

Processes determining plant species diversity in restored tallgrass prairie

by

Andrew David Kaul

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Program of Study Committee:
Brian J. Wilsey, Major Professor
Brent Danielson
Crystal Lu
Lisa Schulte Moore
Haldre Rogers

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

A fundamental goal of restoration ecology is to restore biological diversity in degraded or fragmented environments. In practice, restorations often have lower diversity than reference communities, highlighting a need to identify theoretical and practical barriers to the restoration of native diversity. North American tallgrass prairie is an ideal system in which to study the restoration of plant diversity. Because remnant prairies are rare, prairie restoration projects are now very common, and the size and longevity of grassland plants makes them easy to measure and manipulate. Here, we present the first synthetic study to test the relative importance of soil characteristics, management actions, seed mix design, and site characteristics for predicting prairie restoration success. We found that across many restorations, invasion by exotic plants was the best predictor of outcomes, significantly reducing beta-, and site-level plant diversity. We also found that seeding more species reduced exotic species to increase diversity. Mowing also tended to increase diversity. We examined how plant diversity, richness, grass-forb ratios, and abundance of milkweeds (an important forb assemblage) differ between restored and remnant prairies and found that remnants are more diverse, less grassy, and have considerably higher milkweed abundances. Within restored prairies, the common milkweed was the most abundant species, and abundances of all milkweeds were correlated with higher soil pH, and variables associated with disturbance, including lower soil density and habitats with more edge. In a new prairie restoration in north-central Iowa, we tested whether the use of cover crops or additions of target prairie seeds were effective methods for increasing plant species diversity. We predicted that cover crop treatments seeded the fall before a diverse prairie mix would suppress weeds, and indirectly facilitate better recruitment of prairie species. Instead, we found that cover crops tended to reduce weeds slightly, but they also reduced or had no effect on prairie

establishment. In a seed addition study, we added diverse seed mixes in the 1st, 2nd, or 3rd year or annually after initial seeding. We found that these addition treatments did not increase total species richness or diversity, indicating low seed limitation on diversity. Together, the results of this work add to the mounting evidence that restorations tend to be compositionally different, and less diverse than remnants, that exotic species play an important role in community assembly during grassland restoration, and that assembly processes that occur early in a restoration are the most important and can have long-lasting effects.

CHAPTER 1. GENERAL INTRODUCTION

In the state of Iowa USA, around 80-85% of the landscape pre-settlement was tallgrass prairie. In only a century's time, between ~1830 -1930, nearly all of it was plowed and converted for agricultural uses (Shimek 1911, Smith 1998). The proportion of remnant prairie is now estimated to be less than 0.001 of original prairie extent in Iowa. Although larger prairie remnant areas persist in some parts of the central extent of the tallgrass prairie region (ex. Flint hills), across the historic range, most of this ecosystem was lost in an incredibly short period of time.

This loss of prairie in the early 20th century was a landmark phenomenon in the events that led to the development of modern restoration ecology. One of the oldest roots of formal ecological restoration can be traced back to the first efforts of Aldo Leopold to restore the native habitats of Wisconsin at the University of Wisconsin Madison. Under Leopold's supervision, one of the first prairie restorations, Curtis prairie (Named for John T. Curtis), was established at the University of Wisconsin Madison Arboretum in 1936, by professors Theodore Sperry, and William Longenecker. Not only was this one of the first ecological restorations of prairie, it was also one of the first restoration ecology experiments aimed at testing the efficacy of restoration methods. They examined how restored prairie composition differed if it was planted with seeds, transplanted seedlings, or transplanted sod (Blewett & Cottam 1984).

In the intervening decades, the practice of restoration of grasslands, and the science of restoration ecology have made impressive progress (Temperton et al. 2004, Wilsey 2018). A search for "prairie restoration" in Google Scholar now yields over 2,000 scientific publications. Focal topics of research have included investigations on disturbances, and how fire (Gibson and Hurlbert 1987, Collins 1992), grazing (Fuhlendorf & Engle 2001), mowing (Williams et al. 2007) and their interactions (Collins et al. 1998, Collins & Smith 2006) affect plant community

composition and species diversity in restored and remnant prairies. We have also come to appreciate how organisms at other trophic levels shape prairie vegetation, with much appreciation for bison (Knapp et al. 1999), and their role as keystone species in the prairie, as well as for mycorrhizae and other plant-microbe interactions (Harnett & Wilson 1999, Koziol & Bever 2017). There have been many studies on how restoration methods influence outcomes, including aspects of seed mix composition, seeding method and timing, and site preparation (Kiehl et al. 2010, Larson et al. 2011). Restoration of native ecosystems in the Anthropocene must also address how the presence of exotic species interact with each of the aforementioned topics, and so far, the ecology of exotics species has proven fascinating, as they are often are markedly different than related native species (Cully et al. 2003, Wilsey & Polley 2006).

Restoration provides a great opportunity to study basic community ecology, and restoration ecologists are increasingly connecting their work to theoretical ecology (Wainwright et al. 2018). In the tallgrass prairie system, restorations are now more abundant than remnants, and are predicted to become even more popular as land is continually purchased or set aside for conservation. On March 1, 2019, the United Nations designated the decade of 2021 to 2030, the “decade on ecosystem restoration”, indicating the global importance of- and interest in restoration ecology. Insofar as restorations fail to achieve their goals, we gain insight about the gaps in our theoretical understanding of community assembly (Bradshaw 1987). For these reasons, restoration ecology has grown in popularity in recent decades, and this trend is likely to continue.

Grasslands are a model study system for the study of restoration ecology, because they have convenient properties that make them amenable to study and experimentation (Wilsey 2020). Some of these properties include having a low canopy, (which makes whole organism

measurements of dominant vegetation feasible), high local and spatial diversity, strong networks between scientists and practitioners, and they are easier to experimentally manipulate compared to aquatic systems or terrestrial systems that are dominated by vegetation that changes at slower temporal scales, such as forests.

As mentioned above, tallgrass prairies in the US have an important place in the history of restoration ecology and have become some of the best-studied grasslands. Remnants are isolated, but there is much active restoration research and practice occurring throughout the range.

Tallgrass prairie is characterized by perennial plants, soils with high organic matter and a deep alpha horizon, and high annual productivity (Weaver 1954). While some large-scale prairie restoration incorporates a grazing disturbance regime with bison or cattle, most managed prairies areas and smaller plantings are un-grazed. Prairie restoration practice and science also benefit from having access to the most diverse commercially available seed for any system (White et al. 2018).

Globally, across many systems, restoration efforts tend to fail to achieve complete recovery to reference communities (Jones et al. 2018). In the tallgrass prairie system, remnant and restored prairies differ in their richness (Allison 2002), vegetation height and structure (Ammann and Nyberg 2005), alpha and beta diversity (Martin et al. 2005), and phylogenetic diversity (Barak et al. 2017). In general, planted prairies have lower richness and diversity than remnants (reviewed in Wilsey 2018). Grassland restoration outcomes have proven to be surprisingly unpredictable (Grman et al. 2013, Brudvig et al. 2017), underscoring the need for research on trends on restoration outcomes broadly, and specifically on what process produce such variability in outcomes and how they may be manipulated to improve restoration outcomes.

Dissertation Organization

The work presented here focuses on restoration of species diversity in prairies because diversity measures are of general interest across community and restoration ecology, and because species diversity is important for the many ecosystem functions and services offered by grasslands (Bengtsson et al. 2019). In grasslands, higher diversity has been linked to more stability of productivity (Tilman & Downing 1994, Tilman et al. 2006, Isbell et al. 2009), and increased soil carbon sequestration (Chen et al. 2018). Insect and plant diversity are typically positively correlated in North American prairie systems (Haddad et al. 2001) and important to practitioners, planted species are more likely to persist in higher diversity prairie restorations (Huang et al. 2013).

This dissertation addresses questions related to the establishment of high plant species diversity and the abundances of target forbs in restored prairies. Specifically, in chapters 2 and 3, I present results from a study of 98 prairies, including 93 restorations and 5 remnants sampled throughout Iowa. We sampled the plant community and soil on these prairies and acquired information from managers on how and when the restorations were established. We ask how soil characteristics, management actions, seed mix design, and site characteristics predict prairie restoration success. Of the 93 restorations, 46 were planted in roadsides, and the other 47 were located on other public and private land, so we collectively refer to them as “conservation” prairies. Along with the 5 remnants, we use these classifications to ask how milkweed abundances differ between prairie habitat types and what site characteristics promote milkweed establishment. In Chapters 4 and 5, I present the results of two experiments conducted on a new prairie restoration. In chapter 4, I ask if cover crop treatments seeded the fall before a prairie mix can reduce weeds and facilitate the establishment of a higher diversity of species from the seed mix. In chapter 5, I use seed additions to test whether there is evidence for seed-limitation,

microsite-limitation, or both during the first three years of establishment in a tallgrass prairie seeded with a high diversity mix. Finally, in Chapter 6, I summarize results from all chapters and offer directions for future research.

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CHAPTER 2. EXOTIC SPECIES DRIVE PATTERNS OF PLANT SPECIES DIVERSITY IN 93 RESTORED TALLGRASS PRAIRIES

Modified from a paper in revision for *Ecological Applications*

Andrew D. Kaul and Brian J. Wilsey

Iowa State University, Department of Ecology, Evolution, and Organismal Biology

Abstract

A primary goal of restoration ecology is to understand the factors that generate variability in species diversity and composition. Plant communities may assemble deterministically towards a common community type, or stochastically, based on weather conditions during establishment, soil legacy effects, or competition with exotic species. To test these alternative hypotheses, we sampled plant communities and soil at 93 randomly selected restored prairies distributed throughout Iowa, USA. Five remnant sites were sampled as a reference. We tested our hypotheses using multiple regressions and investigated the strength of direct and indirect effects on species diversity and richness using structural equation models. The prairie restorations were highly variable in their age, size, diversity, soil characteristics, and how they were managed post-seeding. The strongest predictor of plant species richness and diversity was the degree of invasion, as measured by the abundance of exotic species. Seed mixes that were species-rich had reduced exotic species abundance, which led indirectly to higher species richness of restorations. Sites with higher organic matter and a more linear shape had a direct positive effect on exotic abundance, which in turn, decreased diversity. We found little support for deterministic assembly, and diversity did not increase with the age of planting. Our results indicate that restored prairie communities tend to assemble into states of high or low diversity, driven by

invasion from exotic plant species. Management of exotic species is essential for maximizing species diversity in temperate grassland restorations.

Introduction

A fundamental question in restoration ecology is whether the full biodiversity represented in reference areas can be achieved in restored areas, given enough time (Bradshaw 1987).

Remnant prairies can have 15-20 species in areas $< 0.5 \text{ m}^2$ (Partel and Zobel 1999, Martin et al. 2005, Polley et al. 2005), and restoring this high diversity remains a challenge. The factors that are responsible for reproducing this high diversity in restorations remain understudied (Symstad and Jonas 2011).

Two theories make distinct predictions on how communities will assemble into diverse communities over time during restoration (reviewed by Temperton and Hobbs 2004). The deterministic model predicts that communities will assemble towards a predictable and consistent community composition and diversity over time as determined by local environmental conditions. Many restoration seed-mixes include later successional species at establishment to overcome dispersal limitation and bypass the early colonization stage by design. Under the deterministic model, the composition of the seed mix, time, and local environmental conditions would be the most important variables for predicting restoration outcomes, with diversity expected to increase over time as species recruit from the mix (Figure 1, Wilsey and Martin 2015). However, few studies have tested this assumption, and some find that diversity peaks after a few years and then declines over time as tall grasses dominate (Pimm 1991, Sluis 2002, Grman et al. 2013). The stochastic/alternate state model predicts that factors that are somewhat random set the trajectory of community development, which can lead to alternate compositions. For example, weather during initial years post-seeding can lead to unpredictable outcomes if

conditions are far from the climatic norm (Manning & Baer 2018, Groves & Brudvig 2019), and compositions that form during this time can have long-term impacts through priority effects. Soil legacy or priority effects can have strong influences on assembly, resulting in different species diversities and compositions even when seed-mixes and environmental conditions are similar (Temperton and Hobbs 2004, Martin and Wilsey 2012). If restorations differ between sites that were formerly annual crop fields vs. pastures, or that differed in their exotic species propagule pressure, then it would support the stochastic/alternate states model. Perennial exotic species abundance has been found to be strongly associated with reduced prairie species establishment and diversity in areas with high exotic abundance (Martin and Wilsey 2012, 2014). Here, we test these two models with a random sample of 93 grassland restorations.

A common goal of restorations is to restore high species diversity, or the richness and evenness of relative abundance across species. Species diversity can be partitioned into components of alpha, beta, and gamma, where alpha is a measure of average local diversity, beta diversity is the spatial turnover in species, and gamma is total diversity (Whittaker 1960, Anderson et al. 2011). Alpha diversity is a key integrative measure that has been linked to higher ecosystem functions in grasslands, including productivity, nitrogen mineralization, and litter decomposition (Hooper et al. 2005, Isbell et al. 2011). High alpha diversity is also positively associated with stability, especially the consistency of biomass production across years (Tilman et al. 2001, Tilman et al. 2006, Isbell et al. 2009), and resistance to extreme precipitation events (Isbell et al. 2015). Correlations between diversity and stability are highest when growth across species is asynchronous, meaning species respond in a differential fashion to environmental variability (Bai et al. 2004). Alpha diversity is also important to pollinators in grasslands, because animal-pollinated forbs are more abundant in more diverse sites (Haddad et

al. 2001). Beta diversity is less well studied than alpha, but has been found to be affected by species sorting along abiotic gradients, variation in assembly history (Questad and Foster 2008, Martin and Wilsey 2012), actions by animals such as urine deposition or grazing, and dispersal (Steinauer and Collins 1995, Loreau 2000, Chase 2003, Soininen et al. 2007, Martin and Wilsey 2015). Restoring beta diversity is extremely important for grassland restoration, as higher within-site beta diversity in restored tallgrass prairies has been linked to higher ecosystem multifunctionality (Grman et al. 2018).

Patterns of within-site beta diversity may change over time during ecological restoration, but this has seldom been tested. In early years, beta diversity is expected to be low, since propagules generally are homogeneously distributed throughout the site. Deterministic assembly predicts that over time, beta diversity should increase due to species sorting along environmental gradients (Faber and Markham 2011). The increase in beta diversity, in turn could affect site level diversity. Alternately, the presence of exotic species could influence patterns in beta diversity, and we predict that exotics could lower beta diversity over time if they disperse throughout and homogenize the site (Loreau 2000, Martin and Wilsey 2015). Based on these mechanisms, we predict that beta diversity will increase with site age when exotics are rare, as species sort along environmental gradients, but will decrease with age when exotics are abundant as they disperse and establish throughout the site.

An unresolved question in grassland restoration is how management actions and site characteristics affect restoration success (Grman et al. 2015, Norland et al. 2015 and Larson et al. 2018). Most studies that have compared grassland restorations have focused on a single mechanism, have been conducted at a single location, or have not randomly sampled both “successful and unsuccessful” restorations in a random manner. Contrasting variably successful

restorations can be used to compare the roles of seed-mix, management and site characteristics on restoration success (Ewel 1987, Suding 2011). Tallgrass prairie restorations allow for testing community assembly processes in the absence of dispersal limitation for late-successional species because restored prairies are an example of active restoration, where diverse seed-mixes are seeded on to bare-ground. Here, we test for relationships between measures of restoration success (plant diversity and similarity of restorations to remnants) and predictor variables in a large number of randomly selected tallgrass prairie restorations in the central USA. Restoration success was evaluated by estimating species diversity (both alpha and beta) across 93 restorations and in 5 remnant prairies. We tested the following alternate hypotheses, 1) restoration success measures (alpha and beta diversity, target species establishment, similarity to remnants) increase with age and are associated with environmental variables (Figure 1), or 2) these restoration success measures do not increase with age, but are more strongly predicted by variables associated with the stochastic/alternate state mode of community assembly (e.g. weather, past land use history, and the abundance of exotic species, Figure 1).

Methods

Study System and Site Selection

We sampled restorations within the tallgrass prairie region of the central USA. The tallgrass prairie is a temperate sub-humid grassland, characterized by long-lived perennial plants, mollisol soils with high organic matter, a deep alpha horizon, and high annual productivity. Remnant tallgrass prairies have very high alpha diversity and richness, with 15-20 species occurring in less than a half square meter (Polley et al. 2005, Martin et al. 2005). Restorations in this area benefit by having access to the most diverse commercially available seed for any

system (White et al. 2018). Precipitation in Iowa is highest in the south and southeast (mean 965 mm), and decreases substantially towards the north and northwest (mean 660 mm).

We randomly selected 93 restored conservation and roadside prairies across the state of Iowa for sampling. We sampled 44 during 2015 and 49 during 2016. A random sampling approach was used to obtain an unbiased sample of restorations. We randomly selected conservation restorations from Iowa Department of Natural Resource sites as well as county and university owned sites across Iowa. We randomly selected prairie restorations established by the DNR out of a GIS database of 1,314 mixed grass and forb native restorations. For roadside restorations, we randomly selected sites from among the hundreds of restorations around the state of Iowa by selecting a subset of Iowa counties (excluding the loess hills region in western Iowa), randomly selecting each location on the county map, and then sampling the nearest restoration to that point. Only sites that were seeded with both prairie grasses and forbs, and that were not over-seeded into intact vegetation were included. None of these sites were grazed by bison or cattle. No restorations were immediately adjacent to remnants. Sites varied widely in quality and management. The 93 sites used in our analysis were from 27 counties in Iowa that were located across the four largest Iowa landforms, excluding the Loess Hills and riparian flood plains (Figure 2). Of the 93 total sites, 46 were roadsides restorations, and 47 were “conservation” restorations, of which 43 were managed by the DNR, and 4 were managed by other public entities.

Five remnant prairies (never plowed, heavily grazed by cattle, or over-seeded) in the Iowa preserve system were also sampled to provide a benchmark for establishment success to be evaluated. Two remnant prairies (Cayler and Liska-Stanek) were sampled in July 2015, and three more, (Doolittle, Marietta, and Anderson/Dyas) were sampled in July 2016. These remnants

were selected to represent variability among prairies in the state ranging from clay to sandy soil types.

Sampling Design

A sampling approach was used that could quickly assess the composition and diversity of plants of a given site in order to be able to visit a greater number of sites overall. All sampling was done in July to ensure a full canopy had developed, while still capturing most of the early growing species. At each site, we used point-intercept sampling to estimate cover and the number of species. We dropped a 20 x 50 cm quadrat containing 4 pins at 10-25 random locations within each site to sample plants (Martin et al. 2014, Martin and Wilsey 2015). The number of sampling locations per site varied with area of the site (although species richness was rarified to standardize on number of locations, see below). At each restoration, the identity and number of species present was noted at each sample location, and the number of point contacts was recorded by species. All hits were recorded (i.e., multiple hits per pin were possible) to account for layering, and to ensure that number of hits was as closely related to biomass as possible (Wilsey et al. 2011, Xu et al. 2015). This provided information on percent cover of target species (species in the original seed-mix), and volunteer native and exotic species (species not native to North America).

Management and Site Characteristics

At each site, we collected three soil cores (2.5 cm diameter and to 10 cm deep) from which we measured soil bulk density (g/ml), pH, and organic matter. Soil bulk density provides an estimate of soil compaction, which is expected to be an important predictor of establishment success (Heneghan et al. 2008). Soil pH is a strong indicator of soil history, and includes the

effects of past management (e.g. the amount of N fertilization and liming) (Silvertown 1980). Organic matter is an indicator of soil fertility (correlated with nitrogen mineralization), water availability (due to its ability to adsorb water), and landscape position, with greater organic matter in lower elevation hydric sites, compared to xeric hilltop sites (Burke et al. 1989, Honeycutt et al. 1990, Barrett & Burke 2000).

After sampling the sites, we surveyed managers about the history and management of each restoration. We obtained information on the year the prairie was planted, species that were seeded, method used in seeding, when any burn or mowing management occurred, what is known about the land use prior to seeding with prairie species, and if any other management actions occurred. We were unable to collect information for all variables at all sites, because records were not always available or bulk seed mixes were used. We obtained lists of species in the seed mix for 66 sites, of which 48 indicated the relative abundances of seed sown for each species. We did not know the exact age of the restoration planting at 7 sites. Sites were categorized into 3 groups based on mowing frequency: none (n = 34 sites), establishment (n = 31 sites), or other (n = 28 sites). The 'none' category classified restorations that were never mowed after being seeded. The 'establishment' category described sites that were mowed at least one time in the first two years after the restoration date, and up to several times each year during the first two years. The 'other' category combined sites where the mowing treatment was unknown, or it was known, but received a mowing treatment different than the sites that were only mowed during establishment. Examples of these other treatments were haying or being mowed only after the first two years. We classified burn treatments into 4 categorical classes: none (n = 43), once (n = 16), multiple (n = 27), and unknown (n = 7). Using survey responses about the history of the site prior to restoration, we grouped sites into three classes: perennial (n = 50), crops (n = 33), or

unknown ($n = 10$). The ‘perennial’ category included sites in roadsides, pastures, or CRP, which were previously dominated by perennial plant species. The ‘crops’ category includes all previous land use in row-crops, which would likely have an annual seed bank, but not an extensive perennial seed bank. To examine the effect of the seeding method, we grouped treatments into four categories: broadcasted ($n = 46$), drilled ($n = 31$), combination ($n = 6$), or unknown ($n=10$). The “broadcast” treatment included hand broadcasting, mechanized dry broadcast seeders, and hydro-seeders. The “combination” treatment was for sites planted with both a seed drill, and some form of broadcast seeding.

We acquired climate and establishment weather data from the United States NOAA National Climatic Data Center (NCDC) accessed February 2020. Climate norms (30-year averages from 1981 to 2010) for mean annual temperature and precipitation were downloaded for the weather station nearest to each site. For each site, establishment weather corresponded to mean temperature and precipitation over the first 2 years after seeding, or the first year for one-year old restorations, at the nearest weather station where data was available.

Data Processing

Estimates of total species diversity (all species, including seeded species as well as all volunteers) included richness, Shannon’s diversity, and each of these partitioned into alpha and beta. Richness (total number of species) was standardized across sites by rarefaction to 10 quadrats per site with EcoSim software. Because 10 quadrats was the minimum sampling effort used among all sites, we can rarefy richness in sites with a greater sampling effort (25 quadrats max; average 15.3) to produce comparable measures of richness across restorations. Sites that were only sampled with 10 quadrats, thus have the same observed and rarefied species richness. To measure the diversity of the plant community of each site, we used the relative abundances of

each species to calculate Shannon's diversity index, $H' = -\sum_{i=1}^R p_i \ln p_i$. We used the form $e^{H'}$ as a measure of the effective number of species in the community (number of species if all species were equally abundant). Shannon's index was not rarified because it is independent of sampling effort above very small values of N (Lande 1996). Beta diversity was estimated within sites using the multiplicative model, where $\beta = \gamma / \text{mean } \alpha$. Based on the typology of Anderson et al. (2011), we are interested in non-directional variation in beta diversity within sites, and we are specifically interested in comparing variation in beta diversity among a priori groups of invaded vs. un-invaded grasslands. To test our prediction that beta diversity increases with site age when exotics are rare, but decreases when they are abundant, we classified sites as either being "invaded" with above average (> 50% of biomass being exotic species, Table 1), or "un-invaded" with below average (< 50%, Table 1) exotic species biomass.

We used a perimeter/area ratio to measure site linearity, where a line has a ratio of 2 and a square has a ratio of 1. We calculated linearity using a measure of fractal dimension, with the

equation: Linearity = $\frac{2 \ln(\frac{\text{perimeter}}{4})}{\ln(\text{area})}$ (Olsen et al. 1993, Frohn 1998). Thus, more linear habitats, with a higher ratio of perimeter to area and more edge effects have a higher ratio (closer to 2).

We calculated the number of hits per pin from exotic species at each site and used this as a measure of invasion. Thus, higher exotic abundance refers to proportional abundance rather than absolute abundance. Proportion exotic hits and total exotic hits were highly correlated ($r = 0.93$) and gave similar qualitative results (Appendix A: Fig. S1 vs. Figure 3). We present the

proportion of exotic hits rather than total exotic hits because it is standardized for variation in productivity among sites.

Plant community data were used to calculate the following restoration success variables: species richness and Shannon's diversity, the ratio of these measures compared to remnants, and compositional relatedness between seed mixes and restorations (Bray-Curtis similarity). We calculated two similarity measures between the seed mix and the sampled community, one using all sampled species, and one including only seeded species. We calculated a floristic quality index as $FQI = \bar{c}\sqrt{s}$, where s is total richness and c is a coefficient of conservatism for each species for the state of Iowa, which is designated by local botanists (Wilhelm et al. 1999). Coefficient of conservatism is an expert derived estimate of how restricted species might be to "high quality" remnant areas.

Statistical Analyses

All measures of species diversity or richness include all plant species present. We did not analyze diversity or richness of seeded species alone, because seeded and non-seed species are inter-correlated and are not statistically independent.

Predictor variables were regressed against response variables with multiple regression approaches, and a smaller set of significant variables were then included as covariates in models with categorical variables (e.g. management treatments: mowing, burning). To test the relative importance of continuous predictor variables, including site characteristics (linearity, organic matter, and pH), climatic normals, weather during establishment (mean annual precipitation and temperature during the two years following seeding), abundance of exotic species, and site age, we used general linear models with stepwise selection and Mallows $C(p)$ statistic as the selection

criterion (Proc GLM Select in SAS 9.4) and evaluated significance of each variable with type III sums of squares. To prevent problems of autocorrelation among variables, soil bulk density and log (area) were not included because they were correlated with soil organic matter ($r=-0.61$), and linearity ($r=-0.60$), respectively. The perimeter/area (linearity) measure was used instead of area because it has mechanistic implications and area is incorporated into its formula.

We tested the effect of management variables (seed method, burn treatment, mow treatment), site type (roadside vs. conservation planting), and previous land use (legacy) as fixed effects. We used *a priori* contrasts to test hypotheses about the effects of categorical management variables on plant species richness and diversity. We tested whether diversity was higher with establishment mowing versus no mowing treatment, since mowing should suppress annual weeds that may establish before the target species. Contrasts compared cropped vs. perennial legacy, burned vs. unburned prairies, and restorations burned multiple times vs. just once.

We tested our prediction that beta diversity will increase with site age using an ANCOVA, which compared slopes of beta diversity – age regressions between invaded and non-invaded restorations using Proc GLM in SAS 9.4. A significant interaction between the effect of age and invaded status on beta diversity or beta richness would support the hypothesis that beta is developing differently over time in invaded vs. less invaded sites. Beta diversity was also regressed against exotic species relative abundance and tested with polynomial regression, testing for linear and quadratic effects.

We used structural equation models to test hypotheses about direct and indirect effects of measured variables using Proc CALIS in SAS 9.4, using a confirmatory approach. We tested whether higher seed-mix richness and diversity resulted in more diverse restorations through

direct effects or indirectly through its effects on exotics using structural equation models. Specifically, we tested the predictions that species diversity measures increased directly with the diversity of the seed-mix, or indirectly through seed-mix diversity effects on exotic species. We included the ratio of graminoids to forbs in these models, because the effect of dominant grasses on the degree of invasion or recruitment of species from the mix may be important. Graminoid-forb ratio was not significantly correlated with seed mix richness ($r = -0.18$) nor diversity ($r = 0.15$). Preliminary analyses suggested that exotic species were important in predicting restoration success, so we developed structural equation models to test for direct effects of age, linearity, soil organic matter on diversity vs. their indirect effects on diversity through their effects on exotic species abundance. We developed a prediction *a priori* that edge effects and higher soil fertility would favor exotic species, and this in turn, would indirectly influence plant diversity and richness. T scores were used to evaluate significance, and were calculated as $\hat{\beta} / \hat{\sigma}$ where beta is an estimated path coefficient, and sigma was the standard error associated with that estimate. All models presented are saturated and have perfect fit by definition (Grace 2006).

Results

We found variation in management practices and site characteristics for all of the predictor variables. Variation in predictor variables is a requirement for meaningful regression models. Age of the restorations averaged 10 years and ranged from 1 to 26 years old, capturing the full age range of most tallgrass prairie restorations (Table 1). The number of species in the seed-mixes averaged 38, with a wide range of 10 - 80 species. Sites averaged 5.4 ha (13.7 acres), and ranged from <1 – 60 ha. We sampled restorations with extremely low diversity (5% of remnant levels), and more diverse sites, which approached and in two cases exceeded the

average diversity of the five remnants we sampled. On average, restorations had 39% of the diversity and 49% of the richness of remnants (Table 1). In restorations, exotic species abundance averaged 50%, with a range from 0 – 98%. The restorations we sampled also displayed high variation of seed-mix graminoid: forb ratios (Table 1). We were also able to sample sites on a wide range of soils over several Iowan landforms, including wetter and drier sites (Figure 2).

Predictors of Species Diversity in Restorations

When evaluating environmental predictors of restoration success, exotic species abundance was, by far, the strongest predictor of species richness and diversity across restorations (Table 2). Exotic species abundance was strongly negatively correlated with the diversity of restorations (Figure 3). Perimeter/area (linearity) was also a significant predictor of species diversity and richness, with higher diversity in more linear habitats after accounting for other variables (Table 2). Other variables tested were non-significant. Age of restoration was not significantly related to the diversity of the restoration (i.e., their diversities did not increase over time, $p > 0.15$).

