ASSESSING PLANT-SOIL FEEDBACKS FOLLOWING

BIOLOGICAL INVASION

By

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Abstract: Alteration in ecosystem processes following biological invasion is likely to occur if the invasive plant species exhibit different physical and physiological traits than those of the native species. The alteration of soil chemical properties has enormous potential for the modification of other plant species and microbial communities. Therefore, understanding plant-soil feedbacks by biological invasion may be a critical aspect of the restoration of native ecosystems. Two non-native plant species, *Tamarix* sp. (saltcedar) and *Lespedeza cuneata* (sericea lespedeza) were selected for the study. I conducted separate field and greenhouse studies to assess potential plant-soil feedbacks for each species. In field study, soil samples were collected 1) beneath non-native plants in highly invaded areas; 2) from areas where non-native plants have been removed (restoration areas); and 3) from adjacent native prairie. Soil was processed for abiotic (pH, N, P, K) and biotic (microbial communities) properties. My greenhouse study assessed plant-soil feedbacks indirectly through biomass production of different native and non-native plant species grown in soil collected from the same three sites as field study. Plants were grown for 16 weeks, at which time biomass production was determined. Percent root colonization by arbuscular mycorrhizal (AM) fungi was determined microscopically. Greater soil salinity, pH, nitrate-nitrogen, potassium, and phosphorus and greater soil microbial communities from *Tamarix* invaded sites were observed relative to native prairie sites. Greater nitrate-nitrogen and phosphorus were observed in soil from *L. cuneata* invaded sites compared to soil from native areas. The legacy of invasion persisted five years and a year after removal of *Tamarix* and *L. cuneata* respectively with similar trend in soil abiotic and biotic properties as in invaded sites. Both native and non-native plant species produced greater biomass in soils collected with a history of biological invasion, as compared to production in soil from native sites. Different plant species showed different percentage of AM fungal root colonization when grown in soil with a history of biological invasion compared to soil from native areas.

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CHAPTER I

ASSESSING THE SPATIAL VARIABILITY OF SOIL ABIOTIC AND BIOTIC PROPERTIES FOLLOWING SALTCEDAR INVASION

ABSTRACT

Saltcedar (*Tamarix* sp.), a non-native facultative phreatophyte that increases soil salinity has invaded riparian zones of the western United States replacing dominant native tree species, particularly cottonwood (*Populus* sp.) and willow (*Salix* sp.). Saltcedar have been known to increase soil nutrients beneath the canopy. However, research focused on understanding the alteration of soil microbial communities following saltcedar invasion is scarce. Alteration in soil abiotic and biotic properties may lead to modification of native plant community composition. Effects of invasive species on ecosystem processes may be dependent on the spatial distribution of the invader. Therefore, a better understanding of environmental variables will benefit from spatial studies that take into account the footprint of individual invasive plants for successful site-specific restoration efforts. I conducted a field study to determine the spatial variability and footprint of saltcedar individuals on soil abiotic and biotic properties. I assessed soil salinity, soil nutrients, pH, soil microbial abundance, and the herbaceous plant community. To determine the footprint of saltcedar, I randomly selected twelve individual trees within the size class of

approximately 3 m diameter canopy. For each tree, six consecutive 1 m^2 plots were established along a 6 m transect, starting at the base of the tree and expanding outward through dripline of saltcedar to outside the saltcedar canopy. I hypothesized that soil salinity and nutrients would be greater beneath the saltcedar canopy compared to areas outside the canopy. As a consequence, soil microbial biomass would be lowered. Due to the fertilizing effect of saltcedar, I hypothesized greater plant species richness would be present beneath the saltcedar canopy relative to areas outside the canopy. My results showed greater soil salinity beneath the saltcedar canopy. In fact, salinity was <4mmhos cm^{-1}, lower than values reported in previous studies. Contrary to my hypotheses, all other measured variables were similar either beneath or outside the saltcedar canopy. This study indicates the footprint of saltcedar at an individual scale has minimal effects on soil abiotic and biotic properties.

INTRODUCTION

Several mechanisms have been identified by which non-native plants alter the physical, chemical, and biological properties of soils (Vitousek et al. 1987, Hobbie 1992, Daehler and Strong 1996, DiTomaso 1998, Kourtev et al. 2002, Ehrenfeld 2003). Non-native plant species can alter soil abiotic properties of the invaded environment by releasing compounds that alter the soil's suitability for other species of plants (Dukes and Mooney 2004), resulting in shifts in the plant community composition. For example, invasion by the non-native, nitrogen-fixing *Myrica faya* in Hawaii increases soil nitrogen promoting the growth of other introduced plants (Vitousek et al. 1987, Vitousek and Walker 1989, Adler et al. 1998). The exotic *Mesembryanthemum crystallinum* (ice plant) in California accumulates salt from throughout the rooting zone and reduces soil fertility (Vivrette and Muller 1977). A change in soil structure following invasion by *Casuarina equisetifolia* (Australian pine) has resulted in forests on some of Florida's formerly treeless coastlines with increased erosion rates resulting from exclusion of native soil stabilizers such as *Uniola paniculata* (sea oats), *Scaevola plumier* (inkberry), and *Coccoloba uvifera* (seagrape) (Schmitz et al. 1997, Schmid et al. 2008).

Despite the ubiquity of plant-mediated changes in soil physical and chemical properties, there has been little research documenting effects on soil biological properties following invasions (Vitousek et al. 1987, Kourtev et al 2002, Wolfe and Klironomos 2005, Hawkes et al. 2006). Invasion by non-native plant species could initiate a process of

changing the structure and function of the soil biota (Pinton et al. 2001, Kourtev et al. 2002). Soil harbors a wide variety of micro- and macro-organisms such as mycorrhizae, nitrogen-fixing bacteria, pathogens, and nematodes. Invasive plants can alter the soil microbial communities through release of root exudates or anti-microbial compounds, facilitation of symbiotic relationships between roots and soil microbes, or displacement of native plants having unique soil microbial communities (Wolfe and Klironomos 2005).

Biological invasion can lead to potentially new, species-specific effects on ecosystem processes (Vitousek 1990, Levine et al. 2003, Dukes and Mooney 2004). As a consequence, effects of invasive species on ecosystem processes may be dependent on the spatial distribution of the invader. Therefore, a better understanding of environmental variables will benefit from spatial studies that take into account the footprint of individual plant species. The alteration of soil chemical properties has enormous potential for the modification of other plant species and microbial communities. Spatial heterogeneity of soil resources is an important feature of all plant communities, and the scale at which this heterogeneity is expressed can have important consequences for both plant community structure and ecosystem-level processes (Robertson et al. 1993, Robertson and Gross 1994). Studies on shrubs have tended to compare soil nutrients under plant canopies with those in interspaces (Charley and West 1975, Burke et al. 1989). Differences in soil chemistry beneath and between shrubs in dry regions are well documented (Charley and West 1975, Charley and West 1977, Burke et al. 1989, Schlesinger et al. 1990, Hook et al. 1991), however, descriptions of small-scale heterogeneity of soil associated with plant invasions are lacking, yet equally important to analyze the influence of plant cover on soil abiotic and biotic properties. Soil chemistry can vary spatially around individual

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plants (Jackson and Caldwell 1993a, 1993b) with large variations in pH, calcium, magnesium, potassium, sodium, clay, and organic matter occurring over relatively short distances (Raupach 1951). For example, in upland areas of North Wales, significant variations in soil calcium, phosphate, and potassium was found within 60 cm of the perennial *Trifolium repens* plants (Snaydon 1962). Similarly, variability in ammonium, nitrate, phosphate, and potassium within a meter of perennial plants, *Artemisia tridentata* and *Pseudoroegneria spicata* were recorded (Jackson and Caldwell 1993a). Study in New South Wales, Australia by Downes and Beckwith (1951) showed that within a distance of 0.3 m, differences as great as 1 pH unit could occur and that difference determined the distribution of plant species with *Stuartina* sp. and *Crassula* sp. establishing in soils of pH 5.5 and *Hordeum leporinum* establishing in soils of pH 6. In addition, studies suggested that microbial biomass may vary at small (< 1 m) or large (> km) scales (Smith et al. 1994, Robertson et al. 1997).

Alteration in ecosystem processes following biological invasion is likely to occur if the invasive plant species exhibit different physical and physiological traits than those of the native species. Saltcedar (*Tamarix* sp.) is a non-native facultative phreatophyte with an ability to exploit deep water tables and access water from either groundwater or vadose zone water (Brotherson and Field 1987, Busch and Smith 1995, Nippert et al. 2010). Saltcedar has invaded riparian zones of the western United States and northwestern Mexico replacing dominant native tree species, particularly *Populus* sp. (cottonwood) and *Salix* sp. (willow) (Frasier and Johnsen 1991, Glenn and Nagler 2005). Saltcedar, native to Eurasia (southern Europe, northern Africa, and eastern Asia) (Frasier and Johnsen 1991) was introduced to the United States during the 1800s for the stabilization of stream

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banks, to provide windbreaks, and as shade landscaping (Neill 1985). In the United States, over 6 million ha of riparian floodplains and wetlands are currently invaded by saltcedar (Stenquist 2000, Zavaleta 2000, Gaskin and Schaal 2002, Shafroth and Briggs 2008). Saltcedar alters a wide range of characteristics within the invaded habitat such as native vegetation, wildlife habitat, flooding and erosion patterns, and fire frequency (DiTomaso 1998). Common control methods (mechanical, chemical, and biological) to reduce populations of saltcedar have been used to meet a wide range of goals, such as restoring native species in riparian communities, protecting habitat for endangered species, or improving stream water efficiency (Shafroth et al. 2005).

Removal of invasive plants is the objective of restoration efforts. However, removal alone does not restore native ecosystem properties (Harms and Hiebert 2006). Thorough site evaluations are necessary for the appropriate and cost-effective restoration program (Shafroth et al. 2005). Restoration can be challenging due to site differences (Sudbrock 1993, Shafroth et al. 2008). Therefore, understanding site-specific and spatial scale effect on abiotic and biotic variables is important for effective restoration efforts (Shafroth et al. 2008).

Saltcedar accumulates salts from the soil and exudes them through glands on both the adaxial and abaxial surfaces of leaves as well as on young stem surfaces (Decker 1961, Wilkinson 1966, Thomson et al. 1969, Berry 1970, Neill 1985, Sookbirsingh et al. 2010). Previous studies show that salt exudates from saltcedar cause salinization of the soil beneath their canopy relative to areas outside their canopy (Brotherson and Field 1987, Lesica and DeLuca 2004, Ladenburger et al. 2006, Yin et al. 2010). Lesica and DeLuca (2004) compared soil salinity beneath saltcedar to that of native vegetation. Paired

samples were taken within 30 m of each other. They found more than two times the

salinity under saltcedar canopy relative to native vegetation. Ladenburger et al. (2006)

compared soil salinity under the saltcedar canopy, interspaces of saltcedar, and under the

canopy of native species. In addition, previous studies showed greater inorganic nitrogen

and phosphate under saltcedar canopy compared to outside canopy thus showing

fertilizing effect of saltcedar (Lesica and DeLuca 2004, Ladenburger et al. 2006, Yin et

al. 2010). However, characterizing the footprint of saltcedar at an individual plant scale

on soil abiotic and biotic properties is needed to better inform site-specific restoration

efforts.

The objectives of the study were to determine the spatial variability and footprint of

saltcedar individuals on soil abiotic and biotic properties. Specifically, I quantified 1) soil

salinity measured in terms of electrical conductivity (hereafter referred to as EC), 2) soil

nutrients, 3) soil pH, 4) soil microbial biomass, and 5) herbaceous plant communities

from 6 plots established beneath the saltcedar canopy and extended outside the canopy.

Based on previous studies that have reported saltcedar alters soil chemistry, I

hypothesized that soil under saltcedar canopy would have greater soil EC and soil

nutrients as compared to soils outside the canopy. In addition, I hypothesized that

herbaceous plant species richness under the saltcedar canopy would be greater compared

to areas outside the canopy due to the fertilizing effect of saltcedar. I hypothesized that

biomass of different major functional groups of soil microbial communities would be

reduced under the saltcedar canopy due to salt excretions and nutrient accumulation

compared to areas outside the canopy.

MATERIALS AND METHODS

The study site was located adjacent to the Cimarron River, 17 km south of Ashland, Kansas, USA (37°11'19"N, 99°45'55"W). Saltcedar first invaded the site after a flood in 1939 (Nippert et al. 2010). The site is situated over a shallow unconfined aquifer that is connected to the river (Nippert et al. 2010). The soil is coarse-textured. Common herbaceous species at the site are *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalograss), *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Sorghastrum nutans* (L.) Nash (indiangrass), *Sporobolus asper* (Michx.) Kunth (tall dropseed), and *Sporobolus cryptandrus* (Torr.) A. Gray (sand dropseed). Nomenclature of all species was based on USDA Plant Database (2012).

I randomly selected twelve individual saltcedar trees that were approximately the same size (~3 m diameter canopy) and were isolated from the canopy of surrounding trees to avoid any influence from neighboring trees. For each individual tree, six contiguous 1 m^2 plots were placed along a 6 m long transect at every meter, from the base of the tree and extending outward through the dripline of the saltcedar into the area outside saltcedar canopy. To prevent potential interference from adjacent trees, transects were extended from the base of each tree in only one direction. Plant species richness was assessed in each plot and canopy cover of all plant species was determined using the modified Daubenmire 7 cover classes: 1 = < 1% cover, 2 = 1%-5%, 3 = 6%-25%, 4 = 26%-50%, 5

= 51%-75%, 6 = 76%-95%, 7 = 96%-100%. Midpoints of cover classes were used to calculate Shannon species diversity (Magurran 1988, Hickman and Derner 2007). Plants were identified to species (if possible) or genus. All plant species were kept as voucher specimens at the Oklahoma State University, Department of Natural Resource Ecology and Management, Stillwater, Oklahoma.

Soil samples were collected from 10 sampling points at 0-5 cm depth using a 15.70 cm^3 soil corer within the same plots as used for the vegetation study and were composited into one sample from each plot. A 50 g subsample of soil from each plot was analyzed separately for the determination of soil pH, salinity (in terms of electrical conductivity), nutrients (nitrate-nitrogen, ammonium-nitrogen, plant-available phosphorus, and potassium), and soil microbial biomass of major functional groups (total gram positive bacteria, total gram negative bacteria, arbuscular mycorrhizal (AM) fungi, and saprophytic fungi). Soils were dried at room temperature and sieved through a 2 mm sieve before testing pH, EC, and nutrients.

Soil EC and pH were determined through 1:1 soil to water extraction method (Rhoades 1982) using an Accumet AB 30 conductivity meter and Titralab 865, respectively. For nitrate-nitrogen and ammonium-nitrogen, soil samples were extracted with 1M KCl solution and analyzed using a LACHAT Quickchem 8000 Flow Injection Autoanalyzer (LACHAT 2000, Zhang and Kress 2001). Plant-available phosphorus and potassium were extracted with Mehlich III solution and analyzed using inductively coupled plasma emission spectroscopy (ICP) (Zhang and Kress 2001).

Based on results of soil EC, soil pH, and nutrient data that showed no significant differences among plots outside the canopy, soil microbial community composition was determined only for plots located at 1 m, 2 m, and 6 m along each transect. Soil microbial biomass was determined through phospholipid fatty acid analysis (PLFA) (Kourtev et al. 2002, Batten et al. 2006, White and Rice 2009). Certain groups of microorganisms with different signature fatty acids are used to differentiate taxa or estimate bacterial or fungal biomass (Zelles 1999). Unknown fatty acids can be useful in estimating total soil microbial biomass. Microbial lipids were extracted from 5 g freeze-dried soil with a solvent system of methanol, chloroform, and a phosphate buffer. The soil-solvent mixture was separated by centrifugation and the supernatant was decanted. The centrifugation was repeated with the addition of 1:2 (v/v) chloroform-methanol and the supernatant was collected. Phosphate buffer was then added and the mixture separated overnight. The chloroform layer containing the lipids after phase separation was recovered and reduced by nitrogen flow at 60°C. Total extracted lipids were separated into neutral, glyco-, and polar lipids with chloroform, acetone, and methanol through silic acid chromatography. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector.

Fatty acid nomenclature used was that described by Frostegård et al. (1993): total number of carbon atoms: number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. *Cis* and *trans* isomers were indicated by c, and t, respectively. Anteiso- and isobranching were designated by the prefix a or i. Cy indicated cyclopropane fatty acids. The fatty acids i15:0, a15:0, i16:0, i17:0 were chosen to represent gram positive bacteria; 3-OH 14:0, 16:1ω7, cy17:0, 2-OH 16:0, 18:1ω9c,

cy19:0 for gram negative bacteria; 16:1ω5c for AM fungi, 18:2ω9,12c, 18:1ω9c for saprophytic fungi; 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 for non-specific microbes (McKinley et al. 2005). The abundance of individual fatty acid was expressed as nmol g^{-1} dry soil.

Statistical analysis Soil characteristics (EC, pH, ammonium-nitrogen, nitrate-nitrogen, phosphorus, potassium, and biomass of major functional groups within soil microbial communities) and plant species richness and species diversity were analyzed with one-way ANOVA using General Linear Models (GLM) between each 1 m^2 plot. Mean differences of soil characteristics were compared using least square differences (LSD) grouping. Mean soil characteristic values were presented for each plot. All data were analyzed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). A significance level of 0.05 was used for all statistical tests.

RESULTS

Vegetation study The total herbaceous plant species richness and species diversity were similar in all plots either under the saltcedar canopy or outside the canopy with no statistical difference between plots (Fig. 1-1 and Fig. 1-2). The mean plant species richness and species diversity in all plots were approximately 6 and 1.2 per m^2, respectively. Similar plant species were found in all the plots (Table 1-1).

Soil properties Greater soil EC (approximately 590 µmhos per cm = 0.590 mmhos cm^{-1}) was observed in the plot located under the saltcedar canopy compared to plots outside the canopy (Fig. 1-3). Soil pH was at around the neutral range (i.e., 7) in all plots with no statistical differences between plots (Fig. 1-4). ANOVA results showed that all measured soil nutrients (nitrate-nitrogen, ammonium-nitrogen, plant-available phosphorus, and potassium) were similar in all plots located either under the saltcedar canopy or outside the canopy (Figs. 1-5, 1-6, 1-7, 1-8). The results showed no alteration in biomass of any major functional group of the soil microbial communities (total gram positive bacteria, total gram negative bacteria, AM fungi, and saprophytic fungi) in plots under or outside the saltcedar canopy (Fig. 1-9).

DISCUSSION

The footprint of saltcedar at the individual tree scale was not evident in soil chemical or biological properties with the exception of soil salinity. In the study area, no differences in any soil chemical or biological properties could be due to the density of saltcedar trees. EC was higher only directly beneath the saltcedar canopy, as compared to all plots beyond the canopy.

Similar to previous studies (Lesica and DeLuca 2004, Ladenburger et al. 2006), the footprints of individual saltcedar on EC is greater beneath the canopy compared to areas beyond the canopy. However, the soil salinity level in this study was lower than those documented in other riparian sites (e.g., 12.8 mmhos cm^{-1} along the Colorado River) (Busch and Smith 1995). Therefore, results from my study indicate that saltcedar trees of approximately the same size (~3 m diameter canopy) increase soil salinity directly beneath the canopy, but do not alter soil salinity beyond the canopy. Importantly, soil salinity was not increased to a level (> 4 mmhos cm^{-1}) that negatively affected plant species richness and diversity (US Salinity Laboratory Staff 1954, Lesica and DeLuca 2004). The lower salt accumulation in this study (< 4 mmhos cm^{-1}) beneath saltcedar probably reflects the relatively coarse texture of the soils allowing less accumulation of salts. Soil EC is negatively correlated with percent sand and positively correlated with percent clay and silt (Shafroth et al. 1998, Glenn et al. 2012).

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Texture is strongly correlated with soil's ability to adsorb or desorb chemical ions (exchange capacity) (Miller and Donahue 1995). Coarse-textured soils with their substantially larger particle size have less total surface area, and therefore fewer exchange sites for excess sodium binding (Miller and Donahue 1995, Sumner et al. 1998). My results indicated similar soil pH and nutrients among plots beneath and beyond the saltcedar canopy. Thus, my results contradict previous studies with higher inorganic nitrogen and phosphorus and lower soil pH beneath saltcedar canopy as compared to areas beyond the canopy (Lesica and DeLuca 2004, Ladenburger et al. 2006, Yin et al. 2010). Soil texture is among the most important physical properties that influence many biogeochemical processes due to the ability of soils to retain water and nutrients (Jenny 1980, Schoenholtz et al. 2000). Fine-textured soils are positively associated water and nutrients holding capacities relative to coarse-textured soils (Silver et al. 2000, Sher and Marshall 2003, Brady and Weil 2008). In addition, coarse-textured soils have lower ability to hold water and nutrients due to large pore spaces between particles and low surface area relative to fine-textured soils (Brady and Weil 2008). Thus, the similarity in soil pH and nutrients among all plots might be due to similar soil texture (i.e., coarse-textured) in all plots.

Excessive soil salinity is common in saltcedar invaded areas and might only be suitable for the growth of salt-tolerant plant species (Brotherson and Field 1987). However, in my study site, native plant species reestablishment may be less problematic after saltcedar control and removal due to lower soil EC (< 4 mmhos cm^{-1}) compared to other sites in southwestern United States (12.8 mmhos cm^{-1} along the Colorado River) (Busch and Smith 1995). Lesica and DeLuca (2004) found that soils beneath the canopy of saltcedar

with EC <4 mmhos cm^{-1} increased the growth of native grass *Agropyron smithii*. Therefore, lower soil EC among all the plots under or outside the saltcedar canopy could be a possible reason for the similar plant species richness and species diversity in my study. My vegetation study therefore, agreed with previous studies that soil EC <4 mmhos cm^{-1} was not high enough to prevent vegetation growth.

Restoration of saltcedar invaded areas need to ensure that plants other than saltcedar occupy after control methods have been implemented. Invasion by other non-native plant species following invasion by invasive species has been observed in various ecosystems (Adler et al. 1998, Yelenik et al. 2004). Undesirable plants, such as *Lepidium latifolia* (pepperweed), *Kochia scoparia* (kochia), *Elaeagnus angustifolia* (Russian-olive), or *Centaurea* spp. (knapweed) might recolonize the area after saltcedar eradication (Weeks et al. 1987, Shafroth et al. 2005). Revegetation following saltcedar removal is required for restoration of the invaded area. However, the presence of native plant species in the current study indicated that abiotic and biotic properties are still favorable for their growth, suggesting natural revegetation. My results therefore agree with Bay and Sher (2008) that native plant species may reestablish in saltcedar restoration sites over time without any revegetation efforts. Therefore, it is likely that saltcedar control in the current study area might not require a revegetation effort to encourage the return of native species. Dense canopy is associated with lower understory plant species richness in many riparian areas (Pabst and Spies 1998, Zimmerman et al. 1999). In my study, no reduction in herbaceous understory species was found which could be due to the scattered distribution and the small size class (i.e., ~ 3 m canopy) selected for my study.

Invasive plant species may bring about alterations in composition and function of soil microbial communities (Klironomos 2002, Kourtev et al. 2002, Ehrenfeld 2004, Reinhart and Callaway 2006). Despite the importance of soil microbes for ecosystem processes, very few studies have been published to date on saltcedar and soil microbes (Beauchamp et al. 2005, Meinhardt and Gehring 2012). Soil microbes may vary spatially (Smith et al. 1994, Robertson et al. 1997, Aguilera et al. 1999). For example, Yannarell et al. (2011) provided evidence that invasive *Lespedeza cuneata* alters soil bacterial communities at the scale of sites while the fungal communities are altered at the individual plant scale. In my study, the similar abundance of major functional groups within the soil microbial communities could be due to similar soil pH, nutrients, and vegetation in all plots as physical, chemical, and biological factors all affect soil microbial communities (Grayston et al. 1998, Buyer et al. 1999, Gelsomino et al. 1999, Buyer et al. 2002, Cavigelli et al. 2005). Invasive plant species are known to alter the community composition of arbuscular mycorrhizal (AM) fungi (Mummey and Rillig 2006), reduce the viability and infectivity of AM fungal spores (Roberts and Anderson 2001, Callaway et al. 2008), or reduce the AM fungal root colonization of native plant species (Roberts and Anderson 2001, Stinson et al. 2006). Saltcedar is non-mycotrophic (Beauchamp et al. 2005) thus, dominance by such species can change the composition and function of mycorrhizal communities (Stinson et al. 2006, Wolfe et al. 2008). Saltcedar reduced ectomycorrhizal (EM) and AM fungal colonization of native cottonwoods in the presence of saltcedar in a study conducted in Arizona, US (Meinhardt and Gehring 2012). However, Yang et al. (2008) showed greater AM fungal infectivity in saltcedar in Northwest China. Hence, the alteration in AM fungal communities as mediated by saltcedar has not been found to be

consistent across sites. In my study, no footprint of saltcedar on biomass of major functional groups of soil microbial communities doesn't preclude that saltcedar has no effect on composition or function of soil microbes. Diversity and composition of soil microbial communities in saltcedar invaded areas need to be assessed to detect any alteration.

This study provided key details associated with restoration of saltcedar invaded areas including both the soil abiotic and biotic properties that are central for successful restoration. Soil salinity in this site seems less problematic than other riparian sites in southwestern United States (> 4 mmhos cm^{-1}) for restoration efforts. My results suggested that if saltcedar at the size of about 3 m canopy is removed, the change in EC will not negatively affect the established herbaceous vegetation. These findings implied that the consequences for ecosystem properties of saltcedar invasion will largely depend on the site and abundance of saltcedar. Based on the parameters assessed in this study, I suggested that saltcedar removal in the current study site may restore native riparian properties. It is important to consider saltcedar density, native species presence, and soil chemical and biological properties for the successful restoration of saltcedar invaded areas.

REFERENCES

Adler P.B., C.M. D'Antonio, J.T. Tunison. 1998. Understory succession following a dieback of *Myrica faya* in Hawai'i Volcanoes National Park. Pacific Science 52(1):69-78.

Aguilera L.E., J.R. Gutiérrez, P.L. Meserve. 1999. Variation in soil micro-organisms and nutrients underneath and outside the canopy of *Adesmia bedwellii* (Papilionaceae) shrubs in arid coastal Chile following drought and above average rainfall. Journal of Arid Environments 42:61-70.

Batten K.M., K.M. Scow, K.F. Davies, S.P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8:217-230.

Bay R.F., A.A. Sher. 2008. Success of active revegetation after *Tamarix* removal in riparian ecosystems of the southwestern United States: A quantitative assessment of past restoration projects. Restoration Ecology 16(1):113-128.

Beauchamp V.B., J.C. Stromberg, J.C. Stutz. 2005. Interactions between *Tamarix ramosissima* (saltcedar), *Populus fremontii* (cottonwood), and mycorrhizal fungi: Effects on seedling growth and plant species coexistence. Plant and Soil 275:221-231.

Berry W.L. 1970. Characteristics of salts secreted by *Tamarix aphylla.* American Journal of Botany 57:1226-1230.

Brady N.C., R.R. Weil. 2008. The nature and properties of soils. Upper Saddle River, NJ:Prentice Hall.

Brotherson J.D., D. Field. 1987. Tamarix: Impacts of a successful weed. Rangelands 9(3):110-112.

Burke I.C., W.A. Reiners, D.S. Schimel. 1989. Organic matter turnover in a sagebrush steppe landscape. Biogeochemistry 7(1):11-31.

Busch D.E., S.D. Smith. 1995. Mechanims associated with decline of woody species in riparian ecosystems of the Southwestern US. Ecological Monographs 65(3):347-370.

Buyer J.S., D.P. Roberts, E. Russek-Cohen. 1999. Microbial community structure and function in the spermosphere as affected by soil and seed type. Canadian Journal of Microbiology 45:138-144.

Buyer J.S., D.P. Roberts, E. Russek-Cohen. 2002. Soil and plant effects on microbial community structure. Canadian Journal of Microbiology 48:955-964.

Callaway R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson, J. Klironomos. 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89(4):1043-1055.

Cavigelli M.A., L.L. Lengnick, J.S. Buyer, D. Fravel, Z. Handoo, G. McCarty, P. Millner, L. Sikora, S. Wright, B. Vinyard, M. Rabenhorst. 2005. Landscape level variation in soil resources and microbial properties in a no-till corn field. Applied Soil Ecology 29:99-123.

Charley J.L., N.E. West. 1975. Plant-induced soil chemical patterns in some shrub-dominated semi-desert ecosystem of Utah. Journal of Ecology 63(3):945-963.

Charley J.L., N.E. West. 1977. Micro-patterns of nitrogen mineralization activity in soils of some shrub-dominated semi-desert ecosystem of Utah. Soil Biology and Biochemistry 9:357-365.

Daehler C.C., D.R. Strong. 1996. Status, prediction and prevention of introduced cordgrass *Spartina* spp. Invasions in Pacific Estuaries, USA. Biological Conservation 78:51-58.

Decker J.P. 1961. Salt secretion by *Tamarix pentandra* Pall. Forest Science 7:214-217.

DiTomaso J.M. 1998. Impact, biology, and ecology of saltcedar (*Tamarix* spp.) in the Southwestern United States. Weed Technology 12:326-336.

Downes R.G., R.S. Beckwith. 1951. Studies in the variation of soil reaction. I. Field variations at Barooga, N.S.W. Australian Journal of Agricultural Research 2:60-72.

Dukes J.S., H.A. Mooney. 2004. Disruption of ecosystem processes in western North America by invasive species. Revista Chilena de Historia Natural 77:411-437.

