AN ABSTRACT OF THE THESIS OF

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Title: The influence of proximity and land-use legacy on the allelopathic potential of *Lespedeza cuneata*

Abstract approved: ______________________________

Sericea lespedeza (*Lespedeza cuneata*), an invasive plant, displaces native vegetation in North American tallgrass prairie. *L. cuneata* is allelopathic to some species and able to engage in a mutualistic relationship with N-fixing bacteria. The mutualism between *L. cuneata* and rhizobia likely improves the nutrient status of *L. cuneata*, and plants in restored prairie sites with a row-crop agricultural history have higher rhizobia densities that may affect a plant's ability to produce allelopathic compounds. Because of this *L. cuneata* is a good candidate to test two ecological hypotheses of biological invasions, the novel weapons hypothesis and the mutualism facilitation hypothesis. Therefore, I tested if the novel weapons hypothesis (allelopathy) and mutualism facilitation hypothesis explain invasion of *L. cuneata,* and I hypothesized that soil from the rhizosphere of *L. cuneata* from restored prairie sites would be more allelopathic than from native prairie. Soil samples were collected from inside *L. cuneata* rhizosphere and 1 m away from a *L. cuneata* at restored and native prairie sites. Soil extracts were used to germinate seed of 12 different species. Tomato (*Lycopersicum esculentum* var. *Rutgers VF*) seed was used to test for the potential allelopathic effects of the soil extracts. The remaining species tested were grasses and forbs native to the region where soils were collected from including two congeneric species. Seed germination rate and seedling size were measured to evaluate the strength of allelopathy in soils from each site. The total soil N and C from each site were also measured. Soils from the rhizosphere of *L. cuneata* in restored prairie sites reduced germination rates of *L. esculentum*, Indian grass (*Sorghastrum nutans*), round-headed bush clover (*Lespedeza capitata*), and slender bush clover (*Lespedeza virginica*) more than soil from native prairies. Soils from the rhizosphere of *L. cuneata* in restored prairie sites also reduced the seedling length of *L. esculentum*, big blue stem (*Andropogon gerardii*), *L. capitata*, and *L. virginica*. *L. cuneata* did not affected germination and seedling size of forbs except for congeneric legumes, nor was it self-inhibitory. Allelopathy is more pronounced in restored prairie sites, and this may originate from a more robust symbiotic relationship with rhizobia in restored prairie. In the locations tested, *L. cuneata* has no effect of total soil N. Past row-crop agriculture reduced total soil N by up to half, and *L. cuneata* presence has not increased it. The invasion of *L. cuneata* and its production of allelochemicals provides support for the novel weapons hypothesis. Additionally, the different allelopathic responses of *L. cuneata* between native and restored prairie also provides support for the mutualism facilitation hypothesis because invasion is likely enhanced by mutualists that facilitate production of allelochemicals.

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THE INFLUENCE OF PROXIMITY AND LAND-USE LEGACY ON THE ALLELOPATHIC POTENTIAL OF *LESPEDEZA CUNEATA*

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PREFACE

This thesis was prepared following the publication style of *Invasive Plant Science and Management*.

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CHAPTER 1

History of *Lespedeza cuneata* invasion

Background

The term allelopathy is created from the Greek words “allelon” and “pathos,” which means “mutual” and “suffer” respectively (Inderjit and Duke 2003). Allelopathy is described as a biochemical interaction between one plant and other plants that reduces growth of the target plants to the benefit to the plant producing the allelopathic compounds (Field et al. 2006). The definition was broadened to suggest harmful effects of plants and their associated microorganisms on the growth and survival of other plants from releasing toxic secondary metabolic substances (allelopathic compounds) into the environment (Inderjit and Duke 2003). Over the past few decades, allelopathy studies have investigated the effects on plant growth and productivity, interaction between plants and microorganisms, impact on ecology, and composition of allelochemicals.

Allelopathic compounds can exist in any plant structure, and are primarily released through three processes: foliar leaching, volatilization, and residue decomposition (Inderjit and Duke 2003). Plant roots, however, are the primary plant structure that produces allelopathic chemicals, and those compounds are often released into the soils through root exudation (Inderjit and Duke 2003). Once the allelopathic compounds are released into the environment, they can directly suppress growth in other plant species, or indirectly affect the target plant through chemical oxidation, immobilization or microorganism activities, where the microorganism first modifies the allelopathic compounds (Bertin et al. 2003).

Many studies suggest allelopathy as one key mechanism contributing to the invasion success of many plants (Callaway and Aschehoug 2000; Ridenour and Callaway 2001; Orr et al. 2005). Allelopathic compounds produced by invasive plants affect the growth and development of neighboring plants to provide a competitive advantage to the invasive plant. Allelopathy can inhibit other species in germination rate, radicle and coleoptile elongation, respiration, photosynthesis, mineral uptake, protein synthesis, and lipid and organic acid metabolism (Rice 1979). Several previous studies showed that many plants have allelopathic effects on other species by producing chemical inhibitors. Examples of such are garlic mustard (*Alliaria petiolate*) (Roberts and Anderson 2001), spotted knapweed (*Centaurea maculosa*) (Ridenour and Callaway 2001; Bais et al. 2002), tree-of-heaven (*Ailanthus altissima*) (Pisula and Meiners 2010), tall fescue *(Festuca arundinacea*) and autumn olive (*Elaeagnus umbellata*) (Orr et al. 2005).

Allelopathy in legumes

A legume is in the plant family Fabaceae, which includes growth forms from small herbs to large trees (Long 1989). Legumes have the ability to cooperate with soil bacteria, rhizobia (including *Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium*, and *Sinorhizobium*), to establish a legume-rhizobium mutualistic symbiosis. Rhizobia invade the roots of a legume and develop root nodules that the rhizobia inhabit (O'Hare and Daniel 1985). Mutualism has been described as a "market" where a plant and its symbiotic microorganism can exchange their metabolic substances with each other (Noë and Hammerstein 1994). In this "market" the rhizobia can reduce atmospheric nitrogen into ammonium and export it to the legume in exchange for carbohydrates and other nutrients (Long 1989), and as a nitrogen-fixing mutualism provides a large advantage to legume growth compared to non-legumes. In this mutualism, rhizobia helps host plants survive in low nitrogen soil and contributes to the nutrient status of the host plant (Miki and Kondoh 2002).

N-fixing symbiosis could also increase the ability of the host plant to produce defensive compounds such as alkaloids (Clay 2001). Swainsonine, which causes the toxicity of white locoweed (*Oxytropis sericea*), is an alkaloid produced by the fungal endophyte *Embellisia* sp. inside *O. sericea* leaves and flowers (Braun et al. 2003). *O. sericea* seedlings inoculated with rhizobia have greater biomass and production of swainsonine than non-inoculated controls, likely because rhizobia increases available nitrogen in the plant, thus providing resources to synthesize this secondary metabolite (Valdez Barillas et al 2007).

Some legumes have been found to have allelopathic effects on other plants. For example, kudzu (*Pueraria thunbergiana*) leaves suppress the germination and growth of lettuce (Rashid, et al. 2010). Pea (*Pisum sativum*) shoot extract has inhibitory effects on cress radicle and hypocotyl extension (Hisashi 2003). Research of allelopathy of the legume sericea lespedeza (*Lespedeza cuneata*) has been previously documented. Soils extract from where *L. cuneata* was present reduces the germination of ryegrass (*Lolium multiflorum*) and inhibits the radicle and coleoptile length of rye (*Secale cereal*) and *F. arundinacea* (Kalburtji and Mosjidis 1993). In addition, *L. cuneata* residues also affect the growth of warm season grasses such as Bermuda grass (*Cynodon dactylon*) and Bahia grass (*Paspalum notatum*) (Kalburtji and Mosjidis 1992). *Lespedeza cuneata* stem and leaf extract can reduce seed germination of big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), and Kentucky bluegrass (*Poa pratensis*), and inhibit the radicle and coleoptile length of *A. gerardii* and *P. pratensis* (Dudley and Fick 2003).

Ecological effects of sericea lespedeza (Lespedeza cuneata)

Lespedeza cuneata is a non-native invasive legume with high tolerance to drought and nutrient poor environments (Hoveland et al.1971). It was introduced into North America from eastern Asia. In the late 1980s, this species rapidly expanded its range and threatened the integrity of the tallgrass prairie ecosystem in Kansas where it displaced the native grasses (Hoveland et al. 1971; Eddy et al 2003). *Lespedeza cuneata* was originally grown for livestock forage and soil conservation purposes, however, it contains a high concentration of tannins, which makes it highly unpalatable to grazers (Gamble et al 1996).

As is the case with other legumes, *L. cuneata* is able to establish a mutualistic symbiosis with rhizobia, which benefits its growth. Mutualism can play a critical role in the legume invasion. When a non-native legume is transported to a new area by seed, the original mutualist may not be transported at the same time; thereby the introduced plant interacts with native rhizobia in the new range (Reinhart and Callaway 2006). This change of symbionts can alter the non-native legume’s competitive ability and facilitate the invasion success of this plant (Richardson et al. 2000; Mitchell et al. 2006). Lack of N-fixing bacteria could also restrict the invasion potential of non-native legume (Parker 2001). One of the possible mechanisms by which rhizobia might affect legume invasion is that the introduced legume might interact with novel potential rhizobia, which could be more effective in fixing nitrogen (Richardson et al. 2000). Invasive legume can either utilize the rhizobia from the invaded range (Reinhart and Callaway 2006) or utilize the introduced rhizobia (Weir et al. 2004; Rodriguez-Echeverria 2010).

Rhizobia density and L. cuneata distribution

Lespedeza cuneata's allelopathic capacity appears to be due to the compounds: p-coumaric acid, protocatechuic acid, ferulic acid, and vanillin, and these compounds are effective in suppressing the growth of oats (*Avena*) by inhibiting the production of IAA (Langdale and Giddens 1967). The presence of rhizobia could affect the ability of *L. cuneata* to produce allelopathic compounds by providing more resources and enhance the invasive ability of *L. cuneata*.

In the Flint Hills region of Kansas, *L. cuneata* densities in restored prairie sites appear to be higher than in native prairie sites. Many restored prairies in this region were once used for row crop agriculture. *Lespedeza cuneata* located in restored prairie sites have a larger proportion of nodules occupied by rhizobia than plants in the native prairie sites, which is potentially a result of the land-use legacy of these sites (Wang 2013). A subsequent greenhouse experiment showed grown plants inoculated with rhizobia isolated from root nodules of *L. cuneata* had more shoot growth than un-inoculated plants, suggesting that rhizobia assists *L. cuneata* growth (Wang 2013). This robust symbiotic relationship between rhizobia and *L. cuneata* in restored prairies may enhance *L. cuneata*'s capacity to produce allelopathic compounds.

