



January 2013

Effects Of The Seed Bank And Interseeding In Reconstructed Tallgrass Prairies

Stephen C. Rossiter

Follow this and additional works at: <https://commons.und.edu/theses>

Recommended Citation

Rossiter, Stephen C., "Effects Of The Seed Bank And Interseeding In Reconstructed Tallgrass Prairies" (2013). *Theses and Dissertations*. 1589.

<https://commons.und.edu/theses/1589>

This Thesis is brought to you for free and open access by the Theses, Dissertations, and Senior Projects at UND Scholarly Commons. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of UND Scholarly Commons. For more information, please contact zeinebyousif@library.und.edu.

EFFECTS OF THE SEED BANK AND INTERSEEDING IN RECONSTRUCTED TALLGRASS PRAIRIES

by

Stephen Cary Rossiter

Bachelor of Arts, Connecticut College, 2009

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota

December

2013

This thesis, submitted by Stephen Rossiter in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

Kathryn A. Yurkonis

Brett J. Goodwin

Marissa A. Ahlering

This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

Wayne Swisher
Dean of the School of Graduate Studies

Date

PERMISSION

Title Effects of the Seed Bank and Interseeding in Reconstructed Tallgrass Prairies
Department Biology
Degree Master of Science

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my thesis work or, in her absence, by the Chairperson of the department or the dean of the School of Graduate Studies. It is understood that any copying or publication or other use of this thesis or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my thesis.

Stephen Rossiter
October 17th, 2013

TABLE OF CONTENTS

LIST OF FIGURES	vi
LIST OF TABLES	vii
ACKNOWLEDGMENTS	viii
ABSTRACT	ix
CHAPTER	
I. INTRODUCTION	1
II. SEED BANK EFFECTS ON RECOVERY AFTER DISTURBANCE IN RECONSTRUCTED TALLGRASS PRAIRIES	9
Introduction	9
Methods	13
Results	18
Discussion	23
III. DISTURBANCE FREQUENCY HAS SITE-DEPENDENT EFFECTS ON INTERSEEDING SUCCESS IN RECONSTRUCTED TALLGRASS PRAIRIES	28
Introduction	28
Methods	31
Results	36
Discussion	41
IV. CONCLUSION	45

REFERENCES50

LIST OF FIGURES

Figure	Page
1. Arrangement of pins used to measure species abundances in A) 2011 and B) 2012	16
2. A) Richness and B) evenness (mean \pm SE) in Wigan differed between control plots and plots with all seedlings removed	21
3. Simpson's diversity (mean \pm SE) in Arneson across plots clipped none (0x), once (1x), or twice (2x, one month intervals) during summer 2011	22
4. Disturbance affected Simpson's diversity differently across time in A) Arneson and B) Wigan, regardless of seed addition	39
5. Number of seedlings (mean \pm SE) within the four microsites in a plot (0.126m ²) of A) less-frequent species in Wigan in September 2012 and B) of all species in Arneson in September 2011	40

LIST OF TABLES

Table	Page
1. Mean germinated seedling densities per 100 cm ² x 5 cm deep soil sample for species with more than one seedling and the frequency (Freq) that each was observed in the 15 samples	20
2. F-values from a repeated measures ANOVA of Simpson's diversity, evenness, and species richness for each site in August 2011, June 2012, and August 2012	21
3. F-values from repeated measures ANOVAs of Simpson's diversity, evenness, and species richness for each site	37
4. F-values from a repeated measures ANOVA of plot averages of photosynthetically active radiation (PAR) transmission to the soil surface during the first growing season (2011) in each site	39
5. F-values from ANOVAs of seedling abundance per plot	40

ACKNOWLEDGMENTS

Funding was provided by the Esther Wheeler Award and the Biology Department APSAC Award to S. Rossiter and the ND-EPSCoR start-up to K. Yurkonis. Thank you to Cami Dixon and Rebecca Esser of USFWS for providing sites, seed suppliers Ric Jasken and Prairie Restorations Inc., Bob Shepard for help in the greenhouse, and undergraduates Sally Markegard and Nick Dunham. I am very grateful for the patience and advice of my committee members. Finally, heartfelt thanks go to Tiffany Gillie of the University Counseling Center. Without her help, this thesis would not have been completed.

ABSTRACT

Disturbances such as fire and mowing temporarily increase available resources for plants, opening a window of opportunity for establishment. During the recovery of vegetation after disturbance in remnant prairies, seedling establishment is often negligible compared to vegetative regrowth. It is unclear if this is the case in reconstructions. I tested the contribution of seedlings to diversity after disturbance in two, 25-year-old, low diversity reconstructed prairies by removing seedlings, allowing seedlings, and adding seed in 1 m² plots. Disturbance frequency effects were tested by clipping each treatment zero, one, or multiple times. After two growing seasons, in the wetter field site, seedlings removed plots had the lowest species richness and highest evenness, plots with seedlings from the seed bank were intermediate, and plots with added seed had the highest richness and lowest evenness. In the drier site, only adding seed impacted richness (higher) and evenness (lower). In both sites, the seeds present in the seed bank were over 80% from non-native species. Seedling establishment was quantified along with light, water, and nitrate availability within each seeded plot. In the wetter site, resource levels did not predict seedling numbers, but seedlings were more numerous in more frequently clipped, lighter plots. In the drier site, seedlings were more numerous in less frequently clipped plots which received less light and may have remained moister. Seedling establishment from any source never affected community diversity suggesting that while some seeds establish, reconstructions are primarily maintained by vegetative reproduction. The

inconsistent effect of clipping highlights the need for management to adapt to varying precipitation, however, given the non-native dominated seed banks, any management intended to increase seedling establishment could result in increased non-native cover.

CHAPTER I

INTRODUCTION

The tallgrass prairie of North America is a highly threatened ecosystem. In places it has lost over 99% of its former land area (Domek 1998). Preserving and managing remnant patches is critical, but so much has been lost that reconstructed prairies will also form a critical part of any long-term conservation strategy. Understanding reconstructions, especially the seed bank and the processes of seedling establishment within them, is important for predicting and managing how these communities recover from disturbances and change through time. My study examined establishment from the seed bank and from added seed after disturbance in old, low diversity reconstructions, both to understand the resource mechanism that regulates establishment from seed in mature vegetation (basic science) and to improve the success of interseeding, a management practice that attempts to increase the diversity of reconstructions (applied science).

