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Influence of Richness and Seeding Density on Invasion Resistance in Experimental Tallgrass Prairie Restorations

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RESEARCH ARTICLE

Influence of Richness and Seeding Density on Invasion Resistance in Experimental Tallgrass Prairie Restorations

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ABSTRACT

In recent years, agricultural producers and non-governmental organizations and agencies have restored thousands of hectares of cropland to grassland in the Great Plains of the United States. However, little is known about the relationships between richness and seeding density in these restorations and resistance to invasive plant species. We assessed the effects of richness and seeding density on resistance to invasive and other unseeded plant species in experimental tallgrass prairie plots in central Nebraska. In 2006, twenty-four 55 m × 55 m plots were planted with six replicates in each of four treatments: high richness (97 species typically planted by The Nature Conservancy), at low and high seeding densities, and low richness (15 species representing a typical Conservation Reserve Program mix, CP25), at low and high seeding densities. There was a significant negative relationship between richness and basal cover of unseeded perennial forbs/legumes and unseeded perennial/annual grasses, abundance of bull thistle (*Cirsium vulgare*), and the number of inflorescences removed from smooth brome (*Bromus inermis*) transplants. Invasion resistance may have been higher in the high richness treatments because of the characteristics of the dominant species in these plots or because of greater interspecific competition for limiting resources among forbs/legumes with neighboring plants belonging to the same functional group. Seeding density was not important in affecting invasion resistance, except in the cover of unseeded grasses. Increasing seed mix richness may be more effective than increasing the seeding density for decreasing invasion by unseeded perennial species, bull thistle, and smooth brome.

Keywords: bull thistle, grasslands, invasibility, sweet clover

Invasive, nonnative plant species can profoundly impact ecosystems and communities, altering ecosystem structure and function, decreasing native plant species richness and phylogenetic diversity, and disrupting reproductive mutualisms of native plant species (Pyšek and Richardson 2010). These impacts can reduce a community's resilience, or its capacity to absorb disturbance without fundamentally changing its essential structure and functions (Holling 1973, Folke et al. 2002). Another concept applied to a community's response to disturbance is resistance, or the ease or difficulty of changing a system (Walker et al. 2004). In terms of invasions, resistance refers to the biotic and abiotic factors that enhance a community's capacity to limit the spread of an invading species (Levine et al. 2004, D'Antonio and Chambers 2006). Understanding the factors that increase community resistance to invasion is important if land managers and conservationists are to reduce the spread and impact of invasive plant species in natural and restored areas.

In recent decades, the role of plant species richness in resisting invasive plant species has been a major focus of invasion research. Experimental and observational studies conducted at small scales (≤ 20 m²), particularly in North American grasslands, have primarily found negative relationships between plant species richness and invasibility (Naeem et al. 2000, Symstad 2000, Dukes 2002, Fargione and Tilman 2005), while observational studies conducted at large scales (>1 km²) in a variety of ecosystems worldwide have mostly reported positive relationships between plant species richness and invasibility (Stohlgren et al. 1999, 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 (Fridley et al. 2007).

Because plant species that arrive first at a disturbed site can strongly influence the trajectory of succession, "priority effects" are an important consideration in establishing seeded species that may reduce the growth of later-arriving unseeded, or invading,

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species (Körner et al. 2008, Martin and Wilsey 2012). Although varying levels of seeding density can affect the establishment success and plant density of seeded species (Burton et al. 2006, Frances et al. 2010), the role of seeding density has received less attention than richness in grassland invasion resistance research. Martin (2006) examined the effect of four seeding density treatments on prairie establishment. The number of unseeded stems did not show a strong relationship with seeding density and the number of unseeded nonnative species did not vary significantly among treatments. Dickson and Busby (2009) studied the effect of varied grass density on forb establishment. The percent cover of unseeded species declined over three years across all treatments and in two of three years they found no significant treatment effect on the cover of unseeded species. Peters and Schottler (2011) tested five seeding rates and altered the ratios of grass to forb seed to study prairie establishment, finding unseeded species density to have a significant negative correlation to forb seeding rate.

Although all of these studies incorporated seeding densities that are typically used in North American grassland seed mixes, to our knowledge no study has manipulated the seeding rates of low and high richness seed mixes that are often used by practitioners within a particular area to test the relative effects of seeding density on invasion resistance. Although studies conducted under realistic restoration conditions have found that increasing the richness of grassland seed mixes is often associated with increased invasive plant resistance (Young et al. 2009, Institute for Applied Ecology 2011, Oakley and Knox 2013), none have compared the resistance of The Nature Conservancy (TNC) and Natural Resources Conservation Service (NRCS) Conservation Reserve Program (CRP) seed mixes, two common sources of seed mixes for restoration efforts in the Great Plains. In addition, few studies have established research plots at an intermediate scale (between 20 m² and 1 km²). Research that incorporates commonly used seeding methods can provide valuable information for improving the success of restoration projects and advance knowledge about the relationship between biodiversity and ecosystem functioning and services (Symstad 2008).

This study compared invasion resistance of 55 m × 55 m research plots seeded with a low richness tallgrass prairie seed mix commonly used in central Nebraska (15 species representing a typical Conservation Reserve Program mix, the CP25 mix), at low and high seeding densities, and a high richness mix (97 species typically planted by The Nature Conservancy), at low and high seeding densities. We assessed the degree to which the four treatments resisted crop field weeds and three non-native plants of different reproductive strategies: bull thistle (*Cirsium vulgare*) and white and yellow sweet clover (*Melilotus officinalis*), which reproduce by seed; and smooth brome (*Bromus inermis*), which reproduces by both seed and rhizomes (Stubbendieck et al. 2003). These species were selected because they are aggressive and commonly encountered in Nebraska grassland restorations. All are undesirable in natural areas because they can become widespread and reduce the cover and growth of native plant species (Forcella and Randall 1994, Wolf et al. 2003, Vinton and Goergen 2006, Otfinowski et al. 2009, Dillemuth et al. 2009, Van Riper and Larson 2009). In addition, the sharp spines of bull thistle can interfere with livestock grazing (Forcella and Wood 1986) and sweet clover may facilitate the growth of other invasive plant species (Wolf et al. 2003, Van Riper and Larson 2009).

