1.4: Mean (± 1SE) aboveground dry biomass after 13 weeks of A) *Chamaecrista nictitans* (CN), B) *Chasmanthium latifolium* (RO), and C) *Solidago altissima* (SA) when grown in uninvaded (U, green), invaded (I, dark blue), or sprayed (S, light blue) soil with either another individual of the same species or with an individual of *Lespedeza cuneata* (LC). Soil type was found to significantly impact the growth of *Cham. nictitans* ($F_{2,136}$=2.72, *P* =0.0696) and *Chas. latifolium* ($F_{2,153}$=3.36, *P* =0.0373), but not *S. altissima* ($F_{2,156}$=0.80, *P* =0.4521). Neighbor type was found to significantly impact the final biomass of *Cham. nictitans* ($F_{2,136}$=8.75, *P* =0.0037) and *S. altissima* ($F_{2,156}$=81.93, *P* <0.0001), but not *Chas. latifolium* ($F_{2,153}$=2.42, *P* =0.1219). The interaction of neighbor type × soil type was not found to significantly impact the final biomass of any species.

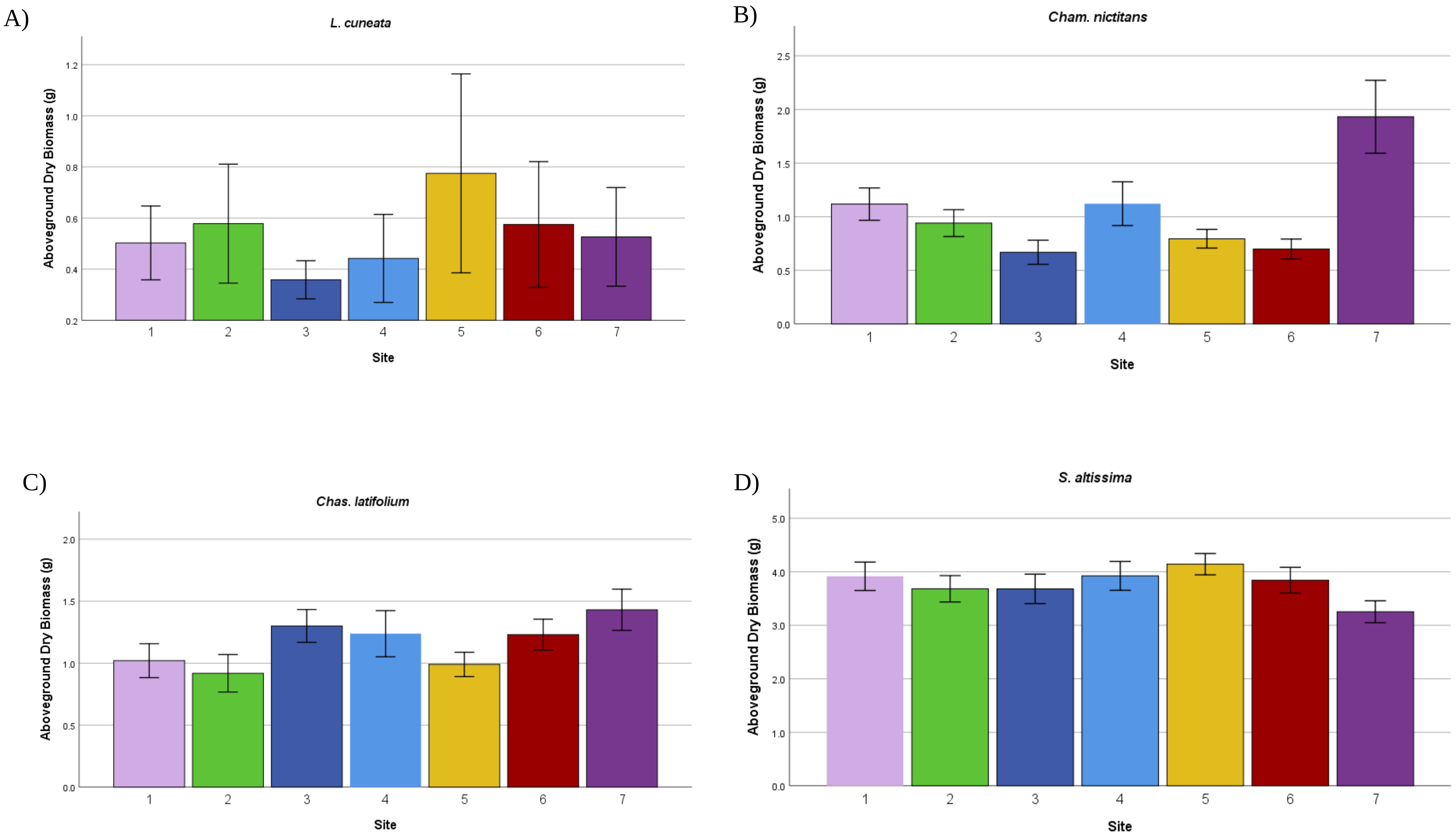


Figure 1.5: Mean (± 1SE) aboveground dry biomass of A) *Lespedeza cuneat*a, B) *Chamaecrista nictitans,* C) *Chasmanthium latifolium,* and D) *Solidago altissima* for each of the seven soil collection sites.

Table 1.3: 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			Survival		
Species	df	*F*	*P*	df	*F*	*P*
L. cuneata	2, 180	1.70	0.1861	2, 107	1.75	0.1785
Cham. nictitans	2, 180	0.55	0.5781	2, 148	0.30	0.7440
Chas. latifolium	2, 180	0.16	0.8492	2, 72	1.19	0.3088
S. altissima	2, 180	7.16	**0.0010**	2, 167	1.99	0.1402

