THE RELATIONSHIP BETWEEN DIVERSITY, SEEDING DENSITY, AND

ECOLOGICAL FUNCTIONS IN TALLGRASS PRAIRIE RESTORATIONS

by

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THE RELATIONSHIP BETWEEN DIVERSITY, SEEDING DENSITY, AND

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In recent decades, agricultural producers and conservation organizations and

agencies have converted thousands of hectares of cropland to grassland in the Great

Plains. Although high diversity seed mixes can cost up to five to ten times as much as

low diversity seed mixes, little information is available on the ecological functions that

may result from the added diversity. Restorations that maintain critical ecological

functions and services may help maintain functional and resilient working landscapes. In

this dissertation I assessed the effects of tallgrass prairie plant diversity and seeding

density on the provision of ecological functions and services including: 1) resistance to

invasive plant species, 2) abundance and diversity of predatory invertebrates, 3)

herbivory levels on two perennial forbs, and 4) soil development. In the spring of 2006,

twenty-four 55m^2-plots were planted to six replicates in each of four treatments: high

diversity sites of 97 species typically planted by The Nature Conservancy planted at a

lower seeding density, high diversity sites at twice this seeding rate, and low diversity

sites using a Natural Resources Conservation Service (NRCS) Conservation Reserve

Program mix (CP25; 15 species), at low and high seeding densities. This study is among

the first to compare the ecological functions provided by grassland seed mixes commonly

used by practitioners. Increasing plant community diversity was found to be more

important than increasing seeding density for enhancing resistance to invasion by unsown

perennial forbs and legumes and in reducing inflorescence production by *Bromus inermis*. There was a significant positive relationship between plant community diversity and the abundance of coccinellid beetles, but the abundance of ants, carabid beetles, and spiders showed no significant response to diversity or seeding density. Seeding density had a positive effect on carabid beetle and spider species richness and Shannon-Weaver diversity. Year was the main significant effect for explaining levels of herbivory damage in *Ratibida columnifera* and *Solidago canadensis* and there was a significant negative relationship between diversity and levels of soil nitrate. Overall, results indicate increasing diversity may be more important than increasing seeding density for provision of the ecological functions studied.

DEDICATION

For John

ACKNOWLEDGMENTS

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CHAPTER 1: INTRODUCTION

Grasslands are among North America's most endangered ecosystems (Samson et al. 2004). Of the three types of grassland found in North America, tallgrass, mixed-grass, and shortgrass prairie, the tallgrass prairie is the most imperiled and is considered a globally endangered resource (Ricketts et al. 1999). Once covering 68.4 million hectares of the Great Plains, more than 97% of the original Northern tallgrass prairie has been lost since European settlement, primarily from conversion to farmland (Samson and Knopf 1994, Samson et al. 2004).

The large-scale conversion of the Great Plains landscape from grassland to cropland has affected ecosystem services, resulting in loss of soil fertility, increased soil erosion, and decreased water quality due to sedimentation and non-point chemical pollution by salts, nutrients, and pesticides (Ostlie et al. 1997, Safriel and Adeel 2005). The loss of grasslands also threatens regional biodiversity. Populations of grassland birds have shown steeper, more consistent, and more geographically widespread declines than any other group of North American bird species (Knopf 1994, Sauer et al. 2005).

Prairie species are impacted not only by the loss of tallgrass prairie but by the deterioration of the prairie fragments that remain. Since European settlement, fire suppression and barriers to fire movement such as roads, agricultural fields, and urban areas, have eliminated fire as a natural component of many native prairies and may explain the loss or decline of some plant species from prairie remnants (Leach and Givnish 1996). Plant species numbers or richness may decline in isolated fragments because propagules from neighboring communities do not reach the fragments (Cully et al. 2003). Fragmentation also makes prairie remnants more vulnerable to invasion by

aggressive species because small areas have a higher ratio of edge to area and thus more points of entry for non-native plant species and increased likelihood of disturbance (Hobbs and Huenneke 1992, Baer et al. 2009). Non-native plant species may affect plant species diversity and ecosystem processes by competing directly with native species of similar life form or phenology for nutrients and moisture (Cully et al. 2003, Vila et al. 2011). The shape of prairie fragments and high edge-to-area ratio can also change other community dynamics, such as the species richness and composition of invertebrates (Stoner and Joern 2004) and birds (Helzer and Jelinksi 1999, Ribic et al. 2009).

Since the mid- to late-1900s organizations and agencies in the United States have attempted to restore grasslands in the Great Plains by planting former croplands with prairie seed mixes. Some organizations focus on promoting habitat diversity and prefer to plant high-diversity seed mixes that consist of 50-100 or more species. Throughout the Great Plains, thousands of hectares have been planted with high-diversity seed mixes (Personal communication, Chris Helzer, The Nature Conservancy). In Nebraska, non-governmental organizations such as The Nature Conservancy and Prairie Plains Resource Institute use a seed mix consisting of local-ecotype seed from more than 100 species collected within a 100-mile radius of the planting site (Steinauer et al. 2003).

The most commonly used Federal grassland program is the U.S. Department of Agriculture's (USDA) Conservation Reserve Program (CRP), initiated by the 1985 USDA Farm Bill. Under this voluntary program, farmers are paid to plant marginally productive or highly erodible cropland with grassland species for 10 years or longer. These plantings may not be considered true grassland restoration because the contracts are temporary and the farmer may convert CRP plantings back to cropland at the end of

the contract. However, CRP plantings are popular and provide wildlife habitat benefits while they are in place (Patterson and Best 1996, McIntyre and Thompson 2003, Riffell et al. 2008). In 2012, 9.8 million hectares of grassland habitat were enrolled in CRP nationwide (USDA 2012). Initially, the main objectives of the CRP program were to lower crop production and decrease soil erosion, thus most seed mixes available in the program were simple, inexpensive seed mixtures consisting of 2-5 species. However, after the 1996 Farm Bill recognized wildlife habitat as an objective of the CRP program, a 15-species combination of grasses and forbs (Conservation Practice (CP)25) mix was developed. In 2012, 649,177 hectares of CRP land were enrolled nationwide in the CP25 mix (USDA 2012).

Although The Nature Conservancy and Prairie Plains Resource Institute high-diversity mixes produce more diverse restorations than CRP seed mixes, they can cost up to five to ten times as much as the CRP mixes. However, very few dollars have been invested to evaluate the success of these restoration activities, how grassland restorations benefit adjacent row crop agriculture, and the benefits provided by the added diversity of higher diversity mixes. Some potential benefits of higher diversity plantings include greater resistance to invasion by aggressive plant species, enhanced soil development, increased diversity of predatory invertebrates, and decreased herbivory on native plants. Restorations that provide these ecological functions and services may help maintain functional and resilient working landscapes that can maintain critical ecological functions and services over a long period of time. Resilience is the capacity of a social-ecological system consisting of humans and their environment to absorb and respond to a disturbance while maintaining its essential structure and functions (Holling 1973, Folke

et al. 2002). One of the characteristics of a resilient agroecosystem is high functional diversity, or the variety of ecosystem services that components of the ecosystem provide, because it allows a range of responses to disturbance (Cabell and Oelofse 2012). In addition, an agroecosystem that possesses ecological components that self-regulate via stabilizing internal feedback mechanisms such as perennials and habitat for predatory invertebrates can reduce the amount of external inputs such as nutrients, water, and energy needed to manage the system (Cabell and Oelofse 2012).

This dissertation focuses on the ecological functions and services provided by $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. In the second chapter, I compare the ability of the treatments to resist invasion by unsown and invasive plant species. The third chapter focuses on the diversity of the predatory invertebrate community (ants, carabid beetles, coccinellid beetles, and spiders) among the treatments. In the fourth chapter, I compare herbivory levels on two native perennial forbs, Canada goldenrod (*Solidago canadensis*) and upright prairie coneflower (*Ratibida columnifera*). In the fifth chapter I assess soil development by measuring the amount of the soil nutrients ammonium and nitrate over time. Finally, in the sixth chapter I conclude with summarizing the results of this study.

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CHAPTER 2: INFLUENCE OF DIVERSITY AND SEEDING DENSITY ON

INVASION RESISTANCE IN TALLGRASS PRAIRIE RESTORATIONS

ABSTRACT

In recent years, agricultural producers and conservation organizations and agencies have converted thousands of hectares of cropland to grassland in the Great Plains. These vary from low plant diversity conservation programs (e.g., those planted through the U.S. Department of Agriculture's Conservation Reserve Program (CRP)) to high plant diversity restorations (e.g., some of those implemented by conservation organizations and agencies). However, little is known about the relationship between diversity and seeding density in these conservation programs and restorations and resistance to invasive and weedy plant species. This study assessed the effects of diversity and seeding density on resistance to invasive and other unsown weedy plant species in experimental tallgrass prairie plots in central Nebraska. In the spring of 2006, twenty-four 55m^2-plots were planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at a lower seeding density, high diversity sites at twice this seeding rate, and low diversity sites using a CRP mix (CP25; 15 species), at low and high seeding densities. The composition of the plant community, abundance of *Cirsium vulgare* and *Melilotus* spp., and the abundance of *Bromus inermis* tillers that spread from transplants and seed and the number of inflorescences removed from transplants were recorded among the treatments. There was a significant negative relationship between plant community diversity and basal cover of unsown perennial forbs/legumes and unsown perennial/annual grasses,

abundance of *C. vulgare*, and the number of inflorescences removed from *B. inermis* transplants. The high diversity treatments may have been more successful in resisting invasion by these species or in reducing the inflorescence production of *B. inermis* transplants because of the characteristics of the dominant species in these plots or because of greater interspecific competition among forbs/legumes with neighboring plants belonging to the same functional group for limiting resources. Seeding density was not important in affecting invasion resistance, except in the cover of unsown perennial/annual grasses. Our findings indicate increasing the plant diversity of a seed mix may be more effective than increasing the seeding density for decreasing invasion by unsown perennial species and *C. vulgare*.

INTRODUCTION

Invasive nonnative plant species can profoundly impact ecosystems, altering ecosystem structure and function (Ehrenfeld 2010, Pysek and Richardson 2010, Vila et al. 2011), decreasing native plant species richness and phylogenetic diversity (Pysek and Richardson 2010), and disrupting reproductive mutualisms of native plant species (Pysek and Richardson 2010). These impacts can reduce an ecosystem's resilience, or its capacity to absorb disturbance without fundamentally changing state (Holling 1973). The invasion of an ecosystem by a new species is influenced by the number of propagules introduced to the ecosystem (propagule pressure), the characteristics of the new species (invasiveness), and the susceptibility of the ecosystem to invasion by new species (invasibility) (Lonsdale 1999). Understanding the factors that increase an ecosystem's ability to resist invasion is particularly important if land managers and conservationists are to reduce the spread and impact of invasive plant species in natural areas, because it is one of the main aspects of the invasion process that can be managed.

In recent decades, the role of plant species diversity in resisting invasive plant species has been a major focus of invasion resistance research. These studies typically define diversity to be species richness, although other components of diversity, such as species evenness and functional group diversity have increasingly been used as metrics of diversity. Elton (1958) created the first framework for community invasion resistance, hypothesizing that species-rich communities are less invasible. According to his biotic resistance theory, species-rich communities are better able to resist invaders because they have a greater variety of methods for capturing resources than simple communities (complimentarity in resource use), and the probability of having the most highly

competitive species for a given resource increases as community diversity increases (sampling effect) (Tilman et al. 1997, Naeem et al. 2000).

However, studies on the biotic resistance theory in plant communities have produced contradictory results. Experimental and observational studies conducted at small scales ($\leq$ 20-m^2), particularly in North American grasslands, have primarily found negative relationships between plant species richness and invasibility (Knops et al. 1999, Naeem et al. 2000, Symstad 2000, Dukes 2002, Fargione and Tilman 2005, Biondini 2007, Petermann et al. 2010), while observational studies conducted at large scales (>1-km^2) in a variety of ecosystems worldwide have mostly reported positive relationships between plant species richness and invasibility (Lonsdale 1999, Stohlgren et al.1999, Herben et al. 2004, White and Houlahan 2007).

The contradictory results between fine- and broad-scale studies may be explained by the different roles of environmental heterogeneity and biotic interactions in structuring plant communities across spatial scales (Davies et al. 2005, Fridley et al. 2007). At smaller scales, environmental heterogeneity is reduced and biotic interactions maintain the negative species richness-invasibility relationship through enhanced competitive interactions that allow native plants to outcompete nonnative plants, and constraints on the number of nonnative and native plant species that can co-exist in a small area (Fridley et al. 2007). At larger scales, environmental heterogeneity increases, overwhelming the role of biotic interactions, and abiotic factors that allow more native species to co-exist at broader scales (e.g., climate, habitat heterogeneity, and disturbance) also increase nonnative species richness (Shea and Chesson 2002, Davies et al. 2005, Fridley et al. 2007, Melbourne et al. 2007).

However, species richness may not always be an important factor in invasion resistance because near complete exploitation of abiotic resources or incomplete resource exploitation can occur in both species-poor and species-rich communities depending on resource supply rates (Davis et al. 2000). According to this theory of fluctuating resource ability, a plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources (Davis et al. 2000). The degree of invasion resistance can also be influenced by functional group diversity, dominant species in the system, and species composition more than species richness (Emery and Gross 2006, Emery and Gross 2007, Lanta and Leps 2008).

Dominant native species in a community can affect resource availability and either facilitate or hinder invasive species depending on whether dominants create a more competitive environment or lessen stressful conditions (Smith et al. 2004, Bulleri et al. 2008). For example, in a native Kansas prairie remnant, invasion of the legume *Melilotus officinalis* appeared to be facilitated by the presence of dominant C_4 grasses, possibly because plots with dominant C_4 grasses had lower light levels that favored *M. officinalis* establishment (Smith et al. 2004). Emery and Gross (2007) found that the identity of dominant species determined invasion by perennial species, with *Coreopsis*-dominated plots having the lowest invasion rates and *Panicum* and *Bromus* plots having the highest invasion rates. The size of the plant species within the community can also affect resource availability as larger plant species self-thin to lower densities than smaller plant species, leaving more areas of unused space suitable for invasion by species adapted to invading open spaces (Schamp and Aarssen 2010).

The role of seeding density has received less attention than diversity in grassland

invasion resistance research. Martin (2006) examined the effect of four seeding density treatments (100, 200, 400, and 800 seeds/m^2) on prairie establishment. The number of all non-planted stems did not show a strong relationship with seeding density and the number of non-planted nonnative species did not vary significantly among treatments. Dickson and Busby (2009) studied the effect of varied grass density on forb establishment, planting a low density mix of 431 seeds/m^2 (151 grass seeds/m^2 and 280 forb seeds/m^2) and a high density mix at four times this rate. The percent cover of unsown species declined over three years across all treatments and in two of three years they found no significant treatment effect on the cover of unsown species. Peters and Schottler (2011) tested seeding rates of 108, 215, 430, 645, and 860 seeds/m^2 and altered the ratios of grass to forb seed to study prairie establishment. They found unsown species density to have a significant negative correlation to forb seeding rate, with weed density decreasing by 9-10% as the forb seeding rate doubled.

Although all of these studies incorporated seeding densities that are typically used in North American grassland seed mixes, to my knowledge no study has manipulated the seeding rates of low and high diversity seed mixes that are often used by practitioners within a particular area, rather than experimentally derived seed mixes, to test the relative effects of diversity and seeding density on invasion resistance. In the central United States, the Natural Resource Conservation Service's (NRCS) Conservation Reserve Program (CRP) is a widely used low diversity conservation program (5-15 plant species), while conservation organizations or agencies such as The Nature Conservancy for example plant high diversity restorations (50-100+ plant species). Although high diversity seed mixes can cost up to five to ten times as much as low diversity seed mixes,

little information is available on the benefits that may result from the added diversity, including resistance to invasive plant species. Because grasslands are often isolated habitats surrounded by agricultural land they are highly vulnerable to invasion by non-target species (Baer et al. 2009). In addition, few studies have established research plots at an intermediate scale (between $20m^2$-$1km^2$) that may better reflect the scale of biological interactions that occur in grasslands.

Therefore, this study compares invasion resistance of $55m^2$-research plots seeded with a low diversity tallgrass prairie seed mix commonly used in central Nebraska, the CRP's CP25 mix (15 plant species) to a high diversity tallgrass prairie seed mix used in the area by The Nature Conservancy (97 plant species), both at two different seeding rates. I assess the degree to which the four treatments displace cropfield weeds and resist three aggressive nonnative plants of different reproductive strategies: *Cirsium vulgare* and *Melilotus* spp. (*Melilotus alba* and *M. officinalis*) which reproduce by seed (Turkington et al. 1978, Forcella and Randall 1994), and *Bromus inermis*, which reproduces by both seed and rhizomes (Otfniowski et al. 2007). All are nonnative to the United States (Kaul et al. 2006) and are of high management interest because they are commonly encountered and aggressive in Nebraska grasslands. I test four null hypotheses: 1) the basal cover of sown nonnative plant species will not differ among the treatments; 2) the basal cover of unsown plant species will not differ among the treatments; 3) the abundance of *C. vulgare* and *Melilotus* spp. will not differ among the treatments; and 4) the abundance of *B. inermis* tillers that have spread from transplants and seed and the number of inflorescences removed from transplants will not differ among the treatments.

METHODS

Study area

The study area lies within the Central Platte River ecosystem, which includes the

Platte River channel and floodplain from mid-Dawson County to mid-Hamilton County

in central Nebraska (NGPC 2005). The Nebraska Game and Parks Commission has

determined the Central Platte River to be a Biologically Unique Landscape (NGPC

2005). The region has a continental climate, with warm, wet summers and cold, dry

winters. Mean annual air temperature is 10.4° C and mean annual precipitation is 63.9

centimeters (High Plains Regional Climate Center 2010).

The study site is located approximately 10 km south of Wood River, Nebraska

(Hall County; 40°44′41″ N, 98°35′11″ W) on a 7.3-ha field owned by The Nature

Conservancy. Soils at the site are of loamy alluvium or sandy alluvium parent material

and include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Calamux

complex, occasionally flooded soils (NRCS 2010). The site is bordered to the south and

east by county roads and Nature Conservancy prairie restorations, to the west by a

cornfield that was seeded to experimental prairie restoration plots in the spring of 2010,

and to the north by trees and the Platte River (Appendix A). The study site was under

cultivation in a corn-soybean rotation in the decades prior to the experiment.

Treatments and experimental design

In late March and early April 2006, the 7.3-ha field was cultivated and divided

into 24, 0.30-ha plots. The plots were seeded from an all-terrain vehicle (ATV) and a

John Deere drop spreader according to a 2 × 2 factorial design, in which two levels of

diversity (low plant diversity and high plant diversity) were applied using two different seeding densities (low and high seeding rates). The experiment was arranged in a randomized block design, with six blocks running west to east across the field and each block containing four plots assigned to the four treatments. Treatments consisted of: 1) a low diversity CRP tallgrass prairie seed mix (CP25 mix, 15 species) used by the NRCS seeded at half the recommended seeding rate (148 grass seeds/m^2, 16 forb seeds/m^2; low diversity/low rate mix); 2) the CP25 mix applied at the recommended seeding rate (297 grass seeds/ m^2, 31 forb seeds/m^2; low diversity/high rate mix); 3) a high diversity tallgrass prairie mix typically used by the local Nature Conservancy (97 species) seeded with a seeding rate typical for Nature Conservancy grassland restorations in the region (129 grass seeds/m^2, 43 forb seeds/m^2; high diversity/low rate), and 4) the Nature Conservancy mix applied at twice the seeding rate (258 grass seeds/m^2, 86 forb seeds/m^2; high diversity/high rate) (Appendix B and C). The second and fourth treatments are at half and double, respectively, NRCS or The Nature Conservancy normal seeding rates because the NRCS normally recommends rates that are about twice as high as The Nature Conservancy uses.

The NRCS CP25 seed mix was designed with the Grand Island, Nebraska NRCS District Conservationist. Grass seed used in the mix was purchased from Arrow Seed in Broken Bow, Nebraska and forb seed was locally harvested from the Platte River area. As of 2012, 74,492 hectares of land throughout Nebraska were restored with a CP25 mix (USDA 2012). The high-diversity seed mix was harvested from local prairies. High-diversity seed mixes have been used to restore more than 3,000 hectares of prairie throughout eastern and central Nebraska (personal communication, Chris Helzer, The

Nature Conservancy).

With the exception of *B. inermis*, which was added in 2008, nonnative species were not seeded in the plots but were allowed to naturally establish through the seed bank or dispersal. Management of the plots was minimal. All of the plots were burned on March 20, 2008. In July 2008, *Achillea millefolium* and *Helianthus maximilliani* that had invaded into the edges of plots where they had not been sown were sprayed with glyphosate and killed in order to reduce the edge effect on the spread of these aggressive species. The inflorescences of *B. inermis* that had naturally invaded the south row of plots from a road ditch and two plots in the northwestern corner of the field were clipped in order to limit the spread of *B. inermis* that had not been experimentally introduced into the plots. Following that effort no plants were intentionally killed or manipulated. Vegetation growing in unseeded 2-m lanes between the plots was mowed several times during the growing season.

Plant community composition

I assessed the composition of the plant community to determine the establishment of sown and unsown species in response to experimental treatments. Within each plot, five 55-m transects located 9.1 m apart were established. Each transect ran north to south and was marked on each edge with a 0.6-m piece of rebar spray-painted orange. I assessed the species composition of the plant community along three of the 55-m long transects within each plot, the middle transect and the two end transects, in mid- to late June 2007-2009. The line-intercept transect method was used because it is an efficient method of collecting cover and species richness (Bonham 1989). Starting at the end of

each transect, I stretched a measuring tape to a length of one meter. I broke the transect

into these smaller one-meter segments, or "subtransects," to keep the measuring tape

from sagging in the wind. I measured the basal cover of any plant touching the top edge

of the measuring tape by recording the distance that the plant covered along the tape to

the nearest 0.2 cm (Elzinga et al. 1998). I took measurements along every twelfth meter

and at the opposite end of the transect for a total of six one-meter subtransects along the

transect (every 0, 12, 24, 36, 48, and 55 meters were recorded).

Cirsium vulgare and *Melilotus* spp.

I assessed the abundance of *C. vulgare* and *Melilotus* spp. when these species

were flowering in September and October of 2006-2009 by walking belt transects along

the five 55–m long transects within each plot (Grant et al. 2004). I placed a 3-m long pole

with flagging tape over the rebar on one end of the transect to ensure the transect was

walked in a straight line. I recorded the number of *C. vulgare* and *Melilotus* plants

observed within 3 meters of either side of the transect. In addition, I recorded the

locations of *C. vulgare* plants within the belt transects with a handheld Garmin eTrex

Legend GPS unit to track changes in the spatial distribution of bull thistle over the course

of the study.

Bromus inermis

In 2008, I added *B. inermis* plants and seeds to each plot in order to compare its

spread from rhizomes and establishment from seeds among the four treatments. In the

two previous growing seasons, I observed small areas of *B. inermis* encroaching into two

plots in the northwest area of the study site and into the row of plots on the southernmost

edge of the study site, which was approximately 15 meters north of a road ditch

dominated by *B. inermis*. All of the plots had been burned on March 20, 2008, facilitating

the addition of the plants and seeds into bare soil. I added plants to the plots on April 13,

2008. I used a shovel to remove blocks of *B. inermis* approximately 13-cm^2 in surface

area and 5-cm deep from the ditch on the southern edge of the study site. Four plants

were transplanted along the middle of the center transect in each plot, with each plant

placed 3 meters apart to form the corners of a square. The east edge of each transplant

was marked with a 1-meter-long stake that was spray painted orange and flagged.

I obtained *B. inermis* seed from Stock Seed Farms in Murdock, Nebraska. I added

seed to the plots in late April, the time of year when farmers often seed *B. inermis*, and in

early September, when *B. inermis* plants naturally drop their seed (personal

communication, Bruce Anderson, University of Nebraska-Lincoln), to determine if

timing of seed addition affected the species' invasiveness. On April 30, 2008, I added

seed to the northwest and southeast corner of each plot by walking 12 paces in a diagonal

line from the corner of the plot. I broadcast seeds over a 1m^2-area using a seeding rate of

120 pure live seeds (PLS)/m^2 and lightly raking in the seed. On September 7, 2008, I

added seed to the northeast and southwest corner of each plot using the same seeding rate

and methods. In both months, I recorded each seeding location with a Trimble GeoXT

handheld GPS unit with submeter accuracy.

I clipped the inflorescences of each planted *B. inermis* on June 29, 2008; on June

24, 2009; and on June 27-June 29, 2010 in order to prevent the plant from dropping seeds

and to ensure it would spread only by rhizomes. The number of inflorescences clipped

from each plant was recorded as a measure of the vigor of the plant. On June 27-29, 2010, I placed a 1-m^2 quadrat frame in each of the four cardinal directions around each *B. inermis* transplant or seeding location and recorded the number of tillers in each 1-m^2 area encompassed by the frame to assess spread of the plant from either rhizomes or seed and rhizomes. The inflorescences of all *B. inermis* plants within the quadrat frame and within 10 meters of the area were removed.

Statistical analyses

Plots were the experimental units in this study. For analyzing plant community composition, plant species recorded along line transects in each plot were grouped into six classes: sown perennial/annual forbs/legumes, sown perennial grasses, unsown perennial forbs/legumes, unsown perennial/annual grasses, unsown annual/biennial forbs/legumes, and invasive species, as defined by the Nebraska Invasive Species Council (2011) (Appendix D). Normality in the response variables, basal cover of plant species aggregated in each functional group; number of *C. vulgare* or *Melilotus* spp. plants; or *B. inermis* inflorescences or tillers, was tested with the Kolmogorov-Smirnov normality test (PROC UNIVARIATE, SAS Version 9.2; SAS Institute 2007) and graphs of predicted values against the residuals. Because the response variables were influenced by fixed and random factors and were not normally distributed, each set of data was fitted with a mixed-effects model using PROC GLIMMIX (SAS Version 9.2; SAS Insitute 2007). Mixed-effects models are appropriate for data that contains both fixed and random factors and the GLIMMIX procedure does not require the response to be normally distributed (Littell et al. 2006). Diversity, seeding density, year, and their interactions

were used as fixed effects and block and plot were used as random effects. Because no *C. vulgare* plants were recorded along belt transects in 2007, this year was omitted from the *C. vulgare* model. Block was subsequently removed from the plant community composition models and from the model analyzing number of *B. inermis* inflorescences removed when results showed it was not necessary in explaining variance. In the *B. inermis* model for the number of tillers established from seeding locations, I combined data from the April and September seeding periods because of the low number of tillers recorded from both time periods. There was an underlying gradient in soil fertility from less fertile to more fertile plots but because of the randomization of the plots it was not confounded by the treatments or by block so I didn't include soil fertility in the analyses (Table 2.1).

The covariance structure that was the best fit for each model covering multiple years of data was determined by comparing Akaike's information criterion (AIC) for the plant community composition models and the pseudo-AIC for the *C. vulgare*, *Melilotus* spp., and *B. inermis* models. The distribution and covariance structures used for each model were: 1) plant community composition - gamma distribution, unstructured covariance structure; 2) *C. vulgare* – negative binomial distribution, autoregressive covariance structure; 3) *Melilotus* spp.– negative binomial distribution, compound symmetry covariance structure; 4) *B. inermis* inflorescences removed – poisson distribution, autoregressive covariance structure; and 5) *B. inermis* spread from seeds and transplants – negative binomial distribution.

RESULTS

Treatment effects on plant community composition

Sown species

Of the seven forb/legume species seeded in the low diversity treatments, two were recorded in the low seeding density treatments and five in the high seeding density treatments (Appendix E and F). *Astragalus canadensis* and *Ratibida columnifera* were the most abundant and widespread forbs and legumes among all of the low diversity plots.

Of the 79 forb/legume species sown in the high diversity plots, 12 were recorded in the low seeding density plots and 11 in the high seeding density plots (Appendix E and F). The only sown annual species was *Plantago patagonica*. The dominant sown forb found was the perennial *Helianthus maximiliani*, which in 2009 accounted for 69% and 75% of the sown perennial forb/legume basal cover in the low and high seeding density plots, respectively, and 22% of the basal cover of all plant species recorded in the high diversity plots. The high density of *H. maximiliani* can be attributed to the grass seed used for the high-diversity plots, which had been mechanically harvested from a restored prairie where *H. maximiliani* was more abundant than is typical for central Nebraska prairie restorations.

There were significant effects of diversity, year, and the diversity × year interaction on sown perennial forb/legume basal cover across all three years of the study (Table 2.2). In 2008 and 2009, the basal cover of sown perennial forbs/legumes in the high diversity treatments was significantly higher than in the low diversity treatments (Table 2.3), reflecting the higher proportion of forbs and legumes that was included in the high diversity seed mix compared to the low diversity seed mix. Year was a significant

effect as the basal cover of sown perennial grasses increased over the three years of the study and was highest in the low diversity, high seeding density plots in 2009 (Figure 2.1).

Unsown species

Four invasive species, *B. inermis*, *P. pratensis*, *Medicago lupulina*, and *Melilotus* spp. (Nebraska Invasive Species Council 2011), were recorded in the plant community line transects. The basal cover of the invasive species was low compared to plant species belonging to the other groups and there were no significant effects of diversity, seeding density, or year, or their interaction terms, on invasive species basal cover (Figure 2.1, Table 2.2). In 2007, the low diversity, high seeding density plots contained significantly greater invasive species basal cover than the high diversity, high seeding density plots and the low diversity, low seeding density plots (Table 2.3) but otherwise there were no significant differences when the data was analyzed by year.

Six of the twelve unsown perennial forb and legume species were internal to the study, having spread from where they were sown in the high diversity plots to the low diversity plots where they had not been seeded. *H. maximiliani* was the most widespread internal unsown perennial forb/legume species, recorded in 75% of the low diversity plots in 2009. *Taraxacum officinale* was the most widespread unsown perennial forb/legume external to the study, recorded in all of the low diversity plots and 83% of the high diversity plots in 2009. Diversity and year were significant effects on the basal cover of unsown perennial forb/legume species, and these effects remained significant when considering only those species that were external to the study (Table 2.2).

Significantly fewer unsown perennial forbs and legumes were recorded in the high diversity, high seeding density treatments compared to the low diversity, low seeding density treatments in 2008 and 2009 and in the high diversity, low seeding density treatments compared to the low diversity, low seeding density treatments in 2008 and 2009 and the low diversity, high seeding density treatments in 2009 (Table 2.3).

Of the eleven unsown annual forbs and legumes, one species, *P. patagonica*, was internal to the study and had spread to the low diversity plots. The large density of unsown annual/biennial forbs and legumes in 2007 (Figure 2.1) was due to the high abundance of *Conyza canadensis* that is common to central Nebraska prairie restorations in their second or third growing season. Year was significant in explaining the basal cover of unsown annual/biennial forbs and legumes, and the diversity × seeding interaction was nearly significant ($P = 0.0649$). Basal cover of unsown annual forbs and legumes was significantly higher in 2007 in the high diversity, high seeding density plots compared to the high diversity, low seeding density plots (Table 2.3), but otherwise there were no significant differences in basal cover in each year.

Of the seven unsown perennial/annual grasses, five were internal to the study and were present in the low diversity plots in low amounts. Diversity, seeding density, year × diversity interaction, and year × diversity × seeding density interactions were significant in explaining the basal cover of unsown perennial/annual grasses (Table 2.2), which in 2009 was significantly higher in the low diversity, high seeding density plots compared to the other three treatments (Table 2.3).

The basal cover of all unsown species did not show an edge effect along transects within the plots. Averaged across all plots and all three years, the basal cover of unsown

species was similar in the two 1-m subtransects at the edge of the plot (0.76 ± 0.065/m

and 0.82 ± 0.076/m) to the two subtransects nearest the center of the plot (1.0 ± 0.075/m

and 0.84 ± 0.061/m). Within the high diversity treatments there was a significant negative

correlation between the basal cover of the sown perennial forbs/legumes and the unsown

annual/biennial forbs/legumes and between unsown perennial/annual grasses and unsown

annual/biennial forbs/legumes (Table 2.4).

Treatment effects on Cirsium vulgare and Melilotus spp. abundance

Diversity, year, and the diversity $\times$ year interaction were significant effects on *C.*

vulgare abundance (Table 2.5). Because *C. vulgare* is a biennial, observed abundances

across all treatments were highest in 2008, when the plants bolted (Figure 2.3). In 2006,

the distribution of bull thistle was clustered in plots on the western edge of the study site

and in 2008 had spread more evenly throughout the plots (Figure 2.4). In both 2008 and

2009, abundance was significantly higher in the low diversity, low seeding density

treatments than in the high diversity treatments seeded to the low and high seeding

densities (Table 2.6).

Year was the only significant effect for *Melilotus* spp., which gradually increased

across the years in all treatments and was more abundant in the low seeding density

treatments than the high seeding density treatments by 2009 (Figure 2.3). In 2007, the

high diversity, low seeding density and low diversity, low seeding density treatments had

significantly more *Melilotus* spp. than the high diversity, high seeding density treatments

(Table 2.6).

Treatment effects on planted and seeded Bromus inermis abundance

Diversity, year, and the diversity × year interaction were significant in explaining the number of inflorescences removed from *B. inermis* transplants in 2008 and 2009 (Table 2.7), with the number of inflorescences removed being higher in the low diversity, low seeding density plots in 2009 compared to the high diversity, high seeding density and high diversity, low seeding density plots (Table 2.8). Fertility, as measured by total soil carbon in 2006, was run as a covariate with the number of inflorescences removed and was not significant in explaining this variable (Table 2.8). There were no significant effects explaining the number of *B. inermis* tillers counted in 1-m^2 quadrat frames placed around seeded and planted areas in 2010 to assess the spread of *B. inermis* (Table 2.7). However, diversity and the diversity × seeding density interaction had low *P* values for the number of tillers counted near planting locations ($P = 0.0995$ and $P = 0.0772$, respectively). In 2010, the number of tillers spreading from plants was significantly higher in the low diversity, low seeding density treatments compared to all three of the other treatments (Table 2.8). However, these differences could largely be attributed to one low diversity, low seeding density plot in the southwestern portion of the field that was an outlier, with an exceptionally high number of *B. inermis* plants. In this plot it was difficult to determine how many of the tillers had spread naturally from the ditch to the south or had spread from the transplants.