We tested four null hypotheses: 1) the basal cover of seeded native plant species will not differ among the different density and richness treatments; 2) the basal cover of unseeded plant species will not differ among the different density and richness treatments; 3) the abundance of bull thistle and sweet clover will not differ among the different density and richness treatments; and 4) the abundance of smooth brome tillers that have spread from transplants and seed and the number of inflorescences removed from transplants will not differ among the different density and richness treatments.

Methods

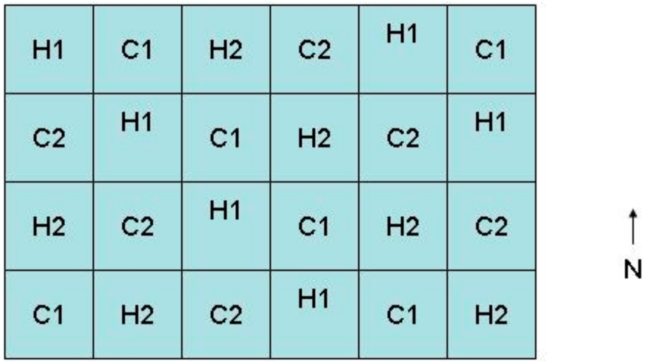
Study Area

The study area lies within the Central Platte River ecosystem, which includes the Platte River channel and floodplain in central Nebraska (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; N 40°44'41", W 98°35'11") on a 7.3 ha field owned by TNC. Soils at the site are of loamy alluvium or sandy alluvium parent material (NRCS 2010). The site is bordered to the south and east by county roads and TNC prairie restorations, to the west by a cornfield that was seeded with experimental prairie restoration plots in the spring of 2010, and to the north by trees and the Platte River. The site was under cultivation in a corn-soybean rotation for decades prior to the experiment, during which it was managed with conventional tillage and chemical inputs.

Treatments and Experimental Design

In late March and early April 2006, the field was cultivated and divided into twenty-four 0.30 ha plots (55 m × 55 m). 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 plant richness (low and high) were applied using two different seeding



H1	C1	H2	C2	H1	C1
C2	H1	C1	H2	C2	H1
H2	C2	H1	C1	H2	C2
C1	H2	C2	H1	C1	H2

Figure 1. Layout of treatments applied to 55 m × 55 m plots in the central Platte River floodplain, Nebraska, USA (C1 = low richness seed mix/low seeding rate; C2 = low richness seed mix/high seeding rate; H1 = high richness seed mix/low seeding rate; H2 = high richness seed mix/high seeding rate).

densities (low and high seeding rates). The experiment was arranged in a systematic design, with six columns running west to east across the field and each column containing four plots assigned to the four treatments (Figure 1). The treatments were applied systematically instead of randomly in order to facilitate seeding with the drop spreader. Treatments consisted of: C1) a low richness CRP tallgrass prairie seed mix (CP25 mix, 15 species) used by the NRCS seeded at half the recommended seeding rate (low richness/low rate mix: grass, 148 pure live seeds (PLS)/m²; forbs, 16 PLS/m²); C2) the CP25 mix applied at the recommended seeding rate (low richness/high rate mix: grass, 297 PLS/m²; forbs, 31 PLS/m²); H1) a high richness tallgrass prairie mix typically used by the local TNC (97 species) seeded with a seeding rate typical for TNC grassland restorations in the region (high richness/low rate: grass, 129 PLS/m²; forbs, 43 PLS/m²); H2) the TNC mix applied at twice the recommended seeding rate (high richness/high rate: grass, 258 PLS/m²; forbs, 86 PLS/m²) (Appendices A and B). The second and fourth treatments are at half and double NRCS or TNC normal seeding rates, respectively, because the NRCS normally recommends rates that are about twice as high as TNC uses.

We designed the CP25 seed mix (Table A1) with the Grand Island, Nebraska NRCS District Conservationist. We purchased grass seed used in the mix from Arrow Seed in Broken Bow, Nebraska and forb seed was locally harvested from the Platte River area. The Nature Conservancy high-richness seed mix (Table A2) was harvested from local prairies.

With the exception of smooth brome, which was added in 2008, we allowed non-native species to naturally establish. All of the plots were burned on March 20, 2008. In July 2008, yarrow (*Achillea millefolium*) and Maximilian sunflower (*Helianthus maximiliani*) that had invaded into the edges of plots where they had not been seeded were sprayed with glyphosate and killed, in order to reduce the edge effect on the spread of these aggressive species. We clipped the inflorescences of smooth brome that had invaded the south row of plots and two plots in the northwestern corner of the field in order to limit the spread of smooth brome 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

Within each plot, we established five 55 m transects located 9.1 m apart. We assessed plant species composition along three of the transects within each plot, the middle transect and the two end transects, in mid- to late June 2007–2009. We used the line-intercept transect method because it is an efficient method of collecting cover and species richness (Bonham 1989). Starting at the end of each transect, we stretched a measuring tape to a length of one meter close to the ground. The transect was broken up into these smaller, one-meter segments, or "sub-transects," to keep the measuring tape from sagging in the wind. We 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). Measurements were taken along every 12th meter and at the opposite end of the transect for a total of six, one-meter subtransects along the transect (data were recorded at 0, 12, 24, 36, 48, and 55 meters).

Bull Thistle and Sweet Clover

We assessed the abundance of bull thistle and sweet clover when these species were flowering in September and October of 2006–2009 by walking belt transects along the five 55 m transects within each plot (Grant et al. 2004). We placed a 3 m pole with flagging tape over the rebar on one end of the transect to ensure a straight line was walked. We recorded the number of bull thistle and sweet clover plants observed within 3 meters on both sides of the transect.

Smooth Brome

In 2008, we added smooth brome plants and seeds to each plot in order to compare its spread from rhizomes and establishment from seeds among the four treatments. We added plants to the plots on April 13, 2008. We used a shovel to remove blocks of smooth brome approximately 13 cm² in surface area and 5 cm deep from the ditch on the southern edge of the study site. We transplanted four plants along the middle of the center transect in each plot, with each plant placed 3 m apart to form the corners of a square. We marked the east edge of each transplant with a flagged 1 m stake.

We clipped the inflorescences of each planted smooth brome on June 29, 2008, June 24, 2009, and June 27–June 29, 2010 in order to prevent these plants from dropping seeds and to ensure they would spread only by rhizomes. We recorded the number of inflorescences clipped from each plant as a measure of the vigor of the plant. On June 27–29, 2010, we placed a 1 m^2 quadrat frame in each of the four cardinal directions around each smooth brome 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. We removed the inflorescences of all smooth brome plants within the quadrat frame and within 10 meters of the area.