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CHAPTER 2

The influence of proximity and land-use legacy on the allelopathic potential of *Lespedeza cuneata*

Abstract: Sericea lespedeza (*Lespedeza cuneata*), an invasive plant, displaces native vegetation in North American tallgrass prairie. *L. cuneata* is allelopathic to some species and able to engage in a mutualistic relationship with N-fixing bacteria. The mutualism between *L. cuneata* and rhizobia likely improves the nutrient status of *L. cuneata*, and plants in restored prairie sites with a row-crop agricultural history have higher rhizobia densities that may affect a plant's ability to produce allelopathic compounds. Because of this *L. cuneata* is a good candidate to test two ecological hypotheses of biological invasions, the novel weapons hypothesis and the mutualism facilitation hypothesis. Therefore, I tested if the novel weapons hypothesis (allelopathy) and mutualism facilitation hypothesis explain invasion of *L. cuneata*, and I hypothesized that soil from the rhizosphere of *L. cuneata* from restored prairie sites would be more allelopathic than from native prairie. Soil samples were collected from inside *L. cuneata* rhizosphere and 1 m away from a *L. cuneata* at restored and native prairie sites. Soil extracts were used to germinate seed of 12 different species. Tomato (*Lycopersicum esculentum* var. *Rutgers VF*) seed was used to test for the potential allelopathic effects of the soil extracts. The remaining species tested were grasses and forbs native to the region where soils were collected from including two congeneric species. Seed germination rate and seedling size were measured to evaluate the strength of allelopathy in soils from each site. The total soil N and C from each site were also measured. Soils from the rhizosphere of *L. cuneata*

in restored prairie sites reduced germination rates of *L. esculentum*, Indian grass (*Sorghastrum nutans*), round-headed bush clover (*Lespedeza capitata*), and slender bush clover (*Lespedeza virginica*) more than soil from native prairies. Soils from the rhizosphere of *L. cuneata* in restored prairie sites also reduced the seedling length of *L. esculentum*, big blue stem (*Andropogon gerardii*), *L. capitata*, and *L. virginica*. *L. cuneata* did not affected germination and seedling size of forbs except for congeneric legumes, nor was it self-inhibitory. Allelopathy is more pronounced in restored prairie sites, and this may originate from a more robust symbiotic relationship with rhizobia in restored prairie. In the locations tested, *L. cuneata* has no effect of total soil N. Past row-crop agriculture reduced total soil N by up to half, and *L. cuneata* presence has not increased it. The invasion of *L. cuneata* and its production of allelochemicals provides support for the novel weapons hypothesis. Additionally, the different allelopathic responses of *L. cuneata* between native and restored prairie also provides support for the mutualism facilitation hypothesis because invasion is likely enhanced by mutualists that facilitate production of allelochemicals.

Nomenclature: sericea lespedeza, *Lespedeza cuneate* (Dumont) G. Don LESCU; tomato, *Lycopersicum esculentum* Mill. var. *Rutgers VF***;** little bluestem, *Schizachyrium scoparium* (Michx.) Nash ANOSC; big bluestem, *Andropogon gerardii* Vitman ANOGE; Indian grass, *Sorghastrum nutans* (L.) Nash ex Small SOSNU; prairie aster, *Aster turbinellus* (Lindl.); yellow coneflower, *Ratibida pinnata* (Vent.) Barnh. PATPI; black-eyed susan, *Rudbeckia hirta* L. RUDHI; purple prairie clover, *Dalea purpurea* Vent.; Illinois bundleflower, *Desmanthus illinoensis* (Michx.) MacM. ex B.L. Robins. & Fern.

DEMIL; round-headed bush clover*,* *Lespedeza capitata* Michx.; slender bush clover, *Lespedeza virginica* (L.) Britton.

Key word: invasive plant, rhizobia-legume mutualism, congeneric allelopathy, novel weapons hypothesis, mutualism facilitation hypothesis

Introduction

Invasive species are non-native species in an ecosystem and often cause ecological and economic problems (Hulme et al. 2009). Several hypotheses have been proposed to explain and predict the invader success. There are ecological and evolutionary hypotheses to explain invasion and the primary ecological hypotheses are the preadaptation hypothesis, inherent superiority hypothesis, biotic resistance hypothesis, empty niche hypothesis, enemy release hypothesis, novel weapons hypothesis and invasional meltdown/ mutualism facilitation hypothesis. However, none of these hypotheses has definitively explained what causes some non-native species to become invasive because of a lack of empirical evidence to support these hypotheses are lacking (Moles et al. 2012).

When a plant is introduced to a new range, it may become established and then become invasive in this new environment. The preadaptation hypothesis implies that for an introduced species to become invasive, it would be more competitive than native species in a human altered ecosystem as long as the introduced species is well adapted to human activities, such as agriculture (Sax and Brown 2000). On the other hand, some explanations for invasions suggest that introduced species are simply superior to native species. Inherent superiority hypothesis implies some species are inherently superior to native species as they might be superior predators or competitors in the invaded range (Sax and Brown 2000). To understand the impact of diversity on a community's ability to resist invasion, biotic resistance hypothesis was proposed (Levine and D'Antonio 1999;

Maron and Vilà 2001). Biotic resistance hypothesis states an ecosystem with high diversity is more resistant against invasion than a less diverse ecosystem (Levine and D'Antonio 1999; Levine et al. 2004; Fridley et al. 2007). In addition, the empty niche hypothesis proposes that invasive species would access resources not utilized by local species (Mac Dougall et al. 2009). Predators, parasites and/or pathogens often keep plants in check within their native range, but may not be present in the invaded range. The enemy release hypothesis predicts that introduced species become invasive because of the escape from enemies like herbivores from the its native range, and now face fewer enemies in the new range (Maron and Vilà 2001; Torchin and Mitchell 2004; Liu and Stiling 2006).

Escaping from the original enemies allows the invasive plants to allocate less resource to defense and more resource to growth and fecundity (Blossey and Notzold 1995). The advantage invasive plants may have is not only caused by the trade-off between growth and defense, but may also be manifested through biochemical mechanisms as explained by the novel weapons hypothesis (Callaway and Ridenour 2004). Novel weapons hypothesis proposes that invasive species possess novel biochemical weapons (such as allelopathic chemicals) to inhibit the competitors, or affect the plant-soil microbial interactions in invaded regions (Callaway and Ridenour 2004). Those chemicals are novel to the species in the new range, but less effective to species in the native range of the invader, where the neighboring competitors have evolved resistance to these allelochemicals (Callaway and Ridenour 2004).

Invasional meltdown may be another important mechanism of invasion for some species. This hypothesis suggests that the establishment of one species would facilitate invasion of another species (Simberloff and Von Holle 1999; Richardson et al. 2000). A subset of invasional meltdown hypothesis involves a special case when mutualists can facilitate invasion. Mutualists can facilitate the invasion of exotic species, where some species may remain noninvasive until their mutualists arrive as described by the mutualism facilitation hypothesis (Richardson et al. 2000). Soil microbes in the new range can facilitate plant invasions because they can be more beneficial than microbes the invasive plant had in its native range (Reinhart and Callaway 2006). When a plant is introduced to a new place, the novel potential mutualists could provide a greater benefit than original mutualists in supplying N or P to host plant (Richardson et al. 2000; Reinhart and Callaway 2006). These mutualist interactions may be especially important for invasive legumes. Some invasive legumes can be nodulated with a wide range of rhizobia strains (Rodríguez-Echeverría et al. 2011). Certain native N- fixing rhizobia strains may more effectively associate with the introduced legume than with native legumes, and the introduced legume may easily establish in the area with more beneficial rhizobia (De Faria et al. 1989; Reinhart and Callaway 2006). Alternatively, legume also can become invasive by utilizing the co-introduced rhizobia (Weir et al. 2004; Chen et al. 2005; Rodriguez-Echeverria 2010).

N-fixing invaders are also able to alter the N cycle and increase N input in soil (Ehrenfeld 2003). Some examples of invasive plants that associate with N-fixing bacteria and alter N dynamics are candleberry myrtle tree (*Myrica faya*), black locust (*Robinia pseudoacacia*) and *Acacia spp. M. faya* invasion has altered ecosystem properties in Hawaii volcanic soil by increasing the annual N input in soil and increasing the rate of N cycling (Vitousek and Walker 1989). *Robinia pseudoacacia* increases soil N and accelerates soil N mineralization rates in a pitch pine (*Pinus tigida*) – scrub oak (*Quercus ilicifolia*) systems (Rice et al. 2004), while *Acacia spp.* increase soil N and P, and enhance N mineralization rate in South African fynbos (Yelenik et al. 2004).

Sericea lespedeza (*Lespedeza cuneata* (Dumont) G. Don) is a non-native invasive legume in North American tallgrass prairie with high tolerance to drought and nutrient poor environments (Hoveland et al. 1971). It was introduced into North America from Eastern Asia. In the late 1980s, this species expanded its range and threatened the integrity of the tallgrass prairie ecosystem in Kansas where it soon displaced the native grasses (Eddy et al 2003). Many mechanisms of *L. cuneata* invasion have been studied. *Lespedeza cuneata* produces more shoot mass than its congers (Allred et al. 2010), that then increases the light competitiveness (Brandon et al. 2004). *Lespedeza cuneata* invasion may also be due to novel weapons such as allelochemicals that have been shown to be allelopathic to some grasses. Soil extracts of *L. cuneata* reduces the germination of ryegrass (*Lolium multiflorum*), and inhibits the radicle and coleoptile length of rye (*Secale cereal*) and tall fescue (*Festuca arundinacea*) (Kalburtji and Mosjidis 1993a). In addition, *L. cuneata* residues also inhibits the growth of warm season grasses such as Bermuda grass (*Cynodon dactylon*) and Bahia grass (*Paspalum notatum*) (Kalburtji and Mosjidis 1992). *Lespedeza cuneata* stem and leaf extract can reduce seed germination of big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), and Kentucky bluegrass (*Poa pratensis*), and inhibit the radicle and coleoptile length of *A. gerardii* and *P. pratensis* (Dudley and Fick 2003). Since it is a legume, *L. cuneata* can engage in a mutualistic relationship with N-fixing rhizobia, and this partnership may facilitate *L. cuneata* invasions as predicted by the mutualism facilitation hypothesis.