DISCUSSION

Recorded seeded plant species richness was approximately twice as large in the high diversity plots compared to the low diversity plots. Over three seasons of sampling

from 2007-2009, I recorded a total of 27 seeded species, with 9, 13, 22, and 22 seeded

species observed among low diversity, low seeding density; low diversity, high seeding

density; high diversity, low seeding density; and high diversity, high seeding density

plots, respectively. Carter and Blair (2012) sampled the plant community in 0.25-m^2

quadrats (1 m^2 total sampling area within each plot) in 2009 and 2010 as part of a study

on the response of restorations to drought. They recorded a total of 37 seeded species,

with 15, 12, 26, and 26 seeded species observed among low diversity, low seeding

density; low diversity, high seeding density; high diversity, low seeding density; and high

diversity, high seeding density plots, respectively.

Two groups of unsown species, unsown perennial forbs/legumes and unsown

perennial/annual grasses, appeared to support Elton's biotic resistance theory that

species-rich plant communities should be less invasible, as there was a significant

negative effect of diversity on basal cover for these groups. This finding was influenced

to some extent by species internal to the experimental seed mix because low diversity

plots were more likely to be "invaded" by sown species from adjacent high diversity

plots. These results reflect other grassland studies in which species internal to the

experimental species pool spread and establish extensively throughout the study site,

particularly in species-poor plots (Roscher et al. 2009, Petermann et al. 2010). However,

the negative relationship between diversity and unsown species still held true when

considering only the unsown perennial forb/legume species that were external to the

study species pool, dominated in basal cover by the perennial forb *Taraxacum officinale*.

An unsown species may be less likely to establish if a species with similar traits is

already present in the community, and high diversity seed mixes have a higher

probability of containing a species with similar resource requirements to that of an unsown species (Turnbull et al. 2005, Funk et al. 2008). The high diversity treatments may have had more perennial forb/legume species with similar niches that were better able to outcompete unsown species belonging to the same functional group.

The number of plant species in Nebraska has increased by approximately 35% in recent decades, primarily because of invasions by annual nonnative species (Decker et al. 2012). Ecosystems with a higher proportion of annuals/biennials may be more vulnerable to invasion because invading species do not have to compete with the established root system and nutrient reserves of perennial species (Wolf et al. 2004). Alternatively, annuals may stabilize soil and reserve space that can be taken over by native, sown perennials as the annuals die and succession proceeds, which is what I observed in this study. The cover of unsown annual/biennial forbs/legumes declined over the course of the study while the cover of sown perennial forbs/legumes and grasses increased. I did not find a significant correlation between the basal cover of unsown annual/biennial forbs/legumes and invasive species. Although the invasive species *B. inermis, P. pratensis, M. lupulina,* and *M. officinalis* can become highly abundant in central Nebraska, their cover in these plots as recorded along the line transects was low compared to species in the other functional groups. These invasive species may also become more abundant in the plots in future years.

Diversity had a negative effect on the abundance of naturally recruited *C. vulgare* measured along belt transects. *C. vulgare* is a short-lived biennial plant that can form dense stands in highly disturbed areas (Forcella and Randall 1994). Unlike many other biennials, *C. vulgare* does not produce long-lived seeds in the seed bank and relies

heavily on wind dispersal to establish local populations (Klinkhamer and DeJong 1988, Klinkhamer et al. 1988). I observed *C. vulgare* to spread from plots in the western portion of the study area to the eastern portion in accordance with the prevailing wind direction.

The performance and density of *C. vulgare* may be related to several factors including the availability of seed, level and spacing of disturbance, and vigor of grass competition, which can suppress the vigor and increase mortality of rosette-forming thistles (Louda and Rand 2003, Jongejans et al. 2006). However, the low diversity treatments, which had a higher proportion of grass cover than the high diversity treatments, had higher abundances of *C. vulgare* in 2008, suggesting grass competition did not reduce the spread of *C. vulgare*, at least in the year that it bolted. Similarly, competition with neighboring plants had little or no effect on growth and seed reproduction of *C. vulgare* rosettes in their final, flowering year in the tallgrass prairie region of eastern Nebraska (Suwa et al. 2010). However, competition can have a greater effect on *C. vulgare* growth in the seedling stage, as interspecific competition with prairie vegetation strongly limits seedling emergence, seedling survival, and seedling size of *C. vulgare* (Suwa 2008).

The high diversity treatments may have been more successful in resisting invasion by *C. vulgare* because of the dominant presence of *H. maximiliani* in these plots. *H. maximiliani* inhibits weed growth allelopathically by exuding chemicals that act as an herbicide (Herz and Kumar 1981, Gershenzon and Mabry 1984, Jackson 1988, Macías et al. 1996). *H. maximiliani* is one of the more successful native species at infiltrating pastures dominated by *P. pratensis* (personal communication, Chris Helzer, The Nature Conservancy). *H. maximiliani* may also inhibit the growth of some native species.

Dickson and Busby (2009) found a significant negative relationship between the percentage canopy cover of *H. maximiliani* and other sown tallgrass prairie species during one year of their study. In this study, cover of unsown annual forbs/legumes and unsown perennial/annual grasses significantly decreased with increasing *H. maximiliani* cover ($P = 0.0004$ and 0.0019, respectively).

Diversity was significant in explaining the number of inflorescences found from *B. inermis* transplants, with low diversity treatments containing significantly more inflorescences per plant than high diversity treatments in 2009. Similarly, HilleRisLambers et al. (2009) found the number of *B. inermis* inflorescences removed per quadrat in experimental prairie plots to be negatively correlated with declining species richness. In this study, the negative relationship between inflorescence production and diversity may have been explained by greater interspecific competition with neighboring plants for limiting resources in the high diversity plots, which reduced the vigor of *B. inermis*.

I found no significant effects explaining the spread of *B. inermis* from seed or rhizomes, as measured by counting tillers. Rhizome production is affected by similar processes to those that affect seed production, such as nutrient availability and interspecific competition (Otfinowski et al. 2007), and is also sensitive to changes in light intensity and quality, with tiller density increasing with increasing light intensity (Biligetu and Coulman 2010). Light conditions may have differed among the treatments, although this variable was not measured. There were no significant differences in the number of tillers around seeded locations. The number of tillers produced from seeded locations was small, making meaningful comparisons among the treatments difficult.

Seeding density was a significant effect only in explaining the basal cover of unsown perennial/annual grasses. By 2009, the higher seeding rate plots had a higher basal cover of sown perennial grasses than the lower seeding rate plots, but the difference was not significant. Other grassland studies that varied seeding densities have also found seeding rate was not a factor in explaining cover or density of unsown species (Martin 2006, Dickson and Busby 2009).

In summary, I found diversity to be more important than seeding density in affecting invasion resistance of experimental tallgrass prairie plots to unsown perennial forbs/legumes, unsown perennial/annual grasses, abundance of *C. vulgare*, and the number of inflorescences removed from *B. inermis* transplants. The high diversity treatments may have been more successful in resisting invasion by these species or in reducing the vigor of transplants because of the characteristics of dominant species in these plots, such as the allelopathic properties of *H. maximiliani*, or because of greater interspecific competition among forbs/legumes with neighboring plants belonging to the same functional group for limiting resources in the high diversity plots. Based on these results, increasing the plant diversity of a seed mix may be more effective than increasing the seeding density for decreasing invasion by unsown perennial species and *C. vulgare*. However, further research is needed on the biotic and abiotic factors that control the seed production and spread of invasive plant species vegetatively in low or high diversity plant communities to better understand the effects of various seed mixes on invasion resistance.

Table 2.1. Results of analysis of variance (ANOVA) for testing the effects of diversity

and seeding density on fertility, as measured by carbon in the plots in 2006. Carbon was

measured in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six

replicates in each of four treatments: high diversity sites of 97 species typically planted

by The Nature Conservancy planted at low and high (2×) seeding densities, and low

diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding

densities. Values in boldface are significant at $P < 0.05$.

Source	df	Sum of squares	Mean square	F	P
Seeding	1	0.07028	0.07028	0.4750	0.5000
Block	5	0.6619	0.1324	0.8947	0.5066
Error	17	2.515	0.1480		
Carbon total	23	3.247			
Density	1	0.03625	0.03625	0.2417	0.6292
Block	5	0.6619	0.1324	0.8827	0.5137
Error	17	2.549	0.1500		
Carbon total	23	3.247			

Table 2.2. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on basal cover of sown and unsown plant species aggregated according to growth form and functional group. Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P <$ 0.05.

Effect	df	F	P
Sown perennial forbs/legumes			
Diversity	1, 23	52.19	**<0.0001**
Seeding density	1, 23	0.89	0.3552
Diversity*Seeding density	1, 23	0.48	0.4969
Year	2, 40.18	40.84	**<0.0001**
Year*Diversity	2, 40.18	4.92	**0.0123**
Year*Seeding density	2, 40.18	1.47	0.2418
Year*Diversity*Seeding density	2, 40.18	0.65	0.5289
Sown perennial grasses			
Diversity	1, 20.61	1.95	0.1771
Seeding density	1, 20.61	0.52	0.4803
Diversity*Seeding density	1, 20.61	0.08	0.7822
Year	2, 38.96	19.04	**<0.0001**

Table 2.2. Continued.

Effect	df	F	P
Sown perennial grasses, continued			
Year*Diversity	2, 38.96	1.26	0.2940
Year*Seeding density	2, 38.96	1.07	0.3521
Year*Diversity*Seeding density	2, 38.96	0.12	0.8859
Unsown perennial forbs/legumes (all)			
Diversity	1, 30.95	9.47	**0.0044**
Seeding density	1, 30.95	2.15	0.1525
Diversity*Seeding density	1, 30.95	0.44	0.5130
Year	2, 46.42	19.44	**<0.0001**
Year*Diversity	2, 46.42	2.39	0.1032
Year*Seeding density	2, 46.42	0.32	0.7301
Year*Diversity*Seeding density	2, 46.42	0.35	0.7043
Unsown perennial forbs/legumes (external)			
Diversity	1, 30.31	4.51	**0.0420**
Seeding density	1, 30.31	1.79	0.1912
Diversity*Seeding density	1, 30.31	0.30	0.5860
Year	2, 46.26	19.95	**<0.0001**
Year*Diversity	2, 46.26	1.08	0.3478
Year*Seeding density	2, 46.26	0.29	0.7500
Year*Diversity*Seeding density	2, 46.26	0.39	0.6761

Table 2.2. Continued.

Effect	df	F	P
Unsown perennial forbs/legumes (internal)			
Diversity	0	.	.
Seeding density	1, 10.45	0.17	0.6902
Diversity*Seeding density	0	.	.
Year	2, 19.15	9.04	**0.0017**
Year*Diversity	0	.	.
Year*Seeding density	2, 19.15	0.04	0.9621
Year*Diversity*Seeding density	0	.	.
Unsown perennial/annual grasses (all)			
Diversity	1, 40.28	7.38	**0.0097**
Seeding density	1, 40.28	7.55	**0.0090**
Diversity*Seeding density	1, 40.28	1.93	0.1726
Year	2, 36.32	0.28	0.7540
Year*Diversity	2, 36.32	3.43	**0.0433**
Year*Seeding density	2, 36.32	0.93	0.4056
Year*Diversity*Seeding density	2, 36.32	3.63	**0.0367**
Unsown perennial/annual grasses (external)			
Diversity	1, 20	0.08	0.7836
Seeding density	1, 20	0.47	0.5023
Diversity*Seeding density	1, 20	2.54	0.1265

Table 2.2. Continued.

Effect	df	F	P
Unsown perennial/annual grasses (external), continued			
Year	2, 40	19.16	**<0.0001**
Year*Diversity	2, 40	5.95	**0.0055**
Year*Seeding density	2, 40	3.68	**0.0340**
Year*Diversity*Seeding density	2, 40	3.52	**0.0390**
Unsown perennial/annual grasses (internal)			
Diversity	1, 20	5.94	**0.0242**
Seeding density	1, 20	5.52	**0.0292**
Diversity*Seeding density	1, 20	4.98	**0.0373**
Year	2, 40	1.57	0.2208
Year*Diversity	2, 40	1.74	0.1885
Year*Seeding density	2, 40	1.29	0.2876
Year*Diversity*Seeding density	2, 40	1.45	0.2470
Unsown annual/biennial forbs/legumes			
Diversity	1, 19.2	1.74	0.2032
Seeding density	1, 19.2	0	1.000
Diversity*Seeding density	1, 19.2	3.83	0.0649
Year	2, 33.7	189.80	**<0.0001**
Year*Diversity	2, 33.7	0.05	0.9499
Year*Seeding density	2, 33.7	0.89	0.4187

Table 2.2. Continued.

Effect	df	*F*	*P*
Unsown annual/biennial forbs/legumes, continued			
Year*Diversity*Seeding density	2, 33.7	0.84	0.4402
Invasives			
Diversity	1, 13.83	2.26	0.1554
Seeding density	1, 13.83	0.01	0.9328
Diversity*Seeding density	1, 13.83	0.60	0.4526
Year	2, 34.36	0.42	0.6599
Year*Diversity	2, 34.36	0.50	0.6090
Year*Seeding density	2, 34.36	1.76	0.1880
Year*Diversity*Seeding density	2, 34.36	2.67	0.0838

Table 2.3. Treatments with significant differences by year in the basal cover of plant species aggregated according to growth form and functional group. Plants were assessed in 55m²-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Comparison	Year	df	t	P
Sown perennial forbs/legumes				
High div, high den vs. low div, high density	2008	56.48	3.53	0.0008
High div, high density vs. low div, low density	2008	56.48	4.94	<0.000
High div, low density vs. low div, high density	2008	56.48	2.37	0.0210
High div, low density vs. low div, low density	2008	56.48	3.79	0.0004
High div, high density vs. low div, high density	2009	56.48	3.79	0.0004
High div, high density vs. low div, low density	2009	56.48	4.72	<0.000
High div, low density vs. low div, high density	2009	56.48	4.75	<0.000
High div, low density vs. low div, low density	2009	56.48	5.68	<0.000
Unsown perennial forbs/legumes				
High div, high density vs. low div, low density	2008	57.75	-2.56	0.0130
High div, low density vs. low div, low density	2008	57.75	0.020	0.0203
High div, high density vs. low div, high	2009	57.75	0.022	0.0229
High div, high density vs. low div, low density	2009	57.75	-3.29	0.0017

Table 2.3. Continued.

Comparison	Year	df	t	P
Unsown perennial forbs/legumes, continued				
High div, low density vs. low div, low density	2009	57.75	-2.41	0.0191
Unsown perennial/annual grasses				
High div, high density vs. low div, high density	2009	48.35	-3.49	0.0010
High div, low density vs. low div, high density	2009	48.35	-3.49	-0.0010
Low div, low density vs. low div, high density	2009	48.35	3.32	0.0017
Unsown annual/biennial forbs/legumes				
High div, high density vs. high div, low density	2007	59.98	2.19	0.0324
Invasives				
High div, high density vs. low div, high density	2008	57.75	-2.56	0.0130
Low div, high density vs. low div, low density	2008	57.75	0.020	0.0203

Table 2.4. Spearman correlation coefficients among basal cover of sown and unsown plant species aggregated according to growth form and functional group. Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at the Bonferroni-corrected $P < 0.0033$.

	Sown perennial grasses	Unsown perennial/ annual grasses	Unsown annual/biennial forbs/legumes	Unsown perennial forbs/legumes	Invasives
Low diversity/low seeding density					
Sown perennial forbs/legumes	0.380	-0.273	-0.485	0.228	-0.148
Sown perennial grasses		-0.024	-0.587	0.122	-0.013
Unsown perennial/annual grasses			0.388	-0.265	-0.328
Unsown annual/biennial forbs/legumes				-0.400	-0.241
Unsown perennial forbs/legumes					0.654

Table 2.4. Continued.

	Sown perennial grasses	Unsown perennial/ annual grasses	Unsown annual/biennial forbs/legumes	Unsown perennial forbs/legumes	Invasives
Low diversity/high seeding density					
Sown perennial forbs/legumes	0.558	0.146	-0.560	**0.826**	-0.206
Sown perennial grasses		0.0072	**-0.884**	0.423	-0.511
Unsown perennial/annual grasses			-0.064	0.225	-0.403
Unsown annual/biennial forbs/legumes				-0.375	0.313
Unsown perennial forbs/legumes					-0.285
High diversity/low seeding density					
Sown perennial forbs/legumes	0.315	**-0.708**	**-0.815**	0.406	-0.027
Sown perennial grasses		-0.293	-0.617	-0.253	-0.583
Unsown perennial/annual grasses			**0.787**	-0.214	0.218
Unsown annual/biennial forbs/legumes				-0.423	0.347

Table 2.4. Continued.

	Sown perennial grasses	Unsown perennial/ annual grasses	Unsown annual/biennial forbs/legumes	Unsown perennial forbs/legumes	Invasives
High diversity/low seeding density, continued					
Unsown perennial forbs/legumes					-0.155
High diversity/high seeding density					
Sown perennial forbs/legumes	0.429	-0.547	**-0.765**	-0.547	-0.232
Sown perennial grasses		-0.451	**-0.636**	0.332	-0.219
Unsown perennial/annual grasses			**0.852**	-0.317	0.085
Unsown annual/biennial forbs/legumes				-0.231	0.067
Unsown perennial forbs/legumes					0.045

Table 2.5. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on abundance of *Cirsium vulgare* and *Melilotus* spp. in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at *P* < 0.05.

Effect	df	F	P
Cirsium vulgare			
Diversity	1, 60	8.14	**0.0059**
Seeding density	1, 60	0.01	0.9321
Diversity*Seeding density	1, 60	1.27	0.2633
Year	2, 60	112.50	**<0.0001**
Year*Diversity	2, 60	4.23	**0.0191**
Year*Seeding density	2, 60	0.17	0.8437
Year*Diversity*Seeding density	2, 60	0.40	0.6693
Melilotus spp.			
Diversity	1, 74	0.56	0.4559
Seeding density	1, 74	2.39	0.1261
Diversity*Seeding density	1, 74	0.14	0.7076
Year	3, 67.8	2.84	**0.0492**
Year*Diversity	3, 67.7	0.18	0.9090

Table 2.5. Continued.

Effect	df	*F*	*P*
Melilotus spp., continued			
Year*Seeding density	3, 67.7	1.53	0.2148
Year*Diversity*Seeding density	3, 58.9	0.64	0.5888

Table 2.6. Treatments with significant differences by year in the abundance of *Cirsium vulgare* and *Melilotus* spp in 55m²-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values are significant at $P < 0.05$.

Comparison	Year	df	t	P
Cirsium vulgare				
High div, high density vs. low div, low density	2008	60	-2.66	0.0101
High div, low density vs. low div, low density	2008	60	-3.14	0.0027
High div, high density vs. low div, low density	2009	60	-2.48	0.0159
High div, low density vs. low div, low density	2009	60	-2.05	0.0451
Melilotus spp.				
High div, high density vs. high div, low density	2007	22.24	-3.12	0.0049
High div, high density vs. low div, low density	2007	22.1	-2.46	0.0222

Table 2.7. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on the number of inflorescences removed from planted *Bromus inermis* in 2008 and 2009 and on number of *B. inermis* tillers recorded in 2010 from quadrats placed adjacent to locations where *B. inermis* had been planted and seeded. *B. inermis* was recorded in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	*F*	*P*
Bromus inermis - inflorescences removed			
Total carbon	1, 39	0.05	0.8232
Diversity	1,39	11.10	**0.0019**
Seeding density	1, 39	0.82	0.3707
Diversity*Seeding density	1, 39	0.06	0.8136
Year	1, 39	9.47	**0.0038**
Year*Diversity	1, 39	6.51	**0.0147**
Year*Seeding density	1, 39	0.33	0.5700
Year*Diversity*Seeding density	1, 39	0.42	0.5221
B. inermis - tillers near planting locations			
Diversity	1, 20	2.98	0.0995
Seeding density	1, 20	2.26	0.1484
Diversity*Seeding density	1, 20	3.47	0.0772

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Table 2.7. Continued.

Effect	df	*F*	*P*
B. inermis – tillers near seeding locations			
Diversity	1, 20	0.01	0.9359
Seeding density	1, 20	3.89	0.0625
Diversity*Seeding density	1, 20	0.32	0.5766

Table 2.8. Treatments with significant differences by year in the number of inflorescences removed from planted *Bromus inermis* in 2008 and 2009 and on number of *B. inermis* tillers recorded in 2010 from quadrats placed adjacent to locations where *B. inermis* had been planted. *B. inermis* was recorded in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values are significant at *P* < 0.05.

Comparison	Year	df	*t*	*P*
Bromus inermis- inflorescences removed				
High div, high density vs. low div, low density	2009	20	-2.27	0.0347
High div, low density vs. low div, low density	2009	20	-2.40	0.0265
B. inermis - tillers near planting locations				
High div, high density vs. low div, low density	2010	19.86	-2.30	0.0326
High div, low density vs. low div, low density	2010	20	-2.55	0.0192
Low div, high density vs. low div, low density	2010	19.92	-2.39	0.0267
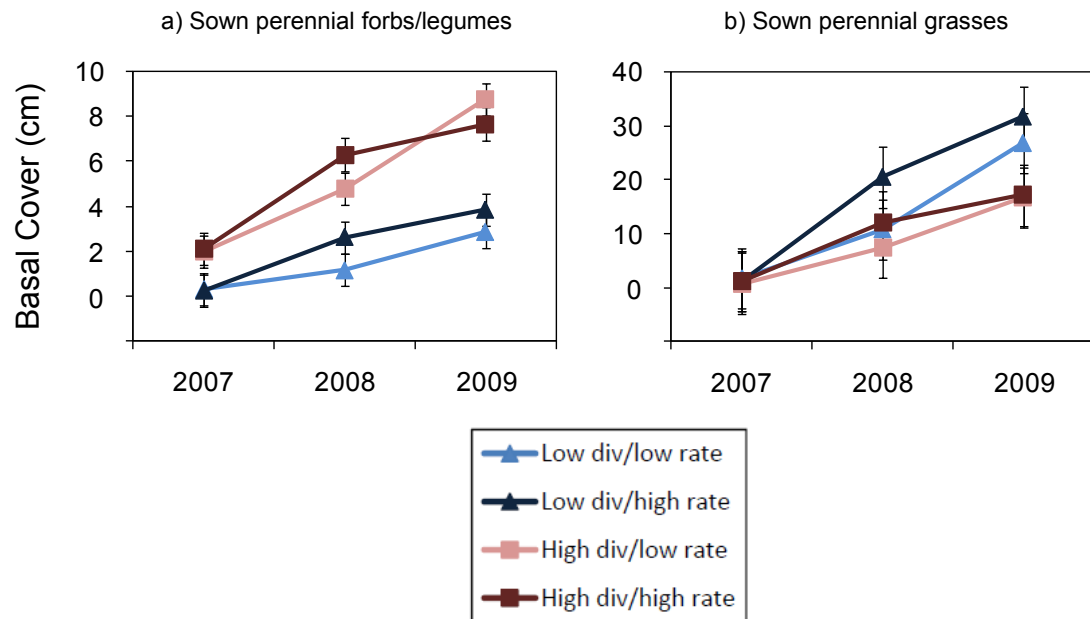
Figure 2.1. Basal cover of plant species aggregated as a) sown perennial forbs/legumes, b) sown perennial grasses, c) unsown perennial forbs/legumes, d) unsown perennial/annual grasses, e) unsown annual/biennial forbs/legumes, and f) invasives during 2007-2009. Plants were assessed in $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.

Figure 2.1. Continued.

a) *Cirsium vulgare*

b) *Melilotus* spp.

Legend:
- Low div/low rate
- Low div/high rate
- High div/low rate
- High div/high rate
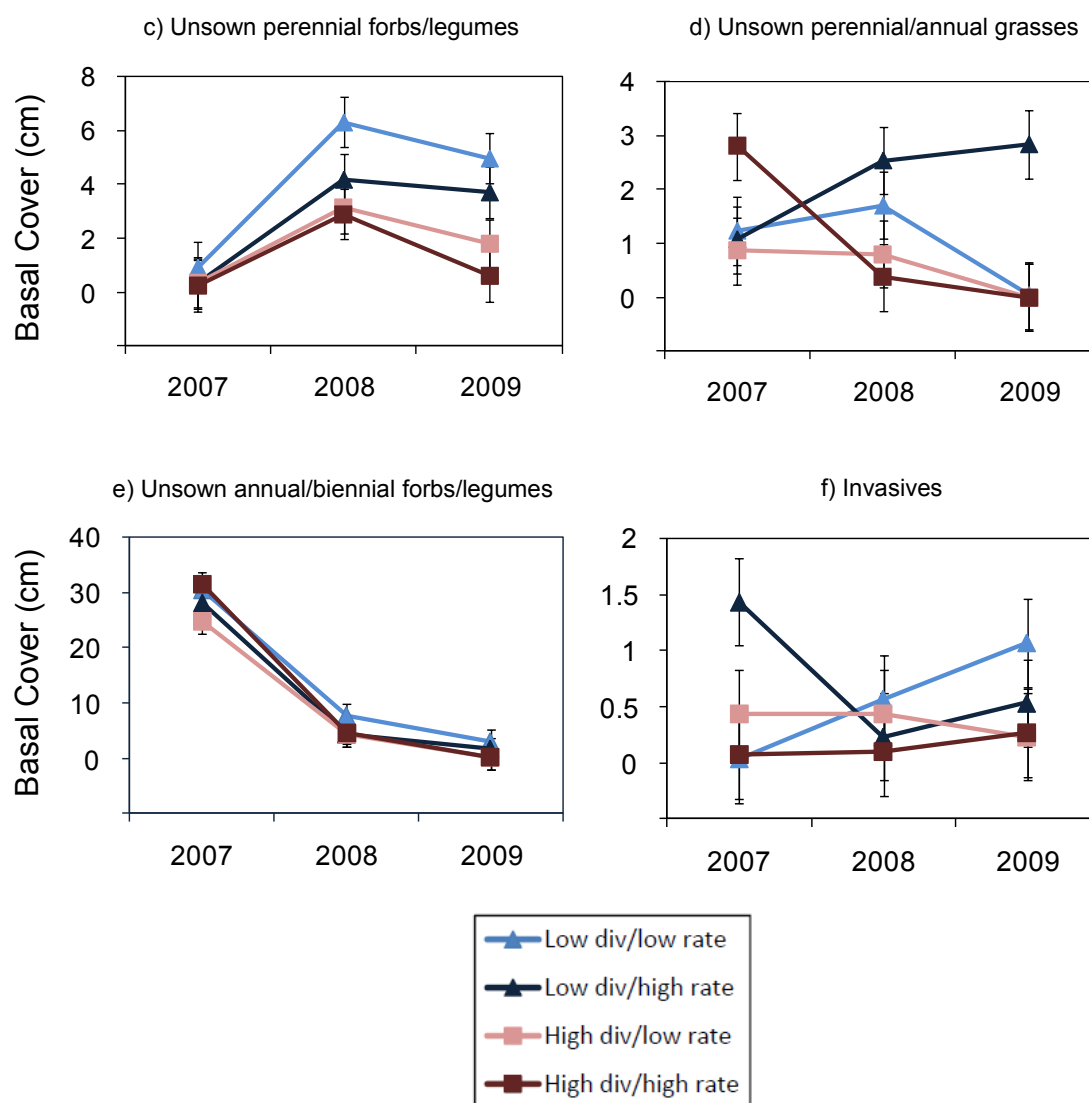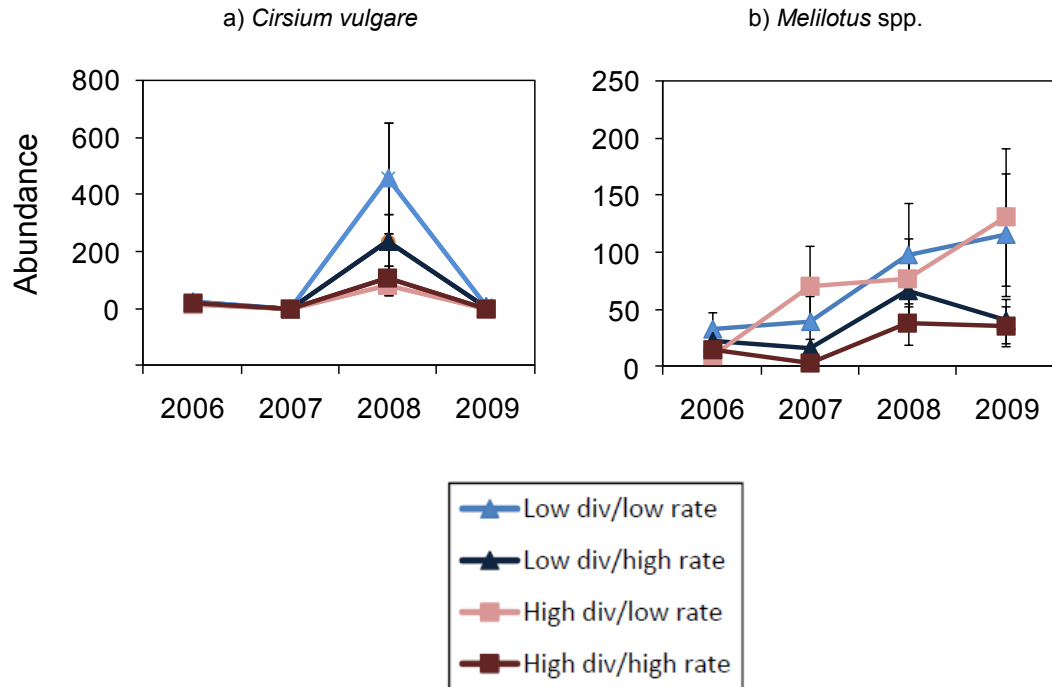
Figure 2.2. Abundance of *Cirsium vulgare* and *Meliltous* spp during 2006-2009. Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.

2006

2008

2009

Legend

Low diversity/low seeding rate
Low diversity/high seeding rate
High diversity/low seeding rate
High diversity/high seeding rate

0   0.05 0.1   0.2 Kilometers
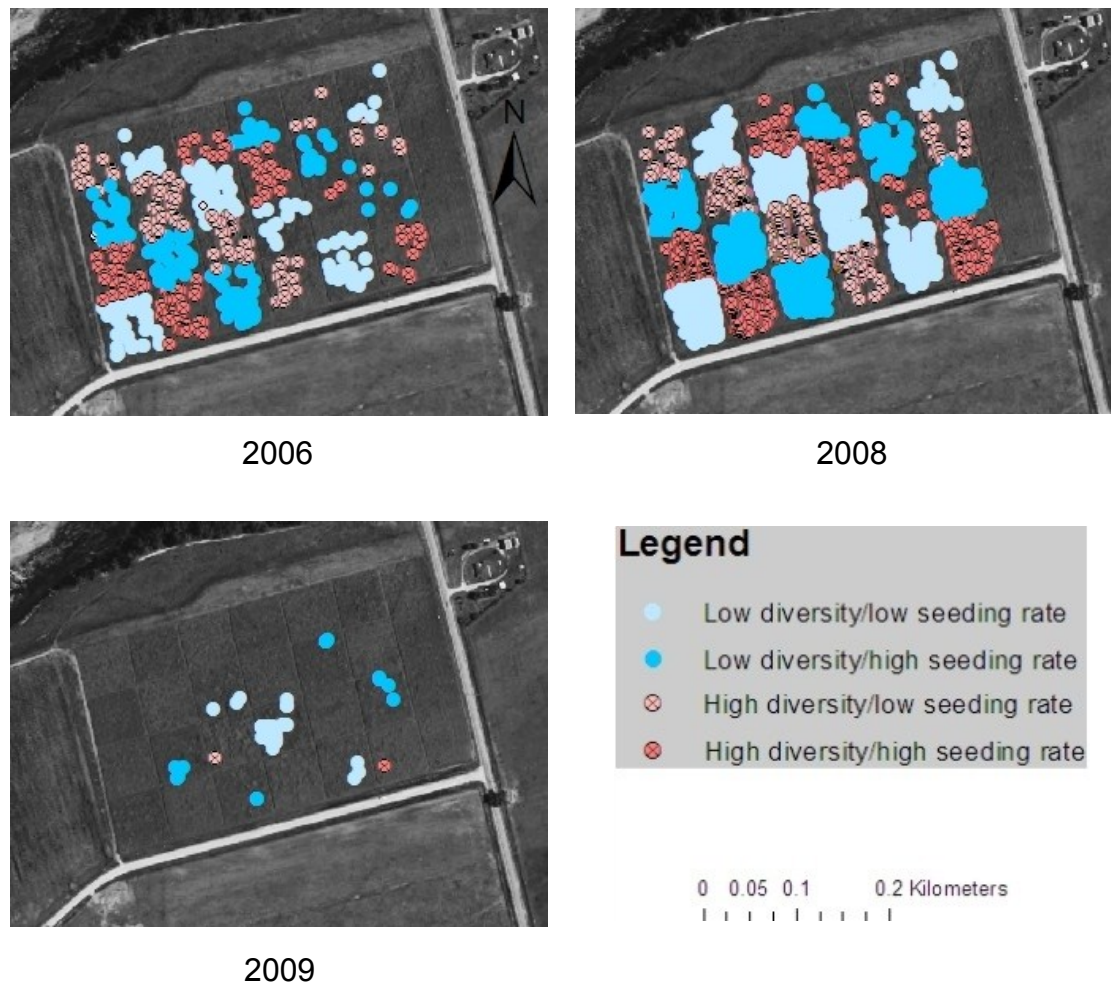
Figure 2.3. Locations of *Cirsium vulgare* plants in 2006, 2008, and 2009 in $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Plant locations were recorded with a Garmin eTrex Legend GPS unit. No *C. vulgare* plants were observed in 2007.

a) *B. inermis* – number of inflorescences clipped per planted individual
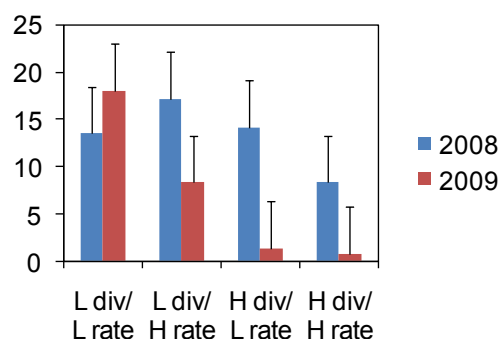
b) *B. inermis* – tillers near planting locations in 2010
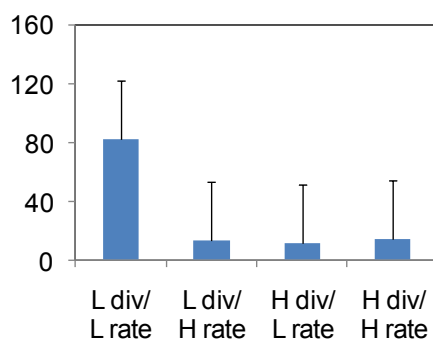
Figure 2.4. Number of seed heads removed per planted *Bromus inermis* individual in 2008-2009 and number of tillers recorded in quadrats adjacent to planting and seeding locations in 2010. *B. inermis* was assessed in $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.

c) *B. inermis* – tillers near seeding locations in 2010
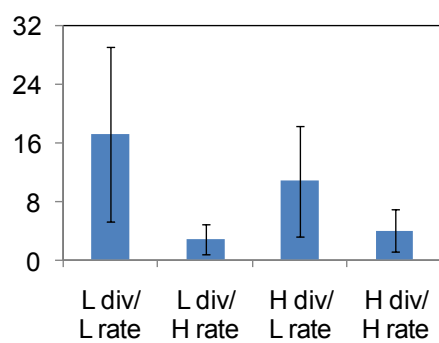
Figure 2.4. Continued.