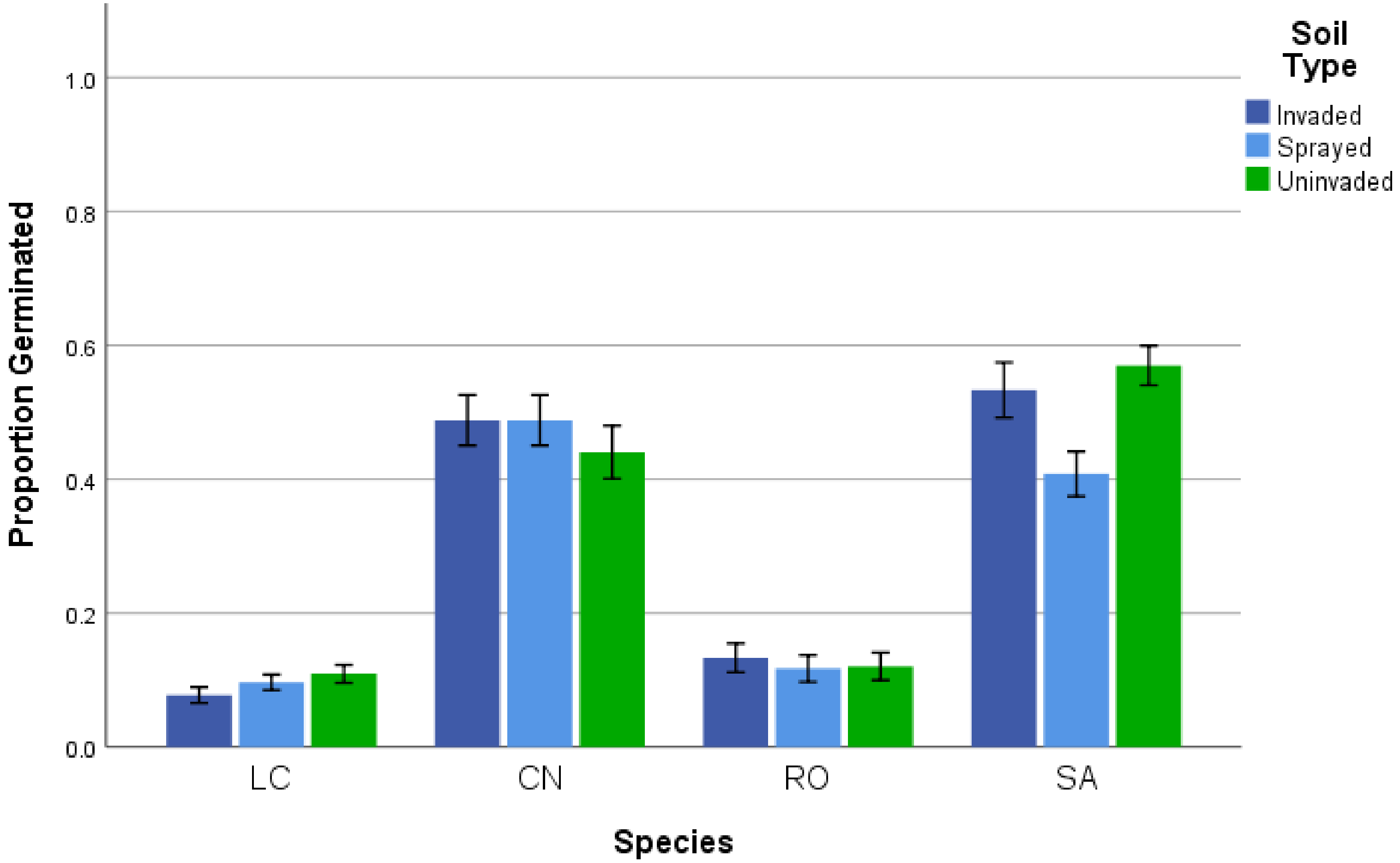


Figure 1.6: Mean proportion (± 1SE) of seeds to successfully germinate in invaded soil (I, dark blue), sprayed soil (S, light blue), and uninvaded (U, green) soil. Soil type did not significantly impact the germination of *Lespedeza cuneata* (LC) ($F_{2,180}$=1.70, *P* =0.1861), *Chamaecrista nictitans* (CN) ($F_{2,180}$=0.55, *P* =0.5781), or *Chasmanthium latifolium* (RO) ($F_{2,180}$=0.16, *P* =0.8492). Soil type significantly influenced the germination success of *Solidago altissima* (SA) ($F_{2,180}$=7.16, *P* =0.0010). Significant differences between soil type, as determined by Tukey's post-hoc procedure ($P < 0.05$), are depicted using different letters.

Table 1.4: A total of 1,431 seedlings representing 86 species emerged over the course of the seed bank experiment. Species marked with (**) are listed as being exotic by the USDA. Native status could not be determined for seedlings not identified to species level. Treatment columns indicate the number of pots out of 35 to contain each species.

		Total # of Individuals	**Invaded Pots**	**Sprayed Pots**	**Uninvaded Pots**
Asteraceae	*Ambrosia artemisiifolia*	2	1	1	0
	Baccharis halimifolia	1	0	0	1
	Bidens bipinnata	3	0	2	1
	Eclipta prostrata	16	1	2	4
	Eupatorium capillifolium	333	24	15	24
	Gamchaeta purpurea	54	9	12	9
	Krigia virginica	19	5	5	4
	Pluchea camphorata	2	0	0	2
	Pseudognaphalium obtusifolium	2	0	2	0
	Solidago altissima	34	5	8	3
	*Soliva sesselis***	9	2	3	3
Brassicaceae	*Cardamine hirsute***	16	1	0	6
	Cardamine pensylvanica	39	2	5	10
Buddlejaceae	*Polypremum procumbens*	5	0	2	3
Campanulaceae	*Triodanis perfoliata*	6	2	0	4
Caryophallaceae	*Cerastium glomeratum***	44	14	2	5
	Sagina decumbens	131	18	16	11
	*Stellaria media***	10	1	1	4
Chenopodiaceae	*Chenopodium alba***	1	0	1	0
Clusiaceae	*Hypericum hypericoides*	3	2	1	0
Cyperaceae	Unknown sedge #1	1	0	1	0
	Unknown sedge #2	1	0	1	0
	Unknown sedge #3	1	0	0	1
	Unknown sedge #4	1	0	1	0
	Unknown sedge #5	20	8	4	2
	Unknown sedge #6	1	0	0	1
	Unknown sedge #7	4	1	1	1
	Unknown sedge #8	1	0	1	0
Euphorbiaceae	*Acalypha rhomboidea*	1	0	0	1
	Chamaesyce maculata	2	0	2	0
Fabaceae	*Lespedeza cuneata***	51	10	10	6
	Trifolium carolinianum	6	0	0	5
	Vicia sp.**	2	0	0	2
Geraniaceae	*Geranium carolinianum*	2	0	0	5
Juncaceae	Unknown rush #1	6	1	0	1

	Unknown rush #2	7	3	0	3
Lamiaceae	*Lamium purpureum***	6	2	0	4
Liliaceae	*Allium vineale***	5	4	0	0
Loganiaceae	*Gelsemium sempervirens*	1	0	0	1
Lythraceae	*Cuphea carthagensis***	74	14	8	7
	Rotala ramosior	3	1	0	2
Molluginaceae	*Mollugo verticillata*	2	0	2	0
Onagraceae	*Ludwigia decurrens*	1	0	0	1
	Ludwigia palustris	10	1	2	4
	Oenothera laciniata	1	0	1	0
Oxalidaceae	*Oxalis dillenii*	22	5	1	9
Phytolaccaceae	*Phytolacca americana*	3	0	0	2
Plantaginaceae	*Plantago heterophyla*	47	8	5	7
	Plantago virginica	1	0	0	1
Poaceae	*Digitaria sanguinalis***	1	1	0	0
	Unknown grass #1	39	8	6	12
	Unknown grass #2	4	0	2	1
	Unknown grass #3	1	0	0	1
	Unknown grass #4	1	0	1	0
	Unknown grass #5	1	0	1	0
	Unknown grass #6	1	0	0	1
	Unknown grass #7	7	4	1	2
	Unknown grass #8	1	1	0	0
	Unknown grass #9	1	1	0	0
	Unknown grass #10	1	0	0	1
Polygonaceae	*Polygonum cespitosum***	27	8	4	6
	*Polygonum persicaria***	47	5	4	7
Portulacaceae	*Portulaca amilis***	1	0	0	1
Ranunculaceae	*Ranunculus abortivus*	3	2	0	0
	Ranunculus pusillus	4	1	0	3
Rosaceae	*Aphanes microcarpa***	8	4	1	1
Rubiaceae	*Diodia virginiana*	2	2	0	0
	Galium sp.	1	1	0	0
Scrophulariaceae	*Mazus pumilus***	3	2	0	1
	Nuttallanthus canadensis	4	0	3	1
	*Veronica arvensis***	4	1	0	2
	Veronica peregrina	30	6	0	11

Smilacaceae	*Smilax rotundifolia*	1	0	0	1
Urticaceae	*Boehmeria cylindrica*	5	4	0	0
Valerianaceae	*Valerianella radiata*	1	0	1	0
Verbenaceae	*Verbena brasiliensis***	196	8	10	14
Violaceae	*Viola sororia*	1	1	0	0
Xyridaceae	*Xyris* sp.	2	0	1	0