In addition to the inherent qualities of invasive species, past land-use practices can also affect the success of invasions. A legacy effect describes an indirect effect of a species on ecosystem after the species has been removed (Cuddington 2011). Agricultural activities have a long legacy effect on the soil microbial communities and vegetative communities (Cuddington 2011). In the Flint Hills region of Kansas, many restored prairies in this region were once used for row crop agriculture. *Lespedeza cuneata* located in restored prairie sites have a larger proportion of nodules occupied by rhizobia than plants in the native prairie sites, and the rhizobia *Bradyrhizobia betae* strain PLTHG1 facilitates greater shoot growth and is only found in *L. cuneata* in restored prairie sites, which suggests that land-use legacy influence the rhizobia mutualists present (Wang 2013). *Lespedeza cuneata* can utilize rhizobia to have more shoot mass than its North American congener, *Lespedeza virginica* (L.) Britton, when they are inoculated with the same rhizobia strains, and those rhizobia strains may only benefit *L. cuneata* (Hu et al. 2014). Removal of the native plant community could also result in more empty niches in restored prairie sites that *L. cuneata* can occupy as predicted by the empty niche hypothesis. Additionally, rhizobia may not only facilitate growth, but can also improve resources to produce defensive chemicals. For example, white locoweed (*Oxytropis sericea*) seedlings inoculated with rhizobia have greater biomass and production of swainsonine than non-inoculated controls because rhizobia increases available nitrogen in the plant, thus providing resource to synthesize this secondary metabolite (Valdez Barillas et al 2007). This raises the question whether rhizobia difference also affects allelopathic chemical production in *L. cuneata.*

In this study, we evaluated the allelopathic effect properties of soil extracts from the rhizosphere of *L. cuneata* in restored and native prairie sites in the Lyon County, Kansas. All restored prairie sites were once used for row crop agriculture. Seed germination and seedling growth tests were employed to evaluate allelopathic effects. Seeds of tomato (*Lycopersicum esculentum var Rutgers VF*), native grasses, native forbs and native legumes were treated with soil extracts from the rhizosphere of *L. cuneata* in restored and native prairies. We predicted that soil from inside the rhizosphere of *L. cuneata* is more allelopathic than outside its rhizosphere, which would provide support for the novel weapons hypothesis. We also predicted soil from inside the *L. cuneata* rhizosphere from restored prairie would be more allelopathic than from native prairie, hereby providing evidence for the mutualism facilitation hypothesis and demonstrate that novel weapons and mutualism facilitation likely work together in *L. cuneata* invasions because a more robust symbiotic relationship between rhizobia and *L. cuneata* would provide more resource to produce allelochemicals.

Materials and Methods

Soil Sample Collection and Preparation

Soil samples were collected from Lyon County, Kansas. Three pairs of adjacent restored and native prairie sites were chosen including one pair from Indian Hills (IH) and one pair from Eagle Creek (EC) at the Flint Hills National Wildlife Refuge in August 2014, and another pair from Melvern Lake managed by the US Corps of Engineers (ML) in March 2015. All restored prairie sites were once used for row crop agriculture. The 30-year (1981-2010) annual precipitation of this region is 963 mm – 980 mm, and the average annual temperature is 12.9 -13.2 °C.

At each site, 10 *L. cuneata* plants were selected. At each plant, soil core samples were taken from the *L. cuneata* rhizosphere and at least 1m away from the plant using a 3cm diameter soil corer. Soil core samples were taken at 0-10 cm level because the allopathic compounds are likely most abundant at the surface of the soil to inhibit seed germination and seedling growth. This sampling protocol resulted in 10 samples of 4 different types at each location: native prairie inside *L. cuneata* rhizosphere, native prairie outside *L. cuneata* rhizosphere, restored prairie inside *L. cuneata* rhizosphere and restored prairie outside *L. cuneata* rhizosphere. Rocks and roots were removed by hand from each soil sample, and then the soils were oven-dried at 105ºC for 48 hours and coarsely ground. Ground soil was mixed in a 1:10 soil (g) to distilled water (ml) ratio (Kalburtji and Mosjidis 1993b). The soil mixture was agitated for 2 hours, left to soak for 24 hours and filtered through filter paper (Whatman #1) to remove soil particulates. Soil extracts were stored at 4 °C until germination tests were performed.

Soil total N and C were also measured on 5 randomly selected soil samples from inside *L. cuneata* rhizosphere and outside rhizosphere at restored and native prairie in at each sample collection location. Each soil sample was homogenized and then sent to the Kansas State University Soil Testing Lab for soil total C and N analysis using the dry combustion method.

Species Tested in Allelopathy Assay

In total, seeds of 12 species were tested in the allelopathy assay. Species included a model species (*L. esculentum*), three native grass species, five forb species, two congeneric legumes and *L. cuneata*. The forbs species included 3 non-legume forbs and 2 non-congeneric legumes. *Lycopersicum esculentum*, which is sensitive to most allelochemicals, was first tested as a model for the allelopathic potential of each soil extraction. Other seeds tested were three native prairie grasses including little bluestem (*Schizachyrium scoparium* (Michx.) Nash), *A. gerardii*, and *S. nutans*; three native non-legumes forbs including prairie aster (*Aster turbinellus* Lindl.), yellow coneflower (*Ratibida pinnata* (Vent.)) and black-eyed susan (*Rudbeckia hirta* L.); two native non-congeneric legumes including Illinois bundleflower (*Desmanthus illinoensis* Vent.) and purple prairie clover (*Dalea purpurea* (Michx.) MacM. ex B.L. Robins. & Fern.); two native congeneric legumes including round-headed bush clover (*Lespedeza capitata* Michx.) and slender bush clover (*Lespedeza virginica* (L.) Britton.), and *L. cuneata*.

Seed Preparation

Seeds of *A. turbinellus*, *R. pinnata* and *R. hirta* were cold stratified for 30 days, and seeds of *L. capitata* and *L. virginica* were cold stratified for 7 days. For the cold stratification, seeds were mixed into moist sand and then stored at 4 °C for duration of the stratification period. The cold stratification procedures followed were the suggested methods provided by Prairie Moon Nursery (Winona, MN) from where these seeds were acquired.

Desmanthus illinoensis seeds were hand collected from Flint Hills National Wildlife Refuge in August 2014. *D. illinoensis* have an impermeable seed coat that reduces germination. To enhance germination, seeds were scratched by sand paper (500 grit) (Call 1985).

Lespedeza cuneata seeds were collected from the Bressner Range Research Unit, Yates Center, KS in late October 2008. *Lespedeza cuneata* seeds were soaked for 1 hour in water brought to a boil and subsequently removed from heat (Wang 2013). Then the seeds were soaked in 0.01M gibberellic acid for 36 hours to break the seed dormancy. After soaking seeds were washed with distilled water.

Lycopersicum esculentum, *A. gerardii*, *S. scoparium*, *S. nutans*, and *D. purpurea* did not require pretreatment for germination. All seeds not collected from field were obtained from Prairie Moon Nursery (Winona, MN) in July 2015.

Allelopathy Assay

For each soil sample collected, 3 replicate petri dishes, each containing 30 seeds each of each species tested (20 for *D. illinoensis* because limited seeds were collected from field) were placed on filter paper (Whatman #3) (Kalburtji and Mosjidis 1992). Filter paper was moistened with 3 ml of soil extract. A control group of seeds were treated with distilled water. Seeds were incubated at 27 °C, which was an appropriate temperature for germination of all seeds tested (Call 1985; Sabo et al. 1979; Qiu et al. 1995; Madakadze et al. 2001). Germination rate and seedling lengths were measured after 1-7 days depending on the inherent growth rate of species. A control group of each species was assayed with distilled water first to determine the optimal length of incubation for each species. Seeds were considered germinated if the radicle emerged from the seed coat (Kalburtji and Mosjidis 1993b). The seedling length included the combined length of the epicotyl and hypocotyl but did not included seed coat. The seedling length was measured using a Vernier caliper.

Statistical Analyses

Two-way ANOVAs (α=0.05) were performed to test for differences in germination and seedling length of seeds treated by soil extracts from each site, and the differences of total soil N in each site. The prairie type and the proximity to *L. cuneata* were the two independent factors of the ANOVA. Germination rate, seedling length, total soil N, total soil C and C:N ratios were the dependent factors. An ANOVA was also used to determine any interaction between prairie type and proximity. Once the general differences were established, a Tukey adjustment was used to compare the differences within same prairie type or same proximity level. A significant level of $\alpha = 0.05$ was used for all statistical tests. All tests were performed using PROC ANOVA in SAS 9.2 (SAS Institute Inc.).

Results and Discussion

Proximity effect of sericea lespedeza (*L. cuneata*) allelopathy

Soil extract from inside *L. cuneata* rhizosphere reduced the germination and seedling growth of *L. esculentum* at all locations tested (Table 1; Figure 1, 2). It also reduced *S. nutans* germination (Table 2; Figure 3c) and *A. gerardii* seedling growth (Table 3; Figure 4b) at each location, but it had no impact on another native tall grass, *S. scoparium* (Table 2, 3; Figure 3a, 4a). There was no proximity effect on germination or seedling growth of five tested forbs, including *A. turbinellus, R. pinnata, R. hirta, D. purpurea,* and *D. illinoensis* (Table 4, 5, 6, 7). My results also showed the germination rate and seedling length of *L. capitata* treated with soil extracts from inside *L. cuneata* rhizosphere was significantly less than outside *L. cuneata* rhizosphere at most locations tested (Table 8; Figure 5a). Soil extracts from inside the rhizosphere of *L. cuneata* reduced the germination rate of *L. virginica* at Eagle Creek and Melvern Lake location (Table 9; Figure 5b), and reduced the seedling length of *L. virginica* at Indian Hills and Eagle Creek (Table 9; Figure 6b). Soil extract had no allelopathic effect on *L. cuneata* itself, which suggests *L. cuneata* was not self-inhibitory (Table 9; Figure 6c).

My results showed soil extracts of *L. cuneata* rhizosphere were allelopathic on germination or seedling growth of *L. esculentum, S. nutans, A. gerardii, L. capitata, L. virginica,* but showed no inhibition of *S. scoparium, A. turbinellus, R. pinnata, R. hirta, D. purpurea* , *D. illinoensis* and *L. cuneata*. Previously, *L. cuneata* residue extracts have been shown to inhibit some warm-season grass (*C. dactylon* and *P. notatum*) growth, but not affect their germination (Kalburtji and Mosjidis 1992). My study corroborates their findings on *A. gerardii*, but this study's results conflict with Kalburtji and Mosjidis's (1992) findings on *S. scoparium* and *S. nutans*. Young (2000) found that *L. cuneata* leaf extract did not have any toxic effects on germination of *S. scoparium*, *A. gerardii* and *S. scoparium*. Dudley and Fick (2003) also found that *L. cuneata* leaf extract did not affect *S. scoparium* germination and radicle growth, but inhibited both the germination and radicle growth of *A. gerardii* as well as germination of *S. nutans*. As the results of these multiple studies suggest, the inhibitory effect of *L. cuneata* can be variable, and as this study shows, the location from where samples are collected can influence the presence of allelochemicals.

Germination and seedling growth of the 3 non-legume forbs, *A. turbinellus*, *R. pinnata* and *R. hirta*, and 2 non-congeneric legume forbs, *D. purpurea* and *D. illinoensis*, were not affected by soil extracts in anyway (Table 4, 5, 6, 7). Unlike the grasses tested, forbs are likely not abundant enough in landscape to inhibit or become a competitive risk to the invader. In a grassland, grasses are found at high densities and compete with *L. cuneata* for resources over time; *L. cuneata* may have evolved the ability to produce allelopathic chemicals against grasses. However, forbs may not have much interaction as grasses with *L. cuneata*, and that might explain why *L. cuneata* soil extract did not inhibit these forb species.