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CHAPTER 3: RESPONSES OF PREDATORY INVERTEBRATES TO DIVERSITY

AND SEEDING DENSITY IN TALLGRASS PRAIRIE RESTORATIONS

ABSTRACT

In recent decades, thousands of hectares of former cropland have been restored to tallgrass prairie in the central United States using low diversity seed mixes through the U.S. Department of Agriculture's Conservation Reserve Program (CRP) or high plant diversity mixes implemented by conservation organizations and agencies. However, the ability of these restorations to attract predatory invertebrates has not been well documented, even though predators provide an important ecosystem service by naturally regulating herbivores. This study assessed the effects of plant diversity and seeding density on the diversity and abundance of surface-dwelling (ants, carabid beetles, spiders) and aboveground (coccinellid beetles) predatory invertebrates. In the spring of 2006, twenty-four 55m^2-plots were planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Ants, carabid beetles, and spiders were sampled using pitfall traps and coccinellid beetles were sampled using sweep netting in 2007-2009. The abundance of ants, carabids, and spiders showed no response to seed mix diversity or seeding density but there was a significant positive relationship between diversity and the abundance of coccinellid beetles. Seeding density had a strong positive effect on carabid and spider species richness and Shannon-Weaver diversity. These results may be related to differences in the plant species composition and relative amount

of grass basal cover among the treatments rather than diversity per se, as recorded plant species richness and Shannon-Weaver diversity index was similar between the low and high diversity treatments in these young restoration plots.

INTRODUCTION

Predatory invertebrates play an important role in regulating insect pest populations within agroecosystems. Natural regulation of agricultural pests by invertebrate predators and parasites is an ecosystem service estimated to provide 5 to 10 times more control of pest species than industrially produced pesticides (Pimental et al. 1992) and is valued at $4.5 billion annually in the United States (Losey and Vaughan 2006). Invertebrate predators that may reduce the densities of herbivorous insects in cropland include spiders (Araneae) (Laub and Luna 1992, Lang et al. 1999, Maloney et al. 2003), carabid beetles (Coleoptera: Carabidae) (Lang et al. 1999, McCravy and Lundgren 2011), ants (Hymenoptera: Formicidae) (Way and Khoo 1992, Choate and Drummond 2011), and coccinellid beetles (Coleoptera: Coccinellidae) (Obrycki and Kring 1998).

In the Midwestern United States, there is a positive relationship between crop pest abundance and the proportion of cropland in a county (Meehan et al. 2011). Because patches of natural or semi-natural non-crop habitats (e.g., hedgerows, field margins, grassland, woodland) are recognized to be important sources of food, shelter, and overwintering habitat for predatory invertebrates in agroecosystems, restoring habitats on former cropland can increase local habitat heterogeneity, the abundance of predatory invertebrates, and the provision of pest control within an agroecosystem (Bianchi et al. 2006, Rusch et al. 2010, Pywell et al. 2011). One of the rarest habitats within North American agroecosystems is native (unplowed) Northern tallgrass prairie, which since the late 1800s has been largely converted to cropland and covers less than 3% of its pre-settlement extent (Samson et al. 2004). Thousands of hectares of tallgrass prairie have

been restored in the central United States in the last few decades, with a focus on restoring ecosystem services such as soil retention, improved water quality, and provision of habitat for wildlife such as birds, butterflies, mammals, and herpetofauna (Dunn et al. 1993, Packard and Mutel 1997). The predatory invertebrate communities of these restorations have received less attention even though restorations may provide a valuable ecosystem service of pest control.

Initial studies indicate tallgrass prairie can provide valuable habitat for some predatory invertebrates such as carabid beetles. In a comparison of carabid beetle assemblages in six different habitats (tallgrass prairie, oats, corn, soybean, old-field, woods) at four sites in northeastern Iowa, tallgrass prairie hosted a significantly more diverse assemblage of ground beetles than was found in the other habitats (Larsen et al. 2003). Prairie also had a higher percentage of habitat specialists in its assemblage than did the less stable agricultural habitats, which were dominated by generalists. In two recent studies, Davis and Utrup (2010) and Orlofske et al. (2011) assessed the invertebrate communities of tallgrass prairie restorations, including some predator families. Davis and Utrup found no difference in total invertebrate abundance, family richness, or diversity of invertebrates between low- and high-diversity plantings of varying sizes and ages in south-central Nebraska. Similarly, Orlofske et al. (2011) found no significant difference in invertebrate abundance and species richness among remnant and restored prairies in Iowa. In both studies, specimens were not identified beyond the family level. To my knowledge, no studies have compared the abundance of predatory invertebrates within experimental tallgrass restorations created with methods typically used to restore prairie in the region. Such an approach may help identify seed mixes that

are most effective in attracting predatory invertebrates.

Therefore, in this study I compare the abundance and diversity of predatory invertebrates in 55m^2-research plots seeded with a low diversity tallgrass prairie seed mix commonly used in central Nebraska, the U.S. Department of Agriculture's Conservation Reserve Program (CRP) CP25 mix (15 plant species) to a high diversity tallgrass prairie seed mix used in the area by The Nature Conservancy (97 plant species), both at low and high seeding rates. I assess the abundance and diversity of four groups of predatory invertebrates: ants, carabid beetles, spiders, and coccinellid beetles that have been identified to species or the lowest taxonomic level feasible and grouped into feeding guilds. Although both omnivorous and carnivorous invertebrates can be effective in pest control (Hunter 2009) and are encompassed by the term "predatory invertebrates" in this paper, feeding guilds may respond differently to habitat manipulation (Harvey et al. 2008). I test two null hypotheses: 1) the abundance of ant, carabid, spider, and coccinellid feeding guilds will not differ among the four treatments; and 2) the diversity of ant, carabid, spider, and coccinellid feeding guilds will not differ among the four treatments.

METHODS

Study area

The study area lies within the Central Platte River ecosystem, which includes the Platte River channel and floodplain from mid-Dawson County to mid-Hamilton County in central Nebraska (NGPC 2005). The Nebraska Game and Parks Commission has determined the Central Platte River to be a Biologically Unique Landscape (NGPC 2005). The region has a continental climate, with warm, wet summers and cold, dry

winters. Mean annual air temperature is 10.4° C and mean annual precipitation is 63.9 cm (High Plains Regional Climate Center 2010).

The study site is located approximately 10 km south of Wood River, Nebraska (Hall County; 40°44′41″ N, 98°35′11″ W) on a 7.3-ha field owned by The Nature Conservancy. Soils at the site are of loamy alluvium or sandy alluvium parent material and include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Calamux complex, occasionally flooded soils (NRCS 2010). The site is bordered to the south and east by county roads and Nature Conservancy prairie restorations, to the west by a cornfield that was seeded to experimental prairie restoration plots in the spring of 2010, and to the north by trees and the Platte River (Appendix A). The study site was under cultivation in a corn-soybean rotation in the decades prior to the experiment.

Treatments and experimental design

In late March and early April 2006, the 7.3-ha field was cultivated and divided into 24, 0.30-ha plots. The plots were seeded from an all-terrain vehicle (ATV) and a John Deere drop spreader according to a 2×2 factorial design, in which two levels of diversity (low plant diversity and high plant diversity) were applied using two different seeding densities (low and high seeding rates). The experiment was arranged in a randomized block design, with six blocks running west to east across the field and each block containing four plots assigned to the four treatments. Treatments consisted of: 1) a low diversity CRP tallgrass prairie seed mix (CP25 mix, 15 species) used by the NRCS seeded at half the recommended seeding rate (148 grass seeds/m^2, 16 forb seeds/m^2; low diversity/low rate mix); 2) the CP25 mix applied at the recommended seeding rate (297

grass seeds/ m^2, 31 forb seeds/m^2; low diversity/high rate mix); 3) a high diversity

tallgrass prairie mix typically used by the local Nature Conservancy (97 species) seeded

with a seeding rate typical for Nature Conservancy grassland restorations in the region

(129 grass seeds/m^2, 43 forb seeds/m^2; high diversity/low rate), and 4) the Nature

Conservancy mix applied at twice the seeding rate (258 grass seeds/m^2, 86 forb seeds/m^2;

high diversity/high rate) (Appendix B and C). The second and fourth treatments are at

half and double, respectively, NRCS or The Nature Conservancy normal seeding rates

because the NRCS normally recommends rates that are about twice as high as The Nature

Conservancy uses.

The NRCS CP25 seed mix was designed with the Grand Island, Nebraska NRCS

District Conservationist. Grass seed used in the mix was purchased from Arrow Seed in

Broken Bow, Nebraska and forb seed was locally harvested from the Platte River area.

The high-diversity seed mix was harvested from local prairies.

Management of the plots was minimal. All of the plots were burned on March 20,

2008. In July 2008, *Achillea millefolium* and *Helianthus maximilliani* that had invaded

the edges of plots where they had not been sown were sprayed with glyphosate and killed

in order to reduce the edge effect on the spread of these aggressive species. The

inflorescences of *B. inermis* that had naturally invaded the south row of plots from a road

ditch and two plots in the northwestern corner of the field were clipped in order to limit

the spread of *B. inermis* that had not been experimentally introduced into the plots.

Following that effort no plants were intentionally killed or manipulated. Vegetation

growing in unseeded 2-m lanes between the plots was mowed several times during the

growing season.

Invertebrate community composition

I collected surface-dwelling invertebrates by randomly placing ten pitfall traps within each plot for a total of 240 samples, or 60 samples per treatment. I used a random number table to place two pitfall traps along each of five 55-m transects that were 9.1 m apart and ran north to south, with the random number representing the number of paces to be walked along the transect before placing the pitfall trap. Each pitfall trap consisted of an 18-mm diameter glass test tube that was filled 2/3 full with Sierra antifreeze (Safe Brands Corporation, Omaha, Nebraska) and inserted into a polyvinyl chloride [PVC] sleeve in the ground. The traps were left open over a 3-day sampling period in late June or early July and in early September 2007-2009. The PVC sleeves remained permanently in the ground and were capped with cork stoppers when not in use, allowing repeated sampling at the same locations over time. Spiders, carabid beetles, and ants were sent to taxonomic experts to be identified to species or the lowest taxonomic level possible.

Coccinellid beetles were collected with a standard 38-cm diameter canvas sweep net along two 55-m transects within each plot. Coccinellids were collected in mid-June, mid-July, and mid-August 2007-2009. Sweep net samples were collected on sunny to partly cloudy days between 1000 and 1600 when the vegetation was dry, the temperature was above 15° C, and the wind speed was less than 24 kph. A total of 60 sweeps were conducted for each transect; after each set of 20 sweeps, contents of the sweep net were transferred to a sealable plastic bag and stored in a cooler in the field. The samples were then frozen until coccinellid beetles were identified to species in the lab.

Plant community composition

Within each plot, five 55-m transects located 9.1 m apart were established. Each transect ran north to south and was marked on each edge with a 0.6-m rebar spray-painted orange. The species composition of the plant community along three of the 55-m long transects within each plot, the middle transect and the two end transects, was assessed in mid- to late June 2007-2009. The line-intercept transect method was used because it is an efficient method of collecting cover and species richness (Bonham 1989). Starting at the end of each transect, a measuring tape was stretched to a length of one meter. The transect was broken up into these smaller one-meter segments, or "subtransects," to keep the measuring tape from sagging in the wind. The basal cover of any plant touching the top edge of the measuring tape was measured by recording the distance that the plant covered along the tape to the nearest 0.2 cm (Elzinga et al. 1998). Measurements were taken along every twelfth meter and at the opposite end of the transect for a total of six one-meter subtransects along the transect (every 0, 12, 24, 36, 48, and 55 meters were recorded).

Statistical analyses

Plots were the experimental units in this study. For analyzing the effects of diversity and seeding density on abundance, insect species were sorted into feeding guild based on literature reports of adults feeding only on animal material (carnivore), feeding only on seeds (granivore), or feeding on both animal and plant material (omnivore) (Appendix G-I). No species were reported to be herbivores, or feeding only on non-seed plant material such as leaves and stems. Because only two granivore species were collected they were not included in the analysis. Twelve other species were not included

in analyses because there was no information available on their diet. Spiders are recognized to be largely carnivorous, feeding on insects and other arthropods (Foelix 2010). Therefore, spiders were grouped into guilds based on foraging strategy and were either web-builders or hunters (Appendix J). The Shannon-Weaver diversity index (H´) (Shannon and Weaver 1949) was calculated using the BIO-DAP software program (Thomas 2000) for plants and for each invertebrate taxon rather than each feeding guild within a taxon because of the low number of carnivorous species recorded for insects.

Normality in the response variables, abundance of predatory invertebrates by feeding guild, and Shannon diversity and species richness of each invertebrate taxon, was tested with the Kolmogorov-Smirnov normality test (PROC UNIVARIATE, SAS Version 9.2; SAS Institute 2007) and graphs of predicted values against the residuals. Because the response variables were influenced by fixed and random factors and were not normally distributed, each set of data was fitted with a mixed-effects model using PROC GLIMMIX (SAS Version 9.2, SAS Institute 2007). Mixed-effects models are appropriate for data that contains both fixed and random factors and the GLIMMIX procedure does not require the response to be normally distributed (Littell et al. 2006). Diversity, seeding density, sampling date, and their interactions were used as fixed effects and plot and block were used as random effects. Block was subsequently removed from the models when results showed it was not necessary in explaining variance. The covariance structure that was the best fit for each model covering multiple years of data was determined by comparing Akaike's information criterion (AIC) for each model. Spearman rank correlations among plant and insect variables recorded in June or July of each year were conducted using SAS and *P*-values for the Spearman rank test were

adjusted using the Bonferroni method (Rice 1989).

RESULTS

A total of 30,320 ants belonging to 18 species, 4,897 carabid beetles belonging to 52 taxa [51 species, 1 identified to genus], and 406 spiders belonging to 51 taxa [36 species, 12 families, 3 genera] were collected from the pitfall traps (Appendix K). A total of 1,273 coccinellid beetles belonging to six species were collected from sweep netting (Appendix K).

Within each invertebrate group, three or fewer taxa accounted for more than 50% of the specimens collected. The dominant ant species was *Lasius neoniger*, which comprised 97.9% of ant specimens collected across all three years of the study. Carabids were dominated by *Notiobia terminata* and *Pterostichus permundus*, which together accounted for 58.7% of the carabid specimens. The most abundant spider taxa were lycosids, linyphiids, and *Agyneta unimaculata*, which accounted for 51.2% of the spider specimens, and the most abundant coccinellid species was *Hippodamia convergens*, which accounted for 78.3% of the coccinellid specimens. The populations of most species fluctuated and showed no clear trends over time. However, two species were abundant either early or late in the course of the study. The spider *Agyneta unimaculata* was only collected in June 2007 and comprised 25% of spiders in this sampling period. The carabid *Amara musculis* was only collected in September 2009 and comprised 25% of carabids in this sampling period.

Treatment effects on abundance of invertebrates within feeding guilds

Most ant, carabid, and coccinellid species were omnivores and most spiders were hunters (Appendix G-J). The number of coccinellids and ants collected increased over the three years of the study, while the number of carabids collected dropped after the first year (Figure 3.1). The number of hunting spiders was generally similar among all three years but web-building spiders were most abundant in the first sampling period (Figure 3.1).

The abundance of all invertebrate feeding guilds except for carnivorous ants varied with sampling date (Table 3.1). Diversity and the sampling date × diversity interaction were significant positive effects on omnivorous coccinellid abundance. The sampling date × diversity interaction was a significant negative effect on omnivorous carabid abundance. The date × seeding density interaction was nearly a significant effect on carnivorous ant abundance using a value of less than 0.05 for significance ($P = 0.0519$). The interaction of seeding density with diversity was a significant effect on hunting spiders, with the largest difference being between the high diversity, high seeding rate plots (mean abundance = 2.08 ± 0.44 spiders/plot) and the high diversity, low seeding rate plots (mean abundance = 1.06 ± 0.23 spiders/plot). The sampling date × seeding density interaction was a significant effect on web-building spiders.

Treatment effects on invertebrate diversity

Because species richness of carnivorous taxa was small relative to omnivorous taxa for ants, carabids, and coccinellids, mean species richness and Shannon-Weaver diversity index were calculated for all species within an invertebrate group rather than by feeding guild. Species richness and Shannon-Weaver diversity of all invertebrate taxa

(Tables 3.2 and 3.3; Figures 3.2 and 3.3) varied with sampling date. Carabid beetle and spider species richness and Shannon-Weaver diversity was higher in plots seeded at higher densities. Total species richness of carabid beetles was highest in 2007, when 42 species were collected, compared to 25 species in 2008 and 24 species in 2009. The total species richness of spiders was variable throughout the study, with the highest number of spider taxa, 38, collected in 2009. Total species richness of coccinellids and ants was steady throughout the study, ranging from five to six for coccinellids and 11-15 for ants in each year of the study.

Correlation between plant community characteristics and invertebrate diversity and abundance

Although in 2007 species richness and Shannon-Weaver diversity of plants was higher in the plots that had been seeded with the high diversity seed mix, by 2009 species richness and Shannon-Weaver diversity was higher in the plots that had been seeded with the low diversity seed mix (Figure 3.4). Within the high diversity treatments, there was a significant negative correlation between plant diversity and ant and carabid beetle diversity and between plant species richness and carabid species richness in the high seeding rate treatment and the low diversity, low seeding rate treatment (Tables 3.4 and 3.5). Within each treatment, there was a significant negative correlation between forb basal cover and ant abundance, and a positive correlation between grass basal cover and ant abundance that was significant for the low diversity/high density treatment (Table 3.6). In contrast, there was a positive correlation between forb basal cover and carabid or

spider abundance and a negative correlation between grass basal cover and carabid or spider abundance (Table 3.6).

DISCUSSION

The densities of invertebrates are influenced by structure of the plant community, prey availability, disturbance, soil moisture, soil type, and presence of competitors or enemies (Kromp 1999, Landis et al. 2000). In this study, I manipulated the structure of the plant community by altering seeding rates and the initial diversity of the seed mix used to restore experimental tallgrass prairie restoration plots. According to ecological theory describing bottom-up forces in communities, more diverse plant communities should support a more diverse array of herbivores than less diverse plant communities because of the more diverse resource base and niches available for specialized consumers (MacArthur 1972). A more diverse plant community may also directly positively influence the diversity of predators and parasites, because these groups obtain some of their nutrition from plant-provided resources such as pollen and nectar (Wäckers et al. 2005). High plant diversity may also indirectly be associated with a high diversity of predators and parasites that feed on the greater variety of herbivores that become available at different times during the growing season and in a variety of microhabitats (Root 1973). In addition, increasing the species diversity of a habitat can increase the structural complexity of the habitat (e.g., variety in vegetation heights or plant architecture; amount of thatch, leaf litter, or mulch) (Langellotto and Denno 2004). Studies that have increased structural complexity at the spatial scale of a single habitat by increasing the amount of detritus or complexity of the living vegetation by no-till or

mowing practices, intercropping, or polyculture have resulted in significant increases in natural enemy abundance (Langellotto and Denno 2004).

Many studies have supported these theories, finding a positive relationship between plant species diversity and predator invertebrate diversity as measured by species richness or diversity indexes such as the Shannon-Weaver diversity index (Crisp et al. 1998, Siemann et al. 1998, Knops et al. 1999, Jonas et al. 2002) or between plant species richness and predator abundance (Haddad et al. 2001, Pywell et al. 2011). However, some studies have contradicted these results. Koricheva et al. (2000) found a significant negative relationship between plant species richness and spider and carabid beetle activity in European grassland communities. Asteraki et al. (2004) sowed different mixtures of simple grasses or complex grasses with or without forbs and found a positive relationship between plant species richness and spider abundance but predatory beetle abundance showed no relationship with the different mixtures. Davis and Utrup (2010) found no significant difference in the abundance of carabids and spiders collected from pitfall traps from prairie restorations in south-central Nebraska that had been sown with low diversity (4-5 prairie grasses) and high diversity (≥ 25 grass and forb species) seed mixes. They also found vegetation characteristics such as percent forb cover, percent grass cover, and percent bare ground cover to be similar between low and high diversity restorations. Their findings are similar to this study in that I did not find the diversity of the seed mix alone to be a significant explanation for the abundance of ants, spiders, or carabids although the diversity $\times$ sampling date interaction had a significant negative effect on omnivorous carabid beetle abundance. The total recorded plant species richness was higher in the high diversity treatments compared to the low diversity treatments

across all three years of the study (see Chapter 2 Discussion). However, the recorded

plant diversity of the treatments seeded with the high diversity seed mix, as measured by

mean species richness and the Shannon-Weaver diversity mix, was only slightly higher

than the diversity of the low plant diversity treatments in 2007 and by 2009 was lower

than the diversity of the low plant diversity treatments. This pattern may have been due to

the high abundance of Maximilian sunflower (*Helianthus maximiliani*), which was

present in unexpectedly high amounts in the combined grass seed used in the high

diversity seed mix and became a dominant plant species in the high diversity treatments

over time, reducing diversity in these plots.

The main difference in vegetation characteristics between the low and high

diversity treatments was the higher basal cover of grasses in the low diversity treatments,

which was expected because grass seed comprised 90% of the low diversity seed mix

compared to 75% of the high diversity seed mix. In addition, although many unsown

weedy species such as mare's tail (*Conyza canadensis*), dandelion (*Taraxacum

officinale*), and common ragweed (*Ambrosia artemisiifolia var. eliator*) were common

among all the treatments, the species composition recorded in low and high diversity

treatments differed, with the more abundant sown species in the low diversity plots being

Virginia wildrye (*Elymus virginiana var. virginiana*), Canada wildrye (*Elymus

canadensis*), western wheatgrass (*Elymus smithii*), and Canada milkvetch (*Astragalus

canadensis*). Dominant sown species in the high diversity plots included big bluestem

(*Andropogon gerardii*), yarrow (*Achillea millefolium*), and Maximilian sunflower.

Therefore, the abundance of surface-dwelling predatory invertebrates in this study

should be interpreted as showing no response to differences in grass basal cover or plant

species composition rather than differences in plant diversity per se. In addition, the abundance and diversity of some invertebrates with large ranges such as spiders and carabids may be influenced more by factors that operate at larger spatial scales (e.g., land use and presence of corridors) rather than within plot factors (e.g., local habitat characteristics), and several studies have found spider and carabid communities to respond strongly to variables at landscape scales on the order of 200 to 500 m (Aviron et al. 2005, Dauber et al. 2005, Hendrickx et al. 2007, Gardiner et al. 2010, Maisonhaute et al. 2010). Ants have been found to respond more strongly to local microclimatic and soil characteristics (e.g., insolation, soil humidity) (Dauber et al. 2005). Because soil structure in tallgrass prairie restorations changes slowly, taking many years to approach the structure of soil in native prairie (Jastrow 1987), differences in soil characteristics in response to the plant communities had likely not emerged among the treatments at the time the study was conducted.

There was a significant positive effect of diversity on coccinellid abundance which was largely driven by the large number of coccinellids that were collected in the high diversity plots in 2008 and 2009. Because coccinellids were collected by sweep netting, their numbers reflect differences in the aboveground cover rather than basal cover of plants. Adult coccinellids are frequent visitors to the extrafloral nectaries and pollen of various plants as well as honeydew excreted from hemipterans (Lundgren 2009). The greater number of coccinellids collected in high diversity treatments may have reflected a stronger preference for pollen and nectar from forb species that were more prevalent in the high diversity plots, although little information is available on the relative attractiveness of the prairie forbs recorded in this study for coccinellids.

In addition to plant diversity, the density of vegetation can affect the densities of invertebrates within a habitat by affecting food resources and the amount of bare ground cover, which influences microclimate (Arnan et al. 2007). Grass basal cover was higher in the low diversity, high seeding rate plots compared to the low diversity, low seeding rate plots, but the basal cover of forbs was similar between low and high seeding rate plots in all treatments. Seeding density had a significant positive effect in explaining the species richness and Shannon-Weaver diversity index of carabids and spiders, and the total species richness of carabids was highest early in the restoration, in 2007. Many carabids are effective seed predators of weed species, and can shape plant diversity and distribution within a habitat (McCravy and Lundgren 2011). The response to seeding density treatments may reflect a greater diversity of carabid beetle species being attracted to the higher density of sown seeds in the higher seeding rate treatments.

My results identified a negative correlation between grass basal cover and carabid abundance. Similarly, Harvey et al. (2008) collected fewer carabids in plots dominated by grasses than in more open plots. The density of vegetation can affect the movement of surface-dwelling invertebrates. Using mark-recapture methods and simulation modeling, Thomas et al. (2006) demonstrated that increased vegetation density impeded the movement of a carabid beetle, markedly reducing the diffusion rate of the beetle and making beetles less likely to be caught in pitfall traps. Conversely, there was a significant negative correlation between forb basal cover and ant abundance. However, the results of pitfall trapping should be interpreted with caution. If more invertebrates are trapped in less densely vegetated plots there may be several interpretations, including: 1) there are more invertebrates in the less densely vegetated plot, 2) invertebrate numbers are similar

in less and more densely vegetated plots but activity is higher in the less densely vegetated plots, or 3) there are more invertebrates in the more densely vegetated plot but activity is higher in the less densely vegetated plots (Thomas et al. 2006). The amount of food within an area also affects the mobility of invertebrates, with invertebrates generally being less mobile, and therefore less likely to be captured, in areas with high amounts of food (Östmann 2004).

I did not detect large differences in the response of feeding guilds to the plant community. This result is expected with omnivores because they have a larger resource base and can feed on plant materials when prey are scarce, whereas carnivores have a more restricted resource base and may be more closely associated with the availability of prey, which is in turn determined by the composition of the plant community in the treatments. However, the proportion of insect species that were carnivores was small, making the detection of any treatment differences difficult for this feeding guild. Harvey et al. (2008) found that the proportion of carnivorous carabids in the community declined with time, herbivores increased, and proportion of omnivores peaked in the second year. We found that the populations of both omnivorous and carnivorous carabids declined with time.

In conclusion, the responses of the invertebrate communities in these experimental tallgrass restoration plots reflect the relatively low diversity of plant species found in both low and high diversity treatments in young (second-fourth growing seasons) restorations that still had a large proportion of unsown, weedy plant species. Over time the high diversity treatments should become more diverse as conservative species that were seeded appear, which may result in greater differences in the

invertebrate communities. Because at the local scale, the composition of invertebrate communities may be best predicted by the species composition of the plant community, future research could use techniques such as co-correspondence analysis to relate differences in plant species composition to invertebrate abundance and diversity in tallgrass prairie restorations (Schaffers et al. 2008). In addition, while natural habitats provide valuable sources of predatory invertebrates for pest control in adjacent cropland, the spillover of predatory invertebrates from managed to natural systems has been the subject of far less research (Blitzer et al. 2012). Future research should focus on the movement of predatory invertebrates between tallgrass prairie restorations and adjacent cropland to determine whether these restorations serve as sources or sinks for different groups of invertebrates, including pest insects. Finally, because a variety of habitats are needed to provide resources for all stages of the life cycles of some invertebrates (Landis et al. 2005), future research could be conducted at larger scales to study the effect of differing diversity levels and configurations of tallgrass prairie and other habitats such as hedgerows on invertebrate assemblages within the tallgrass prairie region.

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Table 3.1. Results of mixed-model analysis for testing the effects of diversity, seeding density, and sampling date on abundance of invertebrate species aggregated according to feeding guild. Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	Df	F	P
Ants			
Omnivores			
Diversity	1, 120	0.12	0.7280
Seeding density	1, 120	1.37	0.2444
Diversity*Seeding density	1, 120	0.25	0.6185
Date	5, 21.79	14.64	**<0.0001**
Date*Diversity	5, 21.79	2.13	0.1005
Date*Seeding density	5, 21.79	0.86	0.5202
Date*Diversity*Seeding density	5, 21.79	2.01	0.1179
Carnivores			
Diversity	1, 20	0.93	0.3474
Seeding density	1, 20	0.93	0.3474
Diversity*Seeding density	1, 20	0.04	0.8493
Date	5, 100	0.46	0.8026

Table 3.1. Continued.

Effect	df	F	P
Ants, continued			
Carnivores			
Date*Diversity	5, 100	1.50	0.1943
Date*Seeding density	5, 100	2.28	0.0519
Date*Diversity*Seeding density	5, 100	1.31	0.2663
Carabid beetles			
Omnivores			
Diversity	1, 24.48	1.14	0.2955
Seeding density	1, 24.48	1.08	0.3099
Diversity*Seeding density	1, 24.48	1.27	0.2714
Date	5, 30.83	34.03	**<0.0001**
Date*Diversity	5, 30.83	4.92	**0.0020**
Date*Seeding density	5, 30.83	1.35	0.2718
Date*Diversity*Seeding density	5, 30.83	1.41	0.2498
Carnivores			
Diversity	1, 35.2	0.41	0.5260
Seeding density	1, 35.2	0.01	0.9063
Diversity*Seeding density	1, 35.2	1.83	0.1850
Date	5, 30.24	26.95	**<0.0001**
Date*Diversity	5, 30.24	0.23	0.9451

Table 3.1. Continued.

Effect	df	*F*	*P*
Carabid beetles, continued			
Carnivores			
Date*Seeding density	5, 30.24	1.29	0.2961
Date*Diversity*Seeding density	5, 30.24	1.42	0.2461
Coccinellid beetles			
Omnivores			
Diversity	1, 34.3	43.08	**<0.0001**
Seeding density	1, 34.3	0.29	0.5931
Diversity*Seeding density	1, 34.3	1.98	0.1680
Date	8, 38.59	51.35	**<0.0001**
Date*Diversity	8, 38.59	5.63	**<0.0001**
Date*Seeding density	8, 38.59	1.17	0.3419
Date*Diversity*Seeding density	8, 38.59	1.22	0.3162
Spiders			
Hunters			
Diversity	1, 35.58	0.86	0.3588
Seeding density	1, 35.58	2.73	0.1072
Diversity*Seeding density	1, 35.58	4.71	**0.0368**
Date	5, 31.09	4.70	**0.0026**
Date*Diversity	5, 31.09	0.35	0.8767

Table 3.1. Continued.

Effect	df	F	P
Spiders, continued			
Hunters			
Date*Seeding density	5, 31.09	0.49	0.7775
Date*Diversity*Seeding density	5, 31.09	1.11	0.3759
Web builders			
Diversity	1, 25.56	0.02	0.9030
Seeding density	1, 25.56	0.97	0.3341
Diversity*Seeding density	1, 25.56	0.24	0.6268
Date	5, 31.21	9.91	**<0.0001**
Date*Diversity	5, 31.21	0.25	0.9357
Date*Seeding density	5, 31.21	3.35	**0.0156**
Date*Diversity*Seeding density	5, 31.21	1.02	0.4227

Table 3.2. Results of mixed-model analysis for testing the effects of diversity, seeding density, and sampling date on species richness of invertebrate species. Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	F	P
Ants			
Diversity	1, 20	0.02	0.8960
Seeding density	1, 20	0.33	0.5727
Diversity*Seeding density	1, 20	0.02	0.8960
Date	5, 100	5.33	**0.0002**
Date*Diversity	5, 100	1.50	0.1961
Date*Seeding density	5, 100	0.62	0.6811
Date*Diversity*Seeding density	5, 100	0.59	0.7067
Carabid beetles			
Diversity	1, 40.21	1.80	0.1878
Seeding density	1, 40.21	4.87	**0.0331**
Diversity*Seeding density	1, 40.21	2.74	0.1057
Date	5, 31.87	35.17	**<0.0001**
Date*Diversity	5, 31.87	2.97	**0.0261**

Table 3.2. Continued.