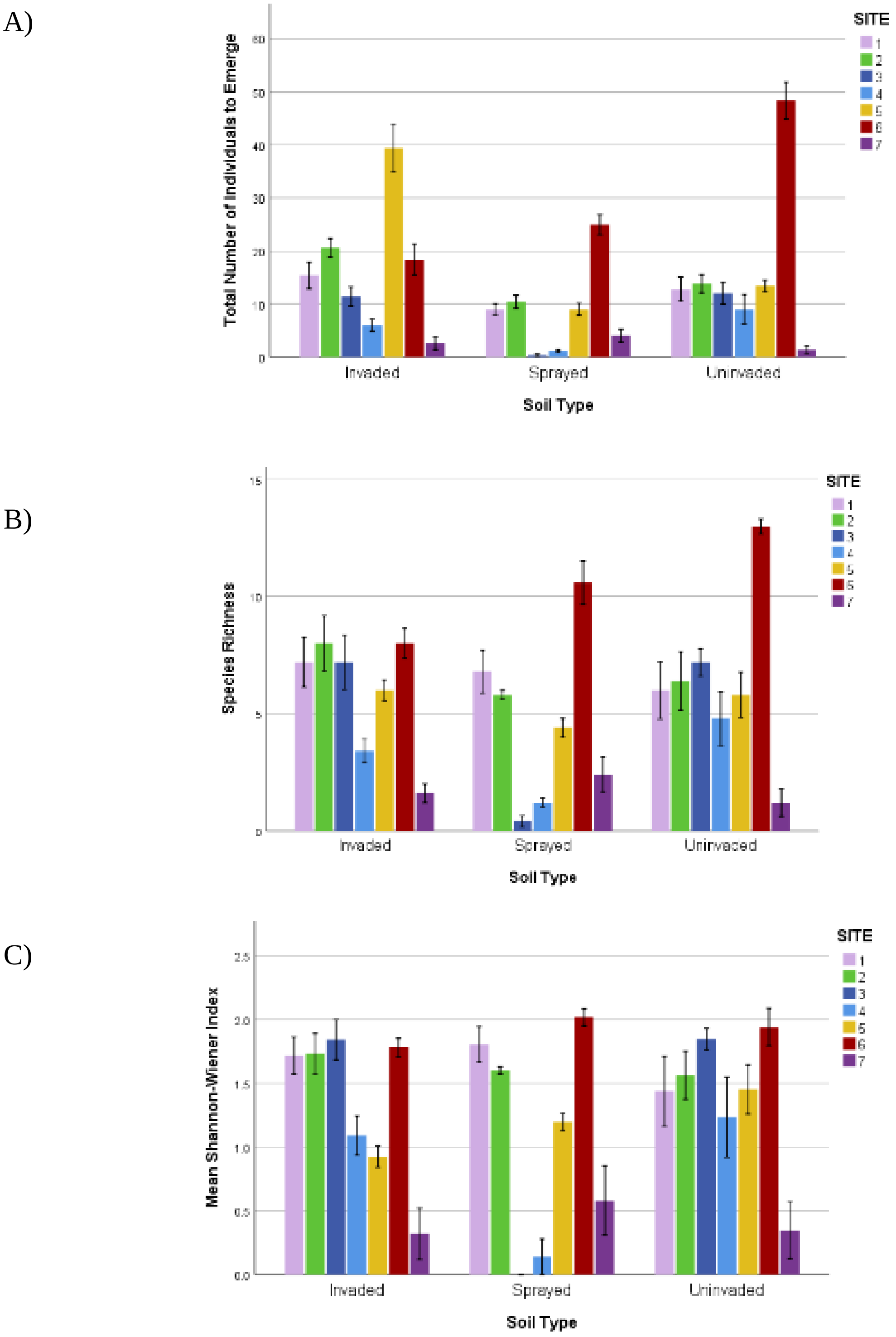


Figure 1.7: Mean (± 1SE) A) total number, B) species richness, and C) Shannon-Wiener Index of seedlings emerging from seed bank for each of the seven sites used in the experiment. Soil type significantly impacted the total number ($F_{2,96}$=9.96, *P* =0.0001), species richness ($F_{2,96}$=6.33, *P* =0.0026,), and Shannon-Wiener index ($F_{2,96}$=4.70, *P* =0.0113).

CHAPTER 2

I. Introduction

The perception of conservation by the general public is often vital to the success of management and conservation efforts. While the ecological aspects of conservation are studied by environmental biologists and the economic aspects are analyzed by public policy makers, the social component of conservation efforts is all too often overlooked (Schüttler et al., 2011). Public support can influence what land and funds are allocated for conservation and often favors species that are perceived as being visually attractive or emotionally appealing (Jacobson et al., 2015). Researchers and the public often have diverging attitudes and beliefs, presenting an obstacle to overcome before serious progress can be made (Fischer et al., 2014).The lay public is typically not fully aware of the biodiversity around them, even in the urban environments where they live and work, and the benefits biodiversity brings (Dallimer et al., 2012). Similarly, the spread of invasive species and its consequences are not always understood by the public. As a result some people take the stance that there is no reason to intervene with management efforts as species invasions are just “nature taking its course” (Schüttler et al., 2011). Many organizations and events whose aims are to promote conservation to the general public focus on five aspects: awareness, knowledge, attitudes, skills, and participation (Jacobson et al., 2015). Researchers are even turning to social media to increase public awareness and education, especially towards younger demographics (Shiffman, 2012). Community engagement and public outreach events provide a means to help bridge this gap in attitudes and perception between researchers and the public.

Citizen engagement and public outreach can be utilized to accomplish conservation tasks. For many programs, citizens contribute towards ecology and conservation by collecting data that are submitted to a larger data base (Kobori et al., 2015). However, an obstacle for many conservation efforts is recruiting the workforce necessary. By participating in these programs and events, citizens become an active part of conservation efforts. Meanwhile, citizens also are educated on conservation concerns, and become more aware of the role they can play in successful conservation efforts and the natural world around them.

Citizen-science has also been successfully implemented with populations of undergraduate students through specifically designed courses. Courses involving citizen-science not only gather data for beneficial research, but also present students with unique class projects and even provide the opportunity for independent research for students outside of the course (Oberhauser and LeBuhn, 2012). Students who participate have had opportunities to serve as coauthors on project publications, apply skills learned in future research endeavors, and gain the satisfaction of knowing data they collected contributed to a greater scientific understanding (Karlin and De La Paz, 2015). Data collected from pre-course and post-course tests illustrate that courses utilizing citizen-science projects impact attitudes of undergraduate students towards not only the course's specific subject matter, but also the student's interest in participating in citizen-science projects again in the future (Vitone et al., 2016). A second approach used to engage with undergraduate students is through service-learning courses. Service-learning activities extend learning beyond the traditional classroom by providing students the opportunity to apply skills and knowledge taught in coursework in real-life situations and projects (Waterman, 1997). Service-learning courses benefit the local community through various projects and community engagement. Although it is more often used to provide services to human communities (Blieszner and Artale, 2001; Carlson and Witschey, 2018), service-learning can also be used to control growing populations of invasive plants (Goodwillie and Jolls, 2018).

One aspect of conservation where citizen-science programs, public outreach events, and service-learning courses have contributed greatly is the detection, management, and removal of invasive flora and fauna. Invasive species are a leading and growing threat to biodiversity and can have negative impacts on the environment, economy, and public health (Wittenburg and Cock, 2001). The management and eradication of invasive species can incur an extreme economic burden, due to the required management efforts and loss of natural resources (Andreu et al., 2009). After being introduced through nurseries, botanical gardens, or contaminants in agricultural seed (Reichard and White, 2001) invasive plants can cause negative impacts that ascend to higher trophic levels (Vilá et al. 2015). Compared to their native counterparts, invasive plants typically produce a higher volume of seeds, experience a lower mortality rate, and other characteristics associated with a higher fitness (van Kleunen et al. , 2010). Fragmented areas (Vilá and Ibáñez, 2011) and areas with a history of land use (Kuhman et al., 2015) are at a higher risk of becoming dominated by invasive plant species. Herbicide is used in some cases to reduce and control populations of invasive plants, but the benefits are only temporary and can have unintended negative effects on the surrounding ecosystem (Wittenburg and Cock, 2001). Likewise, the introduction of a non-native predator to control a target species poses a high risk of damaging species other than the targeted invasive species (Havens et al. 2012, Havens et al., 2019). Simple manual removal is often an effective management strategy, but can require a large workforce (Wittenburg and Cock, 2001). The community can be a diverse source of effective volunteers, as highly detailed knowledge of the target species is unnecessary. Citizens can contribute to the detection, management, and restoration efforts needed to control invasive species (Simberloff, 2009). For example, as the Indo-Pacific lionfish (*Pterois volitans*) expanded its range through the Gulf of Mexico, citizen observation programs detected its presence 1-2 yr earlier and more frequently than traditional monitoring programs (Scyphers et al., 2015).

I sought to involve undergraduates at a local regional public university and assess if undergraduate outreach events are an effective means of improving attitudes of towards conservation. I coordinated an outreach event at East Carolina University (ECU), Greenville, NC, with the members of the ECU Honors College Student Council (HCSC) in which students contributed to the manual removal of an invasive legume from a local greenway. During the event, participants engaged in discussions centered on native flora, conservation, and the threat of invasive species. After the event, participants completed a voluntary survey designed to gauge attitudes of undergraduate students towards conservation and invasive species. Demographic information was also collected to determine if undergraduate major influenced survey responses.

II. Methods

II-A: Study Site

The outreach event was held on the Green Mill Run Greenway in Greenville, NC. This portion of the Greenville Greenway was constructed in 2015 and is located near the ECU Athletic District along Green Mill Run. The native tree community present at the site includes bull bay magnolia (*Magnolia grandiflora* L.), American sycamore (*Platanus occidentalis* L.), and tulip tree (*Liriodendron tulipifera* L.). American beautyberry (*Callicarpa americana* L.), devil’s walking stick (*Aralia spinosa* L.), and jewelweed (*Impatiens capensis* Merrb.) also exist in large populations at the site. Invasions of Chinese privet (*Ligustrum sinense* Lour.), chamber bitter (*Phyllanthus urinaria* L.), Japanese stiltgrass (*Microstegium vimineum* (Trin.) A. Camus), and Chinese lespedeza, (*Lespedeza cuneata* (Dum. Cours.) G. Don) occur throughout the greenway.