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6(6):503-523.

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18:1232-1235.

Frasier G.W., T.N. Johnsen Jr. 1991. Saltcedar (tamarisk): classification, distribution, ecology, and control. *In* James L.F., J.O. Evans, M.H. Ralphs, R.D. Child (eds.). Noxious Range Weeds. Boulder, CO: Westview Press. pp. 377-386.

Frostegård Å., E. Bååth, A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25(6):723-730.

Gaskin J.F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proceedings of the National Academy of Sciences 99(17):11256-11259.

Gelsomino A., A.C. Keijzer-Wolters, G. Cacco, J. D. van Elsas. 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. Journal of Microbiological Methods 38:1-15.

Glenn E.P., K. Morino, P.L. Nagler, R.S. Murray, S. Pearlstein, K.R. Hultine. 2012. Roles of saltcedar (*Tamarix* spp.) and capillary rise in salinizing a non-flooding terrace on a flow-regulated desert river. Journal of Arid Environments 79:56-65.

Glenn E.P., P.L. Nagler. 2005. Comparative ecophysiology of *Tamarix ramosissima* and native trees in western U.S. riparian zone. Journal of Arid Environments 61:419-446.

Grayston S.J., S. Wang, C.D. Campbell, A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30(3):369-378.

Harms R.S., R.D. Hiebert. 2006. Vegetation response following invasive Tamarisk (*Tamarix* spp.) removal and implications for riparian restoration. Restoration Ecology 14(3):461-472.

Hawkes C.V., J. Belnap, C. D'Antonio, M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369-380.

Hickman K.R., J.D. Derner. 2007. Blackland tallgrass prairie vegetation dynamics following cessation of herbicide application. Rangeland Ecology and Management 60(2):186-190.

Hobbie S.R. 1992. Effects of plant species on nutrient cycling. Tree 7(10):336-339.

Hook P.B., I.C. Burke, W.K. Lauenroth. 1991. Heterogeneity of soil and plant N and C associated with individual plants and openings in North American shortgrass steppe. Plant and Soil 138:247-256.

Jackson R.B., M.M. Caldwell. 1993a. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74(2):612-614.

Jackson R.B., M.M. Caldwell. 1993b. Geostatistical patterns of soil heterogeneity around individual perennial plants. Journal of Ecology 81(4):683-692.

Jenny H. 1980. The soil resource: origin and behavior. New York: Springer-Verlag.

Klironomos J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67-70.

Kourtev P.S., J.G. Ehrenfeld, M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83(11):3152-3166.

Lachat. 2000. QuickChem method 10-107-04-1-A. Lachat Instrument. Milwaukee, WI 53218-1239 USA.

Ladenburger C.G., A.L. Hilda, D.J. Kazmer, L.C. Munn. 2006. Soil salinity patterns in *Tamarix* invasions in the Bighorn Basin, Wyoming, USA. Journal of Arid Environments 65:111-128.

Lesica P., T.H. DeLuca. 2004. Is tamarisk allelopathic? Plant and Soil 267:357-365.

Levine J.M., M. Vilà, C.M. D'Antonio, J.S. Dukes, K. Grigulis, S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London B Biological Sciences 270:775-781.

Magurran A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey.

McKinley V.L., A.D. Peacock, D.C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry 37:1946-1958.

Meinhardt K.A., C.A. Gehring. 2012. Disrupting mycorrhizal mutualisms: a potential mechanism by which exotic tamarisk outcompetes native cottonwoods. Ecological Applications 22(2):532-549.

Miller R.W., R.L. Donahue. 1995. Soils in our environment. Seventh Edition. Prudence Hall, Englewood, Cliffs, NJ. pp.323.

Mummey D.L., M.C. Rillig. 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288:81-90.

Neill W.M. 1985. Tamarisk. Fremontia 12:22-23.

Nippert J.B., J.J. Butler, G.J. Kluitenberg, D.O. Whittemore, D. Arnold, S.E. Spal, J.K. Ward. 2010. Patterns of *Tamarix* water use during a record drought. Oecologia 162:283-292.

Pabst R.J., T.A. Spies. 1998. Distribution of herbs and shrubs in relation to landform and canopy cover in riparian forests of coastal Oregon. Canadian Journal of Botany 76:298-315.

Pinton R., Z. Varanini, P. Nannipieri. 2001. The rhizosphere as a site of biochemical interactions among soil components, plants, and microorganisms. *In* Pinton R., Z. Varanini, P. Nannipieri (eds.). The Rhizosphere. New York: Marcel Dekker. pp. 1-17.

Raupach M. 1951. Studies in the variation of soil reaction. II. Seasonal variations at Barooga, N.S.W. III. Variations at the Waite Agricultural Research Institute. Australian Journal of Agricultural Research 2:73-91.

Reinhart K.O., R.M. Callaway. 2006. Soil biota and invasive plants. New Phytologist 170:445-457.

Rhoades J.D. 1982. Soluble salts. *In* Methods of soil analysis. Part 2. Chemical and microbiological properties. Second Edition. American Society of Agronomy Inc. Soil Science Society of America Inc. Publisher Madison, Wisconsin USA. pp. 167-179.

Roberts K.J., R.C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146(1):146-152.

Robertson G.P., J.R. Crum, B.G. Ellis. 1993. The spatial variability of soil resources following long-term disturbance. Oecologia 96(4):451-456.

Robertson G.P., K.L. Gross. 1994. Assessing the heterogeneity of belowground resources: Quantifying pattern and scale. *In* Caldwell M.M., R.W. Pearcy (eds.). Plant Exploitation of Environmental Heterogeneity. Academic Press, New York, New York, USA. pp. 237-253.

Robertson G.P., K.M. Klingensmith, M.J. Klug, E.A. Paul, J.R. Crum. 1997. Soil resources, microbial activity, and primary production across an agricultural ecosystem. Ecological Applications 7(1):158-170.

Schlesinger W.H., J.F. Reynolds, G.L. Cunningham, L.F. Huenneke, W.M. Jarrell, R.A. Virginia, W.G. Whitford. 1990. Biological feedbacks in global desertification. Science 247(4946):1043-1048.

Schmid J.L., D.S. Addison, M.A. Donnelly, M.A. Shirley, T. Wibbels. 2008. The effect of Australian pine (*Casuarina equisetifolia*) removal on loggerhead sea turtle (*Caretta caretta*) incubation temperatures on Keewaydin Island, Florida. Journal of Coastal Research 55:214-220.

Schmitz D.C., D. Simberloff, R.H. Hoffstetter, W. Haller, D. Sutton. 1997. The ecological impact of nonindiengous plants. *In* Simberloff D., D.C., Schmitz, T.C. Brouwn (eds.), Strangers in Paradise. Island Press. pp. 39-61.

Schoenholtz S.H., H. Van Miegroet, J.A. Burger. 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. Forest Ecology and Management 138:335-356.

Shafroth P.B., G.T. Auble, J.C. Stromberg, D.T. Patten. 1998. Establishment of woody riparian vegetation in relation to annual patterns of streamflow, Bill Williams River, Arizona. Wetlands 18(4):577-590.

Shafroth P.B., J.R. Cleverly, T.L. Dudley, J.P. Taylor, C. Van Riper III, E.P. Weeks, J.N. Stuart. 2005. Control of *Tamarix* in the western United States: Implications for water salvage, wildlife use, and riparian restoration. Environmental Management 35(3):231-246.

Shafroth P.B., M.K. Briggs. 2008. Restoration ecology and invasive riparian plants: An introduction to the special section on *Tamarix* spp. in Western North America. Restoration Ecology 16(1):94-96.

Shafroth P.B., V.B. Beauchamp, M.K. Briggs, K. Lair, M.L. Scott, A.A. Sher. 2008. Planning riparian restoration in the context of *Tamarix* control in western North America. Restoration Ecology 16(1):97-112.

Sher A.A., D.L. Marshall. 2003. Seedling competition between native *Populus deltoids* (Salicaceae) and exotic *Tamarix ramosissima* (Tamaricaceae) across water regimes and substrate types. American Journal of Botany 90(3):413-422.

Silver W.L., J. Neff, M. McGroddy, E. Veldkamp, M. Keller, R. Cosme. 2000. Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. Ecosystems 3(2):193-209.

Smith J.L., J.J. Halvorson, H. Bolton Jr. 1994. Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology and Biochemistry 26(9):1151-1159.

Snaydon R.W. 1962. Micro-distribution of *Trifolium repens* L. and its relation to soil factors. Journal of Ecology 50(1):133-143.

Sookbirsingh R., K. Castillo, T.E. Gill, R.R. Chianelli. 2010. Salt separation processes in the saltcedar *Tamarix ramosissima* (Ledeb.). Communitcations in Soil Science and Plant Analysis 41:1271-1281.

Stenquist S.M. 2000. Saltcedar integrated weed management and the endangered species act. *In* Proceedings of the X International Symposium on Biological Control of Weeds, 4-14 July 1999, Montana State University, Bozeman, Montana, USA. Neal R. Spencer (ed.). pp 487-504.

Stinson K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4(5):0727-0731.

Sudbrock A. 1993. Fighting back: An overview of the invasion, and a low-impact way of fighting it. Restoration and Management Notes 11:31-34.

Sumner M., P. Rengasamy, R. Naidu. 1998. Sodic soils: a reappraisal. *In* M. Sumner, R. Naidu (eds.). Sodic Soil: Distribution, Management and Environmental Consquences. Oxford University Press, New York, NY. pp. 3-17.

Thomson W.W., W.L. Berry, L.L. Liu. 1969. Localization and secretion of salt by the salt glands of *Tamarix aphylla*. Proceedings of the National Academy of Sciences of the United States of America 63:310317.

USDA, NRCS (2012). The PLANTS Database (http://plants.usda.gov, 1 November 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

U.S. Salinity Laboratory Staff. 1954. Determination of the properties of saline and alkali soils. USDA Agricultural Handbook No. 60. U.S. Government Printing Office, Washington, DC. pp.7-33.

Vitousek P.M. 1990. Biological inasions and ecosystem processes: Towards an integration of population biology and ecosystem. Oikos 57(1):7-13.

Vitousek P.M., L.R. Walker, L.D. Whiteaker, D. Mueller-Dombois, P.A. Matson. 1987. Biological invasion by Myrica faya alters ecosystem development in Hawaii. Science 238:802-804.

Vitousek P.M., L.R. Walker. 1989. Biological invasion by *Myrica faya* in Hawai'i: Plant demography, nitrogen fixataion, ecosystem effects. Ecological Monographs 59(3):247-265.

Vivrette N.J., C.H. Muller. 1977. Mechanism of invasion and dominance of coastal grassland by *Mesembryanthemum crystallinum*. Ecological Monographs 47:301-318.

Weeks E.P., H.L. Weaver, G.S. Campbell, B.D. Tanner. 1987. Water use by saltcedar and by replacement vegetation in the Pecos River floodplain between Acme and Artesia, New Mexico. US Geological Survey Professional Paper 491-G.

White P.M, C.W. Rice. 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. Soil Sci. Soc. Am. J. 73(1):138-145.

Wilkinson R.E. 1966. Adventitious shoots on saltcedar roots. Botanical Gazette 127(2-3):103-104.

Wolfe B.E., J.N. Klironomos. 2005. Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6):477-487.

Wolfe B.E., V.L. Rodgers, K.A. Stinson, A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology 96:777-783.

Yang Y., Y. Chen, W. Li. 2008. Arbuscular mycorrhizal fungi infection in desert riparian forest and its environmental implications: A case study in the lower reach of Tarim river. Progress in Natural Science 18:983-991.

Yannarell A.C., R.R. Busby, M.L. Denight, D. Gebhart, S.J. Taylor. 2011. Soil bacteria and fungi respond on different spatial scale to invasion by the legume *Lespedeza cuneata*. Frontiers in Microbiology 2:1-12.

Yelenik S.G., W.D. Stock, D.M. Richardson. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12(1):44-51.

Yin C.H., G. Feng, F. Zhang, C.Y. Tian, C. Tang. 2010. Enrichment of soil fertility and salinity by tamarisk in saline soils on the northern edge of the Taklamakan desert. Agricultural Water Management 97:1978-1986.

Zavaleta E. 2000. The economic value of controlling an invasive shrub. Ambio 29(8):462-467.

Zelles L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. 29:111-129.

Zhang H., M. Kress. 2001. Oklahoma state university soil water and forage analytic laboratory procedures manual. Stillwater, OK.

Zimmerman J.C., L.E. DeWald, P.G. Rowlands. 1999. Vegetation diversity in an interconnected ephemeral riparian system of north-central Arizona, USA. Biological Conservation 90:217-228.

Table 1-1 Plant species in different plots (Q1-Q6) with their origin.

Plant Species	Origin	Q1	Q2	Q3	Q4	Q5	Q6
Eriogonum annuum	Native	X	X	X	X	X	X
Astragalus sp.	Native	-	-	-	-	X	X
Cynodon dactylon	Non-native	X	-	-	X	X	X
Solanum rostratum	Native	X	X	X	X	X	X
Bouteloua dactyloides	Native	X	X	X	X	X	X
Conyza canadensis	Native	X	X	X	X	-	X
Prunus angustifolia	Native	X	X	X	X	X	X
Commelina sp.	Native	X	X	-	-	-	-
Physalis pumila	Native	-	-	X	-	-	-
Aristida purpurea	Native	-	-	-	-	-	X
Calamovilfa gigantea	Native	X	X	X	X	X	X
Sorghastrum nutans	Native	X	X	X	X	X	X
Vernonia sp.	Native	-	X	X	X	X	-
Bromus japonicus	Non-native	X	X	X	X	X	X
Poa pratensis	Non-native	-	-	X	-	X	X
Ambrosia bidentata	Native	-	-	X	-	X	X
Amorpha canescens	Native	X	X	X	X	X	X
Schizachyrium scoparium	Native	X	X	X	X	X	X
Coreopsis tinctoria	Native	-	X	X	X	X	-
Plantago sp.	Native	X	-	X	X	X	X
Distichlis spicata	Native	-	-	-	-	-	X
Sporobolus cryptandrus	Native	X	X	X	X	X	X
Carex sp.	Native	X	X	X	X	-	-

Plant Species	Origin	Q1	Q2	Q3	Q4	Q5	Q6
Bothriochloa laguroides	Non-native	X	-	-	-	-	-
Solanum elaeagnifolium	Native	-	-	-	-	-	X
Strophostyles leiosperma	Native	-	X	X	X	-	X
Bromus inermis	Non-native	X	X	X	X	X	X
Panicum virgatum	Native	X	X	X	X	X	X
Sporobolus asper	Native	X	X	X	X	X	X
Ambrosia psilostachya	Native	X	X	X	X	X	X
Artemisia ludoviciana	Native	X	-	X	X	-	-
Panicum capillare	Native	X	X	X	X	X	X
Unknown legume (UK4-I)		X	X	X	X	X	X
Uknown brome (UK-II)		X	X	X	X	X	X

FIGURES
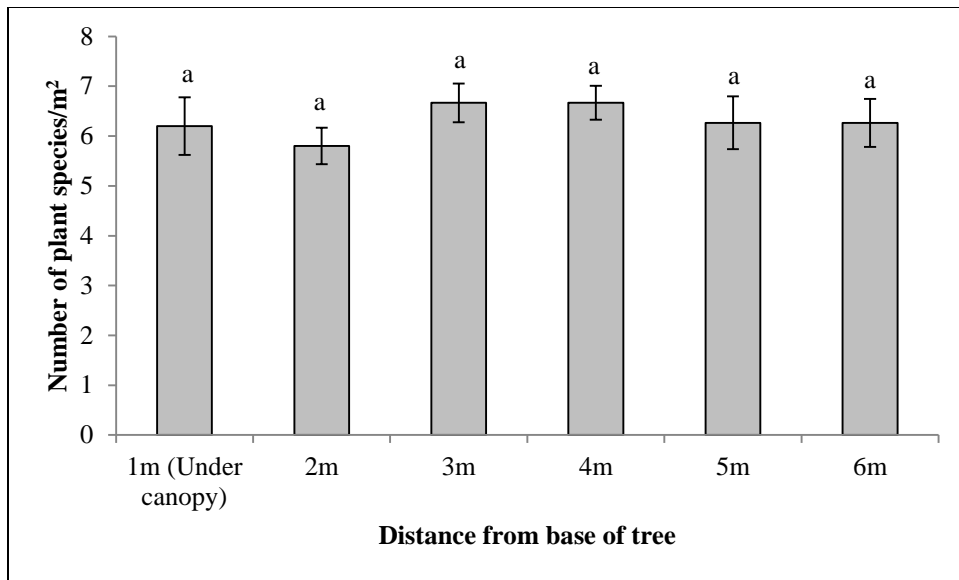
Figure 1-1 Plant species richness (number of species/m^2) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
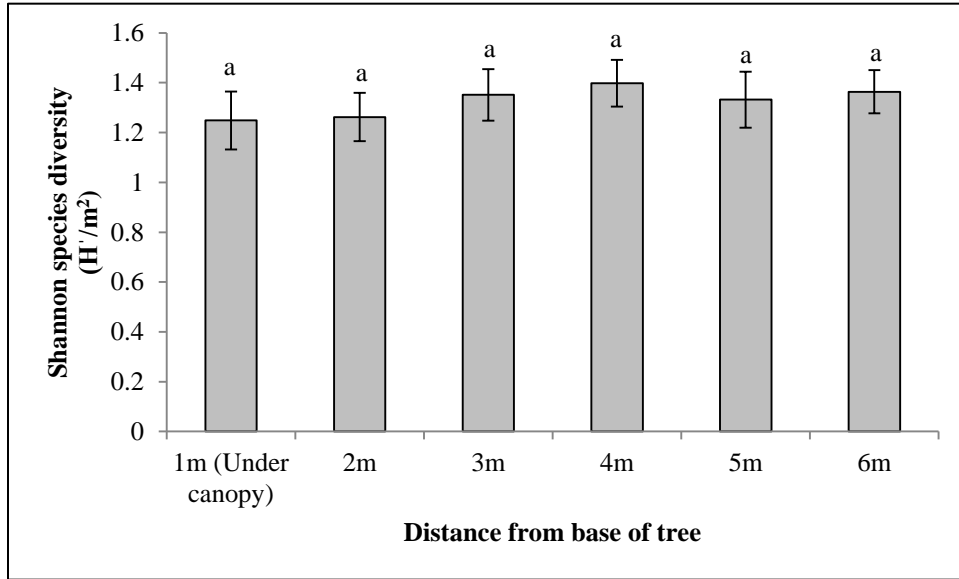
Figure 1-2 Shannon species diversity ($H`/m^2$) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
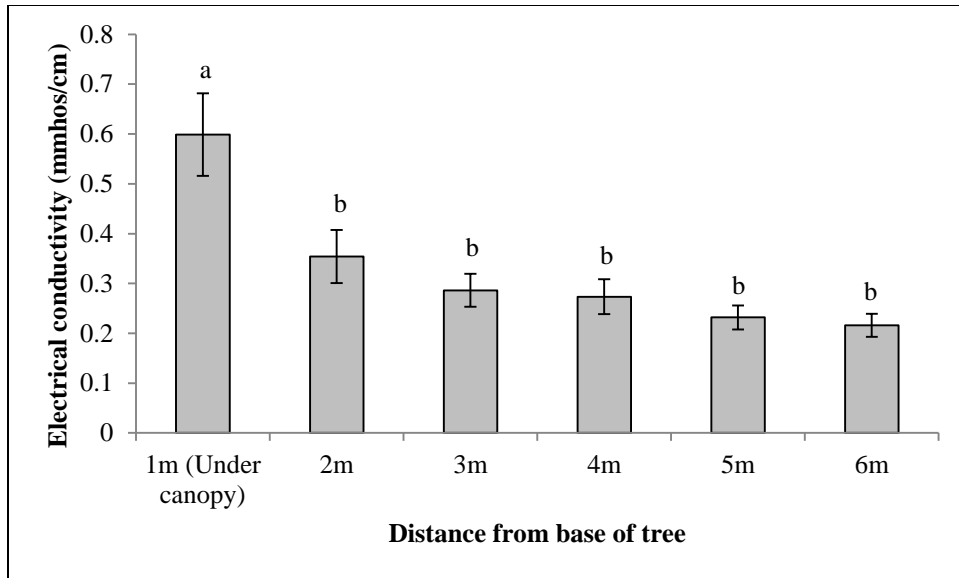
Figure 1-3 Soil electrical conductivity (mmhos/cm) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
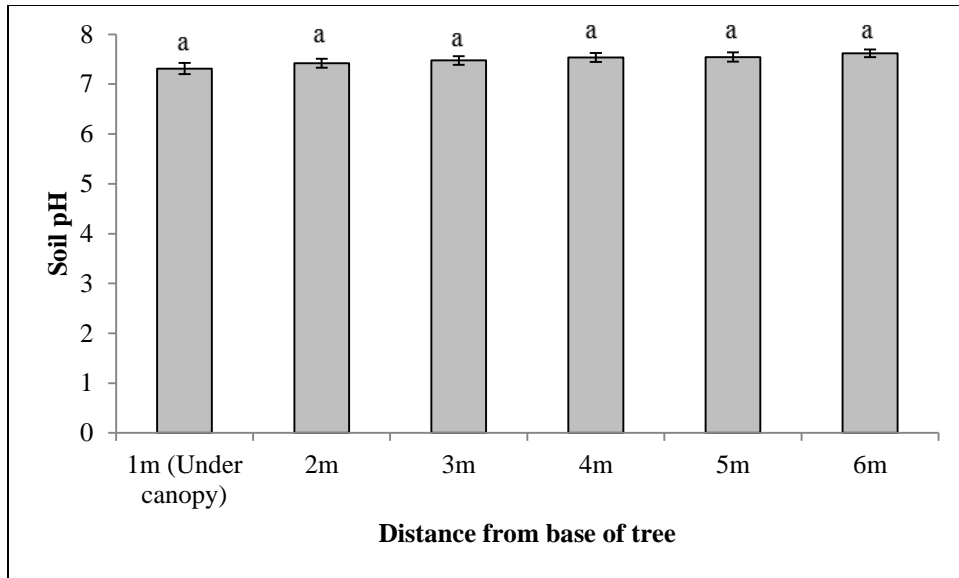
Figure 1-4 Soil pH with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
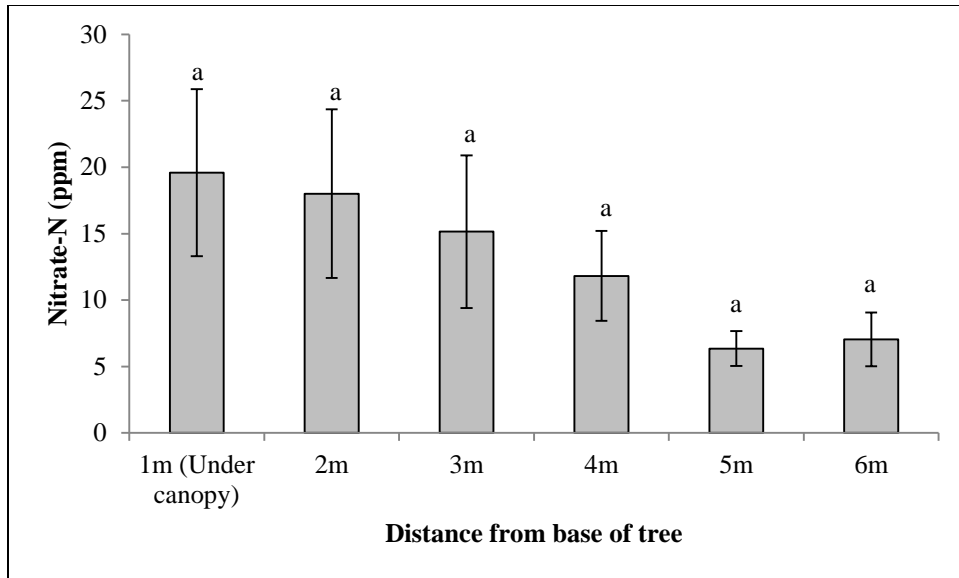
Figure 1-5 Nitrate-nitrogen concentration (ppm) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \le 0.05$).
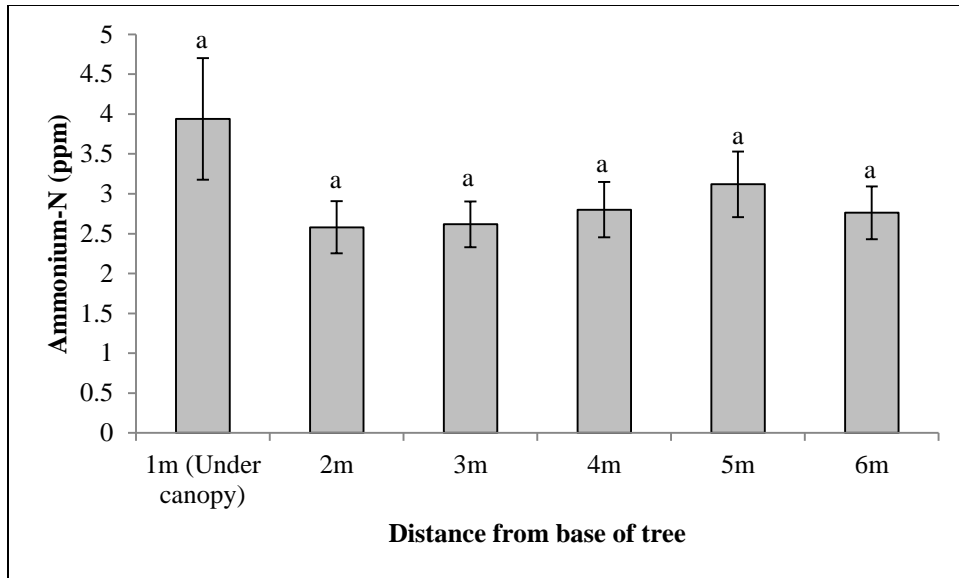
Figure 1-6 Ammonium-nitrogen concentration (ppm) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P{\leq}0.05$).
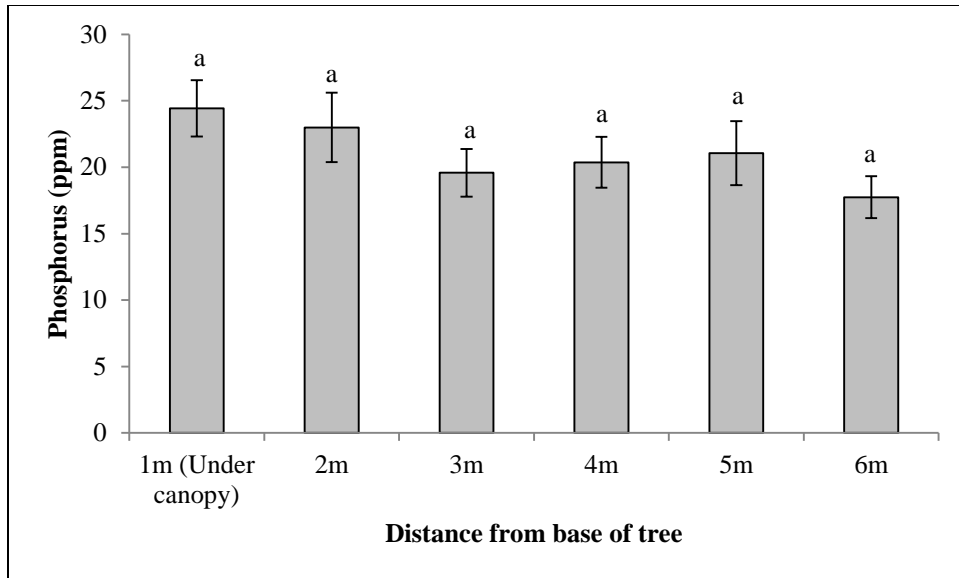
Figure 1-7 Plant-available phosphorus concentration (ppm) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
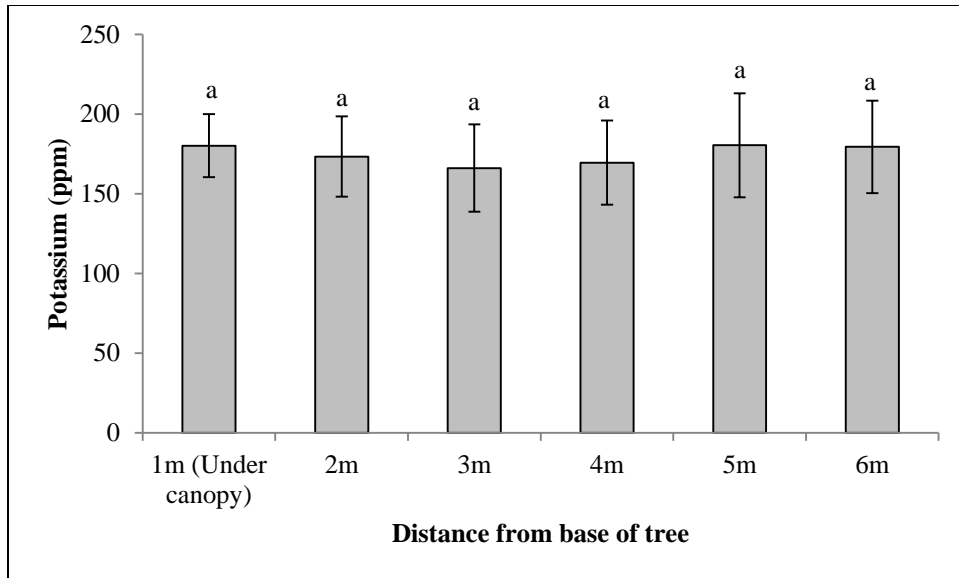
Figure 1-8 Potassium concentration (ppm) with mean values and standard errors of 6 plots located at 1 m intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P{\leq}0.05$).
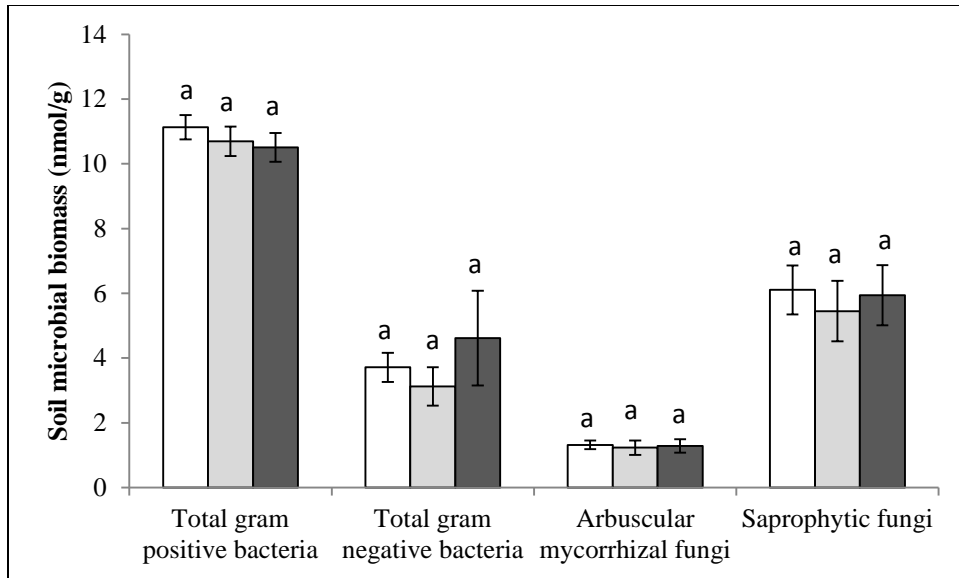
Figure 1-9 Soil microbial biomass (nmol/g) of different communities (total gram positive bacteria, total gram negative bacteria, arbuscular mycorrhizal fungi, and saprophytic fungi) with mean values and standard errors of 3 plots located at 1 m (no filled bar), 2 m (light gray bar), and 6 m (dark bar) intervals starting from base of saltcedar trees near Ashland, Kansas, USA. Bars with the same letter within growth forms are not statistically different ($P \leq 0.05$).