Among the management variables, mowing was the strongest predictor of diversity and richness. Richness was significantly higher in sites mowed during establishment ($p=0.011$ Table 3, Figure 4). Diversity and richness were both higher in roadsides than conservation areas after accounting for other variables in the model. Burn treatment, prior land use, and seeding method were not significant (Table 3).

Environmental predictors affected diversity more strongly through their indirect effects on exotic species abundance. Our SEM indicated that the site perimeter/area (linearity) and soil organic matter had significant effects on diversity by reducing exotic abundance (Figure 5;

Appendix A: Table S1). Linear and more fertile habitats had higher proportional exotic abundance, which in turn, suppressed plant species diversity (Figure 5). Linearity (perimeter/area) was also negatively related to richness and diversity through a direct effect, with fewer species present in more linear habitats with more edge.

Beta Diversity

The abundance of exotic species also significantly affected beta diversity. Neither beta richness nor diversity changed significantly over time in invaded nor less invaded restorations. Invaded sites (over 50% exotic abundance) had higher beta richness, but lower beta diversity than our less invaded sites (Figure 7). Exotic species abundance had a non-linear relationship with beta diversity, with drops in beta diversity only occurring at high levels of exotic abundance.

Effects of Seed-Mixes on Diversity and Composition of Restorations

Seed-mix variables had significant effects on the diversity of the restorations, mostly due to their indirect effects on exotic species abundance (Figure 6; Appendix A: Table S2). Species richness of seed-mixes did not directly affect the species richness of restorations, as predicted by the deterministic model (Figure 6). Seed-mix richness was negatively correlated with exotic species abundance ($r = -0.406$), which in turn was negatively correlated with restoration richness ($r = -0.622$). Thus, the indirect effect of seed richness was positively correlated with richness of the restoration ($r = -0.406 \times -0.622 = 0.2525$).

Seed mix diversity had a small direct effect on diversity, but this effect was cancelled out by an indirect effect on exotic species abundance. The diversity of the restorations was directly correlated with the diversity of the species in the seed-mix (Figure 6, $r = +0.344$), but there was

also a negative indirect effect of diversity through a positive association with exotic species (Figure 6). Somewhat surprisingly, the diversity of the seed-mix was positively associated with exotic species abundance ($r = +0.345$), which was negatively associated with diversity of the restorations ($r = -0.720$). Thus, the overall effect of the diversity of the seed-mix was neutral ($r = +0.344 - [+0.345 \times -0.720] = 0.096$), resulting in no significant effect of the seed mix diversity on the diversity of the restorations.

Predictors of Species Composition in Restorations

Exotic species abundance was also a significant predictor of species composition. Target species establishment (similarity between seed mixes and restorations) was significantly lower in restorations with higher exotic species abundance. Precipitation during the establishment years was the next best predictor of our mix establishment measures, such that restorations with higher precipitation had higher similarity measures (better establishment) (Tables 2 & 3). Age of restoration was unrelated to its compositional similarity to its seed mix or to its floristic quality index ($p > 0.15$, Appendix A: Fig. S2).

Other variables depended on whether only seeded species or all species were used in the analysis. Similarity between seed mixes and restorations using all species increased across sites with pH and with precipitation during establishment years (Table 2). When considering only the seeded species, similarity was lower in drier establishment years (Table 2).

Discussion

The 93 prairie restorations we sampled were highly variable in their site characteristics, management, and similarity to a remnant community. Two sites out of 93 achieved the high diversity of remnants, but most were much less diverse. We found that the abundance of exotic

species was the most important predictor of species diversity and richness. Seed mix variables affected diversity primarily through their indirect effects on exotic abundance. Exotic species were more abundant in sites with higher soil organic matter and more linear site shape, leading to reduced diversity and richness. Similarly, exotic species abundance was the only significant predictor of species composition similarity between the mix and resulting community. Mowing was the only management variable that was significant, with sites that were mowed during establishment having higher species richness than un-mowed sites. Taken together, these results were most consistent with a stochastic model of community assembly (Figure 1). Restoration outcomes were dependent on early management actions and competition with exotic species, which were heavily influenced by variables associated with legacy effects and landscape position. Our results were not consistent with the deterministic model because diversity was not significantly related to age, soil, or climatic averages.

Effects of Exotic Species

Consistent with the stochastic/alternate state model for community assembly, our results indicate that abundance of exotic species is the best predictor of prairie establishment success. Sites can vary in their exotic species abundance due to their past history or management. Exotic species can establish quickly and can suppress native species establishment from seed (Yurkonis et al. 2010, Dickson et al. 2012, Wilsey et al. 2015).

In ecological restoration, exotic species are often assumed to arrive early following disturbance and subsequently drop out of the community due to exclusion from competitively dominant native species (Camill et al. 2004). This is true of annual exotics that disperse readily and grow quickly. However, the three exotic species we sampled most frequently were the perennial grasses *Bromus inermis*, *Poa pratensis*, and *Phalaris arundinacea*. Other common

exotics were perennial legumes, including *Lotus corniculatus*, *Trifolium pratense*, and *Securigera varia*. The most common exotic forbs were *Melilotus* spp, *Daucus carota*, *Pastinaca sativa* (biennials) and *Cirsium arvense*, a perennial. Therefore, in these perennial grasslands, the dominant exotics are not annuals, and we found that they can maintain their abundance for many years.

Multiple drivers can cause ecosystems to lose native species and gain exotic species, but when these processes occur simultaneously, the net change in richness, diversity, and function may be neutral, positive, or negative, depending on the abundance of exotics and how their traits differ from species they replaced (Wardle et al. 2011). Most exotic species do not become invasive. For these reasons, total richness and diversity in these restorations could have increased or decreased with higher proportional abundance of exotic species, but we saw a clear negative relationship indicating exotics are driving diversity declines.

Exotic species dominance could lead to low diversity communities because exotics are ecologically different than native species in several important ways. Most exotic species in grasslands were intentionally introduced by humans (Mack and Lonsdale 2001), and they tend to have different trait values than native species. Exotics often have higher germination rates and emerge from soil earlier than comparable native species (Wilsey et al. 2015), have leaf trait values such as specific leaf area, leaf dry matter and N content that are more on the acquisition end of the leaf economics spectrum (van Kleunen et al. 2010), and can have stronger priority effects than native species (Dickson et al. 2012, Wilsey et al. 2015). Across the tallgrass prairie region, exotic dominated novel grasslands have lower diversities and altered phenologies compared to native dominated grasslands (Wolkovich & Cleland 2011, Martin et al. 2014, Wilsey et al. 2018). Exotics have a different phenology on average, growing earlier in the spring

before the natives start to green up, and then later in the fall after natives have senesced (Wolkovich & Cleland 2011, Wainwright et al. 2012, Wilsey et al. 2018), and this difference may be an important aspect of exotic invasion into the northern tallgrass prairie, since invasibility of prairie has been shown to be dependent on the phenological traits of the existing community (Losure et al. 2007). Once exotic species become established, they are persistent (Kulmatiski 2006, Norland et al. 2015) and generally resist colonization from native species (Kulmatiski 2006, Dickson et al. 2012, Martin and Wilsey 2014).

We found evidence that soil organic matter and edge effects indirectly reduce diversity through positive effects on the degree of invasion. Organic matter is related to nitrogen availability, so its enhanced effect on exotic species may result from greater soil N, which has been shown to decrease plant diversity in fertilization studies (Suding et al. 2005). In an experimental context, soil N reduction has been shown to promote greater native species establishment in restorations (Blumenthal et al. 2003). Organic matter is also highly correlated with topographical landscape position in grasslands, which results in differences in both nutrient and water availability (Burke et al. 1989). In restored grasslands, exotics species may establish throughout the restoration, from propagules in the seed bank, or they may invade from edges, or both. Exotic species have been shown to establish from the edge of remnant grasslands, with exotic abundance positively related to edge proximity (Cilliers et al. 2008). While propagule pressure from the seed bank, which was not measured here, is likely also a major driver exotic abundance in these prairies, our results indicate that pressure from exotic species occupying the matrix surrounding prairie restorations may have been a significant driver of greater exotic invasion into these restored grasslands.

Effect of Mowing on Diversity

Mowing during the first growing season after a prairie has been seeded (establishment mowing) has been suggested to increase establishment of target species (Kurtz 1994), and we found that it significantly increased the species richness of restorations. Establishment mowing in the first 1-2 years post seeding, where the cut material is left in the field, has become a commonly used tool in restorations (Rowe 2010). Mowing can influence vegetation by creating above or belowground gaps for new seedling establishment by increasing light availability (Peltzer and Wilson 2001), and can reduce annual species and prevent them from setting seed. Mowing treatments are sometimes applied after establishment, to continue suppressing exotic species that may grow earlier in the season than most natives. Future studies should investigate establishment-mowing effects in greater detail.

Lack of a Relationship between Site Age and Diversity

Under the deterministic assembly model, we would expect restored prairies to accumulate diversity over time, recruiting more species from the initial mix due to inter-annual variation in biotic and abiotic conditions and differences in species dormancy. However, we found no evidence that species diversity was increasing with age of restorations. Previous work has shown that after initial recruitment and establishment, restored prairies often decrease or are stable in their richness and diversity (McLachlan & Knispel 2005, reviewed in Wilsey 2018). Decreases over time in other studies have been widely attributed to increasing C₄ grass dominance as discussed above (Camill et al. 2004). We did not detect a significant change over time, even after taking into account variation in sown richness and the degree of invasion.

Beta Diversity

We did not find evidence for our predictions that beta diversity would increase over time in un-invaded sites, and decrease over time in invaded sites. The high level of exotic dominance in young sites indicates that exotic species might establish and spread more quickly than previously thought in restorations. We did find evidence that within-site beta diversity was higher in native-dominated than exotic-dominated restorations, consistent with the sampling of sites from Minnesota to Texas by Martin and Wilsey (2015). This may be driven in our sites by the abundance of *Bromus inermis*, which has been shown to reduce diversity in northern prairies by homogenizing plant communities within and across grasslands (Stotz et al. 2019). These results are also inconsistent with a deterministic model of assembly, wherein beta diversity is predicted to increase over-time through environmental sorting.

Effects of Seed Mixes

We found support that the seed-mix design alters restoration outcomes (Sheley and Half 2006, Piper et al. 2007). Grman et al. (2015) found evidence that species sown at higher densities are more likely to establish (see also Larson et al. 2018). However, in contrast to these earlier studies, we found that the number of species in the seed-mix led indirectly to more diverse restorations by suppressing exotic species. In experiments that vary species richness and evenness, diverse plots tend to have fewer invaders than plots that are less diverse (Tilman et al. 2006, Losure et al. 2007, Isbell et al. 2011). Our results add to this body of literature and indicate that seed mixes with a greater number of species have important effects on restorations by suppressing exotic species establishment.

The graminoid to forb ratio in the seed-mix may also influence restored plant diversity. Previous studies found that increasing grass seed density reduces forb cover and richness

(Dickson & Busby 2009), and conversely that increasing forb density increases forb richness, but not total cover (Jaksetic et al. 2018). Our results indicate that managers are generally sowing mixes with high graminoid: forb ratios, and the resulting restorations are more graminoid dominated than remnants (mean of 68% graminoid vs. 52% in remnants, Table 1). However, we did not find that the graminoid: forb ratio significantly predicted establishment after accounting for seeded richness or diversity. Recommendations on optimal grass: forb ratios vary widely, but based on remnant communities, we recommend using a 1:1 ratio of forb to graminoid seeds. Further research is needed on graminoid: forb ratios, invasion resistance, and eventual species diversity of restorations.

Much research has been conducted on how to restore the high levels of plant species and phylogenetic diversity in native grassland systems (Polley et al. 2005, Martin et al. 2005, Barak et al. 2017), with results suggesting that some plant diversity is achievable in restorations, but not to the level of remnant (reference) sites. Our results were consistent with this general finding. Globally, restoration efforts fail to achieve complete recovery to reference communities (Jones et al. 2018). In general, restored grasslands have lower richness and diversity than remnants (reviewed in Wilsey 2018).

Non-Significant Predictors of Restoration Success

Some measures that have been proposed to influence plant diversity in restorations, including burn treatment and seeding method, did not significantly predict diversity. Fire is a natural disturbance in tallgrass prairie and is also predicted to influence species diversity under the deterministic assembly model, yet we did not find evidence that fire drives patterns in restoration success. The method of seeding may also impact establishment outcomes, and some studies show that broadcasting results in more diverse restorations (Applestein et al. 2018,

Norland et al. 2015). Our results are consistent with several experiments that found no significant difference in diversity between broadcast and drilled restored prairies (Newman & Redente 2001, Larson et al. 2011, Yurkonis et al. 2010).

Finally, some potentially informative establishment variables that we were unable to account for may be important and deserve further study. Evidence from a seeding experiment using a 128 species mix, indicates that after the first 2 years of assembly, restorations with higher seeding rates (56, and 78.5kg/ha) had higher richness, seeded diversity, and evenness compared to a low rate of seeding (11.2kg/ha), however it is not clear if these effects are persistent (Goldblum et al. 2013). We were also unable to account for establishment timing due to a lack of variation, which may be an important predictor of established diversity, as there is some evidence that spring seedings tend to have higher diversity than fall seedings (Martin and Wilsey 2012).

Conclusions

Prairie restorations were highly variable in their management and outcomes, with most being less diverse and species rich, and with a higher degree of invasion, than remnants. The abundance of exotic species (relative to natives) was the best predictor of diversity. Thus, the top priority for prairie management should be control of invasive exotics. Targeting restorations for sites with low exotic abundance should be an effective way to establish diverse restorations. Previously, a negative relationship between plant diversity and the abundance of exotics has been documented at the plot scale within a restored grassland (Middleton et al. 2010), but here we demonstrate that this trend is characteristic of restored prairies generally. Mowing during the first two years of establishment, and locating restorations in less fertile soils in less linear habitats are all effective strategies to promote more diverse restorations.

Taken together, our results indicate that community assembly in restorations is most consistent with a stochastic model, where the diversity of restorations does not converge over time but rather is highly variable and contingent on factors influencing the abundance of exotic species and weather conditions during establishment.

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Tables and Figures

Table 1. Summary of site characteristics in 93 tallgrass prairie restorations

	N	Mean	Std. Dev	Range
Continuous Predictor variables				
Age (years)	86	10	6	1 – 26
Seeded Richness	66	37.8	19.8	7 – 81
Seeded Diversity (e^H)	48	11.6	4.4	4.12 – 23.16
Seeded Grass (% mass PLS)	48	76.3	10.3	57.23 – 99.05
Site Size (Ha)	93	5.4	10.8	0.14 – 61.04
Linearity	93	1.2	0.2	1 – 1.55
Soil pH	93	7.4	0.6	6.06 – 8.17
Soil Bulk Density (g/cm^3)	93	1.3	0.2	0.92 – 1.93
Soil Organic Matter (%)	93	9.5	2.8	3.61 – 17.40
Annual Precip. Normals (mm)	93	902.8	57.5	729.2 – 974.1
Annual Temp Normals (C)	93	8.8	0.9	7.17 – 10.72
Establishment Precip. (mm)	82	885.2	146.2	609.2 – 1241.3
Establishment Temp (C)	82	9.0	1.38	5.94 – 12.25
Proportion Exotic	93	0.50	0.29	0.00 – 0.98
Proportion Graminoid	93	0.68	0.18	0.13 – 0.99
Response variables				
Rarefied Richness	93	20.1	6.85	4 – 35.98
Diversity (e^H)	93	9.12	4.85	1.24 – 23.64
Bray-Curtis Similarity (all)	48	0.18	0.17	0 – 0.68
Bray-Curtis Similarity (seeded)	48	0.28	0.20	0 – 0.73
Prop. diversity restored	93	0.39	0.21	0.05 – 1.01
Prop. rarefied richness restored	93	0.49	0.16	0.09 – 0.85
Prop. native restored	93	0.55	0.33	0.02 – 1.13
Prop. graminoid restored	93	1.34	0.35	0.25 – 1.9

Table 2. Multiple regression results from analyses of 93 tallgrass prairie restorations. Effects of environmental variables (soil OM, pH), weather during establishment (temperature and precipitation during the first two years of establishment), and site characteristics (perimeter/area and age of restoration) on species diversity, species richness (rarefied), and Bray-Curtis similarity, which measures how much overlap in richness and abundance of species the sampled community shares with the seed mix. Bray-Curtis used all species, or only species in seed mixes (Seeded Only).

	Diversity			Rarefied Richness			Bray-Curtis			Bray-Curtis Seeded Only		
	<i>P</i>	<i>F</i>	DF	<i>P</i>	<i>F</i>	DF	<i>P</i>	<i>F</i>	DF	<i>P</i>	<i>F</i>	DF
Proportion Exotic	<.0001	47.58	1	<.0001	44.10	1	<.0001	68.16	1	<.0001	31.66	1
Perimeter/Area	0.0044	8.61	1	0.0042	8.70	1	0.0416	4.44	1	>.15		
Age	>.15			>.15			>.15			>.15		
Precipitation (Establishment)	>.15			>.15			0.0131	6.74	1	0.0104	7.21	1
Temperature (Establishment)	>.15			>.15			>.15			>.15		
Precipitation (Normals)	>.15			>.15			>.15			>.15		
Temperature (Normals)	>.15			>.15			>.15			>.15		
Soil Organic Matter	>.15			>.15			>.15			>.15		
Soil pH	>.15			>.15			>.15			0.0427	4.38	1
Total			81			81			43			43
	N=82	R ² =0.435		N=82	R ² =0.419		N=44	R ² =0.733		N=44	R ² =0.563	

Table 3. ANCOVA results from a sampling of 93 tallgrass prairie restorations. Effects of management (mowing, burning, seeding), site characteristics (roadside vs. conservation area, linearity, soil variables), and prior land use history (cropped vs. perennial) on plant species diversity and richness. Bray-Curtis similarity measures how similar the sampled community was from the seed-mix. Bray-Curtis seeded is a measure of similarity between the seed mix and the seeded species in the community. Degrees of freedom were lower for Bray-Curtis measures because many sites used a bulk seed mix or did not know the exact species composition of seed mixes.

	<u>Diversity</u>		<u>Rarefied Richness</u>			<u>Bray-Curtis</u>		<u>Bray-Curtis Seeded</u>			<u>Direction</u>
	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>DF</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>DF</i>	
Deterministic predictors											
Mowing	0.273	1.32	0.033	3.56	2	0.794	0.23	0.900	0.11	2	Mowing +
Seeding	0.858	0.25	0.674	0.51	3	0.487	0.83	0.661	0.54	3	
Burning	0.896	0.20	0.507	0.78	3	0.820	0.20	0.462	0.79	2	
Linearity	0.741	0.11	0.779	0.08	1	0.683	0.17				
Soil pH								0.817	0.05	1	
Stochastic predictors											
Proportion Exotic	<.001	44.04	<.001	41.52	1	0.001	12.85	0.067	3.59	1	Negative
Site Type	0.076	3.24	0.029	4.95	1	0.1931	1.77	0.052	4.07	1	Roadsides +
Prior Land Use	0.111	2.26	0.529	0.64	2						
Temp. during establishment										1	
Precip. during establishment						0.043	4.42	0.034	4.93		Positive
Error					79					32	
Contrasts:											
Mow: none vs. establishment	0.118	2.50	0.011	6.87	1	0.809	0.06	0.788	0.07	1	Establishment +
Prior: cropped vs. perennial	0.959	0.00	0.891	0.02	1						
Burned vs. unburned	0.496	0.47	0.172	1.90	1	0.623	0.25	0.580	0.31	1	
Burned multiple vs. once	0.831	0.05	0.494	0.47	1	0.609	0.27	0.390	0.76	1	
	N=93		N=93			N=44		N=44			
	R ² =0.438		R ² =0.477			R ² =0.776		R ² =0.673			

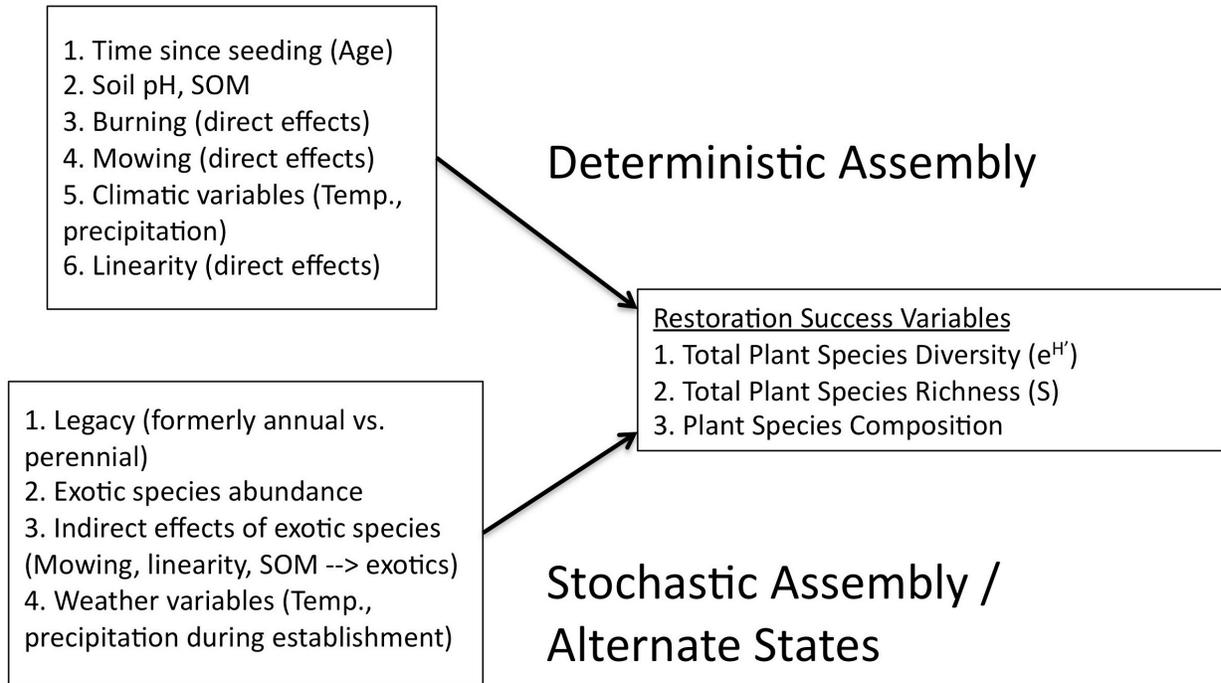


Figure 1. Conceptual relationships between community assembly models and predictors of restoration outcomes in prairie grasslands.

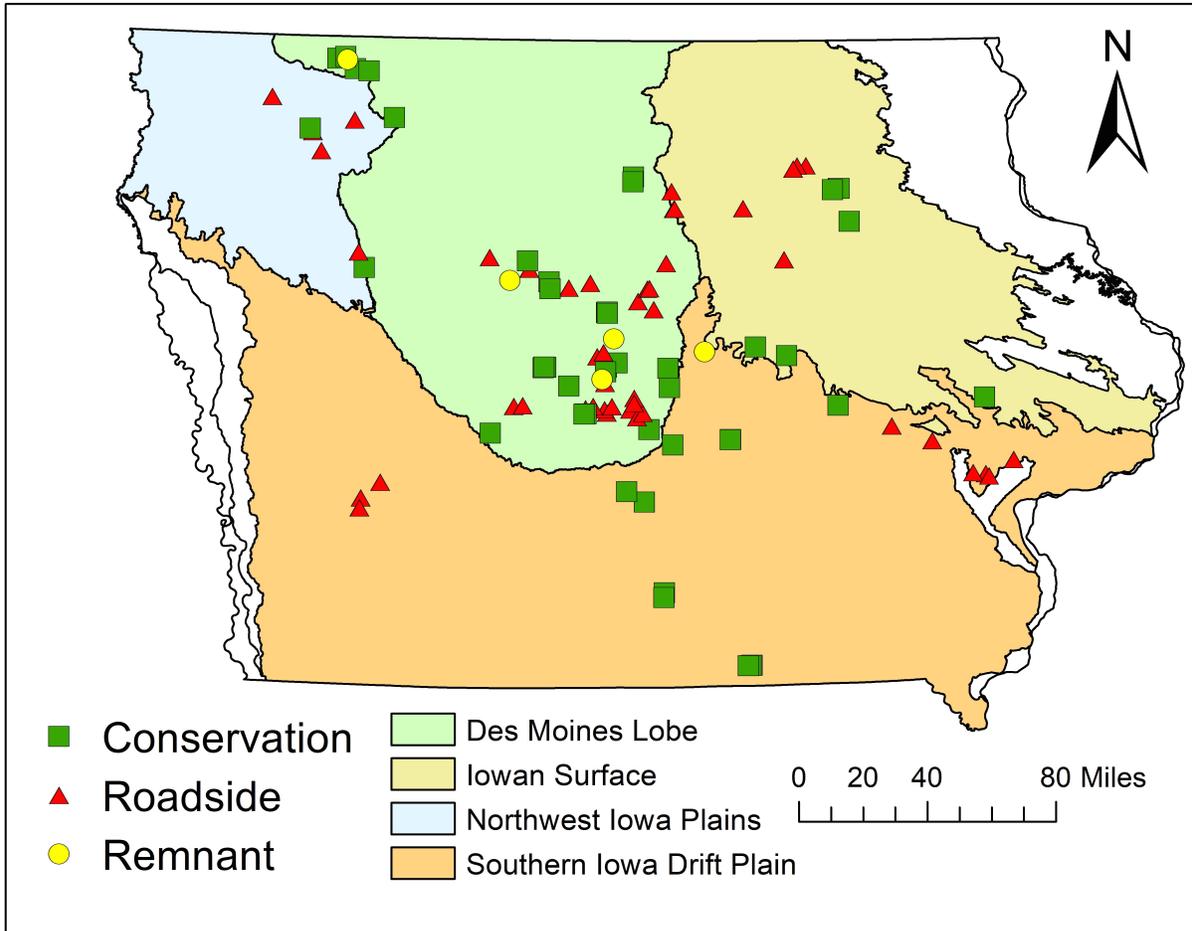


Figure 2. Map of sampled locations.

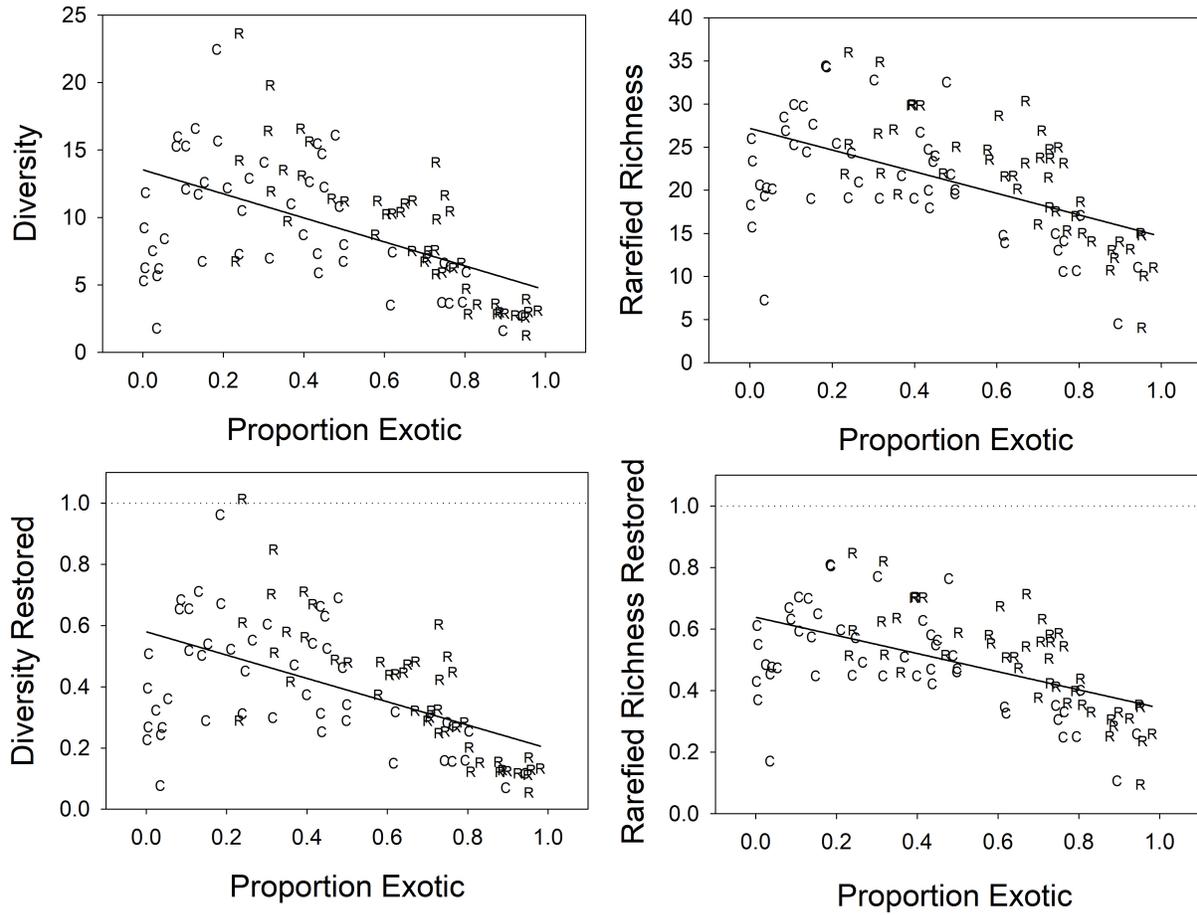


Figure 3. Relationships between diversity (left) and richness (right) and proportion of exotic biomass (R = roadsides, C = conservation areas). Diversity declined significantly as the amount of invasion increased. The bottom panels compared diversity and richness of restorations to the average of the 5 remnants, with a value of 1 equaling equivalence.