All plant communities change through time with the local colonization and extinction of species and the fluctuation of relative abundances of resident species as individuals die and establish. Disturbance often facilitates these changes in plant communities by altering the availability of abiotic resources (Sher & Hyatt 1999; Catford et al. 2009) and opening a window of opportunity for establishment (Gross 1980; Johnstone 1986). In the tallgrass prairie, fire, grazing, drought, and smaller scale events like bison wallows and mammal mounds are all typical disturbances (Gibson 2009). Depending on their frequency or intensity, disturbances remove varying amounts of the existing plants' aboveground biomass. By removing aboveground biomass, a disturbance may temporarily reduce the

use of the resources (i.e. light, water, nutrients) plants need for growth. Grazing and fire both remove aboveground biomass which directly increases the amount of light reaching the soil surface. The reduced leaf area also increases soil moisture in the root zone due to decreased water lost by transpiration (Mikola et al. 2009). However, if enough vegetation is removed, the surface of the soil may become drier due to increased evaporation (Hulbert 1969). The removal of aboveground biomass by grazers also triggers plants to increase carbon allocation to their roots and root exudates, which increases bacterial activity and, thus, nitrogen mineralization (conversion of organic nitrogen to ammonium, NH_4^+ ; Hamilton & Frank 2001), although this may only affect the rhizosphere of resident plants (Mikola et al. 2009). Fire may initially decrease the total amount of nitrogen in the system due to some losses to the atmosphere during combustion (Kenworthy 1963). However, in the weeks following a fire, nitrogen availability increases near the soil surface as the nitrogen-containing ash from burned leaves is mineralized (Johnson & Matchett 2001), although this is not always the case (Blair 1997). Although other mechanisms have been shown to influence plant establishment, such as species-specific safe sites (Grubb 1977) and propagule pressure (Catford et al. 2009), the role of disturbance mediated resource availability in establishment has been theoretically developed by Davis et al. (2000) and experimentally supported by Davis and Pelsor (2001). Their fluctuating resource availability hypothesis states that if individuals can survive through a disturbance, either underground as seeds or rhizome meristems, or as seeds arriving after a disturbance, those individuals will face less resource limitation which will give them a greater chance of establishing.

As a plant community recovers following a disturbance, there is a balance between growth arising from seeds and from vegetative meristems. Seeds are dormant, sexually produced embryos with a protective coat. Seeds fall to the ground, and those that become buried over time are collectively referred to as the seed bank (Gibson 2009). Vegetatively reproduced shoots, sometimes termed ramets,

are produced by clonal, often rhizomatous (Benson et al. 2004), growth from a parent plant. To establish, ramets need the same resources of light, water, and nutrients that seeds need (McKendrick et al. 1975). However, they have easier access to those resources than seedlings because ramets are connected to their parent's root system and its stored carbohydrates (He et al. 2011; Hartnett 1993). This subsidy may allow ramets to establish under conditions that would exclude seedlings. Indeed, studies from remnant prairies found that vegetative regeneration was dominant after fire (0.5% of stems arose from seed; Benson & Hartnett 2006) and small soil disturbances (two to five times as many ramets as seedlings in 6 cm diameter plugs of sterilized soil; Rapp & Rabinowitz 1985). However, I am interested in possible regeneration from seed in reconstructions for two reasons. Establishment from the seed bank can yield undesirable changes in a community if the species in the seed bank are mostly non-native or invasive (e.g. Yurkonis & Meiners 2006). On the other hand, practitioners may add seed to attempt to increase the diversity in a reconstruction and could benefit from knowing how to favor establishment from seed. Better understanding the fine-scale mechanisms regulating seedling establishment would be a valuable step forward.

Although both seedlings and vegetative shoots need access to the same resources, establishment from seed may be favored by more frequent or intense disturbances, which leave more resources available to seedlings (Hofmann & Isselstein 2004). A small disturbance may not release enough resources for a seedling to establish and rhizomes may quickly fill the gap. At the other end of the gradient, if disturbances are frequent enough, the vigor of the resident plants may decrease to the point where they do not have enough carbon reserves to produce ramets (Biswell & Weaver 1933; Ejrnæs et al. 2006; Ilmarinen et al. 2009), reducing or eliminating competition with seedlings. For example, a study of *A. gerardii* found that a new ramet was produced if a shoot meristem was removed by grazing, but if the new ramet was completely grazed again, it would not be replaced (McKendrick et

al. 1975). Disturbance frequency is often manipulated at large scales to affect seedling establishment, but the mechanism that links disturbance to the changes in resources which determine establishment is rarely studied.

Seedling establishment is also affected by the identity of the neighboring species, with seedlings more likely to establish if they belong to a different functional group than the resident plants (Milbau et al. 2005), an impossibility for ramets. The most likely reason for this is that the seedlings and resident plants are competing for slightly different resources in different ways (Tilman 1997; Fargione et al. 2003; Biondini 2007). For instance, the mutualistic relationship legumes have with nitrogen fixing bacteria means that legumes do not have to compete with other species for nitrogen (Eisenhauer & Scheu 2008). Similarly, the different times of peak growth for cool-season and warm-season grasses means that they compete less directly with each other for light and water (Tilman et al. 2001). If a site has a low diversity of species and functional groups, such as a reconstructed prairie, most seeds will likely be functionally different from residents and thus may establish more readily (Fargione et al. 2003; Brown & Rice 2010).