II-B: Lespedeza cuneata

First introduced to North Carolina in 1896 as a means of erosion control and forage (Ohlenbusch and Bidwell., 2007), *Lespedeza cuneata* has established invasive populations in the grasslands of midwestern and eastern United States and is labeled as a noxious weed by the USDA in nearly all states. Distinguished by its trifoliate leaves and small white flowers with purple markings, *L. cuneata* produces five times as many seeds as native congeners in the prairie states of the U.S. (Woods et al., 2009) and establishes seedbanks that may remain viable for several years (Stevens, 2002). On other portions of the greenway, management of the *Lespedeza* invasion is taking place through manual removal performed annually by undergraduate students in a service-learning section of a plant biology course, and application of glyphosate herbicide in select, especially dense locations by Greenville City Recreation and Parks Department.

II-C: Event Design

The outreach event was coordinated with the ECU Honors College Student Council (HCSC) and was designed so that participants assisted in the removal of *Lespedeza cuneata* while also being educated on the local flora. Through this event we hoped to contribute to the management of *L. cuneata* on the greenway, expose students to the diversity of native flora, educate them on the threats invasive species present to native biodiversity, and provide students the opportunity to become better connected with the natural world. Leadership of the ECU HCSC promoted this event at meetings, on social media, and through email. I attended a monthly meeting of the ECU HCSC to explain the purpose of the event and encourage attendance. Members of the ECU Air Force ROTC also attended the event.

The outreach event was conducted on October 12, 2019. Participants gathered in front of the ECU Gateway Residence Hall at 9:00 am. There the 27 participants were debriefed on the purpose of the project, given gloves, provided with insect repellent, and shown the location of the first-aid kit being carried. As a group, participants were led to the Green Mill Run Greenway, where they were shown how to identify *Lespedeza cuneata* and remove it most effectively by grasping the plant at the base of the stem with both hands, pulling straight up, and removing the roots with the rest of the plant if possible. Following this initial demonstration, participants removed invasive individuals from the designated area for approximately ninety minutes. Removed plants were disposed of in garbage bags. Over the course of the event, participants were shown a variety of native plant species and were shown areas of the greenway dominated by invasive plants. Discussion also focused on the importance of the restoration of native flora following the removal of invasive individuals. At the conclusion of the event, participants were led as a group back to Gateway Residence Hall and asked to complete a voluntary survey.

II-D: Participant Survey

Surveys were designed to collect general demographic information, assess attitudes towards conservation, and ascertain opinions of the outreach event. No identifying information was collected from event participants, and all participants were over the age of 18. The survey instrument was submitted to the University and Medical Center Institutional Review Board and was certified as exempt on October 10, 2019 (UMCIRM 19-002167). Following initial demographic questions, the first portion of the survey presented participants with 17 statements concerning invasive species and conservation (Table 2.1). This portion of the survey gauged the participant’s opinion, such as how the participant felt towards all species being conserved, and also the participant’s knowledge of invasive species. Some survey questions from this portion were patterned after the Nature Relatedness Scale, designed to assess an individual’s personal connection with nature, external views of nature, and comfort in the outdoors (Nisbet et al., 2009; Nisbet and Zelenski, 2013). Other questions from this first survey portion were patterned after the Self-Efficacy for Environmental Action survey designed to examine the ability of individuals to effectively address environmental concerns (Porticella et al., 2017). The second portion presented participants with four hypothetical scenarios (Table 2.2) and asked participants to indicate how they felt towards the implementation of suggested conservation efforts. Each scenario concerned either conservation of an endangered species or management efforts of an invasive species. Scenarios were designed to include ecological, social, and economic aspects of conservation, similar to surveys used by other researchers (Bremner and Park, 2007). The final portion of the survey asked participants their opinion of this particular outreach event through nine statements (Table 2.3). These statements concerned the anticipated benefits of participating in the event, such as becoming more aware of plant diversity. This final portion of the survey also addressed desired long-term impacts of the event, including whether participants would attend a similar event in the future. Participants were asked how they feel towards all statements and scenarios from one of five options presented in a Likert scale: “Strongly Disagree” (1), “Disagree” (2), “Neutral” (3), “Agree” (4), or “Strongly Agree” (5).

II-E: Statistical Analysis

For each survey question, the mean and standard error of participant responses were determined using SAS statistical software. To assess if students’ major influenced their opinion toward invasive species and conservation, majors were categorized into two groups. The first group comprised students who were majoring in a social science, which included psychology, public health, philosophy, and criminal justice majors. The second group comprised students majoring in STEM (science, math, engineering, or technology) fields, which included chemistry, physics, exercise physiology, and biology majors. Five students were majoring in a field that did not fit into one of these two groupings, including art and business management, and were not included in this portion of the analysis (Table 2.4). Two-sample two-tailed t-tests were performed for each question to test for a difference in responses between social science and STEM students.

III. Results

Twenty-seven participants attended my outreach event and removed several trash bags of *L. cuneata* from approximately half (0.75 mi) of the Green Mill Run Greenway (1.43 mi). Freshman (n = 7), sophomores (n = 13), juniors (n = 3), and seniors (n = 4) were all in attendance at the event. All participants completed the voluntary survey at the conclusion of the event. Participants agreed most strongly with statements that described their responsibility as an individual to preserve the environment and that it is important to preserve the environment globally (Table 2.1). Participants largely disagreed with the statements concerning their ability to identify flora, both native and invasive (Table 2.1). The scenario concerning the use of herbicide to control an invasive vine and the scenario concerning the restriction of pesticide use to protect an endangered insect both received responses that reflected a neutral stance towards the implementation of proposed conservation efforts (Table 2.2). The scenario concerning the cancelation of a business center to protect an endangered plant and the scenario concerning the eradication of a nonnative reptile received responses that reflected most participants disagreed with conservation efforts (Table 2.2). Finally, for questions asking students about their opinion of the outreach event all mean responses reflected an opinion that was positive (Table 2.3).

While STEM and social science majors had similar responses to all hypothetical scenario statements (Table 2.2) and statements concerning the outreach event (Table 2.3), four of the statements concerning the participant’s opinion towards conservation and invasive species did receive responses that were notably different ($p \leq 0.06$) between the two groups (Table 2.1). Undergraduates majoring in a STEM field agreed more strongly than those majoring in a social science that reducing the number of invasive species is beneficial for the environment. However, students majoring in a STEM field indicated that they were more unsure of where to find resources and how to respond to invasive species than those students majoring in a social science. In addition, social science students agreed more strongly that invasive species were an issue in their area than those students majoring in a STEM field.

IV. Discussion

The removal of invasive flora from the local greenway, the high undergraduate student participation, and the overall responses of the participant surveys all illustrated that the event was successful in achieving its goals. Surveys reflected that participants enjoyed participating in the event, felt more connected to nature, and felt more equipped to help the environment because of this event. Even more promising, surveys indicated that participants were likely to seek out similar events in the future and would recommend that others attend a similar event (Table 2.3). These findings are similar to the outcomes of citizen-science conducted through coursework (Karlin and De La Paz, 2015; Vitone et al., 2016). Survey responses collected during the outreach event illustrated the same generally positive attitudes towards conservation and preserving biodiversity as found in other studies (Nisbet et al., 2009). Previous studies found demographic information concerning pet ownership and membership in nature-oriented organizations explained a considerable amount of variation found in survey responses (Nisbet et al., 2009). Additional demographic questions not included in the survey might have explained some of the variation observed in survey responses of undergraduate students. Responses concerning the scenarios were similar to previous studies in that the conservation efforts involving the eradication of animals or that caused some negative economic impact were the ones participants disagreed with most strongly (Bremner and Park, 2007). Bremner and Park (2007) also found that the species, taxonomic group, and specific means of eradication influenced survey responses. Some results of the survey seem to contradict one another. While participants responded that they had a responsibility to protect the environment and that reducing the number of invasive species is beneficial for the environment (Table 2.1), the overwhelming response to proposed solutions to conservation scenarios was neutral if not disagreement (Table 2.2). These scenarios introduced some of the aspects of invasive ecology that influence decision making for species management, including impacts to economy, agriculture, and business. Survey responses illustrated that while undergraduates acknowledge the ecological threats of invasive species, protection of jobs and agricultural success are still higher priorities. While participants felt that invasive plants threatened native wildlife and that they wanted to preserve their local environment, they did not strongly agree that invasive species were an issue in their area (Table 2.1). While survey responses show that undergraduate students understand the underlying concepts that cause invasive species to threaten local flora or fauna, students did not recognize ongoing invasions that surround them, even after assisting in the control of an invasive plant on a local greenway. This is also illustrated by survey responses indicating participants could not identify native plants, and more so invasive plants (Table 2.1).