Effect	df	*F*	*P*
Carabid beetles, continued			
Date*Seeding density	5, 31.87	0.23	0.9453
Date*Diversity*Seeding density	5, 31.87	0.65	0.6668
Coccinellid beetles			
Diversity	1, 20	3.54	0.0745
Seeding density	1, 20	0.22	0.6431
Diversity*Seeding density	1, 20	0.12	0.7279
Date	8, 160	31.05	**<0.0001**
Date*Diversity	8, 160	1.92	0.0599
Date*Seeding density	8, 160	0.75	0.6473
Date*Diversity*Seeding density	8, 160	0.87	0.5416
Spiders			
Diversity	1, 41.42	5.27	**0.0268**
Seeding density	1, 41.42	6.00	**0.0186**
Diversity*Seeding density	1, 41.42	8.46	**0.0058**
Date	5, 31.36	23.04	**<0.0001**
Date*Diversity	5, 31.36	0.44	0.8196
Date*Seeding density	5, 31.36	1.63	0.1815
Date*Diversity*Seeding density	5, 31.36	1.59	0.1906

Table 3.3. Results of mixed-model analysis for testing the effects of diversity, seeding density, and sampling date on the Shannon-Weaver index (H′) of invertebrate species. Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	F	P
Ants			
Diversity	1, 20	0.17	0.6849
Seeding density	1, 20	2.00	0.1729
Diversity*Seeding density	1, 20	0.00	0.9716
Date	5, 16	14.98	**<0.0001**
Date*Diversity	5, 16	2.45	0.0785
Date*Seeding density	5, 16	1.13	0.3836
Date*Diversity*Seeding density	5, 16	1.53	0.2354
Carabid beetles			
Diversity	1, 41.71	2.40	0.1285
Seeding density	1, 41.71	5.71	**0.0215**
Diversity*Seeding density	1, 41.71	0.94	0.3371
Date	5, 31.25	15.55	**<0.0001**
Date*Diversity	5, 31.25	0.71	0.6232

Table 3.3. Continued.

Effect	df	*F*	*P*
Carabid beetles, continued			
Date*Seeding density	5, 31.25	1.19	0.5611
Date*Diversity*Seeding density	5, 31.25	1.01	0.7549
Coccinellid beetles			
Diversity	1, 42.8	0.35	0.5584
Seeding density	1, 42.8	0.00	0.9481
Diversity*Seeding density	1, 42.8	1.08	0.3048
Date	8, 39.33	22.88	**<0.0001**
Date*Diversity	8, 39.33	1.95	0.0797
Date*Seeding density	8, 39.33	0.88	0.5415
Date*Diversity*Seeding density	8, 39.33	0.24	0.9802
Spiders			
Diversity	1, 20	2.38	0.1390
Seeding density	1, 20	4.78	**0.0409**
Diversity*Seeding density	1, 20	3.80	0.0653
Date	5, 100	17.50	**<0.0001**
Date*Diversity	5, 100	0.59	0.7046
Date*Seeding density	5, 100	1.19	0.3190
Date*Diversity*Seeding density	5, 100	1.01	0.4176

Table 3.4. Spearman correlation coefficients among species richness for plants and surface-dwelling invertebrates (ants, carabid beetles, and spiders). Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at the Bonferroni-corrected $P < 0.0083$.

	Ant richness	Carabid richness	Spider richness
Low diversity/low seeding density			
Plant richness	0.246	**-0.761**	-0.352
Ant richness		-0.076	-0.112
Carabid beetle richness			**0.510**
Low diversity/high seeding density			
Plant richness	0.352	-0.424	**-0.570**
Ant richness		0.086	-0.043
Carabid beetle richness			0.304

Table 3.4. Continued.

	Ant richness	Carabid richness	Spider richness
High diversity/low seeding density			
Plant richness	-0.411	-0.411	-0.193
Ant richness		0.135	0.223
Carabid beetle richness			0.241
High diversity/high seeding density			
Plant richness	-0.256	**-0.572**	-0.121
Ant richness		-0.118	0.263
Carabid beetle richness			0.035

Table 3.5. Spearman correlation coefficients among Shannon-Weaver diversity index (H′) for plants and surface-dwelling invertebrates (ants, carabid beetles, and spiders). Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at the Bonferroni-corrected $P < 0.0083$.

	Ant H′	Carabid H′	Spider H′
Low diversity/low seeding density			
Plant H′	-0.333	-0.500	-0.303
Ant H′		**0.650**	**0.689**
Carabid beetle H′			**0.597**
Low diversity/high seeding density			
Plant H′	-0.147	-0.314	-0.529
Ant H′		0.261	-0.0162
Carabid beetle H′			0.0837

Table 3.5. Continued.

	Ant H′	Carabid H′	Spider H′
High diversity/low seeding density			
Plant H′	**-0.723**	**-0.617**	-0.365
Ant H′		0.482	0.384
Carabid beetle H′			0.159
High diversity/high seeding density			
Plant H′	**-0.710**	**-0.799**	-0.196
Ant H′		0.530	0.390
Carabid beetle H′			0.110

Table 3.6. Spearman correlation coefficients among grass cover for plants and abundance of surface-dwelling invertebrates (ants, carabid beetles, and spiders). Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at the Bonferroni-corrected $P < 0.0083$.

	Ant abundance	Carabid abundance	Spider abundance
Low diversity/low seeding density			
Grass basal cover	0.593	-0.0773	-0.310
Forb basal cover	**-0.903**	0.601	**0.644**
Ant abundance		-0.580	-0.661
Carabid beetle abundance			0.259
Low diversity/high seeding density			
Grass basal cover	**0.637**	**-0.644**	-0.520
Forb basal cover	**-0.601**	0.560	0.291
Ant abundance		**-0.696**	**-0.611**

Table 3.6. Continued.

	Ant abundance	Carabid abundance	Spider abundance
Carabid abundance			0.388
High diversity/low seeding density			
Grass basal cover	**0.637**	**-0.644**	-0.520
Forb basal cover	**-0.601**	0.560	0.291
Ant abundance		**-0.696**	**-0.611**
Carabid abundance			0.388
High diversity/high seeding density			
Grass basal cover	0.450	**-0.606**	-0.278
Forb basal cover	**-0.677**	**0.655**	0.482
Ant abundance		-0.418	-0.502
Carabid abundance			0.250
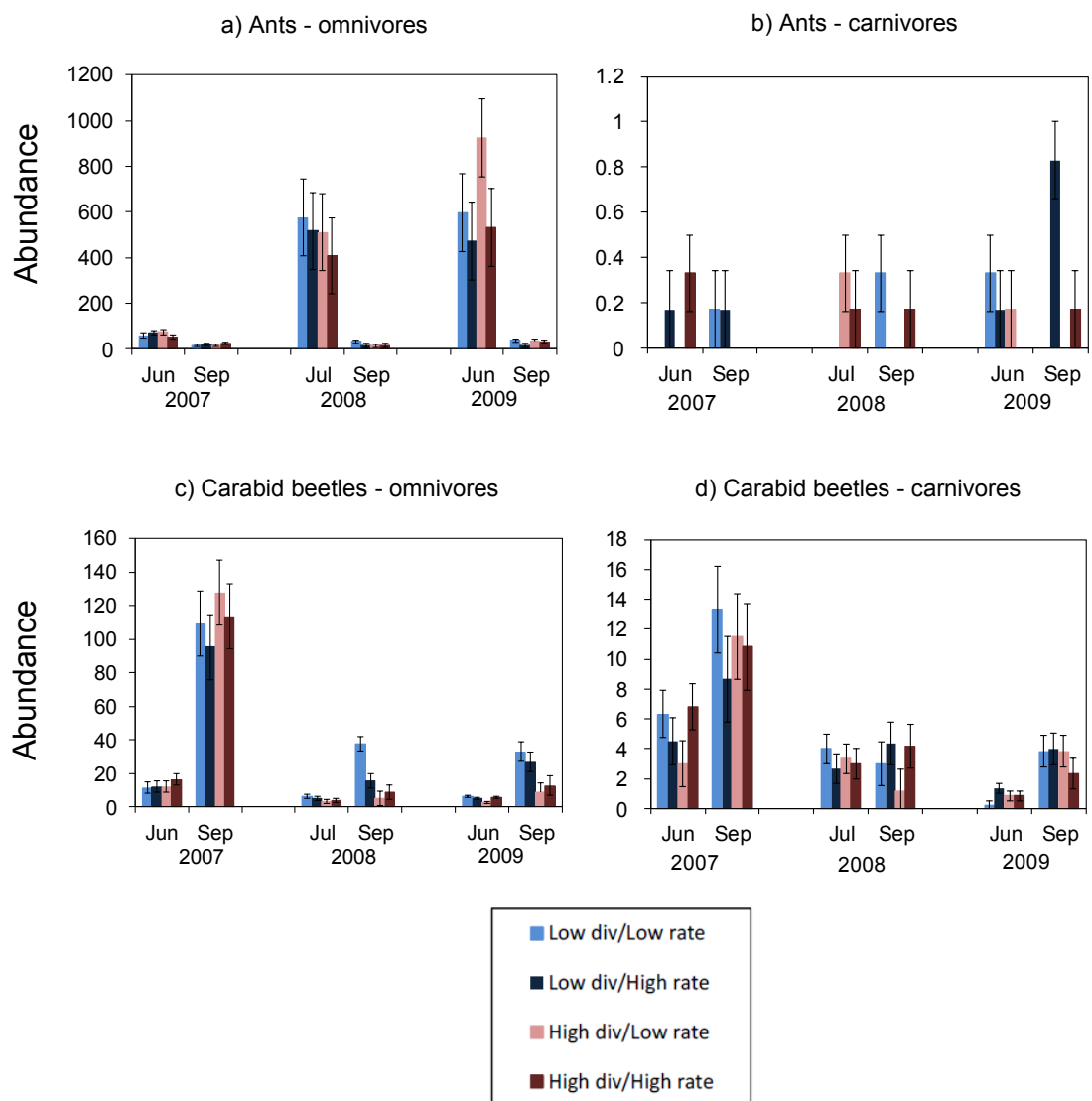
Figure 3.1. Abundance of invertebrate species aggregated according to feeding guild during 2007-2009. Invertebrates were collected in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.

e) Coccinellid beetles - omnivores

f) Spiders - hunters

g) Spiders – web builders

Low div/Low rate

Low div/High rate

High div/Low rate

High div/High rate
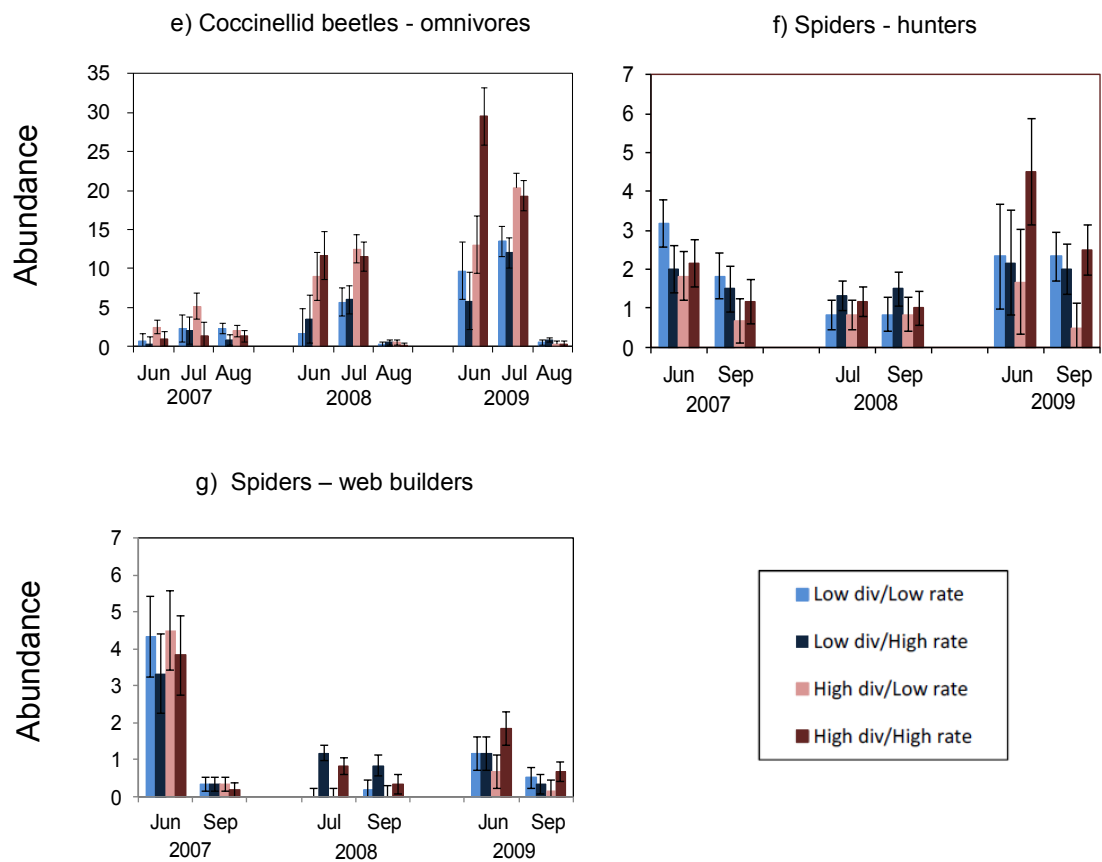
Figure 3.1. Continued.

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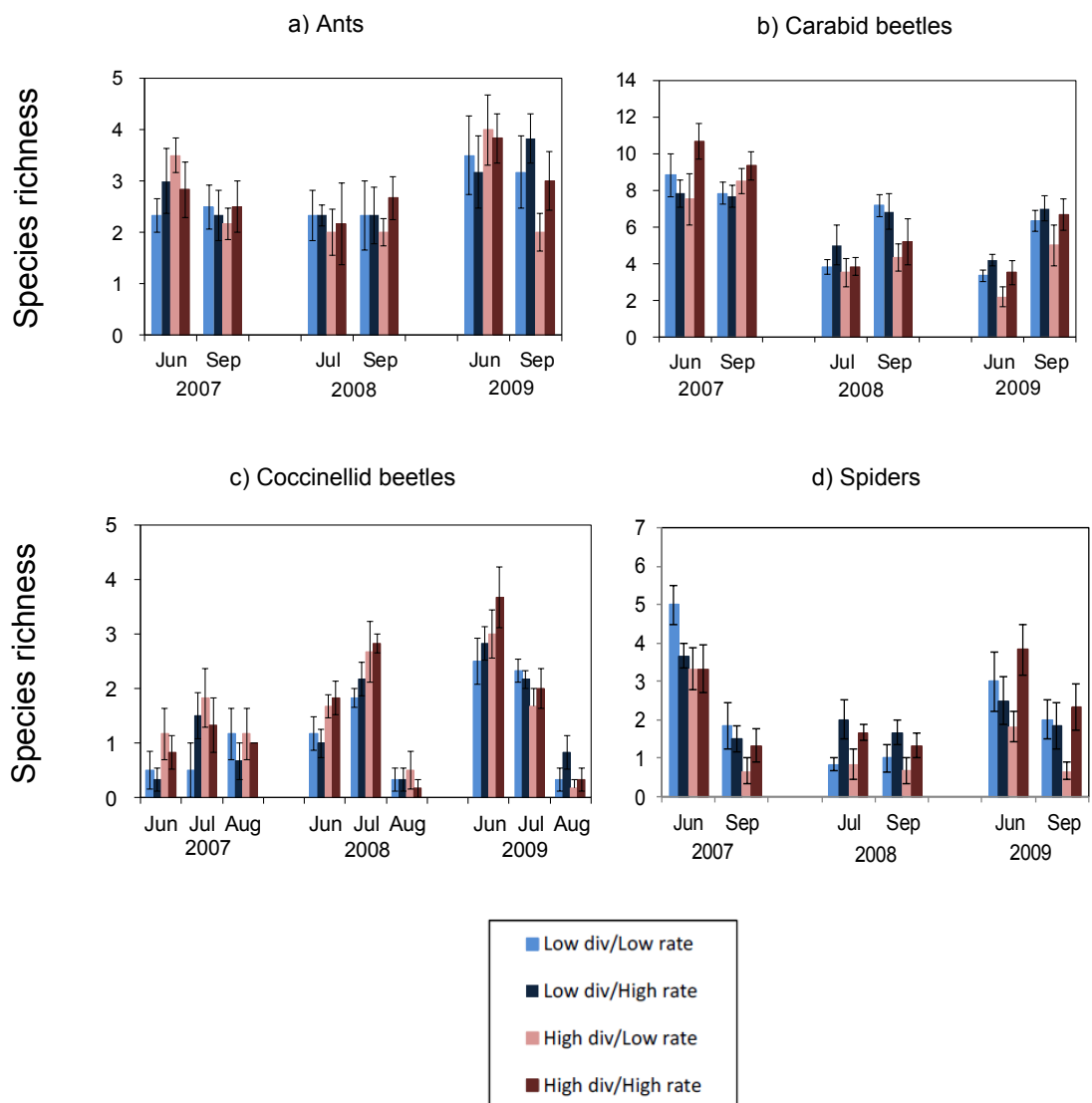
Figure 3.2. Species richness of invertebrate species collected during 2007-2009 in 55m²-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. *N* = 6 plots per treatment.
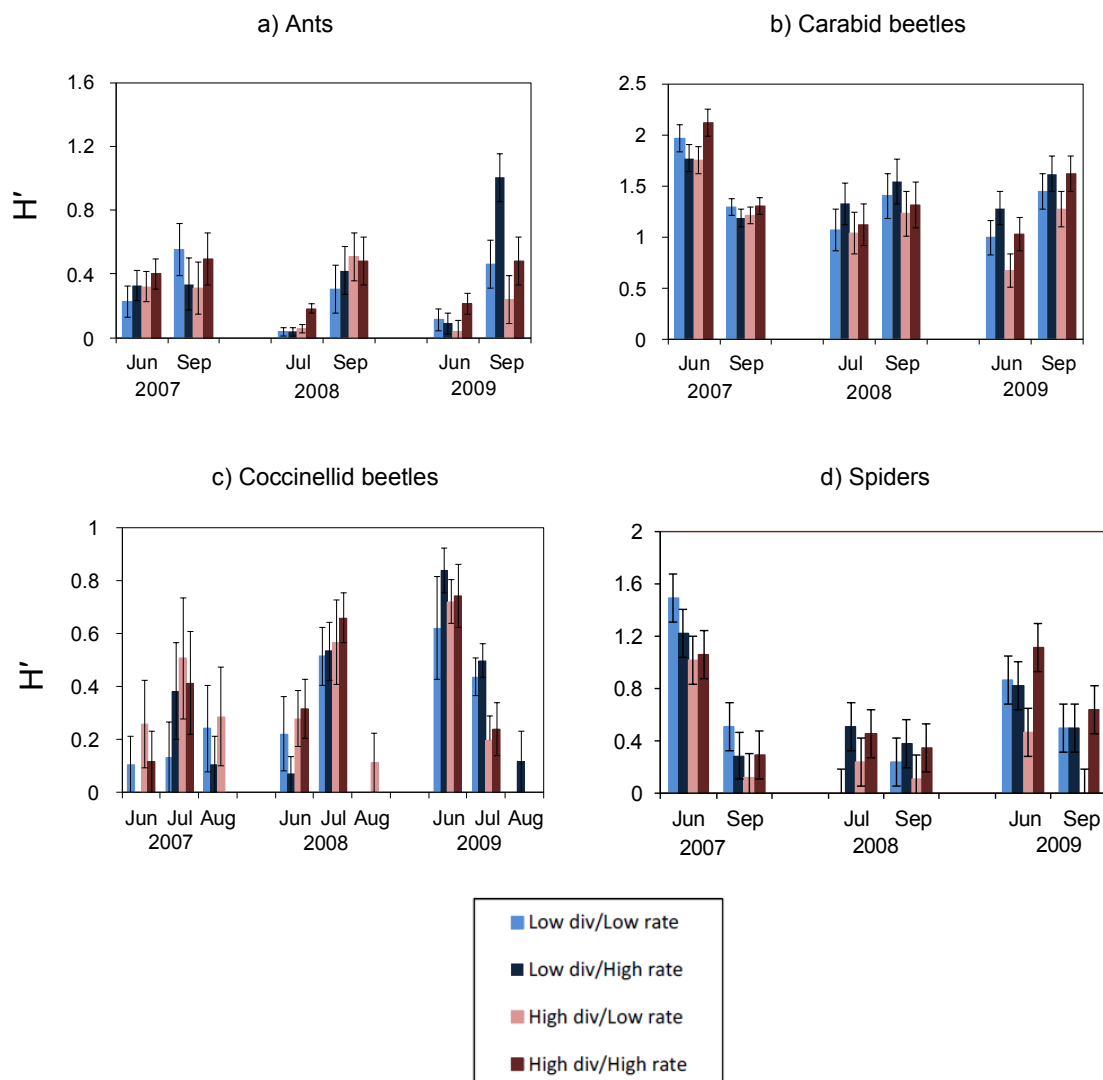
Figure 3.3. Shannon-Weaver diversity index (H′) of invertebrate species collected during 2007-2009 in 55m²-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.
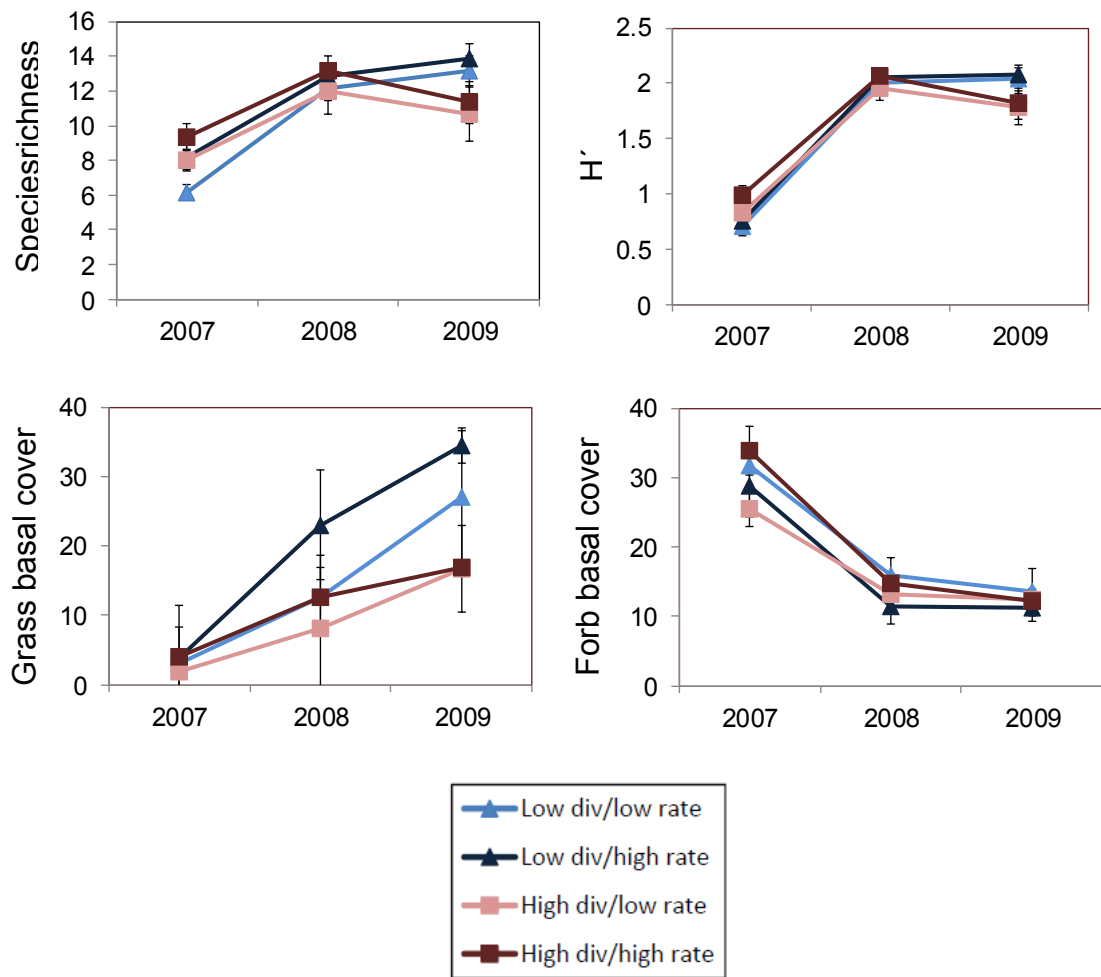
Figure 3.4. Species richness, Shannon-Weaver diversity index (H′), grass basal cover, and forb basal cover of plant species assessed during 2007-2009 in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), also at low and high seeding densities. Values are least-square means (± SE) from mixed-model analysis. $N = 6$ plots per treatment.

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CHAPTER 4: THE RELATIONSHIPS AMONG DIVERSITY, SEEDING DENSITY

AND HERBIVORY DAMAGE IN TALLGRASS PRAIRIE RESTORATIONS

ABSTRACT

Little is known about the relationship between plant diversity and seeding density in grassland restorations and trophic interactions such as herbivory levels in these restorations, which can influence plant succession. I assessed the effects of plant diversity and seeding density on herbivory damage of two perennial forbs, *Ratibida columnifera* and *Solidago canadensis*, in experimental tallgrass prairie plots in central Nebraska, USA. In the spring of 2006, twenty-four 55m^2 plots were planted to six replicates in each of four treatments: high diversity sites of 97 species planted at a low seeding density, high diversity sites at twice this seeding rate using a mix commonly used by The Nature Conservancy, and low diversity sites planted at low and high seeding densities using a U.S. Department of Agriculture Conservation Reserve Program (CRP) mix (CP25; 15 species). In 2010 and 2011, the area of the leaf missing was estimated for leaves of ten individual plants for each species within each plot. Year was the main significant effect for explaining levels of herbivory damage in *R. columnifera and S. canadensis* leaves. Differences in herbivory by year were likely attributed to year-to-year variations in insect populations. Future research should explore additional metrics for measuring herbivory damage effects such as physiological changes in damaged plants or damage from sap-feeding insects. Diversity effects on herbivory damage may also emerge as these restorations age and more conservative species appear in the high diversity plots, enhancing diversity differences among the treatments.

INTRODUCTION

Many studies have considered the community-level relationship between plant diversity and herbivorous insect diversity and abundance in experimental grasslands, generally finding a positive relationship (Siemann et al. 1998, Knops et al. 1999, Mulder et al. 1999, Koricheva et al. 2000, Symstad et al. 2000, Haddad et al. 2001). However, the relationship between grassland plant diversity and trophic interactions such as the interaction between herbivorous insects and individual plant species within a community has received less attention (Scherber et al. 2006, Unsicker et al. 2006).

Although insects may have a minor impact on primary production and rarely consume enough tissue to kill their host plants (Crawley 1997), they can reduce the fitness and competitiveness of host plants (Muller-Scharer and Brown 1995, Tscharntke and Greiler 1995). Herbivorous insects can therefore influence the composition of plant communities by suppressing some species, particularly during the early stages of succession (Louda et al. 1990, Brown and Gange 1992, Carson and Root 1999).

In turn, the composition and diversity of plant communities may influence the abundance of herbivores and possibly the magnitude of their effects on the plant community. Two theories to explain the effect of plant community diversity on herbivory include the resource concentration hypothesis, which was developed in an agricultural system (Root 1973), and the resource dilution hypothesis, which arose from a study in experimental grassland plots (Otway et al. 2005). According to the resource concentration hypothesis, herbivore pressure should be higher in monocultures of plants because specialist herbivores more easily find their host plants in simple habitats than in more diverse plant communities where the density of their host plant species is lower. In

contrast, the resource dilution hypothesis predicts that herbivore pressure is higher on host plant species in higher diversity plant mixtures - although initially herbivores may have a harder time finding their host plants in higher diversity mixtures, over time their populations grow more rapidly on these host plants because herbivores more efficiently use hosts that are sparsely distributed (Yamamura and Yano 1999). Some studies have found the resource concentration hypothesis to be true for certain specialist herbivores (Knops et al. 1999, Koricheva et al. 2000, Haddad et al. 2001) while others support the resource dilution hypothesis (Yamamura 2002, Otway et al. 2005). These studies focused on plant-herbivore interactions at the community level but we know less about the relative herbivore pressure placed on individual plant species in low and high diversity natural habitats. Such knowledge could improve our understanding of the role herbivorous insects may play in affecting the species composition of a plant community.

Two recent studies in Germany have considered the relationship between grassland plant diversity and insect herbivory of individual plant species transplanted into experimental grasslands (Scherber et al. 2006) and semi-natural montane grasslands (Unsicker et al. 2006). Scherber et al. transplanted individuals of a perennial forb, garden sorrel (*Rumex acetosa*), into 82 experimental grassland plots of increasing species richness (1, 2, 4, 8, 16, or 60 plant species). They found the effects of insect herbivory on garden sorrel to be independent of plant diversity. Unsicker et al. transplanted individuals of the perennial forbs ribwort plantain (*Plantago lanceolata*) and red clover (*Trifolium pretense*) into 19 grasslands with the mean plant species richness ranging from 18 to 45 species/m^2 across the sites. Both species also naturally occurred in most of the sites. They quantified leaf damage to the transplanted species and to plants growing along

transects placed in the study sites. In accordance with the resource concentration hypothesis, in June herbivory levels of the plants were significantly lower in the higher diversity sites. However, there was no significant difference between damage and species richness in August and there was no relationship between insect herbivory and plant species richness for the two transplanted species in either month. The transplants also had less herbivore damage compared to individuals of the same species already present in the grasslands.

Similar studies for North American grasslands have not been published. This study is the first to compare herbivore damage of individual plant species among low and high diversity tallgrass prairie sites. I assess herbivory rates on two perennial forbs, upright prairie coneflower (*Ratibida columnifera*), and Canada goldenrod (*Solidago canadensis*) in experimental tallgrass prairie restoration plots seeded with one of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy, planted at a low seeding density (129 grass seeds/m^2, 43 forb seeds/m^2), high diversity sites at twice the low seeding rate (258 grass seeds/m^2, 86 forb seeds/m^2) and low diversity sites using a U.S. Department of Agriculture Conservation Reserve Program (CRP) mix (CP25; 15 species), at low (148 grass seeds/m^2, 16 forb seeds/m^2) and high seeding densities (297 grass seeds/m^2, 31 forb seeds/m^2). The high seeding rate for the high diversity treatments is equivalent of the suggested CRP seeding rate. *R. columnifera* and *S. canadensis* were selected because they are native and relatively abundant among all of the treatments. Known insect herbivores that feed on *R. columnifera* include *Lygus* sp., which are members of Miridae (Fiedler and Landis 2007), and the chrysomelid beetle *Brachypnoea margaretae* (Sauer 2009). Insects that feed on

the leaves of *S. canadensis* include the leaf beetles *Trirhabda canadensis*, *T. borealis*, and *T. virgata* (Werner et al. 1980).

Ratibida columnifera was included in both the low and high diversity seed mixes, but *S. canadensis* was not seeded and emerged from the seed bank. Because herbivory can affect the rate of plant growth (Crawley 1989), I measured the height of sampled plants to see if there was a correlation between plant height and levels of herbivory damage. In 2010, the distance between the sampled *R. columnifera* plant and the nearest *R. columnifera* plants in the four cardinal directions was measured to determine how isolated the sampled plant was, which may influence the ability of herbivores to find the plant. I test three null hypotheses: 1) the extent of leaf damage inflicted upon top, middle, and bottom leaves of *R. columnifera* and *S. canadensis* does not differ among the treatments, 2) the height of sampled plants does not differ among the four treatments, and 3) the mean distance from sampled *R. columnifera* plants to nearby *R. columnifera* plants does not differ among the treatments.

METHODS

Study area

The study area lies within the Central Platte River ecosystem, which includes the Platte River channel and floodplain from mid-Dawson County to mid-Hamilton County in central Nebraska (NGPC 2005). The Nebraska Game and Parks Commission has determined the Central Platte River to be a Biologically Unique Landscape (NGPC 2005). The region has a continental climate, with warm, wet summers and cold, dry winters. Mean annual air temperature is 10.4° C and mean annual precipitation is 63.9

centimeters (High Plains Regional Climate Center 2010).

The study site is located approximately 10 km south of Wood River, Nebraska (Hall County; 40°44′41″ N, 98°35′11″ W) on a 7.3-ha field owned by The Nature Conservancy. Soils at the site are of loamy alluvium or sandy alluvium parent material and include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Calamux complex, occasionally flooded soils (NRCS 2010). The site is bordered to the south and east by county roads and Nature Conservancy prairie restorations, to the west by a cornfield that was seeded to experimental prairie restoration plots in the spring of 2010, and to the north by trees and the Platte River (Appendix A). The study site was under cultivation in a corn-soybean rotation in the decades prior to the experiment.

Treatments and experimental design

In late March and early April 2006, the 7.3-ha field was cultivated and divided into 24, 0.30-ha plots. These plots were seeded from an all-terrain vehicle (ATV) and a John Deere drop spreader according to a 2×2 factorial design, in which two levels of diversity (low plant diversity and high plant diversity) were applied using two different seeding densities (low and high seeding rates). The experiment was arranged in a randomized block design, with six blocks running west to east across the field and each block containing four plots assigned to the four treatments. Treatments consisted of: 1) a low diversity CRP tallgrass prairie seed mix (CP25 mix, 15 species) used by the NRCS seeded at half the recommended seeding rate (148 grass seeds/m^2, 16 forb seeds/m^2; low diversity/low rate mix); 2) the CP25 mix applied at the recommended seeding rate (297 grass seeds/ m^2, 31 forb seeds/m^2; low diversity/high rate mix); 3) a high diversity

tallgrass prairie mix typically used by the local Nature Conservancy (97 species) seeded

with a seeding rate typical for Nature Conservancy grassland restorations in the region

(129 grass seeds/m^2, 43 forb seeds/m^2; high diversity/low rate), and 4) the Nature

Conservancy mix applied at twice the seeding rate (258 grass seeds/m^2, 86 forb seeds/m^2;

high diversity/high rate) (Appendix B and C). The second and fourth treatments are at

half and double, respectively, NRCS or The Nature Conservancy normal seeding rates

because the NRCS normally recommends rates that are about twice as high as The Nature

Conservancy uses. The NRCS CP25 seed mix was designed with the Grand Island,

Nebraska NRCS District Conservationist. Grass seed used in the mix was purchased from

Arrow Seed in Broken Bow, Nebraska and forb seed was locally harvested from the

Platte River area. The high-diversity seed mix was harvested from local prairies.