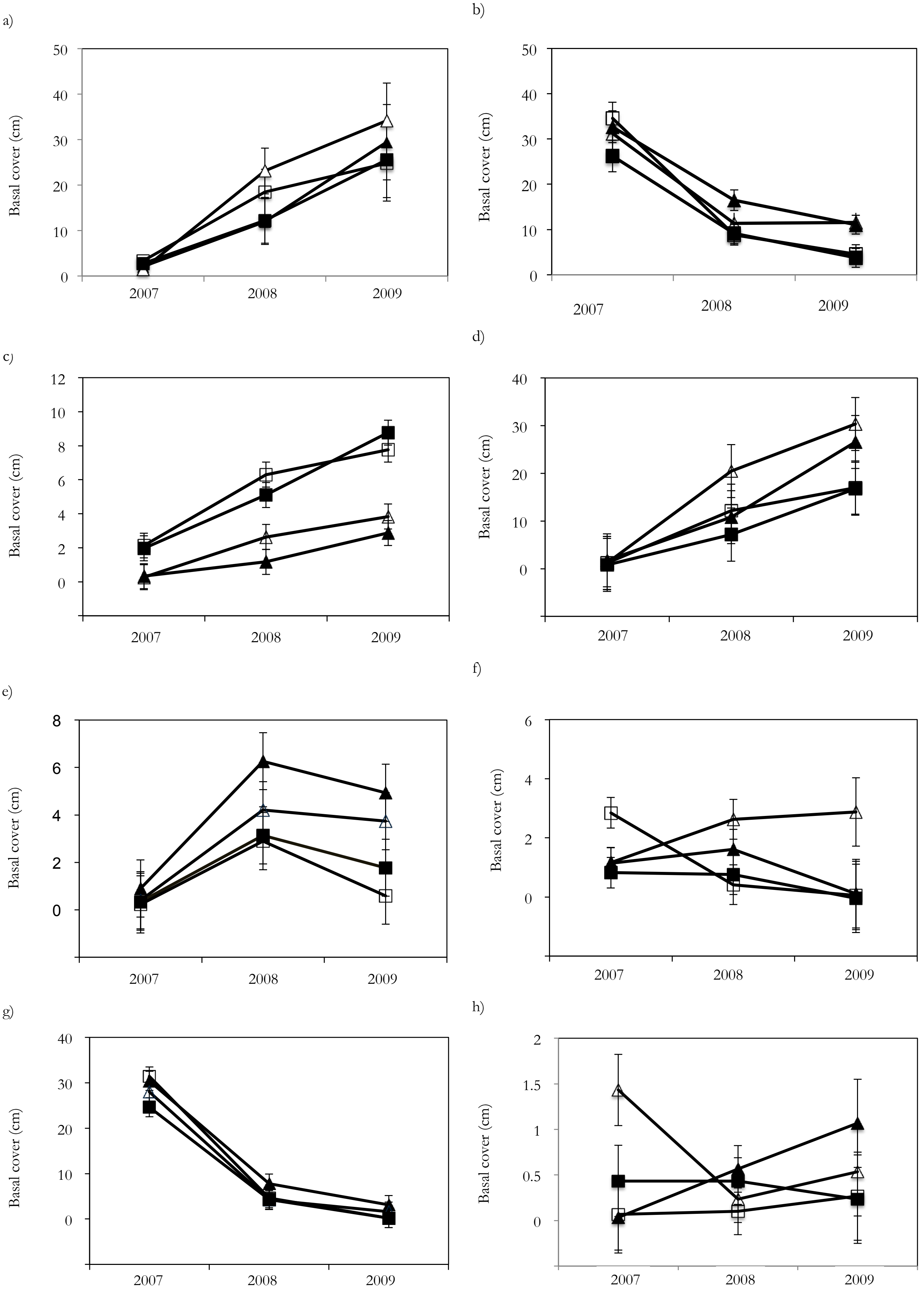
We obtained smooth brome seed from Stock Seed Farms in Murdock, Nebraska. We added seed to the plots in late April, the time of year when farmers often seed smooth brome, and in early September, when smooth brome plants naturally drop their seed (Bruce Anderson, University of Nebraska-Lincoln, pers. comm.), to determine if timing of seed addition affected the species' invasiveness. On April 30, 2008, we 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. We broadcasted seeds over a 1 m^2 area using a seeding rate of 120 PLS/m^2 and lightly raked in the seed. On September 7, 2008, we added seed to the northeast and southwest corner of each plot using the same seeding rate and methods. In both months, we recorded each seeding location with a Trimble GeoXT handheld GPS unit with submeter accuracy.

Statistical Analysis

Plots were the experimental units in this study. For analyzing plant community composition, we grouped plant species recorded along line transects in each plot by summing the basal cover of plants placed into eight classes: all seeded species, all unseeded species, seeded perennial/annual forbs/legumes, seeded perennial grasses, unseeded perennial forbs/legumes, unseeded perennial/annual grasses, unseeded annual/biennial forbs/legumes, and invasive species, as defined by the Nebraska Invasive Species Council (2011) (Table A3). We used these categories because we were interested in identifying the relative success of different growth forms in becoming established and in limiting the spread of unseeded species. The invasive species category was used because we were particularly interested in the ability of the treatments to reduce establishment of plant species that are considered invasive in Nebraska compared to less aggressive unseeded species. We tested normality in the response variables (basal cover of plant species aggregated in each functional group, number of bull thistle or sweet clover plants, or smooth brome inflorescences or tillers) with the Kolmogorov-Smirnov normality test (PROC UNIVARIATE, SAS Version 9.2, SAS Institute 2007). Because the residuals were not normally distributed, we fit each set of data 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). Richness, seeding density, year, and their interactions were used as fixed effects and plot column was used as a random effect to account for observed spatial variation in soil fertility that generally ran from west to east across the field. We ran post-hoc Tukey-Kramer tests comparing significant richness, density, and year interactions. Because no bull thistle plants were recorded along belt transects in 2007, we omitted this year from the bull thistle model. In the smooth brome model for the number of tillers established from seeding locations, we combined data from the April and September seeding periods because of the low number of tillers recorded from both time periods. We determined the covariance structure that was the best fit for each model covering multiple years of data by comparing Akaike's information criterion (AIC) for the plant community composition models and the pseudo-AIC for the bull thistle, sweet clover, and smooth brome. The distribution and covariance structures used for each model were: 1) plant community composition: Gaussian distribution, unstructured covariance structure; 2) *C. vulgare*: negative binomial distribution, autoregressive covariance structure; 3) *M. officinalis*: 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. The Kenward-Roger (1997) degrees of freedom were used in the models.