CHAPTER II

ASSESSING PLANT-SOIL FEEDBACKS FOLLOWING SALTCEDAR (*TAMARIX* SP.) INVASION

ABSTRACT

Saltcedar (*Tamarix* sp.) is a non-native facultative phreatophyte which has escaped intentional plantings and invaded river systems throughout southwestern USA, altering wildlife habitat, flooding patterns, and fire frequency. However, previous studies assessed effects of saltcedar focus on aboveground parameters, with little attention given to the belowground microbial communities. Understanding plant-soil feedbacks by saltcedar invasion may be a critical aspect of the restoration of native ecosystems. I conducted field and greenhouse studies to assess potential plant-soil feedbacks resulting from saltcedar invasion. In field study, soil samples were collected 1) beneath saltcedar trees in highly invaded areas; 2) from areas where saltcedar trees have been mechanically and chemically removed (restoration areas); and 3) from adjacent native prairie. Soil was processed for abiotic (pH, N, P, K, organic matter, and texture) and biotic (microbial communities) properties. Greenhouse study assessed plant-soil feedbacks indirectly through biomass production of six native and four non-native plant species grown in soil collected from the same three sites as field study. Plants were grown for 16 weeks, at which time biomass production was determined. Percent root colonization by arbuscular

mycorrhizal (AM) fungi was determined microscopically. I expected higher soil nutrients and lowered soil microbial composition in saltcedar invaded and saltcedar restoration sites due to fertilizing effect of saltcedar. Additionally, all plant species would have greater biomass when grown in soil from saltcedar invaded and saltcedar restoration sites compared to soil from native sites. Greater soil salinity, pH, nitrate-nitrogen, potassium, and phosphorus in soil and alteration in soil microbial communities from saltcedar invaded sites were observed relative to native prairie sites. The legacy of invasion persisted five years after removal of saltcedar with similar trend in soil abiotic and biotic properties as in saltcedar invaded sites. Both native and non-native plant species produced greater biomass in soils collected from saltcedar invaded or saltcedar restoration sites relative to production in soil from native sites. However, all plant species (except *Andropogoon gerardii, Sorghastrum nutans*, and *Spartina pectinata*) grown in soil from saltcedar invaded or saltcedar restoration sites were less colonized by AM fungi than plants grown in soil from native prairie areas.

INTRODUCTION

Invading plant species can impose undesirable alterations to the structure and functioning

of ecosystems and native biological diversity, therefore costing millions of dollars

through direct losses or control efforts (Vitousek et al. 1997, Pimentel et al. 2000). Most

biological invasion research has focused on aboveground features though invasion affects

both above- and belowground properties (Bardgett and Wardle 2010, Inderjit and van der

Putten 2010). Invasion of aboveground ecosystem components can affect belowground

ecosystem components, and vice versa (Stinson et al. 2006, Wolfe et al. 2008, Bardgett

and Wardle 2010, Inderjit and van der Putten 2010). For example, invasion by non-native

Halogeton glomeratus causes salinization of the soil thus inhibiting the growth of native

Ceratoides lanata in western North America (Harper et al. 1996, Kitchen and Jorgensen

2001). In addition, the non-native grass, *Bothriochloa bladhii* in North America disrupted

mutualistic associations between native grasses and arbuscular mycorrhizal (AM) fungi

(Wilson et al. 2012). The process in which plants alter biotic and abiotic soil environment

resulting in altered plant growth are conceptualized as plant-soil feedbacks (Bever et al.

1997, Ehrenfeld et al. 2005). Plant-soil feedbacks have been proposed as important

factors explaining biological invasion (Reinhart and Callway 2006, Kulmatiski et al.

2008). Positive plant-soil feedbacks develop if soil properties are altered following

invasion which promote growth of the non-native plant, while negative plant-soil

feedbacks develop if growth of the non-native plant is reduced (Bever et al. 1997, Bever 2003, Ehrenfeld et al. 2005). Callaway et al. (2004) provide evidence of positive plant-soil feedbacks for non-native *Centaurea maculosa*, as they detected increased plant growth of the non-native when grown in soils from its invaded range in North America.

Riparian areas, usually forming small parts of the landscape, enhance regional biodiversity (Sabo et al. 2005), and have been invaded by non-native species worldwide (Stohlgren et al. 1998). *Tamarix* spp. (Saltcedar) and *Elaeagnus angustifolia* (Russian olive), non-native riparian shrubs, have invaded and formed dense stands in many areas of southwestern US floodplains, altering the native plant communities (Frasier and Johnsen 1991, Friedman et al. 2005). Different control methods are in practice to remove saltcedar and Russian olive to restore these riparian areas (Katz and Shafroth 2003, Shafroth et al. 2005, Reynolds and Cooper 2011). However, restoration of invaded communities requires removal of the invader followed by subsequent active reestablishment of the native community (Kardol and Wardle 2010). Earlier restoration projects were usually focused on removal of non-native plants and their effects on native plant species and soil nutrients (Maron and Connors 1996, Pickart et al. 1998, Maron and Jefferies 2001). The effects of removal of invasive plant species on belowground properties has only occasionally been explored (Peltzer et al. 2009). Recently, studies recognized the importance of plant-soil feedbacks for ecosystem restoration (Suding et al. 2004, Eviner and Hawkes 2008). Soil legacies following the removal of woody plant species may be persistent due to accumulation of nutrients around the plants (Schade and Hobbie 2005). High levels of nutrients in the soils might cause problems for native plant species, which are not able to grow under such nutrient enriched conditions (Huenneke et

al. 1990, Maron and Jefferies 1999). Greater soil nutrients may remain for several years and alter vegetation composition after non-native plants have been removed (Hughes and Denslow 2005, Marchante et al. 2009). Therefore, this legacy can create obstacles for restoration by facilitating re-invasion by the same or other non-native species, or prevent recovery of native plants (Vinton and Burke 1995, Maron and Connors 1996, Pickart et al. 1998, Maron and Jefferies 1999, Vinton and Goergen 2006). Therefore, an integrated understanding of plant-soil feedback is necessary for the restoration of invaded communities as invasive organisms may alter both above- and belowground ecosystem properties.

Soil harbors a wide variety of micro- and macro-organisms. Invasive plants may alter soil microbial communities through root exudation, release of anti-microbial compounds, facilitation of symbiotic relationships between roots and soil microbes, and displacement of native plants having unique soil microbial communities (Klironomos 2002, Kourtev et al. 2002, Wolfe and Klironomos 2005, Reinhart and Callaway 2006). The common soil organisms that interact with plants are mycorrhizae, nitrogen-fixing bacteria, pathogens, and nematodes. Arbuscular mycorrhizal (AM) fungi form symbiotic associations with up to 80% of vascular plants enhancing their growth and survival (Smith and Read 2008). AM fungal hyphae play a pivotal role in the acquisition of mineral nutrients, specifically phosphorus and nitrogen, from the soil and their subsequent translocation to the plant (George et al. 1995, Hodge et al. 2001). These nutrients are acquired by AM hyphal networks (Leake et al. 2004, Selosse et al. 2006). In addition, AM fungi contributes to soil stability by the aggregation of soil particles, provides resistance to stress, drought, and soil pathogens (Augé 2001, Qiangsheng et al. 2006, Sikes et al. 2009, Wilson et al.

2009). Therefore, mycorrhizal mutualisms have effects on both ecosystem processes and plant communities, suggesting the potential for plant-soil feedbacks.

Saltcedar (*Tamarix* sp.) is a non-native facultative phreatophyte (Brotherson and Field 1987, Busch and Smith 1995) that has subsequently escaped intentional plantings and invaded river systems throughout southwestern USA, replacing native plant species (cottonwood (*Populus* sp.) and willow (*Salix* sp.)), altering wildlife habitat, flooding patterns, and fire frequency (Frasier and Johnsen 1991, DiTomaso 1998, Glenn and Nagler 2005). In the United States, over 6 million ha of riparian floodplains and wetlands have been invaded by saltcedar (Stenquist 2000, Zavaleta 2000, Gaskin and Schaal 2002, Shafroth and Briggs 2008). Saltcedar can tolerate a variety of environments ranging from desert to riparian areas (Horton et al. 2001, Yin et al. 2010). Therefore, saltcedar is known to be more tolerant of drought and salinity relative to native species such as cottonwood (*Populus fremontii*) and willow (*Salix gooddingii*) (Cleverly et al. 1997, Smith et al. 1998, Horton et al. 2001). Saltcedar has an ability to exploit deep water tables due to long tap roots and accesses water from either groundwater or the vadose zone (Brotherson and Field 1987, Busch and Smith 1995, Xu and Li 2006, Nippert et al. 2010). Saltcedar has the ability to excrete salts from glands on its leaves thus increasing soil salinity (Decker 1961, Wilkinson 1966, Thomson et al. 1969, Berry 1970, Neill 1985, Sookbirsingh et al. 2010). Studies have shown greater soil salinity under saltcedar canopy as compared to areas outside the canopy (Brotherson and Field 1987, Lesica and DeLuca 2004, Ladenburger et al. 2006, Yin et al. 2010). Saltcedar contributes to greater nitrogen inputs into the soil due to greater leaf nitrogen concentrations (Tibbets and Molles 2005, Moline and Poff 2008). Saltcedar can grow on a variety of soil textures, from sands to

clays (Nagler et al. 2011) and resprouts readily after fires, which can reinforce its dominance over time (Busch 1995, Busch and Smith 1995). Mechanical, chemical, burning, and biological control methods have been applied to control saltcedar (Hart et al. 2005, Shafroth and Briggs 2008, O'Meara et al. 2010). However, the legacy effects after saltcedar removal may cause problems for restoration of native plant communities. Therefore, examining how plant-soil feedbacks interact with saltcedar invasion and removal may be an important component for the restoration of saltcedar invaded ecosystems.

My study investigated soil nutrients and major functional groups of the soil microbial communities of saltcedar invaded sites, sites following mechanical and chemical removal of saltcedar (hereafter referred to as saltcedar restoration sites), and adjacent native prairie sites. Field and greenhouse studies were conducted to assess plant-soil feedbacks associated with saltcedar invasion. The objectives of the field study were to assess potential differences in soil nutrients and biomass of soil microbial communities from sites with varying stages of saltcedar invasion (saltcedar invaded, saltcedar restoration, and native prairie). In general, I expected higher soil nutrients in saltcedar invaded sites due to fertilizing effect of saltcedar. I hypothesized that soil microbial community composition would be lowered in saltcedar invaded sites as earlier studies have shown that invasive plant species can change the composition and function of soil microbes. Soil legacies may be persistent even after the removal of invasive plant thus, I hypothesized soil nutrients and soil microbial community composition would be more similar between soil from saltcedar restoration sites and saltcedar invaded sites, than in soils from the native prairie.

To assess how soil properties following saltcedar invasion and saltcedar restoration affect the growth of different plant species, I conducted a greenhouse experiment. The objectives of the greenhouse study were to assess plant-soil feedbacks indirectly through biomass production and AM fungal root colonization of six native plant species planted into three different soils collected from the same three sites as in the field study. I hypothesized plant-soil feedbacks function through reduction in AM fungal root colonization and plant growth would be enhanced in soils collected from saltcedar invaded and saltcedar restoration sites relative to native areas not invaded by saltcedar due to fertilizing effect of saltcedar. Soils experiencing alterations following plant invasions may exhibit greater risk of invasion by other non-native species as described by the invasional meltdown hypothesis (Simberloff and Von Holle 1999). To determine if invasion by saltcedar facilitates the growth of other non-native plant species as described by the invasional meltdown hypothesis, I also assessed biomass production and AM fungal root colonization of three non-native plant species. Due to the potential soil nutrient enrichment by saltcedar, I further hypothesized that both native and non-native species would produce greater biomass and exhibit reduced AM fungal root colonization in soil collected from saltcedar invaded and saltcedar restoration sites as compared to soil from native sites.

MATERIALS AND METHODS

Soil for the field and greenhouse studies were collected from the study site located adjacent to the Cimarron River, 17 km south of Ashland, Kansas, USA (37°11'19"N, 99°45'55"W). Saltcedar, the predominant species in the site, first appeared after a flood in 1939 (Nippert et al. 2010). Common herbaceous species in the site are *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalograss), *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Sorghastrum nutans* (L.) Nash (indiangrass), *Sporobolus asper* (Michx.) Kunth (tall dropseed), and *Sporobolus cryptandrus* (Torr.) A. Gray (sand dropseed). Nomenclatures of all species were based on the USDA Plant Database (2012). Three replicates of three different soil sources were selected: 1. Saltcedar invaded "treatment" with no history of attempts to eliminate the saltcedar, 2. Saltcedar restoration "treatment" in which saltcedars were removed through a clear cutting and a 1:4 ratio of herbicide (triclopyr) and diesel mix in 2005 (Communicated by D. Arnold), and 3. Native areas with no history of saltcedar invasion. All sites were located within 2 km of each other.

Field Study: To examine soil nutrients and soil microbial community composition in each of the replicates of each of three different soil sources, I established a total of nine transects. Along each 10 m transect, soil was collected from the top 10 cm at 1 m intervals and homogenized. Soils were sieved through 2 mm sieve to remove large plant

roots and stones. A 50 g subsample of soil from each of the transects (n=9) were analyzed

for pH, electrical conductivity, texture, soil nutrients (nitrate-nitrogen, ammonium-

nitrogen, plant-available phosphorus, and potassium), and soil microbial community

composition. Soil electrical conductivity, pH, texture, and nutrients were tested at the

Oklahoma State University Soil, Water and Forage Analytical Laboratory. Soil microbial

community composition was determined using phospholipid fatty acid analysis (PLFA)

(Kourtev et al. 2002, Batten et al. 2006, White and Rice 2009). Phospolipid fatty acids

are signature molecules and can serve as an important indicator of microbial biomass.

Soil samples sieved through a 2 mm sieve were freeze-dried for 8 hours and ground.

Microbial lipids were extracted from 5 g freeze-dried soil with a solvent system of

methanol, chloroform, and a phosphate buffer. The soil-solvent mixture was separated by

centrifugation and the supernatant was decanted. The centrifugation was repeated with

the addition of 1:2 (v/v) chloroform-methanol and the supernatant was collected.

Phosphate buffer was then added and the mixture separated overnight. The chloroform

layer containing the lipids after phase separation was recovered and reduced by nitrogen

flow at 60°C. Total extracted lipids were separated into neutral, glyco-, and polar lipids

with chloroform, acetone, and methanol through silic acid chromatography. Phospholipid

fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph

with an Agilent 5975C series mass selective detector.

Fatty acid nomenclature used was that described by Frostegård et al. (1993): total number

of carbon atoms: number of double bonds, followed by the position (ω) of the double

bond from the methyl end of the molecule. *Cis* and *trans* isomers were indicated by c,

and t, respectively. Anteiso- and isobranching were designated by the prefix a or i. Cy

indicated cyclopropane fatty acids. The fatty acids i15:0, a15:0, i16:0, i17:0 were selected to represent gram positive bacteria; 3-OH 14:0, 16:1ω7, cy17:0, 2-OH 16:0, 18:1ω9c, cy19:0 for gram negative bacteria; 16:1ω5c for AM fungi, 18:2ω9,12c, 18:1ω9c for saprophytic fungi; 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 for non-specific microbes (McKinley et al. 2005). Fatty acids are expressed as nmol g^{-1} dry soil.

Greenhouse study: I collected soil from the three treatments described in my field study (saltcedar invaded, saltcedar restoration, and native prairie), with three replicate sites at each treatment. Soil was sieved through a 2 mm sieve and 600 g (dry weight) were placed into plastic pots (6 cm diameter X 25 cm deep). Native plant species common in mixed-grass prairie were selected: *Andropogon gerardii* Vitman (big bluestem), *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Sorghastrum nutans* (L.) Nash (indiangrass), *Spartina pectinata* Bosc ex Link (prairie cordgrass), and *Sporobolus cryptandrus* (Torr.) A. Gray (sand dropseed), to examine the growth performance as prairie restoration may require replanting with native plant species. Non-native plant species commonly invasive into native prairies were also selected: *Bothriochloa ischaemum* (L.) Keng (old world bluestem), *Bromus inermis* Leyss. (smooth brome), *Cynodon dactylon* (L.) Pers. (bermudagrass), and *Lespedeza cuneata* (Dum. Cours.) G. Don (sericea lespedeza), to examine if one non-native species facilitates the invasion by other non-native species as per the invasional meltdown hypothesis (Harmoney et al. 2004, Vinton and Goergen 2006, Weir et al. 2009, Simberloff and Von Holle 1999). The experimental design included 3 treatments (saltcedar invaded, saltcedar restoration, and native prairie) x 3 replicates of each

treatment x 10 plant species (6 native and 4 non-native species) x 7 replicate pots per soil source for a total of 630 pots.

Seeds of all plant species were obtained from the Johnston Seed Company, Enid, Oklahoma. Seeds were germinated in vermiculite and seedlings at the second leaf stage were transplanted into pots filled with soil collected from each site. Pots were arranged in a randomized complete block design in a greenhouse maintained at 20-25°C. All pots were watered daily. Plants were harvested after 16 weeks. Roots were washed free of soil. Shoots and roots were oven-dried at 60ºC for 72 hours to determine shoot, root, and total dry weights. To measure the percentage of total root length colonized by AM fungal structures, roots of native and non-native plants species grown in soil from different treatments (saltcedar invaded, saltcedar restoration, and native prairie) were subsampled, stained with trypan blue and examined using a compound microscope. Percent AM fungal root colonization followed the magnified gridline intersect method (McGonigle et al. 1990).

Statistical analysis For the field study, soil characteristics (EC, pH, inorganic ammonium-nitrogen, nitrate-nitrogen, plant-available phosphorus, potassium, and biomass of major soil microbial functional groups) were analyzed with one-way ANOVA using General Linear Models (GLM) with soil treatment as single factor. Mean differences of soil characteristics were compared using least square differences (LSD) grouping. Mean soil characteristic values were presented for each soil sources. All data were analyzed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). A significance level of 0.05 was used for all statistical tests.

Variables quantified in the greenhouse study (i.e., shoot, root, total biomass, and percentage AM fungal root colonization) were analyzed separately for each plant species with one-way ANOVA using GLM for soil treatment as fixed factor. For biomass and percentage AM fungal root colonization, the statistical differences among soil treatments were analyzed using LSD *post hoc* tests. All data were analyzed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). A significance level of 0.05 was used for all statistical tests.

RESULTS

Field study: Soil abiotic properties have been altered following saltcedar invasion, and indicated that the legacy of invasion persisted five years after removal of saltcedar. Soil EC and pH were significantly greater in soil from saltcedar invaded sites as compared to soil from saltcedar restoration and native sites (Fig. 2-1, Fig. 2-2). The cations, sodium and magnesium were greater in saltcedar invaded sites compared to soil from saltcedar restoration and native sites (Fig. 2-3, Fig. 2-4). However, calcium was greater in saltcedar invaded sites and lower in native sites with intermediate value in soil from saltcedar restoration sites (Fig. 2-5). The soil nutrients, inorganic nitrate-nitrogen and potassium, were greater in saltcedar invaded and saltcedar restoration sites relative to native prairie areas (Fig. 2-6, Fig. 2-7). However, soil inorganic ammonium-nitrogen was not significantly different among any soil treatments (0.98-1.47 ppm). Soil inorganic phosphorus was significantly greater in saltcedar invaded and saltcedar restoration sites relative to native prairie sites (Fig. 2-8). Soil from saltcedar restoration sites had the greatest organic matter percent relative to soil from saltcedar invaded and native areas (Fig. 2-9). Regarding soil physical properties, the percentage of silt and clay were greater in saltcedar invaded and saltcedar restoration sites relative to soil from native areas, while a greater percentage of sand was observed in native prairie sites relative to soil from saltcedar invaded and saltcedar restoration sites (Fig. 2-10).

The results showed alteration in the biomass of major functional groups of soil microbial communities in areas invaded by saltcedar and the legacy persisted after the saltcedar removal. There were greater total bacterial, fungal, and total microbial biomass in saltcedar invaded and saltcedar restoration sites relative to soil from native sites (Fig. 2-11). The biomass of major microbial groups, total gram positive bacteria, total gram negative bacteria, AM fungi, and saprophytic fungi were greater in soil from saltcedar invaded and saltcedar restoration sites relative to native prairie sites according to PLFA tests performed (Fig. 2-12).

Greenhouse Study: I indirectly examined plant-soil feedbacks from the invasion of saltcedar and the legacy of the feedbacks five years after removal of saltcedar by growing different plant species into soil from saltcedar invaded, saltcedar restoration, and native sites. Biomass production of both native and non-native plant species increased when grown in soil from saltcedar invaded or saltcedar restoration sites relative to native prairie soil areas (Figs. 2-13, 2-14, 2-15). All plant species (except *A. gerardii, S. nutans*, and *S. pectinata*) grown in soil from saltcedar invaded or saltcedar restoration sites were less colonized by AM fungi than plants grown in soil from native prairie areas (Fig. 2-16).

DISCUSSION

This study demonstrates that saltcedar invasion significantly alters soil abiotic and biotic characteristics and the legacy effect of the invasion persists even after the removal of saltcedar.

Soil abiotic and biotic properties

Soil salinity measured in terms of electrical conductivity (EC) was greater in soil invaded by saltcedar relative to saltcedar restoration and native prairie sites. Saltcedar may contribute to soil salinity by translocating salts from groundwater to surface and also from leaf exudates (Smith et al. 1998, Sookbirsingh et al. 2010). Salts are excreted via salt glands on leaves of saltcedar and assumed as one of the mechanisms to make soil saline thus excluding native cottonwood and willow (Brotherson and Field 1987, DiTomaso 1998). Saline soils have a high concentration of soluble salts and an EC greater than 4 mmhos cm^{-1} (US Salinity Laboratory Staff 1954). Previous studies have concluded that saltcedar does increase soil salinity (Lesica and DeLuca 2004, Landenburger et al. 2006, Yin et al. 2010). Similar to earlier studies, my results provide evidence that saltcedar is increasing salinity of the soil, however after saltcedar removal, the soil salinity is greatly reduced.

My results agreed with Carman and Brotherson (1982) that saltcedar invaded sites have greater sodium concentration compared to other cations, such as calcium and magnesium. The greater soil pH in soil invaded by saltcedar compared to soil from saltcedar restoration and native areas could be attributed to greater cations (Miller and Donahue 1995, Brady and Weil 2008). In agreement with earlier studies that saltcedar increased nutrient availability (Lesica and DeLuca 2004, Ladenburger et al. 2006, Yin et al. 2010), my results showed greater soil nitrate-nitrogen, plant-available phosphorus, and potassium with saltcedar invasion relative to native prairie sites. My results also showed that five years after removal of saltcedar, a legacy effect persisted (i.e., soil nutrient availability remained high). Previous studies have shown greater nitrogen concentrations in saltcedar leaves relative to cottonwood leaves (Tibbets and Molles 2005, Moline and Poff 2008). In my study, increased nitrate-nitrogen in saltcedar invaded sites, could possibly be due to leaf secretions or leaf fall of saltcedar. In my study, greater potassium in soil from both saltcedar invaded and restoration sites relative to soil from native area could be due to greater sodium level, as sodium reduces potassium uptake (Grattan and Grieve 1999). However, I found no significant differences for ammonium-nitrogen among any soil sources, which could be due to increased ammonium uptake by plants (Maathuis 2009). My results supported previous studies that saltcedar enhanced phosphorus accumulation (Bagstad et al. 2006, Ladenburger et al. 2006, Yin et al. 2010) and persisted after the removal of saltcedar thus indicating a legacy effect. Saltcedar leaves have been reported to contain polyphenolic compounds (Sultanova et al. 2001) and such compounds have the potential to increase phosphorus availability through calcium chelation thereby resulting in the solubilization of calcium phosphate (Schlesinger 1997).

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Similar to earlier studies (Stromberg 1998, Bagstad et al. 2006), greater clay content was found in soil from saltcedar invaded areas relative to the native prairie soil which could be due to high stem density of saltcedar thus induce settling of clays.

Non-native plant species that have different functional attributes than native plants can influence composition and function of soil microbial communities (Ehrenfeld 2004, Reinhart and Callaway 2004, Batten et al. 2006, Hawkes et al. 2006, van der Putten et al. 2007). In my study, the analysis of PLFA profiles indicated alteration of soil microbial communities in soil from saltcedar invaded and saltcedar restoration areas compared to soil from native prairie. The total bacterial, fungal, and microbial biomass were greater in the saltcedar invaded and saltcedar restoration soil sources compared to soil from native prairie which could be due to clay soils that have the capacity to preserve microbial biomass (Van Veen et al. 1984, Gregorich et al. 1991). There is evidence of host specialization, in which specific microbial communities, species, or strains associate with specific plant species (Bever 1994, Bais et al. 2006, Badri et al. 2009). Therefore, plant species can affect the composition and activity of the soil microbial community (Belnap and Phillips 2001, Kourtev et al. 2002, Carney and Matson 2006). For example, exotic grass invasion into a California grassland shifted the composition and abundance of the soil microbial community favoring ammonia-oxidizing bacteria (Hawkes et al. 2005). Studies showed that the least beneficial AM fungi are the most competitive (Bever 2002, Bennett and Bever 2009). Greater AM fungal biomass in my study could be due to AM fungal species that are less beneficial to the growth of plants. PLFA profiles only provide an index of soil microbial community structure with no information on specific species

and function therefore, detailed study on species of different soil microbial communities may provide effects of different microbial species on plant growth.

Difference in AM fungal biomass was observed when saltcedar invaded and saltcedar restoration soil sources were compared to soil from native prairie. Alteration in the composition and abundance of the AM fungal community observed for several introduced plant species have been implicated as an important factor in successful invasions, for example, Asian knapweed (*Centaurea maculosa*) and garlic mustard (*Alliaria petiolata*) in North America (Marler et al. 1999, Roberts and Anderson 2001, Klironomos 2002). Hawkes et al. (2006) and Mummey and Rillig (2006) provided further evidence for the different composition of AM fungi in roots of native plants and non-native plants. Therefore, examination on composition of AM fungi in soil from saltcedar invaded, saltcedar restoration, and native sites may provide detail information if saltcedar has altered the AM fungal species. Increased abundance of saprophytic fungi in the soil from saltcedar invaded and saltcedar restoration sites relative to soil from native sites might be due to increased decomposition rates of saltcedar (Bailey et al. 2001) thus reflecting the greater organic matter content (Hršelova et al. 1999).

Greenhouse study

Greenhouse data indicated greater biomass production by both native and non-native plants when planted into soil collected from saltcedar invaded or saltcedar restoration sites compared with soil collected from adjacent native prairie areas. The greater plant biomass in soil with a history of saltcedar invasion could possibly be due to higher soil nutrient availability as elevated concentrations of nitrate-nitrogen and phosphorus for

saltcedar invaded and saltcedar restoration soil sources were observed compared to soil

from native prairie in my study. Lesica and DeLuca (2004) also found greater growth of

native grass *Agropyron smithii* when grown in soils invaded by saltcedar due to greater

inorganic nitrogen and phosphorus thus suggesting a fertilizing effect of saltcedar.

Successful growth of non-native plants grown in soil from saltcedar invaded and

saltcedar restoration sites showed that there would be equal chances for non-native

species to recolonize as predicted by the invasional meltdown hypothesis (Adler et al.