The differences in survey responses between undergraduates majoring in STEM vs. social science fields suggests that underlying attitudes influencing a student’s choice of major or the course of study within their chosen major influences their opinions towards conservation and invasive species. While both of these groups agreed that invasive plants present a threat towards native wildlife, STEM students more strongly agreed that reducing the number of invasive species is beneficial for the environment than did social science students (Table 2.1). This notable difference in survey response can likely be attributed to relative coursework required by undergraduates in a STEM field. However, social science students agreed more strongly than STEM students, whose mean response was nearly neutral, that invasive species are an issue in their area (Table 2.1). This striking result illustrates that undergraduates majoring in the life sciences are not necessarily more aware of the natural world around them.

Data collected through surveys provided insight into the opinions of ECU undergraduate students towards conservation, invasive species, and outreach events. However, these data could be improved greatly by increasing the sample size and including broader representation of different types of students in the survey. Of the over 23,000 undergraduates at ECU only 27 completed the survey. All students who completed the survey were members of either the ECU Honors College or the ECU Air Force ROTC Members of these two subsets of the undergraduate population are possibly not reflective of the opinions of the entire undergraduate population at ECU. Increasing the number of surveys completed would allow for a more accurate depiction of the opinion of undergraduate students towards conservation and invasive species. Some bias may have been introduced because students who volunteered for this outreach event were likely to have had previous outdoor experience and enjoyed engaging in nature. Administering these surveys outside of an outreach event would possibly provide a more representative sample of undergraduate students. Surveys completed by students who did not attend the outreach event would have also provided a control group for comparison to participants. Without this control group, the only effects of the outreach event that are evident are those collected from final portion of participant surveys (Table 2.3). Because the outreach event targeted undergraduates, rather than the entire public, inferences concerning the public and general community members cannot be made. Unlike an outreach event targeting all members of a community, participants of our outreach event were all between the ages of 18-22 and were all in the pursuit of an undergraduate degree. Outreach events promoted to the public would likely see a sample with more diverse ages, education level, and employment status. These criteria have been shown in similar surveys to significantly influence survey responses (Bremner and Park, 2007). A larger and more reflective sample would be necessary to better understand the attitudes and opinions of community members towards invasive species and conservation.

Participants took interest in the local flora that surrounds campus and seemed enthusiastic for similar events in the future. Future endeavors planning for a series of events, possibly monthly throughout the semester, may be more successful in maximizing the removal of invasive plants on local greenway and exposing more undergraduates to the diversity of flora that surrounds them. Having more researchers on site to help manage participant work and take small groups to be shown local flora would aid in the overall execution of the event. A time interval of 90 minutes kept all participants engaged in the event and is likely appropriate for future events. Overall, the design of the outreach event was successful in removing individuals of *L. cuneata* from Greenville Greenway and helped expose more undergraduates at ECU to the diversity of flora that surrounds them.

Table 2.1: Participants were presented with statements pertaining to invasive species and conservation. They were then asked to indicate how they felt towards the statement from “Strongly Disagree” (1) to “Strongly Agree” (5). Responses of undergraduates majoring in a STEM field were compared to those majoring in a social science. The mean response and standard deviation for each question are shown for the total group (n = 27), STEM students (n = 12), and social science students (n = 10). Questions patterned after other surveys marked with * (Nisbet et al., 2009) or ** (H. Vance-Chalcraft, unpublished data). Unmarked questions were self-generated.

Survey Question on Invasive Species and Conservation	**Total (n = 27) Mean ± SE**	**STEM (n = 12) Mean ± SE**	**Social Science (n = 10) Mean ± SE**	**STEM vs. Social Science p-value**
I think more education of invasive species is needed.	4.04 ± 0.14	4.17 ± 0.17	3.70 ± 0.26	$t_{20} = 1.56$; $p = 0.13$
I, as an individual, have a responsibility to do my part in protecting the environment.*	4.30 ± 0.14	4.25 ± 0.22	4.20 ± 0.25	$t_{20} = 0.15$; $p = 0.88$
I believe reducing the number of invasive species is beneficial for the environment.**	4.26 ± 0.15	4.42 ± 0.15	3.80 ± 0.29	$t_{20} = 1.99$; $p = 0.06$
I believe that invasive plants pose a threat to native wildlife. **	4.31 ± 0.15	4.25 ± 0.22	4.22 ± 0.22	$t_{20} = 0.09$; $p = 0.93$
I believe that invasive animal species pose a threat to native wildlife. **	4.30 ± 0.13	4.33 ± 0.19	4.10 ± 0.23	$t_{20} = 0.79$; $p = 0.44$
I would participate in group activities to help the environment.	4.07 ± 0.13	4.00 ± 0.21	4.00 ± 0.26	$t_{20} = 0.00$; $p = 1.00$
I reuse and recycle products when I can.	4.22 ± 0.14	4.17 ± 0.21	4.10 ± 0.28	$t_{20} = 0.20$; $p = 0.85$
I can identify invasive plant species.	2.52 ± 0.14	2.33 ± 0.14	2.70 ± 0.33	$t_{20} = 1.07$; $p = 0.30$
I can identify native plant species.	2.81 ± 0.17	2.75 ± 0.22	2.90 ± 0.35	$t_{20} = 0.38$; $p = 0.71$
I believe we have an obligation to conserve all species.*	3.96 ± 0.17	3.92 ± 0.19	4.00 ± 0.21	$t_{20} = 0.29$; $p = 0.77$
I believe funding should be allocated for conservation.	4.19 ± 0.16	4.08 ± 0.23	4.10 ± 0.18	$t_{20} = 0.06$; $p = 0.96$
I want to preserve my local environment.	4.44 ± 0.14	4.50 ± 0.15	4.30 ± 0.15	$t_{20} = 0.92$; $p = 0.37$
I believe it is important to preserve the environment globally.*	4.52 ± 0.10	4.50 ± 0.15	4.40 ± 0.16	$t_{20} = 0.45$; $p = 0.66$
I want to learn more about invasive species in my area.	3.74 ± 0.15	3.58 ± 0.15	3.60 ± 0.31	$t_{20} = 0.05$; $p = 0.96$
I know where to find resources to learn about invasive species.	3.37 ± 0.19	2.83 ± 0.21	3.60 ± 0.34	$t_{20} = 2.00$; $p = 0.06$
I know what to do if I find an invasive species.	3.26 ± 0.20	2.83 ± 0.21	3.70 ± 0.37	$t_{20} = 2.15$; $p = 0.04$
I think invasive species are an issue in my area.	3.70 ± 0.15	3.33 ± 0.19	4.00 ± 0.26	$t_{20} = 2.13$; $p = 0.05$

Table 2.2: Hypothetic scenarios were presented describing an invasive or endangered species and a proposed plan to control or preserve the species. Participants were asked to indicate how they felt towards the implementation of suggested conservation efforts from “Strongly Disagree” (1) to “Strongly Agree” (5). Responses of undergraduates majoring in a STEM field were compared to those majoring in a social science. The mean response and standard deviation for each question are shown for the total group (n = 27), STEM students (n = 12), and social science students (n = 10). Scenarios patterned after published survey (Bremner and Park, 2007).

Survey Question on Hypothetical Scenarios	**Total (n = 27) Mean ± SE**	**STEM (n = 12) Mean ± SE**	**Social Science (n = 10) Mean ± SE**	**STEM vs. Social Science p-value**
A nonnative plant has established itself within a city. This plant species is a fast growing vine, and is beginning to quickly cover many buildings on private and public property. Conservation efforts would require the use of chemical herbicide to kill the invasive plants.	3.11 ± 0.20	2.92 ± 0.29	3.10 ± 0.35	$t_{20} = 0.41$; $p = 0.69$
A small population of an endangered plant species has been found within an undeveloped parcel of land. This particular parcel of land is intended to be developed into a business center, providing jobs to the local city. Conservation efforts would require construction of the business center to be postponed and possibly canceled permanently.	2.52 ± 0.14	2.50 ± 0.19	2.60 ± 0.31	$t_{20} = 0.29$; $p = 0.78$
A nonnative reptile has been found on an island. It is believed to have been brought in accidently aboard ships importing cargo. The nonnative reptile has been observed eating individuals of several different native bird species. Conservation efforts would require eradication of nonnative reptiles from the island.	2.63 ± 0.20	2.83 ± 0.32	2.50 ± 0.31	$t_{20} = 0.74$; $p = 0.49$
A small population on endangered insects has been found in a wooded area adjacent to an agricultural field. The managers of the agricultural field have been applying pesticides on crops to control an ongoing pest issue. Conservation efforts would require the application of pesticides on the adjacent field to cease.	3.04 ± 0.16	2.92 ± 0.26	3.30 ± 0.26	$t_{20} = 1.03$; $p = 0.31$

Table 2.3: Voluntary surveys presented participants with nine statements pertaining to their opinion of the outreach event. Participants were asked to indicate how they felt towards the statement from "Strongly Disagree" (1) to "Strongly Agree" (5). Responses of undergraduates majoring in a STEM field were compared to those majoring in a social science. The mean response and standard deviation for each question are shown for the total group (n = 27), STEM students (n = 12), and social science students (n = 10). Questions patterned after a published survey marked with * (Nisbet et al., 2009). Unmarked questions were self-generated.