When considering seedling establishment, pre-existing fine-scale resource heterogeneity may mean that seedling establishment varies at the scale of centimeters. The composition and density of adult plants can vary across centimeters, and because each plant has a unique effect on plant-available light, water, and nitrogen, these resources can also vary at centimeter-scales (Augustine & Frank 2001; He et al. 2011). For example, ammonium levels differed up to tenfold between points 4 cm apart within an ungrazed, *Poa*-dominated community in the Czech Republic (Šmilauerová & Šmilauer 2010). In the grasslands of Yellowstone National Park, U.S.A., ammonium and nitrate levels differed between points 40 cm apart in ungrazed areas and points 10 cm apart in grazed areas (Augustine & Frank 2001). Such fine-scale resource variability in the tallgrass prairie could interact with disturbance to drive seedling establishment or failure.

In order to study seed and ramet contributions to regeneration after disturbance, I used a new method that combined inquiry at two different spatial scales. Previous studies have either been conducted at the scale of individuals and have focused on individual survival rates (Rapp and Rabinowitz 1985; Benson and Hartnett 2006) or at such large scales that they do not identify the mechanisms of the community change (Hayes & Holl 2003). Small scale studies typically identify all individuals in an area and either mark them with toothpicks or rings (Rapp & Rabinowitz 1985) or map them (Benson & Hartnett 2006) and subsequently resurvey marked individuals. The alternate method that I propose is more suitable for measuring seedling effects on community diversity on larger plot scales. It is possible to identify individual shoots as either seedlings or ramets (Rapp & Rabinowitz 1985). Removing all the seedlings from an area would isolate the effects of ramets on the community. If areas without seedlings are identical in diversity to areas with seedlings, it would indicate that the seed bank plays no role in recovery after disturbance. To get at this effect one could also remove new ramets. Removing ramets might be possible, but their firm root connections and the potential for regrowth from meristems below the ground makes removing them more difficult than removing seedlings, whose few roots enable them to be gently pulled with minimal disturbance to the soil. Working at micro-site and community scales allows me to address the influence of seeds and ramets on the scale of the community, while still being able to link plot-scale results to resource-driven patterns of establishment in sub-plots.

My research into seedling establishment is in aid of the desire to increase diversity in reconstructions because, in general, they have higher plant density, lower diversity, and are comprised of only a few common species (Polley et al. 2005; Ammann & Nyberg 2005) compared to the never-plowed “remnant” tallgrass prairies that provide desirable ecosystem services (Naeem et al. 2009) and are sometimes used to set goals for reconstructions. My study sites contain very low diversity reconstructed plant communities, dominated by native warm season grasses. These sites are

representative of many others in the landscape that were seeded with only a few species in the 1980s and 1990s (Miller 2013) as part of government programs to increase grassland acreage, such as the Conservation Reserve Program or U.S. Fish and Wildlife Service Waterfowl Production Areas. At that time, seed of most native species was unavailable and the varieties of native grasses that were produced commercially were originally bred for competitiveness and productivity for forage or erosion control purposes (Kedzierski 2013). The current question is how to best manage those low diversity grasslands which are so unlike the moderate to high diversity communities produced by modern reconstruction attempts.

Current reconstruction projects are often based on complex decision trees with numerous considerations (Packard & Mutel 1997; Helzer 2013) but also rely on personal experience and flexibility. The first step is selecting a site. A parcel's habitat connectivity (to be near existing habitat or away from invasive sources), the intactness of its hydrology, its suitability for management with fire and/or grazing, the presence of willing landowners, and its land value may all be considerations. Once a site is chosen, pre-seeding management may be used, such as adding a carbon source to increase microbial nitrogen uptake to decrease soil fertility (Blumenthal et al. 2003), or leasing the land to a farmer to "clean" the field of weed seeds with a few years of Roundup Ready[®] crops. Then, designing a seed mix has many variables. Deciding which species to include and at what density depends on records of historical species occurrence, the abiotic conditions (soil type, moisture) of the site, the availability and cost of their seed, and species composition needed for alternate goals such as erosion control, biofuel production, or use by a threatened species. Whether seed is wild-collected or collected from farmed seed-increase plots, and the distance between the site and the seed source may also be considerations (though how local is "local" is still debated; Broadhurst et al. 2008). Adjusting the ratios of grasses to forbs and aggressive to conservative species may be considered (Weber 1999). For example, it may be

decided to try to add certain conservative forbs in later years and prioritize rapid covering of the ground by sown grasses or aggressive species to reduce invasive establishment. An annual cover crop may also be used. Overall, seed density for each species is a trade-off between seed cost and the-more-seed-the-better for establishment. Before sowing, certain species may require storage under specific stratification conditions, physical scarification (Packard & Mutel 1997), or coating in a mixture of a symbiotic organism (Graham 2005). Multiple seed mixes may be used if the site contains a hydrologic gradient, or if the designer wants to build in spatial heterogeneity. “Strip seeding” is an option to create high density patches of certain species (Rayburn & Laca 2013). Aiming to increase fine-scale diversity is another option to attempt to decrease future invasion (Yurkonis 2013). The actual sowing of seed can be done either in late fall to ensure good seed-soil contact, known as “snow seeding,” or in spring (Kedzierski 2013), and whether sowing is done by drill or broadcast may favor certain species in the mix (Yurkonis et al. 2008). Transfers of soil from remnants containing fungi, nematodes, bacteria, etc. may also be considered. Finally, management following sowing must be considered to both control locally problematic species and form a burning/grazing/mowing plan for perpetuity. Monitoring is also worthwhile, so others can learn from each project’s shortcomings and successes. While my research is directly applicable to low diversity grasslands, the underlying question of resources driving establishment may help us understand recruitment from seed in these higher diversity sites as well.

My questions explored two issues facing old, low diversity prairie reconstructions. In the first data chapter, I investigated the composition of the seed bank and whether it contributes to the recovery after a disturbance. The second data chapter relates to interseeding. I investigated whether disturbance frequency affects the contribution of added seeds to the recovery after disturbance and what mechanism links disturbance frequency to resource availability to seedling survival. Addressing

these questions can advance both ecological science and practice and may help to bridge the gap between them.