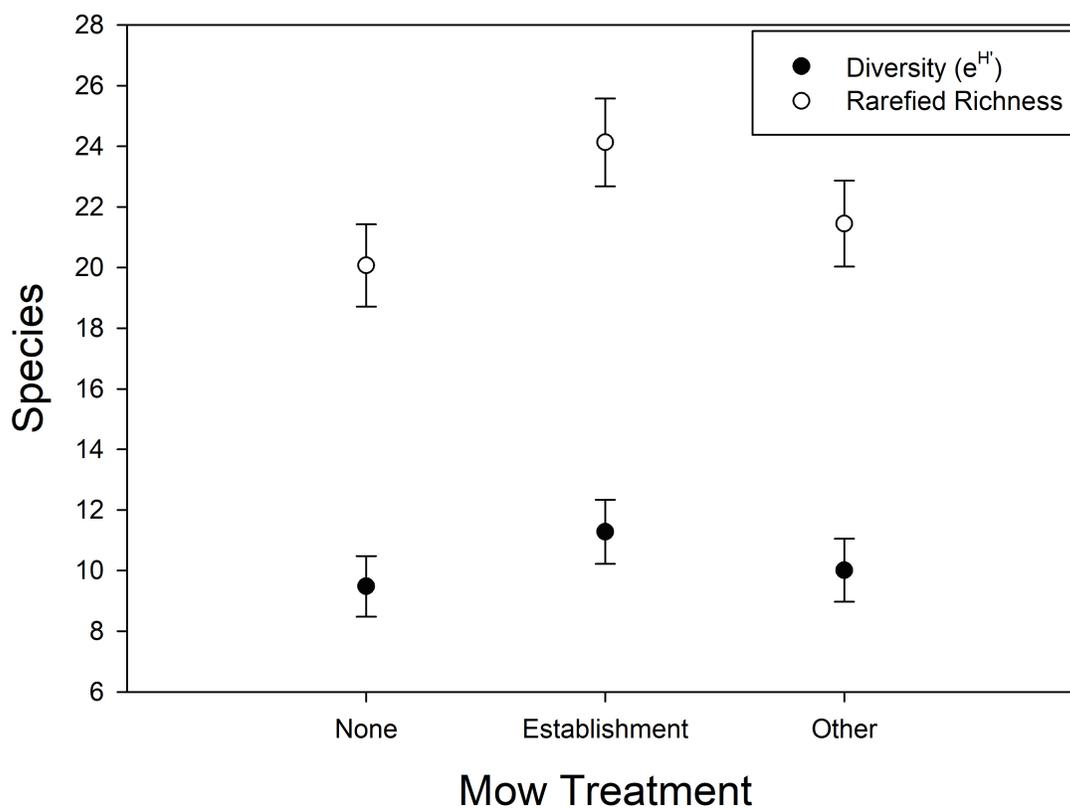


Figure 4. Species diversity and richness of restorations with no mowing, mowing during establishment (first 2 years), or “other” (mostly unknown mowing frequency or mowing after establishment years). Diversity was significantly higher with mowing during establishment than the other two treatments.

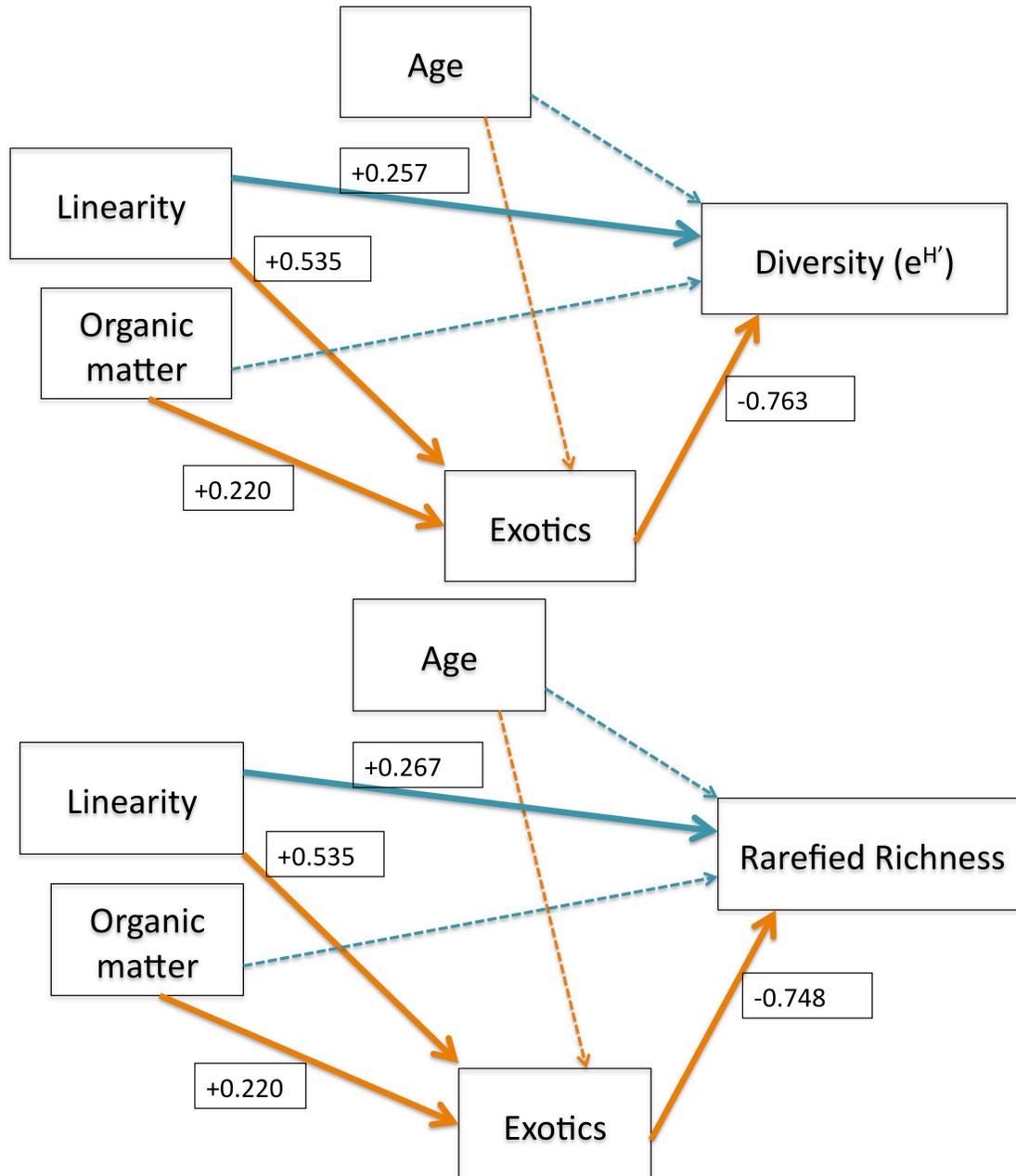


Figure 5. Structural equation model exploring relationships between predictors of plant diversity (top) and richness (bottom) of prairie restorations. Age is the age of restoration, linearity is a measure of linearity, and organic matter is a measure of soil fertility. Significant pathways are marked with a solid line, and non-significant ones are shown with dotted lines. Effect estimates are standardized. Interactions supporting deterministic assembly are shown in blue, and stochastic assembly pathways are shown in orange.

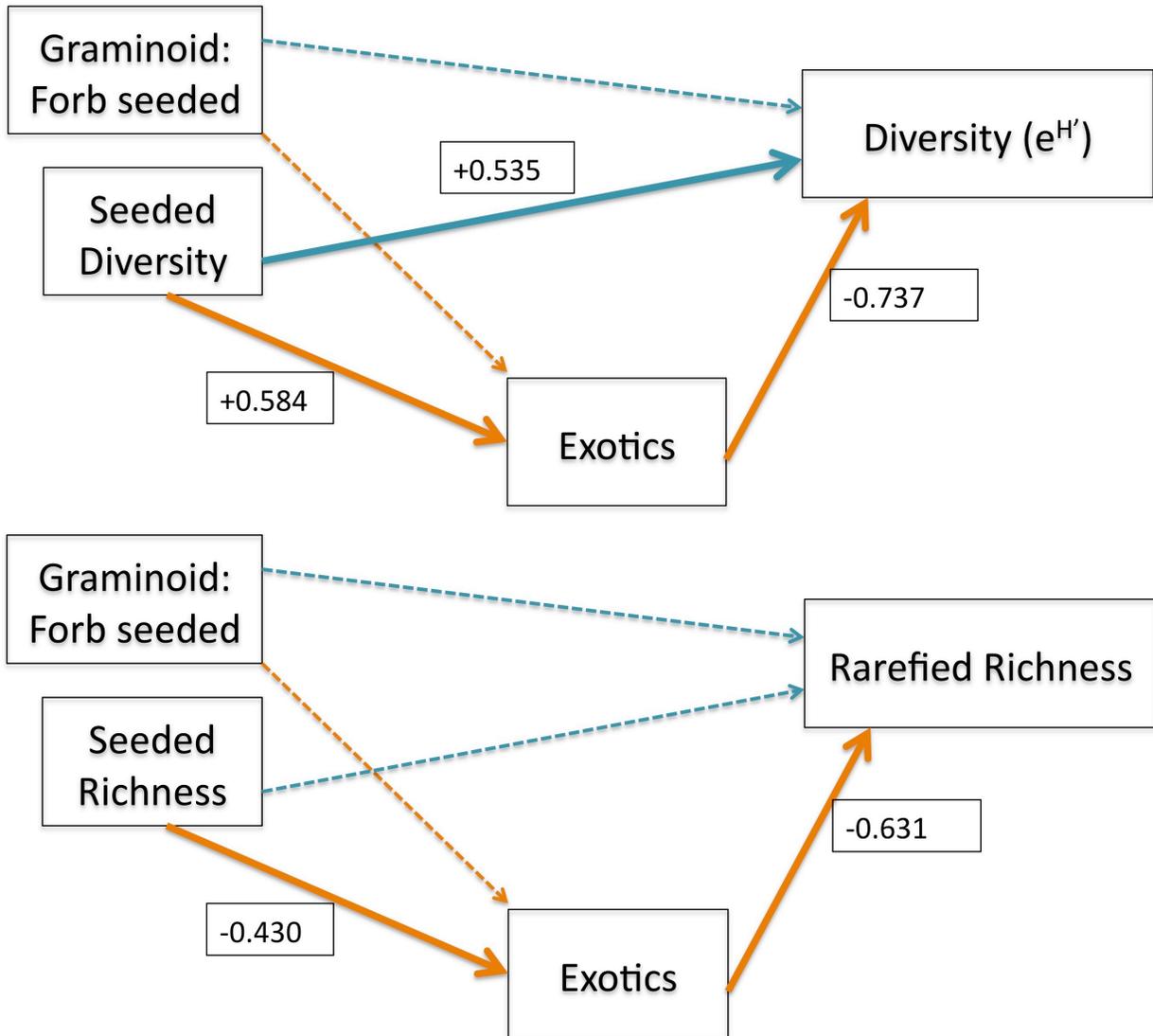


Figure 6. Effect of seed-mix richness, diversity, and graminoid; forb ratio on prairie restorations. Seeded richness increased rarefied richness indirectly by reducing relative abundance of exotic species. Seeded diversity had both a positive direct effect on community diversity, and a negative indirect effect, by encouraging exotic species. Significant pathways are marked with a solid line, and non-significant ones are shown with dotted lines. Interactions supporting deterministic assembly are shown in blue, and stochastic assembly pathways are shown in orange.

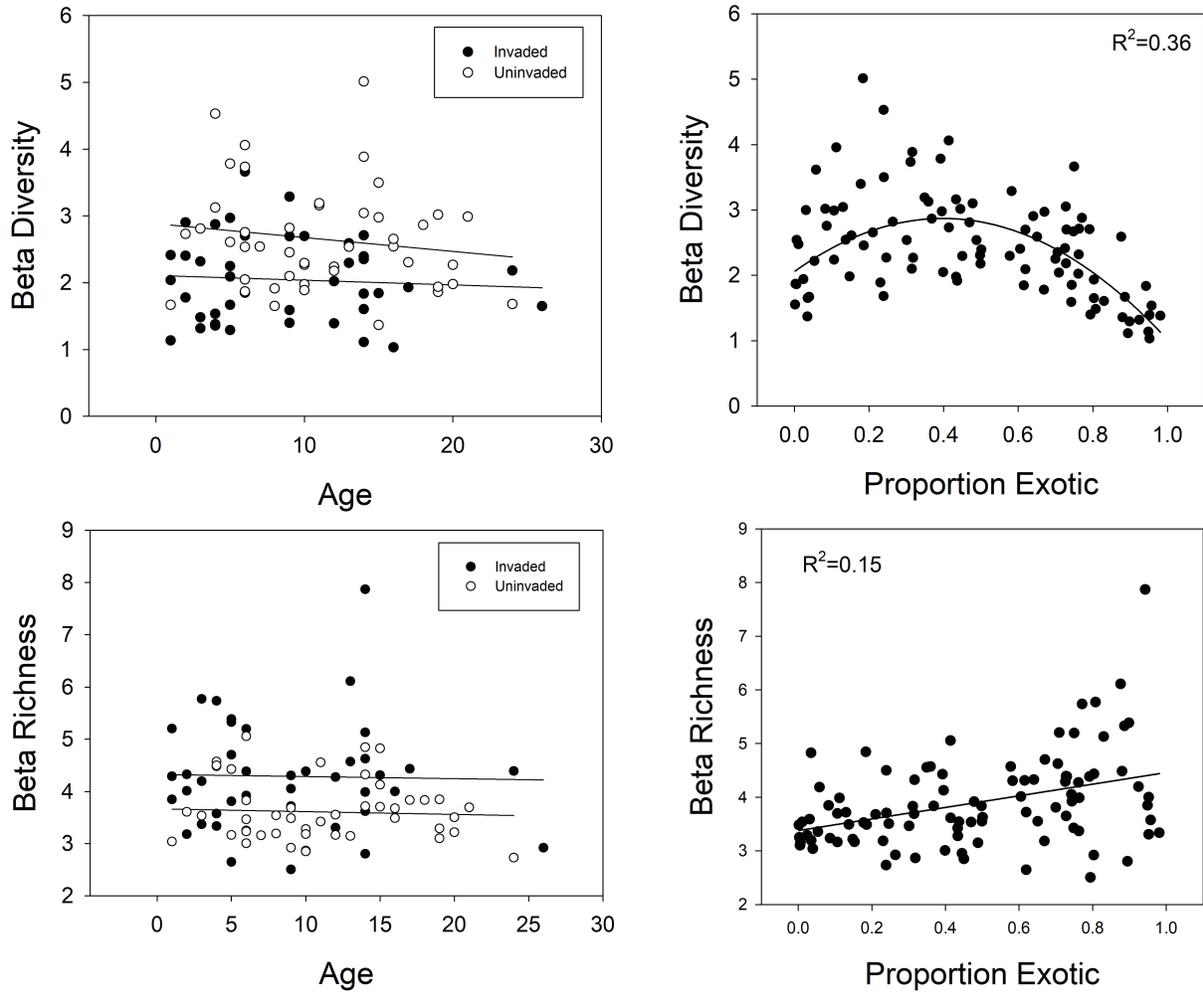


Figure 7. Beta diversity and richness with age of planting (left panels) across 93 restored prairies, and as a function of the amount of exotic species abundance (right panels). “Invaded” sites in left panels have above average exotic abundance (>50%), whereas, un-invaded sites have lower than average exotic abundance.

CHAPTER 3. MONARCH BUTTERFLY HOST PLANT (MILKWEED *ASCLEPIAS* SPP.) ABUNDANCE VARIES BY HABITAT TYPE ACROSS 98 PRAIRIES

Modified from a paper published in *Restoration Ecology*

Andrew D. Kaul and Brian J. Wilsey

Iowa State University, Department of Ecology, Evolution, and Organismal Biology

Abstract

The decline in migratory monarch butterflies (*Danaus plexippus*) over the past 20 years has been attributed to several drivers, including loss of their host plants (milkweeds *Asclepias* spp.). This has sparked widespread interest in milkweed ecology and restoration. We developed a model on environmental and habitat type variables to predict milkweed abundance by sampling 93 prairie plantings (47 conservation plantings and 46 roadsides) and five unplowed prairie remnants throughout the state of Iowa, U.S.A. Milkweeds were censused in 10-25 random locations within each site, and data on plant diversity, age of planting, soil characteristics, and management were tested as predictors of abundance. Milkweed densities of all species combined were highest in remnant prairies (8,705 stems/ha), intermediate in roadside plantings (1,274 stems/ha), and lowest in conservation plantings (212 stems/ha). Most milkweeds were common milkweeds *Asclepias syriaca*, which were more abundant in roadside than conservation plantings. Remnants contained the most milkweed species. Total milkweed and common milkweed abundance were both predicted by higher soil pH, a more linear site shape, and lower soil bulk density across restorations. Our results indicate that common milkweed is maintained by disturbance, and establishes readily in rural roadside habitat. Remnants are important as reservoirs for multiple milkweed species and should be protected.

Introduction

Monarch butterfly (*Danaus plexippus*) populations are in decline across North America (Vidal & Rendón-Salinas 2014). Two populations occur in North America: the eastern population east of the Rocky Mountains (the focus of this paper), and a smaller one along the west coast (Brower 1995). The eastern monarch butterfly population has a unique migratory behavior, travelling from their breeding range in central to northeastern USA down to a single overwintering site in the mountains of central Mexico (Brower 1995). Stable isotope studies have indicated that the source of the individuals in the Mexican overwintering site is primarily from the Midwest (Wassenaar & Hobson 1998, Flockhart et al. 2017). The size of the migratory population declined drastically between 1993 and 2013 (Vidal & Rendón-Salinas 2014), and recent data indicate that the population covered only 2.5 ha of the overwintering site in 2017-18, representing a 56% decline from the 24-year average of 5.7 ha (Monarchwatch.org).

Monarch caterpillars are host specific on multiple milkweed (primarily *Asclepias* in the family *Asclepiadoideae*) species (Brower 1969, Brower et al. 1984, Endress & Bruyns 2000). Within Iowa, there are 17 species of *Asclepias* milkweeds, plus the vining milkweed *Cynanchum leave* (Eilers and Roosa 1994). Oviposition and larval survival studies have examined whether monarchs select and survive preferentially on certain milkweed species. An early study found that pupation length, fecundity, and fertility did not differ among monarchs feeding on one of four different species of milkweed (Erickson 1973). Ladner & Altizer (2005) found evidence for oviposition preference across four host plant species, but monarchs utilized all four species examined at least to some extent. Similarly, a study of nine milkweed species found that monarchs laid eggs on all species, with evidence of oviposition preference for *A. incarnata* (swamp milkweed) and *A. syriaca* (common milkweed) (Pocius et al. 2018). Monarch larvae survived on all nine species (Pocius et al. 2017a,b). Taken together, these studies indicate that,

although there is monarch preference for certain milkweed species, monarchs can utilize all milkweed species, and the abundance of all species should be considered in host plant studies.

Due to the obligate relationship between monarchs and milkweeds, the recent drastic decline in milkweed abundance in Midwestern agricultural land has been proposed as a driver of monarch population declines (Hartzler 2010), i.e., the “milkweed limitation hypothesis” (MLH) (Pleasants and Oberhauser 2013; Flockhart et al. 2015; Pleasants et al. 2017). Since the inception of glyphosate resistant crops, the spraying of glyphosate has led to a large decline in milkweeds in crop fields (Hartzler 2010; Pleasants & Oberhauser 2013). This reduction in milkweeds means that the non-cropped areas in the Midwest are becoming increasingly important in supporting monarchs. Several other mechanisms have been proposed as contributing factors to monarch decline, including loss of nectar habitat, forest loss in the overwintering sites in Mexico, reduced nectar sources during fall in the southern USA, and mortality during migration (Inamine et al. 2016, Pleasants et al. 2017, Agrawal & Inamine 2018).

In 2014, a USA presidential memorandum on pollinator health called for an increase in the migratory eastern population to approximately 225 million butterflies, or 6 ha of cover at the overwintering grounds in Mexico by 2020 (Pollinator Health Task Force 2015). The Midwest currently has ~1.3 billion milkweed stems, supporting 3.2 hectares in Mexico (Pleasants et al. 2017). In order to reach the federal goal of 6 hectares, an estimated 1.6 billion new milkweed stems need to be established in the Midwestern U.S. (Thogmartin et al. 2017b). Because of the ubiquity of glyphosate resistant crops in the Midwestern USA, most of these stems will have to be re-established in non-cropped areas (e.g. grasslands, pastures, suburban areas) embedded within agricultural landscapes (Thogmartin et al. 2017a). However, it is poorly known which species thrive in these habitats, how abundant they are, and what habitat factors predict their

abundance, the objectives of the current study. We present estimates of milkweed densities in three types of non-crop habitat: roadside prairie plantings, non-roadside prairie restorations, and unplowed prairie remnants. Our goal was to estimate milkweed stem densities in these areas and investigate which site-level factors including soil characteristics, and land management practices, best predict their abundance across habitats.

Milkweeds produce numerous small seeds that disperse widely and have very high germination (Morse and Schmitt 1985), suggesting they are favored by disturbance. Common milkweed is abundant in areas with plowing or other disturbances (Evetts & Burnside 1972) including roads, fencerows, and cleared fields (Bhowmik & Bandeen 1976). Based on this, we predicted that common milkweeds would be most abundant in areas with frequent disturbances. Extending this prediction to our measured variables, we predicted that linear areas with greater edge effects, and sites established more recently would have higher milkweed densities. These variables are associated with having greater colonization and persistence opportunities. Common land management practices including disturbance from mowing and burning may also favor milkweed establishment.

We test the following hypotheses: 1) milkweed abundances will vary across habitat types (roadside plantings, conservation plantings, and unplowed remnants), 2) milkweed abundances will decrease with time since planting, and 3) milkweed abundances will be higher in linear, fertile, and more frequently disturbed sites (e.g., from mowing or burning). We tested these hypotheses by sampling 98 prairie plantings (47 conservation plantings and 46 roadsides) and 5 unplowed remnant prairies across Iowa (Figure 1).

Methods

Site Selection

We collected data on milkweed abundances throughout the state of Iowa in 93 prairie plantings and 5 remnants. The 93 planted prairie restorations were selected randomly from plantings conducted by the Iowa Department of Natural Resources, other governmental agencies, and the Department of Transportation. Planted prairies were classified as either roadside restorations or “conservation” plantings. Conservation plantings were treated as a different category from roadsides because they were typically less linear in shape than roadsides and were not near a road (Table S2). Roadside plantings were found along gravel and paved roads, and were typically linear in shape. We sampled a total of 47 conservation plantings and 46 roadsides. For roadsides, we randomly selected 12 counties found within each quadrant of Iowa and then randomly selected sites within each county. This resulted in a range of site ages and soil characteristics. All 93 sites were seeded with prairie grass and forb species. Common milkweed (*Asclepias syriaca*) was very rarely seeded ($n = 3$). Most sites had at least one species of milkweed in the mix, usually either butterfly (*Asclepias tuberosa*) ($n = 41$), or swamp milkweed (*Asclepias incarnata*) ($n = 42$). Remnant prairies were defined as sites that were never plowed or over-seeded, and were dominated by native plant species. Four remnants were protected in the Iowa State Preserve system (Doolittle, Cayler, Liska-Stanek, and Marietta) and one was protected locally (Iowa State University’s Anderson-Dyas Prairie) (Wilsey et al. 2005).

Sampling Design

All sampling was conducted during the month of July during 2015 and 2016. At each site, we censused milkweeds, sampled the overall plant community, and collected soil cores. We located 10-25 sampling locations within each site by following a randomly determined compass

direction in each site, or by walking haphazardly through linear habitats. Quadrat tosses were made at randomly chosen distances between plots, with at least 5 m between locations, and plants were sampled exactly where the quadrat landed. At each location sampled, we estimated all milkweed stems in a 3.14 m² round plot (1 m radius). We also collected plant species composition data as part of a larger study using point intercept sampling. At each location, a pin was dropped at the four corners of a 20 x 50 cm quadrat, and all plant species present were noted. Species present but not hit were assigned 0.5 hits. Plant community data will be presented in detail in another manuscript, but here we incorporate site level measures of plant diversity and proportion exotic biomass as predictors of milkweed abundance.

Environmental data were collected to test for predictors of milkweed abundance. We collected three soil cores to 10 cm depth (diameter = 1.75 cm). Soil samples were then analyzed for soil pH, bulk density (g/cm³), and organic matter (Hendershot 1993). Organic matter was estimated with a muffle furnace by placing soil samples in the oven at 375° C for an hour, and then 600° C for 6 hours (Karam 1993). Age of planting was obtained from site managers.

To account for variation in the shape of sites, we calculated the fractal dimension as an indicator of how linear or square the site is with the following equation: Fractal dimension

$$= \frac{2 \ln\left(\frac{\text{perimeter}}{4}\right)}{\ln(\text{area})}$$

. This measure increases with the linearity of the site, where a line has fractal dimension of two and a square has fractal dimension of one. Thus, more linear habitats have a higher ratio of perimeter to area and have a higher fractal dimension. Site dimensions were calculated using tools in ESRI ArcMaps. After site selection and sampling, we contacted the land managers and obtained information about site age, management history (i.e., mowing and burning regimes), and seed mix used.

Statistical Analyses

We tested each hypothesis separately for both total milkweed stem density, and common milkweed stem density alone at the site level using generalized linear models with a negative binomial distribution and a log-link function (Proc GENMOD in SAS 9.4, Littell et al. 2002). Non-normal distributions were used to model milkweed abundance because many sampled sites had no milkweed present. All models included In area sampled as a covariate.

We initially tested generalized linear models using a Poisson distribution, but this distribution did not fit the data well for total (deviance = 16.5), or common milkweed density (deviance = 13.5). The over dispersion in milkweed counts was likely due to the fact that they are rhizomatous plants, and can grow many stems per genet. The negative binomial distribution fit data well for both total milkweed density (deviance = 1.2) and for common milkweed alone (deviance = 1.1).

To test the hypothesis that milkweed density will vary among habitats, we used a priori contrasts to test whether 1) milkweed abundances differed between remnants and prairie restoration plantings (conservation plantings and roadsides combined), 2) between conservation plantings and roadsides, and 3) between seeded and unseeded restorations. To test whether milkweed density changed with time since planting, we did a separate generalized linear model with linear and quadratic terms for site age including all predictor variables listed below as covariates. Data on site age was not available for 7 of the 93 restorations, so the remaining 86 were used. Sites varied in age from 1 to 26 years since planting, with an average age of 10 years. The age range was similar between the roadside and conservation restorations, but the conservation areas were older on average (mean of 12.5 years) than roadsides (mean of 7.6 years). Partial (Type III) sums of squares were used to evaluate significance.

We tested whether milkweed stem density was related to environmental and management variables with a model that included soil pH, bulk density, and organic matter, and plant diversity (the exponent of Shannon's index ($e^{H'}$) as a measure of nectar plant diversity. Fractal dimension ($r = 0.61$, $p < 0.001$) and proportion exotic species ($r = 0.39$, $p < 0.001$) were correlated with pH, so only pH was included to prevent problems with inter-correlation. All other predictor variables had low inter-correlations. Management variables included mowing and burning regime categories. Mowing was categorized as: 1) none ($n = 34$), 2) early-establishment mowing (mowing only during years one and two, $n = 31$), 3) "other mowing" including spot mowing, haying, or mowing after the first 2 years ($n = 20$), or 4) unknown ($n = 8$). Burn frequency was classified as none, once, multiple times, or unknown. Size of plantings averaged 5.4 ha and ranged from 0.14 to 61 ha. Shannon's species diversity ($e^{H'}$) spanned from very low diversity sites with 1.2 species equivalents, to very diverse restorations with 25.6 species equivalents. Mowing and burning categories were treated as fixed effects in models. Partial (Type III) sums of squares were used to evaluate significance.

Results

We found milkweeds at 65 of the 93 restored sites (70%, Fig. 2). Milkweed densities varied significantly among habitats (Table 1). Common milkweed was the most commonly sampled milkweed species (59 of 65 sites), but other milkweeds were present, especially in remnant areas. Other milkweed species sampled were butterfly weed *A. tuberosa*, whorled *A. verticillata*, and Sullivant's *A. sullivantii* milkweed. All of these species are clonally growing perennials, in which individual stems (ramets) are connected belowground as genets. We estimated stem densities instead of genet densities to align our numbers to restoration stem targets (Tables 1 and 2).