Survey Question on Outreach Event	Cumulative (n = 27) Mean ± SE	STEM (n = 12) Mean ± SE	Social Science (n = 10) Mean ± SE	STEM vs. Social Science p-value
I feel like this event has helped me connect with nature.*	4.26 ± 0.13	4.25 ± 0.18	4.10 ± 0.23	$t_{20} = 0.52$; p = 0.61
I feel more equipped to help the environment because of this event.	4.26 ± 0.13	4.25 ± 0.18	4.10 ± 0.23	$t_{20} = 0.52$; p = 0.61
I am more aware of native plant diversity because of this event.	4.26 ± 0.13	4.33 ± 0.14	4.10 ± 0.28	$t_{20} = 0.79$; p = 0.44
If not for this event, I would not have been outdoors during this time.*	4.15 ± 0.18	4.33 ± 0.26	4.00 ± 0.30	$t_{20} = 0.85$; p = 0.40
I feel more motivated to protect the environment because of this event.	4.04 ± 0.16	3.92 ± 0.26	4.00 ± 0.26	$t_{20} = 0.23$; p = 0.82
I enjoyed participating in this event.	4.41 ± 0.12	4.58 ± 0.15	4.20 ± 0.25	$t_{20} = 1.37$; p = 0.19
I will seek out other opportunities like this in the future.	4.08 ± 0.16	4.08 ± 0.23	4.00 ± 0.26	$t_{20} = 0.24$; p = 0.81
I would encourage others to attend similar events in the future.	4.26 ± 0.14	4.17 ± 0.21	4.20 ± 0.25	$t_{20} = 0.10$; p = 0.92
I would participate in a similar outreach event in the future.	4.22 ± 0.14	4.25 ± 0.18	4.00 ± 0.30	$t_{20} = 0.75$; p = 0.46

Table 2.4: A total of 27 surveys were completed by event participants. Based on major they were placed in either the social science grouping (n = 10), STEM grouping (n = 12), or neither (n = 5). Survey responses of the social science students were compared to the survey responses of the STEM students. Year in school and specific major are shown for all members of each grouping.

		Freshman	Sophomore	Junior	Senior
SOCIAL SCIENCES	Communications	1	0	0	0
	Criminal Justice	1	0	0	1
	Philosophy	0	1	0	0
	Psychology	0	4	0	0
	Public Health	0	1	0	1
STEM	Biochemistry	1	0	0	0
	Biology	1	2	0	1
	Chemistry	1	0	0	0
	Engineering	0	2	0	0
	Exercise Physiology	1	0	0	0
	Information and Computer Technology	0	0	2	0
	Physics	0	0	1	0
OTHER	Art	1	0	0	0
	Business Management	0	2	0	0
	Community and Regional Planning	0	1	0	0
	Management Information Systems	0	0	0	1

REFERENCES

Andreu, J., Vilà, M., & Hulme, P. E. (2009). An assessment of stakeholder perceptions and management of noxious alien plants in Spain. *Environmental Management, 43*(6), 1244-1255. doi:10.1007/s00267-009-9280-1

Argiroff, W. A., Zak, D. R., Lanser, C. M., & Wiley, M. J. (2017). Microbial community functional potential and composition are shaped by hydrologic connectivity in riverine floodplain soils. *Microbial Ecology, 73*(3), 630-644. doi:10.1007/s00248-016-0883-9

Bakker, J. D., & Wilson S. D. (2004). Using ecological restoration to constrain biological invasion. *Journal of Applied Ecology, 41*(6), 1058-1064. doi:10.1111/j.0021-8901.2004.00962.x

Barrett, K. A., & McBride, M. B. (2006). Trace element mobilization in soils by glyphosate. *Soil Science Society of America Journal, 70*(6), 1882-1888. doi:10.2136/sssaj2005.0415

Benbrook, C. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*(1), 1-15. doi:10.1186/s12302-016-0070-0

Bennett, J. A., & Klironomos, J. (2018). Mechanisms of plant–soil feedback: Interactions among biotic and abiotic drivers. *New Phytologist, 222*(1), 91-96. doi:10.1111/nph.15603

Bever, J. D., Dickie, I. A., Facelli, J. M., Facelli, E., Klironomos, J., Moora, M., . . . Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution, 25*(8), 468-478. doi:10.1016/j.tree.2010.05.004

Blieszner, R., Artale, L. M. (2001). Benefits of intergenerational service-learning to human services majors. *Educational Gerontology, 27*(1), 71-87. doi:10.1080/036012701750069058

Bozelli, R. L., Thomaz, S. M., Padial, A., Lopes, P., & Bini, L. M. (2015). Floods decrease zooplankton beta diversity and environmental heterogeneity in an Amazonian floodplain system. *Hydrobiologia, 753*(1), 233-241. doi:10.1007/s10750-015-2209-1

Brandon, A., Gibson, D., & Middleton, B. (2004). Mechanisms for dominance in an early successional old field by the invasive non-native *Lespedeza cuneata* (Dum. Cours) G. Don. *Biological Invasions, 6*(4), 483-493. doi:10.1023/B:BINV.0000041561.71407.f5

Bremner, A., & Park, K. (2007). Public attitudes to the management of invasive non-native species in Scotland. *Biological Conservation, 139*(3), 306-314. doi:10.1016/j.biocon.2007.07.005

Butchart, S. H. M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P. W., Almond, R. E. A., . . . Watson, R. (2010). Global biodiversity: Indicators of recent declines. *Science, 328*(5982), 1164-1168. doi:10.1126/science.1187512

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science, 290*(5491), 521-523. doi:10.1126/science.290.5491.521

Carlson, W., & Witschey, H. (2018). Undergraduate students' attitudes toward individuals with disabilities. *Teaching of Psychology, 45*(2), 189-192. doi:10.1177/0098628318762929

Carino, D. A., & Daehler, C. C. (2002). Can inconspicuous legumes facilitate alien grass invasions? Partridge peas and fountain grass in Hawai'i. *Ecography (Copenhagen), 25*(1), 33-41. doi:10.1034/j.1600-0587.2002.250105.x

Corbin, J. D. & D'Antonio, C. M. (2012). Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. *Invasive Plant Science and Management, 5*(1), 117-124. doi:10.1614/IPSM-D-11-00005.1

Coykendall, K. E., & Houseman, G. R. (2014). *Lespedeza cuneata* invasion alters soils facilitating its own growth. *Biological Invasions, 16*(8), 1735-1742. doi:10.1007/s10530-013-0623-8

Crawford, K. M., & Knight, T. M. (2017). Competition overwhelms the positive plant–soil feedback generated by an invasive plant. *Oecologia, 183*(1), 211-220. doi:10.1007/s00442-016-3759-2

Dallimer, M., Irvine, K. N., Skinner, A. M. J., Davies, Z. G., Rouquette, J. R., Maltby, L. L., . . . Gaston, K. J. (2012). Biodiversity and the feel-good factor: Understanding associations between self-reported human well-being and species richness. *Bioscience, 62*(1), 47-55. doi:10.1525/bio.2012.62.1.9

Dommanget, F., Evette, A., Spiegelberger, T., Gallet, C., Pacé, M., Imbert, M., & Navas, M. (2014). Differential allelopathic effects of japanese knotweed on willow and cottonwood cuttings used in riverbank restoration techniques. *Journal of Environmental Management, 132*, 71-78. doi:10.1016/j.jenvman.2013.10.024