Results

Recorded seeded plant species richness was approximately twice as large in the high richness plots compared to the low richness plots. Over three seasons of sampling from 2007 to 2009, we recorded a total of 27 seeded species, with 9 species observed in the low richness, low seeding density plots; 13 species observed in the low richness, high seeding density plots; 22 species observed in the high richness, low seeding density plots; and 22 seeded species observed in the high richness, high seeding density plots.


a)
Basal cover (cm)
50
40
30
20
10
0
2007
2008
2009
b)
Basal cover (cm)
50
40
30
20
10
0
2007
2008
2009
c)
Basal cover (cm)
12
10
8
6
4
2
0
2007
2008
2009
d)
Basal cover (cm)
40
30
20
10
0
2007
2008
2009
e)
Basal cover (cm)
8
6
4
2
0
2007
2008
2009
f)
Basal cover (cm)
6
4
2
0
2007
2008
2009
g)
Basal cover (cm)
40
30
20
10
0
2007
2008
2009
h)
Basal cover (cm)
2
1.5
1
0.5
0
2007
2008
2009

Treatment Effects on Plant Community Composition

Seeded Species

Year was the only variable that had a significant effect on the basal cover of all seeded species across the treatments ($p < 0.0001$) (Figure 2a, Table A4.1). The dominant seeded forb found in the high richness plots was the perennial Maximilian sunflower, which in 2009 accounted for 69% and 75% of the seeded 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 richness plots.

There were significant positive effects of richness ($p < 0.0001$), year ($p < 0.0001$), and the richness × year interaction ($p = 0.0036$) on seeded perennial forb/legume basal cover, which increased across all three years of the study (Figure 2c, Table A4.1). Year had a significant effect on the basal cover of seeded grasses ($p = 0.0002$), which increased over the three years of the study (Figure 2d).

Unseeded Species

Year ($p < 0.0001$) and richness ($p = 0.0073$) had significant effects on the cover of all unseeded species, with higher cover of all unseeded species being recorded in the low richness treatments (Figure 2b, Table A4.1).

Richness ($p = 0.0115$), year ($p < 0.0001$), and the year × richness interaction ($p = 0.0005$) had significant effects on the basal cover of unseeded perennial forb/legume species, with greater cover recorded in the low richness plots (Figure 2e, Table A4.1). These effects remained significant when considering only those species that were external to the study (not present in the seed mixes). Six of the twelve unseeded perennial forb and legume species were internal to the study, having spread from where they were seeded in the high richness plots to the low richness plots where they had not been seeded. Maximilian sunflower was the most widespread internal unseeded perennial forb/legume species, recorded in 75% of the low richness plots in 2009. Dandelion (*Taraxacum officinale*) was the most widespread unseeded perennial forb/legume external to the study, recorded in all of the low richness plots and 83% of the high richness plots in 2009. Of the eleven unseeded annual forbs and legumes, one species, woolly plantain (*Plantago patagonica*), was internal to the study and had spread to the low richness plots. The large density of unseeded annual forbs and legumes in 2007 (Figure 2g) was due to the high abundance of mare's tail (*Conyza canadensis*) that is common to central Nebraska prairie restorations in their second or third growing season. Year had a significant effect ($p < 0.0001$) and the richness × seeding density had a marginally significant effect ($p = 0.0742$) on the basal cover of unseeded annual forbs and legumes, which decreased across all three years, particularly between the first and second year of the study (Figure 2g).

Figure 2, opposite. Basal cover of plant species aggregated as a) all seeded species, b) all unseeded species, c) seeded perennial forbs/legumes, d) seeded perennial grasses, e) unseeded perennial forbs/legumes, f) unseeded perennial/annual grasses, g) unseeded annual/biennial forbs/legumes, and h) invasives during 2007–2009. Values are least-square means (± SE) from mixed model analysis and represent the basal cover in cm recorded along eighteen 1 m sub-transects within each plot. N = 6 plots per treatment. Low richness treatments are represented by triangles and high richness treatments are represented by squares. Low seeding rate treatments are represented by filled shapes and high seeding rate treatments are represented by open shapes.

Of the seven unseeded perennial/annual grasses, five were internal to the study and were present in the low richness plots in low amounts. Richness ($p = 0.0118$), seeding density ($p = 0.0110$), year × richness interaction ($p = 0.0068$), and year × richness × seeding density interactions ($p = 0.0158$) were significant in explaining the basal cover of unseeded perennial/annual grasses, largely because of the high cover present in the low richness treatments in 2008 and 2009 (Figure 2f, Table A4.1).

Four invasive species, smooth brome, Kentucky bluegrass (*Poa pratensis*), black medic (*Medicago lupulina*), and sweet clover, 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 richness, seeding density, year, or their interaction terms, on invasive species basal cover, although richness had marginal significant effects on invasive species basal cover (Figure 2h, Table A4.1).

Treatment Effects on Bull Thistle and Sweet Clover Abundance

Richness ($p = 0.0059$), year ($p < 0.0001$), and the richness × year interaction ($p = 0.0191$) had significant effects on bull thistle abundance, with more bull thistle recorded in the low richness plots (Figure 3a, Table A4.2). Because bull thistle is a biennial, observed abundances across all treatments were highest in 2008, when the plants bolted (Figure 3a).

Year had the only significant effect on sweet clover ($p = 0.0492$), which gradually increased across the years across treatments and was more abundant in the low seeding density treatments than the high seeding density treatments by 2009 (Figure 3b, Table A4.2). However, standard error was also large for sweet clover and sampling effort may not have been large enough to reveal density effects.