CHAPTER II

SEED BANK EFFECTS ON RECOVERY AFTER DISTURBANCE IN RECONSTRUCTED TALLGRASS PRAIRIES

Introduction

Disturbances can increase plant available resources and open a window of opportunity for plant establishment (Gross 1980; Johnstone 1986; Davis et al. 2000). Establishment can occur either from the local propagule pool or via vegetative reproduction (Cattelino et al. 1979), and establishment from either pathway may alter community composition as a result of introduction of new species, or changes in the relative abundances of established species. This process is particularly important to understand in the context of older reconstructed prairies, which are often species-poor. Such communities may not have the seed bank or the established plants to compete with intense propagule pressure from the surrounding landscape, and it is unclear how their composition may change as they are exposed to disturbances. To understand the potential for community change, I asked whether seeds from the seed bank contribute to the recovery after a disturbance in low diversity tallgrass prairie reconstructions.

Whether any seed can establish depends on the three general factors reviewed by Catford et al. (2009). A seed must first be able to either survive in the seed bank or disperse to the site. Second, abiotic conditions must be suitable throughout establishment. In the tallgrass prairie, the sequence of temperature, light, and moisture conditions must be sufficient to break the seed's dormancy and trigger germination (Baskin & Baskin 2001; Bewley et al. 2012). Water must be available in the soil for seed imbibition and water availability must be maintained until the seedling has developed enough adventitious roots. Enough light and mineral nutrients, particularly nitrate, must then be available by

the time the seedling's stored reserves are depleted. Finally, establishment depends on the biology of the species involved, whether they are more competitive or stress tolerant (Wilson & Lee 2000). The number and identities of a seedling's neighbors can either promote or deter establishment, for example, with the presence of facilitative or strongly competitive species (Crawley et al. 1999; Fargione & Tilman 2005).

Following a disturbance, both abiotic resource availability and competition from neighboring plants are affected, and those factors are combined in Davis et al.'s (2000) fluctuating resource availability hypothesis of vegetation change. The theory suggests that invasion, or for my purposes seed establishment and growth (Johnstone 1986; Davis et al. 2001), is more likely when there is an increase in available resources, such as light, water, or nutrients. In tallgrass prairie, disturbances such as fire or grazing can cause this increase in resource availability by damaging existing individuals enough to reduce their resource uptake (i.e. their competitiveness) or by depositing more of a resource (e.g. nitrogen rich urine). This hypothesis was supported in an old-field system by an experiment that used herbicide and drought as disturbances and found that greater establishment occurred where water availability was higher and competition from resident plants was lower (Davis & Pelsor 2001). Similar increases in establishment have been found with increasing nitrogen concentration when soil has been exposed by disturbance (Maron & Connors 1996) and with increasing light availability (McCain et al. 2010). The importance of available resources for determining establishment has been well supported (Bartha et al. 2003; Colautti et al. 2006; James et al. 2006), but whether those resources will be captured by establishing seedlings or vegetative growth is uncertain.

If available resources are taken up by recruitment from the seed bank, this could result in a substantial change in community composition. The species in tallgrass prairie seed banks are often different from the resident species, usually with more ruderal (annual and biennial) and invasive species

(Johnson & Anderson 1986; Abrams 1988). If the seed bank contains mostly seeds of invasive species, and if conditions change in such a way that favors establishment from the seed bank, the native community could degrade with successive disturbances (Yurkonis & Meiners 2006; Renne & Tracy 2007).

The resources available after disturbance can be captured by rhizomatous vegetative growth as well as seedlings. Prior studies have quantified the balance between these two forms in remnant prairies. In a Kansas remnant, Benson & Hartnett (2006) determined the origin of all stems in plots on annually burned prairie as from either a seed or a vegetative meristem (rhizome). They found an average of 8 seedlings/m² at the end of one growing season which comprised less than 0.5% of total stems. Although this level of seedling establishment may be enough to maintain genetic diversity (Watkinson & Powell 1993), seedling establishment was rare compared to vegetative recovery. In a Missouri remnant, few seedlings colonizing exposed soil surfaces (5.8 cm diameter soil cores filled with sterilized soil) survived their first year, and a majority of stems present after four years colonized the sites via vegetative growth (Rapp & Rabinowitz 1985). Both studies suggest that although some seedlings can establish, vegetative reproduction accounts for most new individuals in remnant tallgrass prairies. However, this relative scarcity of seedling establishment may not be the case in reconstructed prairies or after other types and sequences of disturbance.

Benson and Hartnett (2006) and Rapp and Rabinowitz (1985) only observed establishment after single disturbances, but seedlings may benefit from more frequent disturbances. Repeated defoliating disturbances reduce resource uptake and the capacity for rhizomatous growth by established resident plants which compete with seedlings. For example, seedling establishment was similar between the control and the disturbance used by Rapp and Rabinowitz (1985) because their 5.8 cm diameter disturbances were overgrown by neighboring plants. Seedlings have limited competitive ability because all their initial growth must come from energy stored in their seed. By contrast, vegetative growth is

continuously connected to its parent's energy reserves (Hartnett 1993; He et al. 2011). Because of their access to resources, rhizomes are typically able to rapidly recolonize a disturbed area. However, as disturbance frequency increases, resources available to seedlings increase proportionally as uptake by resident plants decreases. For example, increasing the frequency of defoliating disturbances increases light transmission to the ground, decreases transpiration, and increases water availability (Owensby et al. 1970). Soil nitrogen availability has also been reported to increase with repeated disturbances, but the increase may only be available in the rhizosphere of resident plants (Hamilton & Frank 2001; Mikola et al. 2009). Studies that manipulated clipping frequency (Hofmann & Isselstein 2004) and grazing intensity (Martin & Wilsey 2006) have found increased seedling success with increasing disturbance.

Differences in structure and composition between reconstructions and remnants may also affect the balance between seed and vegetative establishment between these types of communities.