Duke, S. O., & Powles, S. B. (2008). Glyphosate: A once-in-a-century herbicide. *Pest Management Science, 64*(4), 319-325. doi:10.1002/ps.1518

Fischer, A., Selge, S., van der Wal, R., & Larson, B. M. H. (2014). The public and professionals reason similarly about the management of non-native invasive species: A quantitative investigation of the relationship between beliefs and attitudes. *PloS One, 9*(8), e105495. doi:10.1371/journal.pone.0105495

Flory, S. L. & Clay, K. (2009). Invasive plant removal method determines native plant community responses. *Journal of Applied Ecology, 46*(2), 434-442. doi:10.1111/j.1365-2664.2009.01610.x

Fridley, J. D., Stachowicz, J. J., Naeem, S., Sax, D. F., Seabloom, E. W., Smith, M. D., . . . Von Holle, B. (2007). The invasion paradox: Reconciling pattern and process in species invasions. *Ecology (Durham), 88*(1), 3-17. doi:10.1890/0012-9658(2007)88[3:TIPRPA]2.0.CO;2

Furtak, K., Grządziel, J., Gałązka, A., & Niedźwiecki, J. (2020). Prevalence of unclassified bacteria in the soil bacterial community from floodplain meadows (fluvisols) under simulated flood conditions revealed by a metataxonomic approachss. *Catena (Giessen), 188*, 104448. doi:10.1016/j.catena.2019.104448

Gioria, M., & Osborne, B. (2009). The impact of *Gunnera tinctoria* (Molina) Mirbel invasions on soil seed bank communities. *Journal of Plant Ecology, 2*(3), 153-167. doi:10.1093/jpe/rtp013

Gioria, M., & Osborne, B. (2009). Assessing the impact of plant invasions on soil seed bank communities: Use of univariate and multivariate statistical approaches. *Journal of Vegetation Science, 20*(3), 547-556. doi:10.1111/j.1654-1103.2009.01054.x

Glusczak, L., Miron, D. d. S., Moraes, B. S., Simões, R. R., Schetinger, M. R. C., Morsch, V. M., & Loro, V. L. (2007). Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comparative Biochemistry and Physiology, Part C, 146*(4), 519-524. doi:10.1016/j.cbpc.2007.06.004

Goodwillie, C., & Jolls, C. L. (2018). Combating plant blindness and a plant invasion through service-learning. *Plant Science Bulletin*

Gucker, C. (2010). *Lespedeza cuneata*. in: Fire effects information system. *U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory*

Grains Research and Development Corporation. (2013). Rhizobial innoculants fact sheet. *Fact Sheet,*

Greulich, S., Chevalier, R., & Villar, M. (2019). Soil seed banks in the floodplain of a large river: A test of hypotheses on seed bank composition in relation to flooding and established vegetation. *Journal of Vegetation Science, 30*(4), 732-745. doi:10.1111/jvs.12762

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., . . . Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncology, The, 16*(5), 490-491. doi:10.1016/S1470-2045(15)70134-8

Harms, R. S., & Hiebert, R. D. (2006). Vegetation response following invasive tamarisk (*Tamarix spp.)* removal and implications for riparian restoration. *Restoration Ecology, 14*(3), 461-472. doi:10.1111/j.1526-100X.2006.00154.x

Havens, K., Jolls, C. L., Marik, J. E., Vitt, P., McEachern, A. K., & Kind, D. (2012). Effects of a non-native biocontrol weevil, *Larinus planus,* and other emerging threats on populations of the federally threatened pitcher's thistle, *Cirsium pitcheri*. *Biological Conservation, 155,* 202. doi:10.1016/j.biocon.2012.06.010

Havens, K., Jolls, C. L., Knight, T. M., & Vitt, P. (2019). Risks and rewards: Assessing the effectiveness and safety of classical invasive plant biocontrol by arthropods. *Bioscience, 69*(4), 247-258. doi:10.1093/biosci/biz015

Hess, M. C. M., Mesléard, F., & Buisson, E. (2019). Priority effects: Emerging principles for invasive plant species management. *Ecological Engineering, 127,* 48-57. doi:10.1016/j.ecoleng.2018.11.011

Hobbs, R. J., & Norton, D. A. (1996). Towards a conceptual framework for restoration ecology. *Restoration Ecology, 4*(2), 93-110. doi:10.1111/j.1526-100X.1996.tb00112.x

Jackson, L. L., Lopoukhine, N., & Hillyard, D. (1995). Ecological restoration: A definition and comments. *Restoration Ecology, 3*(2), 71-75. doi:10.1111/j.1526-100X.1995.tb00079.x

Jacobson, S. K., McDuff, M. D., & Monroe, M. C. (2015). *Conservation Education and Outreach Techniques* (1. publ. ed.). Oxford [u.a.]: Oxford University Press.

Jiraungkoorskul, W., Upatham, E. S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S., & Pokethitiyook, P. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environmental Toxicology, 18*(4), 260-267. doi:10.1002/tox.10123

Johnson, S. E., Mudrak, E. L., & Waller, D. M. (2014). Local increases in diversity accompany community homogenization in floodplain forest understories. *Journal of Vegetation Science, 25*(3), 885-896. doi:10.1111/jvs.12147

Kalburtji, K. L. & Mosjidis, J. A. (1993). Effects of sericea lespedeza root exudates on some perennial grasses. *Journal of Range Management, 46*(4), 312-315. doi:10.2307/4002464

Karlin, M., & De La Paz, G. (2015). Using camera-trap technology to improve undergraduate education and citizen-science contributions in wildlife research. *The Southwestern Naturalist, 60*(2/3), 171-179. doi:10.1894/SWNAT-D-14-00005.1

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature, 417*(6884), 67-70. doi:10.1038/417067a

Kobori, H., Dickinson, J., Washitani, I., Sakurai, R., Amano, T., Komatsu, N., . . . Miller-Rushing, A. (2015). Citizen science: A new approach to advance ecology, education, and conservation. *Ecological Research, 31*(1), 1-19. doi:10.1007/s11284-015-1314-y

Komatsu, K. J., & Simms, E. L. (2019). Invasive legume management strategies differentially impact mutualist abundance and benefit to native and invasive hosts. *Restoration Ecology,* doi:10.1111/rec.13081

Kuhman, T. R., Pearson, S. M., & Turner, M. G. (2013). Why does land-use history facilitate non-native plant invasion? A field experiment with *Celastrus orbiculatus* in the southern Appalachians. *Biological Invasions, 15*(3), 613-626. doi:10.1007/s10530-012-0313-y

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant-soil feedbacks: A meta-analytical review. *Ecology Letters, 11*(9), 980-992. doi:10.1111/j.1461-0248.2008.01209.x

Lau, J. A., & Suwa, T. (2016). The changing nature of plant–microbe interactions during a biological invasion. *Biological Invasions, 18*(12), 3527-3534. doi:10.1007/s10530-016-1245-8

Liu, H., & Stiling, P. (2006). Testing the enemy release hypothesis: A review and meta-analysis. *Biological Invasions, 8*(7), 1535-1545. doi:10.1007/s10530-005-5845-y

Lonsdale, W. M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology (Durham), 80*(5), 1522. doi:10.2307/176544

Mangla, S. & Callaway, R. M. (2008). Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology, 96*(1), 58-67. doi:10.1111/j.1365-2745.2007.01312.x

Mayr, M. J., Besemer, K., Sieczko, A., Demeter, K., & Peduzzi, P. (2020). Bacterial community composition and function along spatiotemporal connectivity gradients in the Danube floodplain (Vienna, Austria). *Aquatic Sciences, 82*(2), 28. doi:10.1007/s00027-020-0700-x

Moravcová, L., Pyšek, P., Jarošík, V., & Pergl, J. (2015). Getting the right traits: Reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. *PloS One, 10*(4), e0123634. doi:10.1371/journal.pone.0123634

Nisbet, E. K., & Zelenski, J. M. (2013). The NR-6: A new brief measure of nature relatedness. *Frontiers in Psychology, 4,* 813. doi:10.3389/fpsyg.2013.00813

Nisbet, E. K., Zelenski, J. M., & Murphy, S. A. (2009). The nature relatedness scale. *Environment and Behavior, 41*(5), 715-740. doi:10.1177/0013916508318748

Nsikani, M. M., Novoa, A., Wilgen, B. W., Keet, J., & Gaertner, M. (2017). *Acacia saligna*'s soil legacy effects persist up to 10 years after clearing: Implications for ecological restoration. *Austral Ecology, 42*(8), 880-889. doi:10.1111/aec.12515

Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N2-fixing woody species: Implications for ecological restoration. *Restoration Ecology, 26*(2), 235-244. doi:10.1111/rec.12669

Oberhauser, K., & LeBuhn, G. (2012). Insects and plants: Engaging undergraduates in authentic research through citizen science. *Frontiers in Ecology and the Environment, 10*(6), 318-320. doi:10.1890/110274

Ohlenbusch, P. D., & Bidwell, T. (2007). Sericea Lespedeza: History, characteristics, and identification. *Kansas State University Experiment Station and Cooperative Extension Service*. 7

Ooka, J. K., & Owens, D. K. (2018). Allelopathy in tropical and subtropical species. *Phytochemistry Reviews, 17*(6), 1225-1237. doi:10.1007/s11101-018-9596-7

Paynter, Q., Downey, P. O., & Sheppard, A. W. (2003). Age structure and growth of the woody legume weed *Cytisus scoparius* in native and exotic habitats: Implications for control. *Journal of Applied Ecology, 40*(3), 470-480. doi:10.1046/j.1365-2664.2003.00817.x

Porticella, N., Phillips, T., & Bonney, R. (2017). Self-efficacy for environmental action (SEEA, generic). *Technical Brief Series,* (Technical Brief Series)

Radford, A. E., Ahles, H. E., & Bell, C. R. (1968). *Manual of the Vascular Flora of the Carolinas*. Chapel Hill, NC: University of North Carolina Press.

Reichard, S. H., & White, P. (2001). Horticulture as a pathway of invasive plant introductions in the United States. *Bioscience, 51*(2), 103-113. doi:HAAPOI]2.0.CO;2

Reichenborn, M. M., Houseman, G. R., & Foster, B. L. (2020). Plant community recovery following sericea lespedeza (*Lespedeza cuneata*) removal: Testing for a soil legacy effect. *Restoration Ecology,* doi:10.1111/rec.13185

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist, 170*(3), 445-457. doi:10.1111/j.1469-8137.2006.01715.x

Remaley, T. (1998). Plant Conservation Alliance, Alien Plant Working Group: Chinese Lespedeza

Richardson, D. M., Allsopp, N., D'antonio, C. M., Milton, S. J., & Rejmánek, M. (2000). Plant invasions - the role of mutualisms. *Biological Reviews, 75*(1), 65-93. doi:10.1017/S0006323199005435

Sax, D. F. (2002). Native and naturalized plant diversity are positively correlated in scrub communities of California and Chile. *Diversity & Distributions, 8*(4), 193-210. doi:10.1046/j.1472-4642.2002.00147.x

Schüttler, E., Rozzi, R., & Jax, K. (2011). Towards a societal discourse on invasive species management: A case study of public perceptions of mink and beavers in Cape Horn. *Journal for Nature Conservation, 19*(3), 175-184. doi:10.1016/j.jnc.2010.12.001

Scyphers, S. B., Powers, S. P., Akins, J. L., Drymon, J. M., Martin, C. W., Schobernd, Z. H., . . . Switzer, T. S. (2015). The role of citizens in detecting and responding to a rapid marine invasion. *Conservation Letters, 8*(4), 242-250. doi:10.1111/conl.12127

Shiffman, D. S. (2012). Twitter as a tool for conservation education and outreach: What scientific conferences can do to promote live-tweeting. *Journal of Environmental Studies and Sciences, 2*(3), 257-262. doi:10.1007/s13412-012-0080-1

Simberloff, D. (2009). We can eliminate invasions or live with them. successful management projects. *Biological Invasions, 11*(1), 149-157. doi:10.1007/s10530-008-9317-z

Skórka, P., Lenda, M., & Tryjanowski, P. (2010). Invasive alien goldenrods negatively affect grassland bird communities in eastern Europe. *Biological Conservation, 143*(4), 856-861. doi:10.1016/j.biocon.2009.12.030

Sprent, J. I., Sutherland, J. M., de Faria S. M., Dilworth, M. J., Corby, H. D. L., Becking, J. H., . . . J. W. Drozd. (1987). Some aspects of the biology of nitrogen-fixing organisms [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 317*(1184), 111-129. doi:10.1098/rstb.1987.0051

Stevens, S. (2002). Element Stewardship Abstract for *Lespedeza cuneata* (Dumont-Cours.) G. Don Sericea Lespedeza. The Nature Conservancy.

Tejada, M. (2009). Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate+diflufenican herbicides. *Chemosphere (Oxford), 76*(3), 365-373. doi:10.1016/j.chemosphere.2009.03.040

Thomaz, S., Bini, L. M., & Bozelli, R. L. (2007). Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia, 579*(1), 1-13. doi:10.1007/s10750-006-0285-y

Unger, I. M., Kennedy, A. C., & Muzika, R. (2009). Flooding effects on soil microbial communities. *Applied Soil Ecology : A Section of Agriculture, Ecosystems & Environment, 42*(1), 1-8. doi:10.1016/j.apsoil.2009.01.007

United States Department of Agriculture. (2019). PLANTS database.

van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters, 13*(2), 235-245. doi:10.1111/j.1461-0248.2009.01418.x

Vilà, M., & Ibáñez, I. (2011). Plant invasions in the landscape. *Landscape Ecology, 26*(4), 461-472. doi:10.1007/s10980-011-9585-3

Vilà, M., Maron, J. L., & Marco, L. (2005). Evidence for the enemy release hypothesis in *Hypericum perforatum*. *Oecologia, 142*(3), 474-479. doi:10.1007/s00442-004-1731-z

Vilà, M., Rohr, R. P., Espinar, J. L., Hulme, P. E., Pergl, J., Le Roux, J. J., . . . Pyšek, P. (2015). Explaining the variation in impacts of non-native plants on local-scale species richness: The role of phylogenetic relatedness. *Global Ecology and Biogeography, 24*(2), 139-146. doi:10.1111/geb.12249

Vilà, M., & Gimeno, I. (2007). Does invasion by an alien plant species affect the soil seed bank? *Journal of Vegetation Science, 18*(3), 423-430. doi:10.1111/j.1654-1103.2007.tb02554.x

Vitone, T., Stofer, K., Steininger, M. S., Hulcr, J., Dunn, R., & Lucky, A. (2016). School of ants goes to college: Integrating citizen science into the general education classroom increases engagement with science. *Journal of Science Communication, 15*(1) doi:10.22323/2.15010203

Waterman, A. S. (1997). *Service-learning: Applications from the Research* Routledge.

Weakley, A. (2010). *Flora of the Southern and Mid-Atlantic States.* Chapel Hill, NC: University of North Carolina Press.

Williamson, M. H., & Fitter, A. (1996). The characters of successful invaders. *Biological Conservation, 78*(1), 163-170. doi:10.1016/0006-3207(96)00025-0

Wittenberg, R., & Cock, M. J. W. (2001). *Invasive Alien Species: A Toolkit of Best Prevention and Management Practices*. Wallingford, UK: CABI Publishing. doi:10.1079/9780851995694.0000

Wolfe, B. E., & Klironomos, J. N. (2005). Breaking new ground: Soil communities and exotic plant invasion. *Bioscience, 55*(6), 477-487. doi:BNGSCA]2.0.CO;2

Wolfgang, J., Bayley, P. B., & Sparks, R. E. (1989). The flood pulse concept in river-floodplain systems. *Canadian Journal of Fisheries and Aquatic Sciences,*

Woods, T. M., Hartnett, D. C., & Ferguson, C. J. (2009). High propagule production and reproductive fitness homeostasis contribute to the invasiveness of *Lespedeza cuneata* (Fabaceae). *Biological Invasions, 11*(8), 1913-1927. doi:10.1007/s10530-008-9369-0

Yannarell, A. C., Busby, R. R., Denight, M. L., Gebhart, D. L., & Taylor, S. J. (2011). Soil bacteria and fungi respond on different spatial scales to invasion by the legume *Lespedeza cuneata*. *Frontiers in Microbiology, 2,* 127. doi:10.3389/fmicb.2011.00127

Yelenik, S. G., & Levine, J. M. (2011). The role of plant-soil feedbacks in driving native-species recovery. *Ecology, 92*(1), 66-74. doi:10.1890/10-0465.1

Żmihorski, M., Pärt, T., Gustafson, T., Berg, Å, & Rohr, J. (2016). Effects of water level and grassland management on alpha and beta diversity of birds in restored wetlands. *Journal of Applied Ecology, 53*(2), 587-595. doi:10.1111/1365-2664.12588