Treatment Effects on Planted and Seeded Smooth Brome Abundance

Richness ($p = 0.0057$), year ($p = 0.0104$), and the richness × year interaction ($p = 0.0300$) had significant effects on the number of inflorescences removed from smooth brome transplants in 2008 and 2009, with more inflorescences produced by plants that had been planted in the low richness plots (Figure 4a, Table A4.3). No variables had significant effects on

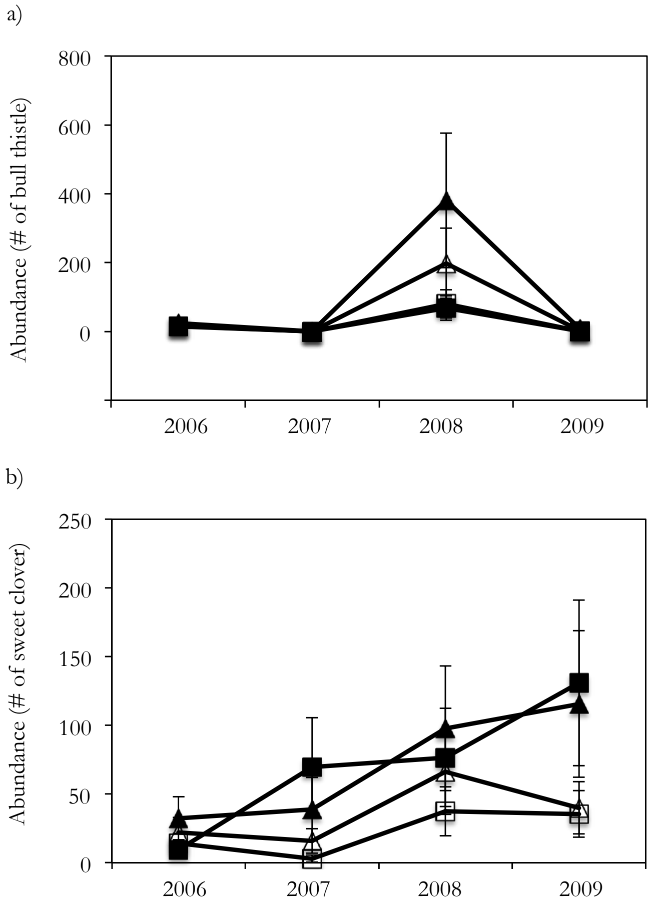


Figure 3. Abundance of bull thistle (a) and sweet clover (b) during 2007–2009. Values are least-square means (± SE) from mixed model analysis. N = 6 plots per treatment. Low richness treatments are represented by triangles and high richness treatments are represented by squares. Low seeding rate treatments are represented by filled shapes and high seeding rate treatments are represented by open shapes.

the number of smooth brome tillers counted in 1 m^2 quadrat frames placed around seeded and planted areas in 2010 to assess the spread of smooth brome (Figure 4b, 4c, Table A4.3). However, there were marginal richness effects on the number of tillers recorded near plugs ($p = 0.0995$ for richness and $p = 0.0772$ for the richness × seeding density interaction) and marginal density effects on the number of tillers recorded near seeding locations ($p = 0.0625$ for seeding density), largely driven by the number of tillers recorded in the low richness, low seeding rate plots (Figure 4c).

Discussion

We found richness to be more important than seeding density in increasing invasion resistance of experimental tallgrass prairie plots to unseeded perennial forbs/legumes, unseeded perennial/annual grasses, bull thistle, and smooth brome. These results support other studies conducted under realistic restoration conditions, which have generally found increasing richness confers greater invasion resistance (Young et al. 2009, Institute for Applied Ecology 2011, Oakley and Knox 2013). The high richness 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 and competitive advantage of Maximilian sunflower, or because of greater interspecific competition among forbs/legumes with neighboring plants belonging to the same functional group for limiting resources in the high richness plots (see Norland et al. 2013, *this issue*).

Seeding density had a significant effect only on the basal cover of unseeded perennial/annual grasses, which were a minor component of all unseeded species. Density had no significant effect on unseeded species. There was a marginal effect of density on the number of tillers removed near seeding locations, with more tillers recorded near locations where smooth brome was seeded in low richness plots. Other grassland studies have found that seeding rate is not a factor in explaining cover or density of unseeded species (Martin 2006, Dickson and Busby 2009).

Two groups of unseeded species, perennial forbs/legumes and perennial/annual grasses, appeared to support Elton's biotic resistance theory (1958) that species-rich plant communities should be less invasible, as there was a significant negative effect of richness on basal cover for these groups. This finding was influenced to some extent by species internal to the experimental seed mix because low richness plots were more likely to be "invaded" by seeded species from adjacent high richness plots. Our 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 richness and unseeded species still held true when considering only the unseeded perennial forb/legume species that were external to the study species pool.

An unseeded species may be less likely to establish if a species with similar traits is already present in the community, and high richness seed mixes have a higher probability of containing a species with similar resource requirements to that of an unseeded species (Funk et al. 2008). We recorded roughly twice as many seeded species in the high richness plots compared to the low richness plots, similar to other studies (Carter and Blair 2012). The high richness treatments may have had more perennial forb/legume species with similar niches that were better able to outcompete unseeded species belonging to the same functional group such as Canada milkvetch (*Astragalus canadensis*), purple prairie clover (*Dalea purpurea*), giant goldenrod (*Solidago gigantea*), and Missouri goldenrod (*Solidago missouriensis*). Young et al. (2009) also found that communities with species functionally similar to an invasive species had greater invasion resistance than functionally dissimilar species. More species-rich communities may also provide greater insurance against environmental variability than communities with fewer species, termed the "portfolio effect" (Doak et al. 1998, Tilman et al. 1998).

Richness had a negative effect on the abundance of naturally recruited bull thistle. The performance and density of bull thistle may be related to several factors including the availability of seed, disturbance, and vigor of grass competition (Louda and Rand 2003). However, the low richness treatments, which had a higher amount of grass cover than the high richness treatments, had higher abundances of bull thistle in 2008, suggesting grass competition did not reduce the spread of bull thistle, at least in the year that it flowered. The high richness treatments may have been more successful in resisting invasion by bull thistle because of the dominant presence of Maximilian sunflower in these plots. Maximilian sunflower inhibits weed growth allelopathically by exuding chemicals that act as an herbicide (Herz and Kumar 1981, Gershenzon and Mabry 1984, Macías et al. 1996). Dickson and Busby (2009) found a significant negative relationship between the percentage canopy cover of Maximilian sunflower and other seeded tallgrass prairie species during one year of their study.