1998, Simberloff and Von Holle 1999, Hughes and Denslow 2005). In spite of above-

and belowground biomass increasing in soil from saltcedar invaded or saltcedar

restoration areas relative to soil from native prairie, percentage root colonization by AM

fungi was significantly lowered for most native and non-native plants with the exception

of native *A. gerardii, S. nutans,* and *S. pectinata*. The level of AM fungal colonization of

plant roots and its effect on plant growth varies depending on the composition and

abundance of the AM fungal species (van der Heijden et al. 1998) and the available

nutrients (Sanders and Sheikh 1983, Blanke et al. 2005). A study of mycorrhizal

responses to nitrogen enrichment with higher soil phosphorus availabilities showed

decreased AM colonization (Sylvia and Neal 1990). In this 16 week greenhouse study,

the lower biomass production of plants when grown in soil from native prairie does not

preclude a mycorrhizal response as mycorrhizal colonization does not always increase

plant biomass (Johnson et al. 2010). The plant biomass though not enhanced by the

symbiosis, mycorrhizae can account for phosphorus uptake (Smith et al. 2003), increase

in tiller production (e.g., *Pascopyrum smithii*) (Miller et al. 1987), protection from plant

pathogens (Fitter and Garbaye 1994, Newsham et al. 1995), or enhance drought tolerance (Ruiz-Lozano et al. 2001, Kaya et al. 2003).

Conclusion

In summary, saltcedar invasion had important consequences belowground through influences on soil nutrients and soil biota. The alteration in soil nutrients and major microbial functional groups lasted 5 years after removal of saltcedar, and might persist in soil as a long lasting legacy. The greenhouse results showed that if saltcedar is removed from the current study site, the vegetation can establish utilizing greater nutrients from saltcedar restoration areas with no legacy effects of soil salinity.

REFERENCES

Adler P.B., C.M. D'Antonio, J.T. Tunison. 1998. Understory succession following a dieback of *Myrica faya* in Hawai'i Volcanoes National Park. Pacific Science 52(1):69-78.

Augé R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3-42.

Badri D.V., N. Quintana, E.G. El Kassis, H.K. Kim, Y.H. Choi, A. Sugiyama, R. Verpoorte, E. Martinoia, D.K. Manter, J.M. Vivanco. 2009. An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiology 151(4):2006-2017.

Bagstad K.J., S.J. Lite, J.C. Stromberg. 2006. Vegetation, soils, and hydrogeomorphology of riparian patch types of a dryland river. Western North American Naturalist 66(1):23-44.

Bailey J.K., J.A. Schweitzer, T.G. Whitham. 2001. Saltcedar negatively affects biodiversity of aquatic macroinvertebrates. Wetlands 21(3):442-447.

Bais H.P., T.L. Weir, L.G. Perry, S. Gilroy, J.M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57:233-266.

Bardgett R.D., D.A. Wardle. 2010. Aboveground- belowground linkages. Biotic interactions, ecosystem processes, and global change. Oxford series in Ecology and Evolution.

Batten K.M., K.M. Scow, K.F. Davies, S.P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8:217-230.

Belnap J., S.L. Phillips. 2001. Soil biota in an ungrazed grassland: Response to annual grass (*Bromus tectorum*) invasion. Ecological Applications 11(5):1261-1275.

Bennett A.E., J.D. Bever. 2009. Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. Oecologia 160:807-816.

Berry W.L. 1970. Characteristics of salts secreted by *Tamarix aphylla.* American Journal of Botany 57:1226-1230.

Bever J.D. 1994. Feedback between plants and their soil communities in an old field community. Ecology 75(7):1965-1977.

Bever J.D. 2002. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society B Biological Sciences 269:2595-2601.

Bever J.D. 2003. Soil community feedback and the coexistence of competitiors: conceptual frameworks and empirical tests. New Phytologist 157:465-473.

Bever J.D., K.M. Westover, J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561-573.

Blanke V., C. Renker, M. Wagner, K. Füllner, M. Held, A.J. Kuhn, F. Buscot. 2005. Nitrogen supply affects arbuscular mycorrhizal colonization of *Artemisia vulgaris* in a phosphate-polluted field. New Phytologist 166(3):981-991.

Brady N.C., R.R. Weil. 2008. The nature and properties of soils. Upper Saddle River, NJ:Prentice Hall.

Brotherson J.D., D. Field. 1987. Tamarix: Impacts of a successful weed. Rangelands 9(3):110-112.

Busch D.E. 1995. Effects of fire on Southwestern riparian plant community. The Southwestern Naturalist 40(3):259-267.

Busch D.E., S.D. Smith. 1995. Mechanims associated with decline of woody species in riparian ecosystems of the Southwestern US. Ecological Monographs 65(3):347-370.

Callaway R.M., G.G. Thelen, A. Rodriguez, W.E. Holben. 2004. Soil biota and exotic plant invasion. Nature 427:731-733.

Carman J.G., J.D. Brotherson. 1982. Comparisons of sites infested and not infested with saltcedar (*Tamarix pentandra*) and Russian olive (*Elaeagnus angustifolia*). Weed Science 30(4):360-364.

Carney K.M., P.A. Matson. 2006. The influence of tropical plant diversity and composition on soil microbial communities. Microbial Ecology 52(2):226-238.

Cleverly J.R., S.D. Smith, A. Sala, D.A. Devitt. 1997. Invasive capacity of *Tamarix ramosissima* in a Mojave Desert floodplain: the role of drought. Oecologia 111(1):12-18.

Decker J.P. 1961. Salt secretion by *Tamarix pentandra* Pall. Forest Science 7:214-217.

DiTomaso J.M. 1998. Impact, biology, and ecology of saltcedar (*Tamarix* spp.) in the Southwestern United States. Weed Technology 12:326-336.

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18:1232-1235.

Ehrenfeld J.G., B. Ravit, K. Elgersma. 2005. Feedback in the plant-soil system. Annual Review of Environment and Resources 30:75-115.

Eviner V.T, C.V. Hawkes. 2008. Embracing variability in the application of plant-soil interactions to the restoration of communities and ecosystems. Restoration Ecology 16(4):713-729.

Fitter A.H., J. Garbaye. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159:123-132.

Frasier G.W., T.N. Johnsen Jr. 1991. Saltcedar (tamarisk): classification, distribution, ecology, and control. *In* James L.F., J.O. Evans, M.H. Ralphs, R.D. Child (eds.). Noxious Range Weeds. Boulder, CO: Westview Press. pp. 377-386.

Friedman J.M., G.T. Auble, P.B. Shafroth, M.L. Scott, M.F. Merigliano, M.D. Freehling, E.R. Griffin. 2005. Dominance of non-native riparian trees in western USA. Biological Invasions 7:747-751.

Frostegård Å., E. Bååth, A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25(6):723-730.

Gaskin J.F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. PNAS 99(17):11256-11259.

George E., H. Marschner, I. Jakobsen. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Critical Reviews in Biotechnology 15:257-270.

Glenn E.P., P.L. Nagler. 2005. Comparative ecophysiology of *Tamarix ramosissima* and native trees in western U.S. riparian zone. Journal of Arid Environments 61:419-446.

Grattan S.R., C.M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. Scientia Horticulturae 78:127-157.

Gregorich E.G., R.P. Voroney, R.G. Kachambk. 1991. Turnover of carbon through the microbial biomass in soils with different textures. Soil Biology and Biochemistry 23(8):799-805.

Harmoney K.R., P.W. Stahlman, K.R. Hickman. 2004. Herbicide effects on established yellow old world bluestem (*Bothriochloa ischaemum*). Weed Technology 18(3):545-550.

Harper K.T., R. Van Buren, S.G. Kitchen. 1996. Invasion of alien annuals and ecological consequences in salt desert shrublands of Western Utah. *In* Barrow J.R., E. McArthur, R.E. Sosebee, R.J. Tausch, J. Robin. Comps. Proceedings: shrubland ecosystem dynamics in a changing environment, Las Cruces, NM. Gen. Tech. Rep. INT-GTR-338. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station.

Hart C.R., L.D. White, A. McDonald, Z. Sheng. 2005. Saltcedar control and water salvage on the Pecos river, Texas, 1999-2003. Journal of Environmental Management 75:399-409.

Hawkes C.V., I.F. Wren, D.J. Herman, M.K. Firestone. 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8:976-985.

Hawkes C.V., J. Belnap, C. D'Antonio, M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369-380.

Hawkes C.V., J. Belnap, C. D'Antonio, M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369-380.

Hodge A., C.D. Campbell, A.H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413(20):297-299.

Horton J.L., T.E. Kolb, S.C. Hart. 2001. Responses of riparian trees to interannual variation in ground water depth in a semi-arid river basin. Plant, Cell and Environment 24:293-304.

Hršelová H., I. Chvatalová, M. Vosátka, J. Klír, M. Gryndler. 1999. Correlation of abudance of arbuscular mycorrhizal fungi, bacteria and saprophytic microfungi with soil carbon, nitrogen and phosphorus. Folia Microbiologica 44(6):683-687.

Huenneke L.F., S.P. Hamburg, R. Koide, H.A. Mooney, P.M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71(2):478-491.

Hughes R.F., J.S. Denslow. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forest of Hawaii. Ecological Applications 15(5):1615-1628.

Inderjit, W.H. van der Putten. 2010. Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology and Evolution 25:512-519.

Johnson N.C., G.W.T. Wilson, M.A. Bowker, J.A. Wilson, R.M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107(5):2093-2098.

Kardol P., D.A. Wardle. 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends in Ecology and Evolution 25(11):670-679.

Katz G.L., P.B. Shafroth. 2003. Biology, ecology and management of *Elaeagnus angustifolia* L. (Russian olive) in western North America. Wetlands 23(4):763-777.

Kaya C., D. Higgs, H. Kirnak, I. Tas. 2003. Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. Plant and Soil 253:287-292.

Kitchen S.G., G.L. Jorgensen. 2001. Winterfat decline and Halogeton spread in the Great Basin. *In* McArthur E.D., D.J. Fairbanks. Comps. Shrubland ecosystsem genetics and biodiversity: proceedings; Provo, UT. Proc. RMRS-P-21. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Klironomos J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67-70.

Kourtev P.S., J.G. Ehrenfeld, M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83(11):3152-3166.

Kulmatiski A., K.H. Beard, J.R. Stevens, S.M. Cobbold. 2008. Plant-soil feedbacks: a meta-analytical review. Ecology Letters 11:980-992.

Ladenburger C.G., A.L. Hilda, D.J. Kazmer, L.C. Munn. 2006. Soil salinity patterns in *Tamarix* invasions in the Bighorn Basin, Wyoming, USA. Journal of Arid Environments 65:111-128.

Leake J., D. Johnson, D. Donnelly, G. Muckle, L. Boddy, D. Read. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Canadian Journal of Botany 82:1016-1045.

Lesica P., T.H. DeLuca. 2004. Is tamarisk allelopathic? Plant and Soil 267:357-365.

Marchante E., A. Kjøller, S. Struwe, H. Freitas. 2009. Soil recovery after removal of the N_2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Biological Invasions 11:813-823.

Marler M.J., C.A. Zabinski, R.M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80(4):1180-1186.

Maron J.L., P.G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105:302-312.

Maron J.L., R.L. Jefferies. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80(2):443-454.

Maron J.L., R.L. Jefferies. 2001. Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11(4):1088-1100.

Maathuis F.J.M. 2009. Physiological functions of mineral macronutrients. Current opinion in Plant Biology 12:250-258.

McGonigle TP, MH Miller, DG Evans, GL Fairchild, JA Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501.

McKinley V.L., A.D. Peacock, D.C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry 37:1946-1958.

Miller R.M., A.G. Jastfer, J.K. Pillai. 1987. Biomass allocation in an *Agropyron smithii-Glomus* symbiosis. American Journal of Botany 74(1):114-122.

Miller R.W., R.L. Donahue. 1995. Soils in our environment. Seventh Edition. Prudence Hall, Englewood, Cliffs, NJ. pp.323.

Moline A.B., N.L. Poff. 2008. Growth of an invertebrate shredder on native (*Populus*) and non-native (*Tamarix, Elaeagnus*) leaf litter. Freshwater Biology 53:1012-1020.

Mummey D.L., M.C. Rillig. 2006. The invasive plant species *Centaurea maculosa* alters arbusculary mycorrhizal fungal communities in the field. Plant Soil 288:81-90.

Nagler P.L., E.P. Glenn, C.S. Jarnevich, P.B. Shafroth. 2011. Distribution and abundance of saltcedar and Russian olive in the Western United States. Critical Reviews in Plant Sciences 30:508-523.

Neill W.M. 1985. Tamarisk. Fremontia 12:22-23.

Newsham K.K., A.H. Fitter, A.R. Watkinson. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. Journal of Ecology 83(6):991-1000.

Nippert J.B., J.J. Butler, G.J. Kluitenberg, D.O. Whittemore, D. Arnold, S.E. Spal, J.K. Ward. 2010. Patterns of *Tamarix* water use during a record drought. Oecologia 162:283-292.

O'Meara S., D. Larsen, C. Owens. 2010. Methods to control saltcedar and Russian olive. *In* Saltcedar and Russian Olive Control Demonstration Act Science Assessment, pp. 65-102. Shafroth P.B., C.A. Brown, D.M. Merritt, eds., U.S. Geological Survey Scientific Investigations Report 2009-5247. USGS, Washington, D.C.

Peltzer D.A., P.J. Bellingham, H. Kurokawa, L.R. Walker, D.A. Wardle, G.W. Yeates. 2009. Punching above their weight: low-biomass non-native plant species alter soil properties during primary succession. Oikos 118:1001-1014.

Pickart A.J., L.M. Miller, T.E. Duebendorfer. 1998. Yellow bush lupine invasion in northern California coastal dunes I. Ecological impacts and manual restoration techniques. Restoration Ecology 6(1):59-68.

Pimentel D., L. Lach, R. Zuniga, D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50(1):53-65.

Qiangsheng W., X. Renxue, H. Zhengjia. 2006. Effect of arbuscular mycorrhiza on the drought tolerance of *Poncirus trifoliate* seedlings. Fron. For. China 1:100-104.

Reinhart K.O., R.M. Callaway. 2004. Soil biota facilitate exotic Acer invasions in Europe and North America. Ecological Applications 14(6):1737-1745.

Reinhart K.O., R.M. Callaway. 2006. Soil biota and invasive plants. New Phytologist 170:445-457.

Reynolds L.V., D.J. Cooper. 2011. Ecosystem response to removal of exotic riparian shrubs and a transition to upland vegetation. Plant Ecology 212:1243-1261.

Roberts K.J., R.C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146(1):146-152.

Ruiz-Lozano J.M., C. Collados, J.M. Barea, R. Azcón. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytologist 151(2):493-502.

Sabo J.L., R. Sponseller, M. Dixon, K. Gade, T. Harms, J. Heffernan, A. Jani, G. Katz, C. Soykan, J. Watts, J. Welter. 2005. Riparian zones increase regional species richness by harboring different, not more, species. Ecology 86(1):56-62.

Sanders F.E., N.A. Sheikh. 1983. The development of vesicular-arbuscular mycorrhizal infection in plant root systems. Plant and Soil 71:223-246.

Schade J.D., S.E. Hobbie. 2005. Spatial and temporal variation in islands of fertility in the Sonoran Desert. Biogeochemistry 73(3):541-553.

Schlesinger W.H. 1997. Biogeochemistry. An analysis of global change. Academic Press. pp. 98-100.

Selosse M-A, F. Richard, X. He, S.W. Simard. 2006. Mycorrhizal networks: des liaisons dangereuses? TRENDS in Ecology and Evolution 21(11):621-628.

Shafroth P.B., J.R. Cleverly, T.L. Dudley, J.P. Taylor, C. Van Riper III, E.P. Weeks, J.N. Stuart. 2005. Control of *Tamarix* in the western United States: Implications for water salvage, wildlife use, and riparian restoration. Environmental Management 35(3):231-246.

Shafroth P.B., M.K. Briggs. 2008. Restoration ecology and invasive riparian plants: An introduction to the special section on *Tamarix* spp. in Western North America. Restoration Ecology 16(1):94-96.

Sikes B.A., K. Cottenie, J.N. Klironomos. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97:1274-1280.

Simberloff D., B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological Invasions 1:21-32.

Smith S.D., D.A. Devitt, A. Sala, J.R. Cleverly, D.E. Busch. 1998. Water relations of riparian plants from warm desert regions. Wetlands 18(4):687-696.

Smith S.E. F.A. Smith, I. Jakobsen. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133(1):16-20.

Smith S.E., D. Read. 2008. Mycorrhizal Symbiosis. Third Edition. Elsevier Ltd.

Sookbirsingh R., K. Castillo, T.E. Gill, R.R. Chianelli. 2010. Salt separation processes in the saltcedar *Tamarix ramosissima* (Ledeb.). Communitcations in Soil Science and Plant Analysis 41:1271-1281.

Stenquist S.M. 2000. Saltcedar integrated weed management and the endangered species act. *In* Proceedings of the X International Symposium on Biological Control of Weeds. 4-14 July 1999, Montana State University, Bozeman, Montana, USA. Neal R. Spencer (ed.). pp.487-504.

Stinson K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4(5):0727-0731.

Stohlgren T.J., K.A. Bull, Y. Otsuki, C.A. Villa, M. Lee. 1998. Riparian zones as havens for exotic plant species in the central grasslands. Ecology 138(1):113-125.

Stromberg J.C. 1998. Functional equivalency of saltcedar (*Tamarix chinensis*) and fremont cottonwood (*Populus fremontii*) along a free-flowing river. Wetlands 18(4):675-686.

Suding K.N., K.L. Gross, G.R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 19(1):46-53.

Sultanova N., T. Makhmoor, Z.A. Abilov, Z. Parween, V.B. Omurkamzinova, A-u-Rahman, M.I. Choudhary. 2001. Journal of Ethnopharmacology 78:201-205.

Sylvia D.M., L.H. Neal. 1990. Nitrogen affects the phosphorus response of VA mycorrhiza. New Phytologist 115(2):303-310.

Thomson W.W., W.L. Berry, L.L. Liu. 1969. Localization and secretion of salt by the salt glands of *Tamarix aphylla*. Botany 63:310-317.

Tibbets T.M., M.C. Molles Jr. 2005. C: N: P stoichiometry of dominant riparian trees and arthropods along the Middle Rio Grande. Freshwater Biology 50:1882-1894.

USDA, NRCS (2012). The PLANTS Database (http://plants.usda.gov, 1 November 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

U.S. Salinity Laboratory Staff. 1954. Determination of the properties of saline and alkali soils. USDA Agricultural Handbook No. 60. U.S. Government Printing Office, Washington, DC. pp.7-33.

van der Heijden M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

van der Putten W.H., J.N. Klironomos, D.A. Wardle. 2007. Microbial ecology of biological invasions. The International Society for Microbial Ecology Journal 1:28-37.

Van Veen J.A., J.N. Ladd, M.J. Frissel. 1984. Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76:257-274.

Vinton M.A., E.M. Goergen. 2006. Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. Ecosystems 9:967-976.

Vinton M.A., I.G. Burke. 1995. Interactions between individual plant species and soil nutrient status in shortgrass steppe. Ecology 76(4):1116-1133.

Vitousek P.M., C.M. D'Antonio, L.L. Loope, M. Rejmanek, R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21(1):1-16.

Weir J.R., T.G. Bidwell, R.D. Elmore, K.R. Hickman, S.D. Fuhlendorf, D.M. Engle. 2009. Weed control on rangelands. Oklahoma Cooperative Extension Service NREM-2882. pp. 1-6.

White P.M, C.W. Rice. 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. Soil Sci. Soc. Am. J. 73(1):138-145.

Wilkinson R.E. 1966. Adventitious shoots on saltcedar roots. Botanical Gazette 127(2-3):103-104.

Wilson G.W.T., C.W. Rice, M.C. Rillig, A. Springer, D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12:452-461.

Wilson G.W.T., K.R. Hickman, M.M. Williamson. 2012. Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22:327-336.

Wolfe B.E., J.N. Klironomos. 2005. Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6):477-487.

Wolfe B.E., V.L. Rodgers, K.A. Stinson, A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology 96:777-783.

Xu H., Y. Li. 2006. Water-use strategy of three central Asian desert shrubs and their responses to rain pulse events. Plant and Soil 285:5-17.

Yin C.H., G. Feng, F. Zhang, C.Y. Tian, C. Tang. 2010. Enrichment of soil fertility and salinity by tamarisk in saline soils on the northern edge of the Taklamakan desert. Agricultural Water Management 97:1978-1986.

Zavaleta E. 2000. The economic value of controlling an invasive shrub. Ambio 29(8):462-467.