Reconstructions often have fewer total species than remnants (Polley et al. 2005; Ammann & Nyberg 2005) and fewer species with early-season phenology (Carter & Blair 2012). If richness is very low, other species arriving by seed may have greater establishment due to less competition from their own functional group (Dukes 2001; Fargione et al. 2003; Mwangi et al. 2007). Alternatively, reconstructions that have higher stem densities (Ammann & Nyberg 2005) or an abundant dominant species (sampling effect) may prevent most seedling establishment. For example, the warm-season (C4) grasses commonly used in reconstructions can decrease the establishment of other functional groups as severely as they decrease establishment of C4 species (Fargione et al. 2003). The only study that I am aware of that measured establishment from the seed bank in reconstructions found decreasing seedling establishment as site age increased from 3 to 11 years (Willand et al. 2013). This suggests seedlings play little role in old reconstructions, however, spring fire was the only disturbance that study evaluated.

Seeding low diversity grassland communities was a common management strategy on Conservation Reserve Program and Waterfowl Production Area lands in the upper Midwest around the 1980s (Cami Dixon 2011, U.S. Fish and Wildlife Service, Jamestown, ND, personal communication). There are now thousands of acres of low diversity reconstructions in the northern tallgrass prairie (Miller 2013) and interactions between disturbance and the seed bank may affect the trajectory of these communities, particularly with respect to non-native species composition. In two circa 25-year-old native warm-season grass reconstructions I asked: 1) What is the composition of the seed bank? 2) Do seeds from the seed bank contribute to the diversity (richness and evenness) of the plant community after disturbance? 3) How does disturbance frequency affect the availability of light, water, and soil nitrate? and 4) Does disturbance frequency affect the contribution of seeds to recovery? Better understanding the role of the seed bank in the recovery after disturbance will help us to better plan for the longevity of our reconstructed tallgrass prairies.

Methods

Study sites

I established experimental plots in two U.S. Fish and Wildlife Service Waterfowl Production Areas, Wigan (Steele County, North Dakota; lat 47°33'12.5892"N, long -97°37'42.6678"W) and Arneson (Becker County, Minnesota; lat 46°51'13.8816"N, long -95°56'28.1934"W). Wigan was in row crop production for at least 5 years before being seeded with *Andropogon gerardii* (7.4 kg pure live seed/ha) and *Panicum virgatum* (1.2 kg pure live seed/ha) sometime between the late 1980s and early 1990s (Cami Dixon 2011, U.S. Fish and Wildlife Service, Jamestown, ND, personal communication; no original records available). Wigan is a flat 3.5 ha site with a high water table and has subsequently been managed with occasional burning and broadleaf herbicide applications to control Canada thistle (*Cirsium*

arvense). The soil is predominately Brantford loam, a Hapludoll (Soil Survey Staff 2011). Wigan was last burned before the study in spring 2010. Arneson was similarly in row crop production before being seeded with unknown rates of *A. gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*, and possibly *Dalea purpurea*, in the late 1980s or early 1990s (Rebecca Esser 2011, U.S. Fish and Wildlife Service, Detroit Lakes, MN, personal communication). Arneson is a 1.8 ha site on drier hilly uplands with predominantly Formdale-Langhei complex clay loam (Soil Survey Staff 2011). Arneson was last burned before the study on 10 May 2011. To mimic the litter removing effect of fire, on 13 May 2011, plots in Wigan were gently raked by hand to remove litter without disturbing the soil.

Mean growing season (May-August) temperature in the region is 22.8°C (NOAA National Climate Data Center 2013). Near Wigan, mean growing season precipitation since 1900 was 29 ± 10 cm SD (Mayville, ND). The 2011 growing season was particularly wet at Wigan, 40 cm of precipitation was recorded across 43 days, with 15 days with more than 1 cm of precipitation. In 2012, 22 cm of precipitation fell across 38 days. Near Arneson, mean growing season precipitation since 1900 was 36 ± 11 cm SD (Detroit Lakes, MN). In 2011, a near-average 37 cm was recorded across 50 days, with 10 days with more than 1 cm of rain. In 2012, 32 cm fell across 44 days.

Experimental Design

Two seed treatments, seedlings removed or seedlings not removed (control), crossed with three clipping disturbance frequencies were assigned to plots (1 x 1 m with 2 m spacing) in a randomized complete block design with five replicate blocks in each site. In seedlings removed plots, seedlings were identified and removed by hand every 4–6 weeks in summer 2011. Grass seedlings were distinguished from stems arising from rhizomes by the remains of the palea and lemma and their thinner leaves. Forb seedlings were distinguished by the presence of cotyledons and their height. Plots were either

unclipped (0x), clipped once by hand (1x), or clipped multiple times (2-3x). The first clipping was done when less than 50% of the photosynthetically active radiation (PAR) was transmitted to the soil surface (approximately mid-June). Plots were clipped for a second time in both sites about 4 weeks later. By mid-September, vegetation had regrown (<50% soil surface PAR) in Wigan, but not Arneson, to necessitate a third clipping. Vegetation was initially clipped at 5–10 cm above the soil surface because that is near the lower limit of a mower that would be used on a large-scale project. Clipping height was increased to 10 cm, then 20 cm, to reduce damage to growing seedlings (Williams et al. 2007). All vegetation was clipped within the plot and 50 cm beyond the plot edge to avoid edge effects. Cut vegetation was gently raked away.

Sampling

Each unclipped and clipped once plot was divided into four quadrants and available light, soil moisture, and soil nitrate were measured near the center of each quadrant in 2011. The percent of photosynthetically active radiation (PAR or light) reaching the soil surface was measured with an AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, U.S.A.). The ceptometer recorded PAR along a 20 cm segment which was inserted along a north-south axis through the center of each quadrant. Measurements were taken on clear days between 10:30 and 2:30 CST. PAR was measured at the start of the growing season, immediately before and after each clipping, and at the peak of biomass in August 2011. Soil nitrate was measured by collecting a 10 cm soil core (1.9 cm diameter) from the center of each quadrant. One core was taken at the start of the growing season and another at peak biomass at least 6 cm away from the previous core location. Soil samples were kept at -2°C for 6 months before they were sent to Kansas State Agronomy Soil Testing Lab (Manhattan, KS, U.S.A.) for analysis of nitrate concentration (ppm) by KCl extraction. Volumetric soil water content (%) of the top 11 cm of soil

was measured with a FieldScout 100 time domain reflectometry probe (Spectrum Technologies; Plainfield, IL, U.S.A.) inserted flush with the soil surface. Measurements were taken at least 6 cm away from soil core locations at the start of the growing season, at each clipping, and at peak biomass. For each resource measure, the four values per plot were averaged to generate a single plot measurement.