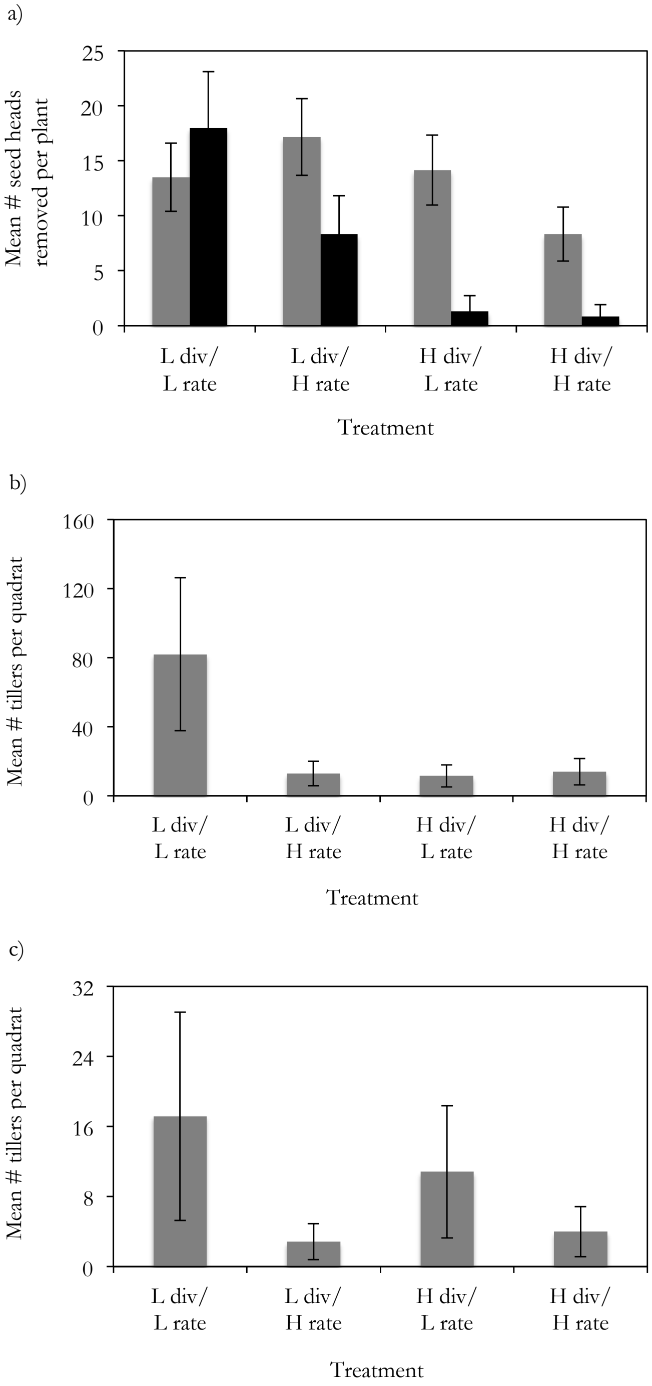


Figure 4. a) Number of smooth brome seed heads removed per planted smooth brome individual in 2008 and 2009. b) Number of smooth brome tillers recorded in quadrats adjacent to planting locations in 2010. c) Number of smooth brome tillers recorded in quadrats adjacent to seeding locations in 2010. Values are least-square means (± SE) from mixed model analysis. N = 6 plots per treatment. The year 2008 is represented by light gray bars and 2009 is represented by black bars.

Richness also explained the number of inflorescences found from smooth brome transplants, with low richness treatments containing significantly more inflorescences per plant than high richness treatments in 2009. Similarly, Hille Ris Lambers et al. (2009) found the number of smooth brome inflorescences removed per quadrat in experimental prairie plots to be negatively correlated with declining species richness. In our study, the negative relationship between inflorescence production and richness may have been explained by greater interspecific competition with neighboring plants for limiting resources in the high richness plots, which reduced the vigor of smooth brome.

No variables had a significant effect on the spread of smooth brome from seed or rhizomes. 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.

In conclusion, the results from these 3,025m^2 plots support studies conducted at smaller (< 20 m^2) scales in which negative relationships have been observed between plant species richness and invasibility (Naeem et al. 2000, Symstad 2000, Dukes 2002, Fargione and Tilman 2005). Environmental heterogeneity was controlled for by a systematic block design in our study and similar to other studies, smaller-scale competitive biotic interactions appeared to be most important in contributing to invasion resistance. 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 richness plant communities to better understand the effects of various seed mixes on invasion resistance. However, based on our results, increasing the plant richness of seed mixes for restoration efforts may be more effective than increasing the seeding density for decreasing invasion by unseeded perennial species and bull thistle. Increasing the richness of a seed mix may reduce the establishment of unseeded species due to species complementing each other in their resource use or by a portfolio effect. In the long run, the initially higher costs of higher richness seed mixes may be offset by reduced time and effort in managing unseeded species.

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Table A1. Low richness seed mix used in the study for experimental restoration of tallgrass prairie in Nebraska, USA.

Species	Low seeding density Pure live seeds/m^2	High seeding density Pure live seeds/m^2	% 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 virginicus	7.5	15.1	4.6
Panicum virgatum	13.4	26.9	8.2
Pascopyrum smithii	14.5	29.0	8.8
Schizachyrium scoparium	26.9	53.8	16.4
Sorghastrum nutans	20.4	40.9	12.5
Total grass	148.2	297.0	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

Table A2. High richness seed mix used in the study for experimental restoration of tallgrass prairie in Nebraska, USA. Because the entire mix was not planted, the % forb column best describes the relative amounts of each forb species seeded. Messy: includes stems and seed heads mixed in with the seed.