FIGURES
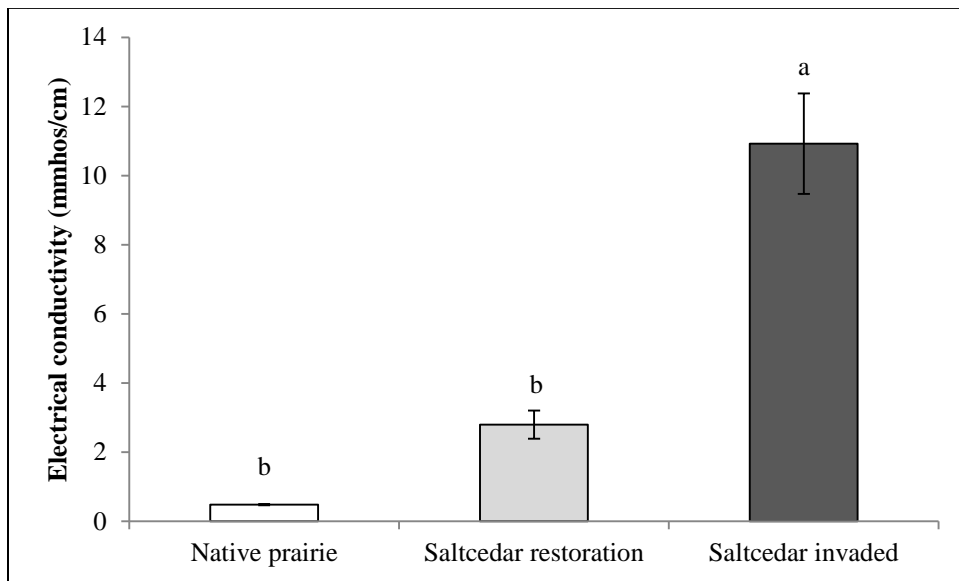
Figure 2-1 Soil EC (mmhos/cm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
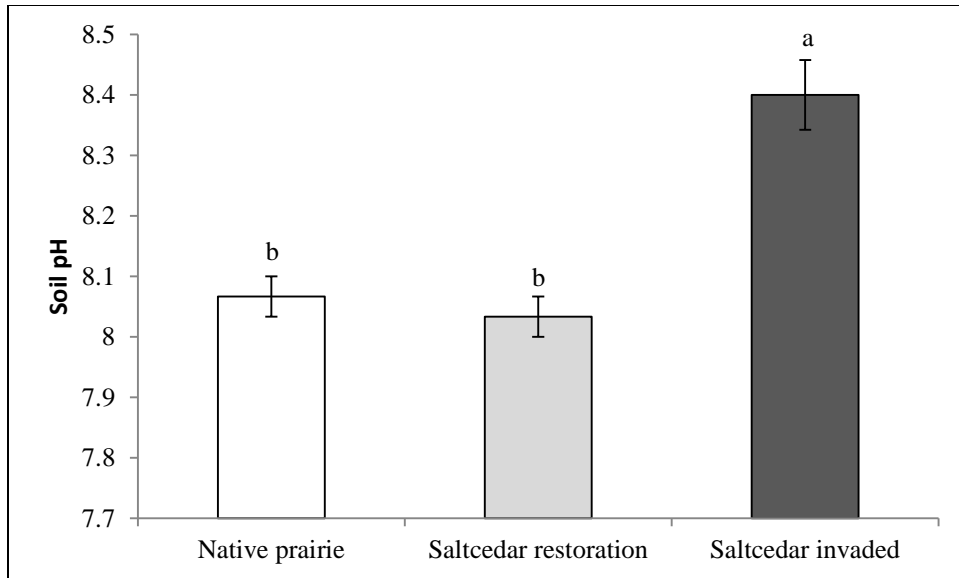
Figure 2-2 Soil pH with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
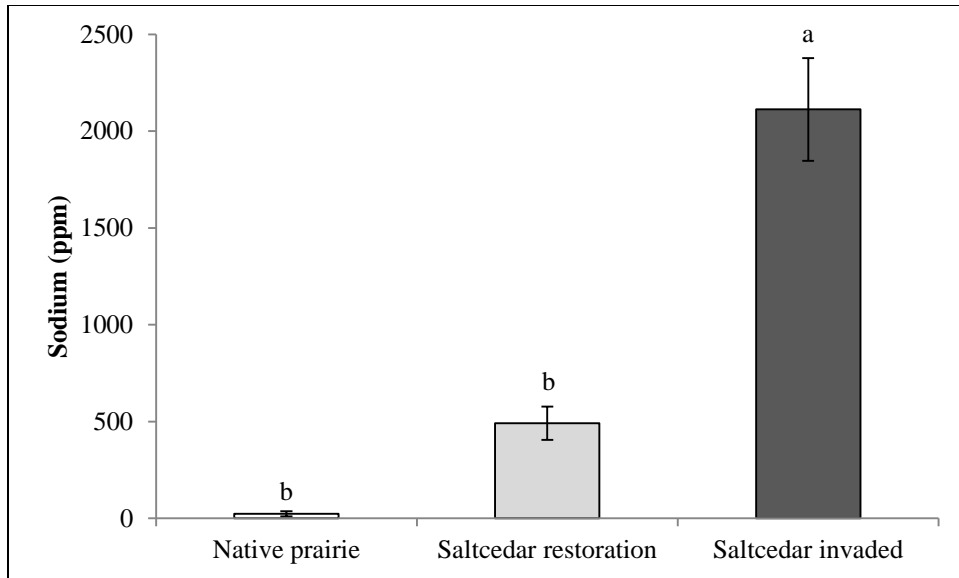
Figure 2-3 Sodium concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
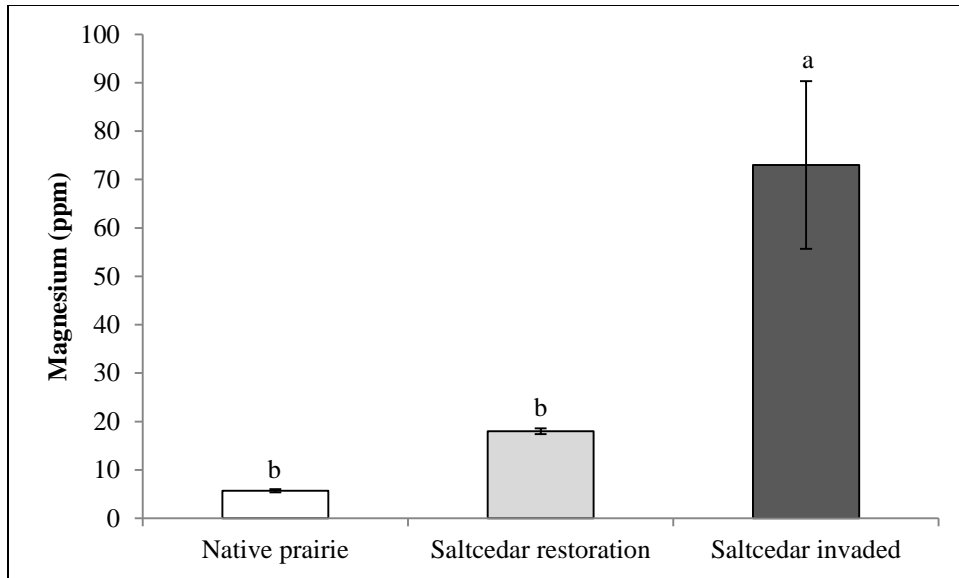
Figure 2-4 Magnesium concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
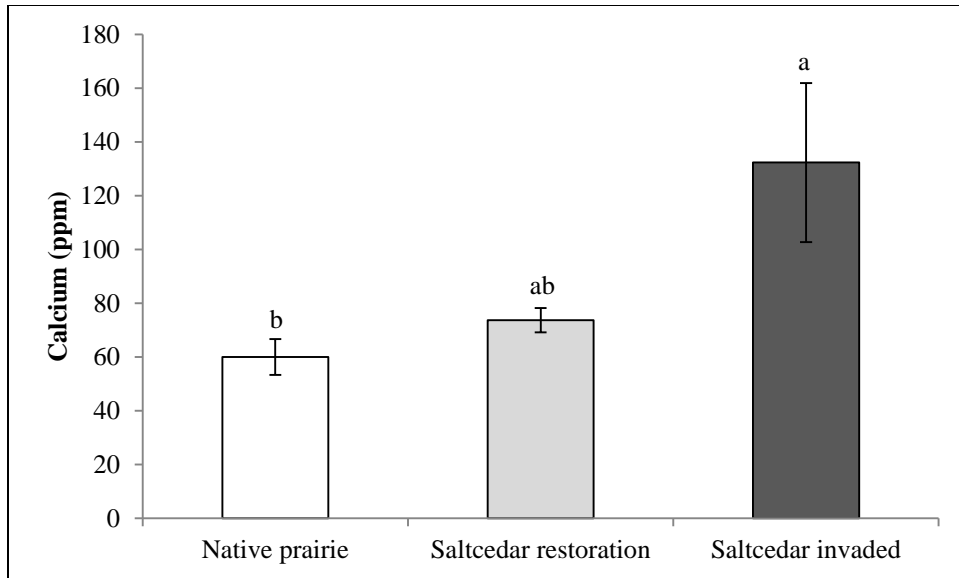
Figure 2-5 Calcium concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
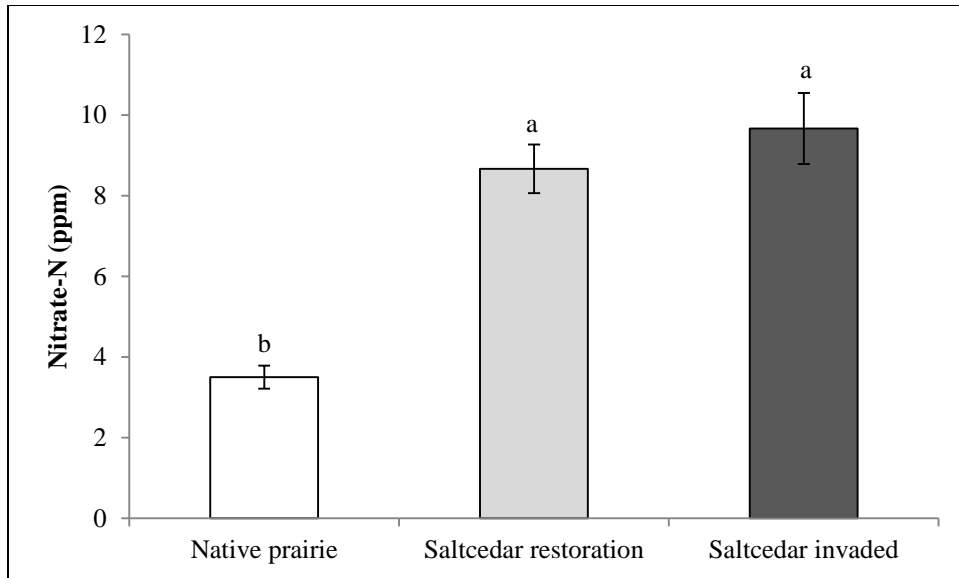
Figure 2-6 Nitrate-nitrogen concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
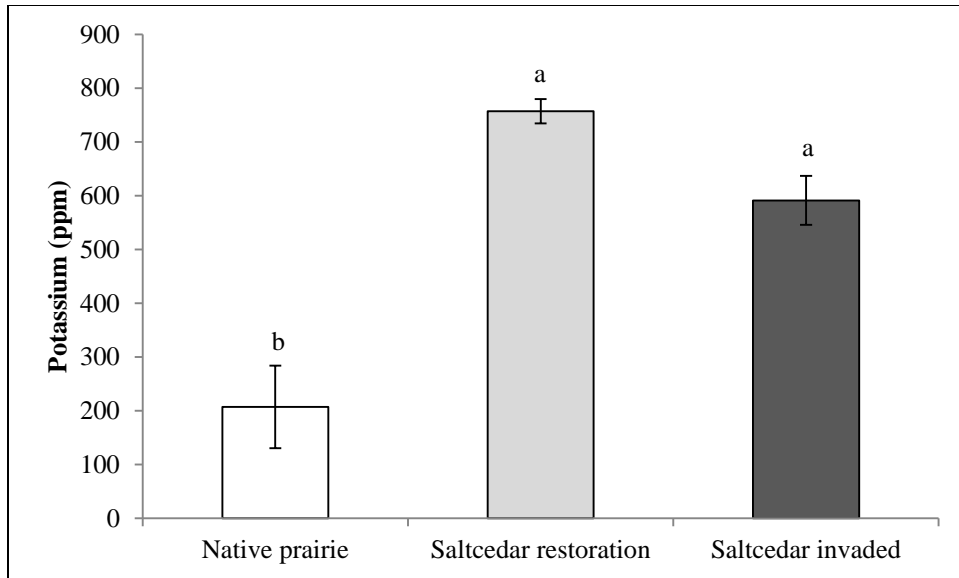
Figure 2-7 Potassium concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
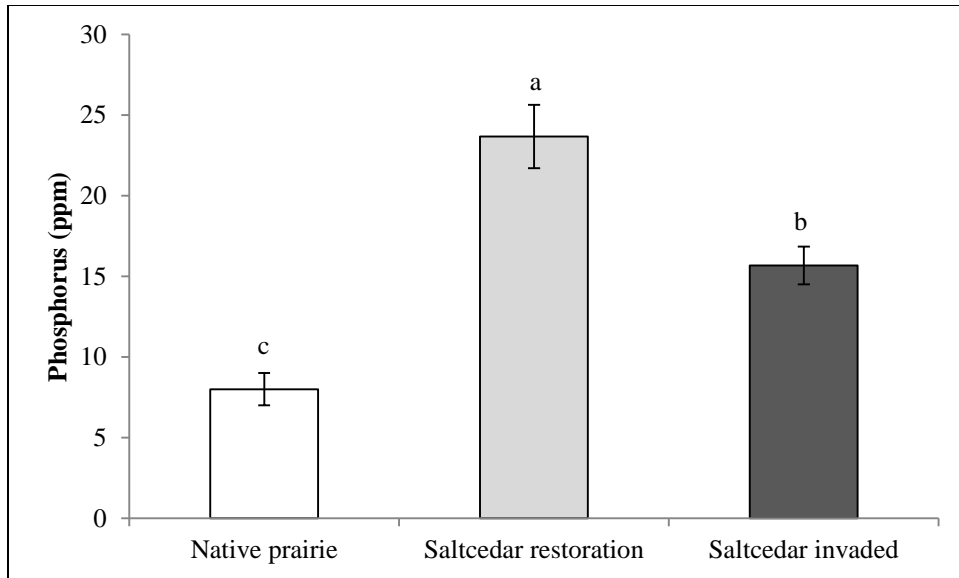
Figure 2-8 Phosphorus concentration (ppm) with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
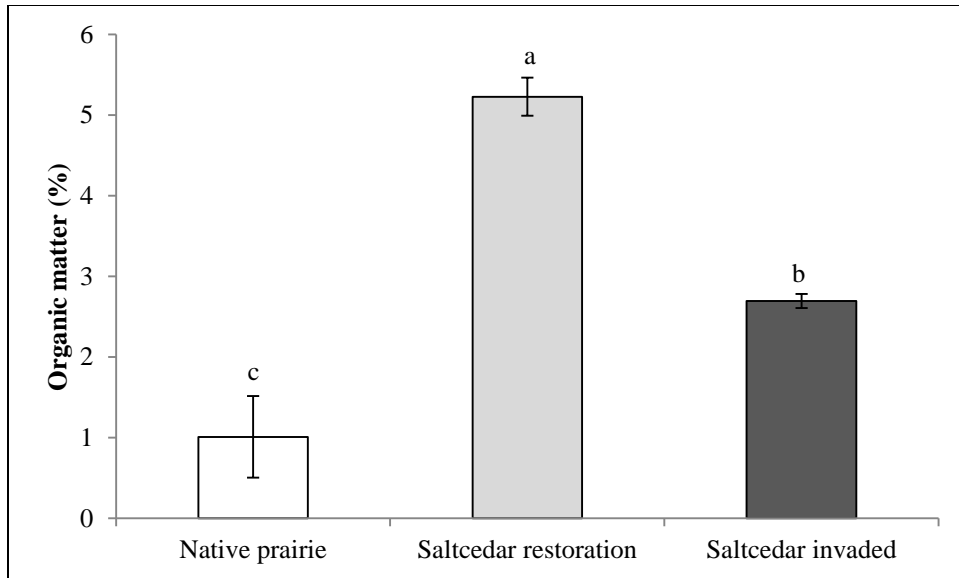
Figure 2-9 Soil organic matter percentage with mean values and standard errors in three soil sources: native prairie, saltcedar restoration, and saltcedar invaded near Ashland, Kansas, USA. Bars with the same letter are not statistically different ($P \leq 0.05$).
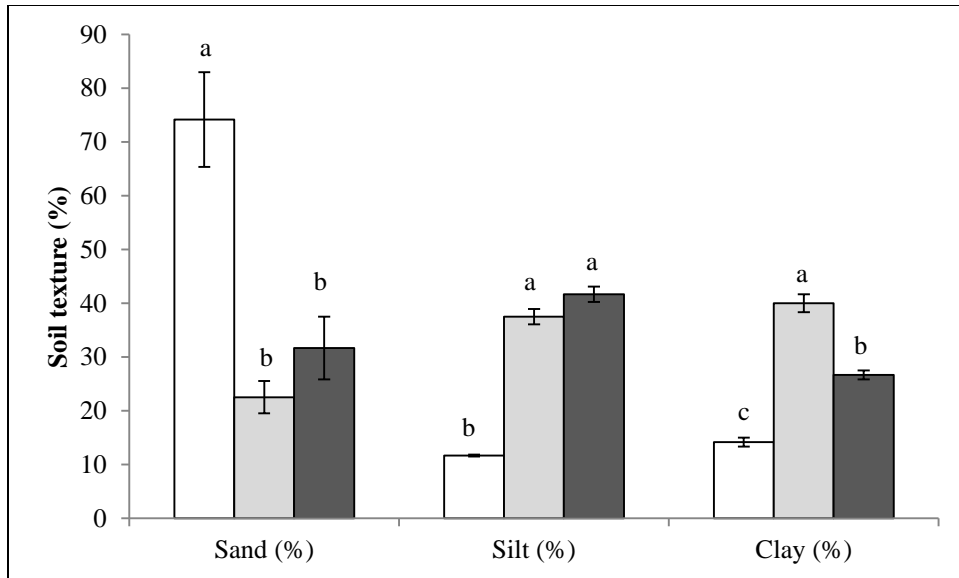
Figure 2-10 Percent soil particles with mean values and standard errors in three soil sources: native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) near Ashland, Kansas, USA. Bars with the same letter within sites are not statistically different ($P \leq 0.05$).
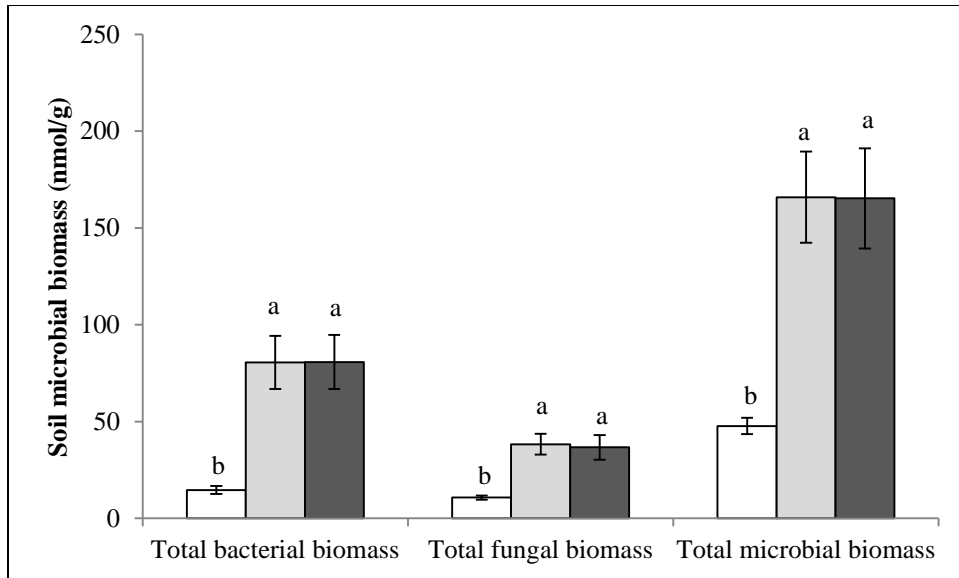
Figure 2-11 Total soil microbial biomass (nmol/g) with mean values and standard errors in three soil sources: native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) near Ashland, Kansas, USA. Bars with the same letter within growth forms are not statistically different ($P \leq 0.05$).
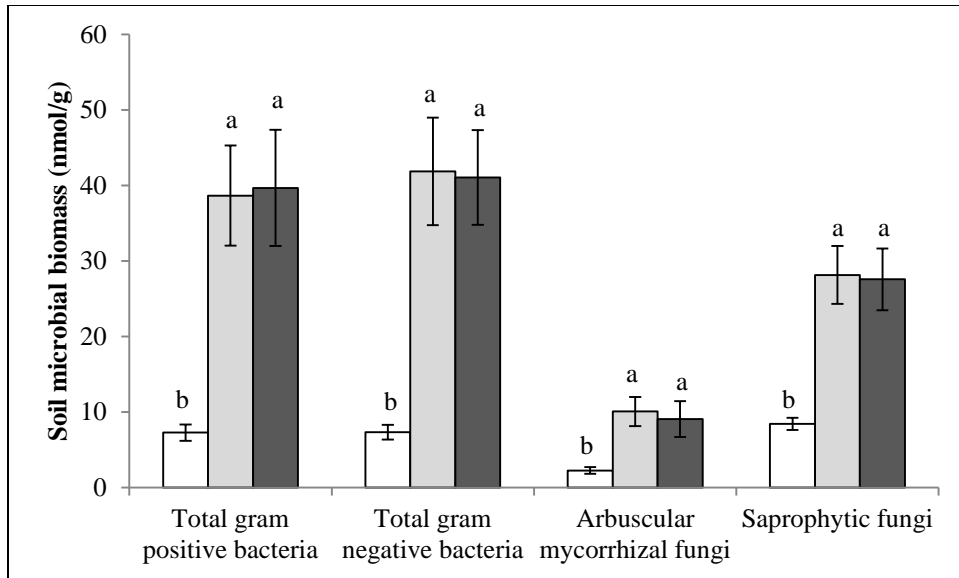
Figure 2-12 Soil microbial biomass (nmol/g) of different communities (total gram positive bacteria, total gram negative bacteria, arbuscular mycorrhizal fungi, and saprophytic fungi) with mean values and standard errors in three soil sources: native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) near Ashland, Kansas, USA. Bars with the same letter within growth forms are not statistically different ($P \leq 0.05$).
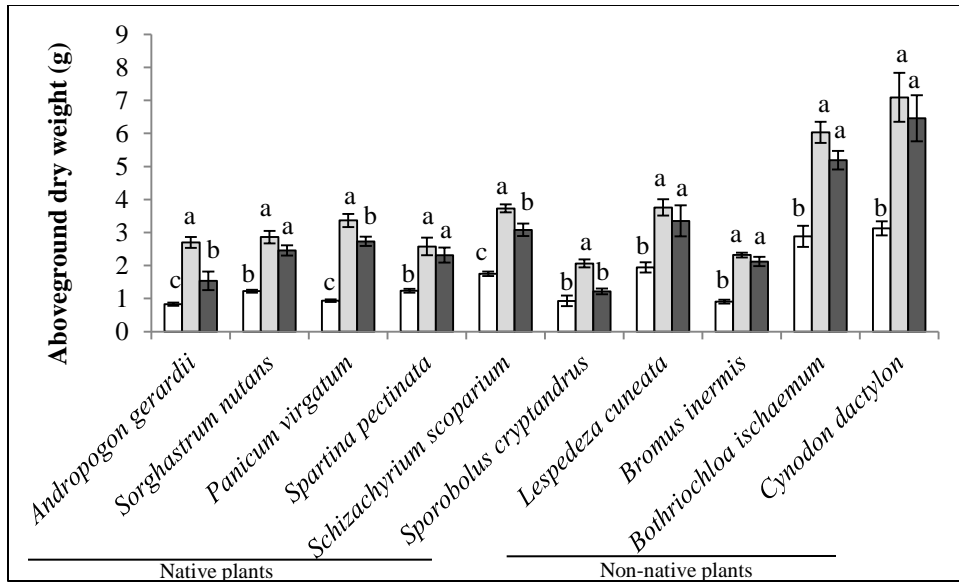
Figure 2-13 Aboveground plant dry weight (g) of native and non-native plants grown in native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) soil sources with mean values and standard errors near Ashland, Kansas, USA. Bars with the same letter for each species in native prairie, saltcedar restoration, and saltcedar invaded soil sources are not statistically different ($P \leq 0.05$).
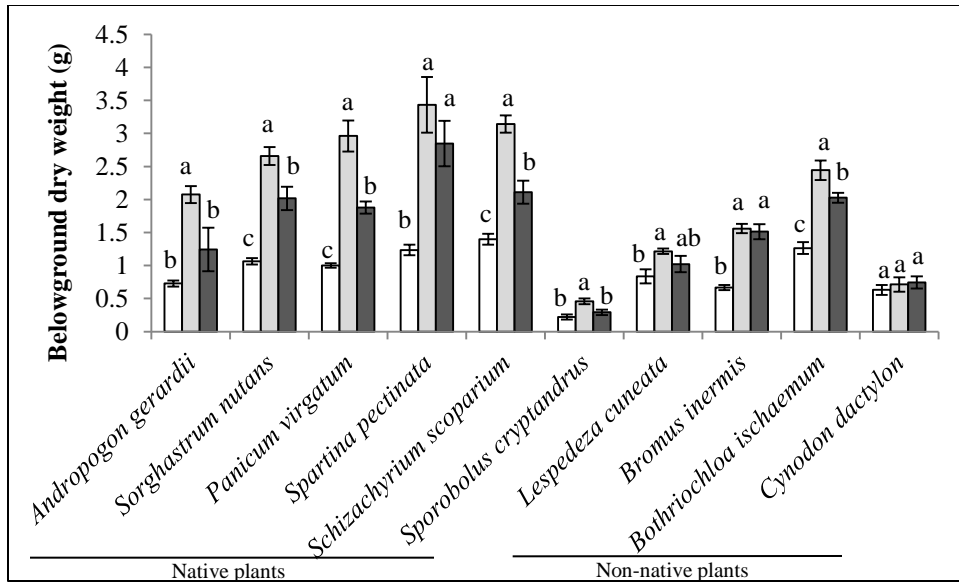
Figure 2-14 Belowground plant dry weight (g) of native and non-native plants grown in native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) soil sources with mean values and standard errors near Ashland, Kansas, USA. Bars with the same letter for each species in native prairie, saltcedar restoration, and saltcedar invaded soil sources are not statistically different (*P*≤0.05).
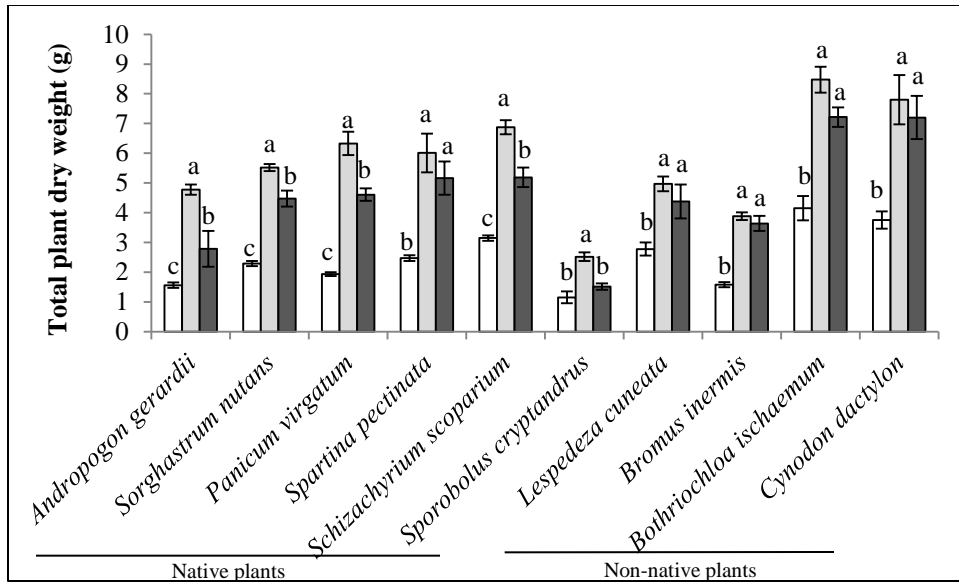
Figure 2-15 Total plant dry weight (g) of native and non-native plants grown in native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) soil sources with mean values and standard errors near Ashland, Kansas, USA. Bars with the same letter for each species in native prairie, saltcedar restoration, and saltcedar invaded soil sources are not statistically different ($P \leq 0.05$).
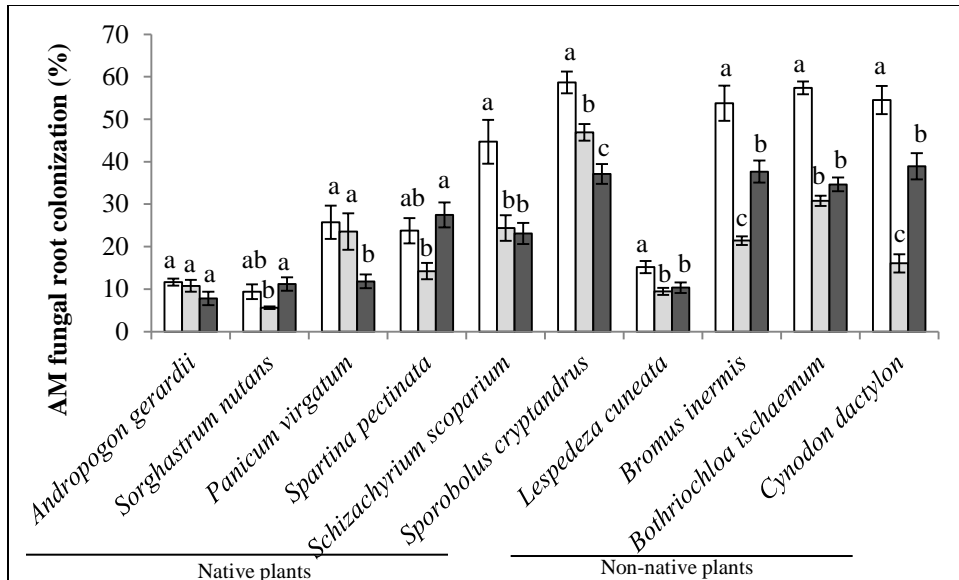
Figure 2-16 Percentage arbuscular mycorrhizal (AM) fungal root colonization of native and non-native plants grown in native prairie (no filled bar), saltcedar restoration (light gray bar), and saltcedar invaded (dark bar) soils near Ashland, Kansas, USA. Bars with the same letter for each species in native prairie, saltcedar restoration, and saltcedar invaded soil sources are not statistically different ($P \leq 0.05$).

CHAPTER III

ASSESSING PLANT-SOIL FEEDBACKS FOLLOWING *LESPEDEZA CUNEATA* (DUMONT) G. DON. (SERICEA LESPEDEZA) INVASION

ABSTRACT

Lespedeza cuneata (sericea lespedeza) is a nitrogen-fixing perennial legume, well known in the southern and midwestern United States as a highly invasive plant of grasslands and other habitats. Little is known about belowground alterations following *L. cuneata* invasion. Understanding potential plant-soil feedbacks may be a critical aspect of the restoration of native ecosystems. I conducted both field and greenhouse studies to assess plant-soil feedbacks. Field study assessed abiotic (N, P, K, organic matter, and soil pH) and biotic (vegetation and microbial communities) soil properties. Soil was collected from areas with: 1) vegetation dominated by *L. cuneata*; 2) *L. cuneata* removed using herbicide (restoration areas); and 3) non-invaded native prairie. Greenhouse study assessed plant-soil feedbacks indirectly through biomass production of six native and three non-native grasses planted into soil collected from the same areas as the field study. Plants were grown for 16 weeks, at which time total biomass was determined. Percent root colonization by arbuscular mycorrhizal (AM) fungi was determined microscopically. I hypothesized plant-soil feedbacks function through alterations in soil nutrients and

microbial communities following *L. cuneata* invasion. Non-native plants compared to native plants grown in soil from *L. cuneata* invaded areas compared to growth in soil from native areas would have greater biomass. I hypothesized that the legacy effect will persist after the removal of *L. cuneata.* My results indicated higher nitrate-nitrogen, lower soil organic matter, and lower pH in soil from *L. cuneata* invaded and *L. cuneata* restoration sites relative to native prairie. Phospholipid fatty acid analysis indicated lower AM fungal biomass in *L. cuneata* invaded and *L. cuneata* restoration sites relative to native prairie. The total plant species richness in native prairie and *L. cuneata* restoration sites was over twice that of *L. cuneata* invaded sites. All plant species (native and non-native) produced greater total biomass when grown in soils with a history of *L. cuneata* invasion, as compared to production in soil from native prairie. All plants (except *Panicum virgatum*) grown in soils from *L. cuneata* invaded and *L. cuneata* restoration areas had lower AM fungal root colonization than plants grown in soil from native prairie.

INTRODUCTION

Biological invasion by non-native plants results in negative economic and environmental effects (Vitousek et al. 1997, Pimentel et al. 2000). Invading species alter the structure and functioning of ecosystems, as well as native biological diversity, with significant economic costs either through direct losses or control efforts (Vitousek et al. 1997, Pimentel et al. 2000). Most previous studies on biological invasion in terrestrial ecosystems focus on aboveground features, with little attention given to the belowground properties, although invasive organisms affect both above- and belowground properties (Bardgett and Wardle 2010, Inderjit and van der Putten 2010). In addition, biological invasion of aboveground ecosystem components can affect belowground ecosystem components, and vice versa (Stinson et al. 2006, Wolfe et al. 2008, Bardgett and Wardle 2010, Inderjit and van der Putten 2010). For example, invasion by non-native nitrogen-fixing *Myrica faya* in Hawaii increased soil nitrogen and thereafter enhanced the growth of introduced plants (Vitousek et al. 1987, Vitousek and Walker 1989, Adler et al. 1998). In addition, the non-native plant *Alliaria petiolata* in North America disrupted mutualistic associations between native tree seedlings and arbuscular mycorrhizal (AM) fungi (Stinson et al. 2006).

Interactions between plants and their biotic and abiotic soil environment are conceptualized as plant-soil feedbacks (Bever et al. 1997, Ehrenfeld et al. 2005). Plant-

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soil feedbacks have gained attention as a mechanism that could explain biological

invasion (Reinhart and Callway 2006, Kulmatiski et al. 2008). Plant-soil feedbacks have

two phases: plants change soil properties and plants respond to these changes (Bever

1994, Ehrenfeld et al. 2005). Plant-soil feedbacks can be positive if the growth of the

non-native plant increases with plant induced alterations in soil conditions or negative, if

non-native plant growth is reduced following alterations in soil biotic or abiotic

properties (Bever et al. 1997, Bever 2003, Ehrenfeld et al. 2005). Wilson et al. (2012)

provide evidence of a negative indirect plant-soil feedback on native grasses

(*Andropogon gerardii* and *Schizachyrium scoparium*) in sites invaded by non-native

Bothriochloa bladhii or *B. ischaemum.*

Restoration of communities invaded by invasive species requires removal of the invader,

typically followed by subsequent active reestablishment of the native community (Kardol

and Wardle 2010). Recent reviews have recognized the importance of interactions

between plants and soils for ecosystem restoration (Suding et al. 2004, Eviner and

Hawkes 2008). Most work on community-level restoration has focused on plants and

belowground abiotic factors (e.g., nutrients) that directly affect plant communities

(Maron and Connors 1996, Pickart et al. 1998, Maron and Jefferies 2001). However,

recent work has recognized soil biota as key determinants of plant community properties

(Wolfe and Klironomos 2005, Kardol et al. 2006). The effects of removal of invasive

plant species on belowground properties has only occasionally been explored (Peltzer et

al. 2009). Soil legacies after the removal of invasive plant species can be persistent and

have been observed in areas cleared of nitrogen-fixing invaders (Marchante et al. 2009).

For instance, nitrogen mineralization rates in South African fynbos (natural shrubland

vegetation) invaded by *Acacia saligna* or in coastal prairies invaded by *Lupinus arboreus* were not different from areas where the invaders had been removed (Maron and Jefferies 1999, 2001, Yelenik et al. 2004). This legacy can potentially create obstacles for restoration by facilitating re-invasion by the same or other non-native species, or preventing recovery of native plants (Vinton and Burke 1995, Maron and Connors 1996, Pickart et al. 1998, Maron and Jefferies 1999, Vinton and Goergen 2006).

Invasive plants with different physiological traits than local plants provide the mechanistic basis for feedback. The most frequently cited example is the invasion by the nitrogen-fixing species, *Myrica faya* into Hawaii which resulted in an increase in the amount of soil nitrogen, thus influencing nitrogen availability and subsequent invasion by other non-native plant species (*Schizachyrium condensatum* and *Andropogon virginicus*) (Vitousek et al. 1987, Vitousek and Walker 1989, Adler et al. 1998). Soils experiencing alterations following plant invasions may exhibit greater risk of invasion by other non-native species as described by the invasional meltdown hypothesis (Simberloff and Von Holle 1999). High levels of nutrients in the soils might cause problems for native plant species which are not able to grow under such nutrient enriched conditions (Huenneke et al. 1990, Maron and Jefferies 1999). Other examples of this include the non-native nitrogen-fixing *Acacia saligna* invasion in fynbos of South Africa which enhanced secondary invasion by the weedy grass *Ehrharta calycina* (Adler et al. 1998, Yelenik et al. 2004). Therefore, an integrated understanding of plant-soil feedback with invasive plants is necessary to manage and restore communities invaded by invasive plant species.