Management of the plots was minimal. All of the plots were burned on March 20,

2008 and on May 2, 2011. In July 2008, *Achillea millefolium* and *Helianthus

maximilliani* that had invaded into the edges of plots where they had not been sown were

sprayed with Glyphosate and killed in order to reduce the edge effect on the spread of

these aggressive species. The inflorescences of *B. inermis* that had naturally invaded the

south row of plots from a road ditch and two plots in the northwestern corner of the field

were clipped in order to limit the spread of *B. inermis* that had not been experimentally

introduced into the plots. Following that effort no plants were intentionally killed or

manipulated. Vegetation growing in unseeded 2-m lanes between the plots was mowed

several times during the growing season.

Measurement of herbivory damage

A random numbers table was used to determine the starting sampling point within each plot. The nearest individuals of *R. columnifera* and *S. canadensis* to the starting point were marked. From the marked plant a line was walked in a random direction until another plant of the same species was observed, and a line was again walked in a random direction until the next individual was encountered. Ten individuals of each species were sampled within each plot. On each plant, three leaf ages were sampled by randomly selecting one leaf from the bottom, middle, and top portions of the plant. For each leaf, herbivory was quantified by visually estimating the percentage of leaf that was missing from the entire leaf area, in one of six classes: 0, 1-4%, 5-25%, 26-50%, 51-75%, and 76-99%. To assess the health of the plant, the height of each plant was measured with a meter stick. In 2010, the distance between each sampled *R. columnifera* plant and the nearest *R. columnifera* plant in each of the four cardinal directions was recorded with measuring tape. Sampling was conducted July 22-25, 2010 and July 24-25, 2011 for *R. columnifera* and September 11-12, 2010 and September 5-6, 2011 for *S. canadensis*.

Data analysis

Because many leaves belonged to the 0 and 1-4% classes and each of the higher herbivory damage classes contained relatively few leaves, the classes were consolidated into 0-4% and 5-99% damage classes. For each area of the plant (top, middle, and bottom), the mean number of individual leaves belonging to the low herbivory damage class of 0-4% and the high herbivory damage class of 5-99% in each treatment ($n = 6$, the number of plots per treatment) was used in data analysis. Normality in the response variables, leaf damage, plant height, and mean distance, was tested with the Kolmogorov-

Smirnov normality test (PROC UNIVARIATE, SAS Version 9.2; SAS Institute 2007)

and graphs of predicted values against the residuals. Each set of data was fitted with a

mixed-effects model using PROC GLIMMIX (SAS Version 9.2; SAS Institute 2007).

Mixed-effects models are appropriate for data that contains both fixed and random factors

and the GLIMMIX procedure does not require the response to be normally distributed

(Littell et al. 2006). Plant height and mean distance data were normally distributed, but

leaf damage data was not. Diversity, seeding density, year, and their interactions were

used as fixed effects and plot and block were used as random effects. Block was

subsequently removed from the models when results showed it was not necessary in

explaining variance. For herbivory damage and height, the covariance structure that was

the best fit for each model covering multiple years of data was determined by comparing

Akaike's information criterion (AIC) for each model.

RESULTS

Ratibida columnifera

Herbivory on *Ratibida columnifera* was widespread, with 78.8% of top leaves,

84.8% of middle leaves, and 58.3% of bottom leaves sampled missing part of the leaf due

to herbivores over both years of the study. In addition, 4.8% of the middle leaves and

35.4% of the bottom leaves sampled were dead, and herbivory damage was not

determined for these leaves. Although herbivory levels were generally similar across both

years, the proportion of the bottom leaves that were dead was higher in 2010 (28.5%)

than in 2011 (6.9%).

For each area of the plant, most leaves had 0-4 percent of the leaf missing although in 2010 most of the bottom leaves sampled were dead (Figure 4.1). Year was the only significant effect for explaining herbivory damage in *R. columnifera*, with more middle and bottom leaves exhibiting low levels of damage and more bottom leaves being heavily damaged in 2011 than in 2010 (Tables 4.1 and 4.2, Figure 4.1).

The height of *R. columnifera* plants differed among the plots, with diversity, the diversity × seeding density interaction and year having significant effects on height (Table 4.3). In 2010, the low diversity, high seeding density treatments contained the tallest *R. columnifera* plants (mean height = 77.9 ± 3.4 cm/plot) and in 2011, the low diversity, low seeding density treatments contained the tallest *R. columnifera* plants (mean height = 68.4 ± 1.8 cm/plot). In 2010, diversity and seeding density had a significant effect on mean distance from the sampled *R. columnifera* plant to the nearest other *R. columnifera* plant (Table 4.4). The shortest mean distance among neighboring plants was recorded in the low diversity, low seeding density plots, indicating *R. columnifera* was most abundant in these plots (44.6 ± 5.7 cm) (Table 4.4, Figure 4.4).

Solidago canadensis

Herbivory damage on *S. canadensis* was less than that on *R. columnifera*, with 50.2% of top leaves, 51.0% of middle leaves, and 14.2% of bottom leaves sampled exhibiting herbivory damage. Dead leaves comprised 10.2% of the middle leaves and 70.2% of the bottom leaves. As with *R. columnifera*, the proportion of bottom leaves that were dead was higher in 2010 (91.7%) than in 2011 (48.8%).

For each area of the plant, most leaves with herbivory damage were missing 0-4 percent of the leaf although in 2010 most of the bottom leaves sampled were dead (Figure 4.2). Year was a significant effect for high herbivory damage of the top, middle, and bottom *S. canadensis* leaves, with more damage recorded in 2010 for top and middle leaves and more damage recorded in 2011 for bottom leaves (Table 4.1, Figure 4.2). The only other significant effect for high herbivory damage in *S. canadensis* was the year × diversity × seeding density interaction for the bottom leaf, with high herbivory levels being significantly more in 2011 than in 2010 (Table 4.1, Figure 4.2). Year was also significant low herbivory damage on all three areas of *S. canadensis* plants, with more leaves belonging to the low herbivory group in 2011 than in 2010 (Table 4.2, Figure 4.2). Diversity, year, and the year × diversity interaction were significant in explaining the height of *S. canadensis* among the treatments. Although in 2011, the height of sampled *S. canadensis* plants was similar among all four treatments, in 2010 plants in the low diversity treatments were significantly taller than in the high diversity treatments, with the tallest plants recorded in the low diversity, high seeding density treatment (Table 4.3, Figure 4.3).

DISCUSSION

The levels of leaf damage observed in this study, which was largely in the range of 0-4% per leaf on two perennial forbs, was similar to levels found in grassland and other herbaceous community studies worldwide. Carson and Root (1999) found that leaf area damage on most herbaceous species they sampled in early successional plant communities in New York state, including *S. altissima*, a subspecies of *S. canadensis*

(Kaul et al. 2006), was almost never greater than 3%. Mulder et al. (1999) observed herbivory levels below 5% on grasses, forbs and legumes in a grassland biodiversity experiment in Switzerland and Sweden. Scherber et al. (2006) found herbivory levels of 2-6% on forbs and legumes in experimental grassland plots in Germany. Mean damage levels on forbs, legumes, and grasses in German hay meadows were usually below 6% (Unsicker et al. 2006). Individual plant species can experience higher levels of herbivory. For example, two species belonging to the genus Rumex, *Rumex crisipus* and *Rumex acetosa*, experienced extensive damage (more than 15-20% of the leaf damage) (Carson and Root 1999, Unsicker et al. 2006).

However, none of these studies differentiated between herbivory levels on the top, middle, or bottom areas of the plants sampled, and instead estimated herbivory damage on all leaves of a sampled plant (Scherber et al. 2006, Unsicker et al. 2006) or a subset of randomly selected leaves (Mulder et al. 1999) or leaves at regular intervals along the length of the plant (Carson and Root 1999). The effects of herbivory on plant fitness can depend not only on the amount of leaf area removed but on the spatial pattern of damage, which affects the movement of resources and chemical defenses within different areas of a plant (Avila-Sakar et al. 2003). Areas of leaf damage concentrated on a particular area of plant are generally more detrimental to plant growth and reproduction than areas of leaf damage dispersed throughout a plant, although some studies have found no effect of the pattern of damage (Mayer 1998, Avila-Sakar et al. 2003, Avila-Sakar and Stephenson 2006). These results may reflect physiological differences in plant species (Avila-Sakar et al. 2003). I observed herbivory levels to be dispersed rather than highly concentrated throughout individuals of both species, although levels were greater on the older (middle

and bottom) leaves. L. Reinarz also found herbivory damage to increase with leaf age in remnant prairies in central Nebraska (unpublished data).

The results for this study are similar to those of others (Scherber et al. 2006, Unsicker et al. 2006, Scherber et al. 2010) that have mostly found no relationship between plant species richness and herbivory levels in experimental restorations, with the exception of significantly lower herbivory levels being observed in high diversity sites in one sampling month (Unsicker et al. 2006). Although I recorded a higher density of *R. columnifera* plants in the low diversity plots, likely because *R. columnifera* comprised a larger proportion of the low diversity seed mix than the high diversity seed mix, I did not record greater herbivory damage in the low diversity plots. Therefore my results did not support the resource concentration hypothesis, in which herbivory damage is expected to be greater in areas with a high density of host plants. Because *S. canadensis* emerged from the seed bank, it may have been more evenly distributed throughout the plots regardless of the seed mix used although the distance between *S. canadensis* plants was not measured.

Greater differences in herbivory damage may be observed as the restoration plots age and more conservative species that were seeded in the high diversity seed mix emerge, which could increase the species richness of these plots. Sampling of the plant community in 2007-2009 indicated differences in the species and functional group composition of the treatments, with a higher proportion of grass basal cover in the low diversity treatments, but averaged across all three years recorded species richness (of both sown and unsown species) was similar among the treatments (Chapter 3). Differences in herbivory levels may be more apparent in native, intact plant communities.

In a similar study in south-central Nebraska, the perennial forbs *Verbena stricta* and *Physalis longifolia* experienced significantly greater herbivory damage in low diversity remnant sites compared to high diversity remnant sites (L. Reinarz, unpublished data).

To my knowledge, no studies have looked at the effects of seeding density on herbivory levels. Seeding density was not a significant explanation for herbivory damage in this study. Seeding density was also not a significant effect in explaining the composition of the plant community, with the exception of basal cover of unsown perennial/annual grasses (Chapter 2).

A plant's growth rate can be slowed by herbivory damage (Crawley 1989). Conversely, plants with slower growth rates may be more vulnerable to attack by insects (Crawley 1989). While there were significant negative diversity effects on plant height for both *R. columnifera* and *S. canadensis*, there were no significant diversity effects for herbivory levels of most leaves, indicating there was little connection between herbivory damage and effect on plant growth or attractiveness of slower-growing plants to insect herbivores.

Future research could consider more than the area of leaf missing when comparing herbivory damage between low and high diversity treatments. Plants can also be stunted by insects that do not feed on leaves, such as sap-feeding insects (Abrahamson and Weis 1997). Goldenrods are especially vulnerable to damage by spittlebugs and other sap feeders (Carson and Root 1999). In addition, because this and other research indicates plant species richness and plant functional diversity are poorly associated with leaf damage by invertebrate herbivores (Scherber et al. 2006, Unsicker et al. 2006, Scherber et al. 2010), future research should focus more on the relationship between the identity of

functional groups and herbivory damage, and how damage from insect herbivores

changes the composition of the plant community (Scherber et al. 2010). The effects of

defloliation can also vary depending on the insect species that feed on the plant (Marquis

1992). The ecophysiological properties of plants may also differ in low versus high

diversity treatments, which can influence trophic interactions. For example, Mraja et al.

(2011) found plants growing in communities of different species richness and

composition to differ in their chemical defenses against insect herbivores. The influence

of diversity on trophic interactions and the ecophysiology of plants is a new area of

research with many possibilities.

Table 4.1. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on number of randomly picked top, middle, and bottom leaves of *Ratibida columnifera* and *Solidago canadensis* exhibiting high invertebrate herbivory damage levels (5-99% of leaf removed). Plants were assessed in $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	*F*	*P*
Ratibida columnifera			
Top leaf			
Diversity	1, 20	1.03	0.3222
Seeding density	1, 20	0.32	0.5790
Diversity*Seeding density	1, 20	0.62	0.4390
Year	1, 20	1.74	0.2023
Year*Diversity	1, 20	1.74	0.2023
Year*Seeding density	1, 20	0.32	0.5784
Year*Diversity*Seeding density	1, 20	0.89	0.3577
Middle leaf			
Diversity	1, 20	1.45	0.2433
Seeding density	1, 20	0.74	0.4007
Diversity*Seeding density	1, 20	0.47	0.5000

Table 4.1. Continued.

Effect	df	F	P
Ratibida columnifera, continued			
Middle leaf			
Year	1, 20	2.63	0.1203
Year*Diversity	1, 20	3.33	0.0829
Year*Seeding density	1, 20	0.04	0.8413
Year*Diversity*Seeding density	1, 20	0.66	0.4267
Bottom leaf			
Diversity	1, 20	0.16	0.6956
Seeding density	1, 20	2.28	0.1470
Diversity*Seeding density	1, 20	0.06	0.8141
Year	1, 20	25.04	**<0.0001**
Year*Diversity	1, 20	0.22	0.6420
Year*Seeding density	1, 20	2.01	0.1721
Year*Diversity*Seeding density	1, 20	0.44	0.5163
Solidago canadensis			
Top leaf			
Diversity	1, 20	1.98	0.1746
Seeding density	1, 20	0.88	0.3593
Diversity*Seeding density	1, 20	1.98	0.1746
Year	1, 20	11.79	**0.0026**

Table 4.1. Continued.

Effect	df	*F*	*P*
Solidago canadensis, continued			
Top leaf			
Year*Diversity	1, 20	0.74	0.4008
Year*Seeding density	1, 20	0.74	0.4008
Year*Diversity*Seeding density	1, 20	2.95	0.1014
Middle leaf			
Diversity	1, 40	1.47	0.2320
Seeding density	1, 40	0.89	0.3509
Diversity*Seeding density	1, 40	0.16	0.6880
Year	1, 40	11.36	**0.0017**
Year*Diversity	1, 40	0.45	0.5041
Year*Seeding density	1, 40	4.09	0.0498
Year*Diversity*Seeding density	1, 40	0.89	0.3509
Bottom leaf			
Diversity	1, 20	1.88	0.1861
Seeding density	1, 20	1.88	0.1861
Diversity*Seeding density	1, 20	1.88	0.1861
Year	1, 20	9.80	**0.0060**
Year*Diversity	1, 20	0.20	0.6657
Year*Seeding density	1, 20	0.20	0.6657

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Table 4.1. Continued.

Effect	df	F	P
Solidago canadensis, continued			
Bottom leaf			
Year*Diversity*Seeding density	1, 20	0.34	**0.0403**

Table 4.2. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on number of randomly picked top, middle, and bottom leaves of *Ratibida columnifera* and *Solidago canadensis* exhibiting low invertebrate herbivory damage levels (0-4% of leaf removed). Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	F	P
Ratibida columnifera			
Top leaf			
Diversity	1, 40	1.17	0.2858
Seeding density	1, 40	2.79	0.1024
Diversity*Seeding density	1, 40	0.24	0.6256
Year	1, 40	1.17	0.2858
Year*Diversity	1, 40	0.09	0.7695
Year*Seeding density	1, 40	2.79	0.1024
Year*Diversity*Seeding density	1, 40	0.47	0.4952
Middle leaf			
Diversity	1, 40	0.50	0.4855
Seeding density	1, 40	1.03	0.3153
Diversity*Seeding density	1, 40	0.30	0.5870

Table 4.2. Continued.

Effect	df	*F*	*P*
Ratibida columnifera, continued			
Middle leaf			
Year	1, 40	4.46	**0.0410**
Year*Diversity	1, 40	3.24	0.0795
Year*Seeding density	1, 40	0.06	0.8156
Year*Diversity*Seeding density	1, 40	0.06	0.8156
Bottom leaf			
Diversity	1, 40	0.88	0.3547
Seeding density	1, 40	1.41	0.2426
Diversity*Seeding density	1, 40	0.00	0.9505
Year	1, 40	12.66	**0.0010**
Year*Diversity	1, 40	2.06	0.1588
Year*Seeding density	1, 40	0.66	0.4219
Year*Diversity*Seeding density	1, 40	1.72	0.1973
Solidago canadensis			
Top leaf			
Diversity	1, 20	1.96	0.1772
Seeding density	1, 20	0.87	0.3622
Diversity*Seeding density	1, 20	1.96	0.1772
Year	1, 20	12.31	**0.0002**

Table 4.2. Continued.

Effect	df	*F*	*P*
Solidago canadensis, continued			
Top leaf			
Year*Diversity	1, 20	0.77	0.3909
Year*Seeding density	1, 20	0.77	0.3909
Year*Diversity*Seeding density	1, 20	3.08	0.0947
Middle leaf			
Diversity	1, 20	3.70	0.0688
Seeding density	1, 20	0.06	0.8045
Diversity*Seeding density	1, 20	0.17	0.6803
Year	1, 20	62.22	**<0.0001**
Year*Diversity	1, 20	2.83	0.1083
Year*Seeding density	1, 20	4.83	**0.0399**
Year*Diversity*Seeding density	1, 20	0.42	0.5253
Bottom leaf			
Diversity	1, 20	1.04	0.3189
Seeding density	1, 20	0.08	0.7833
Diversity*Seeding density	1, 20	0.01	0.9269
Year	1, 20	64.40	**<0.0001**
Year*Diversity	1, 20	3.59	0.0728
Year*Seeding density	1, 20	0.01	0.9291

Table 4.2. Continued.

Effect	df	*F*	*P*
Solidago canadensis, continued			
Bottom leaf			
Year*Diversity*Seeding density	1, 20	0.40	0.5351

Table 4.3. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on height of *Ratibida columnifera* and *Solidago canadensis* plants sampled for herbivory damage. Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P <$ 0.05.

Effect	df	*F*	*P*
Ratibida columnifera			
Diversity	1, 40	4.69	**0.0364**
Seeding density	1, 40	0.03	0.8727
Diversity*Seeding density	1, 40	5.80	**0.0207**
Year	1, 40	45.63	**<0.0001**
Year*Diversity	1, 40	1.35	0.2516
Year*Seeding density	1, 40	2.09	0.1558
Year*Diversity*Seeding density	1, 40	3.82	0.0576
Solidago canadensis			
Diversity	1, 40	18.34	**0.0001**
Seeding density	1, 40	0.78	0.3816
Diversity*Seeding density	1, 40	0.00	0.9550
Year	1, 40	65.30	**<0.0001**

Table 4.3. Continued.

Effect	df	*F*	*P*
Solidago canadensis, continued			
Year*Diversity	1, 40	12.36	**0.0011**
Year*Seeding density	1, 40	0.26	0.6148
Year*Diversity*Seeding density	1, 40	0.08	0.7746

Table 4.4. Results of mixed-model analysis for testing the effects of diversity and seeding density on mean distance of *Ratibida columnifera* sampled for herbivory damage to the nearest *R. columnifera* plant in 2010. Plants were assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at *P* < 0.05.

Effect	df	*F*	*P*
Ratibida columnifera			
Diversity	1, 15	11.43	**0.0041**
Seeding density	1, 15	8.52	**0.0106**
Diversity*Seeding density	1, 15	0.07	0.7986
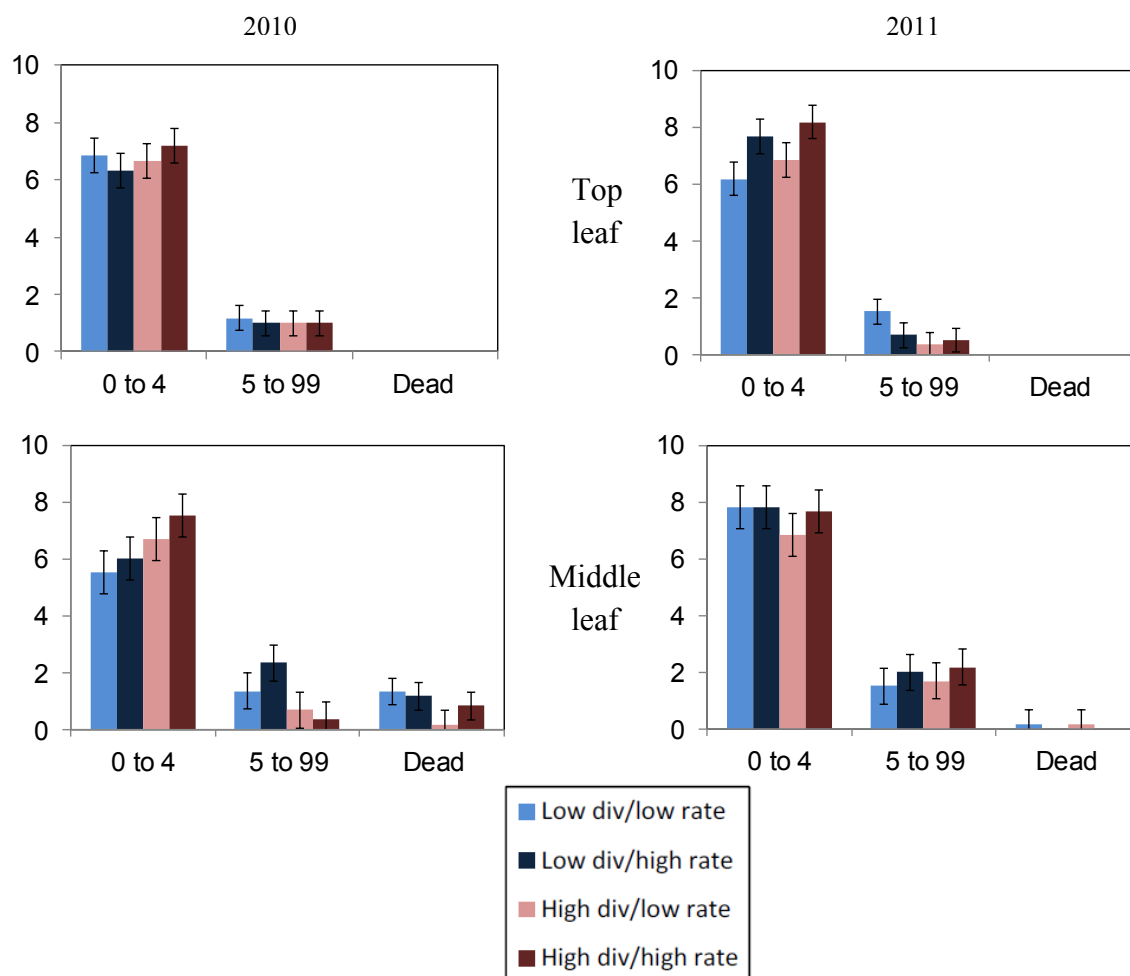
Figure 4.1. Mean number of *Ratibida columnifera* leaves per treatment belonging to each of three classes for estimating the percent of the leaf damaged by herbivory: 0-4%, 5-99%, and dead leaves. Herbivory damage is evidenced by parts of the leaf missing from chewing. The sample size for each treatment consists of the number of plots within the treatment; $n = 6$. Herbivory damage was assessed in $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.

2010

2011

Bottom
leaf

Figure 4.1. Continued.

Figure 4.2. Mean number of *Solidago canadensis* leaves per treatment belonging to each of three classes for estimating the percent of the leaf damaged by herbivory: 0-4%, 5-99%, and dead leaves. Herbivory damage is evidenced by parts of the leaf missing from chewing. The sample size for each treatment consists of the number of plots within the treatment; $n = 6$. Herbivory damage was assessed in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.

Figure 4.2. Continued.
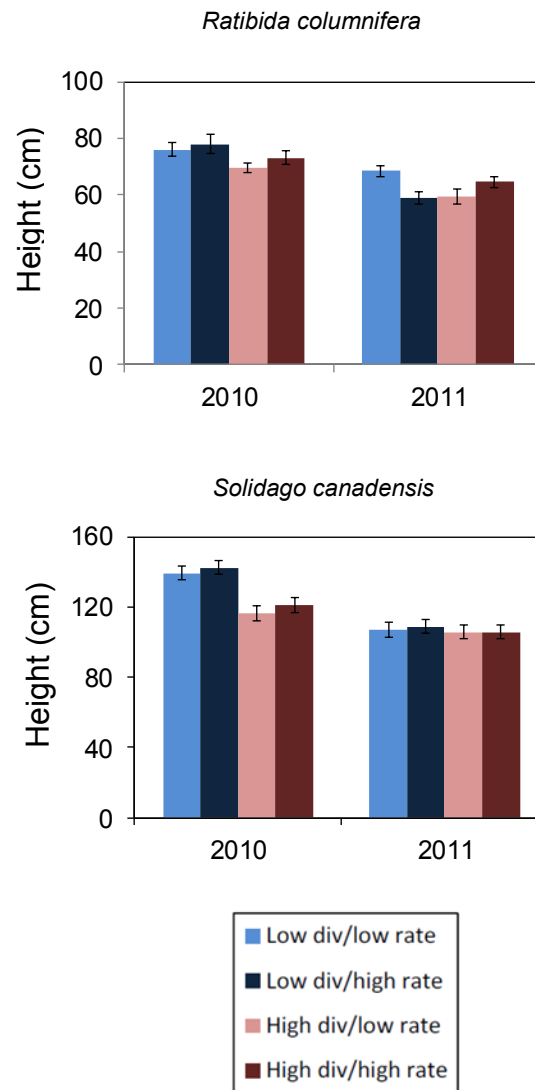
Figure 4.3. Mean height of *Ratibida columnifera* and *Solidago canadensis* plants per treatment randomly sampled for herbivory damage. The sample size for each treatment consists of the number of plots within the treatment; $n = 6$. Plants were assessed in 55m²-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.

Ratibida columnifera
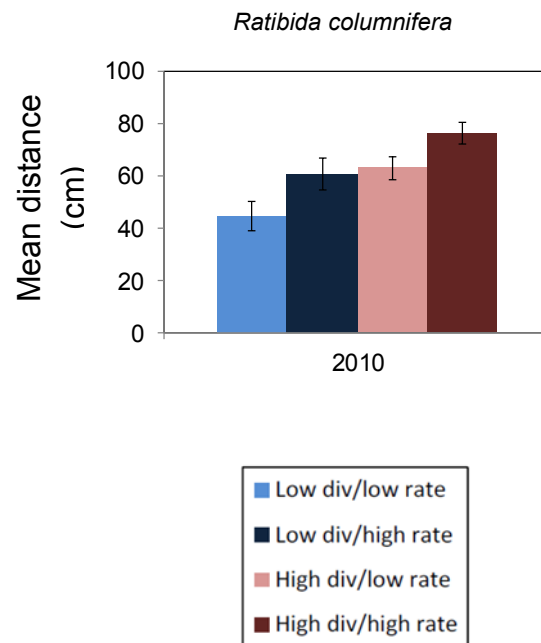
Figure 4.4. Mean distance of *Ratibida columnifera* plants per treatment from neighboring

R. columnifera plants in 2010. The sample size for each treatment consists of the number

of plots within the treatment; $n = 6$. Plants were assessed in 55m^2-research plots 10 km

south of Wood River, Nebraska planted to six replicates in each of four treatments: high

diversity sites of 97 species typically planted by The Nature Conservancy planted at low

and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15

species), also at low and high seeding densities.

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CHAPTER 5: SOIL NUTRIENT RESPONSE TO DIVERSITY AND SEEDING

DENSITY IN TALLGRASS PRAIRIE RESTORATIONS

ABSTRACT

The conversion of grassland to cropland can negatively affect the ecosystem
service of soil production and resulting soil fertility, decreasing nutrient inputs because of
annual removal of crop biomass and increased decomposition rates of soil carbon and soil
nitrogen. Some agricultural practices such as conservation tillage and soil amendments
can minimize loss of soil nutrients in cropland. Soil production and fertility can also be
enhanced in agroecosystems by restoring grassland to the landscape. I assessed the
effects of plant diversity and seeding density on soil nutrient levels in experimental
tallgrass prairie plots in central Nebraska, USA. In the spring of 2006, twenty-four $55m^2$
plots were planted to six replicates in each of four treatments: high diversity sites of 97
species planted at a low seeding density, high diversity sites at twice this seeding rate
using a mix typically used by The Nature Conservancy, and low diversity sites planted at
low and high seeding densities using a Natural Resources Conservation Service (NRCS)
Conservation Reserve Program (CRP) mix (CP25; 15 species). Total carbon and total
nitrogen were recorded in 2006 and nitrate and ammonium were recorded in 2006, 2008,
2009, and 2011. Total carbon and total nitrogen were highest in the high diversity, high
seeding rate plots in 2006 but diversity and seeding density were not significant in
explaining levels of these nutrients. Year and the year × diversity interaction were
significant in explaining ammonium and nitrate levels. Total carbon also had a significant
positive association with nitrate and diversity had a significant negative association with

nitrate. From 2006 to 2011, soil ammonium levels increased while soil nitrate levels decreased across the treatments, likely because developing root systems in these restoration plots are using more nitrate and because decaying roots retain ammonium.

INTRODUCTION

Since the mid-1800s, over 97% of the Northern tallgrass prairie has been lost from the central United States, with most of it plowed and converted to agricultural crops (Samson et al. 2004). Agricultural practices such as tillage, fertilization, irrigation, drainage, and changes in plant biomass have altered hundreds of thousands of hectares of former grassland soil (Huggins et al. 1998, Samson et al. 2004, McLauchlan 2006). Agricultural land management may affect soil by increasing erosion, leaching dissolved soil carbon, decomposing soil nitrogen and soil organic carbon, and by decreasing nutrient input from plant biomass because of annual crop removal (Brye et al. 2002, McLauchlan 2006). Over several decades, 30-60% of soil organic matter and 18-75% of nitrogen can be depleted in soil that has been converted from grassland to cropland, with higher losses reported for sandy soils compared to clay soils (Tiessen et al. 1982, Knops and Tilman 2000, Matamala et al. 2008). Conversely, some agricultural practices such as no-tillage, ridge-tillage or mulch-tillage that conserve crop residue and the increased application of fertilizers since the 1950s may reduce loss of carbon, nitrogen, and phosphorous levels in cropland (Haas et al. 1961, Vitousek et al. 1997, Haynes and Naidu 1998, Follett 2001, Kucharik et al. 2001, Bernacchi et al. 2005).

In the last few decades, conservationists and agricultural producers have attempted to restore some ecosystem services such as soil development and the resulting provision of soil fertility, soil retention, and wildlife habitat to Great Plains agroecosystems by seeding hundreds of thousands of hectares of former cropland with native prairie grasses and forbs (Dunn et al. 1993, Packard and Mutel 1997, USDA FSA 2012). Plant-soil interactions are an important consideration when designing and

managing terrestrial ecosystem restorations, including grassland restorations (Eviner and Hawkes 2008). Soil nutrient availability influences the species composition of plant communities, and can affect the success of invasive plant species (Huenneke 1990, Davis et al. 2000). Nitrogen is the most limiting nutrient affecting the structure and function of grassland ecosystems (Risser and Parton 1982, Seastedt et al.1991, Hooper and Johnson 1999, McCulley et al. 2009). For example, in a three-year-old Kansas tallgrass prairie restoration, aboveground net primary productivity (ANPP) was positively correlated with soil nitrogen levels and plant species diversity and species richness were negatively correlated with soil nitrogen levels (Baer et al. 2003). Others have also found ANPP to increase significantly and species richness to decline with nitrogen fertilization in experimental tallgrass prairie restoration plots (Wedin and Tilman 1996, Foster and Gross 1998, Camill et al. 2004). Plant species diversity may be lower in nutrient enriched grasslands, including restored grasslands that have the residual effects of nutrient-rich agricultural soils, because increased ANPP associated with high nitrogen levels increases plant biomass, which increases shading and competition for light (Tilman and Pacala 1993, Collins et al. 1998, Baer and Blair 2008).

Native prairie soils have lower soil nitrogen availability and greater species richness than the soils of grassland restorations that have been seeded on high-nutrient sites, likely because producers and decomposers in native prairie rapidly use and immobilize the biologically available forms of nitrogen, soil nitrate and ammonium (Risser and Parton 1982). As prairie restorations age, increased soil organic carbon from increased biomass of developing root systems can result in greater plant uptake of nitrogen and microbial immobilization (Baer et al. 2003). Over time nitrate levels in the

soil may decrease because as an anion, nitrate is more mobile than ammonium and more readily taken up by plants (Baer et al. 2003, Robertson and Groffman 2007). Because it is a positively-charged cation, ammonium can be held on cation-exchanges sites in organic matter and clay particles and is less mobile in the soil and less available to plants than nitrate (Robertson and Groffman 2007). Therefore, as organic matter increases with prairie restoration age soil ammonium levels would be expected to increase.

The higher soil fertility in restored grasslands that have a legacy of agricultural nutrient enrichment may partially account for the higher cover of invasive species often observed in restorations compared to remnants (Vinton and Goergen 2006, Faber and Markham 2012). High levels of nutrients increase weed invasion, possibly because aggressive species depend on high levels of available soil nitrogen to support their rapid growth, allowing them to outgrow slower growing prairie species (Averett et al. 2004). Grassland restoration sites are vulnerable to invasion by aggressive weeds because they are often located on recently abandoned agricultural land with relatively high levels of available soil nitrogen and seed banks containing a variety of aggressive species (Averett et al. 2004, Baer et al. 2009). Invasive plant species can also alter soil conditions such as the composition of the soil microbial community, soil moisture, and nutrient cycling, creating conditions that may facilitate their growth and survival (Corbin and D'Antonio 2004, Jordan et al. 2011).