All Milkweed Species Combined

Milkweeds were much more abundant in remnant prairies when all species were considered together (Fig. 2B). The density of all milkweed species was significantly higher in remnants (median 8,705 stems/ha) than restored prairies (median 425 stems/ha) ($\chi^2 = 9.86$; $p = 0.002$) (Fig. 2B; Appendix B Fig. S1). Within restorations, milkweed density was higher in roadsides than conservation areas, with 1,274 vs. 212 stems/ha respectively ($\chi^2 = 11.58$; $p = 0.001$). Total milkweed stem density was not significantly higher in sites that received milkweed seed (531 stems/ha; $n=57$) than those that did not (425 stems/ha; $n=9$) ($\chi^2 = 2.63$; $p = 0.1050$).

Total stem density was strongly correlated with soil variables and weakly correlated with site age. It was positively related to soil pH ($\chi^2 = 12.68$; $p = 0.0004$; Fig 3A) and negatively related to soil bulk density ($\chi^2 = 5.48$; $p = 0.0192$; Fig 3C). Soil pH was higher in roadsides than restoration areas, but bulk density was similar among habitat types (Table S2). Milkweed density changed significantly with site age with slightly higher densities at intermediate ages. (Fig. S2A, linear $\chi^2 = 5.63$; $p = 0.0177$; quadratic $\chi^2 = 4.83$; $p = 0.0279$). No other management or environmental variables were significant (Appendix B: Table S1).

Common Milkweed

Common milkweed was more common in roadsides (Fig. 2A) than in conservation plantings, with median stem densities of 1,062 vs. 127 stems/ha in roadsides and conservation plantings, respectively ($\chi^2 = 8.98$; $p = 0.0027$). Common milkweed was not significantly different between remnants and restored prairies ($\chi^2 = 0.07$; $p = 0.7901$). Similar to total counts, common

milkweed density was not significantly different in areas where it was planted than those where it was not, with median 0, and 319 stems/ha respectively ($\chi^2=1.98$; $p=0.1590$), although sample sizes were small with $n=3$ seeded sites compared to 63 not seeded with common milkweed.

Common milkweed density also varied with soil variables, being strongly positively related to soil pH ($\chi^2=9.98$; $p=0.0016$; Fig. 3B), and negatively with bulk density ($\chi^2=4.50$; $p=0.0338$; Fig. 3D). Common milkweeds did not vary significantly with site age (Fig. S2B, linear $\chi^2=1.79$; $p=0.1807$; quadratic $\chi^2=1.73$; $p=0.1885$). No other variables were significant predictors (Appendix B: Table S1).

Discussion

Declines in milkweed abundance throughout the Midwestern United States have been implicated as an important driver of monarch butterfly population collapse and quasi extinction risk (Semmens et al. 2016). Efforts to increase milkweed stems will rely on information about where milkweeds are currently located, and how to manage land to promote milkweed abundance and persistence (Pleasants 2017). Previous work has documented milkweed densities in several Midwestern habitats, but here we filled in the remaining gaps, and report densities for prairie remnants, restored prairies, and planted roadsides in Iowa (Table 2). The strength of this study is that we present data from 93 prairie plantings and five remnants throughout Iowa, covering a large portion of the central tallgrass prairie region. We assume Iowa is representative of the Midwestern breeding land and reflects similar milkweed densities in habitats across the range. We found that common milkweeds were most common in planted roadsides, and in areas with high soil pH and low bulk density. All milkweed species combined were most abundant in remnant prairies. The planted roadsides we sampled had considerably higher milkweed densities than the conservation areas.

Most milkweeds sampled were common milkweed, which was present at 59 of the 65 restorations (91%) where milkweeds were detected. Common milkweed accounted for 77% of all stems sampled at restored sites, but only 11% at remnants, representing a combined 62%. Among all restorations, we also found *A. tuberosa*, *A. incarnata*, and *A. verticillata*. Among the 5 remnants sampled, we found the four species previously mentioned, as well as *A. sullivantii* and *A. amplexicaulis*. All of these species are potential host plants for monarchs, but this relationship has not been tested for *A. amplexicaulis* explicitly (Pocius et al. 2017). As these other milkweed species are much more common in remnants, future studies should address how they differ from common milkweed in their habitat preferences and response to management practices. The greater number of milkweed species in remnants indicates their value as reservoirs for milkweed species and they should continue to be protected for their value to monarch butterflies. Diversity of milkweed host plants may be an important feature of habitat for monarchs as it may provide more phenological diversity throughout the breeding season (Kaul et al. 1991) or in the case one species fails, having other milkweeds present may help maintain their habitat value for monarchs.

We expected common milkweed density to be highest in younger or medium aged plantings, associated with establishment disturbance, and found some evidence to support this. We found a quadratic relationship between total milkweed density and age after accounting for site management and soil characteristics, where middle-aged planted prairies had more milkweeds. However, this relationship was weaker than relationships with other variables (Fig. S2A).

Consistent with our predictions, we found higher milkweed densities in prairies with higher forb diversity. Zaya et al. (2017) found that milkweeds were more common in species

diverse areas in Illinois. When all milkweed species were combined, remnant areas had the highest densities of milkweeds. This effect was weak, but indicates that prairie habitats with more nectar producing plants are also better habitats for milkweeds.

We compared milkweed densities in our sampled habitats to previous estimates from other land uses in the literature (Table 2), including unplanted roadsides, Conservation Reserve Program plantings (CRP), and agricultural habitat (Kasten et al. 2016; Pleasants and Oberhauser 2013; and Pleasants 2017). Estimates of hectares of Iowa roadsides were estimated by Mark Masteller (personal communication), and hectares for remnant areas were from Samson & Knopf (1994), and Wilsey et al. (2005). Planted prairie area was calculated as the sum of planted areas at National Wildlife refuges, Department of Natural Resource lands and county conservation areas (Karen Viste-Sparkman and Thomas Hazelton personal communications). Our estimates of stem density in planted roadsides were about 80 times higher than previous estimates for unplanted roadsides (Table 2). Milkweed densities may be higher in planted than unplanted roadsides due to the disturbances associated with seeding during establishment. Common milkweed is weedy, and we found that variables related to disturbance were positively correlated with their abundance. Despite this much higher density, they only contributed around five times as many total milkweed stems as unplanted roads due to their smaller coverage in Iowa (Table 2.). We found that restored conservation plantings have about twice the density of a previous estimate for CRP land. However, CRP makes up the largest portion of any habitat type, accounting for over half of the milkweed stems in the state. Agricultural cropland has the lowest density of milkweeds of any habitat surveyed to date, so it contributes very little to the total milkweed stem estimate, despite the extremely high proportion of cropland in the Midwest.

Remnants in Iowa have by far the highest density estimates of any habitat type. Despite being extremely rare on the landscape, we estimate that remnants have about a fifth of the total milkweed stems in the state.

Our results indicate that roadsides could be excellent habitat for new milkweed plantings. We found that planted roadside habitats have orders-of-magnitude higher densities of common milkweed than unplanted roads (Table 2). Because most of the milkweed in restored prairies is common milkweed, roadsides offer a great opportunity for establishing large populations of this monarch butterfly host species. Previous work indicated that planting roadsides with prairie species provides value to rare butterflies due to increased flowering plant species richness (Ries et al. 2001). Increasing larval host-plant density could enhance the value of roadside habitat for monarch butterflies, as long as densities are below saturation levels (Kasten et al. 2016), as most of our densities are here.

Our roadsides differed from previous studies in that they were primarily rural and not urban. McKenna et al. (2001) found high Lepidoptera mortality in roadsides near a Midwestern city, especially near roads with high traffic rates. They suggested that traffic is a major form of mortality, which suggests some roadsides may be poor habitat for monarchs. However, in their study, country roads, which are more similar to our rural roadsides, had little to no mortality. Based on this, we suggest that roadsides in rural areas might make the best habitat for milkweed establishment. Future plantings of common milkweeds in rural roadside habitats may be an effective way of establishing these important host plants.

The strong correlation between milkweed density and soil pH needs to be studied further. Soil pH was higher in roadsides than conservation areas, and this may be the primary mechanism explaining the higher milkweed densities we found in roadsides. The relationship

between milkweed density and pH could be explained by three different mechanisms, and further work is needed to test among these possibilities. The relationship between milkweeds and soil pH could be a direct cause and effect relationship. If this is so, then fertilizing fields could be leading to greater acidity as NH_3 is nitrified to NO_3^- . Fertilizing fields without liming leads to reduced soil pH (Silvertown et al. 2006), and reduced pH could have reduced milkweed abundance. However, the relationship is correlative, and pH is an integrative measure that is correlated with other aspects of soil fertility. A second possibility is that higher pH is associated with higher calcium and mineralized nitrogen availability (Donahue et al. 1971), consistent with the negative relationship between milkweed abundance and soil bulk density. This could be tested with fertilizer studies that add N and Ca without altering the soil pH. A final possibility is that rocks from gravel roads, distributed by snowplows and other trucks could be causing local soil disturbance, and common milkweed could be responding positively to this disturbance. Further work is needed to separate the correlated effects of linear habitat, disturbance, nutrient availability, and soil pH on milkweed abundance with an experimental approach. Future studies with liming and fertilizer additions could assess what factors are causing milkweed abundances to increase or decrease over time.

In conclusion, we found that the abundance of common milkweed, the most common species sampled, did not vary across habitat types the same way that all milkweed species did. Butterfly, swamp and Sullivant's milkweed were found primarily in highly diverse remnant prairie areas. Thus, remnants are important as reservoirs for these species and should be protected. Common milkweed on the other hand, was most abundant in planted roadsides. Roadside habitat is very abundant in the Midwest. This indicates that the most economically feasible option may be to establish milkweeds in rural roadsides in the Midwest.

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Tables and Figures

Table 1. Estimates of stem densities per hectare for all milkweeds, and only common milkweed in remnant prairie, and seeded and unseeded roadside, conservation, and all combined restored prairie plantings. Medians are presented with 25% and 75% quantiles in parentheses, and means are presented with standard errors. Estimates for total milkweed densities by habitat include sites that were seeded with any milkweed species, not seeded with milkweed, and sites where it was unknown if milkweed was seeded.

	Statistic	Remnant	Conservation	Roadside	Restorations Combined
Seeded	N		20	37	57
All Milkweeds	Median		194.6 (0 – 743.1)	1273.9 (212.3 – 4140.1)	530.8 (0 – 3184.7)
	Mean		845.3 (369.1)	3126.5 (733.5)	2326.1 (511.7)
Seeded	N		1	2	3
Common	Median		0 (0)	424.6 (0 – 849.3)	0 (0 – 849.3)
	Mean		0 (0)	424.6 (424.6)	283.1 (283.1)
Unseeded	N		7	2	9
All Milkweeds	Median		127.4 (0 – 424.6)	1804.7 (424.6 – 3184.7)	424.6 (0 – 3184.7)
	Mean		1390.7 (855.6)	1804.7 (1380.0)	1482 (695.5)
Unseeded	N		26	37	63
Common	Median		127.4 (0 – 530.8)	1061.6 (212.3 – 3184.7)	318.5 (0 – 1910.8)
	Mean		850.3 (337.9)	2330.4 (572.2)	1719.6 (373.1)
Total	N	5	47	46	93
All Milkweeds	Median	8704.8 (2070.1 – 14649.7)	212.3 (0 – 1433.1)	1273.9 (212.3 – 4246.3)	424.6 (0 – 2707.0)
	Mean	9522.3 (3660.4)	920.7 (225.7)	3259.5 (664.2)	2077.5 (366.7)
Total	N	5	47	46	93
Common	Median	849.3 (159.2 – 1592.4)	127.4 (0 – 636.9)	1061.6 (212.3 – 3184.7)	318.5 (0 – 1592.4)
	Mean	934.2 (400.2)	736.6 (212.0)	2486.0 (553.5)	1601.9 (306.2)

Table 2. Estimates of milkweed stems in Iowa, including new data from this study (*). Data are presented as means and standard errors (SE) to be comparable to earlier published estimates.

	stems/ha	SE	ha in Iowa	total # stems	SE
Planted Roadsides (total)	3259.5*	664.2	21,892	71,356,337	14,541,050
<i>Asclepias syriaca</i>	2486.0*	553.5			
Other <i>Asclepias</i>	773.5*	381.1			
Conservation Areas (total)	920.7*	225.7	39,014	35,920,847	8,804,687
<i>Asclepias syriaca</i>	736.6*	212.0			
Other <i>Asclepias</i>	184.1*	93.7			
Remnants (total)	9,522.3*	3,660	12,400	118,076,433	45,389,014
<i>Asclepias syriaca</i>	934.2*	400			
Other <i>Asclepias</i>	8588.1*	4,044			
Published studies					
Corn/ Soybeans	0.12		9,360,000	1,130,000	
CRP	413.4		663,000	274,084,200	
Unplanted Roadsides	36		365,189	13,146,804	
Sum				513,714,621	

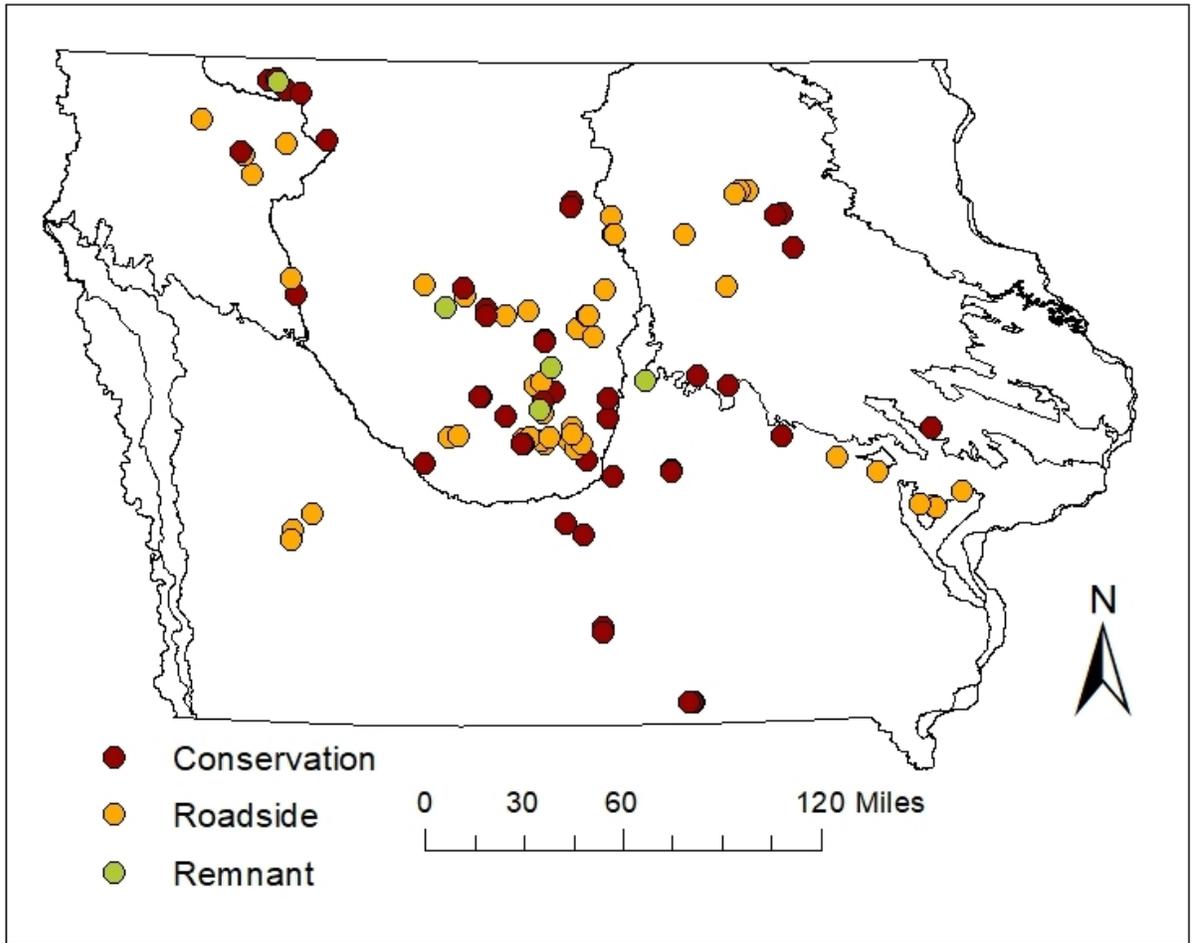


Figure 1. Site locations. Background map outlines major Iowa Landforms.

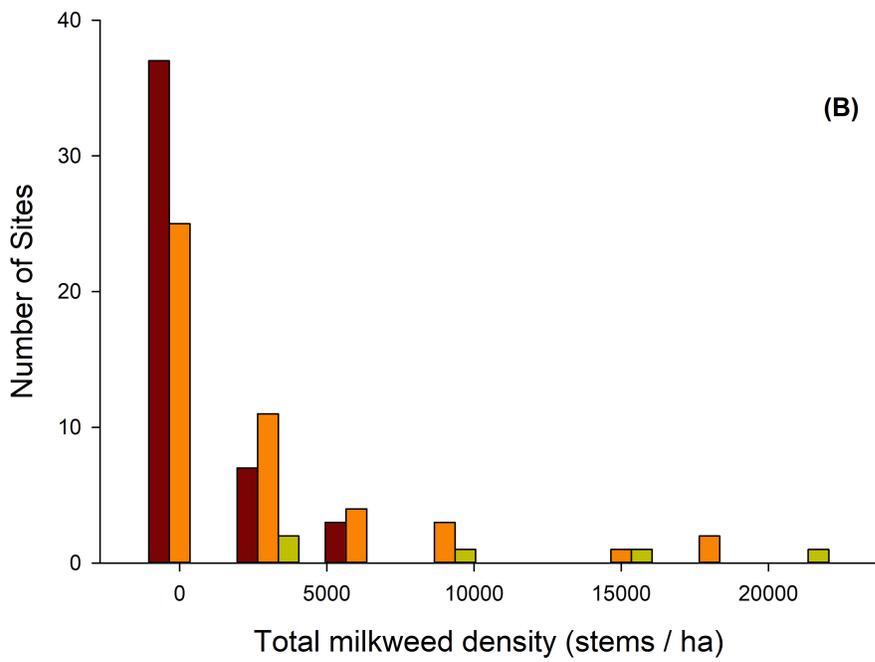
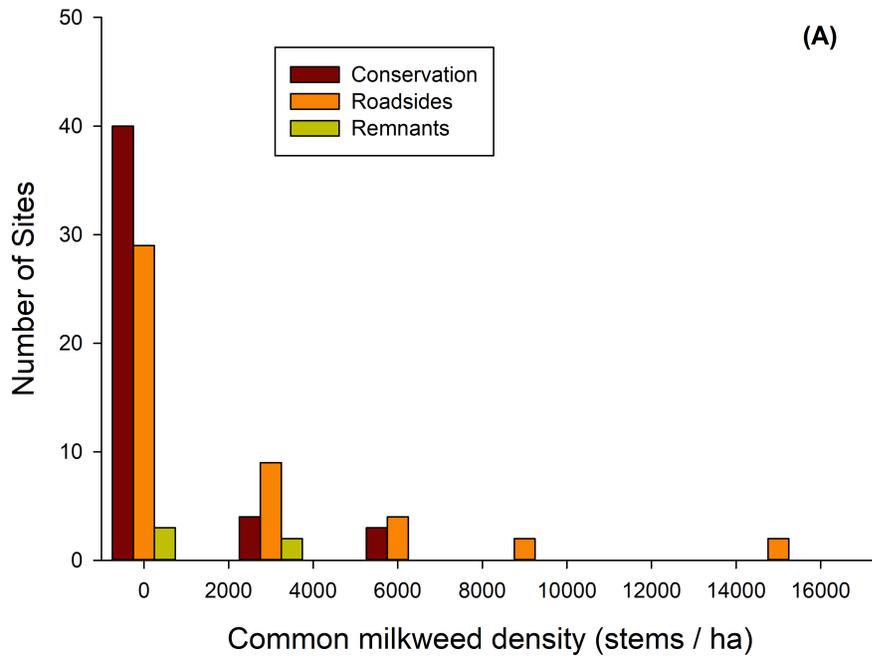


Figure 2. Stem densities (x axis) and number of sites (y axis) containing common milkweed (A), and all milkweeds (B) across 93 restored prairies and five remnants.

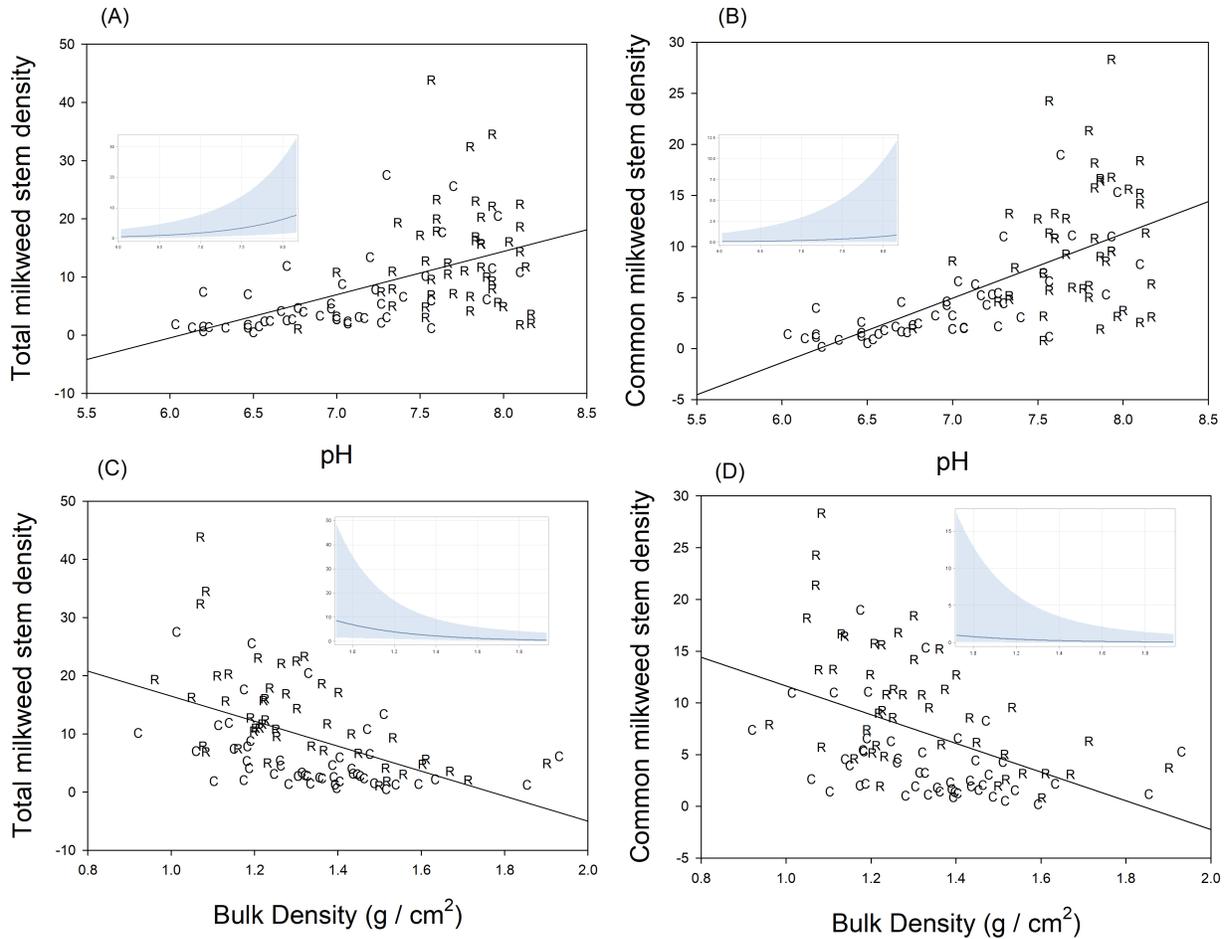


Figure 3. Relationships between soil pH and bulk density and all milkweed species (A, C) and common milkweed density (B, D). Milkweed densities are adjusted for other variables in the model. Insets show fitted model with 95% confidence interval.

**CHAPTER 4. PRIORITY EFFECTS FROM COVER CROP SPECIES
DIFFERENTIALLY ALTER GRASSLAND COMMUNITY ASSEMBLY IN A TALL
GRASS PRAIRIE RESTORATION**

Andrew D. Kaul and Brian J. Wilsey

Iowa State University, Department of Ecology, Evolution, and Organismal Biology

Abstract

Priority effects occur when the presence or abundance of early arriving species restrict the establishment of later arriving species. We examined the role of early arriving species on later community composition using cover-crop treatments and control plots. When used as a tool for ecological restoration, cover-crops are hypothesized to facilitate establishment of target species by reducing weed abundances without competing with the target mix. Our cover-crop treatments included seeding one annual or biennial cover-crop species (tillage radish (*Raphanus sativus*), oats (*Avena sativa*), or gray primrose (*Oenothera biennis*)), a perennial species Canada wild rye (*Elymus canadensis*), two grass-forb species combinations, or nothing as a control in fall 2015. All plots received a diverse prairie seed mix in March 2016. Community composition and species diversity were sampled in the following three years and were compared among treatments with perMANOVAs and ANOVA. Species composition and species diversity differed among cover-crop treatments in all four sampling dates suggesting the existence of strong priority effects. Cover-crop species varied in their effectiveness at reducing weed abundances, with Canada wild rye being especially effective at reducing weeds. However, Canada wild rye also greatly reduced establishment and species diversity of the prairie-seed mix. The other cover-crops were largely neutral in their effects on prairie establishment.

Cover-crops can have beneficial effects in early years, but they may also reduce prairie establishment, especially when the cover-crop species is perennial. We found strong evidence that priority effects induced by a persistent perennial cover-crop did influence the outcome of community assembly. This was most evident in terms of the functional composition of species recruiting under different treatments, with the C₃ perennial grass, Canada wild rye, reducing abundance of C₄ grasses and increasing forbs and other C₃ grasses. Our results indicate that using cover-crops in prairie restorations can be useful for reducing weeds, but it can also have the overall effect of reducing prairie establishment when the cover-crop is a perennial.

Introduction

Species differ strongly in when they arrive and become established in assembling communities. Species that arrive early can affect the establishment of later arriving species, an example of a priority effect. Priority effects can be defined in two ways: 1) the effect of early arriving species on later arriving ones, or 2) the differential effect of a species on a community, if it arrives earlier vs. if it arrives later (Shulman et al. 1983, Morin 1999, Fukami 2015). Here, we discuss priority effects following the first definition as it relates to changes in community composition or species diversity. Priority effects may have facilitative or inhibitory effects on later arriving species (Fukami 2015). Inhibitory, or negative priority effects occur when the early arriving species utilizes resources such that it asymmetrically competes with later ones (Huston 1979). Facilitative priority effects (sometimes called “Inverse priority”) are common in harsher environments where germination or seedling survival drives community dynamics, and occur when early arriving “nurse plants” alter the environment through changes to edaphic properties or the microclimate (shading), such that it creates more favorable conditions for later arriving species to establish (Padilla & Pugnaire 2006).

Negative priority effects can be produced by two primary mechanisms: asymmetric competition (Ejrnaes et al. 2006, Körner et al. 2008), or soil legacy effects (Grman & Suding 2010) which reduce colonization success for later arriving species. Asymmetric competition often occurs when early arriving species outcompete later arriving ones due to their larger size (Platt 1975, Alford & Wilbur 1985). Negative priority effects through competition are predicted to be strongest in productive (less harsh) environments where pioneer species can grow rapidly, resulting in more asymmetric competition and niche pre-emption (Chase 2003, Kardol et al. 2013, Fukami 2015), and there is some evidence for this effect in grassland priority experiments with variable nutrient or water availability (Young et al. 2014, Lang et al. 2017, Young et al. 2017, Fry et al. 2017). Priority effects through niche preemption are also predicted to be strongest when pioneer and later arriving species have greater niche overlap (Vanette and Fukami 2014).

There are two alternate predictions about the functional composition of species entering a community where there are strong priority effects. If competition for resources is driving assembly, limiting similarity may select for recruitment of species into the community that are more dissimilar from the pioneer species than random (MacArthur & Levins 1967, Fargione et al. 2003). An extension of predictions from limiting similarity, Fox's assembly rule (guild proportionality) predicts that the next species to recruit to a community is most likely to belong to a guild (functional group) that is underrepresented in the current community (Fox 1987). Alternately, if environmental filtering is driving assembly, early arriving species act through niche modification, creating an environment that is favorable to species similar to themselves, and facilitate their recruitment (Bazzaz 1996). Early arriving species can cause niche

modification by affecting abiotic variables such as light striking the soil surface. When they create shade, it can lead to poor establishment from shade intolerant species.