Invasive plants can alter the soil microbial communities through root exudation, release of anti-microbial compounds, facilitation of symbiotic relationships between roots and

soil microbes, and displacement of native plants having unique soil microbial communities (Wolfe and Klironomos 2005). Therefore, invasive species may bring about new interactions with soil microbial communities (Klironomos 2002, Kourtev et al. 2002, Reinhart and Callaway 2006). An alteration in soil microbial communities has been observed with the invasion of non-native nitrogen-fixing plants *Acacia holosericea* in Senegal and *Falcataria moluccana* in Hawaii (Allison et al. 2006, Remigi et al. 2008). Dominance by bacteria was observed after invasion of nitrogen-fixing *Falcataria moluccana* in Hawaii (Allison et al. 2006). Soil harbors a wide variety of micro- and macro-organisms. The profound effect of soil microbes on plant growth depends on the composition of various functional groups of soil organisms (e.g., bacteria, fungi, and nematodes) present in the system (Bever et al. 1997). Most vascular plants form mycorrhizal associations with arbuscular mycorrhizal (AM) fungi and many plants are highly dependent on this association for their growth and survival (Smith and Read 2008). AM fungi can benefit plants by enhancing mineral uptake, specifically phosphorus and nitrogen, and by improving drought tolerance (George et al. 1995, Hodge et al. 2001, Qiangsheng et al. 2006). AM fungal hyphae extend into the soil surrounding the roots and this hyphal network increase uptake of nutrients and water, as well as increase soil structure (Marschner and Dell 1994, Wilson et al. 2009). Therefore, mycorrhizal mutualisms have effects on both ecosystem processes and plant communities, suggesting the potential for plant-soil feedbacks.

Lespedeza cuneata (Dumont) G. Don. (sericea lespedeza) is a nitrogen-fixing perennial legume, well known in the southern and midwestern United States as a highly invasive plant of grasslands and other habitats (Eddy and Moore 1998, Brandon et al. 2004,

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Cummings et al. 2007). It was introduced into the United States in 1896 from eastern

Asia for the purposes of forage production, land reclamation, and erosion control

(Brandon et al. 2004, Cummings et al. 2007). *L. cuneata* is common in highly disturbed

habitats and quality remnant plant communities such as oak savannas and prairies

(Brandon et al. 2004). Different invasive traits help explain invasion success of *L.*

cuneata. For example, *L. cuneata* can produce five times as many seeds per plant relative

to native congeners (Woods et al. 2009), and although palatable early in its phenology, *L.*

cuneata in the later growth stages is avoided by grazers due to high phenolic polymers

(lignin and tannin) production throughout the plant (Donnelly 1954**,** Hawkins 1955,

Mosjidis et al. 1990). Other possible mechanisms for successful invasion by *L. cuneata*

may include shading native vegetation, allelopathic effects on neighboring plants, and

resistance to herbivory (Kalburtji and Mosjidis 1992, 1993, Eddy and Moore 1998,

Dudely and Fick 2003, Brandon et al. 2004, Schutzenhoffer and Knight 2007, Allred et

al. 2010). Common control methods to reduce populations of *L. cuneata* are mechanical,

chemical, and fire/grazing management practices (Brandon et al. 2004, Cummings et al.

2007). However, the legacy effects after *L. cuneata* removal may be similar to other

nitrogen-fixing non-native plants (e.g., *A. saligna* in fynbos, *L. arboreus* in coastal

prairies), and may lead to challenges for restoration. Therefore, examining how plant-soil

feedbacks are influenced by *L. cuneata* invasion and *L. cuneata* removal may be an

important component for the restoration of *L. cuneata* invaded ecosystems.

My study investigated vegetation, soil nutrients, and soil microbial communities of *L.*

cuneata invaded sites, sites following chemical removal of *L. cuneata* (hereafter referred

to as *L. cuneata* restoration), and adjacent native prairie sites. Field and greenhouse

studies were conducted to assess plant-soil feedbacks associated with *L. cuneata* invasion. The objectives of the field study were to assess potential differences in plant species richness and canopy cover, soil nutrients, and biomass of soil microbial communities from sites with varying stages of *L. cuneata* invasion (*L. cuneata* invaded, *L. cuneata* restoration, and native prairie). In general, I expected greater plant biomass, reduced plant species richness, greater soil nitrogen availability, and altered soil microbial biomass in the *L. cuneata* invaded sites. I hypothesized that greater specific leaf area and canopy cover of *L. cuneata* would lead to reductions in native plant species survival, thereby reducing plant species richness. The nitrogen-fixing ability of *L. cuneata* would lead to increased soil nitrogen with a concomitant alteration in soil microbial biomass production. Soil legacies after the removal of non-native nitrogen-fixing plants can be persistent thus, I hypothesized that soil nutrients and microbial communities would not change in *L. cuneata* restoration sites compared to *L. cuneata* invaded sites.

Because restoration efforts may be hindered by alterations in soil properties, I assessed growth of native grass species planted into soil collected from *L. cuneata* invaded, *L. cuneata* restoration, and native sites. The objectives of this greenhouse study were to assess plant-soil feedbacks indirectly through biomass production and AM fungal root colonization of six native warm-season grasses planted into three different soils collected from the same three sites as examined in my previously described field study. To determine if *L. cuneata* facilitates the growth of other non-native plant species as described by the invasional meltdown hypothesis, I also assessed biomass production and AM fungal root colonization of three non-native plant species. I hypothesized that non-

native plant species would produce greater biomass in soil collected from *L. cuneata* invaded and *L. cuneata* restoration sites compared to soil from native sites as those non-native plants are able to grow under nutrient enriched conditions compared to native plants. Given that *L. cuneata* is of different functional form (i.e., nitrogen-fixing legume) than the dominant native grasses, invasion by *L. cuneata* could alter composition and function of soil microbial communities. Therefore, I hypothesized that both native and non-native species planted into soil from *L. cuneata* invaded and *L. cuneata* restoration sites would have lowered AM fungal root colonization compared to soil from native sites.

MATERIALS AND METHODS

Soil for the field and greenhouse studies were collected from pastures within the

Oklahoma State University Range Research Station, about 21 km southwest of Stillwater,

Oklahoma, USA (latitude: 36°N, longitude: 97°W). This study area has been burned

historically to minimize the encroachment of eastern redcedar (*Juniperus virginiana* L.)

(McCollum et al. 1999, Fuhlendorf and Engle 2004). Several hundred hectares within

these pastures have been invaded by *L. cuneata*, while the rest of the area exists as a

matrix of native tallgrass prairie within oak-cedar woodlands. Dominant tallgrass prairie

grasses include *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Andropogon*

gerardii Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), *Panicum*

virgatum L. (switchgrass), and *Sporobolus asper* (Michx.) Kunth (tall dropseed).

Dominant forbs are *Ambrosia psilostachya* DC. (western ragweed) and *Gutierrezia*

dracunculoides (DC.) S.F. Blake (common broomweed). Oak-cedar woodland include

Quercus stellata Wang. (post oak), *Q. marilandica* Münch. (blackjack oak), and *Celtis*

spp. (hackberry). Nomenclature of all species was based on USDA Plant Database

(2012). Three replicates of three different soil sources were selected: 1. *L. cuneata*

invaded "treatment" with more than 60% coverage and no history of attempts to

eliminate the invasive, 2. *L. cuneata* restoration "treatment" in which a foliar application

of PastureGard ® (1.75 liters per ha) was applied in June 2010, resulting in over 85%

removal of *L. cuneata*, and 3. Native tallgrass prairie with no history of *L. cuneata* invasion. All sites were located within 2 km of each other. Soil within all study sites was characterized as clay loam.

Field study: Plant-soil feedbacks were evaluated by assessing plant species composition and soil chemical and biological components. To characterize the plant species richness and canopy cover in three replicates in each "treatment", we established a total of nine transects. Along each 10 m transect, five 1 m^2 quadrats were established at random points. Canopy cover for all plant species present was assessed using the Daubennmire method (Daubenmire 1959).

Based on canopy coverage data, dominant plant species at the native sites were determined to be *S. scoparium*, while *A. psilostachya* was dominant in the *L. cuneata* restoration sites, and *L. cuneata* invaded sites were dominated by *L. cuneata*. Dominant plant species were identified for further assessment as they will drive ecosystem processes and are the major drivers of ecological properties according to "mass ratio hypothesis" (Grime 1998). Sampling of shoot and root biomass and arbuscular mycorrhizal (AM) fungal root colonization was targeted using the dominant species of each site. Aboveground biomass of the dominant plant species at three different random points was clipped by selecting corresponding plant along the same transect used for plant species composition estimates. Belowground biomass of the same plant was assessed by collecting roots using a 237.5 cm^3 (5.5 cm diameter and 10 cm deep) soil corer and washing soil from the roots. Both above- and belowground components were dried in an oven at 60ºC for 3 days to determine dry weight. To determine AM fungal root colonization, three other random soil core samples of each dominant plant species on

the same transect were collected. Roots from these soil cores were washed free of soil with tap water. To measure the percentage of total root length colonized by AM fungal structures, roots were stained with trypan blue and examined under a compound microscope following the magnified gridline intersect method (McGonigle et al. 1990).

To assess soil nutrient composition, a 10 m transect was established across each site (*L. cuneata* invaded, *L. cuneata* restoration, and native prairie). At each site, three transects were established, along which soil was collected from the top 10 cm at 1 m intervals and homogenized. Soils were sieved through 2 mm sieve to remove large plant roots and stones. A 50 g subsample of soil from each 10 m transect at each site was analyzed separately for the determination of soil pH, nutrients (nitrate-nitrogen, ammonium-nitrogen, soil organic matter, total nitrogen, plant-available phosphorus, and potassium), and soil microbial biomass and community composition. Soil pH and nutrient analyses were conducted at the Oklahoma State University Soil, Water and Forage Analytical Laboratory. Soil samples were dried at room temperature, sieved through 2 mm sieve, and ground. Soil pH was determined through 1:1 soil to water extraction method using Titralab 865 pH electrode (Rhoades 1982). For nitrate-nitrogen and ammonium-nitrogen, soil samples were extracted with 1M KCl solution and analyzed using a LACHAT Quickchem 8000 Flow Injection Autoanalyzer (LACHAT 2000, Zhang and Kress 2001). Soil phosphorus and potassium were extracted with Mehlich III solution and analyzed using inductively coupled plasma emission spectroscopy (ICP) (Zhang and Kress 2001).

Effects of *L. cuneata* soil feedback on soil microbial composition was determined using phospholipid fatty acid analysis (PLFA) (Frostegård et al. 1993, Kourtev et al. 2002, Batten et al. 2006, White and Rice 2009). Fatty acids are components of cell membranes

and generally constitute a relatively constant proportion of the biomass of an organism. Certain groups of microorganisms have different "signature fatty acids" and are used to differentiate different taxa or estimate bacterial or fungal biomass (Zelles 1999). Other fatty acids that cannot be distinguished between taxonomic groups can be useful in estimating total microbial biomass. Soil samples were sieved through 2 mm sieve and were freeze dried for 8 hours and ground. Microbial lipids were then extracted from 5 g freeze-dried soil with a solvent system that included methanol, chloroform, and a phosphate buffer. The soil-solvent mixture was separated by centrifugation and the supernatant was decanted. The centrifugation was repeated with the addition of 1:2 (v/v) chloroform-methanol and the supernatant was collected. Phosphate buffer was then added and the mixture separated overnight. After phase separation, the chloroform layer containing the lipids was recovered and reduced by nitrogen flow at 60˚C. Total extracted lipids were separated into neutral, glyco-, and phospholipids with chloroform, acetone, and methanol through silic acid chromatography. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector.

Fatty acid nomenclature used was that described by Frostegård et al. (1993): total number of carbon atoms: number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. *Cis* and *trans* isomers were indicated by c, and t, respectively. Anteiso- and isobranching were designated by the prefix a or i. Cy indicated cyclopropane fatty acids. The fatty acids i15:0, a15:0, i16:0, i17:0 were chosen to represent gram positive bacteria; 3-OH 14:0, 16:1ω7, cy17:0, 2-OH 16:0, 18:1ω9c, cy19:0 for gram negative bacteria; 16:1ω5c for AM fungi, 18:2ω9,12c, 18:1ω9c for

saprophytic fungi; 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 for non-specific microbes (McKinley et al. 2005). Fatty acids are expressed as nmol g^{-1} dry soil.

Greenhouse study: I collected soil from the three sites previously described in my field study (*L. cuneata* invaded, *L. cuneata* restoration, and native prairie), with three replicates in each site. Soil was sieved through a 2 mm sieve and 600 g (dry weight) was placed into plastic pots (6 cm diameter X 25 cm deep). Native warm-season grasses common in tallgrass prairie: *Andropogon gerardii* Vitman (big bluestem), *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalograss), *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Sorghastrum nutans* (L.) Nash (indiangrass), and *Sporobolus asper* (Michx.) Kunth (tall dropseed), were selected (Conant and Risser 1974, Anderson 2006) to assess plant-soil feedbacks indirectly through biomass production and AM fungal root colonization. Non-native plant species: *Bothriochloa ischaemum* (L.) Keng (old world bluestem), *Bromus inermis* Leyss. (smooth brome), and *Cynodon dactylon* (L.) Pers. (bermudagrass), were also selected for biomass production and AM fungal root colonization, as these species are invading into native prairies (Harmoney et al. 2004, Vinton and Goergen 2006, Weir et al. 2009) in this region. Nomenclature of all species was based on USDA (2012). The experimental design included 3 sites (*L. cuneata* invaded, *L. cuneata* restoration, and native prairie) x 3 replicates of each site x 9 plant species (6 native and 3 non-native species, common in Central Great plains) x 10 replicate pots per soil source for a total of 810 pots.

Seeds of all plant species were obtained from a local commercial seed company (Johnston Seed Company, Enid, Oklahoma). Seeds were germinated in vermiculite and seedlings at the second leaf stage were transplanted into pots filled with 600 g (dry

weight) soil collected from each site. Pots were arranged in a randomized complete block design in a greenhouse maintained at 20-25°C. Plants were harvested after 16 weeks. All pots were watered daily. Roots washed free of soil and shoots and roots were oven-dried at 60°C for 72 hours to determine shoot, root, and total dry weights. To measure the percentage of total root length colonized by AM fungal structures, roots were subsampled, stained with trypan blue, and examined using a compound microscope. Percent AM fungal root colonization followed the magnified gridline intersect method (McGonigle et al. 1990).

Statistical analysis For the field study, soil characteristics (pH, inorganic ammonium-nitrogen, nitrate-nitrogen, soil organic matter, total nitrogen, plant-available phosphorus, potassium, and biomass of soil microbial communities) and plant species richness were analyzed with one-way ANOVA using General Linear Models (GLM) of the three soil sites (*L. cuneata* invaded, *L. cuneata* restoration, and *L. cuneata* non-invaded native prairie) with soil site as single factor. Mean differences of soil characteristics were compared using least square differences (LSD) grouping. Mean soil characteristic values were presented for each soil source. All data were analyzed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). A significance level of 0.05 was used for all statistical tests.

Biomass and percentage AM fungal root colonization of dominant plant species in each soil types were analyzed with one-way ANOVA using GLM. I performed Canonical Correspondence Analysis (CCA) in Canoco for Windows 4.5 to evaluate differences in plant species composition among the three different soil sites (ter Braak and Šmilauer 2002). Since the presence of *L. cuneata* was the primary factor distinguishing among

three soil sites (*L. cuneata* invaded, *L. cuneata* restoration, and native prairie), this species was excluded from the input data for multivariate tests.

For the greenhouse study, shoot, root, total biomass, and percentage AM fungal root colonization were analyzed separately for each plant species with one-way ANOVA using GLM for soil source as fixed factor. For biomass and percentage AM fungal root colonization, the statistical differences among soil sites were analyzed using LSD *post hoc* tests. All data were analyzed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). A significance level of 0.05 was used for all statistical tests.

RESULTS

Soil abiotic and biotic properties I assessed alterations in soil pH, soil nutrients and biomass of major functional groups of the soil microbial communities. Soil pH and soil nutrients of *L. cuneata* invaded and *L. cuneata* restoration sites were not significantly different from one another (Figs. 3-1 – 3-3). Soil pH was significantly greater in the soil from native prairie compared to *L. cuneata* invaded and *L. cuneata* restoration sites (Fig. 3-1). Soil inorganic nitrate-nitrogen was greater in both *L. cuneata* invaded and *L. cuneata* restoration sites, compared to native prairie sites (Fig. 3-2). Soil inorganic phosphorus was significantly greater in *L. cuneata* invaded sites compared to native prairie sites, but soil from *L. cuneata* restoration sites was intermediate between the *L. cuneata* invaded and native sites (Fig. 3-3). However, soil inorganic ammonium-nitrogen and potassium were not significantly different among soils from any of the sites (2.54-3.5 ppm for ammonium-nitrogen and 82-130 ppm for potassium). Percentage of soil organic matter, soil organic carbon, and total nitrogen were all significantly greater in native prairie sites compared to either *L. cuneata* invaded or *L. cuneata* restoration sites (Figs. 3-4 – 3-6).

The results showed alteration in the biomass of major functional groups of soil microbial communities in soil from *L. cuneata* invaded and *L. cuneata* restoration areas (Figs. 3-7 – 3-8). Total fungal and microbial biomass were significantly greater in native prairie and

L. cuneata invaded sites compared to *L. cuneata* restoration sites, as determined by PLFA

assessments (Fig. 3-7). However, both total bacterial and fungal biomass were not

different between native prairie and *L. cuneata* invaded sites (Fig. 3-7). There was greater

biomass of all major functional groups of soil microbial communities (total gram positive

bacteria, total gram negative bacteria, AM fungi, and saprophytic fungi) in native prairie

compared to *L. cuneata* restoration sites (Fig. 3-8). Biomass of total gram negative

bacteria and AM fungi differed between native prairie and *L. cuneata* invaded sites (Fig.

3-8). AM fungal biomass was greatest in native prairie sites compared to *L. cuneata*

invaded or *L. cuneata* restoration sites (Fig. 3-8). Saprophytic fungal biomass was

significantly lower in *L. cuneata* restoration sites compared to *L. cuneata* invaded and

native prairie soils (Fig. 3-8).

The total plant species richness of native prairie or *L. cuneata* restoration sites was more

than twice that of *L. cuneata* invaded sites. Total plant species richness was highest in the

native and lowest in the *L. cuneata* invaded sites with about 16 and 6 species per m^2,

respectively (Fig. 3-9). Plant species composition in different sites was evaluated using

CCA. The first two canonical axes explained 83.3% and 100 % of variance of the species

composition in three treatments, respectively (Fig. 3-10). Native prairie soil was at the

left side of axis 1 and soil from *L. cuneata* restoration on the right side of axis. The

second axis represented *L. cuneata* invaded soil on the upper axis. The plant species

composition in *L. cuneata* restoration sites were different compared to native prairie.

Non-native *Bromus* sp. was observed in *L. cuneata* restoration sites.

In the field study, *L. cuneata* showed the greatest shoot and root dry weight and *A.

psilostachya* had the lowest relative to *S. scoparium* (Fig. 3-11 – 3-12). However,

dominant species, *S. scoparium* in native prairie had the greatest percentage of root colonized by AM fungi with no differences between *L. cuneata* and *A. psilostachya* (Fig. 3-13).

Greenhouse Study I examined plant-soil feedbacks following *L. cuneata* invasion and *L. cuneata* restoration indirectly through biomass production and arbuscular mycorrhizal (AM) fungal root colonization of native and non-native grasses grown in soils from the different sites. Biomass production of both native and non-native grasses were increased when seedlings were grown in soil from *L. cuneata* invaded or *L. cuneata* restoration sites compared to soil collected from native prairie not invaded by *L. cuneata* (Fig. 3-14 – 3-16). However, all plants (except *P. virgatum*) grown in soils collected from *L. cuneata* invaded and *L. cuneata* restoration sites were less colonized by AM fungi than plants grown in soil from native prairie sites (Fig. 3-17).

DISCUSSION

Plant-soil feedbacks can operate through different pathways involving both abiotic and biotic processes. This study demonstrates that *L. cuneata* invasion significantly alters soil abiotic and biotic characteristics and the legacy effect of the invasion persists at least one year following the removal of *L. cuneata*.

Soil abiotic and biotic properties

The efficacy of restoration practices that remove non-native nitrogen-fixing plants will depend on how long elevated levels of nitrogen persist. My study indicated that the legacy effect of *L. cuneata* on soil properties is likely to remain for at least a year after removal of the non-native legume.

The presence of non-native nitrogen-fixing plants has been shown to profoundly alter nitrogen cycling, differentially affect the growth of native and non-native plant species, and alter other soil properties (Vitousek et al. 1987, Vitousek and Walker 1989). My study is in agreement with earlier studies that non-native nitrogen-fixing plants increase available nitrogen in an ecosystem (Vitousek et al. 1987, Vitousek and Walker 1989, Yelenik et al. 2004); my results showed greater soil nitrate-nitrogen from *L. cuneata* invaded sites compared to native prairie. My results also showed that a year after removal of *L. cuneata*, the soil nitrogen availability was still much higher than native areas thus

indicating that invasion by *L. cuneata* alters soil nitrogen and subsequent removal does

not immediately eliminate that legacy of invasion. Previous studies have shown that the

alteration in soil properties induced by a non-native nitrogen-fixing species (e.g., *Acacia*

longifolia), persists for several years after removal leaving a legacy of altered soil

properties (Maron and Jefferies 1999, Maron and Jefferies 2001, Yelenik et al. 2004,

Marchante et al. 2009). The greater soil nitrate-nitrogen in *L. cuneata* restoration sites

might be due to increased decomposition of dead plant materials, leading to nitrogen

mineralization, therefore, higher nitrate-nitrogen available in the soils (Knicker 2004).

However, I found no significant differences for soil ammonium-nitrogen among any

sites, which suggested that ammonium might be taken up by plants and microbes

(Maathuis 2009). My results showed greater soil pH in the soil from native sites

compared to *L. cuneata* invaded and *L. cuneata* restoration sites. Soil pH in *L. cuneata*

invaded and *L. cuneata* restoration sites was about 5, in an acidic range. Plant phenolic

compounds affect different soil properties and some functional groups of phenolic

compounds may be the source of H^+ after dissociation and thus lowered soil pH (Inderjit

and Malik 1997, Brady and Weil 2008). *L. cuneata* contains phenolic compounds

(Langdale and Giddens 1967). Therefore, low pH in *L. cuneata* invaded and *L. cuneata*

restoration areas could be due to production of phenolic acids by *L. cuneata*. Soil from *L.*

cuneata invaded sites had greater phosphorus than native prairie areas and a trend toward

greater phosphorus concentrations (although not statistically significant *P*>0.05) in *L.*

cuneata restoration soil sources. *L. cuneata* contains phenolic compounds thus, carboxyl

or hydroxyl groups of phenolic acids might have enhanced phosphorus solubilization via

the chelation of Fe or Al (Ae et al. 1990, Heim et al. 2000) thus increasing the concentration in soil from *L. cuneata* invaded sites.

Increased nitrogen availability can stimulate labile material decomposition but may retard decomposition of recalcitrant soil organic matter (Knorr et al. 2005). The greater percentage of soil organic matter and soil organic carbon in native prairie soil as compared to soil from *L. cuneata* invaded and *L. cuneata* restoration areas could be due to highly stabilized soil organic matter that is less susceptible to changes in mineralization rates (Anderson 1991). A possible explanation for reduced soil carbon in soil from *L. cuneata* invaded and *L. cuneata* restoration areas could be due to enhance soil organic matter decomposition through changes in nitrogen availability (Kirschbaum et al. 2008). The increasing distribution and abundance of non-native plant species is well documented with few studies addressing the consequences for carbon storage (Jackson et al. 2002, Bradley et al. 2006, Litton et al. 2006) thus, detailed studies may explain carbon storage with biological invasion.

Non-native plant species have been reported to alter both the composition and functional properties of soil biota within 1 to 2 years of invasion (Ehrenfeld 2004). Two years following the invasion of *Bromus tectorum* in an arid grassland in Utah, significant changes in microbial community function occurred, as indicated by altered nitrogen cycling and shifts in soil community composition (Belnap and Phillips 2001, Evans et al. 2001). In my study, the analysis of PLFA profiles indicated alterations of soil microbial communities of *L. cuneata* invaded and *L. cuneata* restoration sites, as compared to soil from native prairie. Total gram negative bacteria and AM fungal biomass were lower in *L. cuneata* invaded sites compared to native sites. No significant differences for total

fungal biomass and total soil microbial biomass were observed between *L. cuneata* invaded and native sites. However, total soil fungal biomass and total soil microbial biomass, and major functional groups of soil microbial communities (total gram positive bacteria, total gram negative bacteria, and saprophytic fungi) were lowered in soil from *L. cuneata* restoration areas. Herbicides used to control invasive plants may also exert effects on soil microbial communities (Weidenhamer and Calloway 2010, Gupta et al. 2011). Baarschers et al. (1988) showed toxicity of triclopyr to fungi and bacteria in laboratory experiments. In my study, reduction in biomass of major functional groups of soil microbial communities (total gram negative bacteria, and saprophytic fungi) and total microbial biomass in *L. cuneata* restoration sites compared to *L. cuneata* invaded and native sites suggested negative impacts of herbicide used to remove *L. cuneata*. However, no significant difference was observed in biomass of total gram positive bacteria and AM fungi between *L. cuneata* invaded and *L. cuneata* restoration sites, which indicated that the herbicide triclopyr, used to remove *L. cuneata* in the present study had minimal or no effect at least one year post treatment. Herbicides other than triclopyr, such as glyphosate and alachlor in soil growing medium were not detrimental to AM fungi at recommended field application rates (Pasaribu et al. 2011). However, detailed study may explain the direct effect of triclopyr at various concentrations on AM fungi.

Previous studies showed host specialization, in which specific microbial communities or species, or strain associate with specific plant species (Bever 1994, Bais et al. 2006, Badri et al. 2009). Therefore, plant species can impact the composition and activity of the soil microbial community (Belnap and Phillips 2001, Kourtev et al. 2002, Carney and Matson 2006). Yannarell et al. (2011) observed different bacterial communities in *L. cuneata*

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invaded sites from those of uninvaded areas and different fungal communities between native plants and *L. cuneata.*

Although soil harbors a wide variety of micro- and macro-organisms, in this study I focused on soil microbial communities with an emphasis on arbuscular mycorrhizal (AM) fungi. Most vascular plants form mycorrhizal associations with AM fungi and many plants are highly dependent on this association for their growth and survival (Smith and Read 2008). AM fungi can benefit plants by enhancing mineral uptake, specifically phosphorus, nitrogen, and by improving drought tolerance (George et al. 1995, Hodge et al. 2001, Qiangsheng et al. 2006). Non-native plants can alter the mycorrhizal fungal community and composition (Reinhart and Callaway 2004, Batten et al. 2006, Hawkes et al. 2006), which may lead to positive feedback and subsequent spread of the non-native species (Bever et al. 1997, Bever 2002, 2003). Previous studies showed that alteration in the composition and abundance of the AM fungal community observed for several introduced plant species have been also implicated as an important factor in the successful invasion, for example, *Centaurea maculosa* (Asian knapweed), *Alliaria petiolata* (garlic mustard) in North America (Marler et al. 1999, Roberts and Anderson 2001, Klironomos 2002).

AM fungal hyphae extend into the soil surrounding roots increasing uptake of nutrients and water (Smith and Read 2008). AM fungi aid in both phosphorus and nitrogen acquisition (Hartnett and Wilson 2002, Govindarajulu et al. 2005). Experiments focusing on the individual or combined effects of nitrogen and phosphorus have indicated that AM fungal abundance and root colonization may demonstrate positive, negative, or even neutral responses to soil nutrients (Mosse and Phillips 1971, Bååth and Spokes 1989,

Sylvia and Neal 1990, Johnson 1993, Corkidi et al. 2002, Treseder and Allen 2002, Johnson et al. 2003). In my study, both nitrogen and phosphorus were in greater concentrations in *L. cuneata* invaded and *L. cuneata* restoration sites compared to native prairie sites and therefore a reduction in AM fungal biomass in *L. cuneata* invaded soil would be expected.

Vegetation study

A greater reduction in species richness in the *L. cuneata* invaded area might be due to *L. cuneata* shading other plant species as explained by earlier studies (Eddy and Moore 1998, Brandon et al. 2004, Allred et al. 2010). One year following *L. cuneata* removal, I observed different plant species dominance in *L. cuneata* restoration sites relative to native sites. Through the results from CCA, the biplots showed different plant species composition between native and *L. cuneata* restoration sites. In native prairie sites, the plant species were more similar to native tallgrass prairie species such as *Schizachyrium scoparium*, *Panicum virgatum*, and *Symphyotrichum ericoides*. These common tallgrass prairie species were absent in *L. cuneata* restoration sites. However, removal of *L. cuneata* allowed the successful establishment of native forb species, *Ambrosia artemisiifolia, A. psilostachya, Gutierrezia dracunculoides* which presumably occurred due to increased soil nitrate-nitrogen available, similar to changes which occurred following removal of nitrogen-fixing *Lupinus arboreus*, which led high levels of ammonium and nitrate-nitrogen available to weedy grasses and forbs (Maron and Connors 1996). Non-native *Bromus* spp. also colonized *L. cuneata* restoration areas. Maron and Jefferies (1999) showed similar trends with re-invasion by non-native grasses after massive die-offs of the invasive nitrogen-fixing plant *Lupinus arboreus*. Although

annual forbs dominated areas after *L. cuneata* removal, presence of *L. cuneata* in these

areas might be due to an abundant and long-lived seed bank of *L. cuneata* (Logan et al.

1969, Woods et al. 2009). The lack of presence/dominance of native tallgrass species in

L. cuneata invaded and *L. cuneata* restoration sites was most likely due to the absence of

remnant native plants or nearby sources for dispersal, as *L. cuneata* has invaded this site

for more than 20 years (personnel communication with J. Chris Stansberry, station

superintendent at Oklahoma State University, Range Research Station, Stillwater, OK,

USA).