The effects of the diversity of the plant community as a whole on soil conditions is another important aspect of plant-soil feedbacks that has received increasing attention in recent years. The quality of plants, or amount of carbohydrates, proteins, and water in tissue and amount of secondary metabolites (Mattson 1980, Boege and Marquis 2005)

influences the carbon available to soil microbes through plant detritus (Knops et al. 2002). Soil microbes in turn control nitrogen cycling by releasing, or mineralizing, excess nitrogen to the soil when they obtain enough nitrogen from the plant detritus they consume and by immobilizing nitrogen from the soil when plant detritus does not contain enough nitrogen to meet their needs (Robertson and Groffman 2007). Plant communities with a high diversity of species would be more likely to include species characterized by a range of plant quality. In addition, plant communities that include plants with a diversity of phenologies can provide a more consistent source of organic matter over the growing season than low diversity plant community containing a few species (Bach et al. 2012).

The level of diversity in a community can influence nitrogen cycling in grasslands, with more species-rich communities more fully using soil mineral nitrogen (Tilman et al. 1996) and greater loss of nitrogen due to soil leaching occurring under low diversity plant communities (Tilman et al. 1996, Bingham and Biondini 2011). Other research has found the identity of plant functional groups to be more important than species richness in influencing nitrate and ammonium levels in European grasslands (Gastine et al. 2003) but some have found both species richness and functional group identity to affect nitrate levels (Oelmann et al. 2007) or no relationship between diversity and nitrate leaching in serpentine California grasslands (Hooper and Vitousek 1998). In experimental Minnesota grassland restorations, C4 grasses and legumes were associated with greater C and N accumulation in both low and high diversity mixes (Fornara and Tilman 2008). In Europe, soil carbon storage significantly increased with increasing levels of plant species richness in experimental grasslands (Steinbess et al. 2008).

Most previous studies have considered the effects of experimentally-derived seed mixes on soil properties. Although thousands of hectares in the Great Plains have been seeded with low-diversity (5-15 plant species) seed mixes through the Natural Resources Conservation Service's (NRCS) Conservation Reserve Program (CRP) and high-diversity (50-100+ plant species) by conservation organizations and agencies, little is known about how these restoration methods may affect soil development. Therefore, this study compares the soil nutrients of $55m^2$-research plots seeded with a low diversity tallgrass prairie seed mix commonly used in Nebraska, the CP25 mix (15 species) to a high diversity tallgrass prairie seed mix used in the area by The Nature Conservancy (TNC) (97 plant species), both at different seeding rates. To my knowledge, there has been no research comparing differences in soil properties under seeding densities commonly employed by these two seeding methods. The study tested three null hypotheses: 1) total carbon and nitrogen will not differ among the treatments; 2) ammonium and nitrate will not differ among the treatments; and 3) ammonium and nitrate will not change over time.

METHODS

Study area

The study area lies within the Central Platte River ecosystem, which includes the Platte River channel and floodplain from mid-Dawson County to mid-Hamilton County in central Nebraska (NGPC 2005). The Nebraska Game and Parks Commission has determined the Central Platte River to be a Biologically Unique Landscape (NGPC 2005). The region has a continental climate, with warm, wet summers and cold, dry

winters. Mean annual air temperature is 10.4° C and mean annual precipitation is 63.9 cm
(High Plains Regional Climate Center 2010).

The study site is located approximately 10 km south of Wood River, Nebraska
(Hall County; 40°44′41″ N, 98°35′11″ W) on a 7.3-ha field owned by The Nature
Conservancy. Soils at the site are of loamy alluvium or sandy alluvium parent material
and include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Calamux
complex, occasionally flooded soils (NRCS 2010). The site is bordered to the south and
east by county roads and Nature Conservancy prairie restorations, to the west by a
cornfield that was seeded to experimental prairie restoration plots in the spring of 2010,
and to the north by trees and the Platte River (Appendix A). The study site was under
cultivation in a corn-soybean rotation in the decades prior to the experiment.

Treatments and experimental design

In late March and early April 2006, the 7.3-ha field was cultivated and divided
into 24, 0.30-ha plots. The plots were seeded from an all-terrain vehicle (ATV) and a
John Deere drop spreader according to a 2×2 factorial design, in which two levels of
diversity (low plant diversity and high plant diversity) were applied using two different
seeding densities (low and high seeding rates). The experiment was arranged in a
randomized block design, with six blocks running west to east across the field and each
block containing four plots assigned to the four treatments. Treatments consisted of: 1) a
low diversity CRP tallgrass prairie seed mix (CP25 mix, 15 species) used by the NRCS
seeded at half the recommended seeding rate (148 grass seeds/m^2, 16 forb seeds/m^2; low
diversity/low rate mix); 2) the CP25 mix applied at the recommended seeding rate (297

grass seeds/m^2, 31 forb seeds/m^2; low diversity/high rate mix); 3) a high diversity tallgrass prairie mix typically used by the local Nature Conservancy (97 species) seeded with a seeding rate typical for Nature Conservancy grassland restorations in the region (129 grass seeds/m^2, 43 forb seeds/m^2; high diversity/low rate), and 4) the Nature Conservancy mix applied at twice the seeding rate (258 grass seeds/m^2, 86 forb seeds/m^2; high diversity/high rate) (Appendix B and C). The second and fourth treatments are at half and double, respectively, NRCS or The Nature Conservancy normal seeding rates because the NRCS normally recommends rates that are about twice as high as The Nature Conservancy uses.

The NRCS CP25 seed mix was designed with the Grand Island, Nebraska NRCS District Conservationist. Grass seed used in the mix was purchased from Arrow Seed in Broken Bow, Nebraska and forb seed was locally harvested from the Platte River area. The high-diversity seed mix was harvested from local prairies.

Management of the plots was minimal. All of the plots were burned on March 20, 2008 and on May 2, 2011. In July 2008, *Achillea millefolium* and *Helianthus maximilliani* that had invaded the edges of plots where they had not been sown were sprayed with Glyphosate and killed in order to reduce the edge effect on the spread of these aggressive species. The inflorescences of *B. inermis* that had naturally invaded the south row of plots from a road ditch and two plots in the northwestern corner of the field were clipped in order to limit the spread of *B. inermis* that had not been experimentally introduced into the plots. Following that effort no plants were intentionally killed or manipulated. Vegetation growing in unseeded 2-m lanes between the plots was mowed several times during the growing season.

Soil sampling

Soil samples were collected in October 2006, November 2008, July 2009, and July 2011. Within each plot, the soil sampling location was located 21 m diagonal to the northwest and northeast corners of the plot. In 2006, a bucket auger was used to remove and discard the top 15 cm of the soil and a 15-cm long, 2.5-cm diameter soil probe was used to remove soil from 15-30 cm below the soil surface. Four soil samples 0-15 cm below the soil surface were also collected with a 2.5cm diameter soil probe two m in each direction from the 15-30 cm sampling location. These two 0-15cm samples were composited into a single sample. In 2008, 2009, and 2011, two soil samples 0-15 cm below the soil surface approximately two m apart were removed. In 2006, the year the study plots were seeded, four samples were collected in the cornfield to the west of the study site. Each cornfield sample was collected parallel to one of the four rows of plots in the study area. The soil samples were placed in plastic bags and refrigerated until analyzed (within 48 hours).

In the lab, approximately 25 grams of soil from each depth (0-15 cm and 15-30 cm for 2006 and only the 0-15 cm depth for 2008, 2009, and 2011) was extracted and mixed with 50 ml of 1 molar KCl solution, shaken for 30 minutes, and refrigerated overnight. The clear extract was then placed in a plastic vial and frozen until analysis. The extract was analyzed on a 2-track autoanalyzer for ammonium and nitrate concentrations (Ecosystem Analysis Lab, School of Biological Sciences, University of Nebraska – Lincoln). These solution concentrations were converted to N as ammonium or nitrate per unit dry soil (mg N per kg dry soil, i.e. ppm).

The remainder of each 0-15 cm and 15-30 cm sample was ground into coarse particles with a mortar and pestle. These particles were passed through a 2-mm sieve and a subsample of about 40 g was ground and homogenized in a coffee grinder. This sample was analyzed by combustion on a CHN analyzer for total carbon (C) and total nitrogen (N) (Ecosystem Analysis Lab, School of Biological Sciences, University of Nebraska – Lincoln). Because total soil C and N was not measured in all years and changed little during the 6 year study, only the first year results for total soil C and N are presented here. These values show the underlying differences in soil fertility across the plots. For statistical analyses, average values for NO3, NH4, total soil C and total soil N were calculated for each of the 24 plots from the subsamples within plots.

Data analysis

Plots were the experimental units in this study. Normality in the response variables, total nitrogen, total carbon, ammonium, and nitrate, were tested with the Kolmogorov-Smirnov normality test (PROC UNIVARIATE, SAS Version 9.2; SAS Institute 2007) and graphs of predicted values against the residuals. Because the response variables were influenced by fixed and random factors and were not normally distributed, each set of data was fitted with a mixed-effects model using PROC GLIMMIX (SAS Version 9.2; SAS Insitute 2007). Mixed-effects models are appropriate for data that contains both fixed and random factors and the GLIMMIX procedure does not require the response to be normally distributed (Littell et al. 2006). Diversity, seeding density, year, and their interactions were used as fixed effects and block and plot were used as random effects. We used total soil carbon recorded from 2006 as a covariate because total soil

carbon changes slowly. Some studies report no significant changes in soil carbon even after several years of restoration (Baer et al. 2000, Brye and Kucharik 2003, Camill et al. 2004). Contour plots for the distribution of nutrients were generated using the program JMP (JMP, Version 8.0.2. SAS Institute Inc., Cary, NC, 1989-2007).

RESULTS

Soil properties varied spatially across the plots (Figures 5.2 and 5.3). The highest levels of nitrate were concentrated in the eastern plots in 2006 and 2008, but in 2009 high concentrations of nitrate were recorded in the western portion of the field as well (Figure 5.3). Ammonium was also initially highest in the easternmost plots in 2006 but was more evenly distributed across the field in 2009 and 2011 (Figure 5.2).

In 2006, total nitrogen and total carbon were highest in the high diversity, high seeding density plots but diversity and seeding density did not significantly explain levels of these nutrients (Table 5.1, Figure 5.1). There was a strong correlation between total carbon levels and nitrate levels (Figure 5.2). The relationship between nitrate and soil carbon became more variable over time (Figure 5.4). Diversity, year, and the diversity × year interaction explained ammonium and nitrate levels, with higher levels being recorded in the low diversity treatments (Table 5.1, Figure 5.1). Additionally, the diversity × seeding density interaction and year × diversity × seeding density interaction explaned ammonium levels (Table 5.1). Levels of nitrate were generally highest in 2008, while levels of ammonium were highest in 2011 (Figure 5.1).

DISCUSSION

Early in the study, nitrate was unusually low for a disturbed site, with values ranging from 0.25-0.40 ppm in 2006. In comparison, soil nitrate in newly planted experimental plots to the west of the the research plots had values of 0.5-2.5ppm during their first growing season. It is possible that the soil and weed heterogeneity in the 2006 research plots was so large early in the experiment in this first growing season that it minimized any treatment differences in nitrate uptake. Similarly, total carbon and total nitrogen did not differ significantly among the treatments in 2006. Nitrate is a better measure of the nitrogen that is biologically available to plants than ammonium (Wedin and Tilman 1993, Robertson and Groffman 2007). Levels of soil nitrate were initially lower in the higher diversity plots, possibly because plants in the higher diversity plots were more efficiently taking up nitrate because of resource complimentarity (Fargione and Tilman 2005). Across all of the treatments nitrate levels would be expected to decline over time due to increasing competition for nitrate as C4 grasses establish their deep root systems (Tilman and Wedin 1991) and due to increasing immobilization potential of the soil for nitrogen (Schimel 1986). Several studies have observed lower nitrate availability in soil restored to grasses for 5-10 years through the CRP relative to unrestored cropland (Baer et al. 2002). Nitrate levels also declined from 2006 to 2011 across all treatments (Figure 1) and by 2011 nitrate levels were similar among all of the treatments.

Because decaying roots provide more exchange sites for positively charged ammonium, ammonium levels would be expected to increase over time concurrent with increasing development of root systems. While ammonium levels were slightly higher in 2009 compared to 2006 in the low diversity treatments, they had declined from 2006 to 2009 in the high diversity treatments before sharply increasing in 2011 across all four

treatments (Figure 5.1). The large increase in recorded ammonium in 2011 may be attributed to soil moisture patterns around the time of sampling since the increase was too sudden to be attributed to development of root systems. The only significant effects on soil ammonium levels were year and year × diversity interactions.

Overall, these results confirm the expected increases in ammonium and decreases in nitrate that occur over time in grassland restorations. Long-term research would help determine if these trends continue with shifts in the composition of the plant community over time. Changes in dominant functional groups of plants over the course of a restoration can affect carbon and nitrogen cycling and this correlation would be worth exploring in these research plots. For example, because C4 grasses have a greater C:N ratio and lower decomposition rate than many C3 grasses and forbs (Wedin and Tilman 1990), they have different effects on C and N storage and cycling in native and restored grasslands (Knops and Tilman 2000, Kucharik et al. 2001). Including a high proportion of legumes in CRP restorations can increase N cycling (Robles and Burke 1997). Nitrate levels became more variable over time and less associated with soil fertility, indicating nitrate was influenced more by the developing plant community rather than soil carbon levels.

Additionally, nitrogen availability can vary seasonally, with high concentrations of extractable soil nitrogen observed early in the growing season that then decreases by mid-growing season, followed by a post-growing season increase in extractable soil N following accumulation of plant biomass and N (Turner et al. 1997). In the future reducing temporal variability by collecting soil at the same time each year may facilitate data interpretation.

Table 5.1. Results of mixed-model analysis for testing the effects of diversity, seeding density, and year on soil properties in restoration plots seeded with low diversity or high diversity plant mixes seeded at low and high seeding rates. Soil was removed from 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. Values in boldface are significant at $P < 0.05$.

Effect	df	F	P
NH4			
Total C	1, 19	0.24	0.6268
Diversity	1, 19.29	2.63	0.1214
Seeding density	1, 19.2	0.08	0.7750
Diversity*Seeding density	1, 19.86	2.89	0.1046
Year	3, 18	64.43	**<0.0001**
Year*Diversity	3, 18	7.19	**0.0023**
Year*Seeding density	3, 18	0.72	0.5533
Year*Diversity*Seeding density	3, 18	3.09	0.0530
NO3			
Total C	1, 19	13.12	**0.0018**
Diversity	1, 18.03	16.59	**0.0007**
Seeding density	1, 17.92	2.01	0.1735

Table 5.1. Continued.

Effect	df	F	P
NO3, continued			
Diversity*Seeding density	1, 18.68	0.00	0.9643
Year	3, 18	10.35	**0.0003**
Year*Diversity	3, 18	6.33	**0.0040**
Year*Seeding density	3, 18	0.13	0.9379
Year*Diversity*Seeding density	3, 18	0.45	0.7185
Total C			
Diversity	1, 19	0.59	0.4537
Seeding density	1, 19	0.30	0.5892
Diversity*Seeding density	1, 19	2.41	0.1374
Total N			
Diversity	1, 19	0.46	0.5077
Seeding density	1, 19	1.12	0.3036
Diversity*Seeding density	1, 19	2.63	0.1210
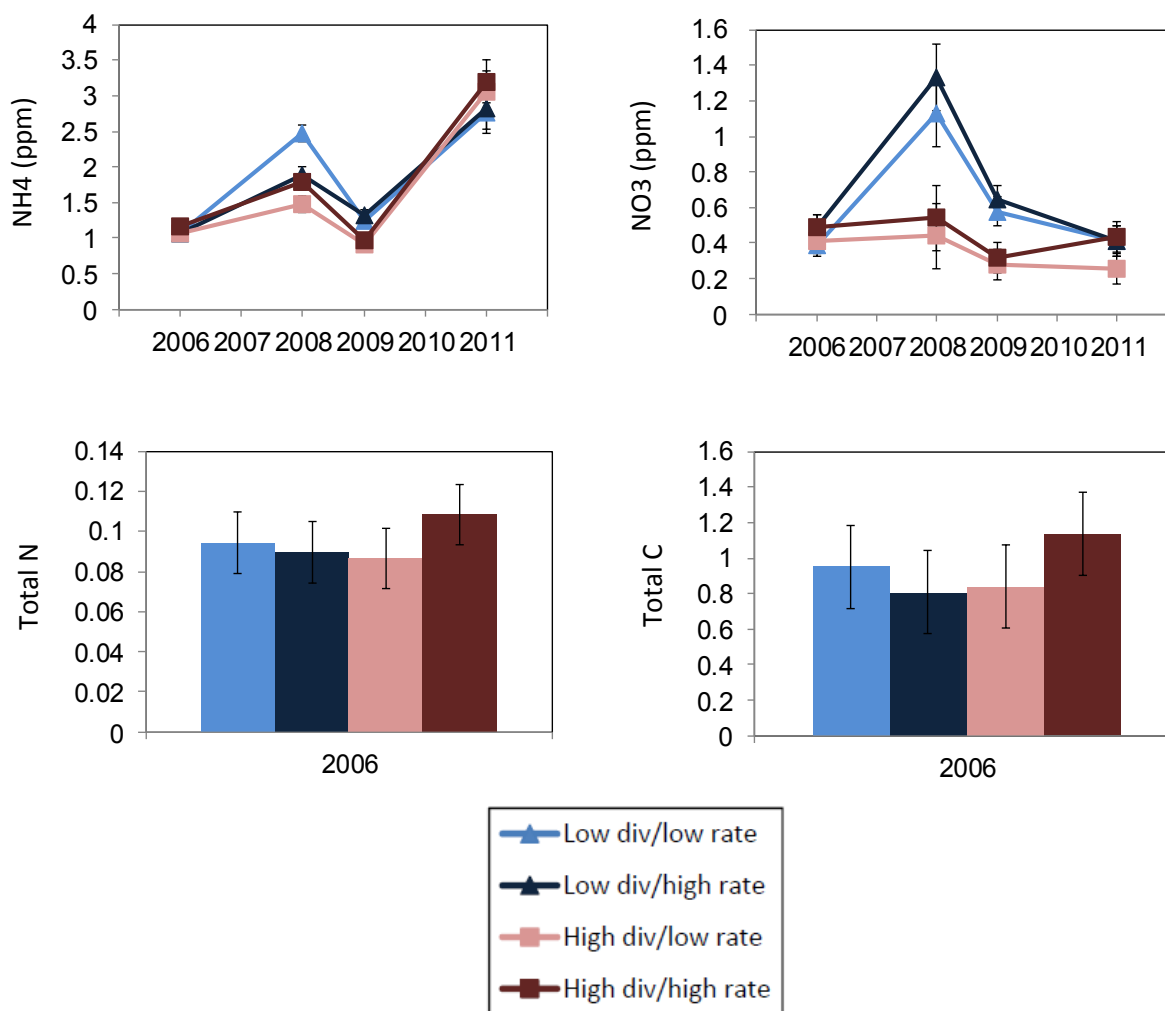
Figure 5.1. Mean levels of soil ammonium and nitrate and mean levels of total carbon and total nitrogen per treatment. The sample size for each treatment consists of the number of plots within the treatment; $n = 6$. Soil was analyzed from $55m^2$-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high ($2\times$) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.
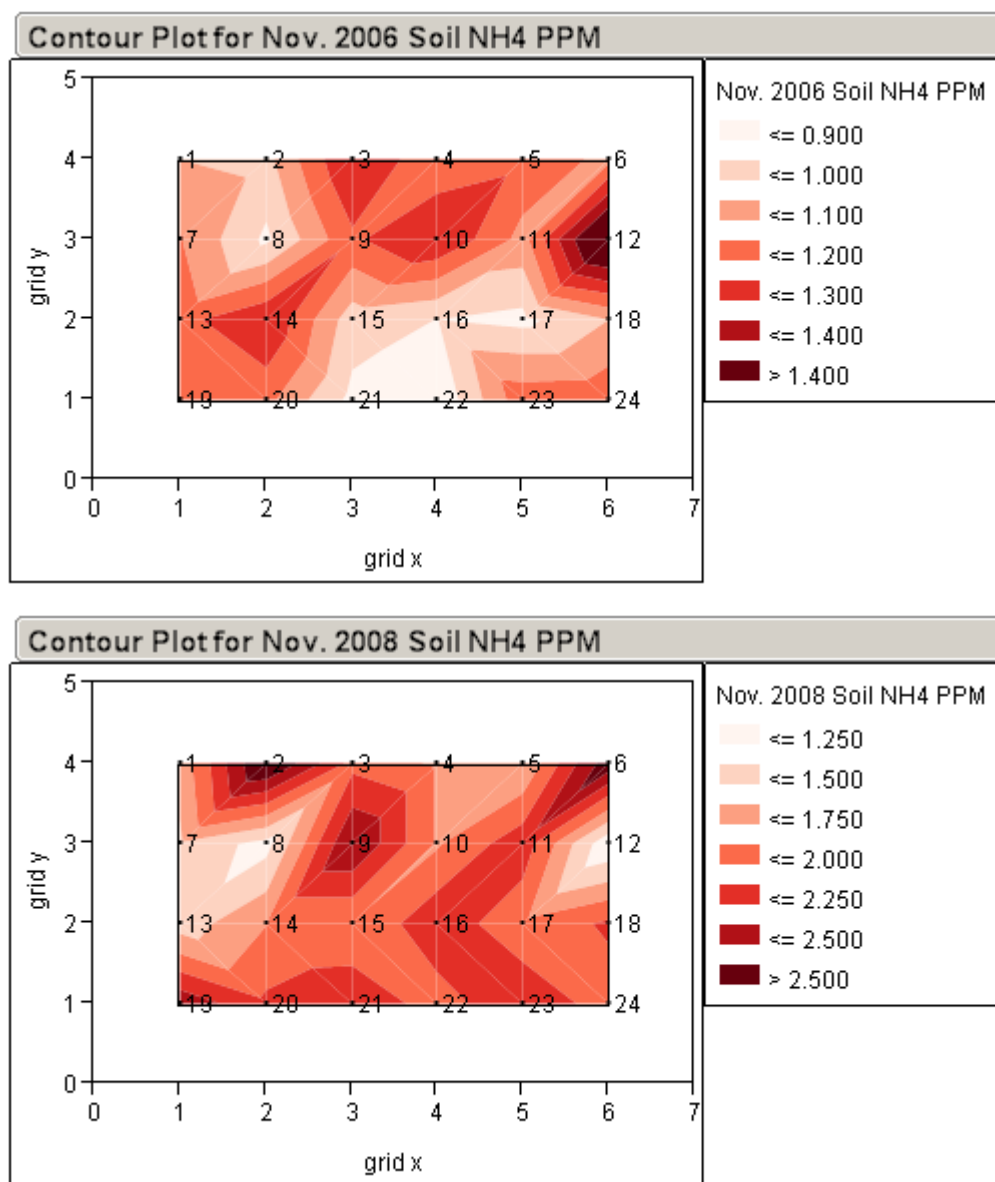
Figure 5.2. Spatial varation in soil ammonium (NH4) in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.
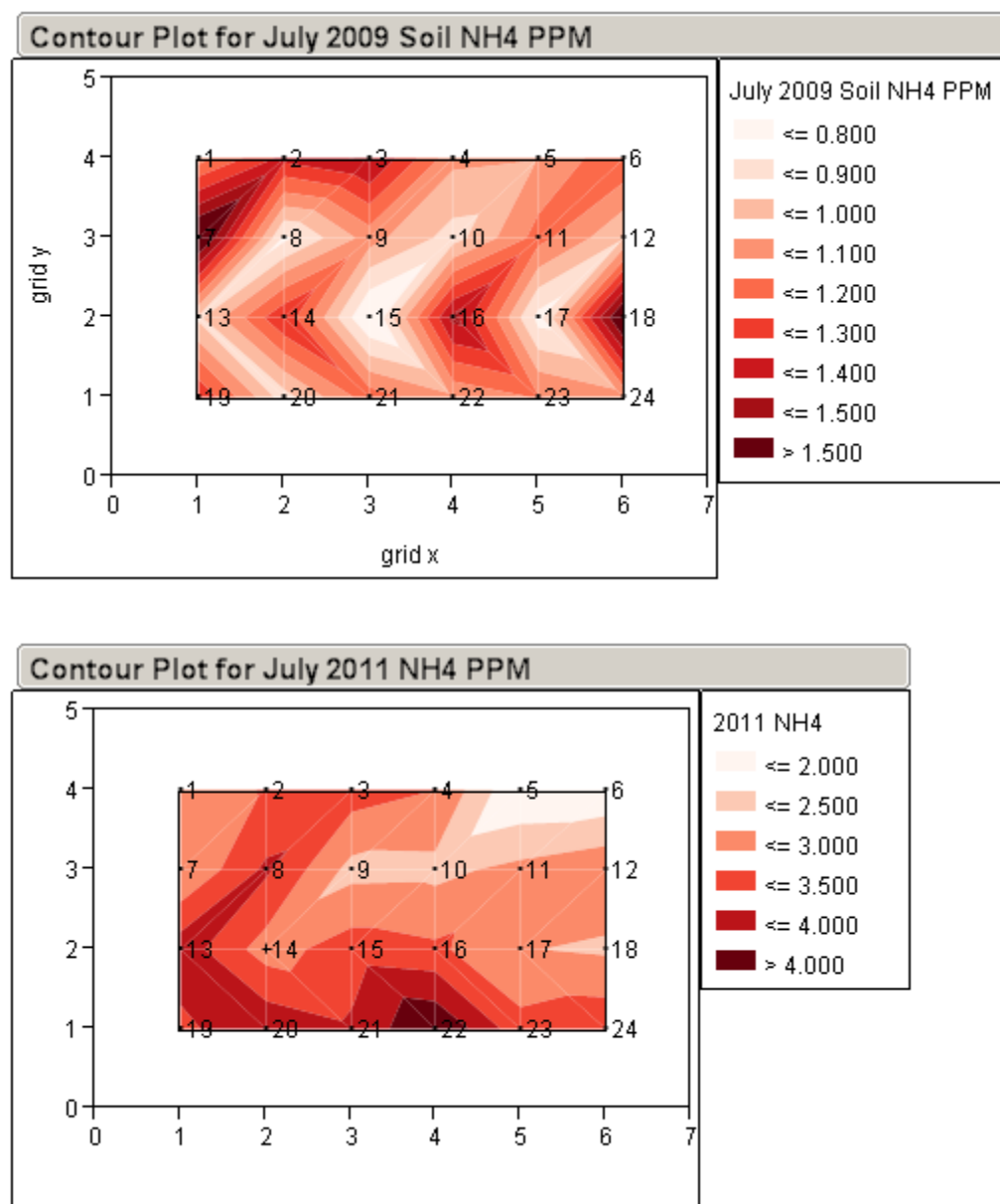
Figure 5.2. Continued.
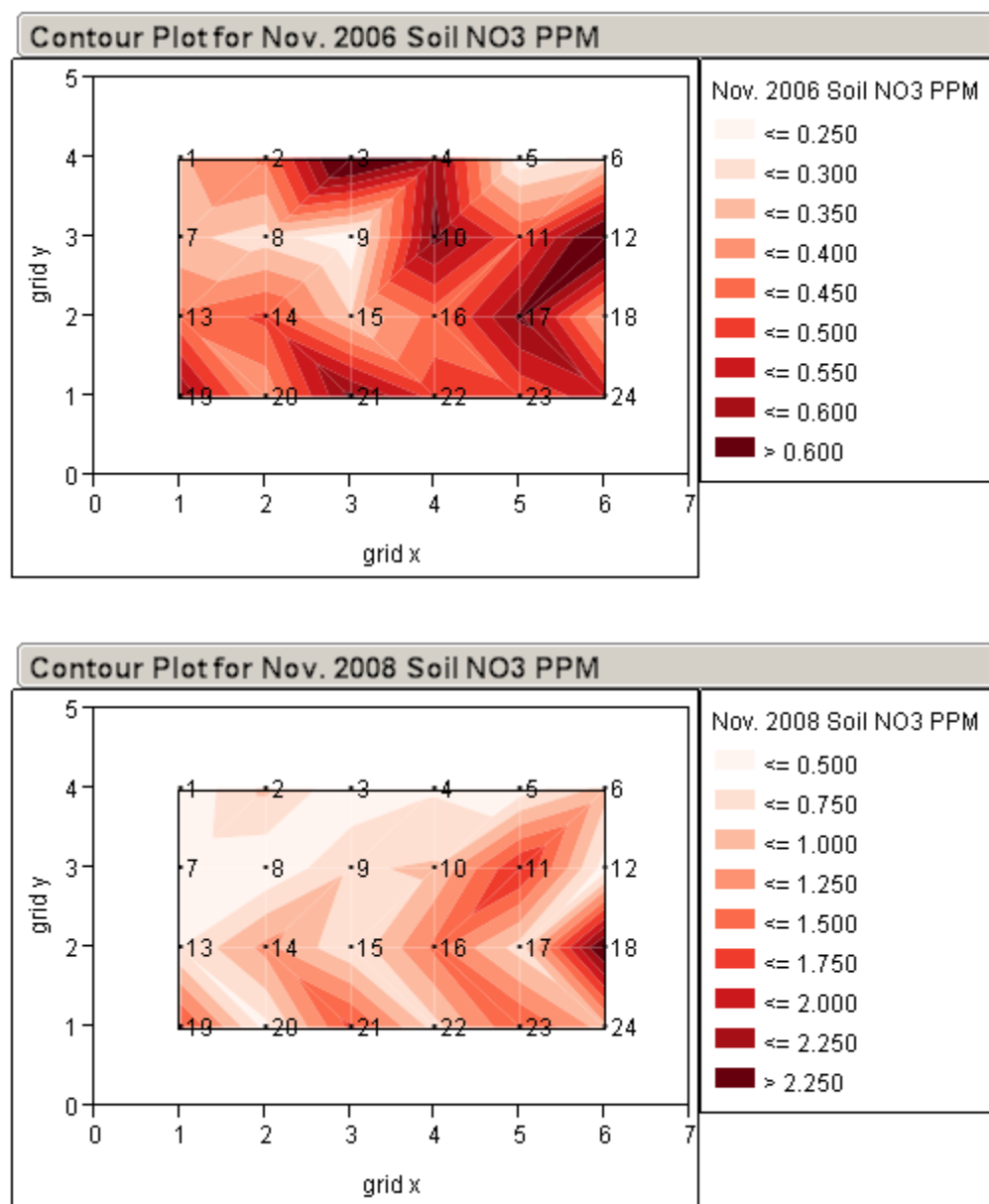
Figure 5.3. Spatial varation in soil nitrate (NO3) in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities.
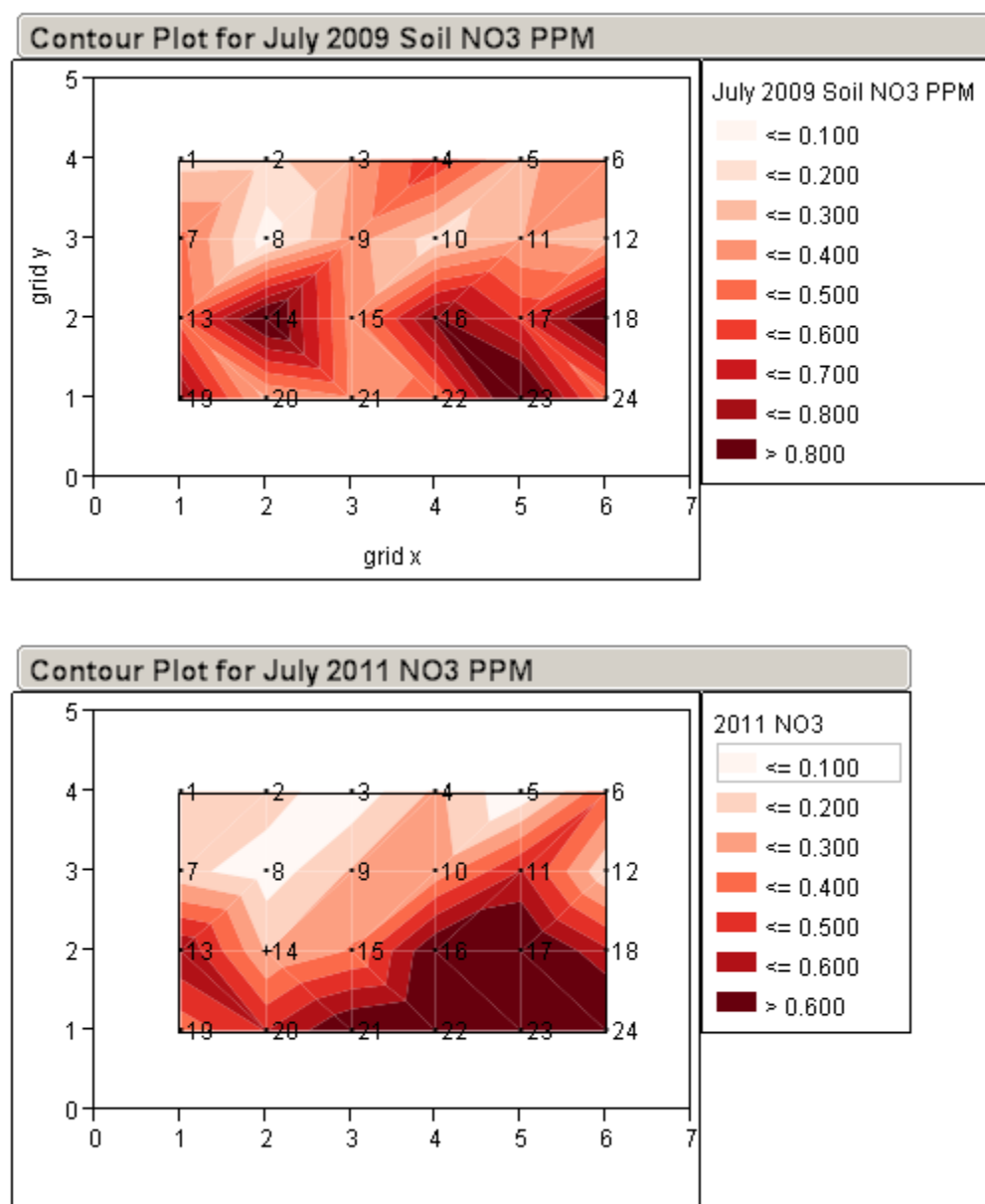
Figure 5.3. Continued.
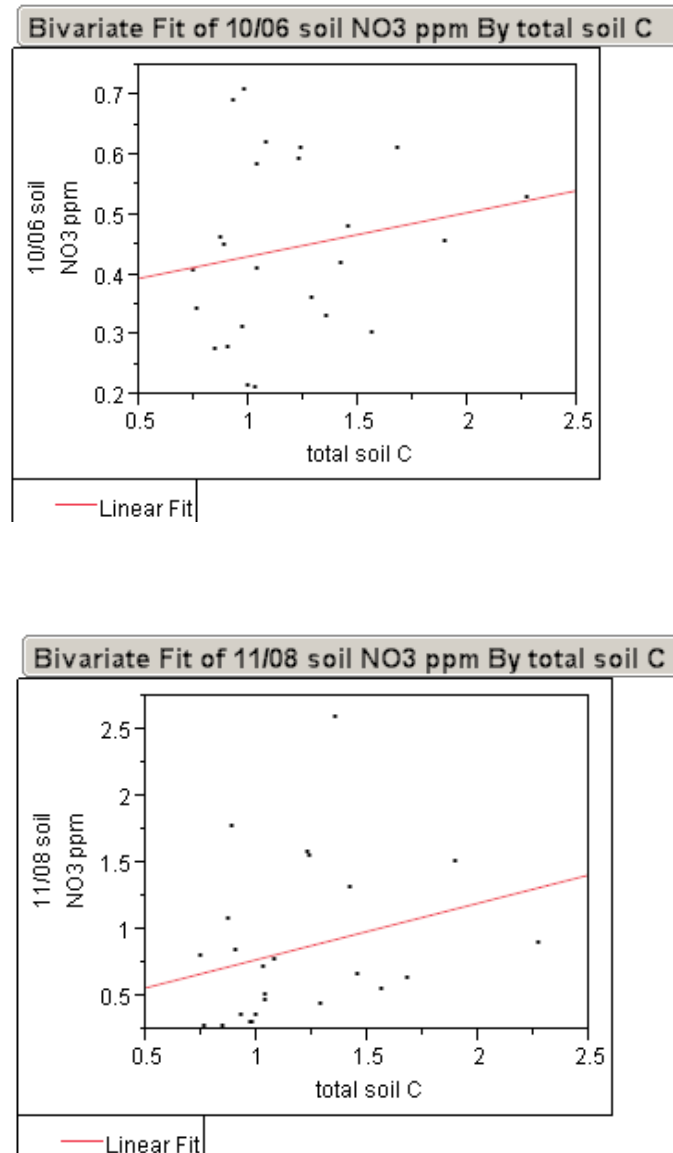
Figure 5.4. Bivariate fit between soil nitrate (NO3) and carbon (C) in 55m^2-research plots 10 km south of Wood River, Nebraska planted to six replicates in each of four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at low and high (2×) seeding densities, and low diversity sites using a CRP mix (CP25; 15 species), also at low and high seeding densities. R-squared values were 0.0343 (2006), 0.0733 (2008), 0.170 (2009), and 0.556 (2011).
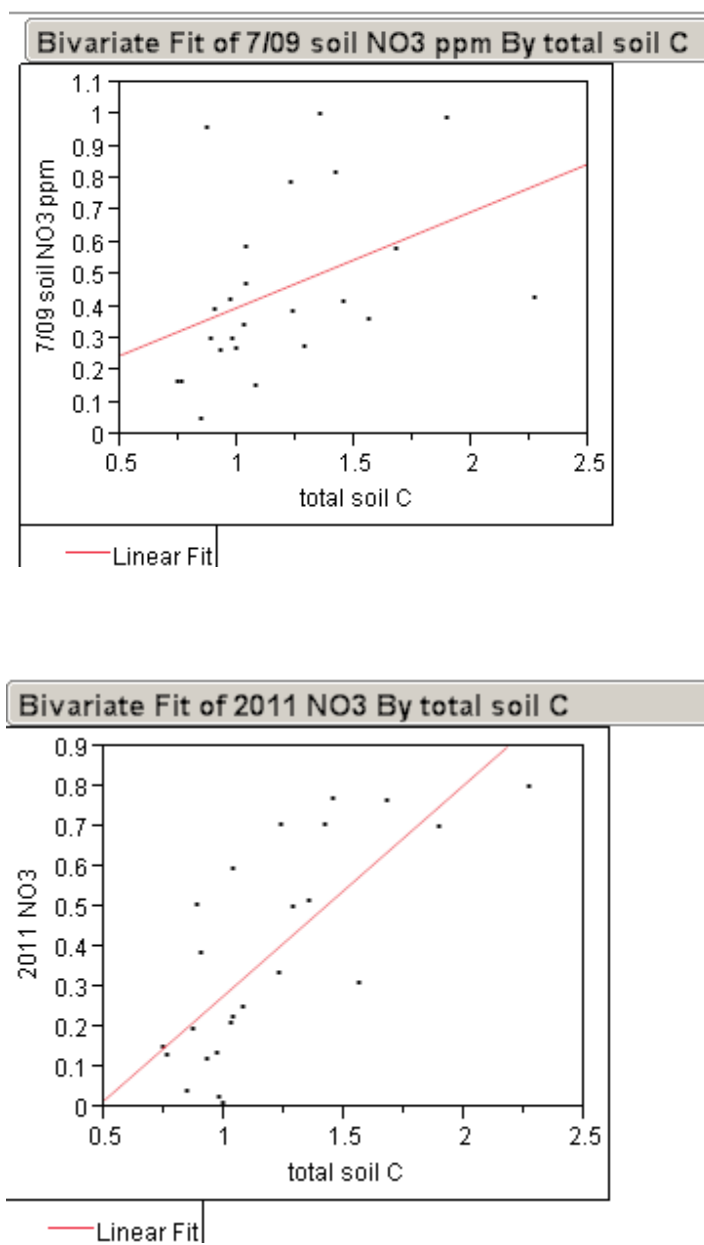
Figure 5.4. Continued.