Lespedeza cuneata may utilize a biochemical weapon that is novel to native species and inhibit their germination and growth, but as shown, not all species are affected. According to novel weapons hypothesis, some invasive species gain advantages over native plants by releasing novel allelopathic compounds (Callaway and Ridenour 2004). For example, spotted knapweed (*Centaurea maculosa*) can release phytotoxin (±)-

catechin to inhibit and displace native plants (Bais et al.2002; Bais et al. 2003). My results showed *L. cuneata* inhibits 7 of 12 tested species which provide preliminary support for the novel weapons hypothesis. To confirm that allelopathy is novel in the invasive range, allelopathy of *L. cuneata* would need to be tested against the dominant vegetation in its native range. In addition, it would also be useful to test the allelopathy of *L. cuneata* from both its native range and its invaded range.

Land-use legacy effect on sericea lespedeza (*L. cuneata*) allelopathy

Lycopersicum esculentum seed germination and seedling growth were significantly reduced by the land-use legacy of row crop agriculture at all the sites tested (Table 1; Figure 1, 2). There was a significant interaction between prairie type and proximity to *L. cuneata* where the allelopathic effect of the soil extract was greater for soil collected from the rhizosphere of *L. cuneata* in restored prairie than native prairie (Table 1). Past agricultural land-use legacy also significantly increased allelopathy of *L. cuneata* on *S. nutans* and *A. gerardii* (Table 2, 3). Germination of *S. nutans* seeds treated with soil extract from *L. cuneata* rhizosphere at restored prairie site was significantly lower than from native prairie sites at all locations. The soil extracts from restored prairie at location Melvern Lake showed reduced *A. gerardii* seedling growth more than native prairie, and comparing within the same proximity level, the soil extracts from restored prairie inside *L. cuneata* rhizosphere were also most inhibitory to *A. gerardii* seedling growth at Eagle Creek (Table 3, Figure 4b). Forbs, including *A. turbinellus*, *R. pinnata, R. hirta, D. purpurea* and *D. illinoensis* were not affected by soil extracts (Table 6, 7). Germination rate and seedling growth of *L. capitata* treated by soil extracts from restored prairie site was significantly less than from restored prairie site at Eagle Creek and Melvern Lake (Table 8, 9; Figure 5b, 6a). Germination rate of *L. virginica* treated by soil extracts from restored prairie site was also significantly less than from restored prairie site at Indian Hills and Melvern Lake (Table 8; Figure 5b), and the seedling length of *L. virginica* was significantly inhibited by soil extract from restored prairie site at all locations (Table 9, Figure 6b).

The interaction between proximity to *L. cuneata* and prairie type was not consistent at all locations. The inconsistency of interaction may be a function of the time of soil sample collection. Soil samples were collected in August at the Indian Hills and the Eagle Creek locations and in March at the Melvern Lake location. *Lespedeza cuneata* roots are likely producing more allelopathic compounds during the growing season in August than during the dormancy season in March. The allelochemicals are likely still present at Melvern Lake since *Lespedeza cuneata* proximity still inhibited germination and growth regardless of when soil samples were collected (Table 1).

The results suggest the presence of more allelopathic compounds in soil within the *L. cuneata* rhizosphere as shown by reduction in seed germination and/or seedling growth. *Lespedeza cuneata* growing in restored prairie sites were likely able to produce more allelopathic compounds than in native prairie sites. The possible reason for greater allelopathic capacity at restored prairie sites might be due to either a symbiont availability differences or N availability differences between prairie types because of cultivation history.

According to Wang (2013), in the Flint Hills of Kansas, *L. cuneata* located in restored prairie, with a history of row-crop agriculture has a larger proportion of nodules occupied by rhizobia than in native prairie, and the rhizobia *Bradyrhizobia betae* strain PLTHG1 was only found in *L. cuneata* at restored prairie sites. They also found *B. betae* is more effective in allocating to shoot biomass than *Bradyrhizobia liaoningense* strain 2281, which was present in *L. cuneata* at both restored and native prairie (Wang 2013). This rhizobia strain only present in restored prairie may provide *L. cuneata* with increased access to N therefore allowing for greater aboveground growth and resources to produce secondary metabolites. Another legume, *O. sericea* inoculated with rhizobia was shown to have greater biomass and production of swainsonine, a secondary metabolite, than non-inoculated controls because rhizobia increases available nitrogen in the plant, thus providing resources to synthesize this secondary metabolite (Valdez Barillas et al 2007). The results of these studies suggest that allelopathic compound production by *L. cuneata* may be dependent on, or at least enhanced by, symbiont availability at each site.

According to mutualism facilitation hypothesis, mutualists facilitate another species' invasion (Simberloff and Von Holle 1999; Richardson et al. 2000). Legumes invading new habitats are facilitated by rhizobia (Richardson et al. 2000), and invasion might be enhanced by the presence of certain rhizobia strains that more effectively associate with the introduced legume than with the native legume (Reinhart and Callaway 2006; Rodriguez-Echeverria. 2010). The findings of this study in combination with the finding of Wang (2013) not only provide preliminary support for novel weapons hypothesis, but also provide support for mutualism facilitation hypothesis. Restored prairie sites with a row-crop agricultural legacy may have a greater availability of symbionts and increase *L. cuneata*'s ability to produce allelopathic compounds. Unlike the study by Valdez Barillas et al. (2007), where the allelochemical is a N containing alkaloid, the most likely candidates for *L. cuneata*'s allelochemicals are p-coumaric acid, protocatechuic acid, ferulic acid, and vanillin (Langdale and Giddens 1967). All four allelochemicals are phenols that do not contain N unlike a N containing alkaloid, so a N-fixing symbiont would assist *L. cuneata* with resource allocation and subsequent distribution of resources to secondary metabolites rather than fixed N directly contributing to allelochemical production. Mutualism not only assists growth but also likely facilitates the ability of *L. cuneata* to produce chemical weapons. To confirm this, further study is required. Because the sites I chose were not the same as Wang's (2013), future studies could measure the concentration of allelopathic compounds in plant and soil samples collected from rhizosphere of *L. cuneata*, and confirm the identity of rhizobia in root nodules as well.

Some plant species may be sensitive to different allelochemicals produced by *L. cuneata*, and each allelochemical may not be present in equal concentrations at all locations. In species like oats (*Avena spp.*), protocatechuic acid at higher concentrations is less effective in inhibiting auxin activity of IAA than p-coumaric acid, ferulic acid, and vanillin (Langdale and Giddens 1967). The main four allelopathic compounds in *L. cuneata* may also not increase equally in response to landuse legacy. Rhizobia may assist production of some of the allelochemicals more so than others, and each allelochemical's inhibition of a test species may differ. In one location, if a certain allelochemical increases more than the others, the species sensitive to that chemical would be reduced more than species less sensitive to a different allelochemical. This may explain the different pattern of inhibition between different species at a single location. To confirm this, a future study could measure the concentration and proportion of each allelochemical at each sampling location with HPLC or gas chromatography.

Soil N and C

The legacy effect of agriculture significantly reduced total soil N and C at Eagle Creek and Melvern Lake (Figure 7, 8). At Eagle Creek and Melvern Lake locations, soil from the native prairie sites have significantly more soil N than restored prairie sites ($p < 0.001$, Figure 7). At Eagle Creek, the native prairie has nearly twice as much soil N as restored prairie. In contrast, the Indian Hills location does not exhibit the expected difference between native and restored prairies. Potential reasons could be because there was a greater level of N fertilization in the restored prairie site at Indian Hills, or because this site was only cultivated for short period of time.

Invasive N-fixing species, such as *M. faya*, peacock plume (*Albizia falcataria*) and *R. pseudoacacia* are able to increase total N input and mineralization rate, and increase N cycling rates in the invaded range (Vitousek and Walker 1989; Binkley 1997; Rice et al. 2004). Because of *L. cuneata* N fixing capability, total soil N is expected to be greater within the *L. cuneata* rhizosphere. Surprisingly, *L. cuneata* had no detectable effect on soil N at any of the sites. The samples collected outside of the *L. cuneata* rhizosphere were only collected 1 m outside of the rhizosphere and that distance may not be far enough away from the plant’s zone of influence on N cycling. Invasive N fixing plants may also affect the N cycling at entire site where it is present. To test if *L. cuneata* has impact on soil N in its invaded range, a further investigation of total soil N of a nearby location without *L. cuneata* is needed.

At Eagle Creek and Melvern Lake locations, soil from the native prairie sites have significantly more soil C than restored prairie sites ($p < 0.005$, Figure 8). We expected total soil C would be lower in the restored prairie site because cultivation aerates soil and accelerates respiration by soil microbes, and thereby substantially reduces soil C (Davidson and Ackerman 1993). However, similar to soil total N in Indian Hills, the soil total C is not different between restored and native prairie site. One possible reason is that the restored prairie site in Indian Hills may have been cultivated for a very short time. The C/N ratio is also not significantly different between native and restored prairie sites in Indian Hills, Eagle Creek and Melvern Lake. The C/N ratios were all below 20 (Table 10), which indicates N mineralization is greater than immobilization and thereby suggests that these locations are not highly N limited over long term timeframes (Parnas 1975).

Soil from the *L. cuneata* rhizosphere in restored prairie significantly reduced *A. gerardii* and *L. capitata* seedling growth or germination at Eagle Creek and Melvern Lake, where native prairie sites had higher soil total N than restored prairie sites, but there was no significant difference found in Indian Hills where soil N was not different between restored and native prairie. At Eagle Creek and Melvern Lake, possible N limitations in the restored prairie site could increase competition for N, and would make production of allelochemicals more cost effective than when N is not limiting. Total soil N likely remains steady over time, but available N may vary seasonally and be more limiting in restored prairie sites like Eagle Creek and Melvern Lake. Intensity of below ground competition is intense under low N conditions, and fertilization decreases below ground competition (Wilson and Tilman 1993). The high competition intensity under low resource availability may encourage plant allelopathy. In an aquatic ecosystem, *Prymnesium parvum* increases allopathic substances when growing under N deficient conditions (Granéli and Johansson 2003). There may be a similar phenomenon in land-based ecosystems.

Allelopathy against congeneric species

Soil extract from inside the *L. cuneata* rhizosphere reduced the germination and seedling growth of *L. capitata* and *L. virginica,* but had no allelopathic effect on *L. cuneata* itself. Allelopathy of congeneric species have been studied, but previous studies have focused on comparing the allelopathic ability of multiple species within a genera, not allelopathy of members of a genera against one another. However, my result showed the *L. cuneata* inhibits germination and growth of its North American congeners. *Lespedeza cuneata* evolved in eastern Asia where there are numerous *Lespedeza spp*. (Xu et al. 2012). Congeners often compete for resources in shared niche space because congeners may be close competitors. According to Beaton (2011), the invasive genotype of *L. cuneata* is a better competitor than the native genotype collected from Asia, and ancestral genotype planted at North American in 1930s, and has greater biomass than the native and ancestral genotype when grown together. Even through the invasive genotype of *L. cuneata* has evolved to become more competitive, it still likely occupies similar niches with native and ancestral genotypes, and reduces growth of other genotypes to compete with resources (Beaton 2011). Regardless of the competitive differences within *L. cuneata* genotypes, *L. cuneata* is also more productive than its North American congeners (Allred et al. 2010). *Lespedeza* diverged into North American species and East Asian species at about 8.2 million years ago, however, *L. cuneata* is a young species at less than 0.5 million years old (Xu et al. 2012). If all *Lespedeza* species are allelopathic against one another, then allelopathy is an ancestral trait of the common ancestor to North American and Asia species. If not all *Lespedeza* species are allelopathic to each other, allelopathy may have evolved recently in *L. cuneata* and possibly other *Lespedezas*, and the allelochemicals work on an ancestral metabolic mechanism to both North American and Asia species. To determine to origin of allelopathy in the genus, further investigation should test the allelopathy of all *Lespedeza* species against each other. If *L. cuneata* is only allelopathic in its invaded range, then *L. cuneata* has newly evolved this trait after its transport to North America and allelopathy against congeners may be the unique character of *L. cuneata* in its invaded range. A new selection pressure or mutualism in the invaded communities may drive this evolution. To confirm this hypothesis, further investigation is needed to test if the *L. cuneata* from it native range is allelopathic on other *Lespedeza* species in its native range.