Cover-crops are plants that are used in agricultural practices, planted for purposes other than harvesting, during the dormant season (over winter) when growing cash crops is not possible (Unger & Vigil 1998). Cover-cropping can have many environmental and economic benefits, with the primary function usually being to prevent soil erosion and water runoff when a field would otherwise be bare (Unger & Vigil 1998). Cover-crops provide numerous benefits, including increasing soil carbon, reducing water runoff, sediment loss, nitrogen leaching, wind erosion, and soil compaction, improving soil structural properties, and suppressing weeds (Blano-Canqui et al. 2015). Cover crop roots stabilize soil, and create pores to increase water infiltration, and aboveground biomass shades the soil and covers it from the impact of rain. These benefits, compared to leaving a bare fallow field, can often be achieved without affecting crop yields, but the type and magnitude of effects depends on the species used (Snapp et al. 2005, Tonitto et al. 2006). Cover-crop species are generally annuals or biennials, chosen because they are fast growing, so they will quickly recruit to and produce biomass above and below ground (Tribouillois et al. 2015).

Priority effects are now being manipulated in various creative ways as a management tool in the restoration of grassland ecosystems, including through the use of cover-crops (Young et al. 2017, reviewed in Hess et al. 2019). In restoration/ re-vegetation projects, cover-crops are also used to stabilize soil and competitively exclude weeds (Packard and Mutel 1997). They are assumed to be beneficial if they lower weed invasion, and if the benefits exceed any negative competitive effects on establishing prairie (Figure 1, Wilsey 2020). In restorations, the most

commonly used cover-crop species are annuals like oats and cereal rye, and presumably short-lived perennials such as Canada wild rye (Martin and Wilsey 2012).

Grassland restoration is now very common in the tallgrass prairie region of the United States (Smith 1998, Kaul and Wilsey 2019), but most restorations fail to reach the diversity of remnants (Martin et al. 2005, Polley et al. 2005), or even of their own seed mixes (Kaul and Wilsey in review; chapter 2). The largest barrier to establishment of high diversity is invasion by exotic species, so a primary question becomes how to exclude non-target species early in restoration sites (Kaul and Wilsey Chapter 2). Put simply, managers seek strategies to achieve more natives and fewer invasives (Hess et al. 2019). Ecosystem restoration often begins by assembling a plant community on bare soils, and the initial establishment order of species in these communities could have long lasting impacts on the community composition, diversity, and functioning of the restored ecosystem. Considering that early differences in restored grassland community composition often persist for many years (Fukami et al. 2005, Martin and Wilsey 2012, Plückers et al. 2013, Werner et al. 2016, Švamberková et al. 2019), there is now considerable interest in the potential for cover-crops to be used to exclude exotic species in the earliest stages of community assembly.

The identity and species richness of the cover-crop treatment can be important in restorations. With respect to negative priority effects produced by asymmetric competition, grasses often have stronger priority than forbs (Werner et al. 2016, Stuble and Young 2020). Additionally, more diverse groups of species may exert stronger niche preemption than monocultures, due to the greater niche breadth being occupied by species through complementarity (Fargione & Tilman 2005). Cover-crop species can also differ in their effect based on their functional group and growth patterns with annual species growing faster and their

effects dissipating sooner than biennials or perennials (Jarchow et al. 2012). Irrespective of functional group, negative priority effects should be stronger in more productive environments (Chase 2003), when species have a longer time to grow (Gillhaussen et al. 2014), and should favor establishment of species with dissimilar traits from the cover-crop species.

In the context of a grassland restoration in a productive (high precipitation) landscape, we test predictions that 1) cover-crop treatments will significantly affect plant community composition, diversity, and establishment from the mix, 2) plots with cover treatments will have higher establishment success, total richness, and diversity, and lower abundances of exotic species compared to controls, 3) the effect of the cover-crop will depend on the cover-crop's functional group, with annual and biennial cover-crops affecting early arriving species, and the effect of perennials lasting for several years, and 4) cover-crop mixes of two species have stronger effects than either one in monoculture. We test these hypotheses in a field experiment that established cover-crops in the fall before a diverse mix of prairie species was established.

Methods

Study Design

To test these predictions, we conducted a cover-crop experiment with six cover-crop treatments and control plots, which received no cover-crop. Our cover treatments include 1) the native perennial prairie grass, *Elymus canadensis* (Canada wild rye), 2) a biennial forb *Oenothera biennis* (Common evening primrose), 3) a combination of *Elymus canadensis* and *Oenothera biennis*, 4) an annual grass commonly used as a cover-crop, *Avena sativa* (Oats), 5) the biennial forb, *Raphanus sativus* (tillage radish), and 6) a combination of *Avena sativa* and *Raphanus sativus*. We chose Canada wild rye and oats because they are commonly used native cover-crop species in restorations (Packard and Mutel 1997, Martin and Wilsey 2006, 2012).

Tillage radish is a new cover-crop that is starting to be used more frequently by land managers (citations). We tested a new proposed cover-crop species, evening primrose, because it is one of the few native biennial prairie species, and being a biennial, is expected to grow quickly, but not be persistent. Each of these 7 treatments was applied to a plot using a randomized design. Plots were 6 x 12 m (20' x 40'), with 10 replicates per treatment. A buffer of at least 15 m between plots prevented species from spreading from one plot to another. Cover-crop treatments were added the autumn prior to seeding a prairie mix.

Each treated plot was seeded with cover-crops at a rate of 300,000 seeds per acre (741,000 seeds / ha) after a soybean crop was harvested in 2015 (September 29 & 30). We standardized treatments based on seed number rather than mass because the seeds vary tremendously in size across species, and to use the treated densities recommended for land management in the area. Unseeded plots provide a controlled comparison of the effectiveness of each cover treatment. The study was conducted at Brushy Creek Recreation Area (Iowa, USA) in a 6.5 Ha field previously in corn/soy rotation. The entire field, including all research plots, received a diverse prairie seed mix in winter in 2016. The mix included variable abundance of > 15 graminoid species, and 52 species of forbs and legumes. The entire area was mowed during the first growing season (2016), and once during the spring of the second year (2017), and burned in late April of the third year (2018).

Sampling Design

To test whether cover-crop treatments stabilized soil and prevented erosion, we measured displaced soil in two locations in each plot using 30 cm² handmade mesh bags (Hsieh et al. 2009). We put the bags out in 2015, concurrent with the cover-crop seeding, and removed them from the field in April 2016. We weighed the soil caught in each bag.

Aboveground biomass of cover-crop treatments was estimated in November 2015, by harvesting biomass in two 20 x 50 cm quadrats from random locations in each plot. We sampled the plant community in each plot in September 2016, June 2017, September 2017, and June 2018. We used point-intercept sampling to estimate cover and the number of species (Jonasson 1988). We dropped a 20 x 50 cm quadrat containing 4 pins at 2 random locations within each plot, and the identity and number of species present were noted at each quadrat, and the number of point contacts was recorded by species. All hits were recorded such that multiple hits per pin were possible, in order to account for layering, and to ensure that number of hits was as closely related to biomass as possible (Wilsey et al. 2011, Xu et al. 2015). Species that were present in the quadrat, but did not touch any of the 4 pin-drops, received 0.5 hits for the quadrat. This sampling method provided information on the percent coverage of cover-crop species, target seeded species in the prairie mix, and volunteer species.

For each plot in each sampling period, we calculated total species richness, Simpson's species diversity (presented as $1/D$), proportional abundance of target species from the seed mix, and functional group proportionality. Plot-level measures were calculated as mean measures from two sampled quadrats. To investigate the effects of priority treatment on functional composition of the prairie community, we calculated the proportional abundance of five functional groups in each plot in each sample. Each species was classified according to its functional group, including forbs, C_3 annuals, C_4 annuals, C_3 perennial grasses, and C_4 perennial grasses. Legumes were only present in very low abundances in any sample, so they were combined with forbs. Biennial species were also present at low abundances, so they were combined accordingly with either C_3 or C_4 annuals.

In the third year of sampling, we measured vegetation structure and biomass of two important weeds, in order to evaluate differences in community-level responses 3 years after initial treatments. During May 2018, we sampled total above ground biomass in two clip subplots per plot. Subplots were averaged before analysis to prevent pseudoreplication. We sorted biomass to isolate dandelions and thistles from everything else. Simultaneously, we measured canopy height using a Robel pole (Robel et al. 1970), and calculated the average between two measurements in each plot.

Statistical Analyses

All measures of species diversity or richness include all plant species present, excluding only the cover-crops. We did not analyze diversity or richness of the 67 seeded prairies species alone, because seeded and unseeded species are inter-correlated and are not statistically independent.

To evaluate initial responses to cover-crop addition, we compared cover-crop biomass and soil displacement after the first winter and spring. For later samples, we compared species diversity and establishment measures to track changes among treatments over time. We tested if cover-crop biomass in the fall after it was seeded, or if soil displacement the following spring differed between treatments using ANOVA (Proc GLM, SAS 9.4). We tested whether diversity measures (total richness or Simpson's diversity) or establishment success differed among treatments using one-way repeated measures ANOVA with a priori contrasts. Contrasts compared control vs. all cover-crop treatments together, grasses vs. forbs, two cover-crop species vs. one, and Canada wild rye vs. Oats. Similarly, we tested predictions about community structure and weed suppression in year three using one-way ANOVA with a priori contrasts as

above. Significance was evaluated with type III sums of squares. Abundances of thistles and dandelions were log transformed to achieve normality.

To test alternate predictions of niche preemption vs. niche modification on the functional composition of colonizing species, we compared functional group proportions with a MANOVA for each sampling period. To test for treatment by time interactions for differences in functional groups, we analyzed significant variables with repeated measures ANOVA with a priori contrasts as above.

We used PerMANOVA with pair-wise comparisons to test whether species composition differed between treatments in each of the four samples using PC-ORD version 7. Cover-crop species were excluded from compositional data. To test if compositions began to converge over the 4 samples as predicted by deterministic assembly, we conducted a symmetric convergence / divergence test using the ‘vegclust’ package in R (De Cáceres et al. 2019).

We used structural equation models to test our prediction that cover-crops indirectly facilitate target species establishment through their suppression of volunteer weeds using Proc CALIS in SAS 9, using a confirmatory approach (Figure 1).

Results

Early Cover Crop Establishment

Cover-crops are expected to grow during cold periods when most plants are inactive, in order to suppress weeds and stabilize soil. We evaluated initial cover-crop growth, two months after sowing, with biomass clip plots in late November (Figure 2) and found biomass production during fall varied significantly among the cover-crop treatments ($F_{6,63} = 3.55, p < 0.0043$). The native prairie cover-crop species (Canada wild rye and primrose) did not produce any biomass during this time period (Figure 2). The tillage radish produced the greatest amount of cover

during this time, and oats and cereal rye produced intermediate amounts. Weeds did not grow during this period, so control plots were bare ground. The mass of soil displaced between fall 2015 and spring 2016 was highly variable with an average of 23g / bag (0.15g – 100.53g). We were unable to recover mesh bags from 2 of the 70 treatment plots. Mass of displaced soil did not significantly differ between treatments ($F_{6,61}=1.94$; $p=0.09$).

Effects of Treatment on Weed Suppression

We tested the effect of cover-crops on weed suppression and establishment success of target species, using structural equation models, to investigate the relative strength of direct and indirect effects (Figure 1). We modeled cover-crops based on their biomass in fall 2015 after they were seeded (Figure 8A), and based on their relative abundance the following fall (June 2016) (Figure 8B). These models differ because cover-crop biomass in fall was negatively correlated with proportional cover-crop abundance in the next spring ($r = -0.23$; Pearson $p = 0.0487$). Cover-crop biomass from 2015 was positively related to hits from volunteer species (native and exotic species not in the seed mix) in 2016, and volunteer hits were positively related to establishment success in the third growing season, thus the cover-crops had a weak indirect facilitative effect on establishment (Figure 8A). Cover-crop abundance in 2016 had an overall negative effect on prairie establishment. Biomass of cover-crops had a strongly negative direct effect on establishment, which overwhelmed a weak indirect facilitative effect produced by suppression of volunteers (Figure 8B).

The biomasses of the most common weeds and canopy height were significantly different among treatments at the end of our study. The abundances of common dandelion (*Taraxacum officinale*) and Canada thistle (*Cirsium arvense*) significantly differentiated community composition between treatments during September 2017 (Figure 4B), and dandelion was the

most abundant invader during third year of growth in 2018. Since these were dominant weed species early on, we measured dandelion and thistle biomass in clip plots in June 2018, and found that cover-crops significantly reduced the abundances of these weeds compared to control plots (Table 6; Figure 7A & 7B). The biomass of dandelions was also lower in native compared to exotic cover-crop plots (Table 6). Total biomass did not differ significantly between treatments in the third year (Figure 7C), but wild rye treatments had slightly taller average canopy heights compared to oats. Similarly, the grass cover-crop plots were taller on average than those with forb cover-crops (Table 6; Figure 7D).

Effects of Treatment on Establishment Success

When testing for effects of cover-crop treatments on diversity, richness, and establishment success, there was a significant interaction between treatment and sampling period for all three measures (Table 1). Mix establishment increased over time in all treatments except the *E. canadensis* and native mix with *E. canadensis* and *O. biennis* (Figure 3A). Contrasts showed establishment and total species richness were lower in grass treatments compared to forbs, and this was largely driven by effect of *E. Canadensis*, which led to severely reduced establishment compared to oats (Figure 3A, 3C). Treatment differences in species diversity were similar to the effects on total richness, however on average, diversity was lower in cover-crop treatments compared to controls (Figure 3B). Diversity was also higher in treatment plots seeded with two cover-crop species compared to one (Table 1).

Effects of Treatment on Community Composition

Species composition differed among cover-crop treatments on all four sampling dates (Table 2; p values all < 0.001). The cover-crop was not included in the analyses, so the

ordination plots the abundance of species from the seed mix or coming in as volunteers (Figure 4). The first year, Canada wild rye reduced *Amaranthus* spp. and side oats grama from the seed mix (Figure 4A), and this expanded to other species from the seed mix in years 2 and 3. Biplots in the second sample (June 2017) indicate important species differentiating compositions of cover-crop plots were dandelion, Canada thistle, and tall dropseed (Figure 4B). In late summer 2017, the two treatments with Canada wild rye were clearly differentiable from the other treatments, with NMDS loadings indicating differences were driven by little bluestem, side-oats gramma, tall dropseed, Canada goldenrod, and frost aster (Figure 4C). In the final sample from June 2018, the wild rye plots are still distinct, again indicated by differences in common seeded C₄ grasses tall dropseed, little bluestem, and side-oats (Figure 4D). The strength of the effect on cover-crops increased over time (Table 2).

We examined whether cover-crops changed the initial composition to produce distinct community trajectories relative to controls, with treatments expected to differ from controls based on early weed suppression. Treatments containing wild rye clearly initially assembled dissimilar communities from the other cover-crop or control plots, and over the sampling period, these treatments do not converge significantly with the others (Figure 5A). Excluding cover-crop species, the communities in all treatments tended to converge in successional trajectory rather than diverge (Table 3; Figure 5B & 5C). Neither treatment containing wild rye converged towards the control, but all 4 other treatments did (Table 3; Figure 5B & 5C).

We found greater support for the niche modification hypothesis than for the limiting similarity hypothesis. Proportions of functional groups differed significantly between treatments in June 2017, September 2017, and June 2018 (Table 4; Figures 6B, 6C, 6D), but not in September 2016 (Figure 6A). C₄ annuals decreased between each sample, and they did not differ

between treatments (Table 4; Figure 6). C₃ perennial grasses became more abundant over time and were significantly more abundant in plots with cover-crops compared to controls. Grass cover-crops, which were C₃ grasses, had significantly greater amounts of C₃ grasses compared to forbs. Furthermore, Canada wild rye had a greater amount of C₃ grasses compared to oats. There was no significant interaction between treatment and time (Table 4, Table 5). There were significant interactions between sampling period and treatment for C₃ annuals, forbs, and C₄ perennial grasses. Like the C₄ annuals, the C₃ annuals also decreased over time in all treatments, but they were proportionally more abundant in plots with exotic cover-crops (Table 5; Figure 6). Forbs and C₄ grasses both became proportionally more abundant over time in all plots. However, this effect was significantly stronger for forbs, and reduced for C₄ grasses in grass compared to forb, and in wild rye compared to oats cover-crop treatments (Table 5; Figure 6).

Discussion

We found cover-crop species varied in their effects on prairie establishment. Canada wild rye was especially effective at reducing weeds, however, it also greatly reduced establishment from the prairie seed mix, as well as total species richness and diversity. Overall, cover-crops had a net negative effect on prairie establishment, despite evidence that they do reduce weed abundance compared to controls. This effect was largely driven by the perennial species Canada wild rye, whereas other cover-crops were largely neutral in their effects on prairie establishment. When given priority, Canada wild rye significantly suppressed C₄ grasses from establishing from the mix, and improved proportional recruitment of forbs and other C₃ grasses. This is strong evidence for the niche modification hypothesis for early establishing prairie species.

Effects of Cover Treatment on Weed Suppression

While the goal of cover-crops is to reduce weeds, we found variability among cover-crop species in their ability to suppress weeds. The relationship between cover-crop variables (biomass in 2015, and proportional abundance in 2016) and volunteer plant abundance differed based on which cover-crop variable we used in our model (Figure 8). When we consider the mass of cover-crop species growing in the late fall when we planted them, this measurement is actually positively related to the proportional weed abundance in the following spring. This is likely explained by the fact that cover-crop biomass in fall led to lower proportional cover-crop abundance in the spring. There were few weeds growing in fall 2015, when tillage radish and oats were abundant (Figure 2). This is not surprising, considering the phenological aspects oat and radish growth that make them popular cover-crops. *Avena sativa* is used widely as a cover-crop, because it is cheap and establishes quickly, but it typically does not compete well with target perennials (Espeland and Perkins 2013). The effect of biennial radish (*Raphanus sativus* L.) on weed suppression comes primarily from competition with weeds during the fall, with these persisting in to the following spring (Lawley et al. 2012). We found that in the spring the cover-crops were added, the native species - primrose, and Canada wild rye - became established and they strongly competed with both volunteers and target prairie species. Thus, the net effect of all cover-crop treatments was to suppress prairie establishment, despite a slight indirect facilitative effect through competition with weeds (Figure 8B).

Effects of Treatment on Establishment Success

Canada wild rye likely had much stronger effects due to its perennial habit, consistent with the prediction that functional group may be the best predictor of long-term effects from species with temporal priority (Jarchow et al. 2012). Persistence of the cover-crop is clearly

important in determining its effect on the resulting community (Blong 2007). Perennial cover-crops may also be beneficial to prairie restorations that are managed with burning, since they provide a larger fuel load compared to annuals or controls, and the added fuel can lead to hotter and more complete burns which suppress invasive exotics such as smooth brome (Martin and Wilsey 2012).

The impressive establishment, persistence, and priority effects exhibited by Canada wild rye are consistent with previous work on this species specifically, and on perennial grasses generally. Canada wild rye is a better competitor than two of the most abundance invasive grasses in northern tallgrass prairie, *Bromus inermis*, and *Poa pratensis*, and this effect can be extreme when coupled with temporal priority (Ulrich and Perkins 2014). Also consistent with our findings, Burkle et al. (2015) found stronger priority effects of grasses, compared to a forb in terms of influence on community composition, and they found a strong negative relationship between pioneer grass productivity and species diversity. Similar to our results, in a study on functional group priority, a forb/grass combo did not perform better than the grass only treatment (Wohlwend et al. 2019). Several non-exclusive mechanisms may produce stronger priority effects from grasses compared to forbs, including higher germination rates (Hillhouse & Zedler 2011), higher productivity than forbs (McCain et al. 2010), grass-specific plant-soil feedbacks, or spatial niche-preemption due to various forms of clonal growth.

We did not find strong evidence that 2-species grass/forb combinations had stronger priority effects than monocultures. Two species of cover-crops are predicted to be better at suppressing weeds due to greater occupied niche breadth, but monocultures may actually provide better cover if total productivity is not higher in mixes (Florence et al. 2019).

Consistent with our results, a similar study in a European grassland, found a lack of strong priority effects on total richness from groups of dominant or subdominant species seeded 4 weeks before the target seed mix, but these treatments both suppressed non-target species richness and cover (Torrez et al. 2017). However, Torrez et al. (2017) found changes in functional composition over time were not related to priority treatments and their treatments began to converge in community composition. Our study area may be more fertile, which could potentially explain the stronger priority effects and lack of convergence we found.

Implications for Restoration and Community Assembly

Deterministic theory based on competitive hierarchies predicts that dissimilar communities should converge if propagules of the dominant species, here C₄ grasses (Wedin and Tilman 1993), are present. We did not find evidence for this, since the C₄ grasses *Bouteloua curtipendula*, and *Sporobolus asper*, occurred at high densities in other treatments, but were nearly absent from plots seeded to the native C₃ grass, *Elymus canadensis*. Relatedly, limiting similarity predicts that Canada wild rye (C₃) would reduce other C₃ grasses (Fargione et al. 2003), but we did not find support for this. Instead, ordinations showed that C₄ species were knocked back by Canada wild rye, likely due to shading (niche modification). C₄ grasses are not shade tolerant and grow best in full sun locations (Edwards et al. 2010). Niche modification would predict lower C₄ abundance, and fewer annuals recruiting in shadier environments.

We did not find evidence for a nurse plant effect, facilitating the establishment of seeded species compared to control plots. If there was facilitation by cover-crop treatments, these effects were not seen in the establishment measures over the first three years post-seeding.

Other Considerations

We did not measure differences in belowground production, but this is an important area of research that could have significant implications for use of cover-crops in grassland restoration (Körner et al. 2008, Weidlich et al. 2018). Additionally, species-specific priority effects can change with mycorrhizae inoculation (Burkle et al. 2015), and this may be an important management action available to restorationists.

Our experiment was conducted on land previously in row crops, which likely led to an annual-dominated rather than perennial weed-seed bank (Chapter 2). Because of this difference, land use history may interact strongly with cover-crop treatments and their effect on weed suppression. Future studies should examine the effects of cover-crop functional groups on weed suppression under variable resource availability.

Future work should also include more in situ tests of native legume cover-crops (Martin and Wilsey 2012), which may facilitate establishment of target species better than a grass or forb cover-crop (Körner et al. 2008, von Gillhaussen et al. 2014, Weidlich et al. 2017).

Conclusions

We provide evidence that cover-crops do tend to suppress weeds during early assembly of a grassland restoration, but they have a stronger effect on reducing target species establishment, especially when the cover-crop species is perennial. Annual and biennial cover-crops were mostly neutral in their effects on prairie establishment. Priority effects produced by cover-crop treatments significantly influenced community composition, functional group proportions, and species diversity through niche modification in the first three years of a tallgrass prairie restoration.

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Tables and Figures

Table 1. Results of one-way repeated measures ANOVA with *a priori* contrasts comparing restoration success measures among cover crop treatments over 3 years. Diversity corresponds to Simpsons 1/D, and mix establishment measures the proportional abundance of species from the seed mix.

	d.f.	<u>Richness</u>		<u>Diversity</u>		<u>Mix Establishment</u>	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treat	6	14.6	<.0001	22.95	<.0001	10.94	<.0001
Time	3	18.49	<.0001	10.48	<.0001	29	<.0001
Treat * Time	18	5.04	<.0001	2.98	<.0001	6.21	<.0001
Error	42						
<u>Contrast Treat</u>							
Control vs. all others	1	3.06	0.0852	6.11	.0162	0.71	0.4017
Two vs. One	1	2.22	0.1416	5.61	.0209	1.67	0.2005
Grasses vs. Forbs	1	15.53	.0002	25.49	<.0001	18.99	<.0001
Oats vs. Wild Rye	1	51.69	<.0001	64.35	<.0001	23.63	<.0001
Error	63						

Table 2. Results of permutational MANOVA comparing plant species composition among cover crop treatments in four samples over three years.

Source	d.f.	Sept. 2016		June 2017		Sept. 2017		June 2018	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
treat	6	5.2	<0.001	6.3	<0.001	12.1	<0.001	7.64	<0.001
Residual	63								
Total	69								
		<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
Control vs. Oats		3.15	0.0002	3.66	0.0002	5.49	0.0002	4.17	0.0002
Control vs. Radish		3.49	0.0002	3.27	0.0002	3.67	0.0002	3.63	0.0002
Control vs. Oats & Rad.		2.67	0.0002	3.66	0.0002	5.42	0.0002	3.19	0.0002
Control vs. Wild Rye		2.78	0.0002	3.06	0.0002	4.41	0.0002	2.94	0.0006
Control vs. Primrose		2.46	0.0002	2.27	0.0014	3.10	0.0002	2.23	0.0028
Control vs. W. Rye & Prim.		3.30	0.0002	3.90	0.0002	5.81	0.0002	4.24	0.0004
Oats vs. Radish		1.29	0.1004	1.28	0.0704	1.68	0.0078	1.34	0.0852
Oats vs. Oats & Rad.		1.79	0.0018	1.79	0.0052	2.49	0.0002	2.23	0.0008
Oats vs. Wild Rye		1.28	0.1182	1.28	0.0964	1.67	0.0050	1.61	0.0184
Oats vs. Primrose		2.97	0.0002	3.53	0.0002	5.48	0.0002	4.34	0.0002
Oats vs. W. Rye & Prim.		1.04	0.3670	1.25	0.1084	1.74	0.0050	1.37	0.1054
Radish vs. Oats & Rad.		1.89	0.0026	1.55	0.0142	2.24	0.0002	1.63	0.0118
Radish vs. Wild Rye		1.21	0.1654	1.15	0.2008	1.48	0.0404	1.17	0.2156
Radish vs. Primrose		3.33	0.0004	3.21	0.0002	3.80	0.0002	3.77	0.0002
Radish vs. W. Rye & Prim.		1.62	0.0084	1.63	0.0104	1.80	0.0102	1.41	0.0856
Oats & Rad. vs. Wild Rye		1.43	0.0500	1.34	0.0786	1.66	0.0128	1.34	0.0746
Oats & Rad. vs. Primrose		1.98	0.0002	3.09	0.0002	5.10	0.0002	2.90	0.0002
Oats & Rad. vs. W. Rye & Prim.		2.08	0.0002	2.12	0.0002	2.61	0.0002	2.16	0.0048
Wild Rye vs. Primrose		2.74	0.0002	3.03	0.0002	4.63	0.0002	3.11	0.0002
Wild Rye vs. W. Rye & Prim.		1.55	0.0224	1.73	0.0022	2.04	0.0002	1.63	0.0248
Primrose vs. W. Rye & Prim.		3.15	0.0002	3.61	0.0002	5.81	0.0002	4.30	0.0002

Table 3. Results of symmetric convergence / divergence test for a prairie community assembling into control plots or plots that received a cover crop treatment. Values above diagonal correspond to the statistic (τ) of the Mann-Kendall test. Negative values indicate trajectories are converging, and positive values indicate they are diverging. Significance level is indicated by p-values below the diagonal.

Treatment	C	O	R	O&R	W	P	W&P
Control (C)	.	-.55	-.35	-.34	-.21	-.48	-.12
Oats (O)	<.001	.	-.39	-.25	-.13	-.37	-.10
Radish (R)	.0014	.0004	.	-.14	-.21	-.34	-.06
Oats & Radish (O&R)	.0018	.0261	.1999	.	-.24	-.23	-.15
Wild Rye (W)	.0574	.2344	.0575	.0293	.	-.21	-.16
Primrose (P)	<.001	.0008	.0022	.0391	.0586	.	-.02
Wild Rye & Prim. (W&P)	.2837	.3821	.6082	.1764	.1498	.8338	.

Table 4. Results of MANOVA comparing functional composition of prairies seeded into one of six cover crop treatments. Comparisons were conducted independently in four samples over three years. Test statistics are presented for Pillai's Trace.