To determine plot diversity, species abundances in each plot were quantified using the point intercept method (Jonasson 1988) at peak warm-season biomass in August 2011 and 2012, as well as at the peak of cool-season biomass in June 2012. A frame was used to position 2 mm diameter pins vertically through the vegetation and the number of leaves touched by pins were recorded by species. In 2011, 20 pins per plot were arranged with pins at the corners of 10 x 10–cm squares centered in each quadrant and the corners of a 14 x 14–cm square at the plot center. In 2012, 40 pins per plot were distributed uniformly across each plot at each intersection of gridlines spaced 14.1 cm apart to better sample establishing seedlings (Fig. 1). Simpson’s diversity, denoted $1/D$, was calculated for each plot as $1/D = 1 / \sum (p_i^2)$ where p_i is the proportion of total pin touches in the plot made by species i . All species in each plot were identified and counted (richness, S) and used to calculate evenness (E) as $E = 1/D/S$. To directly estimate regrowth, leaf heights (cm) of the dominant grasses were recorded at peak

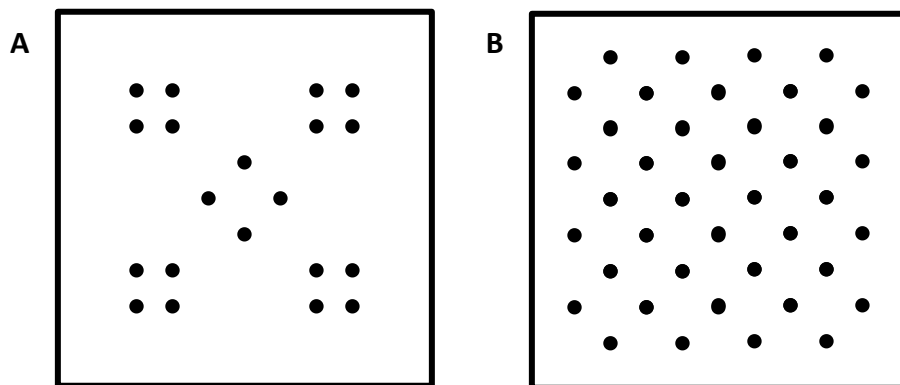


Figure 1. Arrangement of pins used to measure species abundances in A) 2011 and B) 2012. Plots (black squares) were 1m per side.

biomass. Finally, vegetative stems (tillers) of the dominant grass species were counted separately by species 1 month after the first clipping in 30 cm diameter subplots at each quadrant center.

Seed Bank

In each block, three 100 cm² x 5 cm deep soil samples were collected (based on methods described in Galatowitsch & van Der Valk 1996; Wang et al. 2010; Jacquemyn et al. 2011) in May 2011 and July 2012 and stored at 4°C prior to testing. Samples were collected from aisles among plots and were at least 4.5 m from each other. Each sample was halved. Sample halves were prepared by rinsing them through a 2 mm sieve over a 75 µm sieve to remove roots and debris. Halves were then spread in a thin layer (<5 mm) in 12 x 12 cm pots of Sunshine Mix #4 (Sun Gro Horticulture, Bellevue, WA, U.S.A.) and placed in a greenhouse with 14 hours of light per day and mean temperatures of 29°C during the day and 23°C at night. One control pot per five pots was placed among the sample pots to detect contaminating seeds from the greenhouse. Once a seed germinated, the seedling's identity was recorded and it was removed. Unidentifiable seedlings were transplanted at the 2-3 cm stage and grown for identification (Hutchings & Booth 1996). After 1 month with no new seeds germinating in any pot, each half sample was mixed, allowed to dry for 1 week, then rewetted (Dreber & Esler 2011). The experiment was concluded after another 2 weeks with no germination. The halves of each 2011 sample were run at different times to allow adjustment of greenhouse conditions. The first halves, which ran from June 2011 through February, were watered to field capacity daily which resulted in substantial moss and algae growth. The second halves, and all 2012 samples, ran from July 2012 through November and were watered only enough to soak the top of the soil and were not watered again until the soil surface had dried. Data from both halves of each sample were summed. Samples from both years were averaged to calculate seedling density.

Data Analysis

Sites were analyzed separately due to differences in site history and characteristics. Repeated measures ANOVAs (proc glm, SAS Version 9.3, SAS Institute Inc.) with model terms block (fixed), seed, disturbance, seed x disturbance and each term's interaction with time were used to analyze the community indices: diversity, richness, and evenness, and the resources measurements: PAR, soil moisture, and soil nitrate. ANOVA assumptions were met and transformations were not needed on the responses. Post-hoc Tukey's tests were used to test for differences among levels. Single measurements of leaf heights and tiller counts were analyzed with ANOVAs with terms for block and disturbance.

Results

Community Composition

The standing vegetation in Wigan was comprised of a continuous overstory of *Andropogon gerardii* (41% of all pin touches ± 3.7 ; mean \pm standard error hereafter) and a nearly continuous understory of non-native *Poa pratensis* (55 \pm 5.0%) with eight non-native species (2.1 \pm 1.2%) and nine native species (1.7 \pm 0.6%) comprising the remaining portion of the established community. The seedling community that was removed (14 species, 0.065 \pm 0.013 seedlings/cm²) was dominated by *A. gerardii* (94 \pm 3% of all seedlings removed) and secondarily *Asclepias syriaca*, *Oxalis* sp., and the non-natives *Cirsium arvense* and *Sonchus arvensis*. I also removed eight species from the field that did not appear in the greenhouse germination study, my second measure of seed bank composition. Germinating from soil samples in the greenhouse, I observed a range of one to six species per sample. *A. gerardii* and *P. pratensis* comprised 2 \pm 1% and 48 \pm 6% of seedlings/sample, respectively, of a mean density of 0.201 \pm 0.028 seedlings/cm². *Typha angustifolia* was present adjacent to the site and accounted for 37 \pm 6% of seedlings. Three other non-native species (6 \pm 2%), eleven native species (6 \pm

2%), and seven unknown species ($2 \pm 1\%$) comprised the remainder of the seedlings from the seed bank (Table 1).