Conclusion

Allelopathy of *L. cuneata* soil extracts is not uniform against all species. Some grasses are sensitive, but forbs, both legume and non-legume, are insensitive except for congeneric species. Native *Lespedeza* species are sensitive to allelochemicals from *L. cuneata*, but *L. cuneata* is not self-inhibitory. Allelopathy is more pronounced in restored prairie, and this may originate from a more robust symbiotic relationship with rhizobia in restored prairie. Introduced legumes often change N cycling in the introduced system, but in the locations tested, *L. cuneata* has no effect of total soil N. Past row-crop agriculture reduced total soil N by up to half, and *L. cuneata* has not increased it. *Lespedeza cuneata* invasion can be explained by novel weapons hypothesis that releasing allelopathic compounds successfully inhibit several grass species that are dominant in the tallgrass prairie. Mutualism facilitation hypothesis also well supports *L. cuneata* invasion. Mutualism may facilitate the invasion of *L. cuneata* not only by assisting growth but also increased the ability of *L. cuneata* produce the chemical weapons. *Lespedeza cuneata* invasion is likely enhanced by mutualists that facilitate the production of allelochemicals.

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Table 1. Interaction of site type and proximity for germination rate and seedling length of *Lycopersicum esculentum* seeds treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Germination rate						
Prairie type	45.25	**<0.001**	9.66	**0.004**	4.61	**0.039**
Proximity	22.23	**<0.001**	117.82	**<0.001**	5.50	**0.025**
Prairie type * Proximity	26.76	**<0.001**	31.56	**<0.001**	0.30	0.586
Seedling size						
Prairie type	5.60	**0.023**	45.42	**<0.001**	11.69	**0.002**
Proximity	61.09	**<0.001**	50.87	**<0.001**	61.99	**<0.001**
Prairie type * Proximity	12.34	**0.001**	22.42	**<0.001**	20.78	**<0.001**

Table 2. Interaction of site type and proximity for germination rate of tall grass species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Schizachyrium scoparium						
Prairie type	1.96	0.170	0.21	0.653	0.94	0.338
Proximity	0.74	0.394	0.33	0.857	0.11	0.748
Prairie type * Proximity	1.96	0.170	0.67	0.42	2.62	0.114
Andropogon gerardii						
Prairie type	0.33	0.571	0.18	0.673	0.07	0.794
Proximity	2.48	0.124	2.10	0.156	0.43	0.515
Prairie type * Proximity	3.46	0.071	0.01	0.933	0.07	0.794
Sorghastrum nutans						
Prairie type	10.06	**0.03**	5.57	**0.024**	13.60	**0.001**
Proximity	130.32	**<0.001**	10.05	**0.030**	67.66	**<0.001**
Prairie type * Proximity	0.10	0.753	0.22	0.640	10.64	**0.002**

Table 3. Interaction of site type and proximity for seedling length of tall grass species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie site. Site type are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Site type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Schizachyrium scoparium						
Prairie type	1.42	0.241	3.40	0.073	1.11	0.298
Proximity	0.35	0.558	0.60	0.445	0.66	0.442
Prairie type * Proximity	0.71	0.406	1.32	0.259	0.51	0.478
Andropogon gerardii						
Prairie type	2.07	0.159	3.05	0.089	7.51	**0.010**
Proximity	20.08	**<0.001**	21.42	**<0.001**	12.28	**0.001**
Prairie type * Proximity	1.01	0.322	6.41	**0.016**	0.32	0.573
Sorghastrum nutans						
Prairie type	0.20	0.659	2.47	0.125	<0.01	0.958
Proximity	<0.01	0.948	1.66	0.205	0.47	0.497
Prairie type * Proximity	3.60	0.066	1.17	0.288	<0.01	0.984

Table 4. Interaction of site type and proximity for germination rate of forb species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Aster turbinellus						
Site type	0.87	0.357	0.42	0.520	3.20	0.082
Proximity	0.01	0.926	0.03	0.860	0.26	0.613
Site type * Proximity	0.14	0.711	0.28	0.599	0.07	0.799
Ratibida pinnata						
Site type	0.33	0.568	0.86	0.361	0.35	0.560
Proximity	0.04	0.849	2.81	0.102	1.03	0.317
Site type * Proximity	0.20	0.657	3.52	0.069	0.64	0.428
Rudbeckia hirta						
Site type	0.23	0.635	0.16	0.691	2.20	0.147
Proximity	1.38	0.248	0.23	0.634	0.93	0.340
Site type * Proximity	<0.01	0.966	8.31	**0.007**	3.03	0.090
Dalea purpurea						
Site type	0.50	0.482	0.42	0.523	0.43	0.519
Proximity	<0.01	1.000	0.05	0.831	0.15	0.698
Site type * Proximity	0.32	0.860	0.05	0.831	2.058	0.160
Desmanthus illinoensis						
Site type	0.19	0.670	1.67	0.205	0.52	0.476
Proximity	3.63	0.650	0.42	0.522	0.52	0.476
Site type * Proximity	0.64	0.430	2.61	0.115	0.29	0.592

Table 5. Germination rate of forb species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie site. Data are presented as the mean ± standard error. There were no significant differences in germination treated with any soil extracts between any treatments.

		Restored		Native	
Unit: (cm)		Inside rhizosphere	Outside rhizosphere	Inside rhizosphere	Outside rhizosphere
Aster turbinellus	IH	22.67±1.50	23.22±0.91	22.00±1.27	21.67±0.98
	EC	24.00±0.94	24.67±1.19	25.11±0.94	24.78±0.60
	ML	26.89±0.93	27.56±0.86	25.56±0.91	25.78±0.77
Ratibida pinnata	IH	67.67±0.75	67.89±0.67	67.89±1.03	67.00±0.97
	EC	66.44±2.03	74.33±2.73	72.67±2.37	72.22±1.59
	ML	70.11±1.00	72.00±0.77	71.56±1.16	71.78±1.17
Rudbeckia hirta	IH	64.67±1.31	66.11±0.90	65.22±1.07	66.78±1.68
	EC	68.33±1.34	63.67±1.26	64.89±1.51	68.22±1.42
	ML	64.78±0.95	64.11±0.81	62.00±1.07	64.33±0.51
Dalea purpurea	IH	96.00±0.76	95.89±0.62	96.33±0.60	96.44±0.49
	EC	97.67±0.45	97.89±0.51	97.44±0.53	97.44±0.57
	ML	98.44±0.41	98.89±0.33	98.78±0.31	98.00±0.59
Desmanthus illinoensis	IH	93.67±1.24	95.17±0.84	92.00±2.08	95.67±0.90
	EC	95.33±0.85	94.33±1.00	92.33±1.27	94.67±0.96
	ML	94.50±0.93	95.67±0.79	94.33±0.83	94.50±1.11

Table 6. Interaction of site type and proximity for seedling length of forb species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
Unit: (cm)	F	*P*	F	*P*	F	*P*
Aster turbinellus						
Site type	0.89	0.352	0.24	0.625	0.01	0.937
Proximity	0.03	0.867	0.41	0.527	0.66	0.421
Site type * Proximity	1.10	0.300	0.01	0.906	0.59	0.448
Ratibida pinnata						
Site type	0.09	0.769	3.26	0.080	0.95	0.337
Proximity	5.03	0.31	7.92	**0.008**	0.08	0.783
Site type * Proximity	0.63	0.432	0.19	0.667	0.02	0.899
Rudbeckia hirta						
Site type	2.04	0.162	0.01	0.924	0.69	0.413
Proximity	<0.01	0.974	0.17	0.680	0.05	0.819
Site type * Proximity	<0.01	0.987	0.02	0.888	0.10	0.755
Dalea purpurea						
Site type	0.13	0.717	0.35	0.557	0.25	0.621
Proximity	1.02	0.320	1.28	0.266	0.86	0.360
Site type * Proximity	5.95	**0.020**	0.07	0.795	0.32	0.574
Desmanthus illinoensis						
Site type	2.15	0.151	0.44	0.513	2.04	0.162
Proximity	1.75	0.195	0.30	0.587	0.18	0.678
Site type * Proximity	0.14	0.711	0.08	0.773	4.43	**0.042**

Table 7. Seedling length of forb seeds treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie site. Data are presented as the mean ± standard error. There were no significant differences in seedlings treated with any soil extracts between any treatments.

Unit: (cm)		Restored		Native	
		Inside rhizosphere	Outside rhizosphere	Inside rhizosphere	Outside rhizosphere
Aster turbinellus	IH	0.63±0.04	0.60±0.04	0.63±0.04	0.68±0.04
	EC	0.66±0.04	0.69±0.03	0.65±0.05	0.67±0.03
	ML	0.66±0.03	0.72±0.04	0.69±0.04	0.69±0.05
Ratibida pinnata	IH	2.01±0.09	2.43±0.22	2.07±0.11	2.28±0.08
	EC	1.93±0.13	2.31±0.10	2.15±0.07	2.68±0.27
	ML	2.51±0.12	2.50±0.06	2.60±0.07	2.57±0.08
Rudbeckia hirta	IH	0.74±0.02	0.74±0.02	0.76±0.02	0.76±0.01
	EC	0.81±0.02	0.82±0.02	0.81±0.02	0.82±0.02
	ML	0.65±0.01	0.65±0.01	0.66±0.01	0.66±0.01
Dalea purpurea	IH	1.08±0.02	1.17±0.03	1.15±0.03	1.12±0.02
	EC	1.18±0.03	1.23±0.04	1.21±0.03	1.24±0.03
	ML	1.16±0.04	1.21±0.02	1.13±0.03	1.17±0.03
Desmanthus illinoensis	IH	1.55±0.04	1.48±0.05	1.60±0.03	1.56±0.03
	EC	1.76±0.04	1.76±0.04	1.72±0.03	1.75±0.04
	ML	1.91±0.04	1.77±0.04	1.87±0.06	1.96±0.07

Table 8. Interaction of site type and proximity for germination rate of congeneric legume treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Lespedeza capitata						
Site type	1.89	0.178	6.73	**0.014**	5.31	**0.027**
Proximity	15.08	**<0.001**	5.89	**0.020**	39.27	**<0.001**
Site type * Proximity	0.11	0.748	0.03	0.868	5.31	**0.027**
Lespedeza virginica						
Site type	4.69	**0.037**	0.71	0.406	10.56	**0.003**
Proximity	1.61	0.212	12.79	**0.001**	9.51	**0.004**
Site type * Proximity	6.57	**0.015**	0.01	0.943	4.75	**0.036**
Lespedeza cuneata						
Site type	0.01	0.929	0.17	0.681	0.70	0.408
Proximity	0.01	0.929	0.42	0.522	0.55	0.465
Site type * Proximity	0.03	0.859	1.16	0.289	0.70	0.408

Table 9. Interaction of site type and proximity for seedling length of congeneric legume treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Prairie types are restored or native prairie. Proximity are inside rhizosphere or outside rhizosphere. Prairie type * Proximity represents the interaction between site type and proximity. Significant interactions are shown in bold ($p<0.05$).