Species	**Low seeding density Dry liters (L)**	**High seeding density Dry liters (L)**	**% forb**
Grass mix			
Andropogon gerardii	N/A	N/A	
Bouteloua curtipendula	N/A	N/A	
Calamagrostis canadensis	N/A	N/A	
Digitaria cognata	N/A	N/A	
Elymus canadensis	N/A	N/A	
Elymus trachycaulus	N/A	N/A	
Elymus virginicus	N/A	N/A	
Eragrostis spectabilis	N/A	N/A	
Eragrostis trichodes	N/A	N/A	
Koeleria macrantha	N/A	N/A	
Panicum virgatum	N/A	N/A	
Paspalum setaceum	N/A	N/A	
Sorghastrum nutans	N/A	N/A	
Spartina pectinata	N/A	N/A	
Sphenopholis obtusata	N/A	N/A	
Sporobolus compositus	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 millefolium	4.4	8.8	2.7
Allium canadense	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
Astragalus canadensis	0.55	1.1	0.34
Brickellia eupatorioides	2.2	4.4	1.4
Callirhoe involucrata	3.3	6.6	2.1
Calylophus serrulatus	3.3	6.6	2.1
Carex brevior	0.24	0.47	0.15
Carex duriuscula	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 candida	0.47	0.95	0.30
Dalea purpurea	11.0	22.0	6.9
Delphinium carolinianum	0.0074	0.0074	0.0023
Desmanthus illinoensis	2.2	4.4	1.4
Desmodium illinoense	0.24	0.47	0.15
Eleocharis 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

Species	Low seeding density Dry liters (L)	High seeding density Dry liters (L)	% forb
Gaura parviflora	0.12	0.24	0.075
Geum canadense	0.06	0.12	0.037
Geum vernum	0.06	0.12	0.037
Glycyrrhiza lepidota	0.36	0.71	0.22
Helianthus grosseserratus	0.08	0.16	0.050
Helianthus pauciflorus	4.4	8.8	2.7
Helianthus petiolaris	0.12	0.24	0.075
Helianthus tuberosus	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
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
Mimosa nuttallii	0.24	0.47	0.15
Mirabilis nyctaginea	0.12 (messy)	0.24 (messy)	0.075
Monarda fistulosa	4.4 (messy)	8.8 (messy)	2.7
Oenothera biennis	0.12	0.24	0.075
Oenothera rhombipetala	0.55	1.1	0.34
Oligoneuron rigidum	11.0	22.0	6.9
Onosmodium bejariense	4.4	8.8	2.7
Packera plattensis	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
Silphium integrifolium	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
Symphyotrichum ericoides	4.4	8.8	2.7
Symphyotrichum novae-angliae	4.4	8.8	2.7
Symphyotrichum lanceolatum	2.2	4.4	1.4
Teucrium canadense	0.12	0.24	0.075
Tradescantia bracteata	0.24	0.47	0.15
Tradescantia occidentalis	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

Table A3. Plant species recorded in plots by seeded/unseeded status and growth form and life cycle. Species marked with * were seeded or unseeded in high richness plots, and species marked with ** were seeded or unseeded in low richness plots; a species may appear in multiple lists if it was found both in plots where it was seeded and where it was not.

Species	Common Name
Seeded perennial forb/legume	
Achillea millefolium	yarrow*
Astragalus canadensis	Canada milkvetch
Dalea purpurea	purple prairie clover
Desmanthus illinoensis	Illinois bundleflower
Geum canadense	white avens*
Helianthus maximiliani	Maximilian sunflower*
Plantago patagonica	woolly plantain*
Ratibida columnifera	upright prairie coneflower
Rudbeckia hirta	black-eyed Susan*
Solidago gigantea	giant goldenrod*
Solidago missouriensis	Missouri goldenrod
Symphyotrichum ericoides	heath aster*
Verbena stricta	hoary vervain*
Seeded perennial grass	
Andropogon gerardii	big bluestem
Bouteloua curtipendula	sideoats grama
Dichanthelium oligosanthes	Scribner's panic grass*
Elymus canadensis	Canada wildrye
Elymus trachycaulus	slender wheatgrass*
Elymus virginicus	Virginia wildrye
Koeleria macrantha	Junegrass*
Panicum virgatum	switchgrass
Pascopyrum smithii	western wheatgrass**
Schizachyrium scoparium	little bluestem
Sorghastrum nutans	Indiangrass
Spartina pectinata	prairie cordgrass*
Sphenopholis obtusata	wedge grass*
Sporobolus compositus	tall dropseed*
Unseeded perennial forb/legume (internal to study, from a seed mix)	
Achillea millefolium	yarrow**
Dalea candida	white prairie clover**
Eupatorium altissimum	tall white joe pye**
Helianthus maximiliani	Maximilian sunflower**
Solidago gigantea	giant goldenrod**
Verbena stricta	hoary vervain**
Unseeded 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

Species	**Common Name**
Unseeded annual forb/legume	
(internal to study)	
Plantago patagonica	woolly plantain**
(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
Unseeded biennial forb/legume	
Cirsium altissimum	tall thistle
Cirsium vulgare	bull thistle
Conium maculatum	poison hemlock
Unseeded perennial/annual grass	
(internal to study)	
Dichanthelium oligosanthes	Scribner's panic grass**
Elymus trachycaulus	slender wheatgrass**
Koeleria macrantha	Junegrass**
Spartina pectinata	prairie cordgrass**
Sporobolus compositus	tall dropseed**
(external to study)	
Setaria pumila	yellow foxtail
Setaria species	foxtail
Invasive species	
Bromus inermis	smooth brome
Medicago lupulina	black medic
Melilotus officinalis	sweet clover
Poa pratensis	Kentucky bluegrass

Table A4.1. Results of mixed-model analysis for testing the effects of richness, seeding density, and year on basal cover of seeded and unseeded plant species aggregated according to growth form and functional group. Internal = species included in the study seed mix; external = species not included in the study seed mix. Values in boldface are significant at $p < 0.05$.