Source	d.f.	Sept. 2016		June 2017*		Sept. 2017		June 2018	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Numerator	24	1.52	.0595	1.86	.0103	2.86	<.0001	3.16	<.0001
Denominator	252/248*								
Total	280								
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
C ₃ Annuals		1.54	.1800	3.08	.0105	2.67	.0225	4.22	.0012
C ₄ Annuals		0.89	.5077	1.08	.3848	0.88	.5159	0.74	.6204
Forbs		1.93	.0902	1.77	.1196	9.09	<.0001	6.35	<.0001
C ₄ Perennial Grasses		2.13	.0619	1.94	.0878	9.93	<.0001	4.66	.0006
C ₃ Perennial Grasses		1.00	.4347	2.66	.0231	1.91	.0932	3.73	.0031

Table 5. One-way repeated measures ANOVA with *a priori* contrasts comparing proportions of plant functional groups between cover-crop treatments.

	d.f.	<u>C₃ Annuals</u>		<u>C₄ Annuals</u>		<u>Forbs</u>		<u>C₄ Per. Grass</u>		<u>C₃ Per. Grass</u>	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treat	6	1.91	.0928	.66	.6858	8.07	<.0001	5.80	<.0001	5.24	.0002
Time	3	13.58	<.0001	140	<.0001	26.09	<.0001	44.34	<.0001	6.53	.0003
Treat * Time	18	2.40	.0018	1.00	.4659	2.77	.0003	2.87	.0002	1.58	.0675
Error	42										
<u>Contrast Treat</u>											
Control vs. all others	1	1.22	.2733			0.17	.6793	0.48	.4890	4.87	.0309
Two vs. One	1	1.00	.3207			3.75	.0572	1.61	.2086	0.25	.6183
Grasses vs. Forbs	1	0.23	.6346			4.47	.0385	9.17	.0036	9.42	.0032
Oats vs. Wild Rye	1	2.17	.1459			12.78	.0007	10.68	.0018	12.41	.0008
Error	63										

Table 6. Statistical results of one-way ANOVA with *a priori* contrasts comparing weed abundance and vegetation structure between cover-crop treatments 3 years after establishment.

	d.f.	<u>Dandelion</u>		<u>Thistle</u>		<u>Total Biomass</u>		<u>Canopy Height</u>		
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Treat	6	3.12	.0096	1.82	.1086	0.61	.7189	1.83	.1076	
Error	63									
<u>Contrast Treat</u>										
Control vs. all others		12.81	.0007	4.16	.0457	0.78	.3798	0.12	.7286	
Two vs. One		1.12	.2942	0.76	.3853	0.02	.8899	1.47	.2296	
Grasses vs. Forbs		0.01	.9053	2.69	.1061	1.23	.2711	4.64	.0351	
Oats vs. Wild Rye		2.71	.1049	2.74	.1028	1.35	.2500	4.02	.0492	
		R ² =0.23		R ² =0.15		R ² =0.06		R ² =0.14		

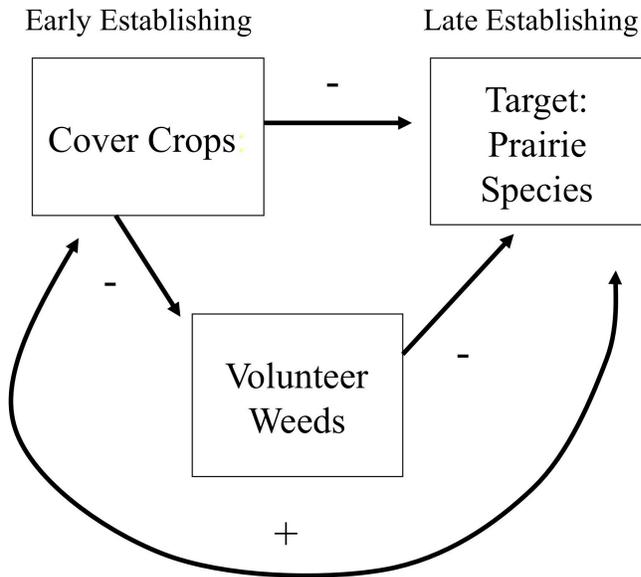


Figure 1. Conceptual diagram illustrating the potential net effect of cover crop treatments through both direct competitive interactions with seeded prairie species and indirect effect of weed suppression, which may facilitate establishment of target species (Wilsey 2020).

Biomass production of cover crops during late Fall 2015

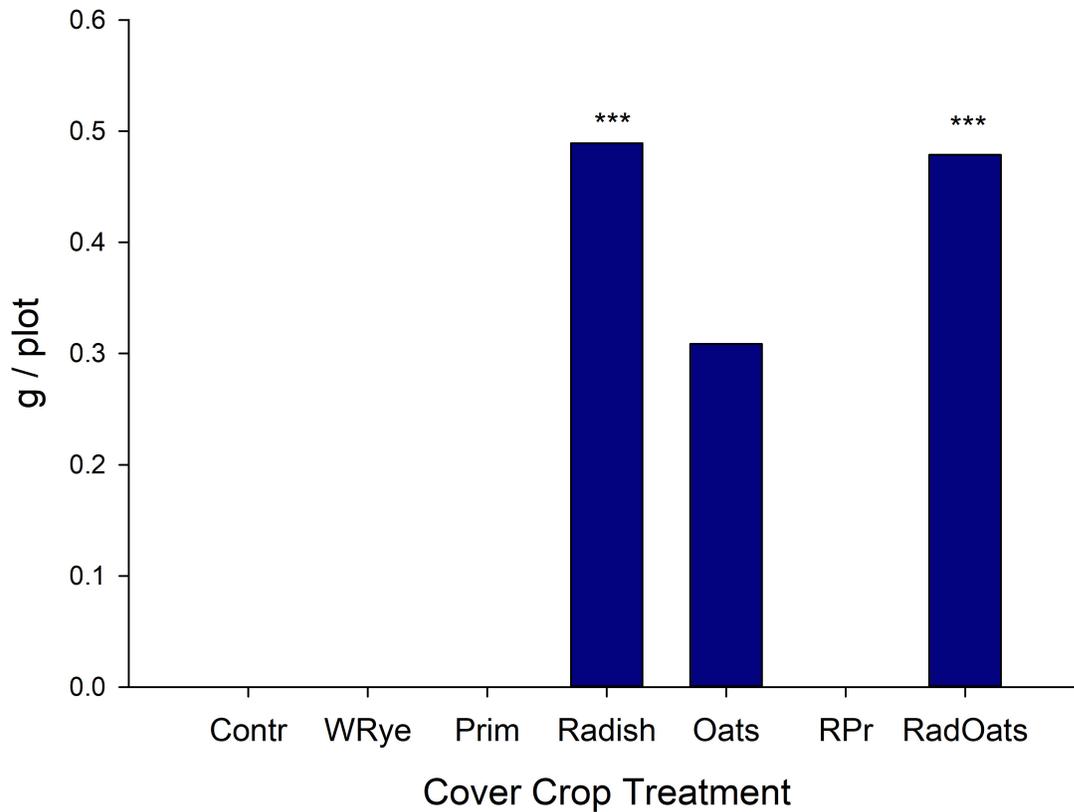


Figure 2. Biomass during late fall/ early winter 2015 in our cover crop experiment at Brushy Creek Recreation Area. There was no biomass in control plots, Canada wild rye (WRye = *Elymus canadensis*), primrose (Prim = *Oenothera biennis*), or mixed Canada wild rye and primrose plots (RPr). *** denotes treatments that were significantly greater than the control plots, which occurred in Radish, Oats, Radish + Oats (RadOats).

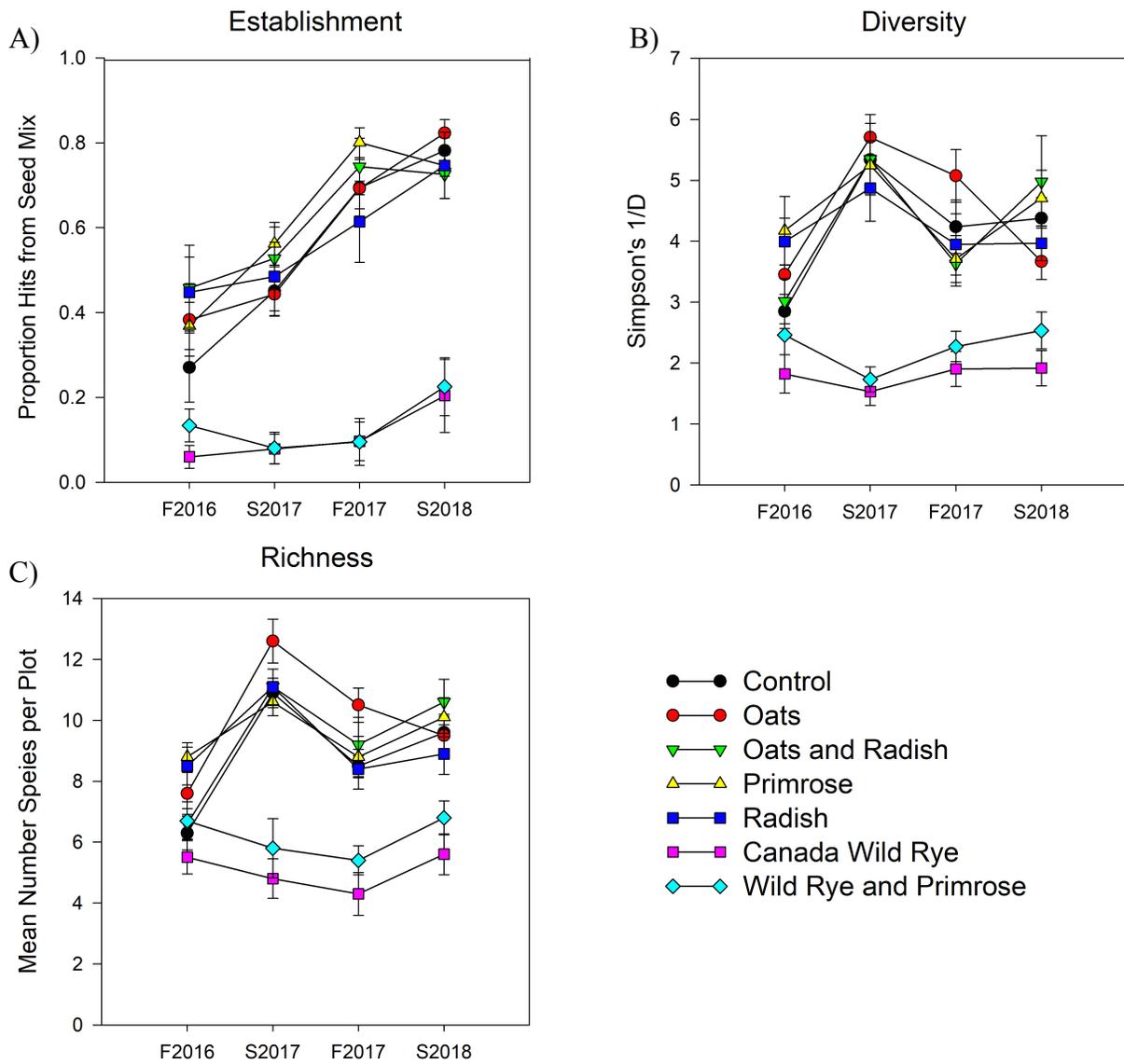


Figure 3. Cover Crop community response measures over two years. Error bars denote standard error. F2016 and F2017 samplings took place in September. S2017 and S2018 took place in June.

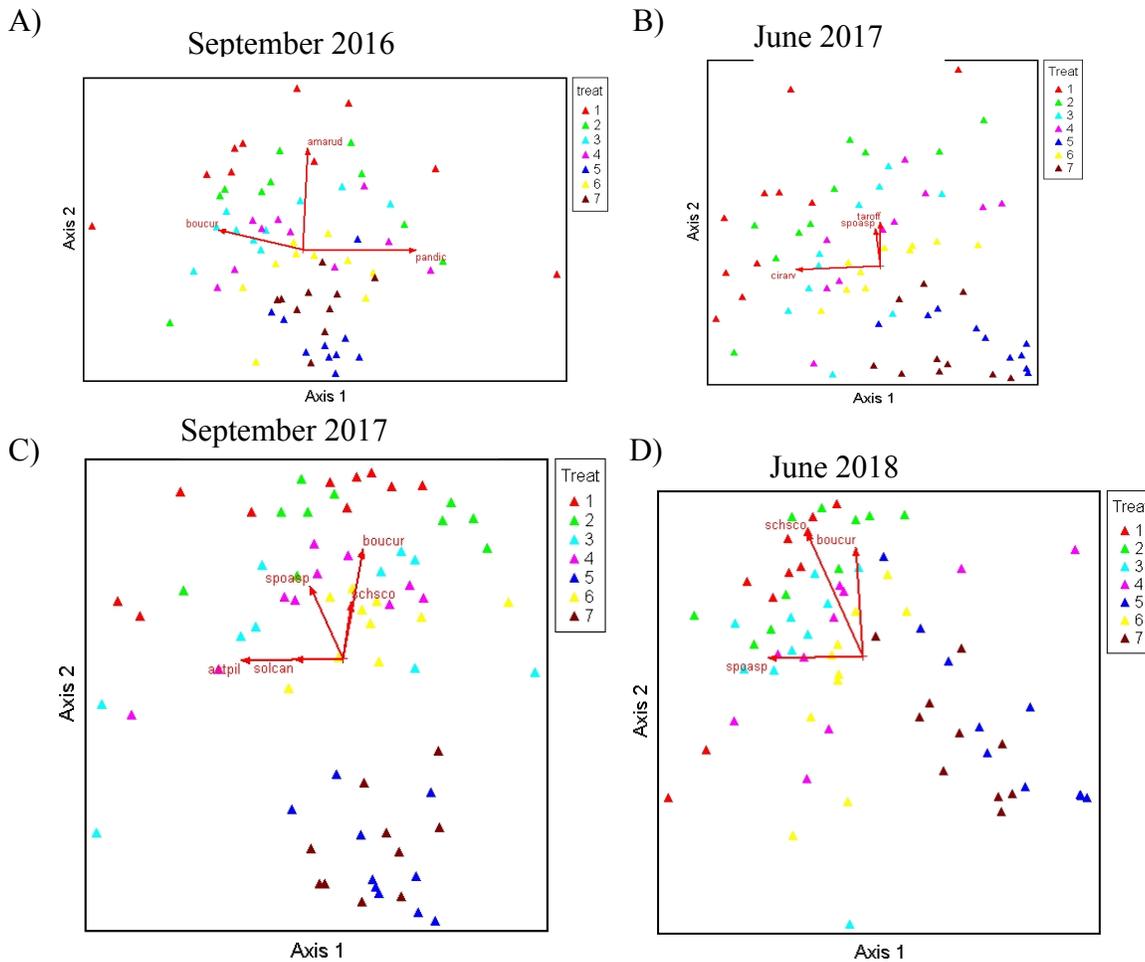


Figure 4. Plots of plant species composition among cover crop treatments in June and September of each year. Axes were most related to the abundance of *Bouteloua curtipendula* (side-oats grama), *Amaranthus rudis* (water hemp), *Cirsium arvense* (thistle), *Taraxicum officinale* (Dandelion), *Sporobolus asper* (Tall dropseed), *Aster pilosus* (Frost Aster), *Solidago canadensis* (Canada goldenrod), and *Schizachyrium scoparium* (little bluestem). Treatment numbers displayed in the legends correspond to the following treatments: 1 – Control, 2 – Oats, 3 – Radish, 4 – Oats & Radish, 5 – Wild Rye, 6 – Primrose, 7 – Wild Rye & Primrose.

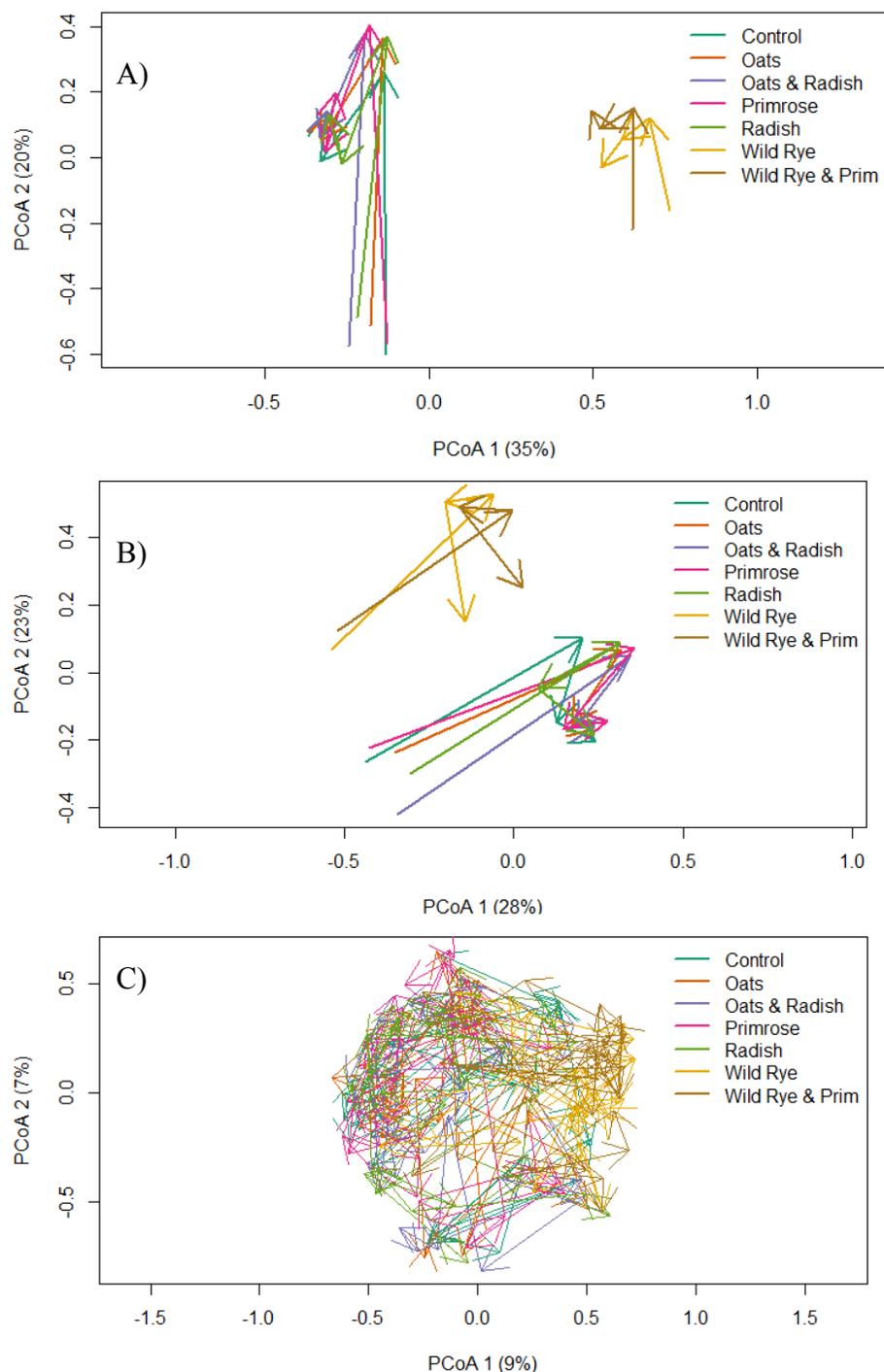


Figure 5. Principal Coordinates plots showing trajectories in community composition in four samples taken over three years in a tallgrass prairie with 6 cover crop priority treatments and control plots. A) shows each treatment including the cover crop species added. B) excluding cover crop species C) shows all 70 plots over time, not summed by treatment.

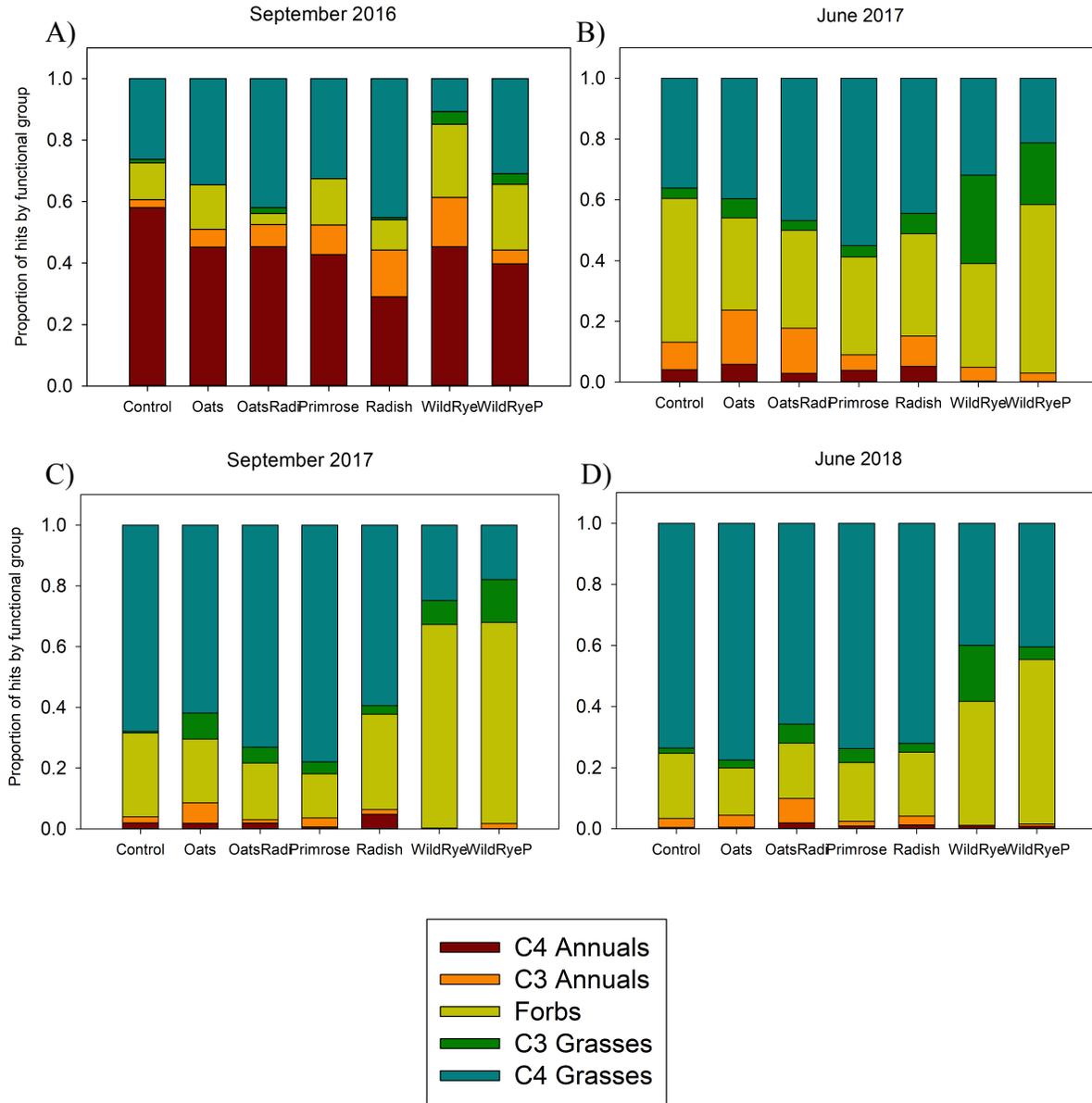


Figure 6. Composition differences in proportionality of functional groups by cover crop treatment in four samples over three years in a prairie restoration.

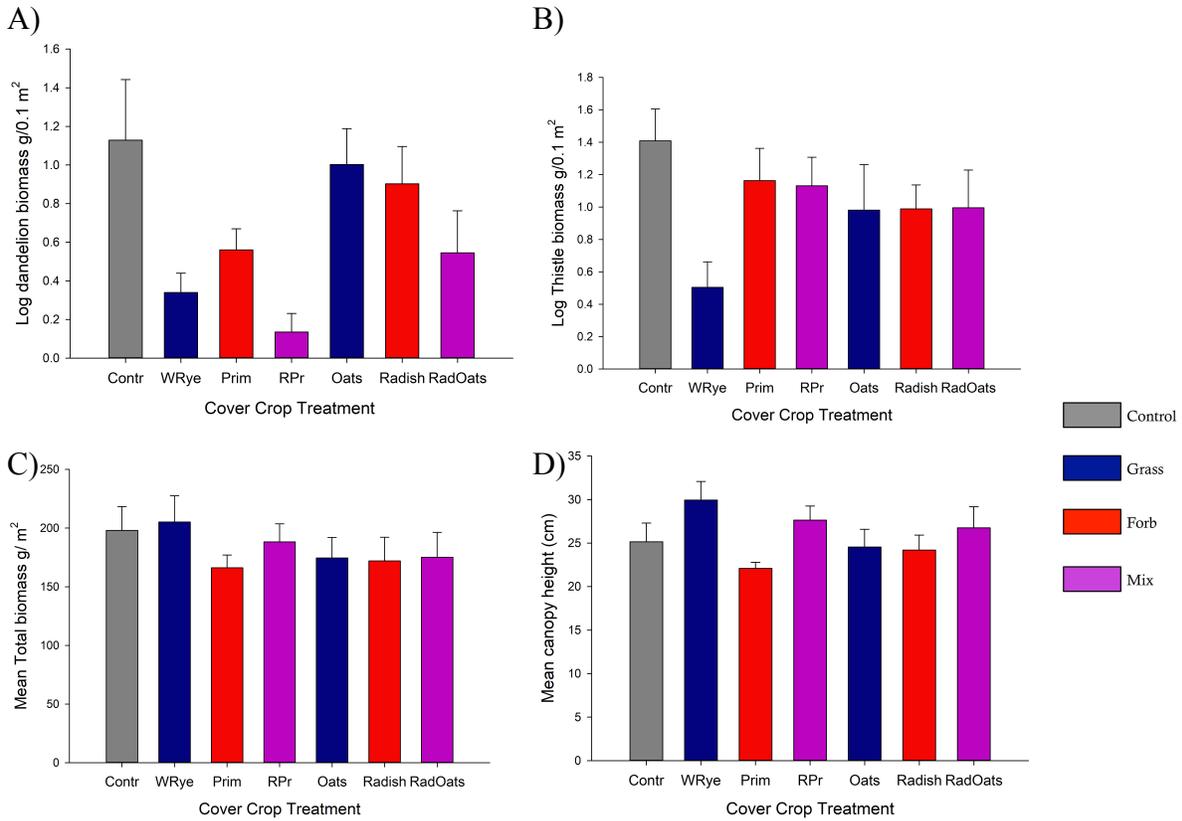


Figure 7. Biomass of dandelion (the most common invader at the time) in cover crop treatments (means+SE). Cover crops also reduced the biomass of thistles in the plots. Treatment abbreviations are: WRye = Canada wildrye, RPr = Canada Wildrye + Primrose, Prim = Primrose alone, Radish = Tillage Radish, Oats, RPr = Radish + Oats, and DOT = DOT mix.

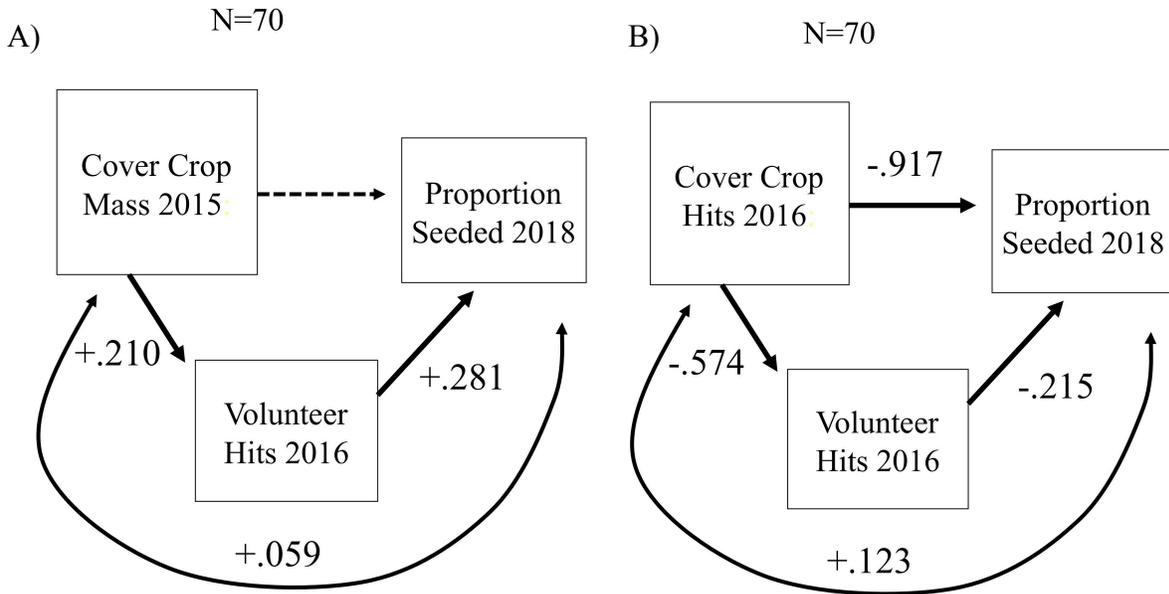


Figure 8. Path diagrams showing direct effect of cover crop treatment on establishment success as measured by proportion of hits from target seeded species, and indirect effects on establishment mediated through suppression of volunteer weeds early in establishment.

CHAPTER 5. LIMITED EVIDENCE FOR SEED LIMITATION DURING THE FIRST THREE YEARS OF ASSEMBLY IN A TALL GRASS PRAIRIE RESTORATION

Andrew D. Kaul and Brian J. Wilsey

Iowa State University, Department of Ecology, Evolution, and Organismal Biology

Abstract

Prairie grassland restorations often do not achieve the diversity of the target seed mix, due to a combination of failure to recruit all species from seeds, and because warm season C₄ grasses often dominate the community after only a few years. Species diversity in these prairies may be dispersal limited, microsite limited, or both. If dispersal limitation is primarily restricting assembly, then seed additions should increase diversity. Adding seeds in small amounts yearly should lead to establishment of a greater number of species due to niche sorting, where establishment matches environmental conditions of a given year. However, in cases where microsites are limiting, adding seeds will not increase diversity without some form of disturbance that creates openings. Dominant grasses, which are C₄ grasses in tallgrass prairie, can affect microsites. We tested whether species diversity during restoration was limited by seed additions or dominant grasses. We use a novel approach to testing seed and microsite limitation on species diversity during restoration. We added a 32 species seed mix either in the 1st, 2nd, or 3rd year after initial seeding, or 1/3 of the seed mix added annually, to test whether additions increase diversity. This treatment was crossed with C₄ grasses to test the alternative hypothesis that establishment is impacted more by dominant species effects on microsite formation. After three years, no seed addition treatments significantly changed total species richness or diversity compared to controls. When only considering the species from the seed addition, annual and single-year addition treatments did not increase their species richness compared to controls. We

did not find evidence for additions significantly altering the trajectory of community development, and the C₄ grass addition had negligible effects on community composition and diversity. Together, these results highlight the importance of establishing target species early during restoration, as microsite limitation can occur quickly, even in the absence of dominant C₄ grasses.