	Indian Hills		Eagle Creek		Melvern Lake	
	F	*P*	F	*P*	F	*P*
Lespedeza capitata						
Site type	0.27	0.605	5.15	**0.029**	7.31	**0.010**
Proximity	33.12	**<0.001**	56.85	**<0.001**	126.81	**<0.001**
Site type * Proximity	2.94	0.095	2.13	0.153	6.34	**0.016**
Lespedeza virginica						
Site type	9.56	**0.004**	6.87	**0.013**	4.75	**0.036**
Proximity	35.85	**<0.001**	10.59	**0.002**	1.21	0.278
Site type * Proximity	0.68	0.416	3.06	0.089	1.04	0.314
Lespedeza cuneata						
Site type	0.09	0.762	0.34	0.566	0.11	0.740
Proximity	1.32	0.258	0.52	0.477	0.73	0.400
Site type * Proximity	0.52	0.477	0.90	0.350	0.01	0.935

Table 10. C:N ration of soils from Lyon County, Kansas. Soils were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites. Data are presented as the mean ± standard error. There were no significant differences in C:N ration between any soil types in each location.

	Restored		Native	
	Inside rhizosphere	Outside rhizosphere	Inside rhizosphere	Outside rhizosphere
Indian Hills	11.63±0.08	11.45±0.30	11.08±0.45	11.65±0.27
Eagle Creek	12.57±0.36	12.34±0.20	12.81±0.36	12.44±0.11
Melvern Lake	11.39±0.12	11.64±0.40	11.63±0.27	11.55±0.23

Figure 1. Germination rate of *Lycopersicum esculentum* seeds treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of germination treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of germination treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of germination treated by soil extracts from restored prairie sites, hatched bars represent the means of germination treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

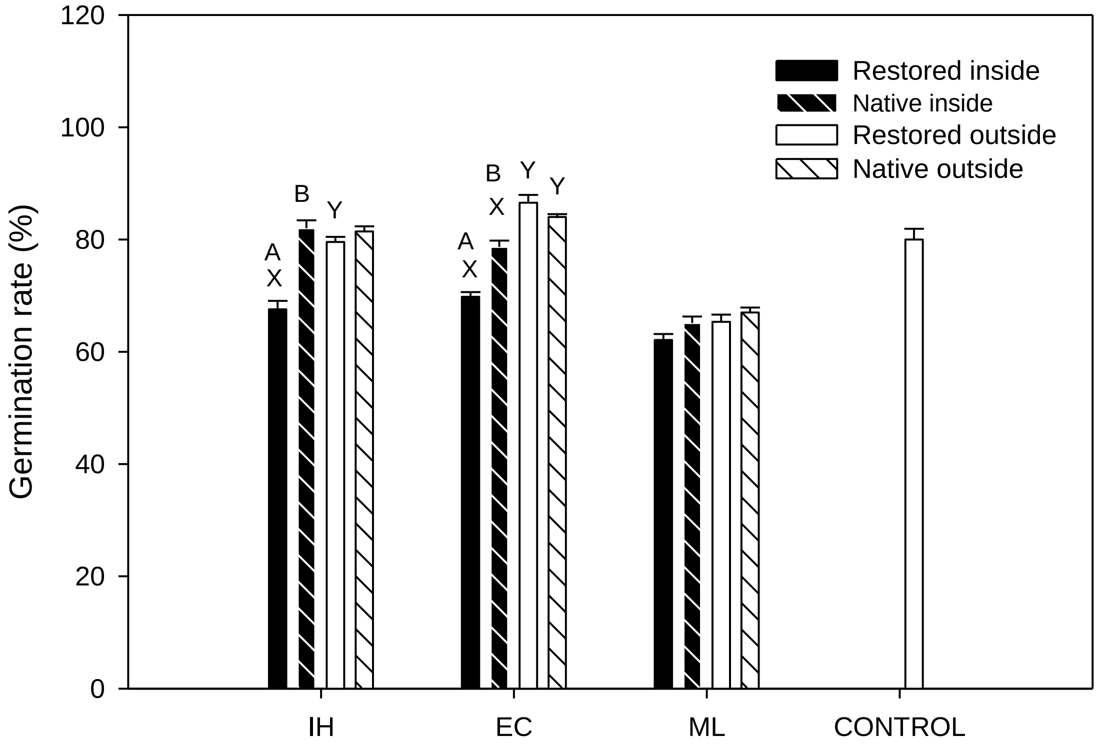

Germination rate (%)
0
20
40
60
80
100
120
IH
EC
ML
CONTROL
Restored inside
Native inside
Restored outside
Native outside
A
X
B
Y
A
X
B
X
Y
Y

Figure 2. Seedling length of *Lycopersicum esculentum* seeds treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of seedling length treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of seedling length treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of seedling length treated by soil extracts from restored prairie sites, hatched bars represent the means of seedling length treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

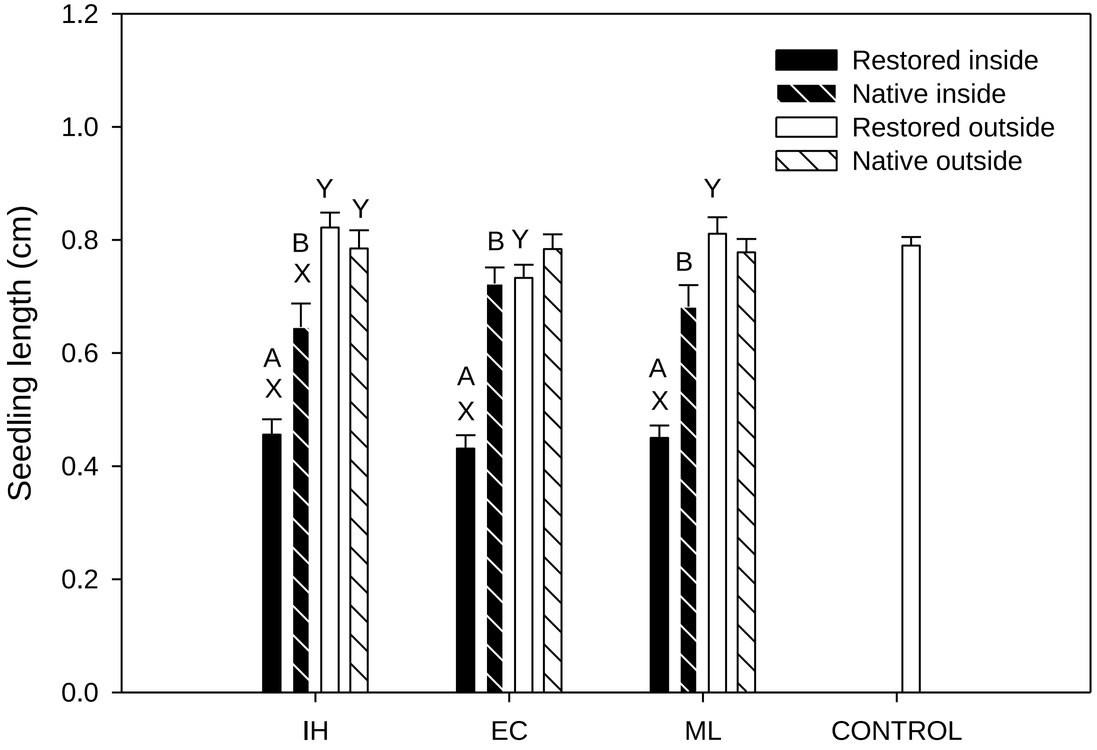

Seedling length (cm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
IH
EC
ML
CONTROL
Restored inside
Native inside
Restored outside
Native outside
A
X
B
X
Y
Y
A
X
B
Y
A
X
B
Y

Figure 3. Germination rate of tall grass species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of germination treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of germination treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of germination treated by soil extracts from restored prairie sites, hatched bars represent the means of germination treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