Effect	df	*F*	*p*
Seeded perennial forbs/legumes			
Richness	1, 20	46.62	<**0.0001**
Seeding density	1, 20	0.79	0.3832
Richness*Seeding density	1, 20	0.43	0.5216
Year	2, 19	60.80	<**0.0001**
Year*Richness	2, 19	7.66	**0.0036**
Year*Seeding density	2, 19	1.38	0.2747
Year*Richness*Seeding density	2, 19	0.72	0.5009
Seeded perennial grasses			
Richness	1, 19.93	2.17	0.1564
Seeding density	1, 19.93	0.57	0.4576
Richness*Seeding density	1, 19.93	0.09	0.7710
Year	2, 19	14.28	**0.0002**
Year*Richness	2, 19	0.93	0.4118
Year*Seeding density	2, 19	1.42	0.2658
Year*Richness*Seeding density	2, 19	0.14	0.8678
Unseeded perennial forbs/legumes (all)			
Richness	1, 20	7.75	**0.0155**
Seeding density	1, 20	1.76	0.1995
Richness*Seeding density	1, 20	0.36	0.5561
Year	2, 19	41.63	<**0.0001**
Year*Richness	2, 19	11.50	**0.0005**
Year*Seeding density	2, 19	1.24	0.3118
Year*Richness*Seeding density	2, 19	0.21	0.8122
Unseeded perennial forbs/legumes (external)			
Richness	1, 20	3.58	**0.0729**
Seeding density	1, 20	1.42	0.2472
Richness*Seeding density	1, 20	0.24	0.6289
Year	2, 19	25.12	<**0.0001**
Year*Richness	2, 19	3.77	**0.0418**
Year*Seeding density	2, 19	0.99	0.3911
Year*Richness*Seeding density	2, 19	0.31	0.7358
Unseeded perennial forbs/legumes (internal)			
Richness	0	.	.
Seeding density	1, 10	0.17	0.6892
Richness*Seeding density	0	.	.
Year	2, 9	6.82	**0.0157**
Year*Richness	0	.	.
Year*Seeding density	2, 9	0.05	0.9541
Year*Richness*Seeding density	0		
Unseeded perennial/annual grasses (all)			
Richness	1, 20	7.68	**0.0118**
Seeding density	1, 20	7.85	**0.0110**
Richness*Seeding density	1, 20	2.01	0.1721
Year	2, 19	0.28	0.7557
Year*Richness	2, 19	6.56	**0.0068**
Year*Seeding density	2, 19	0.59	0.5638
Year*Richness*Seeding density	2, 19	5.20	**0.0158**

Effect	**df**	***F***	***p***
Unseeded perennial/annual grasses (external)			
Richness	1, 19.8	0.08	0.7848
Seeding density	1, 19.8	0.46	0.5048
Richness*Seeding density	1, 19.8	2.51	0.1288
Year	2, 19	29.49	<**0.0001**
Year*Richness	2, 19	4.73	**0.0215**
Year*Seeding density	2, 19	2.45	0.1133
Year*Richness*Seeding density	2, 19	2.42	0.1161
Unseeded perennial/annual grasses (internal)			
Richness	1, 19.86	6.00	**0.0237**
Seeding density	1, 19.86	5.57	**0.0286**
Richness*Seeding density	1, 19.86	5.02	**0.0366**
Year	2, 19	2.36	0.1213
Year*Richness	2, 19	2.95	0.0763
Year*Seeding density	2, 19	2.15	0.1437
Year*Richness*Seeding density	2, 19	2.73	0.0907
Unseeded annual/biennial forbs/legumes			
Richness	1, 20	1.61	0.2195
Seeding density	1, 20	0	1.000
Richness*Seeding density	1, 20	3.55	0.0742
Year	2, 19	159.21	<**0.0001**
Year*Richness	2, 19	0.18	0.8327
Year*Seeding density	2, 19	0.60	0.5605
Year*Richness*Seeding density	2, 19	1.25	0.3084
Invasives			
Richness	1, 17.46	3.51	0.0779
Seeding density	1, 17.46	0.01	0.9160
Richness*Seeding density	1, 17.46	0.93	0.3486
Year	2, 19	0.88	0.4299
Year*Richness	2, 19	1.05	0.3680
Year*Seeding density	2, 19	1.61	0.2258
Year*Richness*Seeding density	2, 19	1.60	0.2272
Seeded			
Richness	1, 20	0.39	0.5418
Seeding density	1, 20	0.76	0.3937
Richness*Seeding density	1, 20	0.14	0.7095
Year	2, 19	20.32	<**0.0001**
Year*Richness	2, 19	0.43	0.6554
Year*Seeding density	2, 19	2.01	0.1619
Year*Richness*Seeding density	2, 19	0.17	0.8476
Unseeded			
Richness	1, 20	8.91	**0.0073**
Seeding density	1, 20	0.09	0.7654
Richness*Seeding density	1, 20	2.68	0.1173
Year	2, 20	55.50	<**0.0001**
Year*Richness	2, 20	0.82	0.4536
Year*Seeding density	2, 20	1.24	0.3117
Year*Richness*Seeding density	2, 20	0.63	0.5446

Table A4.2. Results of mixed-model analysis for testing the effects of richness, seeding density, and year on abundance of bull thistle and sweet clover. Values in boldface are significant at $p < 0.05$.

Effect	df	*F*	*p*
Bull thistle			
Richness	1, 60	8.14	**0.0059**
Seeding density	1, 60	0.01	0.9321
Richness*Seeding density	1, 60	1.27	0.2633
Year	2, 60	112.50	<**0.0001**
Year*Richness	2, 60	4.23	**0.0191**
Year*Seeding density	2, 60	0.17	0.8437
Year*Richness*Seeding density	2, 60	0.40	0.6693
Sweet clover			
Richness	1, 74	0.56	0.4559
Seeding density	1, 74	2.39	0.1261
Richness*Seeding density	1, 74	0.14	0.7076
Year	3, 67.8	2.84	**0.0492**
Year*Richness	3, 67.7	0.18	0.9090
Year*Seeding density	3, 67.9	1.53	0.2148
Year*Richness*Seeding density	3, 58.9	0.64	0.5888

Table A4.3. Results of mixed-model analysis for testing the effects of richness, seeding density, and year on the number of inflorescences removed from planted smooth brome in 2008 and 2009 and on number of smooth brome tillers recorded in 2010 from quadrats placed adjacent to locations where smooth brome had been planted and seeded. Values in boldface are significant at $p < 0.05$.

Effect	df	*F*	*p*
Smooth brome - inflorescences removed			
Richness	1, 22.97	9.31	**0.0057**
Seeding density	1, 22.97	0.70	0.4114
Richness*Seeding density	1, 22.97	0.07	0.7992
Year	1, 22.97	7.78	**0.0104**
Year*Richness	1, 22.97	5.35	**0.0300**
Year*Seeding density	1, 22.97	0.27	0.6086
Year*Richness*Seeding density	1, 22.97	0.34	0.5639
Smooth brome—tillers near planting locations			
Richness	1, 20	2.98	0.0995
Seeding density	1, 20	2.26	0.1484
Richness*Seeding density	1, 20	3.47	0.0772
Smooth brome—tillers near seeding locations			
Richness	1, 20	0.01	0.9359
Seeding density	1, 20	3.89	0.0625
Richness*Seeding density	1, 20	0.32	0.5766