Introduction

To date, restorations of tallgrass prairie generally fail to achieve the diversity of remnants (Polley et al. 2005, Martin et al. 2005). This may indicate an incomplete understanding of the key community assembly processes in this system. Plant species diversity is often used as a measure of prairie restoration success because higher species diversity in grasslands has been linked to a suite of ecosystem functions and services including stability of productivity, nutrient cycling, value to pollinators, and cultural value (Tilman & Downing 1994, Bengtsson et al. 2019). Conventional restoration practices assume species diversity will increase over time as more species have opportunities to establish during years with favorable conditions, but previous work has found that diversity often peaks in the first few years after planting and decreases thereafter (Sluis 2002, Wilsey 2018).

These declines in diversity in tallgrass prairie restorations are often driven by perennial grass dominance, which can develop quickly in the first few years following initial seeding (Sluis 2002; Camill et al. 2004; Grman et al. 2013). This effect is mostly due to perennial warm-season C₄ grasses, which are the tallest and most productive prairie plants (Kindscher & Tieszen 1998, Baer et al. 2004). This dominance by a few species leads to species poor restorations, since the majority of species diversity in prairies comes from the large species pool of forbs and cool-season C₃ grasses (Collins & Glenn 1990, Howe 1999). Key questions in prairie restoration

ecology are thus, how to recruit higher species diversity during assembly, and how to suppress dominant C₄ grasses, in favor of opening up microsites for other species.

Community composition during restoration is ultimately determined by dispersal constraints, environmental constraints (abiotic filtering), and internal constraints (biotic filtering), such as competition (Belyea & Lancaster 1999). Dispersal limitation describes how much species richness and diversity are constrained by the availability of seeds. Alternately, microsite limitation (sometimes ‘establishment limitation’) occurs when seed availability is not a constraint on species diversity, but rather niche availability constrains it, such that when they arrive, seeds of novel or rare species do not germinate or establish, due to unfavorable abiotic, or biotic conditions (Eriksson & Ehrlén 1992). Each species from the local pool can be seed limited, microsite limited, or both (Eriksson & Ehrlén 1992, Zobel et al. 2000, Foster et al. 2004).

Foster et al. (2004) describe three ways the relative importance of these limitations may change as a function of standing biomass in the plant community. First, under the niche limitation hypothesis, irrespective of standing biomass, microsites are limiting and seed additions have little effect because there is low availability of space for seedlings to recruit. Secondly, the species pool hypothesis predicts that there is strong seed limitation and low microsite limitation, irrespective of biomass. The third view, the shifting limitations hypothesis, predicts that communities with less standing biomass will have more microsites and thus seed additions are predicted to have strong effects on diversity, but as biomass increases, microsite limitation increases and seed additions are less effective (Foster et al. 2004).

In addition to standing biomass, the effect of seed additions may be determined strongly by niche sorting in response to extrinsic abiotic factors, causing “year effects” when there is

significant inter-annual variation in temperature and precipitation which affect which species establish well that year (Stuble et al. 2017). Each species may respond differently to weather variation, affecting germination, survival, or growth (Zavaleta et al 2003). Restorations seeded with the same mix, but in different years, often produce difference communities (Bakker et al. 2003, Vaughn & Young 2010, Grman et al. 2013, Stuble et al. 2017), and this effect likely also applies to seed additions, however that has not been previously tested.

Seed limitation, and thus seed additions, may be very important for grassland restorations on land with a legacy of agricultural use (Tilman 1997). In former agricultural fields, there is likely no seed bank for target prairie species, and many prairie restorations occur in a matrix of land being cropped, so there is very low probability that target species will disperse into a given restoration site (Foster et al. 2004, Damschen et al. 2006). Due to lower germination rates, on average, native forbs and legumes establish at lower rates than grasses (Hillhouse & Zedler 2011), but with continued additions, sufficient propagule pressure may lead to recruitment of rare forbs. Dispersal-limitation is often only tested with a single addition (Martin & Wilsey 2014, Pinto et al. 2014), yielding mixed results, however the effect of an addition may depend on the year, as described above. Multiple additions may result in more recruitment of missing species from the mix than a single one because this practice could lead to each species having an opportunity to recruit in multiple years with variable growing-season weather.

When seed arrival is not limiting recruitment, establishment may be restricted by microsite limitation from dominant species, especially C₄ grasses (Wilsey & Martin 2015), and seed additions will have little or no effect (Sluis 2002, Camill et al. 2004, Dickson & Busby 2009). Several studies have investigated the effects of reducing or removing C₄ grasses on prairie composition and species diversity (Collins 1987, Hartnett et al. 1996, Howe 1999, Smith &

Knapp 2003, Silletti et al. 2004, McCain et al. 2010), finding that removal of these dominant species can lead to greater light availability, forb recruitment and species richness and diversity (McCain et al. 2010). Instead of removing these species, we conducted a seed addition study in a restoration that contained only trace amounts of 3 dominant C₄ grass species from the mix at the outset. We then added these C₄ species along with addition treatments in the second year of a restoration to test the importance of their abundance on seed limitation.

Specifically, we seek to answer three questions. 1) Is seed (dispersal) limitation reducing diversity in a new prairie restoration? 2) Does C₄ grass dominance early in reconstruction reduce the establishment of other seeded species? 3) Is there evidence for strong niche sorting which would lead to higher diversity in plots seeded annually compared to a single year?

Methods

We tested dispersal limitation as a mechanism for reducing diversity in a restored tallgrass prairie by experimentally adding propagules in the form of a diverse seed mix, and examined whether the effect of addition varied based on the year the seed was added comparing plots seeded in one of 3 successive years of addition, or plots seeded in each of these 3 years (annually). We simultaneously tested whether the addition of dominant C₄ grasses reduced diversity, changed composition, or interacted with timing of seed additions.

Study Design

The study was conducted at Brushy Creek Recreation Area (Iowa, USA) in a 6.5-hectare field previously in corn/soy rotation. The entire field, including all research plots, received a diverse prairie-seed mix in winter of early 2016. The mix included 15 graminoid species, and 52 species of forbs and legumes. The entire area was mowed during the first growing season (2016),

and once during the spring of the second year (2017), and burned in late April of the third year (2018).

We established 60 circular experimental plots, 2 m in diameter, in March of 2017. Using a two-way factorial design, plots were randomly assigned to one of 5 seed addition treatments, and half of the plots in each of these treatments received a C₄ grass addition in the first year, thus we produced 6 replicates for each of the 10 possible treatment combinations. The 5 addition treatments included a diverse mix seeded 1) in the first year after the prairie was established (2017; year 1), 2) an addition in year 2 (2018), 3) an addition in year 3 (2019), 4) an annual addition in years 1, 2, and 3 with each addition being 1/3 of the one-year treatments, or 5) control plots with no addition.

The seed mix we added included a subset of the species that were in the initial restoration seed mix. We developed a 32-species mix based on the reliability of obtaining all species in sufficient quantities for all treatment years (Appendix C: Table S1). This mix included 6 graminoid species (4 grasses and 2 sedges), and 26 species of forbs and legumes. Each species was seeded to treatment plots at 7x its background seeding rate, so species were not equally abundant in the mix, but the relative abundances of these 32 species in the addition mix was the same as their relative abundances in the initial 67 species mix. At this rate, each single-year addition plot received 20.75 g of seeds. Based on mean seed mass by species, we estimate this treatment contained over 4,000 seeds of forbs and legumes. The 12 plots seeded annually for our three treatment years received a third of this 7x mix, and thus received 3 total additions of 32 species, with each at 2.3x the background rate.

The C₄ grass addition was seeded simultaneously with the year 1 additions, and included 3 species: *Andropogon gerardii* (Big bluestem), *Sorghastrum nutans* (Indian grass), and

Panicum virgatum (Switchgrass). These species were included in the initial mix, but at low abundances. We added a mix of these three species at rates of 11lb/acre (1.12kg/ha) for *Andropogon gerardii*, and 10 lb/acre (1.1203 kg/ha) for *Sorghastrum nutans*, and *Panicum virgatum*. This translated to 7.39 g of this mix/ plot, or 2.35 g/m², which is approximately 10 x the recommended rate for sowing these grasses to avoid their dominance (Dickson & Busby 2009).

Sampling Design

We sampled the plant community in each plot in June and September of 2017, 2018, and 2019. We used point-intercept sampling to estimate cover and the number of species (Jonasson 1988). We placed a 1 m² sampling-frame, 1 m tall, with 9 pins directly over each plot, and recorded the identity and number of species present, and the number of point contacts for each species. The summation of hits layered vertically in the canopy for each species provides a good proxy for biomass (Xu et al. 2015). Species that were present in the plot, but did not touch any of the 9 pin-drops, received 0.5 hits for the plot. This sampling method provided information on the relative abundance of target, seeded species and other species including volunteers and species seeded in the initial mix, but not in the additions.

For each plot in each sampling period, we calculated total species richness, Simpson's species diversity (presented as 1/D), and then only considering the species in the addition mixes, we calculated added richness, and abundance of added species (total richness = richness of added species + richness of non-added species). Plot-level measures were calculated as mean measures from the two samples within each year. To evaluate the strength of the C₄ grass addition treatment, we also calculated the abundance and richness of those three species in each plot.

Statistical Analyses

We used a two-way ANOVA with a priori contrasts to test our predictions about our four establishment variables in each sample year. Comparisons were specified for each treatment in each year, since the structure of appropriate controls for each treatment differed by year. For example, in the first addition year, there were 12 plots that received a full addition, 12 plots received a 1/3 addition, and 36 plots were controls, including plots that were controls in all three years, as well as plots that would later receive additions in year 2 or 3. A two-way ANOVA allowed us to test for effects of target seed mix addition, *C*₄ grass seed addition, or their interactive effects on diversity and richness. In analyses for 2018 and 2019, we used contrasts to test for “lag effects” where a treatment is compared to appropriate controls for differences appearing 1, or 2 years after an addition treatment was applied.

We tested predictions about treatment effects on community composition using PerMANOVA and community trajectory analysis (CTA). We analyzed effects each year independently with PerMANOVA using Bray-Curtis distances in a two-way factorial design. Tests of significance were conducted using a randomization test of pseudo *F* values based on 4999 randomizations in PC-ORD 5.31 (Anderson 2001). To test if compositions began to diverge from controls over the 3 years, we conducted a symmetric convergence / divergence test using the ‘vegclust’ package in R (De Cáceres et al. 2019).

Results

Species Diversity and Mix Establishment

Seed addition treatments did not increase species diversity or the abundance of added species compared to controls, but some treatments did increase richness of added species, and

total species richness in later samples (Table 1). No treatments differed in their diversity, richness, or recruitment of added species in the first year.

In the second sample year (2018) there was higher total richness in annually seeded plots compared to control plots, or those that were seeded that spring (2018 treatment) (Figure 1A). Richness of added species was higher in annually seeded, and year 1 plots, than controls, and in year 1 compared to year 2 plots (Figure 1C). Diversity in annually seeded plots was slightly higher than controls, but this effect was not significant (Figure 1E).

In the third sample year (2019), no significant differences in total richness between treatments was detected (Figure 1B), but richness of the added species was higher in plots seeded annually, or the previous 1 or 2 years compared to controls or those plots with additions in 2019 (Figure 1D). Seeded species richness was also higher in the plots with species added one year prior, compared to those seeded annually, or two years prior (Figure 1D). Again, species diversity was slightly higher in the annual addition treatments compared to controls but this effect was not statistically significant (Figure 1F).

The only response variable that shows evidence of a lag effect is the richness of added species. In year 1 (2017), the year 1 plots were not significantly different from controls, but they were in 2018 (Figure 1C) and this effect persisted into 2019 (Figure 1D). Similarly, the plots seeded in year 2 (2018) were not different from controls in 2018 (Figure 1C), but they were in 2019 (Figure 1D).

There was no significant interaction between mix and C₄ grass addition treatments in any samples (Table 1), but in the third year, we found a slightly lower richness of added species in the plots that also received C₄ grass additions compared to plots that did not (Figure 2).

Species Composition

C₄ grass additions did not significantly affect species composition (Figure 3B, 3D, 3E), but addition treatments did, with these effects being dependent on sampling year (Table 2). No treatments differed in their composition in the first sampling year (2017) (Figure 3A). The second year, plots seeded that spring differed in composition from control plots, with seeded plots having higher abundances of sown grasses *Schizachyrium scoparium*, and *Bouteloua curtipendula*, and lower abundances of volunteer species including Canada thistle and native goldenrods (Figure 3C). In 2019, the 2018 addition treatment differed from controls similarly as in the previous year, and additionally the 2018 addition treatment was also distinct from plots with additions in 2017 or 2019. The 2018 plots differed from those with additions a year earlier, or later due to having higher abundances of the two added native grasses mentioned above, and less of the volunteer species frost aster, or giant goldenrod (Figure 3E).

Although some treatments differed in community composition in some years as described above, we did not find evidence for any treatments significantly converging or diverging over the three years this experiment was conducted (Table 3, Figure3).

Discussion

We found a lack of evidence for seed limitation on plant diversity in this prairie restoration. Surprisingly, there was also little effect on recruitment of seeds from three dominant C₄ grasses. At the end of our study, species richness did not differ between seed addition treatments, and additions never significantly altered the abundance of added species compared to controls or based on their timing. However, additions did increase added-species richness compared to controls, and annual additions increased species diversity slightly, through an effect on evenness. Community composition differed in the plots seeded right before a burn in the

second year, favoring added grasses over volunteer forbs. Our results indicate that microsite rather than seed limitation on recruitment of plant species diversity was more important during the first three years of restoration post seeding in a tallgrass prairie.

It is not well understood when local productivity influences the extent to which seed availability constrains diversity in grasslands. However, the shifting-limitations hypothesis predicts that the effect of seed additions on richness will decrease under conditions of higher standing biomass or productivity (Foster et al. 2004). Previous work has shown that even established grasslands can be seed limited, and vary greatly in their microsite limitation (Zeiter et al. 2006). Consistent with a meta-analysis on similar seed addition experiments, we found evidence for seed limitation, but the effect size was small, indicating stronger microsite limitation on richness, perhaps because prairies establish a full canopy rapidly, reducing light availability for seedlings (Clark et al. 2007). These trends stand in contrast to grassland addition experiments in drier or less productive sites, which have shown large increases in richness due to seed addition, this effect being stronger than competition or niche limitation (Tilman 1997, Zeiter et al. 2006, Pinto et al. 2014). A seed addition experiment in a mesic prairie, found aboveground biomass in the year of addition significantly reduced the effect of additions on species richness (Russell and Roy 2008). Our study took place in central Iowa, in comparatively productive, mesic grassland. Under the shifting limitations hypothesis, the higher water availability in our site may explain the minimal effect of seed additions on species richness.

Seed addition studies that include disturbances (creating available microsites) generally find higher recruitment than without disturbance. This is consistent with co-limitation of seed and microsite availability (Foster et al. 2004, Clark et al. 2007, Long et al. 2014, Wilsey and Martin 2015). We did not include disturbance in our study, but tested whether the addition of

three species of dominant grasses would affect diversity or interact with effects of seed addition. The C₄ grass addition was predicted to increase biomass (the inverse of disturbance) because these species are typically exceptionally productive. We did not find strong evidence for this effect, perhaps because in the absence of these three typically dominant grasses, other grass species, including *Schizachyrium scoparium* (C₃), and *Bouteloua curtipendula* (C₄), became very abundant early in assembly, contributing to low recruitment of the added C₄ grasses and the target seed additions.

In contrast to seed limitation based on dispersal, many processes can contribute to microsite limitation in the broad context of niche limitation. Seeds need physical space to recruit, but other biotic and abiotic constraints reduce the potential of a viable seed to recruit, including being blown away, being eaten by vertebrates (Howe & Brown 1999, Pellish et al. 2018), or invertebrates (Linabury et al. 2019), failing to germinate, succumbing to disease (Clark & Wilson 2003), or being outcompeted (Carrington 2014). The likeliness of each of these fates may differ by species based on seed or vegetative traits. Successful seedling establishment is very rare in tallgrass prairie, with over 99% of new shoots in burned and unburned prairie coming from vegetative reproduction (Benson & Harnett 2006). When soil is disturbed consistent with small mammal activity, this provides opportunities for recruitment of more ruderal or rare species, (Platt 1975) however, vegetative recruitment and re-growth still dominate re-vegetation, even with seed additions (Rogers & Hartnett 2001). Considering this, competition may be the most important driver of microsite limitation, but many factors may interact to reduce the likeliness of recruitment from seeds in the context of seed additions.

We observed a trend consistent with inter-annual niche sorting, where species diversity was slightly higher in plots with seeds added each year, but this effect was not statistically

significant. This suggests that repeated seed additions may help to increase diversity in a restored prairie, but this effect may be very small, especially compared to the costs associated with seed addition. Seed rain from existing vegetation can also be extremely high in native prairies, with the highest estimates around 37,200 – 91,200 seeds per m² (Schott & Hamburg 1997). This means that seedlings from added seeds must compete not just with established vegetation, but also with the propagule pressure from those already established plants. Martin & Wilsey (2006) found that seed additions were important for seedling emergence 4 months after seeding, but in a follow-up study, they found these effects did not last, and there was no effect of addition on rare species recruitment (Wilsey & martin 2015). Considering that grassland community states tend to be stable and resistant to change once established (Martin & Wilsey 2014), and seed additions in the absence of disturbance often fail to produce measurable differences in richness or composition, this underscores the necessity of early recruitment of target species in prairie restoration (Piper & Pimm 2002).

Conclusions

In mesic grasslands, restoration efforts should be focus on initial seeding, as we find lack of evidence for recruiting missing species from a mix with later addition. In other systems, connectivity and dispersal may be more important, but in high productivity prairie, there is very limited evidence for recruitment from inter-seeding leading to higher diversity plant communities. Even with addition of species in annually for three years, we did not find strong evidence for substantially increased their recruitment compared to a single addition or controls. These results are consistent with previous work showing strong microsite limitation in productive grasslands, and thus the need for disturbance to create microsites, which added seeds may utilize. Interestingly, we found that three typically dominant warm-season grasses remain at

low abundances when seeded at a high rate a year after establishing a diverse prairie. This may be an effective way to prevent dominance-diversity declines in future prairie restorations.

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Tables and Figures

Table 1. Two-way ANOVA with *a priori* contrasts, based on type III SS, comparing effects of target seed addition timing and presence of dominant C₄ grasses on species diversity over 3 years in a restored tallgrass prairie.

	d.f.	<u>Richness</u>		<u>Richness Added</u>		<u>Diversity</u>		<u>Added Species</u>		
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
2017										
Treat	4	1.60	.1897	2.15	.0880	1.05	.3927	1.53	.2069	
C ₄ addition	1	0.70	.4064	0.32	.5733	1.69	.1990	0.03	.8565	
Treat * C ₄ addition	4	2.30	.0715	1.32	.2763	0.56	.6955	1.02	.4073	
Error	50									
2018										
Treat	4	2.37	.0648	3.96	.0072	1.52	.2118	0.88	.4835	
C ₄ addition	1	0.10	.7504	0.38	.5388	0.19	.6688	0.06	.8139	
Treat * C ₄ addition	4	1.94	.1184	0.57	.6862	2.04	.1030	1.02	.4068	
Control vs. Seeded in Year 1	1	2.51	.1193	10.86	.0018					
Control vs. Seeded in Year 2	1	0.03	.8682	0.72	.4008					
Control vs. Seeded Annually	1	7.13	.0102	8.52	.0053					
Year 1 vs. Annual	1	0.88	.3522	0.11	.7457					
Year 2 vs. Annual	1	6.03	.0176	3.22	.0789					
Year 2 vs. Year 1	1	2.30	.1355	4.49	.0390					
2019										
Treat	4	0.86	.4940	8.88	<.0001	1.52	.2099	0.30	.8764	
C ₄ addition	1	0.02	.8923	4.17	.0465	0.03	.8740	0.01	.9321	
Treat * C ₄ addition	4	0.49	.7456	1.63	.1818	1.23	.3082	0.95	.4413	
Control vs. Year 1	1			12.73	.0008					
Control vs. Year 2	1			25.98	<.0001					
Control vs. Year 3	1			0.46	.4999					
Control vs. Annual	1			9.35	.0036					
Year 1 vs. Annual	1			0.26	.6125					
Year 2 vs. Annual	1			4.16	.0468					
Year 3 vs. Annual	1			5.66	.0212					
Year 2 vs. Year 1	1			2.34	.1325					
Year 2 vs. Year 3	1			19.52	<.0001					
Year 1 vs. Year 3	1			8.34	.0057					

Table 2. Results of permutational MANOVA comparing plant species composition among cover crop treatments in four samples over three years. Pair-wise comparisons ignore insignificant effects of C₄ addition or interaction.

Source	d.f.	2017		2018		2019			
		<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>
Treatment	2	1.35	0.1838	3	1.72	0.0444	4	1.59	0.0286
C ₄ addition	1	1.34	0.2226	1	1.16	0.3056	1	1.22	0.2720
Interaction	2	1.30	0.1964	3	1.38	0.1276	4	0.86	0.6922
Residual	30			40			50		
Total	35			47			59		
		<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>		
Control vs. Annual		1.11	0.2660	1.28	0.1232	0.96	0.4908		
Control vs. 2017		1.28	0.1262	0.97	0.4588	0.93	0.5772		
Control vs. 2018				1.45	0.0482	1.48	0.0242		
Control vs. 2019						1.23	0.1490		
Annual vs. 2017		1.02	0.3968	1.36	0.1086	1.08	0.2878		
Annual vs. 2018				1.30	0.1302	1.22	0.1726		
Annual vs. 2019						1.33	0.0838		
2017 vs. 2018				1.32	0.1114	1.44	0.0476		
2017 vs. 2019						1.32	0.1020		
2018 vs. 2019						1.57	0.0278		

Table 3. Results of symmetric convergence / divergence test for a prairie community assembling into control plots or plots that received seed additions. Values above diagonal correspond to the statistic (τ) of the Mann-Kendall test. Negative values indicate trajectories are converging, and positive values indicate they are diverging. Significance level is indicated by p-values below the diagonal.

Treatment	Control	Annual	2017	2018	2019
Control	.	-.10	-.14	-.20	-.05
Annual	.4060	.	-.07	-.14	-.09
2017	.2360	.5581	.	+.09	+.07
2018	.0837	.2470	.4375	.	-.05
2019	.6928	.4704	.5489	.6928	.

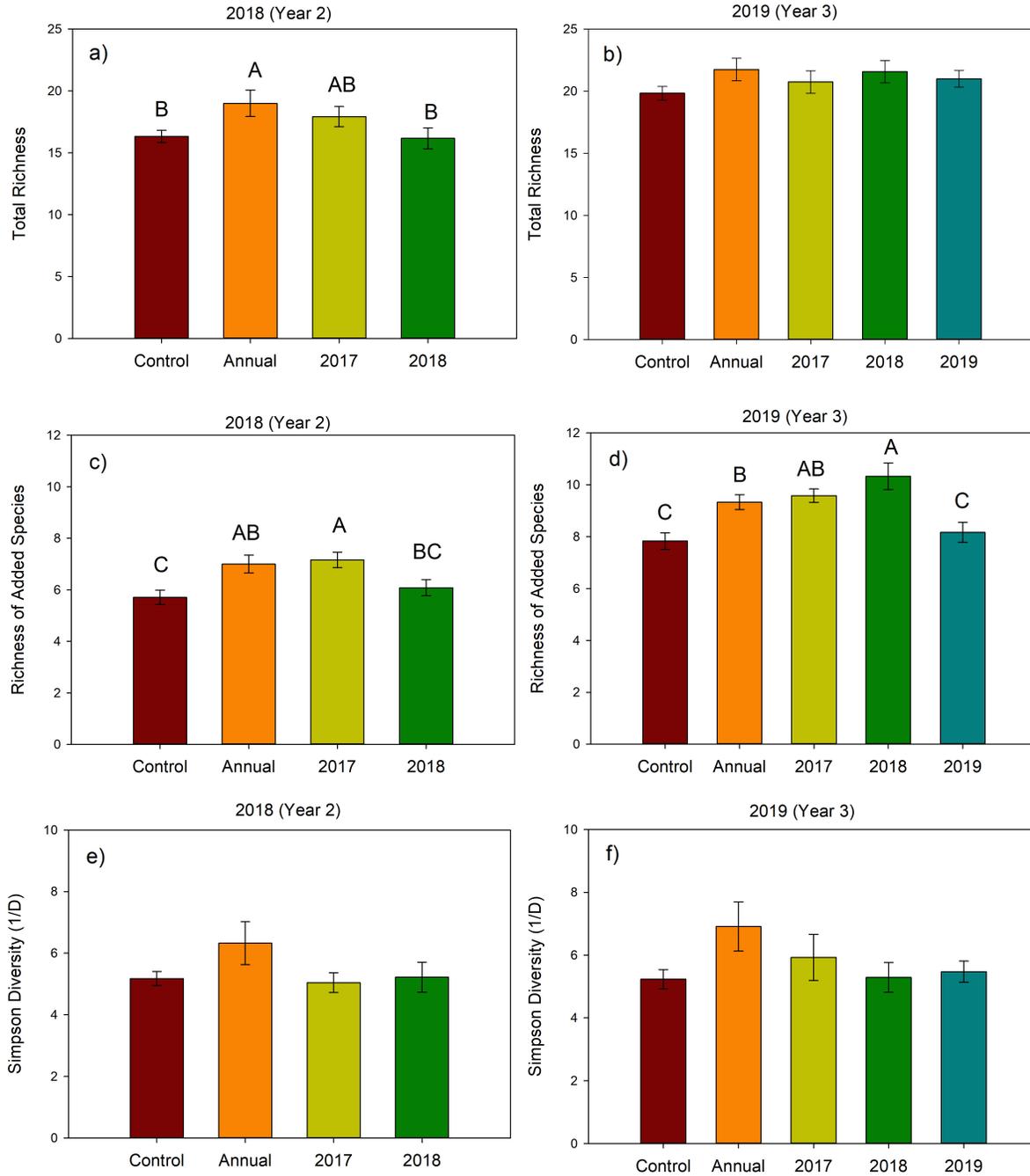


Figure 1. Plant species diversity measures in the 2nd and 3rd year of a 3-year seed addition experiment. Differences among timing of addition treatments are shown for total richness (A, B), richness of only the 32 added species (C, D), and Simpson's reciprocal diversity index for all species present (E, F). Columns with shared letters are not significantly different.

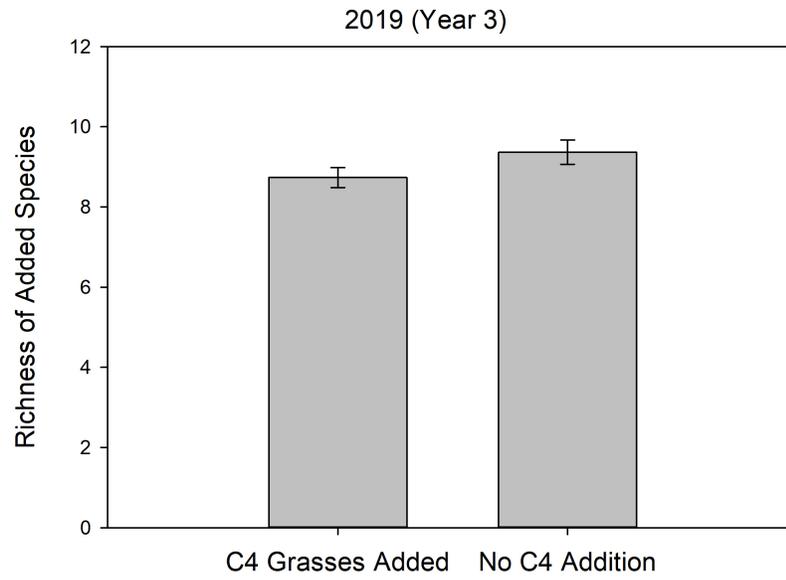


Figure 2. Richness of 32 added species is slightly, but significantly lower in plots with 3 added C₄ grasses, compared to controls in a 4-year-old prairie restoration, where the 3 C₄ grasses were added in year 2.