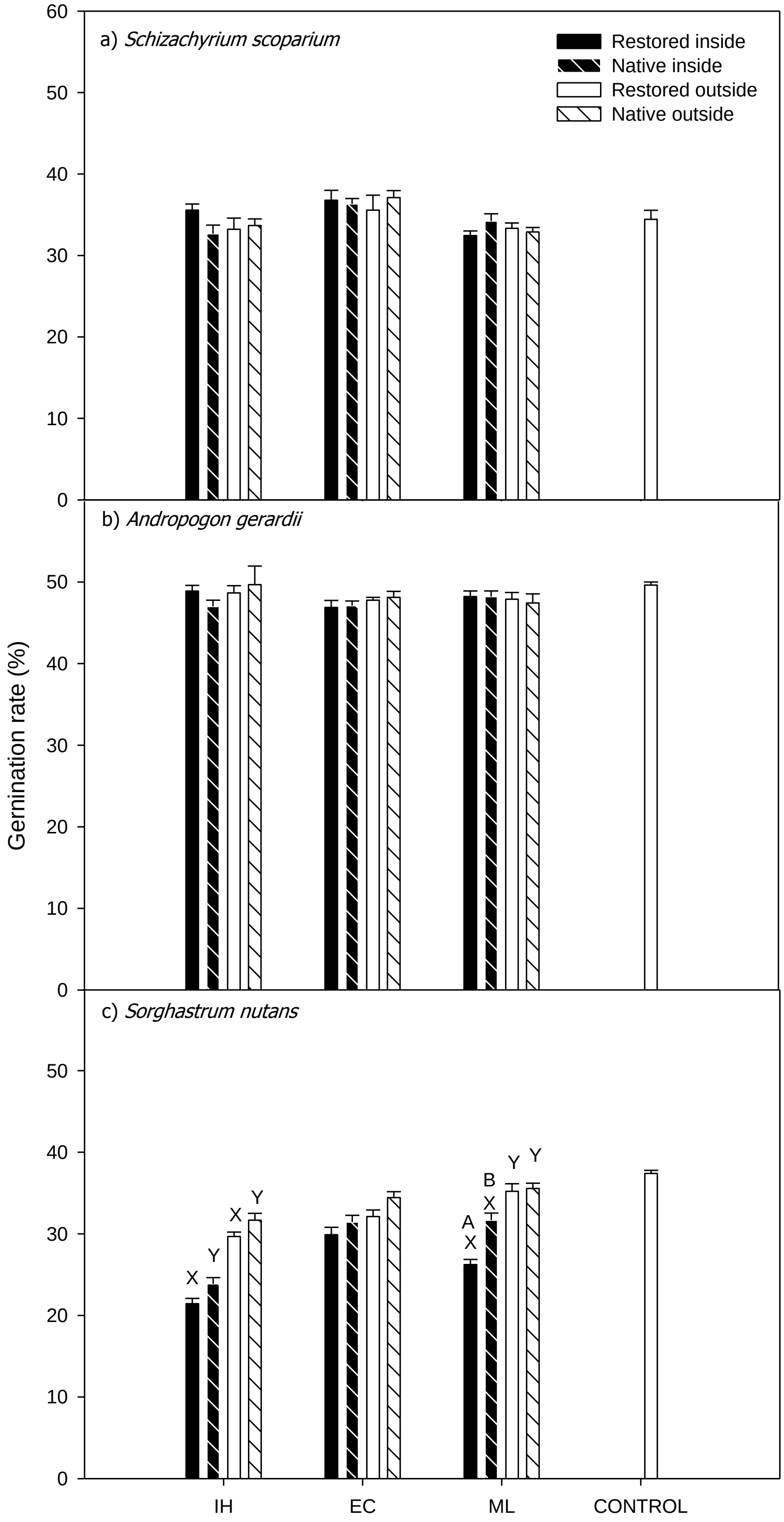

a) Schizachyrium scoparium
b) Andropogon gerardii
c) Sorghastrum nutans
Restored inside
Native inside
Restored outside
Native outside
Gernination rate (%)
0
10
20
30
40
50
60
IH
EC
ML
CONTROL
X
Y
X
Y
A
X
B
X
Y
Y

Figure 4. Seedling length of tall grass species treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of seedling length treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of seedling length treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of seedling length treated by soil extracts from restored prairie sites, hatched bars represent the means of seedling length treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

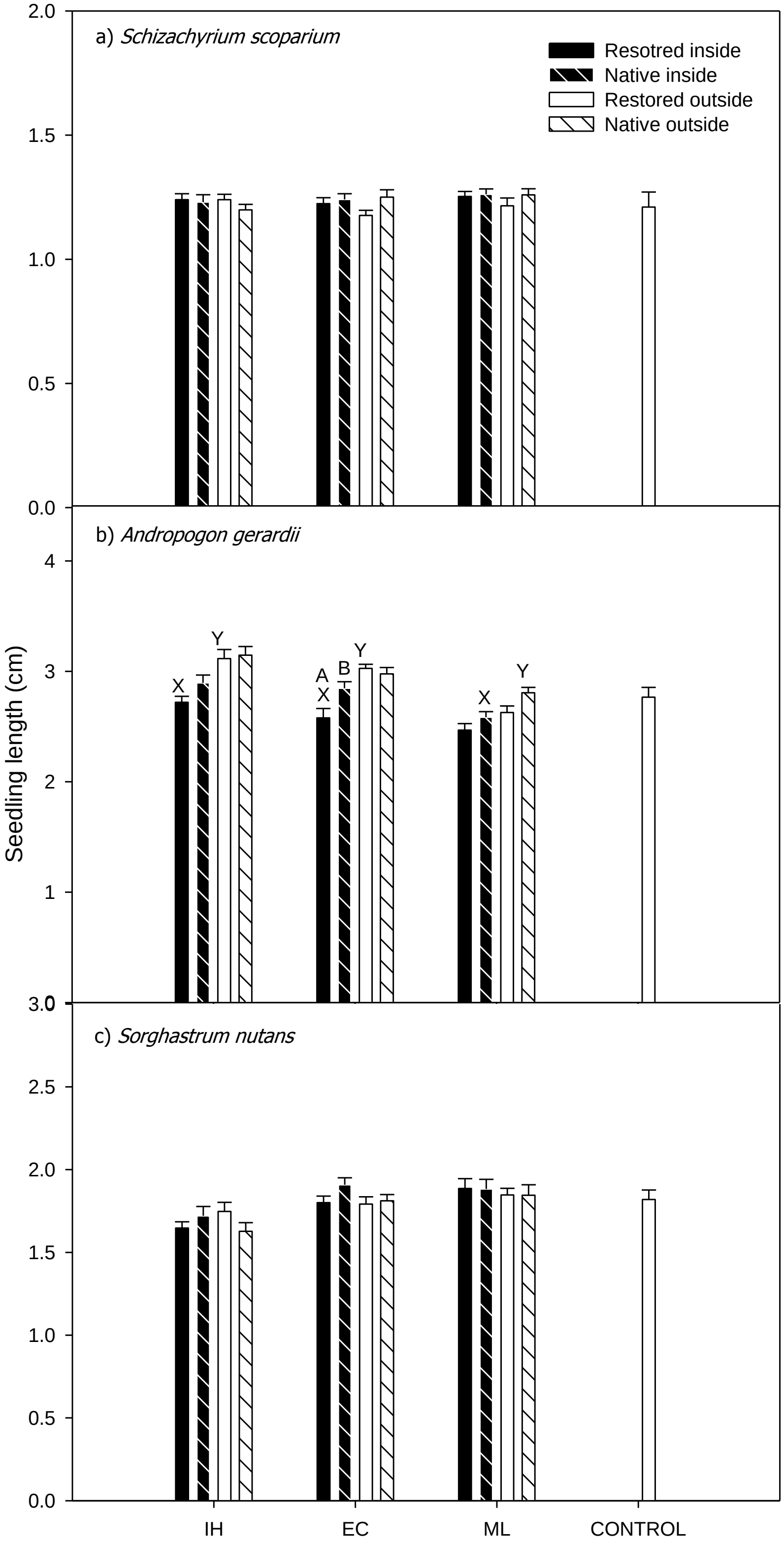

a) *Schizachyrium scoparium*
Resotred inside
Native inside
Restored outside
Native outside
b) *Andropogon gerardii*
c) *Sorghastrum nutans*
Seedling length (cm)
2.0
1.5
1.0
0.5
0.0
4
3
2
1
0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
X
Y
A
X
B
Y
X
Y
IH
EC
ML
CONTROL

Figure 5. Germination rate of congeneric legume treated with soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of germination treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of germination treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of germination treated by soil extracts from restored prairie sites, hatched bars represent the means of germination treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference ($p<0.05$) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference ($p<0.05$) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

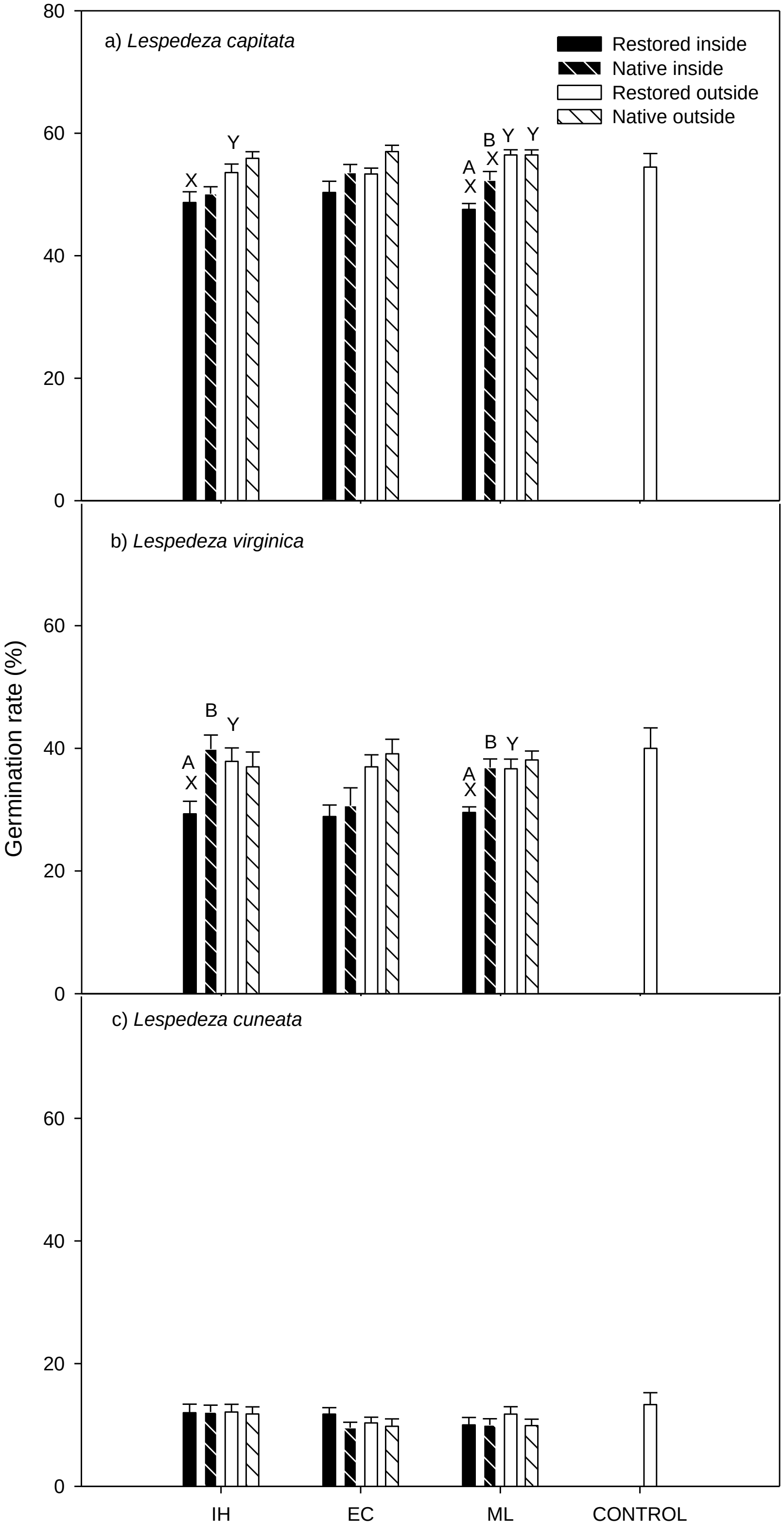

a) *Lespedeza capitata*
b) *Lespedeza virginica*
c) *Lespedeza cuneata*
Restored inside
Native inside
Restored outside
Native outside
Germination rate (%)
0
20
40
60
80
X
Y
A
X
B
X
Y
Y
B
Y
A
X
B
Y
A
X
IH
EC
ML
CONTROL

Figure 6. Seedling length of congeneric legume treated by soil extracts from Lyon County, Kansas. Soil extracts were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil extracts were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie sites**.** For all panels, bars are means ± SE. Gray bars represent the means of seedling length treated by soil extracts from inside *L. cuneata* rhizosphere, and white bars represent the means of seedling length treated by soil extract from outside *L. cuneata* rhizosphere. Solid bars represent the means of seedling length treated by soil extracts from restored prairie sites, hatched bars represent the means of seedling length treated by soil extracts from native prairie sites. Control represents seed treated with distilled water. Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity to *L. cuneata* level is indicated by A and B.