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CHAPTER 6: CONCLUSION

Tallgrass prairie restoration, or the planting of tallgrass prairie seed mixes in former cropland, has been conducted on hundreds of thousands of hectares in the central United States in recent decades. However, little is known about the relative ability of restorations seeded with different methods to provide ecosystem functions and services. An improved understanding of the factors that increase the provision of ecosystem functions and services and grassland resilience will help improve the success of grassland restoration. In this study, I examined the ecological functions and services provided by 55-m^2 research plots 10 km south of Wood River, Nebraska seeded with four treatments: high diversity sites of 97 species typically planted by The Nature Conservancy planted at a lower seeding density, high diversity sites at twice this seeding rate, and low diversity sites using a Conservation Reserve Program mix (CP25; 15 species), at low and high seeding densities.

Recorded seeded plant species richness in this study was approximately twice as large in the high diversity plots compared to the low diversity plots. Over three seasons of sampling from 2007-2009, I recorded a total of 27 seeded species, with 9, 13, 22, and 22 seeded species observed among low diversity, low seeding density; low diversity, high seeding density; high diversity, low seeding density; and high diversity, high seeding density plots, respectively. Carter and Blair (2012) sampled the plant community in 0.25-m^2 quadrats (1 m^2 total sampling area within each plot) in 2009 and 2010 as part of a study on the response of restorations to drought. They recorded a total of 37 seeded species, with 15, 12, 26, and 26 seeded species observed among low diversity, low

seeding density; low diversity, high seeding density; high diversity, low seeding density; and high diversity, high seeding density plots, respectively.

In chapter 2, I assessed the effects of diversity and seeding density on resistance to invasive and other unsown weedy plant species that were allowed to naturally colonize the plots. I examined the overall composition of the plant community, including any seeded and non-seeded plant species, along line transects and the abundance of *Cirsium vulgare* (bull thistle) and *Melilotus* spp. (sweet clover) that had been recorded along belt transects. I also studied the abundance of the one invasive plant species that was intentionally introduced into the plots, *Bromus inermis* (smooth brome), that had spread from transplants and seed and the number of inflorescences removed from *B. inermis* transplants.

I found two groups of unsown species, unsown perennial forbs/legumes and unsown perennial/annual grasses to have significantly less basal cover in high diversity treatments, supporting Elton's (1958) biotic resistance theory that species-rich plant communities should be less invasible. Similarly, the abundance of *C.vulgare* was negatively correlated with diversity. The high diversity treatments may have contained more perennial forb/legume species with similar niches to the unsown species and were better able to outcompete unsown species belonging to the same functional group. The sown perennial forb, *Helianthus maximiliani*, was also a dominant forb species in the high diversity treatments compared to the low diversity treatments and in other studies has been observed to inhibit weed growth allelopathically. Naturally recruited populations of plant species that are considered invasive by the Nebraska Invasive Species Council (2011), *B. inermis*, *Melilotus* spp., *Poa pratensis*, and *Medicago lupulina*

were present in small amounts in the restorations and did not differ significantly in basal cover among the treatments. However, there was a negative relationship between inflorescence production in transplanted *B. inermis* and diversity, which may have been explained by greater interspecific competition with neighboring plants for limiting resources in the high diversity plots, which reduced the health and vigor of *B. inermis*. Seeding density was not an important factor in affecting invasion resistance, except for the basal cover of unsown perennial/annual grasses.

In chapter 3, I compared the abundance and diversity of four groups of predatory invertebrates among the treatments: ants, carabid beetles, spiders, and coccinellid beetles. According to ecological theory, more diverse plant communities should support a more diverse array of herbivores than less diverse plant communiites because of the more diverse resource base and niches available for specialized consumers, and because of increased structural complexity of the habitat (MacArthur 1972, Langellotto and Denno 2004). Because predators obtain some of their nutrition from plant-provided resources such as pollen and nectar (Wäckers et al. 2005), high diversity plant communities may also directly positively influence the diversity of predators by enhancing the variety of plant-provided resources available.

However, in this study, diversity of the seed mix used was not a significant explanation for the abundance of ants, spiders, or carabids although the diversity × sampling date interaction had a significant negative effect on omnivorous carabid beetle abundance. The recorded plant diversity of the treatments seeded with the high diversity seed mix, as measured by species richness and the Shannon-Weaver diversity index, was only slightly higher than the diversity of the low plant diversity treatments in 2007 and by

2009 was lower than the diversity of the low plant diversity treatments. The main difference between the plant communities of the low and high diversity treatments was therefore related to the relative basal cover of grasses and forbs, with higher basal cover of grasses in the low diversity treatments, and in the dominant plant species recorded in the treatments. The abundance of surface-dwelling invertebrates in this study should therefore be interpreted as showing no response to differences in grass basal cover or plant species composition rather than to differences in plant diversity. There was a significant positive effect of diversity on coccinellid abundance which was driven by the large number of coccinellids collected in the high diversity plots in 2008 and 2009. The greater number of coccinellids collected in high diversity treatments may have reflected a stronger preference for pollen and nectar from forb species or more structurally complex habitat provided by large forbs such as *H. maximiliani* in high diversity treatments compared to grasses in low diversity treatments.

In chapter 4, I assessed the effects of plant diversity and seeding density on herbivory damage of two perennial forbs, *Ratibida columnifera* and *Solidago canadensis*. The area of the leaf missing for top, middle, and bottom leaves from ten randomly selected plants in each plot was estimated as evidence of herbivory damage. Year was the main significant effect for explaining levels of herbivory damage, likely because of year-to-year variations in insect populations.

In chapter 5, I compared soil development among the treatments. Diversity was significant in explaining soil nitrate levels, with higher levels of nitrate being recorded in the low diversity treatments early in the restoration (2006-2009). Total carbon and total nitrogen were highest in the high diversity, high seeding rate plots in 2006 but diversity

and seeding density were not significant in explaining levels of these nutrients. Year and the year × diversity interaction were significant in explaining ammonium and nitrate levels. Total carbon also had a significant positive relationship with nitrate and diversity had a significant negative relationship with nitrate. These patterns are most likely related to increasing root biomass as the restorations age, because as roots lengthen plants can become more efficient at removing nitrate from the soil and increases in the amount of decaying roots can increase the amount of ammonium that is held within the soil because of the greater cation exchange sites on the decaying roots.

Overall, these results indicate that increasing the diversity of a tallgrass prairie seed mix may be more effective than increasing the seeding density of a seed mix for providing certain ecosystem services, such as resistance to unsown perennial forbs and legumes, reducing inflorescence production by *Bromus inermis*, increasing abundance of coccinellid beetles, and increasing effectiveness of the plant community in assimilating nitrate. However, the recorded plant diversity of the treatments, as measured by species richness and the Shannon-Weaver index for sown and unsown species, did not differ to the extent expected between the low and high diversity plots in 2006-2009, possibly because *Helianthus maximiliani* became a dominant forb in the high diversity plots. The main recorded differences in the plant communities between the low and high diversity treatments were the functional group composition of the plots, with higher basal cover of grasses recorded in the low diversity treatments and higher basal cover of forbs recorded in the high diversity treatments. As the restorations age and more conservative plant species appear in the high diversity plots, diversity differences might be enhanced among

the treatments, which can affect the ecological functions and services detected in these

restorations in the future.

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Appendix A. Location of study plots 10km south of Wood River, Nebraska in relation to the Platte River and other land owned by The Nature Conservancy (TNC). In the spring of 2010, the TNC cornfield to the west of the plots that had been planted in 2006 was planted with seed mixes to create additional research plots.
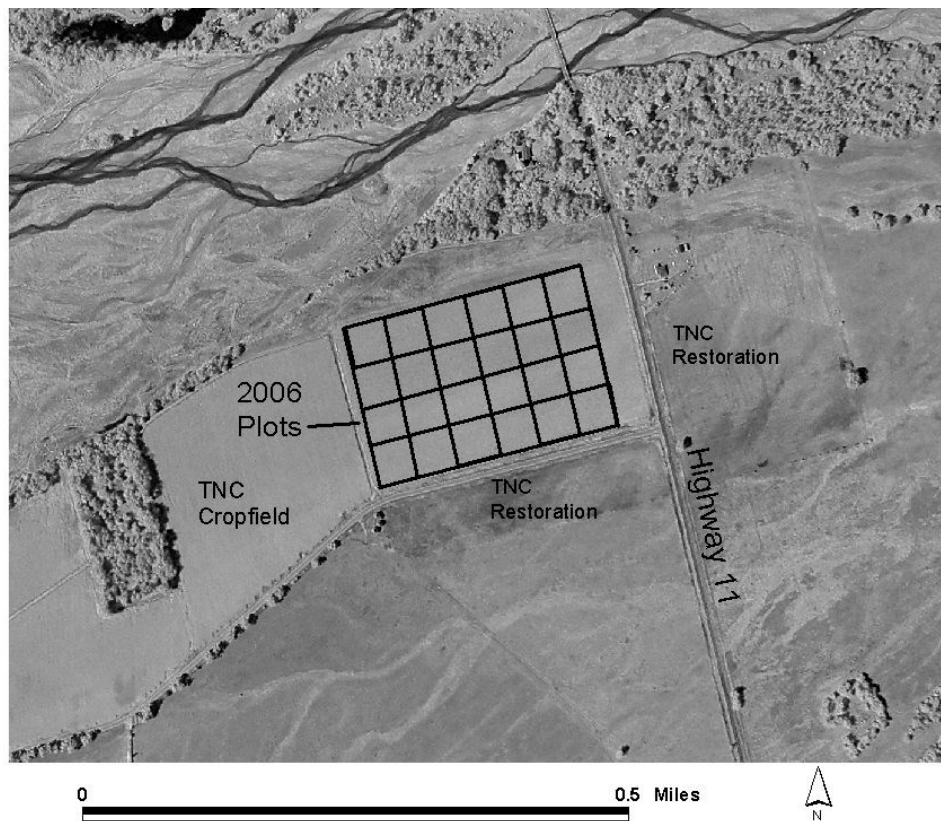
Appendix B. Low diversity seed mix used in the study.

Species	Low seeding density Pure live seeds/m^2	High seeding density Pure live seeds/m^2	Percent of seed mix
Grasses			
Andropogon gerardii	36.0	72.1	22.0
Bouteloua curtipendula	16.1	32.3	9.8
Elymus canadensis	13.4	26.9	8.2
Elymus smithii	14.5	29.0	8.8
Elymus virginiana	7.5	15.1	4.6
Panicum virgatum	13.4	26.9	8.2
Schizachyrium scoparium	26.9	53.8	16.4
Sorghastrum nutans	20.4	40.9	12.5
Total grass	148.2	297	90.5
Forbs			
Amorpha canescens	1.1	2.2	0.67
Astragalus canadensis	4.3	8.6	2.6
Dalea purpurea	1.1	2.2	0.67
Desmanthus illinoensis	5.4	10.8	3.3
Liatris punctata	0.5	1.1	0.33
Ratibida columnifera	1.1	2.2	0.67
Solidago missouriensis	2.2	4.3	1.3
Total forbs	15.7	31.4	9.5

Appendix C. High diversity seed mix used in the study. Because the entire mix was not planted, the percent column best describes the relative amounts of each species seeded.

Species	Low seeding density Dry liters (L)	High seeding density Dry liters (L)	% of forb seed mix
Grass mix			
Andropogon gerardii	N/A	N/A	
Bouteloua curtipendula	N/A	N/A	
Calamagrostis inexpansa	N/A	N/A	
Digitaria cognata	N/A	N/A	
Elymus canadensis	N/A	N/A	
Elymus trachycaulus	N/A	N/A	
Elymus virginiana	N/A	N/A	
Eragrostis spectabilis	N/A	N/A	
Eragrostis trichodes	N/A	N/A	
Koeleria cristata	N/A	N/A	
Panicum virgatum	N/A	N/A	
Paspalum stramineum	N/A	N/A	
Sorghastrum nutans	N/A	N/A	
Spartina pectinata	N/A	N/A	
Sphenopholis obtusata	N/A	N/A	
Sporobolus asper	N/A	N/A	
Sporobolus cryptandrus	N/A	N/A	
Tridens flavus	N/A	N/A	
Forbs included in grass mix			
Desmanthus illinoensis	N/A	N/A	
Helianthus maximiliani	N/A	N/A	
Forbs			
Achillea lanulosa	4.4	8.8	2.7
Allium canadensis	2.2	4.4	1.4
Amorpha canescens	1.1	2.2	0.69
Anemone canadensis	0.36	0.71	0.22
Artemisia ludoviciana	0.36	0.71	0.22
Asclepias speciosa	2.2	4.4	1.4
Asclepais syriaca	2.2	4.4	1.4
Asclepias verticillata	0.18	0.36	0.11
Aster ericoides	4.4	8.8	2.7
Aster novae-angliae	4.4	8.8	2.7
Aster simplex	2.2	4.4	1.4
Astragalus canadensis	0.55	1.1	0.34
Brickellia eupaoroides	2.2	4.4	1.4

Appendix C (continued). High diversity seed mix used in the study. Because the entire mix was not planted, the percent column best describes the relative amounts of each species seeded.

Species	Low seeding density Dry liters (L)	High seeding density Dry liters (L)	% of forb seed mix
Forbs			
Callirhoe involucrata	3.3	6.6	2.1
Calylophus serrulata	3.3	6.6	2.1
Carex brevior	0.24	0.47	0.15
Carex eliocharis	2.2 (messy)	4.4 (messy)	1.4
Carex gravida	0.06	0.12	0.037
Crepis runcinata	0.06	0.12	0.037
Cyperus lupulinus	0.06	0.12	0.037
Cyperus schweinitzii	0.24	0.47	0.15
Dalea candidum	0.47	0.95	0.30
Dalea purpureum	11	22	6.9
Delphinium carolinense	0.0074	0.0074	0.0023
Desmanthus illinoensis	2.2	4.4	1.4
Desmodium illinoense	0.24	0.47	0.15
Eliocharis elliptica	0.12	0.24	0.075
Eupatorium altissimum	6.6	13.2	4.1
Eustoma grandiflorum	0.08	0.16	0.050
Euthamia graminifolia	6.6	13.2	4.1
Gaura parviflora	0.12	0.24	0.075
Geum canadense	0.06	0.12	0.037
Geum vemum	0.06	0.12	0.037
Glycyrrhiza lepidota	0.36	0.71	0.22
Helianthus grosse-serratus	0.08	0.16	0.050
Helianthus laetiflorus	4.4	8.8	2.7
Helianthus petiolaris	0.12	0.24	0.075
Helianthus tuberosa	0.08	0.16	0.050
Heliopsis helianthoides	0.36	0.71	0.22
Hesperostipa comata	0.47	0.95	0.30
Hesperostipa spartea	0.12	0.24	0.075
Heterotheca villosa	4.4	8.8	2.7
Juncus dudleyi	0.022	0.044	0.014
Lespedeza capitata	13.2	26.4	8.2
Liatris lancifolia	6.6	13.2	4.1
Liatris punctata	4.4	8.8	2.7

Appendix C (continued). High diversity seed mix used in the study. Because the entire mix was not planted, the percent column best describes the relative amounts of each species seeded.

Species	Low seeding density Dry liters (L)	High seeding density Dry liters (L)	% of forb seed mix
Forbs			
Liatris squarrosa	4.4	8.8	2.7
Lithospermum caroliniense	0.47 (messy)	0.95 (messy)	0.30
Lithospermum incisum	0.12 (messy)	0.24 (messy)	0.075
Lotus unifoliolatus	0.36	0.71	0.22
Mirabilis nyctaginea	0.12 (messy)	0.24 (messy)	0.075
Monarda fistulosa	4.4 (messy)	8.8 (messy)	2.7
Oenotherea biennis	0.12	0.24	0.075
Oenothera rhombipetala	0.55	1.1	0.34
Onosmodium molle	4.4	8.8	2.7
Penstemon digitalis	0.71	1.4	0.44
Penstemon gracilis	0.015	0.030	0.0094
Penstemon grandiflorus	0.60	1.2	0.37
Plantago patagonica	2.2	4.4	1.4
Potentilla norvegica	0.0074	0.015	0.0094
Prunella vulgaris	0.0074	0.015	0.0094
Pycnanthemum virginianum	0.90	1.8	0.56
Ratibida columnifera	0.8	1.6	0.50
Rosa arkansana	0.70	1.4	0.44
Rudbeckia hirta	0.36	0.71	0.22
Schrankia nuttallii	0.24	0.47	0.15
Senecio plattensis	4.4	8.8	2.7
Silphium speciosum	15.4	30.8	9.6
Sisyrinchium campestre	0.0074	0.015	0.0094
Solidago gigantea	0.12	0.24	0.075
Solidago missouriensis	1.7	3.3	1.0
Solidago rigida	11	22	6.9
Teucrium canadense	0.12	0.24	0.075
Tradescantia bracteata	0.24	0.47	0.15
Tradescantia occidentale	2.2	4.4	1.4
Verbena hastata	0.12	0.24	0.075
Verbena stricta	0.12	0.24	0.075
Vernonia fasciculata	4.4	8.8	2.7
Grasses included in forb mix			
Hesperostipa comata	0.47	0.95	0.30
Hesperostipa spartea	0.12	0.24	0.075

Appendix D. Plant species recorded in the plots according to functional group.

Scientific name	Common name

Sown perennial forb/legume
  Achillea millefolium — Yarrow (high diversity plots)
  Astragalus canadensis — Canada milkvetch
  Dalea purpurea — Purple prairie clover
  Desmanthus illinoensis — Illinois bundleflower
  Geum canadense — White avens (high diversity plots)
  Helianthus maximilliani — Maximilian sunflower (high diversity plots)
  Plantago patagonica — Woolly plantain (high diversity plots)
  Ratibida columnifera — Upright prairie coneflower
  Rudbeckia hirta — Black-eyed susan (high diversity plots)
  Solidago giganta — Giant goldenrod (high diversity plots)
  Solidago missouriensis — Missouri goldenrod
  Sympotrichum ericoides — Heath aster (high diversity plots)
  Verbena stricta — Hoary vervain (high diversity plots)

Sown perennial grass
  Andropogon gerardii — Big bluestem
  Bouteloua curtipendula — Sideoats grama
  Elymus canadensis — Canada wildrye
  Elymus smithii — Western wheatgrass (low diversity plots)
  Elymus trachycaulus — Slender wheatgrass (high diversity plots)
  Elymus virginiana — Virginia wildrye
  Koeleria macrantha — Junegrass (high diversity plots)
  Panicum oligosanthes — Scribner's panic grass (high diversity plots)
  Panicum virgatum — Switchgrass
  Schizachyrium scoparium — Little bluestem
  Sorghastrum nutans — Indiangrass
  Spartina pectinata — Prairie cordgrass (high diversity plots)
  Sphenopholis obtusata — Wedge grass (high diversity plots)
  Sporobolus compositus — Tall dropseed (high diversity plots)

Unsown perennial forb/legume
 (internal to study, from a seed mix)
  Achillea lanulosa — Yarrow (low diversity plots)
  Dalea candidum — White prairie clover (low diversity plots)
  Eupatorium altissimum — Tall white joe pye (low diversity plots)
  Helianthus maximilliani — Maximilian sunflower (low diversity plots)
  Solidago giganta — Giant goldenrod (low diversity plots)
  Verbena stricta — Hoary vervain (low diversity plots)

Appendix D (continued). Plant species recorded in the plots according to functional group.

Scientific name	Common name

Unsown perennial forb/legume
 (external to study, not from seed mix)

Ambrosia psilostachya	Western ragweed
Equisetum arvense	Common horsetail
Physalis longifolia	Common groundcherry
Physalis virginiana	Virginia groundcherry
Solidago canadensis	Canada goldenrod
Taraxacum officinale	Dandelion

Unsown annual forb/legume
(internal to study)

| *Plantago patagonica* | Woolly plantain (low diversity plots) |

(external to study)

Abutilon theophrasti	Velvetleaf
Ambrosia artemisiifolia	Common ragweed
Ambrosia trifida	Giant ragweed
Cannabis sativa	Hemp
Chenopodium album	Lamb's quarters
Conyza canadensis	Mare's tail
Helianthus annuus	Common sunflower
Lactuca serriola	Wild lettuce
Sonchus asper	Prickly star thistle
Xanthium strumarium	Cocklebur

Unsown biennial forb/legume

Cirsium altissimum	Tall thistle
Cirsium vulgare	Bull thistle
Conium maculatum	Poison hemlock

Unsown perennial/annual grass
(internal to study)

Elymus trachycaulus	Slender wheatgrass (low diversity plots)
Koeleria macrantha	Junegrass (low diversity plots)
Panicum oligosanthes	Scribner's panic grass (low diversity plots)
Spartina pectinata	Prairie cordgrass (low diversity plots)
Sporobolus compositus	Tall dropseed (low diversity plots)

(external to study)

| *Setaria pumila* | Yellow foxtail |
| *Setaria* species | Foxtail |

Appendix D (continued). Plant species recorded in the plots according to functional group.

Scientific name	Common name
Invasive species	
Bromus inermis	Smooth brome
Medicago lupulina	Black medic
Melilotus spp.	Sweet clover
Poa pratensis	Kentucky bluegrass

Appendix E. List of plant species recorded and their plot frequencies (percentage of plots occupied; $n = 6$) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Abutilon theophrasti	2007				
	2008	16.7	33.3	16.7	
	2009				
Achillea millefolium	2007			50	33.3
	2008			66.7	16.7
	2009	16.7	16.7	66.7	50
Ambrosia artemisiifolia	2007	16.7	16.7	16.7	50
	2008	50	16.7	66.7	66.7
	2009	50	16.7	16.7	16.7
Ambrosia psilostachya	2007				
	2008				16.7
	2009			16.7	
Ambrosia trifida	2007		16.7		16.7
	2008			33.3	16.7
	2009	33.3	33.3	16.7	
Andropogon gerardii	2007	33.3	50	33.3	33.3
	2008	66.7	83.3	50	66.7
	2009	66.7	66.7	66.7	100
Astragalus canadensis	2007	16.7	16.7	16.7	
	2008	50	83.3	50	50
	2009	100	100	83.3	100
Bouteloua curtipendula	2007		16.7		
	2008				
	2009	16.7	33.3	66.7	66.7
Bromus inermis	2007				
	2008			16.7	16.7
	2009	33.3			
Cannabis sativa	2007	16.7			
	2008	33.3		33.3	16.7
	2009				

Appendix E (continued). List of plant species recorded and their plot frequencies (percentage of plots occupied; $n = 6$) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Chenopodium album	2007				
	2008	100	100	100	100
	2009	33.3	16.7		
Cirsium altissimum	2007	33.3	33.3	33.3	66.7
	2008	16.7			
	2009		16.7		
Cirsium vulgare	2007				
	2008	33.3	16.7		16.7
	2009	16.7			
Conyza canadensis	2007	100	100	100	100
	2008	100	66.7	50	33.3
	2009	83.3	83.3	16.7	
Conium maculatum	2007				
	2008	33.3	33.3		
	2009	33.3			
Dalea candida	2007				
	2008				
	2009		16.7		
Dalea purpurea	2007			16.7	
	2008		16.7	16.7	16.7
	2009				
Desmanthus illinoensis	2007		33.3		16.7
	2008				16.7
	2009				
Elymus canadensis	2007	16.7	50		33.3
	2008	66.7	83.3	33.3	83.3
	2009		50	50	33.3
Elymus smithii	2007				
	2008	33.3	100		
	2009	50	50		

Appendix E (continued). List of plant species recorded and their plot frequencies (percentage of plots occupied; $n = 6$) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Elymus trachycaulus	2007				
	2008		33.3	33.3	50
	2009	16.7			16.7
Elymus virginiana	2007				
	2008	50	66.7	16.7	66.7
	2009	66.7	83.3	66.7	50
Equisetum arvense	2007				
	2008				
	2009	16.7			
Eupatorium altissimum	2007		16.7		
	2008				
	2009				
Geum canadense	2007			16.7	
	2008				
	2009				
Helianthus annuus	2007	16.7		16.7	16.7
	2008	33.3	66.7	33.3	66.7
	2009	50	33.3	16.7	16.7
Helianthus maximiliani	2007	16.7		100	100
	2008	50	16.7	100	83.3
	2009	66.7	83.3	100	100
Koeleria macrantha	2007				
	2008		16.7	50	
	2009		16.7	33.3	16.7
Lactuca serriola	2007	100	83.3	83.3	100
	2008	50	16.7	16.7	33.3
	2009	33.3	16.7		
Medicago lupulina	2007				
	2008	50	16.7	33.3	16.7
	2009	50	33.3	16.7	16.7

Appendix E (continued). List of plant species recorded and their plot frequencies (percentage of plots occupied; $n = 6$) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Melilotus species	2007	16.7	33.3	33.3	16.7
	2008				
	2009				
Panicum oligosanthes	2007				
	2008				
	2009		16.7	16.7	
Panicum virgatum	2007		16.7	16.7	33.3
	2008	33.3	33.3	66.7	50
	2009	50	66.7	33.3	66.7
Physalis longifolia	2007				
	2008		33.3	16.7	16.7
	2009				
Physalis virginiana	2007		16.7		
	2008				
	2009				
Plantago patagonica	2007				16.7
	2008				
	2009	16.7		50	16.7
Poa pratensis	2007		50	66.7	16.7
	2008	16.7			
	2009				
Ratibida columnifera	2007	33.3	33.3		16.7
	2008	33.3	33.3		33.3
	2009	50	50	33.3	16.7
Rudbeckia hirta	2007			16.7	
	2008				
	2009			33.3	16.7
Schizachyrium scoparium	2007		16.7		
	2008	33.3	33.3		33.3
	2009	66.7	83.3	16.7	33.3

Appendix E (continued). List of plant species recorded and their plot frequencies (percentage of plots occupied; $n = 6$) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Setaria pumila	2007	83.3	100	100	83.3
	2008				
	2009				
Setaria species	2007				16.7
	2008	100	100	83.3	83.3
	2009		16.7		
Solidago canadensis	2007				
	2008				33.3
	2009	16.7			
Solidago giganta	2007	16.7			
	2008				
	2009			16.7	
Solidago missouriensis	2007				
	2008			16.7	16.7
	2009		33.3	16.7	
Sonchus asper	2007		16.7		16.7
	2008		16.7		
	2009				16.7
Sorghastrum nutans	2007				
	2008		16.7		
	2009		33.3	16.7	33.3
Spartina pectinata	2007				
	2008				
	2009	16.7	16.7		
Sphenopholis obtusata	2007				
	2008				16.7
	2009				
Sporobolus compositus	2007	16.7	33.3		33.3
	2008				
	2009		33.3		50

Appendix E (continued). List of plant species recorded and their plot frequencies (percentage of plots occupied; *n* = 6) for each treatment.