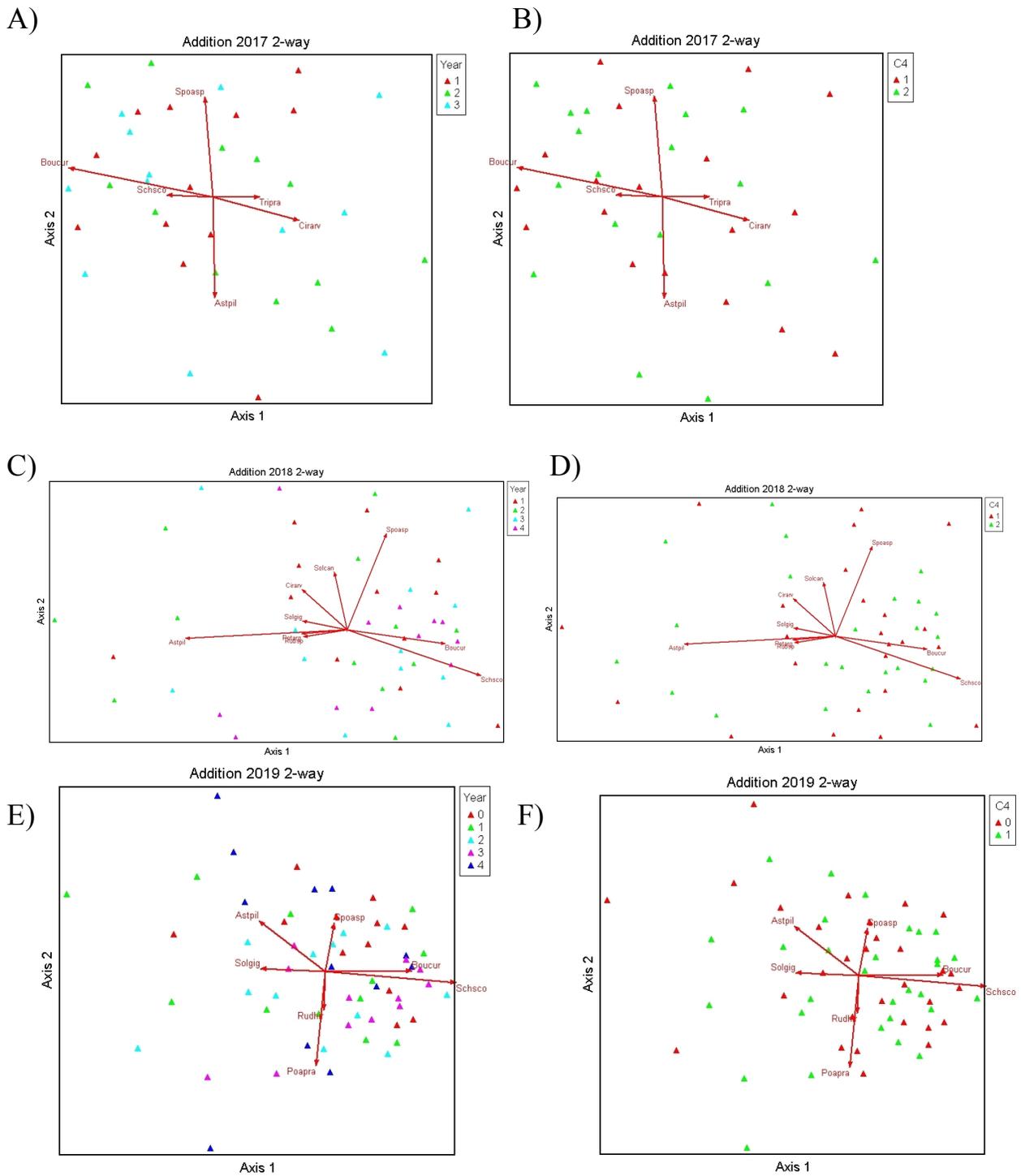


Figure 3. NMDS plots showing plant community composition differences between timing of addition treatments (A, C, E), and with (treatment 1) or without (treatment 2) three added C4 grasses (B, D, F) in three subsequent years. Numeric treatment labels for A, C, and E correspond to treatments as follows: 1 = control; 2 = annual additions; 3 = addition in 2017; 4 = addition in 2018; 5 = addition in 2019.

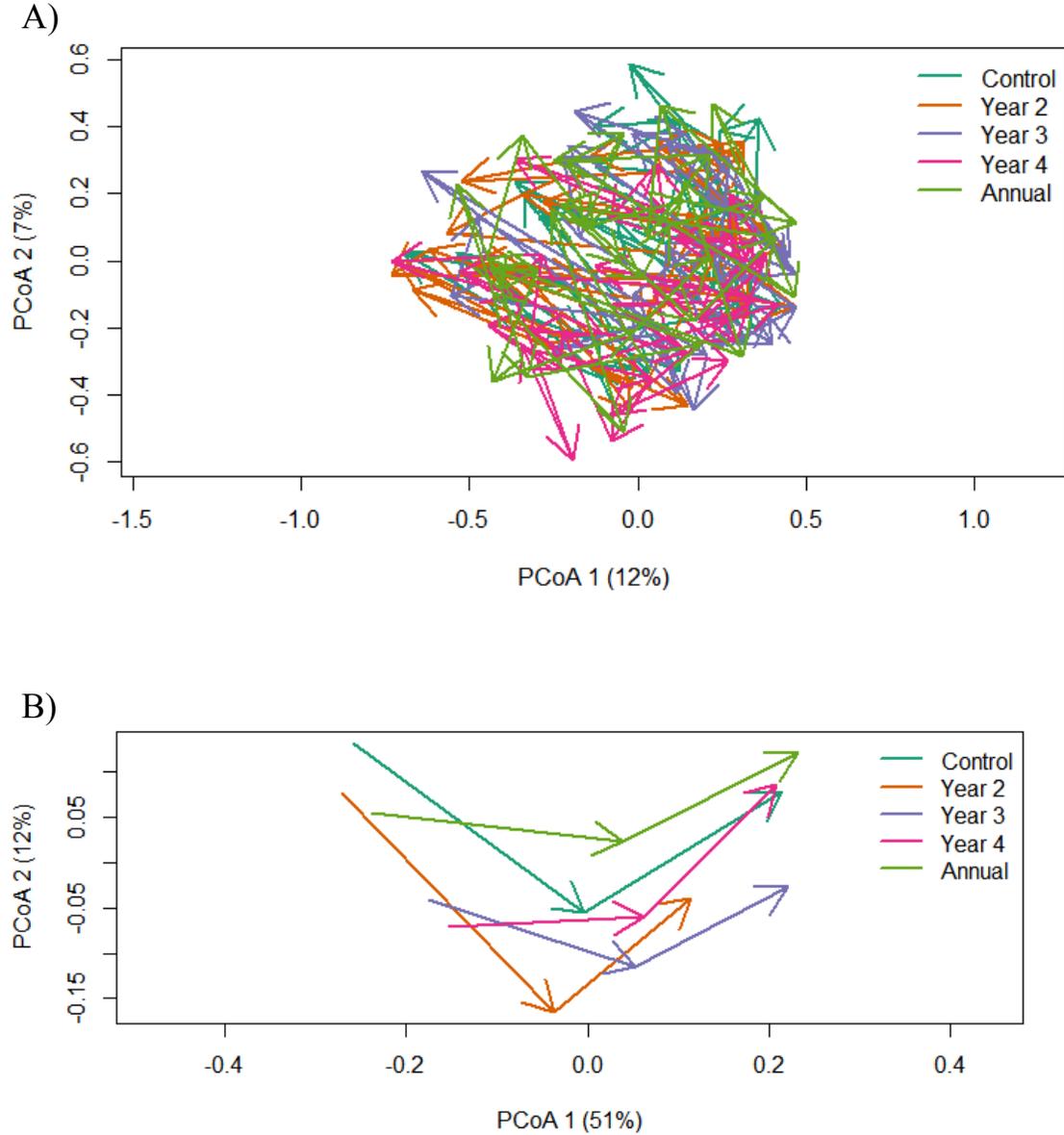


Figure 4. Principal coordinates plots corresponding to community trajectory analysis using Bray-Curtiss dissimilarities in community composition, for all treatment plots (A), or for treatments summed across plots (B). Trajectories for the single-year additions are labeled years 2-4 to indicate that the prairie grew for 1 year before the additions occurred.

CHAPTER 6. GENERAL CONCLUSIONS

Summary

Restoration ecologists ask questions about how to restore degraded, damaged, or heavily human-modified environments. My dissertation work explores restoration in the context of tallgrass prairies in the Midwestern United States. This is an especially interesting and important system in which to study restoration, because so little intact tallgrass prairie exists (Sampson & Knopf 1994), so restoration usually involves attempts to recreate these diverse grasslands from scratch, as opposed to many other habitats that may be restored through management of existing vegetation. The prairies studied in my dissertation were all restored by introducing seeds to bare ground. This process, building an ecosystem from the ground up, provides an excellent opportunity to test theory from community ecology about how species assemble to form communities, how those communities change over time, and how we can manipulate natural processes like dispersal or disturbance in order to favor more desirable restoration outcomes (Wainwright et al. 2018). Specifically, the main aim of this work was to investigate how assembly processes affect plant species diversity in restored tallgrass prairies.

Many hundreds of prairies have been planted, but the results are highly variable with respect to restoration targets (Chapter 2; Table 1). Many restorations “fail” and it is a central goal of grassland restoration ecology to explain why this happens, and how to improve restoration outcomes. In Chapter 2, we investigated which aspects of prairie restoration practices or site attributes were most important for predicting site-level (gamma diversity) species diversity, and spatial diversity (beta diversity) within 93 prairie restorations across Iowa, and compared these to 5 representative prairie remnants. Consistent with previous work, we find that restored prairies tend to be less diverse than remnants in the same landscape (Martin et al. 2005). We show that

the abundance of exotic species strongly reduces total diversity within prairies, and increases homogenization of species within them (beta diversity). More diverse seed mixes produced more diverse prairies, as expected, but surprisingly, we show this pattern mostly arises because more diverse mixes tend to suppress exotic abundance. We found higher rates of invasion in more linear and fertile habitats, and suggest that future restoration efforts are more likely to succeed in areas that are in more nutrient poor soils and with higher area: perimeter ratio.

Milkweeds (*Asclepias* spp.) are an important group of native wildflower species that are of supreme conservation interest among prairie forbs, due to their ecological function as obligate host plants for the larval stage of the charismatic monarch butterfly (*Danaus plexippus*). We surveyed the 98 prairies presented in chapter 2, for milkweed ramets, and in chapter 3 we present the first study of how variably abundant milkweeds are in prairie habitats, and across these habitats, what environmental conditions best promote milkweed abundance (Kaul & Wilsey 2019). Prairie remnants had remarkably more diverse and have more abundant milkweed assemblages than restored prairies, and this provides yet more evidence that remnant prairies need to be preserved. In restored prairie habitats, we find that the common milkweed, *Asclepias syriaca*, is very common, even though it is rarely seeded. Densities of milkweed stems in restored prairies were higher in sites with soil traits associated with disturbance, consistent with the ruderal nature of common milkweed (Evetts & Burnside 1972).

We provide one of the first studies to examine how cover crops may be used in grassland restoration in order to suppress weedy species in the first year, and facilitate establishment of a diverse prairie mix (Chapter 4). This restoration method is predicted to utilize priority effects, such that cover crop species arrive before weeds can germinate, and grow larger in size to exclude them. If cover crop species are short lived, they are then predicted to die off after the

first full growing season, allowing the target prairie species to grow during the second year without a substantial weed presence. We did not find evidence for any cover crop treatments acting on assembly in this way. Rather, based on their functional group, short-lived cover crops weakly suppressed weeds, but had ultimately no effect on prairie establishment, and a perennial treatment heavily suppressed both weeds and prairie species. The effect of these cover-crop treatments on the functional proportionality the resulting community showed evidence for filtering through niche modification rather than limiting similarity.

Further investigating barriers to establishment of species diversity in restored prairie, we tested whether there was strong evidence that a restoration was seed limited during the first three years of development (Chapter 5). Seed limitation may occur for species that do not recruit during the first year and were seeded at a low rate initially, or where the seeds have low persistence in inter-annual viability (Eriksson & Ehrlén 1992). We conducted a seed addition experiment crossed with a treatment adding 3 dominant warm-season grasses, and found that additions had no significant effect on total richness or diversity, indicating a lack of evidence for seed limitation. The added C₄ grasses only recruited to around 5% abundance over three years. These results indicate strong limitation of recruitment for any species from seed even in the second year of community development.

Future Research

Taken together, the work in this dissertation highlights the importance of assembly at the earliest stages during restoration, which for tallgrass prairie restoration occurs within the first 1-3 years. Major barriers to establishment of high diversity restored tallgrass prairies include invasion by exotic species (Chapter 2), limited recruitment of target species from seed mixes (Chapter 2, Sluis 2002, Larson et al. 2018), and grass dominance reducing forb abundance and

diversity (Baer et al. 2004). Future research must address these barriers, especially focusing on dynamics during the early stages of community assembly. Since exotic species tend to have altered phenology compared to natives (Wainwright et al. 2012, Wilsey et al. 2018), using cover crops or mowing during the first two years may help to suppress exotics and provide a more open community for target prairie species.

Future work is also needed to bridge the gap between species diversity in remnants and restorations that addresses the differences in community composition and diversity that arise from two sequential filters on the species found in restored prairies. First, there is an anthropogenic filter on the species selected for a restoration site that likely favors a non-random subset of potential species, based on their showiness, ease of establishment, and costs – which are driven by ease of production. Secondly, a biological filter acts on the sown seeds through community assembly processes. A trait-based approach is needed to address the importance of each of these filters restricting species' presence in restorations compared to the tallgrass prairie species pool. Differences in richness between remnants and restorations indicate that there are species failing to establish in restoration plantings (Martin et al. 2005, Chapter 2). If the species failing to establish in plantings are not random with respect to the species pool (Barak et al. 2017), then examining patterns in the traits common to those that successfully establish, and to those that often fail to recruit when seeded, could suggest what mechanisms are limiting species establishment.

There is also much work to be done in developing a science of seed mix design that is ecologically informed and optimizes desired ecosystem functions. This includes selecting species that are appropriate for the restoration site, while also considering how many species to sow, and in what proportions. Optimal grass: forb ratios are not well studied, and altering these is likely to

affect both exclusion of exotic species, and establishment of the diverse assemblage of subdominant forb species, which are often lacking in restorations (Dickson & Busby 2009, Jaksetic et al. 2018). Additionally, the study of trade-offs and optimization of ecosystem multifunctionality in seed mix design is necessary to improve restoration outcomes. Many commercial and custom prairie seed mixes are designed to optimize diverse functions including value to pollinators, water infiltration rates, carbon sequestration, soil stabilization, forage for herbivores, aesthetic appeal, and more. It is not known to what extent these mixes designed for a single function outperform other mixes at their desired function, or to what extent this single-function optimization produces reductions in other valuable services. Studies of seed-mix design may be especially fruitful for restoration ecology, considering that they provide simultaneous opportunities to test theory, and produce clear recommendations for practitioners.

Future research on grassland restoration should also include the novel, and increasingly popular “pollinator gardens,” or “micro prairies”, which are small native plant gardens in urban and suburban landscapes. In agricultural regions of the world, cities may represent the largest opportunity for biodiversity conservation (Seto et al. 2012). Urban ecology research and practice has been transforming the value of cities for ecosystem function and services and these small prairies are under-studied but provide a rich opportunity to integrate theory from restoration and community ecology with urban ecology and considerations for the social benefits and challenges associated with creating and managing urban ecosystems.

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APPENDIX A. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 2

Table S1. Structural Equation Model testing the direct effects of site age, organic content of the soil, linearity, and the abundance of exotic species on restored plant diversity and richness, and the indirect effect of age, linearity, and soil through their influence on exotic species. T-values, which tested for significance in coefficients, were calculated using maximum likelihood estimates.

Path	Diversity		Rarefied Richness	
	<i>P</i>	<i>T-value</i>	<i>P</i>	<i>T-value</i>
Age → Diversity	0.276	-1.0885	0.127	-1.5271
Fractal → Diversity	0.013	2.4891	0.010	2.5778
Exotic → Diversity	<.001	-9.2187	<.001	-8.8315
Soil OM → Diversity	0.963	-0.0460	0.677	-0.4172
Age → Exotic	0.421	-0.8042	0.421	-0.8042
Soil OM → Exotic	0.012	2.5110	0.012	2.5110
Fractal → Exotic	<.001	6.4308	<.001	6.4308
Diversity / Richness	R ² =0.428		R ² =0.424	
Exotic	R ² =0.346		R ² =0.346	

Table S2. Structural Equation Model testing the direct effects of the richness or diversity the seed mix, the proportion grasses to forbs in the seed mix, and the abundance of exotic species on restored plant diversity and richness, and the indirect effect of seeded diversity/richness and graminoids through their influence on exotic species

Path	Diversity		Rarefied Richness	
	<i>P</i>	<i>T-value</i>	<i>P</i>	<i>T-value</i>
Seeded S / Div. → Diversity	0.047	1.988	0.961	0.049
Seeded graminoid → Diversity	0.173	1.363	0.250	-1.149
Exotic → Diversity	<.001	-8.237	<.001	-6.019
Seeded S / Div. → Exotic	0.002	3.081	<.001	-3.587
Seeded graminoid → Exotuc	0.115	1.575	0.162	-1.399
Diversity / Richness	R ² =0.460		R ² =0.398	
Exotic	R ² =0.162		R ² =0.197	

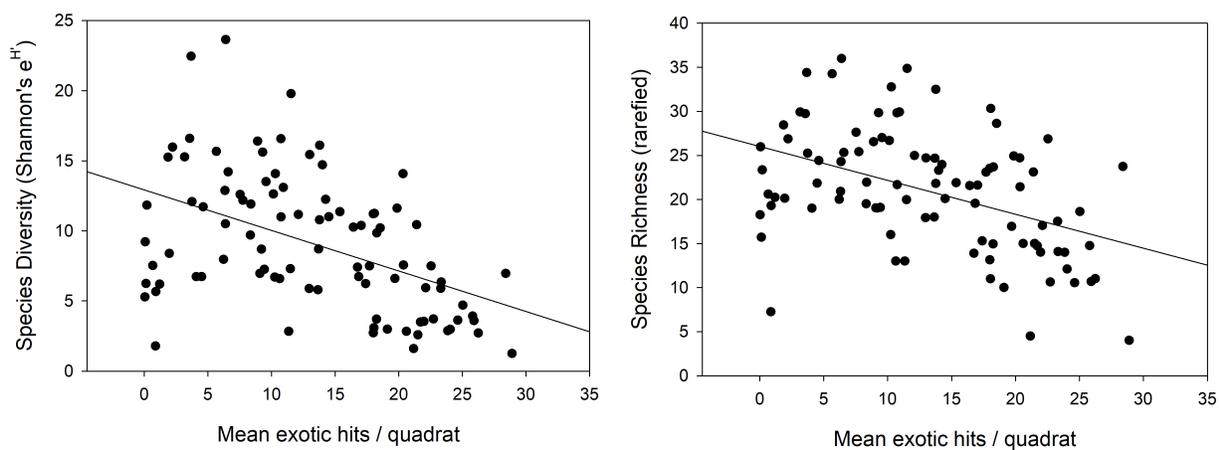


Figure S1. Relationships between species diversity (left) and richness (right) and abundance of exotic species in 93 restored tallgrass prairies.

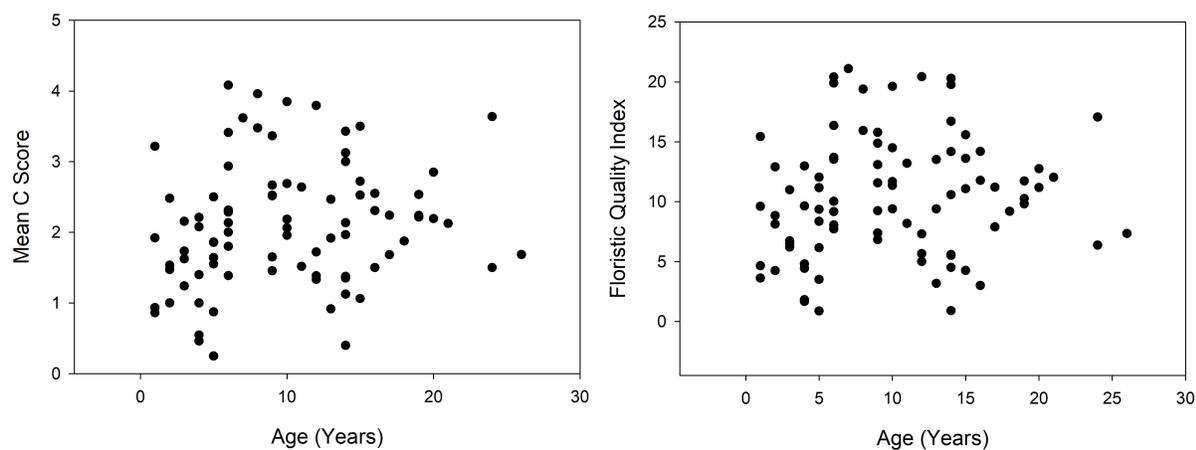


Figure S2. Relationships between average coefficient of conservatism (left) and floristic quality (right) and age of restoration in 93 restored tallgrass prairies.

APPENDIX B. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 3

Table S1. Effects of environmental variables on total and common milkweed stem density in restored praires (based on type III sums of squares).

Effect n=93	Total stem density					Common milkweed stem density			
	df	P	χ^2	estimate	direction	P	χ^2	estimate	direction
Soil pH	1	<.001	12.68	1.22	positive	.002	9.98	1.17	positive
Soil Bulk density	1	.019	5.48	-2.93	negative	.034	4.50	-2.95	negative
Burn treatment	3	.259	4.03			.516	2.28		
Seeded	2	.227	2.97			.337	2.17		
Mowing regime	3	.438	2.72			.671	1.55		
Diversity (e^H)	1	.106	2.62			.441	0.59		
Log area sampled	1	.556	0.35			.499	0.46		
Soil organic matter	1	.775	0.08			.615	0.25		
Residual	79								

Table S2. Distribution of predictor variables among three site types

		Roadsides	Conservation	Restorations	Remnants
Sites	n	46	47	93	5
Continuous					
Age	n	44	42	86	.
	Mean	7.6	12.5	10.0	.
	Range	1 – 24	1 – 26	1 – 26	.
Area (ha)	n	46	47	93	5
	Mean	0.67	10.1	5.43	5.64
	Range	0.14 – 2.71	1.12 – 61.04	0.14 – 61.04	1.56 – 11.43
Fractal	n	46	47	93	5
	Mean	1.30	1.07	1.18	1.01
	Range	1.05 – 1.55	1.00 – 1.21	1 – 1.55	1 – 1.02
Soil pH	n	46	47	93	5
	Mean	7.73	6.97	7.35	7.29
	Range	6.77 – 8.2	6.0 – 8.1	6.0 – 8.2	7.0 – 7.9
Organic matter	n	46	47	93	5
	Mean	9.3	9.7	9.5	13.5
	Range	3.6 – 17.4	4.0 – 15.1	3.6 - 17.4	2.6 – 17.6
Bulk density	n	46	47	93	5
	Mean	1.30	1.34	1.32	1.32
	Range	0.96 – 1.90	0.92 – 1.93	0.92 – 1.93	0.92 – 1.93
Diversity (e ^H)	n	46	47	93	5
	Mean	8.8	9.7	9.2	23.3
	Range	1.2 – 23.6	1.6 – 25.6	1.2 - 25.6	17.3 – 27.3
Exotic Prop.	n	46	47	93	5
	Mean	0.66	0.33	0.50	0.08
	Range	0.23 – 0.98	0.00 – 0.94	0.00 – 0.98	0.01 – 0.18
Categorical					
Mowing	n	46	47	93	.
	Never	27	7	34	.
	Establishment	8	24	31	.
	Other	10	9	20	.
	Unknown	1	7	8	.
Burning	n	46	47	93	.
	Never	33	10	43	.
	Once	7	9	16	.
	Multiple	4	23	27	.
	Unknown	2	5	7	.

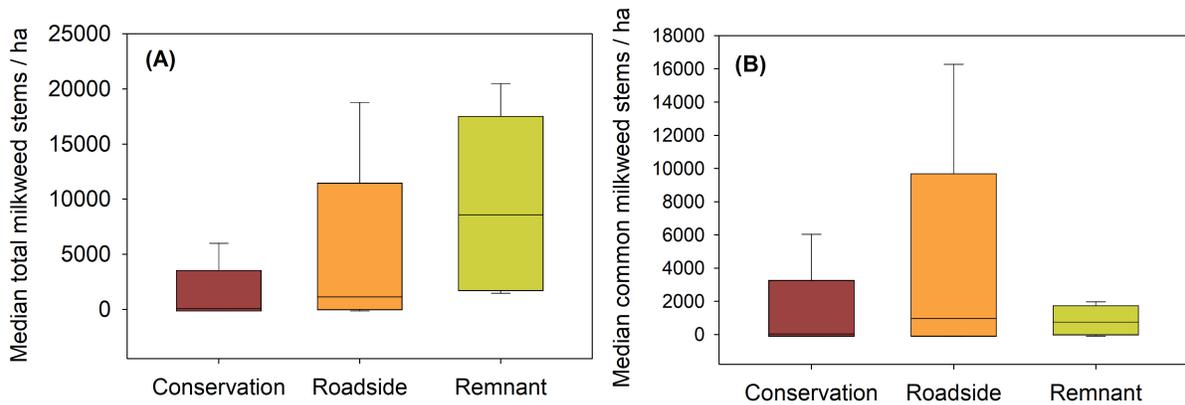


Figure S1. Total milkweed (A), and common milkweed (B) stem densities across habitat types.

Lines denote medians, and boxes show 25th to 75th percentiles (Upper and lower quartiles)

Whiskers denote maximum and minimum values.

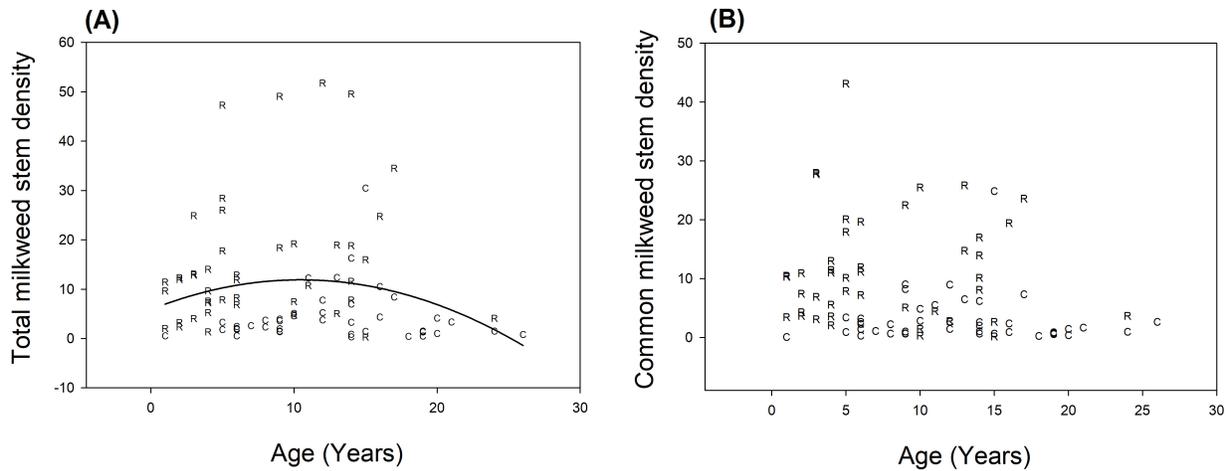


Figure S2. Relationship between total milkweed density (A), and common milkweed (B) stem density and age of restoration planting. Milkweed densities are adjusted for other variables in the model.

APPENDIX C. ADDITIONAL TABLE FOR CHAPTER 5

Table S1. Species included in mixes used for seed addition into prairie vegetation.

Primary Seed Mix	C ₄ Grass Addition
<u>Forbs</u>	<i>Andropogon gerardii</i>
<i>Ceanothus americanus</i>	<i>Panicum virgatum</i>
<i>Coreopsis palmata</i>	<i>Sorghastrum nutans</i>
<i>Echinacea pallida</i>	
<i>Eryngium yuccifolium</i>	
<i>Gentiana alba</i>	
<i>Helenium autumnale</i>	
<i>Heliopsis helianthoides</i>	
<i>Hypericum pyramidatum</i>	
<i>Liatris pycnostachya</i>	
<i>Lobelia siphilitica</i>	
<i>Monarda fistulosa</i>	
<i>Penstemon digitalis</i>	
<i>Potentilla arguta</i>	
<i>Pycnanthemum virginianum</i>	
<i>Ratibida pinnata</i>	
<i>Rudbeckia hirta</i>	
<i>Ruellia humilis</i>	
<i>Silphium perfoliatum</i>	
<i>Solidago rigida</i>	
<i>Symphotrichum novae-angliae</i>	
<i>Tradescantia ohiensis</i>	
<i>Veronicastrum virginicum</i>	
<i>Zizia aurea</i>	
<u>Legumes</u>	
<i>Chamaecrista fasciculata</i>	
<i>Lespedeza capitata</i>	
<u>Graminoids</u>	
<i>Bouteloua curtipendula</i>	
<i>Carex bicknellii</i>	
<i>Carex brevior</i>	
<i>Elymus virginicus</i>	
<i>Schizachyrium scoparium</i>	
<i>Sporobolus aspera</i>	
<i>Sporobolus heterolepis</i>	