The standing vegetation in Arneson was dominated by *A. gerardii* ($74 \pm 2.0\%$ of pin touches) with some *Sorghastrum nutans* ($1.9 \pm 0.6\%$) and *Schizachyrium scoparium* ($1.0 \pm 0.6\%$), and with *P. pratensis* present, but sparse ($17 \pm 2.0\%$). *Galium boreale* and *Dalea purpurea* were occasional along with ten other native species ($4.4 \pm 0.7\%$) and five non-natives ($1.1 \pm 0.2\%$). The seedling community that was removed (17 species, 0.0045 ± 0.0007 seedlings/cm²) was dominated by *Zizia* spp., while *A. gerardii* comprised $13 \pm 3\%$ of the seedlings. I also removed thirteen species from the field that did not appear in the greenhouse germination study. Germinating from soil samples, I observed a range of zero to nine species per sample. *A. gerardii* and *P. pratensis* comprised $10 \pm 4\%$ and $40 \pm 5\%$ of the seedlings/sample, respectively, of a mean density of 0.093 ± 0.010 seedlings/cm². Nearby *Typha* stands again contributed $30 \pm 6\%$ of seedlings. Four other non-native species ($9 \pm 2\%$) and thirteen natives ($8 \pm 2\%$) comprised the remainder of the seedlings from the seed bank (Table 1).

Response to Treatments

After two growing seasons, recruitment from the seed bank increased richness and decreased evenness in the wetter site, Wigan (Table 2; Fig. 2), but this did not affect Simpson's diversity (1.83 ± 0.07 SE treatments pooled; Table 2). In Wigan, light availability increased in the clipped plots (disturbance $F_{[1,12]} = 38.37$, $p < 0.0001$), but soil moisture and nitrate were similar between 0x and 1x plots. Disturbance frequency never influenced the seed bank's effect on any community metric, however, disturbance itself had a varying effect on evenness across time (Table 2). Altering disturbance frequency similarly affected the communities within a time period, but the 0x plots were the most even in August 2011 and the least even in June and August 2012.

Table 1. Mean germinated seedling densities per 100 cm² x 5 cm deep soil sample for species with more than one seedling and the frequency (Freq) that each was observed in the 15 samples. Origin denotes native (N) or introduced (I) species.

Origin	Species	Wigan			Arneson				
		2011		2012		2011		2012	
		Mean (±SE)	Freq	Mean (±SE)	Freq	Mean (±SE)	Freq	Mean (±SE)	Freq
I	<i>Poa pratensis</i> ¹²	3.80 (0.91)	12	11.33 (2.11)	15	5.27 (1.28)	14	3.00 (0.72)	12
I	<i>Typha</i> sp. ¹²	19.60 (4.17)	15	0.73 (0.25)	7	5.33 (0.88)	15	1.13 (0.52)	6
I	<i>Barbarea vulgaris</i>	-	-	-	-	0.20 (0.11)	3	0.33 (0.21)	3
I	<i>Cirsium arvense</i> ¹²	1.07 (0.34)	9	0.20 (0.11)	3	0.33 (0.16)	4	0.13 (0.09)	2
I	<i>Sonchus arvensis</i> ¹	0.53 (0.24)	5	0.07 (0.07)	1	0.07 (0.07)	1	0.27 (0.21)	2
N	<i>Andropogon gerardii</i> ¹²	0.07 (0.07)	1	0.40 (0.24)	3	0.33 (0.16)	4	0.60 (0.21)	7
N	<i>Salix eriocephala</i>	0.13 (0.09)	2	-	-	-	-	-	-
N	<i>Descurainia pinnata</i>	0.13 (0.09)	2	-	-	-	-	-	-
N	<i>Oxalis</i> sp. ¹	0.07 (0.07)	1	0.33 (0.19)	3	0.07 (0.07)	1	-	-
N	<i>Asclepias syriaca</i> ¹	0.07 (0.07)	1	0.13 (0.13)	1	-	-	-	-
N	<i>Solanum ptycanthum</i>	-	-	-	-	-	-	0.13 (0.13)	1
N	<i>Veronica peregrina</i>	0.13 (0.09)	2	0.13 (0.09)	2	-	-	-	-
N	<i>Galium</i> sp. ²	-	-	-	-	-	-	0.13 (0.09)	2
N	<i>Achillea millefolium</i> ²	-	-	-	-	-	-	0.13 (0.13)	1
N	<i>Artemisia biennis</i>	0.20 (0.11)	3	0.13 (0.09)	2	0.07 (0.07)	1	-	-
N	<i>Erigeron philadelphicus</i> ²	-	-	-	-	0.07 (0.07)	1	0.27 (0.21)	2
	All species	26.53 (4.54)		13.67 (2.21)		12.33 (1.41)		6.2 (1.05)	

¹ Presence in the aboveground community in Wigan. ² Presence aboveground in Arneson.

Other species represented by only one seedling, mean of 0.07 (0.07 SE): Wigan 2011: *Epilobium ciliatum* var. *glandulosum* (N), *Chenopodium rubrum* (N), *Melilotus officinalis*¹ (I), *Echinacea* sp. (N), and six species of unidentified dicots. Wigan 2012: Two *Solidago* spp.¹ (N) and one unidentified dicot species. Arneson 2011: *Thlaspi arvense* (I), *Salix interior* (N), *Potentilla rivalis* (N), *Dalea purpurea*² (N), *Oenothera biennis* (N), *Symphoricarum* sp. (N), and one *Solidago* sp.² (N). Arneson 2012: One *Carex* sp. (N)

Table 2. F-values from a repeated measures ANOVA of Simpson’s diversity, evenness, and species richness for each site in August 2011, June 2012, and August 2012. Seed represents control plots versus plots with seedlings removed. Disturbance represents the number of post-fire clipping disturbances: zero, one, or multiple.