Species	Year	Low div Low dens	Low div High dens	High div Low dens	High div High dens
Sympotrichum ericoides	2007				
	2008			16.7	
	2009				16.7
Taraxacum officinale	2007	50	50	66.7	50
	2008	83.3	83.3	83.3	66.7
	2009	100	100	100	66.7
Verbena stricta	2007				
	2008	33.3		16.7	16.7
	2009	33.3	33.3		
Xanthium strumarium	2007	16.7		16.7	33.3
	2008				
	2009				

Appendix F. Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Abutilon theophrasti Velvetleaf	2007	.	.	.	.
	2008	0.0074 ± 0.0045	0.0074 ± 0.0058	0.0037 ± 0.0037	.
	2009	.	.	.	.
Achillea millefolium var. occidentalis Yarrow	2007	.	.	0.022 ± 0.017	0.0093 ± 0.0061
	2008	.	.	0.031 ± 0.018	0.015 ± 0.0086
	2009	0.0037 ± 0.0037	0.0037 ± 0.0037	0.017 ± 0.0084	0.031 ± 0.0095
Ambrosia artemisiifolia var. elatior Common ragweed	2007	0.0019 ± 0.0019	0.0037 ± 0.0037	0.0019 ± 0.0019	0.030 ± 0.012
	2008	0.0093 ± 0.0055	0.0037 ± 0.0037	0.011 ± 0.0064	0.035 ± 0.013
	2009	0.022 ± 0.0080	0.0037 ± 0.0026	0.0019 ± 0.0019	0.0037 ± 0.0037
Ambrosia psilostachya Western ragweed	2007	.	.	.	.
	2008	.	.	.	0.0056 ± 0.0056
	2009	.	.	0.0037 ± 0.0037	.
Ambrosia trifida Giant ragweed	2007	.	0.0019 ± 0.0019	.	0.011 ± 0.0078
	2008	.	.	0.0074 ± 0.0052	0.0074 ± 0.0052
	2009	0.011 ± 0.0069	0.0037 ± 0.0026	0.0019 ± 0.0019	.
Andropogon gerardii Big bluestem	2007	0.057 ± 0.047	0.037 ± 0.014	0.039 ± 0.020	0.019 ± 0.012
	2008	0.18 ± 0.070	0.37 ± 0.13	0.19 ± 0.082	0.25 ± 0.087
	2009	0.48 ± 0.19	0.36 ± 0.15	0.30 ± 0.16	0.38 ± 0.13

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Astragalus canadensis	2007	0.015 ± 0.015	0.0037 ± 0.0037	0.0019 ± 0.0019	.
Canada milkvetch	2008	0.052 ± 0.020	0.12 ± 0.034	0.031 ± 0.020	0.019 ± 0.012
	2009	0.085 ± 0.025	0.17 ± 0.030	0.057 ± 0.021	0.063 ± 0.021
Bouteloua curtipendula	2007	.	0.0037 ± 0.0037	.	.
Sideoats grama	2008	.	.	.	.
	2009	0.0019 ± 0.0019	0.11 ± 0.059	0.19 ± 0.087	0.19 ± 0.079
Bromus inermis	2007	.	.	.	.
Smooth brome	2008	.	.	0.013 ± 0.013	0.0037 ± 0.0037
	2009	0.013 ± 0.011	.	.	.
Cannabis sativa	2007	0.0037 ± 0.0037	.	.	.
Hemp	2008	0.0056 ± 0.0041	.	0.0056 ± 0.0041	0.0019 ± 0.0019
	2009	.	.	.	.
Chenopodium album	2007	.	.	.	.
Lamb's quarters	2008	0.28 ± 0.033	0.16 ± 0.032	0.18 ± 0.032	0.16 ± 0.039
	2009	0.0037 ± 0.0026	0.0019 ± 0.0019	.	.
Cirsium altissimum	2007	0.013 ± 0.0099	0.0093 ± 0.0055	0.0056 ± 0.0041	0.020 ± 0.012
Tall thistle	2008	0.0056 ± 0.0056	.	.	.
	2009	.	.0093 ± 0.0093	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Cirsium vulgare Bull thistle	2007	.	.	.	.
	2008	0.030 ± 0.019	0.0093 ± 0.0093	.	0.0019 ± 0.0019
	2009	0.026 ± 0.026	.	.	.
Conyza canadensis Mare's tail	2007	1.6 ± 0.069	1.5 ± 0.070	1.3 ± 0.068	1.6 ± 0.10
	2008	0.044 ± 0.014	0.011 ± 0.0044	0.015 ± 0.0057	0.0093 ± 0.0041
	2009	0.043 ± 0.011	0.044 ± 0.013	0.0019 ± 0.0019	.
Conium maculatum Poison hemlock	2007	.	.	.	.
	2008	0.011 ± 0.0069	0.0093 ± 0.0076	.	.
	2009	0.0056 ± 0.0041	.	.	.
Dalea candida var. candida White prairie clover	2007	.	.	.	.
	2008	.	.	.	.
	2009	.	0.0019 ± 0.0019	.	.
Dalea purpurea var. purpurea Purple prairie clover	2007	.	.	0.0037 ± 0.0037	.
	2008	.	0.0056 ± 0.0056	0.0074 ± 0.0074	0.0019 ± 0.0019
	2009	.	.	.	.
Desmanthus illinoensis Illinois bundleflower	2007	.	0.0037 ± 0.0026	.	0.0019 ± 0.0019
	2008	.	.	.	0.0019 ± 0.0019
	2009	.	.	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Elymus canadensis	2007	0.039 ± 0.039	0.022 ± 0.011	.	0.033 ± 0.020
Canada wildrye	2008	0.14 ± 0.052	0.20 ± 0.075	0.050 ± 0.033	0.18 ± 0.065
	2009	.	0.0074 ± 0.0045	0.16 ± 0.077	0.033 ± 0.024
Elymus smithii	2007	.	.	.	.
Western wheatgrass	2008	0.048 ± 0.037	0.11 ± 0.056	.	.
	2009	0.076 ± 0.064	0.019 ± 0.0077	.	.
Elymus trachycaulus ssp. trachycaulus	2007	.	.	.	.
Slender wheatgrass	2008	.	0.0056 ± 0.0041	0.041 ± 0.029	0.046 ± 0.031
	2009	0.0019 ± 0.0019	.	.	0.0019 ± 0.0019
Elymus virginiana var. virginiana	2007	.	.	.	.
Virginia wildrye	2008	0.061 ± 0.033	0.21 ± 0.068	0.020 ± 0.020	0.072 ± 0.030
	2009	0.42 ± 0.12	0.67 ± 0.19	0.10 ± 0.058	0.054 ± 0.034
Equisetum arvense	2007	.	.	.	.
Common horse tail	2008	.	.	.	.
	2009	0.024 ± 0.024	.	.	.
Eupatorium altissimum	2007	.	0.0019 ± 0.0019	.	.
Tall white joe pye	2008	.	.	.	.
	2009	.	.	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Geum canadense	2007	.	.	0.0019 ± 0.0019	.
White avens	2008	.	.	.	.
	2009	.	.	.	.
Helianthus annuus	2007	0.0019 ± 0.0019	.	0.0056 ± 0.0056	0.0037 ± 0.0026
Common sunflower	2008	0.022 ± 0.012	0.017 ± 0.0084	0.011 ± 0.0064	0.030 ± 0.016
	2009	0.031 ± 0.012	0.017 ± 0.0079	0.0037 ± 0.0037	0.0037 ± 0.0026
Helianthus maximiliani	2007	0.0019 ± 0.0019	.	0.078 ± 0.023	0.096 ± 0.022
Maximilian sunflower	2008	0.019 ± 0.0097	0.017 ± 0.013	0.19 ± 0.031	0.30 ± 0.038
	2009	0.044 ± 0.015	0.048 ± 0.015	0.34 ± 0.034	0.32 ± 0.041
Koeleria macrantha	2007	.	.	.	.
Junegrass	2008	.	0.065 ± 0.051	0.050 ± 0.029	.
	2009	.	0.020 ± 0.020	0.033 ± 0.027	0.057 ± 0.057
Lactuca serriola	2007	0.081 ± 0.019	0.061 ± 0.018	0.048 ± 0.016	0.078 ± 0.023
Wild lettuce	2008	0.015 ± 0.0086	0.0056 ± 0.0056	0.0019 ± 0.0019	0.0093 ± 0.0055
	2009	0.026 ± 0.012	0.011 ± 0.011	.	.
Medicago lupulina	2007	.	.	.	.
Black medic	2008	0.030 ± 0.011	0.013 ± 0.013	0.011 ± 0.0069	0.0019 ± 0.0019
	2009	0.046 ± 0.021	0.030 ± 0.014	0.013 ± 0.0099	0.015 ± 0.012

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Melilotus officinalis Yellow sweetclover	2007	0.0019 ± 0.0019	0.0074 ± 0.0058	0.0093 ± 0.0066	0.0019 ± 0.0019
	2008	.	.	.	.
	2009	.	.	.	.
Panicum oligosanthes var. scribnerianum Scribner's panic grass	2007	.	.	.	.
	2008	.	.	.	.
	2009	.	0.0019 ± 0.0019	0.028 ± 0.028	.
Panicum virgatum Switchgrass	2007	.	0.0019 ± 0.0019	0.0037 ± 0.0037	0.0056 ± 0.0041
	2008	0.059 ± 0.036	0.080 ± 0.043	0.050 ± 0.025	0.080 ± 0.040
	2009	0.15 ± 0.10	0.15 ± 0.96	0.11 ± 0.071	0.098 ± 0.057
Physalis longifolia Common groundcherry	2007	.	.	.	.
	2008	.	0.0074 ± 0.0045	0.0037 ± 0.0037	0.0019 ± 0.0019
	2009	.	.	.	.
Physalis virginiana Virginia groundcherry	2007	.	0.0019 ± 0.0019	.	.
	2008	.	.	.	.
	2009	.	.	.	.
Plantago patagonica var. patagonica Woolly plantain	2007	.	.	.	0.0093 ± 0.0066
	2008	.	.	.	.
	2009	0.019 ± 0.019	.	0.046 ± 0.020	0.0037 ± 0.0037

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Poa pratensis	2007	.	0.072 ± 0.030	0.015 ± 0.0078	0.0019 ± 0.0019
Kentucky bluegrass	2008	0.019 ± 0.019	.	.	.
	2009	.	.	.	.
Ratibida columnifera	2007	0.0037 ± 0.0026	0.0074 ± 0.0045	.	0.0019 ± 0.0019
Upright prairie coneflower	2008	0.013 ± 0.0080	0.024 ± 0.014	.	0.0074 ± 0.0058
	2009	0.074 ± 0.046	0.037 ± 0.019	0.011 ± 0.0083	0.0019 ± 0.0019
Rudbeckia hirta var. pulcherrima	2007	.	.	0.0019 ± 0.0019	.
Brown-eyed susan	2008	.	.	.	.
	2009	.	.	0.015 ± 0.012	0.0037 ± 0.0037
Schizachyrium scoparium	2007	.	0.0019 ± 0.0019	.	.
Little bluestem	2008	0.12 ± 0.068	0.10 ± 0.060	.	0.039 ± 0.026
	2009	0.35 ± 0.16	0.35 ± 0.14	0.013 ± 0.013	0.067 ± 0.047
Setaria pumila	2007	0.067 ± 0.015	0.054 ± 0.011	0.048 ± 0.12	0.15 ± 0.025
Yellow foxtail	2008	.	.	.	.
	2009	.	.	.	.
Setaria species	2007	.	.	.	0.0019 ± 0.0019
Foxtail	2008	0.094 ± 0.018	0.070 ± 0.017	0.044 ± 0.014	0.020 ± 0.0064
	2009	.	0.0037 ± 0.0037	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Solidago canadensis	2007	.	.	.	.
Canada goldenrod	2008	.	.	.	0.020 ± 0.012
	2009	0.0093 ± 0.0076	.	.	.
Solidago giganta	2007	0.0019 ± 0.0019	.	.	.
Giant goldenrod	2008	.	.	.	.
	2009	.	.	0.0037 ± 0.0037	.
Solidago missouriensis var. fasciculata	2007	.	.	.	.
Missouri goldenrod	2008	.	.	0.0037 ± 0.0037	0.0037 ± 0.0037
	2009	.	0.0093 ± 0.0061	0.0019 ± 0.0019	.
Sonchus asper	2007	.	0.0074 ± 0.0045	.	0.011 ± 0.0083
Prickly star thistle	2008	.	0.0093 ± 0.0093	.	.
	2009	.	.	.	0.0037 ± 0.0037
Sorghastrum nutans	2007	.	.	.	.
Indiangrass	2008	.	0.063 ± 0.058	.	.
	2009	.	0.019 ± 0.013	0.0056 ± 0.0056	0.0056 ± 0.0041
Spartina pectinata	2007	.	.	.	.
Prairie cordgrass	2008	.	.	.	.
	2009	0.0093 ± 0.0066	0.070 ± 0.067	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Sphenopholis obtusata var. obtusata	2007	.	.	.	.
Wedge grass	2008	.	.	.	0.0074 ± 0.0074
	2009	.	.	.	.
Sporobolus compositus	2007	0.0019 ± 0.0019	0.0056 ± 0.0041	.	0.0074 ± 0.0058
Tall dropseed	2008	.	.	.	.
	2009	.	0.13 ± 0.078	.	0.057 ± 0.033
Sympotrichum ericoides var. ericoides	2007	.	.	.	.
Heath aster	2008	.	.	0.019 ± 0.019	.
	2009	.	.	.	0.0056 ± 0.0041
Taraxacum officinale	2007	0.048 ± 0.019	0.017 ± 0.0095	0.019 ± 0.0093	0.013 ± 0.0080
Dandelion	2008	0.33 ± 0.086	0.21 ± 0.064	0.17 ± 0.055	0.13 ± 0.059
	2009	0.17 ± 0.050	0.14 ± 0.044	0.094 ± 0.031	0.033 ± 0.015
Verbena stricta	2007	.	.	.	.
Hoary vervain	2008	0.0056 ± 0.0041	.	0.0019 ± 0.0019	0.0037 ± 0.0037
	2009	0.0093 ± 0.0066	0.011 ± 0.0069	.	.

Appendix F (continued). Mean (+-SE) basal cover of plant species (cm) per 1-m subtransect within each treatment. Decimal points are used in place of zeros for visual clarity.

Species	Year	Low diversity Low seed density	Low diversity High seed density	High diversity Low seed density	High diversity High seed density
Xanthium strumarium	2007	0.0074 ± 0.0074	.	0.019 ± 0.019	0.019 ± 0.019
Cocklebur	2008	.	.	.	.
	2009	.	.	.	.

Appendix G. Ant species collected in the study according to feeding guild.

Scientific Name	Diet of Adults
Omnivores	
Aphaenogaster rudis	Food consists of insects, seeds, pollen of ground nesting bees[1]; live termites, Virginia pine-sawfly larvae[2]; generalist scavenger/ predator[3]
Formica dolosa	Workers [of *F. pallidefulva* group to which this species belongs] are omnivores[3]
Formica incerta	Workers [of *F. pallidefulva* group to which this species belongs] are omnivores[3]; often visits extrafloral nectaries of sunflower, partridge peas, other prairie plants, tends aphids and membracids[4]
Formica montana	Omnivorous, frequently tends aphids and coccids[3]; on bloom of *Solidago* sp., eats honeydew[2]
Formica pallidefulva	Workers [of *F. pallidefulva* group to which this species belongs] are omnivores[3]; on bloom of *Daucus carota*, on apple, extrafloral nectaries[2]
Lasius interjectus	Workers [of *L. claviger* group to which this species belongs] are omnivorous, tend root-feeding aphids and coccids[3]; largely or exclusively honeydew[2]
Lasius neoniger	On bloom of *Pastinaca sativa*, often taken at bait, fosters subterranean honeydew excreting insects, but largely carnivorous[2]
Myrmica americana	Food varied, consists of animal matter and plant juices[2]; ants [of *Myrmica* genus] primarily carnivorous, but also tend aphids and take plant sap and flower nectar[3]
Myrmica brevispinosa	Ants [of *Myrmica* genus] primarily carnivorous, but also tend aphids, take plant sap and flower nectar[3]
Pheidole pilfera	Taken at bait, harvester of grass seeds, will take dead insects but largely granivorous[2]
Prenolepis imparis	On bloom of *Solidago* sp., *Solidago caesia* and *Daucus carota*, on fruit and other baits, honeydew, exudates from galls, earthworms, arthropods, collected on dead phalangid[2]

Appendix G. Continued.

Scientific Name	Diet of Adults
Solenopsis molesta	Dead insects, larvae and pupae of its host, corn kernels[5]; highly predaceous, nearly omnivorous[2]; feed on both dead and live insects, highly granivorous, fond of honeydew, many household foods including bread, ripened fruits[6]
Tapinoma sessile	Honeydew, secretions of floral and extrafloral nectaries, living and dead insects[2,5]

Carnivores

Formica pergandei	Facultative slave-making ants, enslave other Formica[3]; brood of *Aphaenogaster treatae*, *Aphaenogaster ruids*, and *Lasius pallitarsis*[2]
Hypoponera opacior	Workers [of *Hypoponera* genus] prey on small soil invertebrates[3]
Ponera pennsylvanica	Workers [of *Ponera* genus] forage in soil and litter for variety of small soil invertebrates[3]; carnivorous, feeding on small insects[2]

Granivore

Nylanderia parvula	Seeds of myrmecochorous plant *Trillium erectum* collected for elaiosomes; extrafloral nectaries of partridge pea in FL[2]

Unknown

Paratrecina terricola

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Appendix H. Carabid beetle species collected in the study according to feeding guild.

Scientific Name	Diet of Adults
Omnivores	
Agonum crenistriatum	Corn (field); immature stages of ants and beetles, adult carabids, earthworms, pieces of meat and fruit (captivity)[1]
Agonum cupripenne	Flies, coccinellids, orthopterans (field); mealworms, lepidopterous caterpillars, dead beetles, earthworms, pieces of meat, corn kernels (captivity)[1]
Agonum placidum	Dipterous larvae, lepidopterous caterpillars, vegetal matter (field); lepidopterous eggs, slugs, meat (captivity)[1]
Amara carinata	Grass material, seeds, fungi, dipterous larvae (field); vegetal matter (captivity)[1]
Amara musculis	Plant seeds, pollen, animal matter (field)[1]
Anisodactylus harrisii	Plant seeds (field); lepidopterous caterpillars, earthworms, mealworms, meat (captivity)[1]
Anisodactylus merula	Plant seeds/weevils (field); mealworms, earthworms (captivity)[1]
Anisodactylus ovularis	Fly pupae and seeds (captivity) [2]
Anisodactylus rusticus	Vegetal matter, lepidopterous caterpillars and other insects (field); grass seeds, corn kernels, mealworms, earthworms, slugs (captivity)[1]
Anisodactylus sanctaecrusis	Vegetal matter, lepidopterous caterpillars (field); various plant seeds, lepidopterous caterpillars, dipterous pupae, immature stages of weevils, scarabeid larvae, earthworms, mealworms, dog food, chicken eggs (captivity)[1]
Calathus gregarious	Sawfly cocoons, lepidopterous caterpillars, grass pollen (field); mealworms and meat (captivity)[1]
Carabus serratus	Mealworms, shrimps, earthworms, lepidopterous caterpillars, apple (captivity)[1]
Chlaenius tomentosus	Lepidopterous caterpillars, scarabeids, other insects, fungi, vegetal tissue (field); meat, earthworms, dead carabids (captivity)[1]
Cyclotrachelus alternans	Lepidopterous caterpillars, dead and live invertebrates, seeds (captivity)[1]

Appendix H. Continued.

Scientific Name	Diet of Adults
Cyclotrachelus sodalis	Lepidopterous caterpillars, curculionids, lygaeids, flies, ants (field); lepidopterous caterpillars, grasshopper nymphs, pentatomids, circulionids, hymenopteran larvae, weed and corn seeds, cheese (captivity)[1]
Harpalus caliginosus	Seeds and pollen of plants, lepidopterous caterpillars, tenebrionids, coccinellids, chrysomelids, scarabaeids, flies, acridids, ant pupae and adults, and mites (field); plant seeds, rose haws, berries, apple, peach, banana, boiled wheat, mealworms, meat, cheese (captivity)[1]
Harpalus compar	Plant seeds and lygaeids (field)[1]
Harpalus eraticus	Corn, chrysomelids, scarabaeid eggs, and other insects (in field); chrysomelid larvae (in captivity)[1]
Harpalus herbivagus	Grass shoots, grass seeds, fungi, lepidopterous caterpillars, scarabaeids, flies, cabbage worms (field); earthworms and meat (captivity)[1]
Harpalus pensylvanicus	Plant seeds, tissue, pollen, fungi, lepidopterous caterpillars, curculionid larvae, chrysomelid eggs and adults, coccinellids, scarabaeid larvae and adults, carabids, grasshoppers, honeybees, mites (field); aphids (captivity)[1]
Notiobia terminata	Pollen, seeds, tissue of grass and weeds (field); grass seeds, beetles, curculionid larvae, lepidopterous caterpillars and pupae, mealworms (captivity)[1]
Poecilus chalcites	Lepidopterous caterpillars, flies, hymenopterans, other insects, fungi and other vegetal tissue (field); lepidopterous caterpillars, chrysomelid eggs, mealworms, earthworms, slugs, meat, corn kernels, raisins (captivity)[1]
Poecilus lucublandus	Lepidopterous caterpillars, dipterous larvae, pupae, and adults, hymenopterous larvae, chrysomelids, scarabaeid larvae, elaterids, vegetal tissue (field)[1]

Appendix H. Continued.

Scientific Name	Diet of Adults
Pterostichus permundus	Lepidopterous caterpillars, hymenopterans, other insects, fungi, vegetal tissue (field); weevils, hymenopterous larvae, mealworms, dog food (captivity)[1]
Scarites subterraneus	Lepidopterous caterpillars, cetoniid larvae, coccinellids, elaterids, other insects, earthworms, injured salamander, young seedlings in seed bed (field)[1]
Stenolophus comma	Flies, lygaeids, ants, aphids, grasshopper eggs, corn seed, fungi (field); lepidopterous caterpillars, dipterous immature stages, chrysomelid eggs and larvae, grasshopper eggs, dead lygaeids, plant seeds (captivity)[1]

Carnivores

Amara patruelis	Lepidopterous caterpillars (captivity)[1]
Anisodactylus carbonarius	Lepidopterous caterpillars (captivity)[1]
Bembidion quadrimaculatum oppostum	All lepidopterous and dipterous immature stages (field); aphid nymphs, dipterous eggs and larvae, lepidopterous eggs, chrysomelid eggs, immature stages of weevils, larval chrysomoelids (in captivity)[1]
Bembidian rapidum	Lepidopterous immature stages, dipterous larvae and pupae (field)[1]
Calathus opaculus	Ant eggs (captivity)
Chlaenius tricolor tricolor	Lepidopterous caterpillars and slugs (field); carabids, mealworms, earthworms, dead slugs, meat (captivity)
Cicindela cursitans	carnivore[3]
Cicindela punctulata	carnivore[3]
Cratacanthus dubius	Small insects (field)[1]
Dicaleus elongatus	Lepidopterous caterpillars and undetermined animal matter (field)
Harpalus erythropus	Scarabaeid larvae (field); chrysomelid prepupae and pupae (captivity)[1]
Harpalus somnulentus	Lepidopterous caterpillars and grasshopper eggs (field)[1]

Appendix H. Continued.

Scientific Name	Diet of Adults
Scarites quadriceps	Lepidopterous caterpillars and scarabeids (field); weevils, live and dead invertebrates (captivity)[1]
Stenolophus conjunctus	Coccinellids (field); meat (captivity)[1]

Granivore

Amara rubrica	Seed heads of Compositae (Solidago) (field)[1]

Unknown

Acupalpus sp.
Amara coelebs
Cyclotrachelus torvus
Cymindis pilosus
Discoderus parallelus
Elaphropus anceps
Harpalus indianus
Harpalus nigritarsis
Notiophilus semistriatus
Selenophorus ellipticus
Selenophorus opalinus

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Appendix I. Coccinellid beetle species collected in the study according to feeding guild.

Scientific Name	Diet of Adults
Omnivores	
Coccinella septempunctata	Fungal spores from honeydew[1], pea aphids, larvae of alfalfa weevil[2]; euryphagous coccinellid capable of exploiting many aphid species and supplementary food sources such as plant nectaries, pollen of Compositae and Umbellifera and fungal spores when aphids not abundant[3]; aphidophagous beetle that relies heavily on pollen before undergoing diapause[4]
Coleomegilla maculata	Omnivorous predator that eats corn pollen, aphids and eggs of the important corn pests *Ostrinia nubilalis* and *Helicoverpa zea*[5]
Cycloneda munda	Oligophagous, feeds primarily on aphids[6]
Harmonia axyridis	Preys on numerous aphid species, Tetranichidae, Psyllidae, Coccoidea, immature stages of Chrysomelidae, Curculionidae, Lepidoptera, and pollen and nectar[7]
Hippodamia convergens	Consumes prey from several insect orders, but mostly consumes aphids; nectar and pollen when aphid prey are scarce[8]
Hippodamia parenthesis	Aphids, pollen[4,9]

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Appendix J. Spider species collected in the study according to guild[1].

Scientific Name	Family
Hunters	
Agroeca pratensis	Liocranidae
Arctosa rubicunda	Lycosidae
Castianeira variata	Corinnidae
Clubiona abbotti	Clubionidae
Clubionidae sp.	Clubionidae
Corinnidae sp.	Corinnidae
Drassyllus depressus	Gnaphosidae
Drassyllus lepidus	Gnaphosidae
Gnaphosidae sp.	Gnaphosidae
Gnaphosa sericata	Gnaphosidae
Hogna frondica	Lycosidae
Lycosidae sp.	Lycosidae
Marpissa formosa	Salticidae
Mimetus sp.	Mimetidae
Oxiopidae sp.	Oxiopidae
Pardosa milvina	Lycosidae
Pardosa saxatilis	Lycosidae
Phidippus audax	Salticidae
Pirata minutus	Lycosidae
Salticidae sp.	Salticidae
Schizocosa avida	Lycosidae
Schizocosa ocreata	Lycosidae
Sitticus concolor	Salticidae
Thomisidae sp.	Thomisidae
Tibellus sp.	Tibellidae
Xysticus gulosus	Thomisidae
Zelotes aiken	Gnaphosidae
Zelotes fratris	Gnaphosidae
Zelotes hentzi	Gnaphosidae
Zelotes laccus	Gnaphosidae
Web builders	
Agyneta unimaculata	Linyphidae
Araneidae sp.	Araneidae
Argenna obesa	Dictynidae
Bathyphantes concolor	Linyphidae
Bathyphantes pallidus	Linyphidae
Ceratinops crenatus	Linyphidae

Appendix J. Continued.

Scientific Name	Family
Dictynidae sp.	Dictynidae
Erigone atra	Linyphidae
Erigone autumnalis	Linyphidae
Erigone brevidentata	Linyphidae
Euryopis emertoni	Theridiidae
Glenognatha foxi	Tetragnathidae
Grammonota inorata	Linyphidae
Halorates plumosus	Linyphidae
Islandiana flaveola	Linyphidae
Linyphidae sp.	Linyphidae
Mermessus trilobata	Linyphidae
Neoantistea agilis	Hahniidae
Tennesseellum formicum	Linyphidae
Tetragnathidae sp.	Tetragnathidae
Theridiidae sp.	Theridiidae

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Appendix K. Number of specimens collected for each invertebrate species in 2007-2009 at low (L) and high (H) plant diversity plots seeded at low and high seeding rates. For guild, c = carnivore, g = granivore, o = omnivore (ants, carabid beetles, and coccinellid beetles); h = hunter, and w = web-builder (spiders).

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Ants						
Lasius neoniger	o	7,705	6,487	9,304	6,173	29,669
Myrmica americana	o	88	98	60	86	332
Tapinoma sessile	o	22	6	25	23	76
Solenopsis molesta	o	7	7	14	21	49
Formica incerta	o	8	8	7	21	44
Formica pallidefulva	o	11	11	5	8	35
Aphaenogaster rudis	o	13	4	1	7	25
Formica montana	o	4	9	5	7	25
Nylanderia parvula	g	2	8	11	3	24
Ponera pennsylvanica	c	5	5	3	5	18
Formica dolosa	o	0	0	1	7	8
Prenolepis imparis	o	5	0	0	1	6
Hypoponera opacior	c	0	2	0	0	2
Myrmica brevispinosa	o	0	2	0	0	2
Paratrechina terricola	u	0	2	0	0	2
Formica pergandei	c	0	1	0	0	1
Lasius interjectus	o	1	0	0	0	1
Pheidole pilfera	o	0	0	0	1	1
Total		7,871	6,650	9,436	6,363	30,320

Appendix K. Continued.

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Carabid beetles						
Notiobia terminata	o	496	394	507	385	1,782
Pterostichus permundus	o	274	250	247	319	1,090
Calathus opaculus	c	105	88	89	84	366
Anisodactylus rusticus	o	80	71	64	57	272
Harpalus pennsylvanicus	o	128	52	14	16	210
Amara musculis	o	81	63	16	18	178
Amara rubrica	g	36	36	21	20	113
Scarites subterraneus	o	36	28	18	26	108
Harpalus herbivagus	o	34	22	9	22	87
Harpalus somnulentus	c	23	16	9	25	73
Harpalus compar	o	10	9	15	26	60
Amara carinata	o	21	11	9	13	54
Anisodactylus carbonarius	c	18	16	6	12	52
Poecilus lucublandus	o	17	11	9	15	52
Cicindela punctulata	c	15	9	11	9	44
Cicindela cursitans	c	8	9	13	11	41
Poecilus chalcites	o	11	7	5	18	41
Bembidion quadrimaculatum oppostum	c	7	7	5	11	30
Anisodactylus merula	o	10	10	3	5	28
Ansiodactylus ovularis	o	4	4	5	12	25
Harpalus erythropus	c	4	6	5	9	24
Harpalus eraticus	o	9	6	3	2	20
Agonum placidum	o	5	2	9	2	18
Cyclotrachelus alternans	o	1	5	5	6	17

Appendix K. Continued.

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Anisodactylus sanctaecrusis	o	3	0	1	12	16
Harpalus caliginosis	o	3	2	7	2	14
Anisodactylus harrisii	o	2	1	4	4	11
Elaphropus anceps	u	3	5	2	1	11
Bembidion rapdium	c	2	1	2	3	8
Harpalus indianus	u	3	0	0	5	8
Chlaenius tomentosus	o	0	4	1	1	6
Cyclotrachelus torvus	u	1	0	1	3	5
Stenolophus conjunctus	c	1	1	0	2	4
Cyclotrachelus sodalis	o	1	2	0	0	3
Agonum cupripenne	o	0	1	1	0	2
Amara coelebs	u	1	1	0	0	2
Calathus gregarious	o	0	0	1	1	2
Carabus serratus	o	0	2	0	0	2
Cymindis pilosus	u	0	0	1	1	2
Discoderus parallelus	u	0	0	0	2	2
Notiophilus semistriatus	u	0	0	1	1	2
Selenophorus ellipticus	u	1	0	0	1	2
Acupalpus sp.	u	0	1	0	0	1
Agonum crenistriatum	o	0	0	0	1	1
Amara patruelis	c	1	0	0	0	1
Chlaenius tricolor tricolor	c	0	0	1	0	1
Cratacanthus dubius	c	0	0	0	1	1
Dicaleus elongatus	c	0	0	0	1	1
Harpalus nigritarsis	u	1	0	0	0	1
Scarites quadriceps	c	0	0	1	0	1

Appendix K. Continued.

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Selenophorus opalinus	u	0	1	0	0	1
Stenolophus comma	o	0	0	0	1	1
Total		1,456	1,154	1,121	1,166	4,897
Spiders						
Lycosidae sp.	h	32	22	16	38	108
Agyneta unimaculata	w	10	20	13	17	60
Linyphidae sp.	w	10	6	14	10	40
Pardosa saxatilis	h	8	6	6	4	24
Drassyllus depressus	h	7	8	5	3	23
Gnaphosidae sp.	h	4	5	0	4	13
Clubiona abbotti	h	0	5	2	2	9
Gnaphosa sericata	h	1	2	5	1	9
Theridiidae sp.	w	2	4	0	3	9
Erigone autumnalis	w	3	2	1	2	8
Tennesseellum formicum	w	1	4	0	3	8
Ceratinops crenatus	w	6	0	1	0	7
Thomisidae sp.	h	2	3	1	1	7
Salticidae sp.	h	1	2	0	3	6
Pardosa milvina	h	0	1	0	4	5
Agroeca pratensis	h	1	0	0	3	4
Arctosa rubicunda	h	2	0	0	2	4
Erigone atra	w	1	0	1	2	4
Glenognatha foxi	w	0	0	1	3	4

Appendix K. Continued.

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Islandiana flaveola	w	1	0	1	2	4
Schizocosa ocreata	h	2	1	0	1	4
Araneidae sp.	w	1	1	1	0	3
Argenna obesa	w	2	0	0	1	3
Drassyllus lepidus	h	0	1	0	2	3
Xysticus gulosus	h	0	2	0	1	3
Zelotes laccus	h	1	0	0	2	3
Clubionidae sp.	h	0	2	0	0	2
Corinnidae sp.	h	1	1	0	0	2
Halorates plumosus	w	0	2	0	0	2
Hogna frondica	h	1	0	0	1	2
Neoantistea agilis	w	0	1	0	1	2
Zelotes hentzi	h	1	0	0	1	2
Bathyphantes concolor	w	0	0	1	0	1
Bathyphantes pallidus	w	0	1	0	0	1
Castianeira variata	h	0	1	0	0	1
Dictynidae sp.	w	0	0	0	1	1
Erigone brevidentata	w	0	0	0	1	1
Euryopis emertoni	w	1	0	0	0	1
Grammonota inorata	w	1	0	0	0	1
Mermessus trilobata	w	0	1	0	0	1
Mimetus sp.	h	1	0	0	0	1
Oxiopidae sp.	h	1	0	0	0	1
Phidippus audax	h	0	0	0	1	1
Pirata minutus	h	0	0	1	0	1
Schizocosa avida	h	0	1	0	0	1

Appendix K. Continued.

	Guild	L div L rate	L div H rate	H div L rate	H div H rate	Total
Sitticus concolor	h	0	0	1	0	1
Tetragnathidae sp.	w	0	1	0	0	1
Tibellus sp.	h	0	0	0	1	1
Xysticus sp.	h	1	0	0	0	1
Zelotes aiken	h	1	0	0	0	1
Zelotes fratris	h	0	0	1	0	1
Total		107	106	72	121	406
Coccinellid beetles						
Hippodamia convergens	o	172	134	304	375	985
Coccinella septempunctata	o	28	35	51	44	158
Hippodamia parenthesis	o	13	17	27	28	85
Coleomegilla maculata	o	7	5	7	6	25
Unknown species	u	1	1	5	6	13
Cycloneda munda	o	0	0	2	4	6
Harmonia axyridis	o	0	0	1	0	1
Total		221	192	397	463	1,273