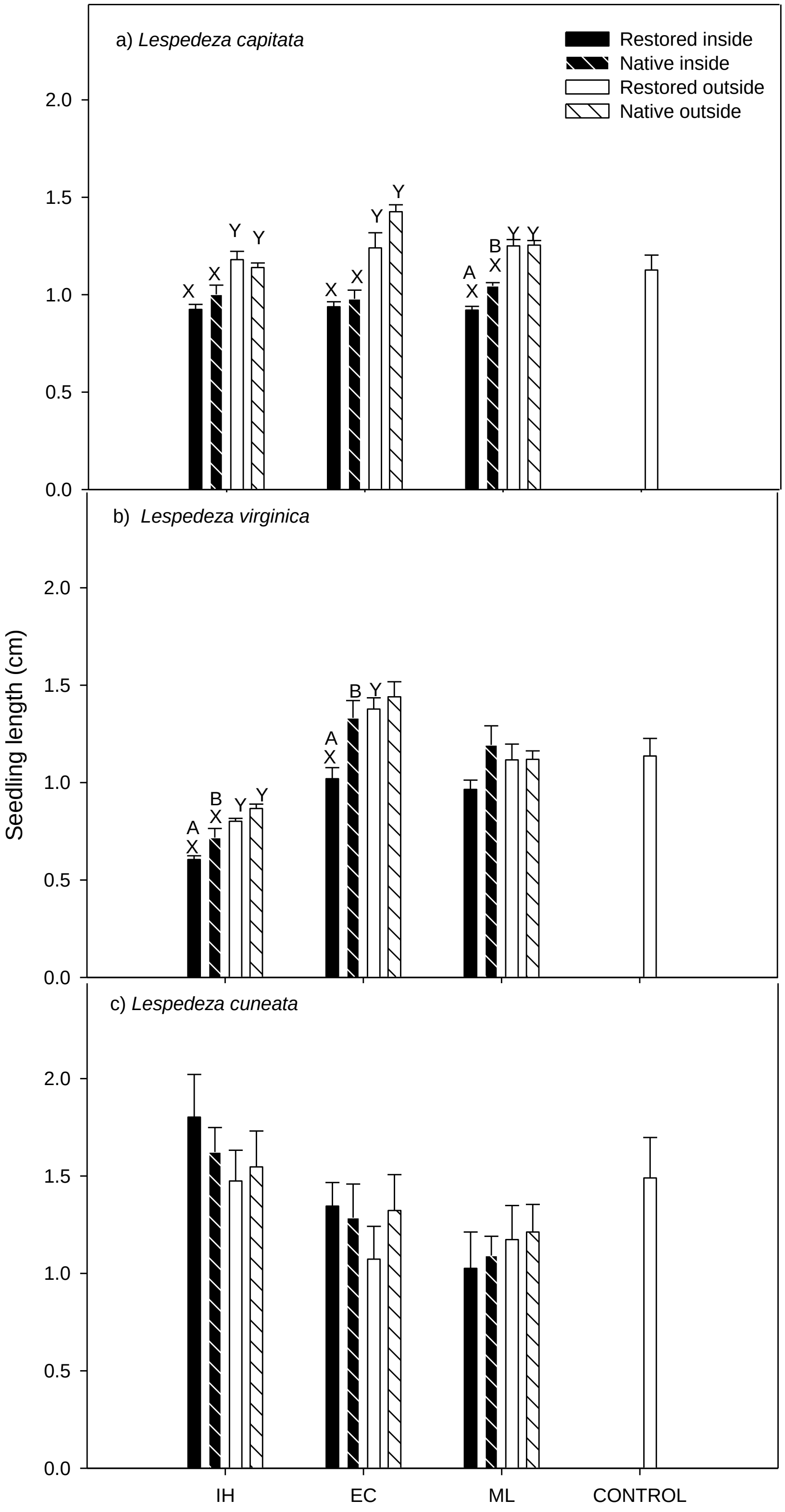

a) *Lespedeza capitata*
Restored inside
Native inside
Restored outside
Native outside
b) *Lespedeza virginica*
c) *Lespedeza cuneata*
Seedling length (cm)
0.0
0.5
1.0
1.5
2.0
X
Y
A
B
IH
EC
ML
CONTROL

Figure 7. Total N content of soil from Lyon County, Kansas. Soil were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie site**.** Significant difference ($p<0.05$) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference ($p<0.05$) between restored and native prairie site at the same proximity level is indicated by A and B.

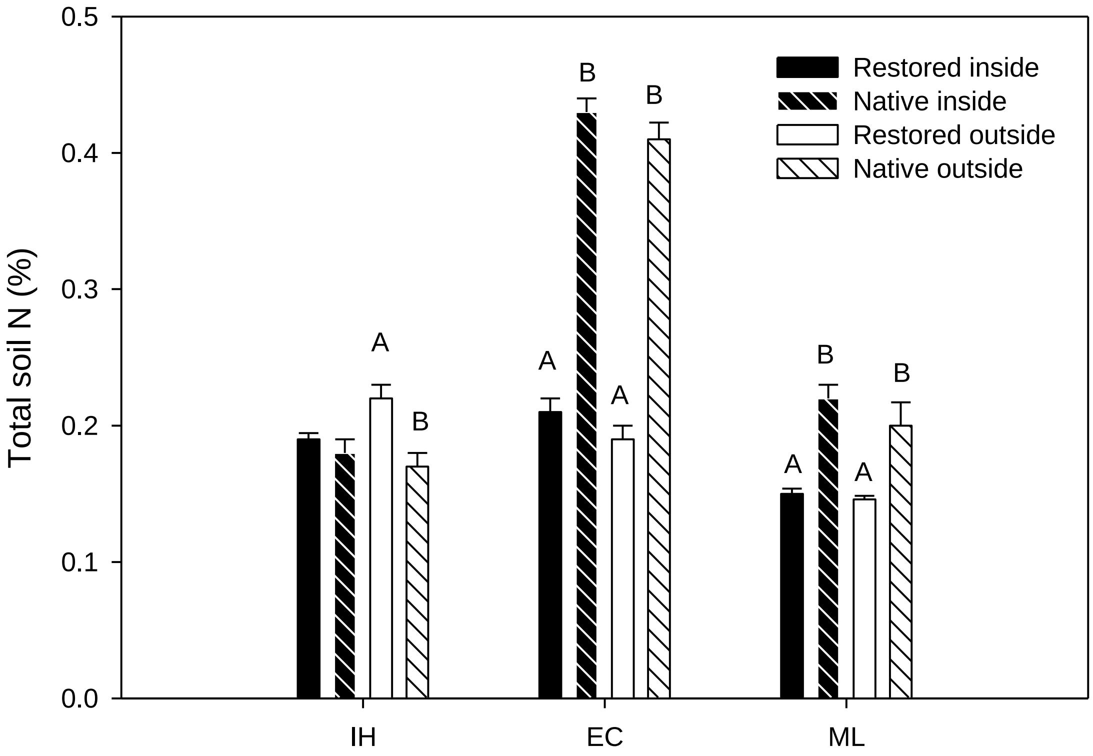
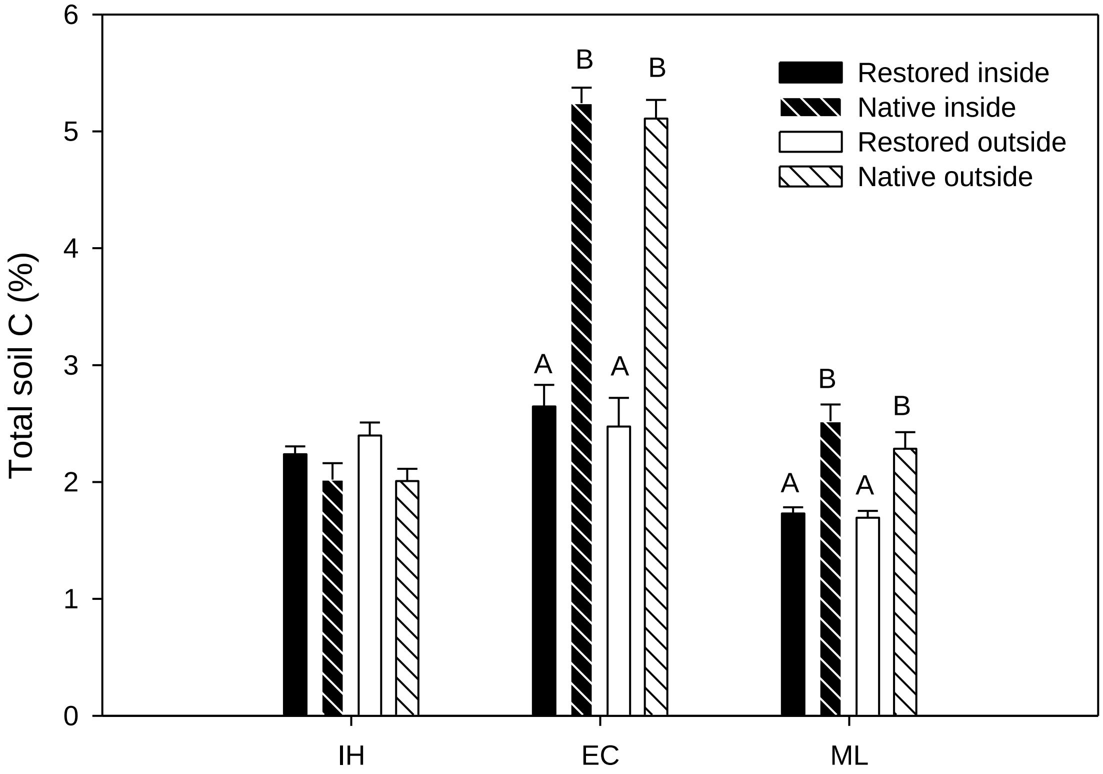

Total soil N (%)
0.0
0.1
0.2
0.3
0.4
0.5
IH
EC
ML
Restored inside
Native inside
Restored outside
Native outside
A
B
A
B
A
B
B
A
B
B
A
A

Figure 8. Total C content of soil from Lyon County, Kansas. Soil were collected from Indian Hills (IH), Eagle Creek (EC) and Melvern Lake (ML). In each location, soil were collected from inside *Lespedeza cuneata* rhizosphere or outside *L. cuneata* rhizosphere at restored and native prairie site**.** Significant difference (p<0.05) between inside and outside *L. cuneata* rhizosphere within a same prairie type is indicated by X and Y. Significant difference (p<0.05) between restored and native prairie site at the same proximity level is indicated by A and B.


Total soil C (%)
0
1
2
3
4
5
6
IH
EC
ML
A
B
A
B
A
B
A
B
Restored inside
Native inside
Restored outside
Native outside