Both shoot and root dry weight were greatest for dominant *L. cuneata* in *L. cuneata*

invaded site, as compared to *A. psilostachya* and *S. scoparium* in *L. cuneata* restoration

and native sites respectively. Allred et al. (2010) also observed greater shoot dry weight

of *L. cuneata*, as compared to native species, *A. psilostachya* and *Andropogon gerardii*.

The greatest dry weight of *L. cuneata* could be due to extensive root system and greater

total and specific leaf area, aiding in greater resource acquisition relative to native species

(Joost and Hoveland 1986, Allred et al. 2010).

When assessing effects of *L. cuenata* invasion in percentage AM fungal root

colonization, *L. cuneata* showed significantly lower colonization relative to the native *S.*

scoparium. The greater root colonization in *S. scoparium* might be explained by a variety

of factors. First, perennial warm-season grasses such as *S. scoparium*, are obligate

mycotrophs and respond positively to mycorrhizal fungi (Wilson and Hartnett 1998).

Second, allelopathic compounds released by *L. cuneata* could be another potential factor

for the reduced AM fungal root colonization of *L. cuneata* and the legacy effect on *A.*

psilostachya. Roberts and Anderson (2001) showed that *Alliaria petiolata* (garlic

mustard) leachates negatively affected the germination of AM fungal spores and inhibited the AM fungal associations with *Lycopersicum esculenteum* (tomato). Therefore, the production of phytotoxic phenolic compounds through *L. cuneata* might have reduced AM root colonization in *L. cuneata* and the legacy of phenolic compounds on *A. psilostachya*.

Greenhouse study

The greenhouse study was conducted to examine potential plant-soil feedbacks through modifications of soil abiotic and biotic properties following *L. cuneata* invasion and *L. cuneata* removal. It was hypothesized that *L. cuneata* invaded soils would promote the growth of non-native grasses due to their ability to better utilize enhanced nitrogen availability compared to native grasses. Contrary to my hypothesis, examination of shoot- and root biomass on an individual plant basis showed growth of both native and non-native grasses were significantly greater when grown in soil from *L. cuneata* invaded and *L. cuneata* restoration sites, as compared to their biomass when grown in soil from native prairie sites. This contradicts the common assumption that high levels of nutrients in the soils might facilitate the establishment of other non-native species relative to native species as predicted by the invasional meltdown hypothesis (Adler et al. 1998, Simberloff and Von Holle 1999, Hughes and Denslow 2005). For example, non-native nitrogen-fixing *Acacia saligna* invasion enhanced secondary invasion by weedy grass *Ehrharta calycina* in the fynbos of South Africa (Yelenik et al. 2004). The greater nitrate-nitrogen in soil from *L. cuneata* invaded and *L. cuneata* restoration sites could have resulted in higher biomass of all grasses as compared to grasses grown in native prairie sites. Therefore, my study agreed with Vitousek and Walker (1989) and Hughes and Denslow

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(2005) that plants grown in soil invaded by a nitrogen-fixing non-native species accumulated more biomass relative to plants grown in soil from native prairie.

Although shoot and root biomass of plant species increased when grown in soils collected from *L. cuneata* invaded and *L. cuneata* restoration sites, compared to growth in soil from native sites, percent AM fungal root colonization of all the grasses was significantly greater when the plants were grown in native prairie areas compared to *L. cuneata* invaded and *L. cuneata* restoration sites, with the native *P. virgatum* being the only exception. The effect of root colonization by AM fungi on plant growth may vary depending on the composition and abundance of AM fungal species (van der Heijden et al. 1998) and the available soil nutrients (Reynolds et al. 2006). Therefore, one possible mechanism for the reduced AM fungal root colonization of all grasses in soil from *L. cuneata* invaded and *L. cuneata* restoration sites could be the result of a shift in AM fungal species by *L. cuneata*. As in previous studies, my results indicated that the level of AM fungal root colonization may vary depending on the available soil nutrients (Sanders and Seikh 1983, Blanke et al. 2005, Reynolds et al. 2006, Smith and Read 2008). The higher mycorrhizal colonization in native prairie is likely to be an interacting effect of lower soil nutrient availability relative to *L. cuneata* invaded and *L. cuneata* restoration sites. In my 16 week greenhouse study, the lower biomass production of plants when grown in soil from native prairie does not preclude a mycorrhizal effect as mycorrhizal colonization does not always increase plant biomass (Johnson et al. 2010). Even if plant biomass is not enhanced by the symbiosis, mycorrhizae can account for phosphorus uptake (Smith et al. 2003), increase in tiller production (e.g., *Pascopyrum smithii*) (Miller et al. 1987), protection from plant pathogens (Fitter and Garbaye 1994, Newsham et al.

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1995), or enhance drought tolerance (Ruiz-Lozano et al. 2001, Kaya et al. 2003). In addition, allelopathic compounds released by non-natives has been reported to directly inhibit the ability of AM fungi to colonize native grasses, or indirectly reduce AM fungal colonization of native grasses (Callaway and Ridenour 2004, Abhilasha et al. 2008, Inderjit et al. 2008). *L. cuneata* has been shown to produce phytotoxic phenolic compounds that are phytotoxic to other plants and these compounds might influence microbial communities and their functioning as well. Studies examining allelopathic effect of *L. cuneata* on soil microbial community may provide information for successful restoration of *L. cuneata* invaded soils.

Conclusion

In summary, the nitrogen-fixing invasive *L. cuneata* can alter plant community composition and have important legacy effects through influences on soil nutrients and soil biota. My results suggest that after removal of *L. cuneata* and subsequent changes in soil nutrient availability and soil microbial community, more than a year is required before soil nutrients and soil microbial community to return to pre-invasion levels.

The findings of this study have major implications for the restoration of native prairie systems since the impacts on soil nutrient enrichment persisted a year after removal of *L. cuneata*. Current restoration practices are almost exclusively focused on aboveground removal, while the soil abiotic and biotic properties are overlooked.

REFERENCES

Abhilasha D., N. Quintana, J. Vivanco, J. Joshi. 2008. Do allelopathic compounds in invasive *Solidago canadensis* s.I. restrain the native European flora? Journal of Ecology 96:993-1001.

Adler P.B., C.M. D'Antonio, J.T. Tunison. 1998. Understory succession following a dieback of *Myrica faya* in Hawai'i Volcanoes National Park. Pacific Science 52(1):69-78.

Ae N., J. Arihara, K. Okada, T. Yoshihara, C. Johansen. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian Subcontinent. Science 248(4954):477-480.

Allison S.D., C. Nielsen, R.F. Hughes. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology and Biochemistry 38:1537-1544.

Allred B.W., S.D. Fuhlendorf, T.A. Monaco, R.E. Will. 2010. Morphological and physiological traits in the success of the invasive plant *Lespedeza cuneata*. Biological Invasions 12:739-749.

Anderson J.M. 1991. The effects of climate change on decomposition processes in grassland and coniferous forests. Ecological Applications 1(3):326-347.

Anderson R.C. 2006. Evolution and origin of the Central Grassland of North America: Climate, fire, and mammalian grazers. Journal of the Torrey Botanical Society 133(4):626-647.

Baarschers W.H., J.G. Donnelly, H.S. Heitland. 1988. Microbial toxicity of triclopyr and related herbicides. Toxicity Assessment 3(2):127-136.Bååth E., J. Spokes. 1989. The effect of added nitrogen and phosphorus on mycorrhizal growth response and infection in *Allium schoenoprasum*. Canadian Journal of Botany 67(11):3227-3232.

Badri D.V., N. Quintana, E.G. El Kassis, H.K. Kim, Y.H. Choi, A. Sugiyama, R. Verpoorte, E. Martinoia, D.K. Manter, J.M. Vivanco. 2009. An ABC transporter

mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiology 151(4):2006-2017.

Bardgett R.D., D.A. Wardle. 2010. Aboveground- belowground linkages. Biotic interactions, ecosystem processes, and global change. Oxford series in Ecology and Evolution.

Bais H.P., T.L. Weir, L.G. Perry, S. Gilroy, J.M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57:233-266.

Batten K.M., K.M. Scow, K.F. Davies, S.P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8:217-230.

Belnap J., S.L. Phillips. 2001. Soil biota in an ungrazed grassland: Response to annual grass (*Bromus tectorum*) invasion. Ecological Applications 11(5):1261-1275.

Bever J.D. 1994. Feedback between plants and their soil communities in an old field community. Ecology 75(7):1965-1977.

Bever J.D., K.M. Westover, J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561-573.

Bever J.D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant and Soil 244:281-290.

Bever J.D. 2003. Soil community feedback and the coexistence of competitiors: conceptual frameworks and empirical tests. New Phytologist 157:465-473.

Blanke V., C. Renker, M. Wagner, K. Füllner, M. Held, A.J. Kuhn, F. Buscot. 2005. Nitrogen supply affects arbuscular mycorrhizal colonization of Artemisia vulgaris in a phosphate-polluted field. New Phytologist 166(3):981-991.

Bradley B.A., R.A. Houghton, J.F. Mustard, S.P. Hamburg. 2006. Invasive grass reduces aboveground carbon stocks in shrublands of the Western US. Global Change Biology 12:1815-1822.

Brady N.C., R.R. Weil. 2008. The nature and properties of soils. Upper Saddle River, NJ:Prentice Hall.

Brandon A.L., D.J. Gibson, B.A. Middleton. 2004. Mechanisms for dominance in an early successional old field by the invasive non-native *Lespedeza cuneata* (Dum. Cours.) G. Don. Biological Invasions 6:483-493.

Callaway R.M., W.M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2(8):436-443.

Carney K.M., P.A. Matson. 2006. The influence of tropical plant diversity and composition on soil microbial communities. Microbial Ecology 52(2):226-238.

Conant S., P.G. Risser. 1974. Canopy structure of a tall-grass prairie. Journal of Range Management 27(4):313-318.

Corkidi L., D.L. Rowland, N.C. Johnson, E.B. Allen. 2002. Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. Plant and Soil 240:299-310.

Cummings C.D., S.D. Fuhlendorf, D.M. Engle. 2007. Is altering grazing selectivity of invasive forage species with patch burning more effective than herbicide treatments? Rangeland Ecol Manage 60:253-260.

Daubenmire R. 1959. A canopy-coverage method of vegetational analysis. Northwest Science 33(1):43-64.

Donnelly E.D. 1954. Some factors that affect palatability in sericea lespedeza (Lespedeza cuneata). Agronomy Journal. 46:96-97.

Dudley D.M., W.H. Fick. 2003. Effects of sericea lespedeza residues on selected tallgrass prairie grasses. Transactions of the Kansas Academy of Science 106(3/4):166-170.

Eddy T.A., C.M. Moore. 1998. Effects of Sericea lespedeza (*Lespedeza cuneata* (Dumont) G. Don) invasion on oak savannas in Kansas. Transactions of the Wisconsin Academy of Sciences, Arts, and Letters 86:57-62.

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18:1232-1235.

Ehrenfeld J.G., B. Ravit, K. Elgersma. 2005. Feedback in the plant-soil system. Annual Review of Environment and Resources 30:75-115.

Evans R.D., R. Rimer, L. Sperry, J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications 11(5):1301-1310.

Eviner V.T, C.V. Hawkes. 2008. Embracing variability in the application of plant-soil interactions to the restoration of communities and ecosystems. Restoration Ecology 16(4):713-729.

Fitter A.H., J. Garbaye. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159:123-132.

Frostegård Å., E. Bååth, A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25(6):723-730.

Fuhlendorf S.D., D.M. Engle. 2004. Application of the fire: grazing interaction to restore a shifting mosaic on tallgrass prairie. Journal of Applied Ecology 41(4):604-614.

George E., H. Marschner, I. Jakobsen. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Critical Reviews in Biotechnology 15:257-270.

Govindarajulu M., P.E. Pfeffer, H. Jin, J. Abubaker, D.D. Douds, J.W. Allen, H. Bücking, P.J. Lammers, Y. Shachar-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819-823.

Grime J.P. 1998. Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. Journal of Ecology 86(6):902-910.

Gupta A., A. Aggarwal, M. Rathi, Khushbu. 2011. Effect of herbicide clodinafop on AM fungal biodiversity of wheat crop. The Ecoscan *In* 3rd International Conference on Climate Change, Forest Resource and Environment (ICCFRE). pp. 195-199.

Harmoney K.R., P.W. Stahlman, K.R. Hickman. 2004. Herbicide effects on established yellow old world bluestem (*Bothriochloa ischaemum*). Weed Technology 18(3):545-550.

Hartnett D.C., Gail W.T. Wilson. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant and Soil 244:319-331.

Hawkes C.V., J. Belnap, C. D'Antonio, M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369-380.

Hawkins G.E. 1955. Consumption and digestibility of Lespedeza sericea hay and Alfalfa hay plus gallotannin. Journal of Dairy Science 38(3):237-243.

Heim A., J. Luster, I. Brunner, B. Frey, E. Frossard. 2000. Effects of aluminium treatment on Norway spruce roots: Aluminium binding forms, element distribution, and release of organic substances. Plant and Soil 126:103-116.

Hodge A., C.D. Campbell, A.H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413(20):297-299.

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Huenneke L.F., S.P. Hamburg, R. Koide, H.A. Mooney, P.M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71(2):478-491.

Hughes R.F., J.S. Denslow. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forest of Hawaii. Ecological Applications 15(5):1615-1628.

Inderjit, A.U. Mallik. 1997. Effect of phenolic compounds on selected soil properties. Forest Ecology and Management 92:11-18.

Inderjit, T.R. Seastedt, R.M. Callaway, J.L. Pollock. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions 10:875-890.

Inderjit, W.H. van der Putten. 2010. Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology and Evolution 25:512-519.

Jackson R.B., J.L. Banner, E.G. Jobbágy, W.T. Pockman, D.H. Wall. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418(8):623-626.

Johnson N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecological Applications 3(4):749-757.

Johnson N.C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185:631-647.

Johnson N.C., D.L. Rowland, L. Corkidi, L.M. Egerton-Warburton, E.B. Allen. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84(7):1895-1908.

Johnson N.C., G.W.T. Wilson, M.A. Bowker, J.A. Wilson, R.M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107(5):2093-2098.

Joost R.E., C.S. Hoveland. 1986. Root development of sericea lespedeza and alfalfa in acid soils. Agronomy Journal 78(4):711-713.

Kalburtji K.L., J.A. Mosjidis. 1992. Effects of sericea lespedeza residues on warm-season grasses. Journal of Range Management 45(5):441-444.

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

Kalburtji K.L., J.A. Mosjidis. 1993. Effects of sericea lespedeza residues on cool-season grasses. Journal of Range Management 46(4):315-319.

Kardol P., D.A. Wardle. 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends in Ecology and Evolution 25(11):670-679.

Kardol P., T.M. Bezemer, W.H. van der Putten. 2006. Temporal variation in plant-soil feedback controls. Ecology Letters 9:1080-1088.

Kaya C., D. Higgs, H. Kirnak, I. Tas. 2003. Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. Plant and Soil 253:287-292.

Kirschbaum M.U.F., L.B. Guo, R.M. Gifford. 2008. Why does rainfall affect the trend in soil carbon after converting pastures to forests? A possible explanation based on nitrogen dynamics. Forest Ecology and Management 255:2990-3000.

Klironomos J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67-70.

Knicker H. 2004. Stabilization of N-compounds in soil and organic-matter-rich sediments-what is the difference? Marine Chemistry 92:167-195.

Knorr M., S.D. Frey, P.S. Curtis. 2005. Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86(12):3252-3257.

Kourtev P.S., J.G. Ehrenfeld, M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83(11):3152-3166.

Kulmatiski A., K.H. Beard, J.R. Stevens, S.M. Cobbold. 2008. Plant-soil feedbacks: a meta-analytical review. Ecology Letters 11:980-992.

Lachat. 2000. QuickChem method 10-107-04-1-A. Lachat Instrument. Milwaukee, WI 53218-1239 USA.

Langdale G.W., J.E. Giddens. 1967. Phytotoxic phenolic compounds in sericea lespedeza residues. Agronomy Journal 59:581-584.

Litton C.M., D.R. Sandquist, S. Cordell. 2006. Effects of non-native grass invasion on aboveground carbon pools and tree population structure in a tropical dry forest of Hawaii. Forest Ecology and Management 231:105-113.

Logan R.H., C.S. Hoveland, E.D. Donnelley. 1969. A germination inhibitor in the seedcoat of sericea (*Lespedeza cuneta* (Dumont) G. Don). Agronomy Journal 61:265-266.

Maathuis F.J.M. 2009. Physiological functions of mineral macronutrients. Current opinion in Plant Biology 12:250-258.

Marchante E., A. Kjøller, S. Struwe, H. Freitas. 2009. Soil recovery after removal of the N$_2$-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Biological Invasions 11:813-823.

Marler M.J., C.A. Zabinski, R.M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80(4):1180-1186.

Maron J.L., P.G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105:302-312.

Maron J.L., R.L. Jefferies. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80(2):443-454.

Maron J.L., R.L. Jefferies. 2001. Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11(4):1088-1100.

Marschner H., B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159:89-102.

McCollum III F.T., R.L. Gillen, B.R. Karges, M.E. Hodges. 1999. Stocker cattle response to grazing management in tallgrass prairie. Journal of Range Management 52(2):120-126.

McGonigle TP, MH Miller, DG Evans, GL Fairchild, JA Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501.

McKinley V.L., A.D. Peacock, D.C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry 37:1946-1958.

Miller R.M., A.G. Jastfer, J.K. Pillai. 1987. Biomass allocation in an *Agropyron smithii-Glomus* symbiosis. American Journal of Botany 74(1):114-122.

Mosjidis C.O., C.M. Peterson, J.A. Mosjidis. 1990. Developmental differences in the location of polyphenols and condensed tannins in leaves and stems of sericea lespedeza, *Lespedeza cuneata.* Annals of Botany 65(4):355-360.

Mosse B., J.M. Phillips. 1971. The influence of phosphate and other nutrients on the development of vesicular-arbuscular mycorrhiza in culture. Journal of General Microbiology 69:157-166.

Newsham K.K., A.H. Fitter, A.R. Watkinson. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. Journal of Ecology 83(6):991-1000.

Pasaribu A., R.B. Mohamad, Y. Awang, R. Othman, A. Puteh. 2011. Growth and development of symbiotic arbuscular mycorrhizal fungi, *Glomus mossea* (Nicol. And Gerd.), in alachlor and glyphosate treated soils. African Journal of Biotechnology 10(55):11520-11526.

Peltzer D.A., P.J. Bellingham, H. Kurokawa, L.R. Walker, D.A. Wardle, G.W. Yeates. 2009. Punching above their weight: low-biomass non-native plant species alter soil properties during primary succession. Oikos 118:1001-1014.

Pickart A.J., L.M. Miller, T.E. Duebendorfer. 1998. Yellow bush lupine invasion in northern California coastal dunes I. Ecological impacts and manual restoration techniques. Restoration Ecology 6(1):59-68.

Pimentel D., L. Lach, R. Zuniga, D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50(1):53-65.

Qiangsheng W., X. Renxue, H. Zhengjia. Effect of arbuscular mycorrhiza on the drought tolerance of *Poncirus trifoliate* seedlings. Fron. For. China 1:100-104.

Remigi P., A. Faye, A. Kane, M. Deruaz, J. Thioulouse, M. Cissoko, Y. Prin, A. Galiana, B. Dreyfus, R. Duponnois. 2008. The exotic legume tree species *Acacia holosericea* alters microbial soil functionalities and the structure of the arbuscular mycorrhizal community. Applied and Environmental Microbiology 74(5):1485-1493.

Reinhart K.O., R.M. Callaway. 2004. Soil biota facilitate exotic Acer invasions in Europe and North America. Ecological Applications 14(6):1737-1745.

Reinhart K.O., R.M. Callaway. 2006. Soil biota and invasive plants. New Phytologist 170:445-457.

Reynolds H.L., K.M. Vogelsang, A.E. Hartley, J.D. Bever, P.A. Schultz. 2006. Variable responses of old-field perennials to arbuscular mycorrhizal fungi and phosphorus source. Oecologia 147:348-358.

Rhoades J.D. 1982. Soluble salts. *In* Methods of soil analysis. Part 2. Chemical and microbiological properties. Second Edition. American Society of Agronomy Inc. Soil Science Society of America Inc. Publisher Madison, Wisconsin USA. Pp. 167-179.

Roberts K.J., R.C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146(1):146-152.

Ruiz-Lozano J.M., C. Collados, J.M. Barea, R. Azcón. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytologist 151(2):493-502.

Sanders F.E., N.A. Sheikh. 1983. The development of vesicular-arbuscular mycorrhizal infection in plant root systems. Plant and Soil 71:223-246.

Schutzehhofer M.R., T.M. Knight. 2007. Population-level effects of augmented herbivory on *Lespedeza cuneata*: Implications for biological control. Ecological Applications 17(4):965-971.

Simberloff D., B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological Invasions 1:21-32.

Smith S.E., D. Read. 2008. Mycorrhizal Symbiosis. Third Edition. Elsevier Ltd.

Smith S.E. F.A. Smith, I. Jakobsen. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133(1):16-20.

Stinson K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4(5):0727-0731.

Suding K.N., K.L. Gross, G.R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 19(1):46-53.

Sylvia D.M., L.H. Neal. 1990. Nitrogen affects the phosphorus response of VA mycorrhiza. New Phytologist 115(2):303-310.

ter Braak C.J.F., P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power (Ithaca , NY,   USA), 500p.

Treseder K.K., M.F. Allen. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. New Phytologist 155(3):507-515.

USDA, NRCS (2012). The PLANTS Database (http://plants.usda.gov, 1 November 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

van der Heijden M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

Vinton M.A., E.M. Goergen. 2006. Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. Ecosystems 9:967-976.

Vinton M.A., I.G. Burke. 1995. Interactions between individual plant species and soil nutrient status in shortgrass steppe. Ecology 76(4):1116-1133.

Vitousek P.M., C.M. D'Antonio, L.L. Loope, M. Rejmanek, R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21(1):1-16.

Vitousek P.M., L.R. Walker, L.D. Whiteaker, D. Mueller-Dombois, P.A. Matson. 1987. Biological invasion by Myrica faya alters ecosystem development in Hawaii. Science 238:802-804.

Vitousek P.M., L.R. Walker. 1989. Biological invasion by *Myrica faya* in Hawai'i: Plant demography, nitrogen fixataion, ecosystem effects. Ecological Monographs 59(3):247-265.

Weidenhamer J.D., R.M. Callaway. 2010. Direct and indirect effects of invasive plants on soil. Journal of Chemical Ecology 36:59-69.

Weir J.R., T.G. Bidwell, R.D. Elmore, K.R. Hickman, S.D. Fuhlendorf, D.M. Engle. 2009. Weed control on rangelands. Oklahoma Cooperative Extension Service NREM-2882. pp. 1-6.

White P.M, C.W. Rice. 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. Soil Sci. Soc. Am. J. 73(1):138-145.

Wilson G.W.T., C.W. Rice, M.C. Rillig, A. Springer, D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12:452-461.

Wilson G.W.T., D.C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85(12):1732-1738.

Wilson G.W.T., K.R. Hickman, M.M. Williamson. 2012. Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22:327-336.

Wolfe B.E., J.N. Klironomos. 2005. Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6):477-487.

Wolfe B.E., V.L. Rodgers, K.A. Stinson, A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology 96:777-783.

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

Yannarell A.C., R.R. Busby, M.L. Denight, D. Gebhart, S.J. Taylor. 2011. Soil bacteria and fungi respond on different spatial scale to invasion by the legume *Lespedeza cuneata*. Frontiers in Microbiology 2:1-12.

Yelenik S.G., W.D. Stock, D.M. Richardson. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12(1):44-51.

Zelles L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. 29:111-129.

Zhang H., M. Kress. 2001. Oklahoma state university soil water and forage analytic laboratory procedures manual. Stillwater, OK.
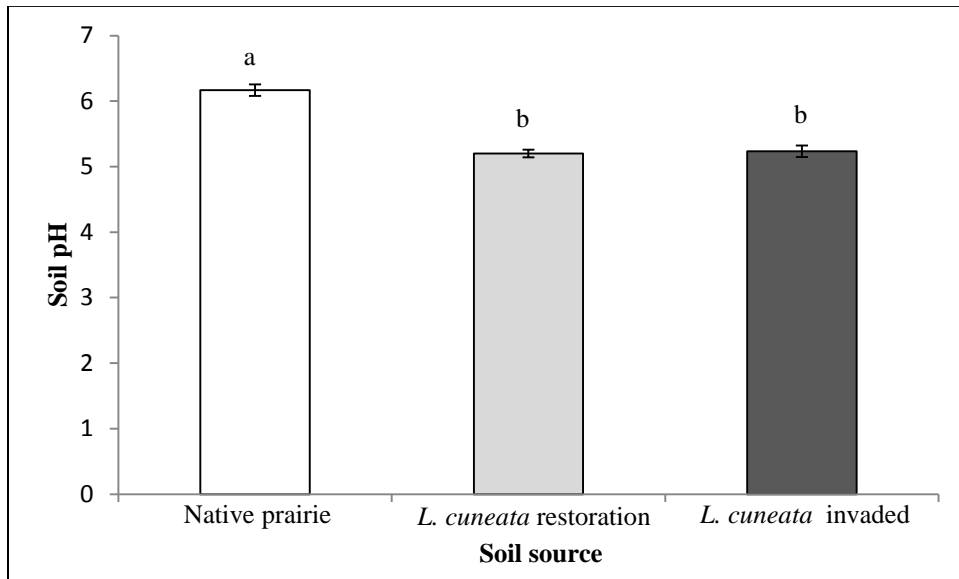
Figure 3-1 Soil pH with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).
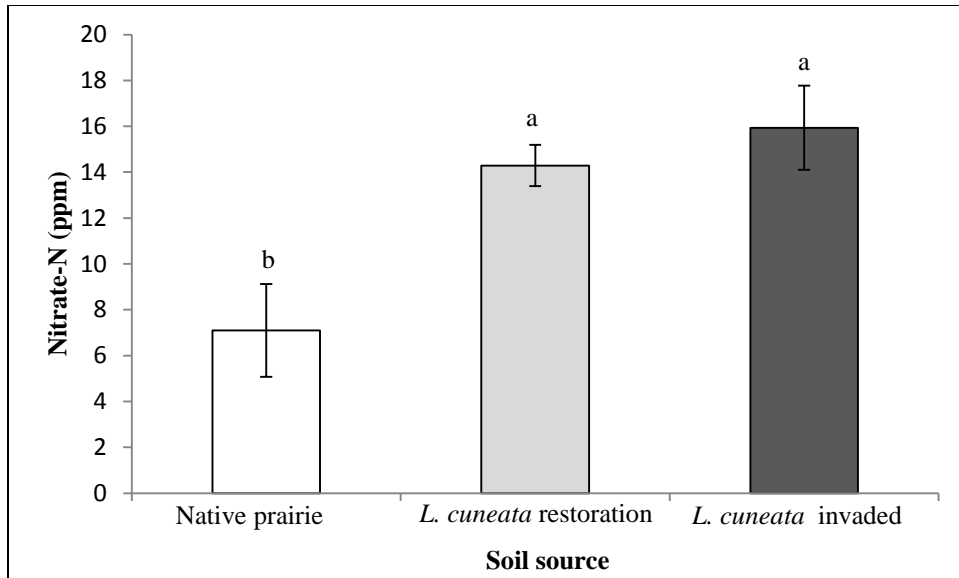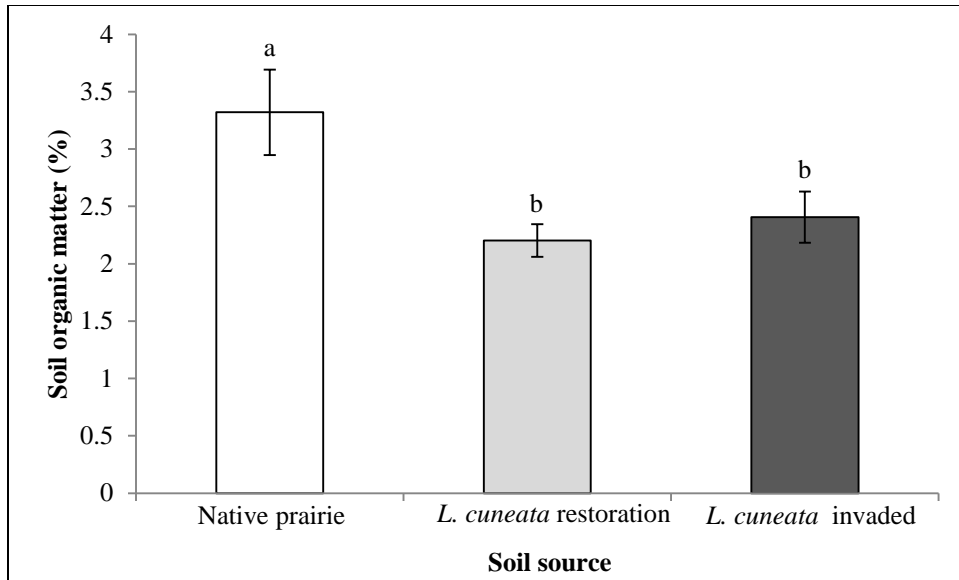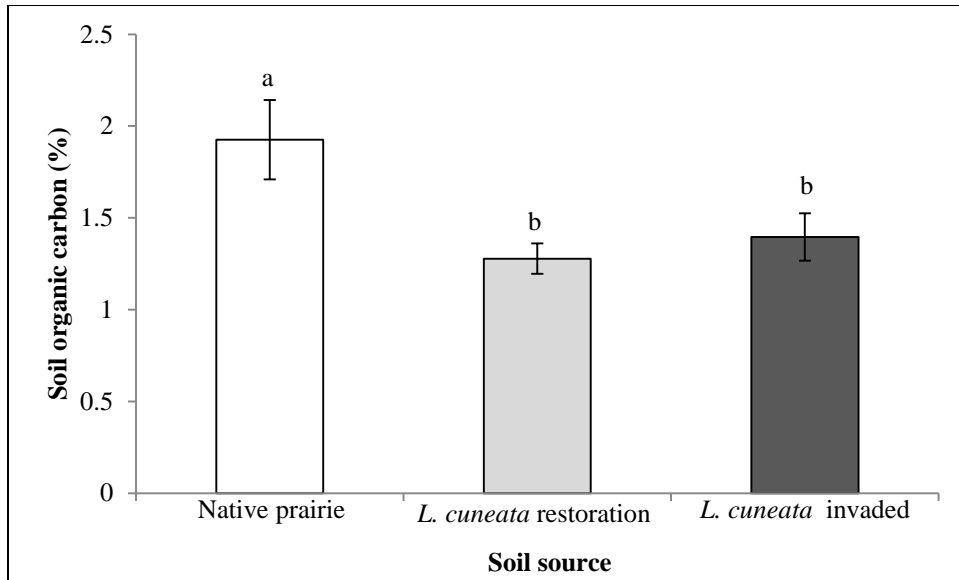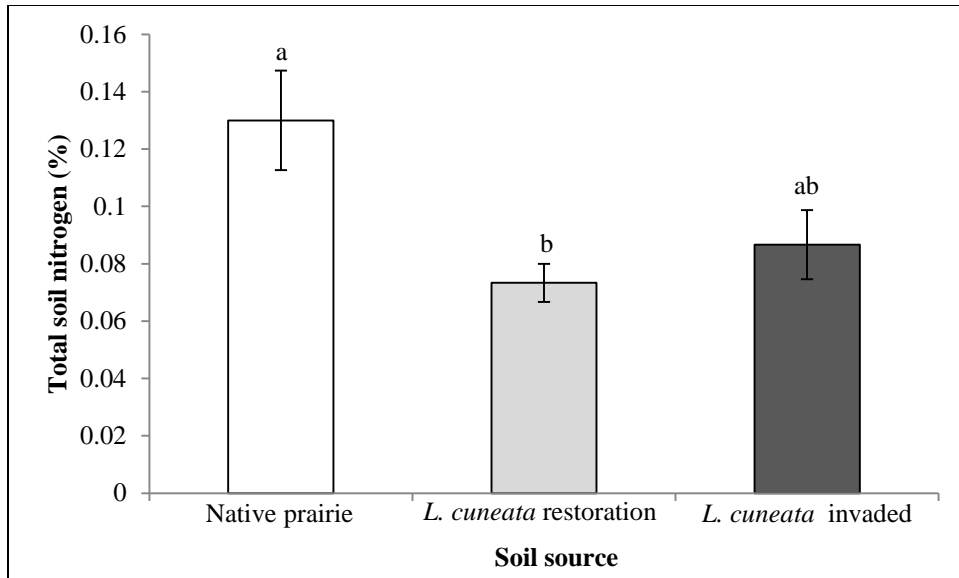
Figure 3-2 Nitrate-nitrogen concentration (ppm) with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different (*P*≤0.05).
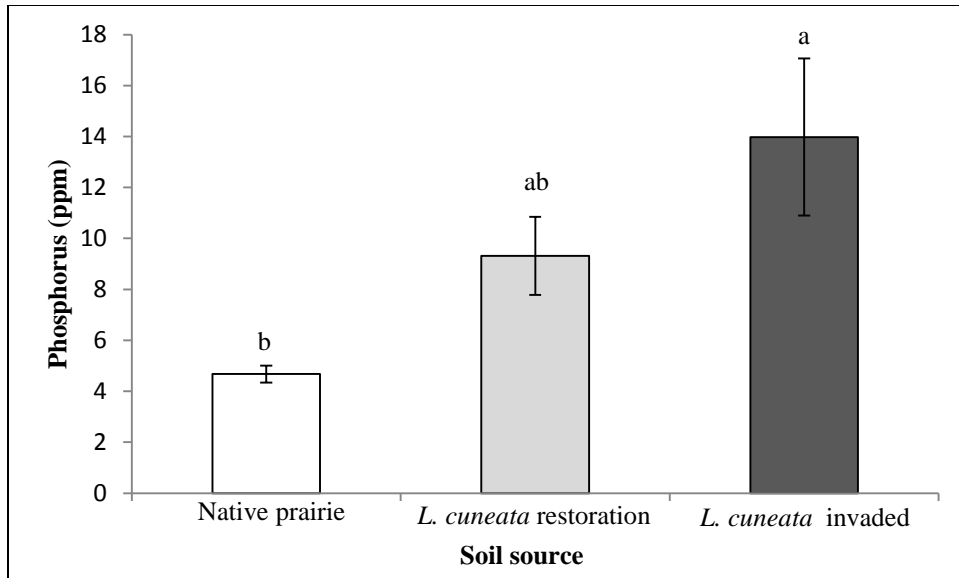
Figure 3-3 Phosphorus concentration (ppm) with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).
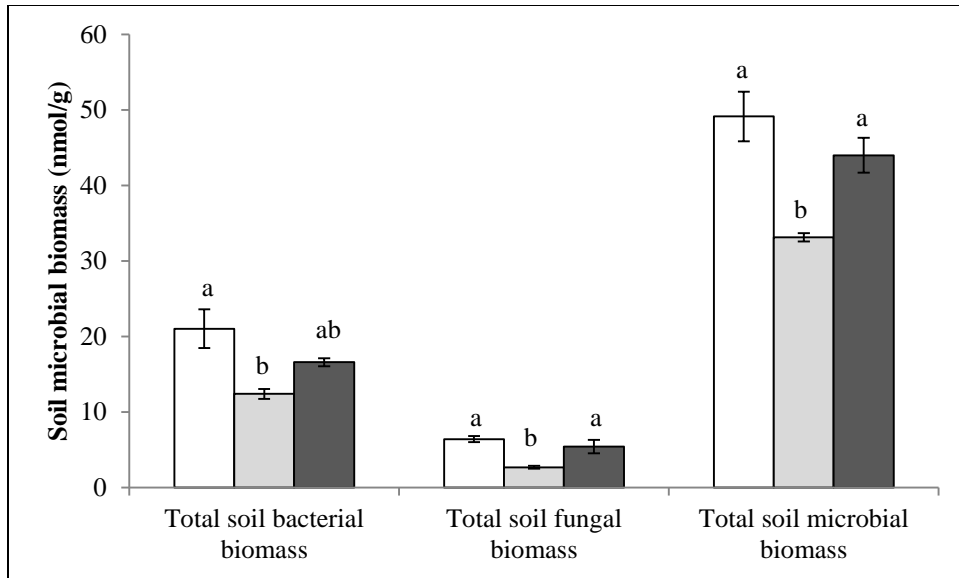
Figure 3-4 Soil organic matter percentage with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).

Figure 3-5 Soil organic carbon percentage with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).

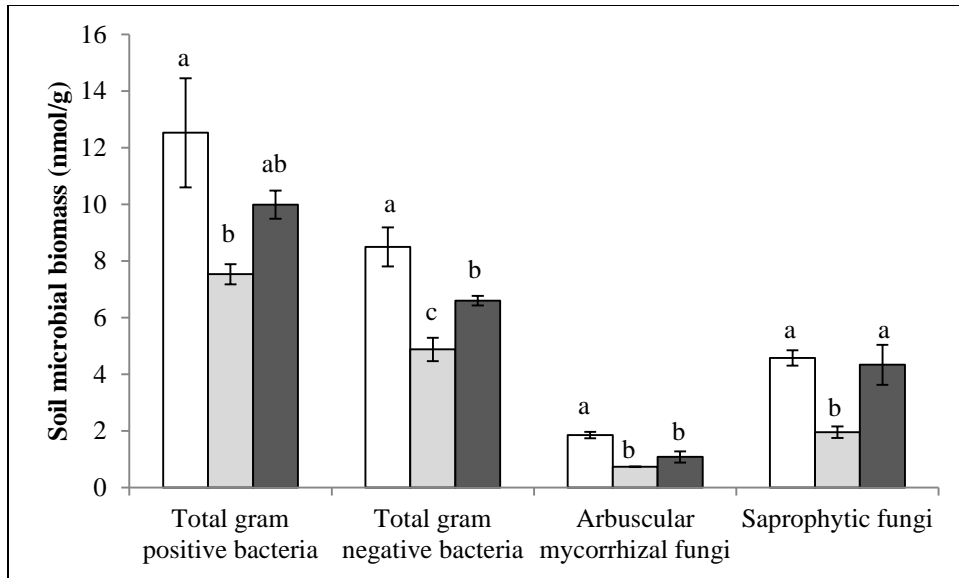Figure 3-6 Total soil nitrogen percentage with mean values and standard errors in three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq .05$).

Figure 3-7 Total soil microbial biomass (nmol/g) with mean values and standard errors in three soil sources: native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter within growth forms are not significantly different ($P \leq 0.05$).

Figure 3-8 Biomass (nmol/g) of soil microbial communities (total gram positive bacteria, total gram negative bacteria, arbuscular mycorrhizal fungi, and saprophytic fungi) with mean values and standard errors in three soil sources: native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter within growth forms are not significantly different ($P \leq 0.05$).
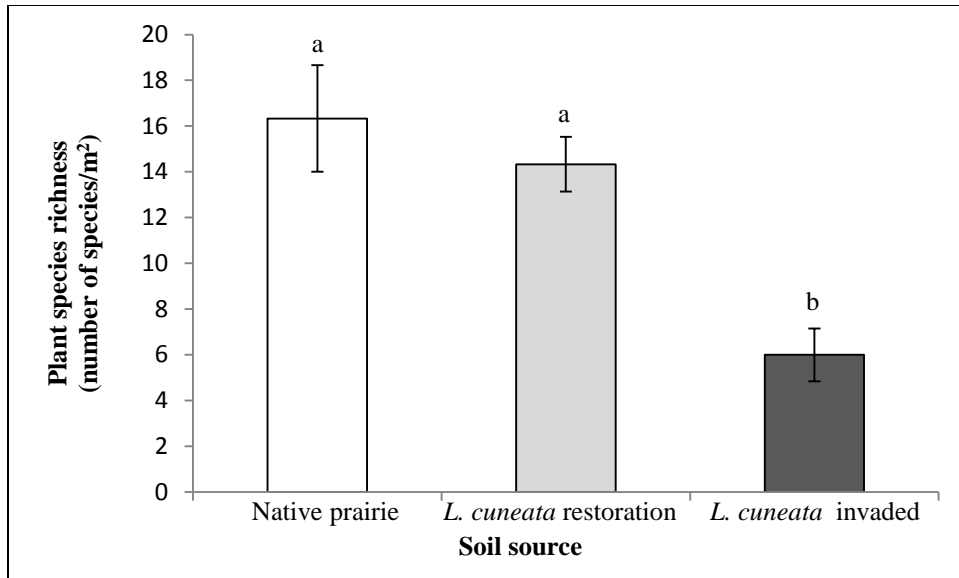
Figure 3-9 Plant species richness (number of species/m^2) with mean values and standard errors of three soil sources: native prairie, *L. cuneata* restoration, and *L. cuneata* invaded at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).
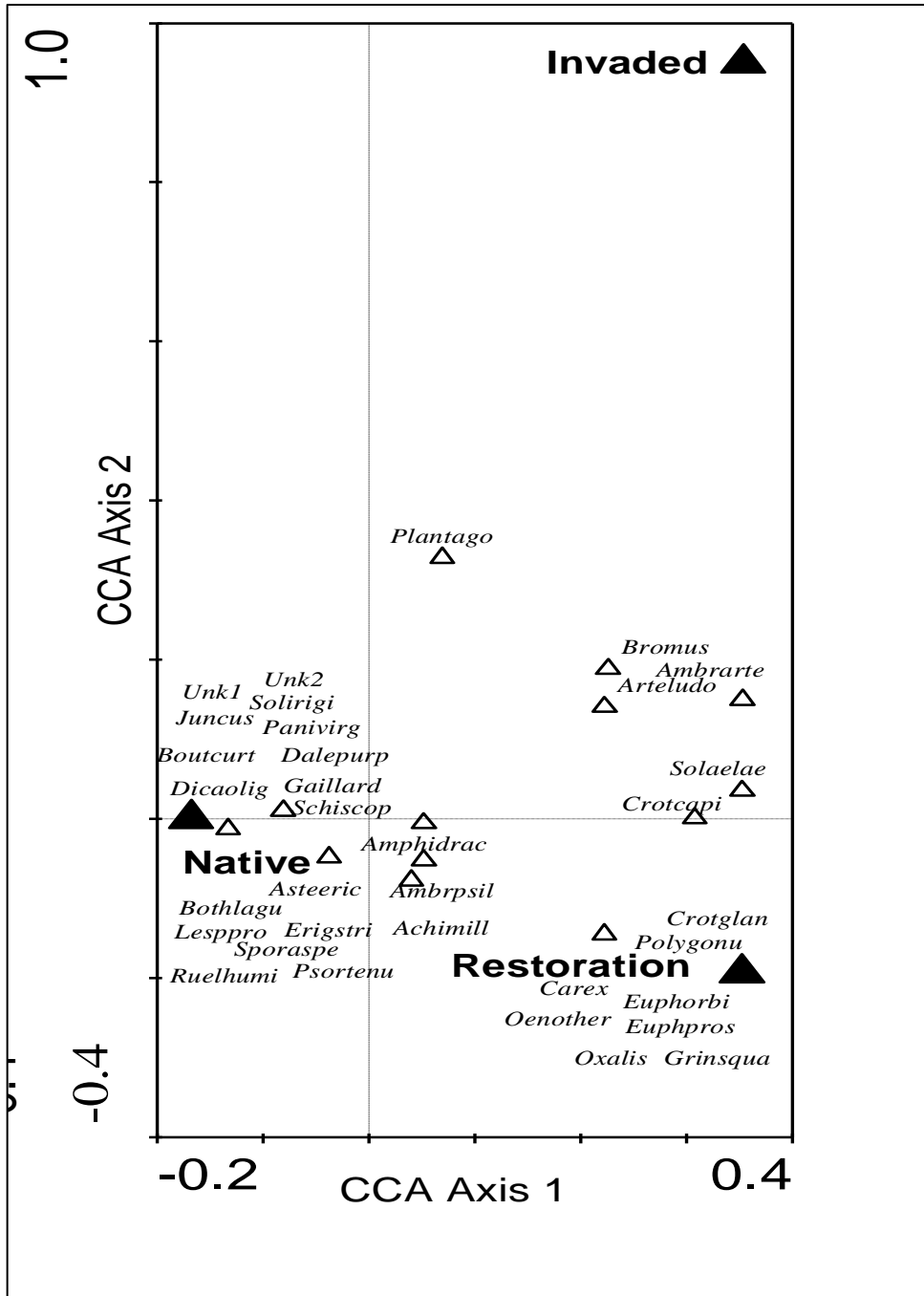
Figure 3-10 Biplots of the CCA ordinations of plant species (opened triangles) composition data with three soil sources (filled triangles) with native prairie on the left gradient of first axis and *L. cuneata* restoration on the right gradient of first axis and *L. cuneata* invaded on the upper second axis with 83.3% and 100% variation explained by first and second axes respectively.
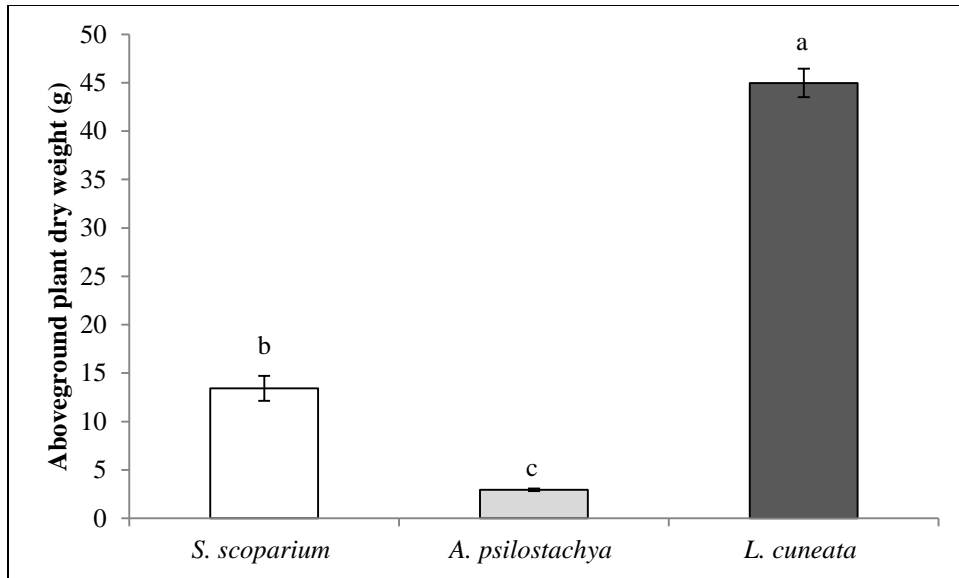
Figure 3-11 Aboveground plant dry weight (g) of dominant plant species; *Schizachyrim scoparium*, *Ambrosia psilostachya*, and *L. cuneata*, in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources respectively at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).
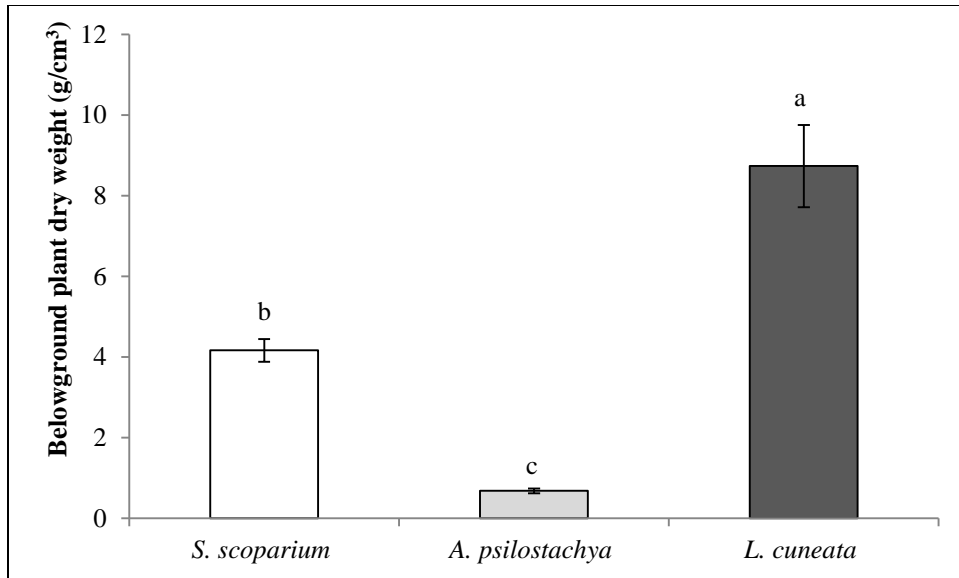
Figure 3-12 Belowground plant dry weight (g/cm³) of dominant plant species; *Schizachyrim scoparium*, *Ambrosia psilostachya*, and *L. cuneata*, in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources respectively at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different ($P \leq 0.05$).
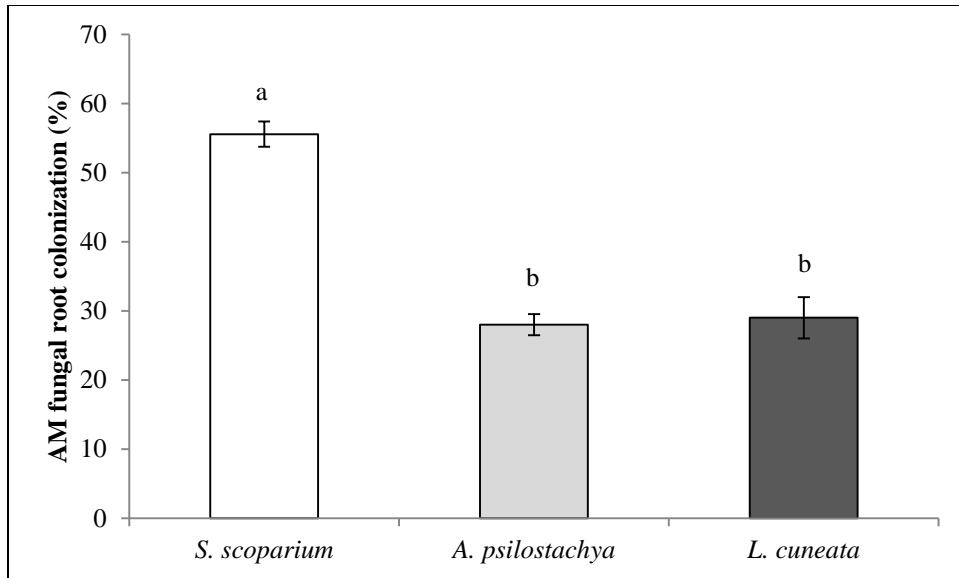
Figure 3-13 Percentage arbuscular mycorrhizal (AM) fungal root colonization of dominant plant species; *Schizachyrim scoparium*, *Ambrosia psilostachya*, and *L. cuneata*, in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources respectively at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter are not significantly different (*P*≤0.05).
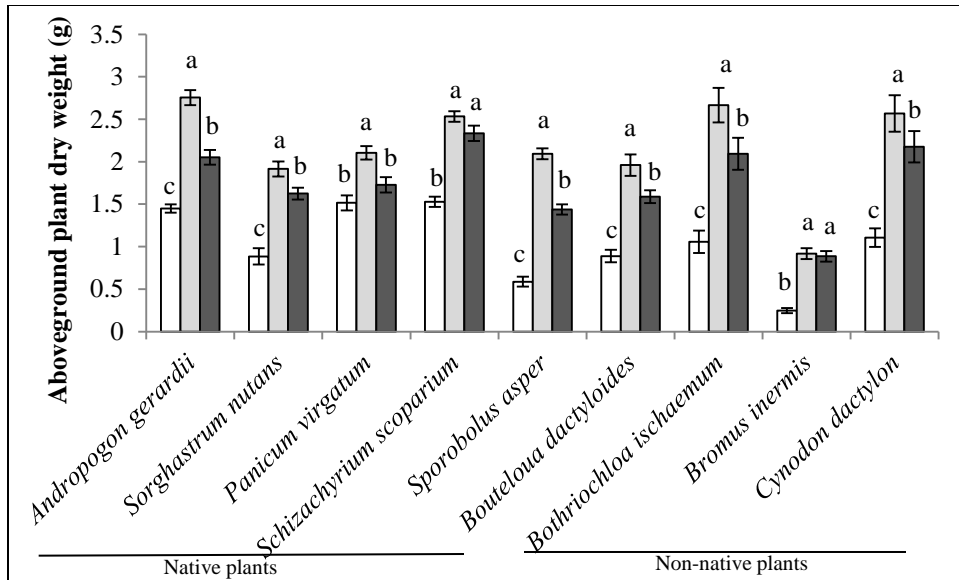
Figure 3-14 Aboveground plant dry weight (g) of native and non-native grasses grown in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter for each grass in native prairie, *L. cuneata* restoration, and *L. cuneata* invaded sites are not significantly different (*P*≤0.05).
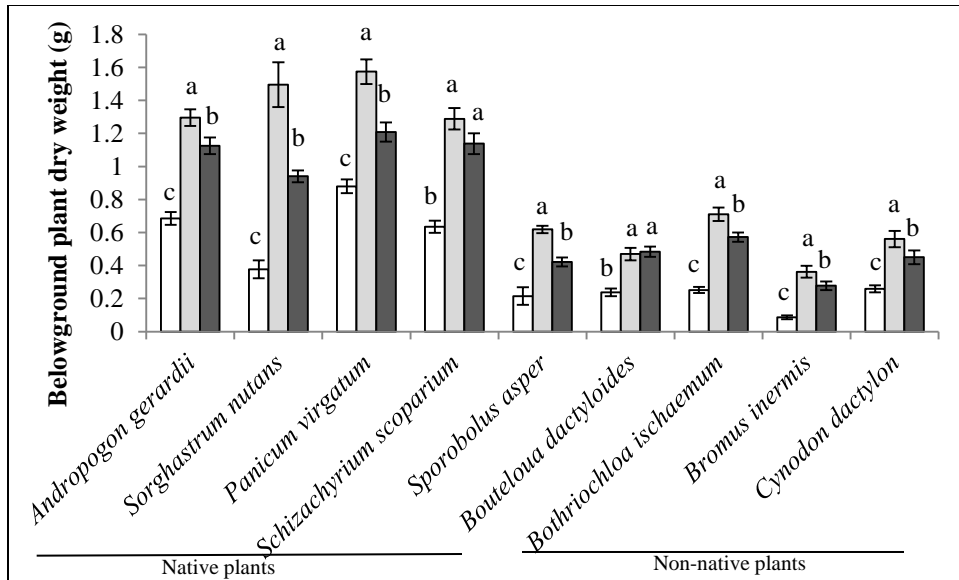
Figure 3-15 Belowground plant dry weight (g) of native and non-native grasses grown in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter for each grass in native prairie, *L. cuneata* restoration, and *L. cuneata* invaded sites are not significantly different (*P*≤0.05).
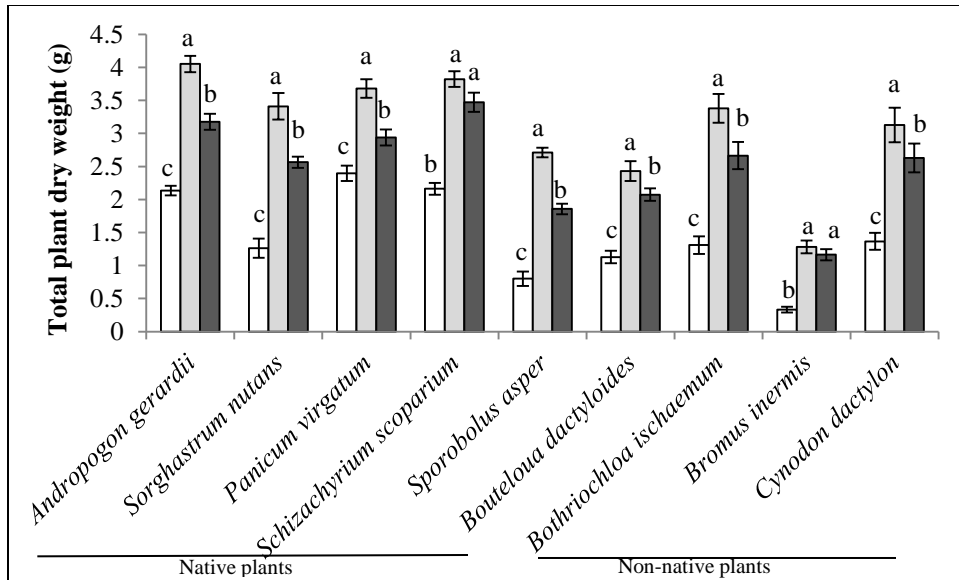
Figure 3-16 Total plant dry weight (g) of native and non-native grasses grown in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter for each grass in native prairie, *L. cuneata* restoration, and *L. cuneata* invaded sites are not significantly different (*P*≤0.05).
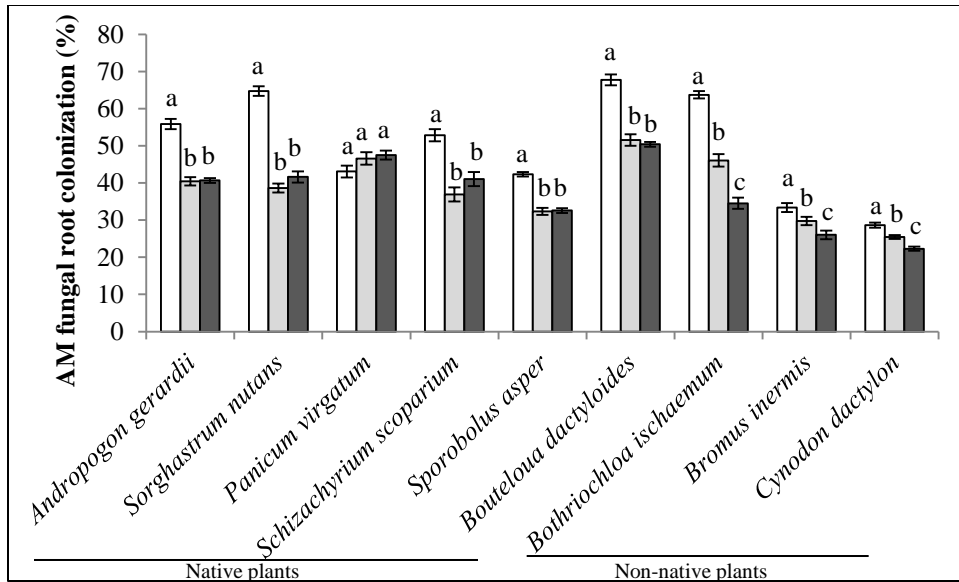
Figure 3-17 Percentage arbuscular mycorrhizal (AM) fungal root colonization of native and non-native grasses grown in native prairie (no filled bar), *L. cuneata* restoration (light gray bar), and *L. cuneata* invaded (dark bar) soil sources at the Oklahoma State University Range Research Station, Stillwater, Oklahoma, USA. Bars with the same letter for each grass in native prairie, *L. cuneata* restoration, and *L. cuneata* invaded sites are not significantly different (*P*≤0.05).

VITA

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