Source	df	Wigan			Arneson		
		SimpD	Even	Rich	SimpD	Even	Rich
Block	4,20	1.56	14.49**	9.95**	1.29	2.85†	3.03*
Seed (S)	1,20	2.23	10.74**	4.89*	0.13	0.21	1.04
Disturbance (D)	2,20	0.88	0.34	0.11	1.44	0.58	1.70
S x D	2,20	0.19	2.25	1.11	0.19	0.08	0.10
Time	2,40	2.93†	7.40**	3.03†	21.58**	4.54*	10.04**
Time x Block	8,40	3.70**	0.53	1.01	1.75	1.56	1.72
Time x S	2,40	0.05	1.34	0.01	1.33	1.56	2.32
Time x D	4,40	0.75	2.90*	0.62	4.47**	2.13†	1.02
Time x S x D	4,40	1.78	0.65	0.20	0.16	2.28†	0.70

† $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

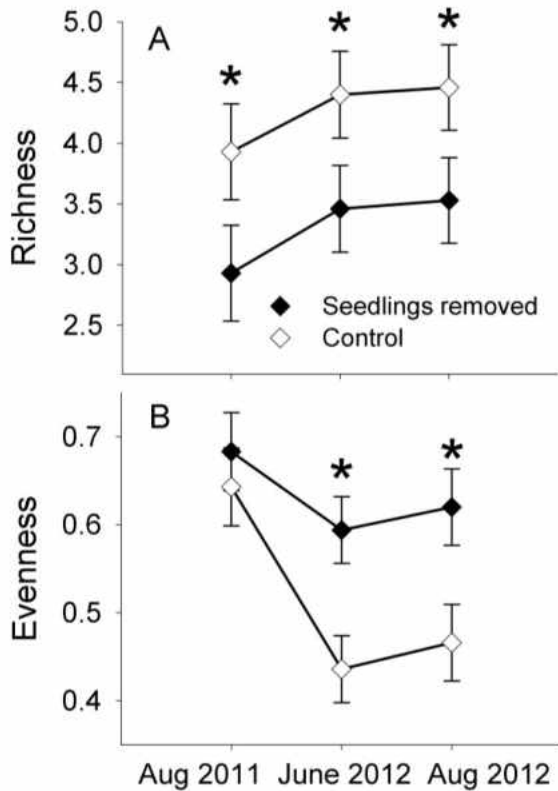


Figure 2. A) Richness and B) evenness (mean ± SE) in Wigan differed between control plots and plots with all seedlings removed. Asterisks denote significant differences ($p < 0.05$) within a time period.

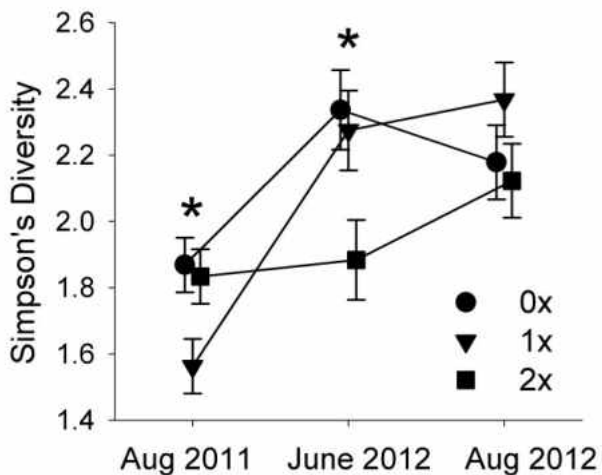


Figure 3. Simpson's diversity (mean \pm SE) in Arneson across plots clipped none (0x), once (1x), or twice (2x, one month intervals) during summer 2011. Asterisks denote significant differences ($p < 0.05$) within a time period.

In Arneson, the seed bank did not contribute to recovery after disturbance, regardless of disturbance frequency, for any community metric. However, vegetative regeneration was affected by disturbance. Tiller densities of *A. gerardii*, but not *P. pratensis*, were greater in 30 cm diameter subplots in clipped than in unclipped plots 1 month after the first clipping (6.2 ± 0.3 vs. 5.0 ± 0.4 , respectively; $F_{[1,80]} = 5.67$; $p = 0.0196$). The same magnitude effect was also observed in Wigan ($F_{[1,114]} = 5.57$; $p = 0.0199$). In Arneson, light availability increased with clipping (disturbance $F_{[1,12]} = 8.25$, $p = 0.0140$), but soil moisture and nitrate were not affected by the first clip.

Independent of seed treatment, disturbance affected Simpson's diversity inconsistently across time. The 1x plots were least diverse in August 2011, but by June 2012, the 2x plots were the least diverse (Fig. 3). This result was caused by a strong negative correlation between diversity and *A. gerardii* touches ($r^2 = 0.94$) in August 2011. In the 1x plots, *A. gerardii* regrew more after the clipping than the cool season *P. pratensis*, leading to more *A. gerardii* touches and lower diversity at the end of the season. The second clipping of the 2x plots and the lack of *A. gerardii* growth afterwards evened out

the abundances by the August 2011 measurement. By September 2011 in Arneson, *A. gerardii* leaves in 2x clipped plots were 20-25 cm tall compared to typically 55 cm in the 0x and 1x plots ($F_{[2,8]} = 30$; $p = 0.0002$).

Discussion

Recruitment from the seed bank affected the subsequent plant community in only one of the two sites. In Wigan, seedling establishment increased richness and decreased evenness through the addition of infrequent non-native forbs. The frequency of my post-fire clipping disturbances did not affect the seed bank's contribution to the community in either site. These results suggest that while seedlings are capable of contributing to the recovery after disturbance, their contribution may often be small. Even small contributions from the seed bank are important to consider because, in both reconstructions, the seed bank was dominated by non-native species.

In Wigan, seedlings removed plots were less species rich and more even. This result is expected if less common species were establishing from seed. Removing the less common species would increase the similarity between the relative abundances of the remaining species, increasing evenness. This appears to be the case in Wigan. Plots were co-dominated by *Andropogon gerardii* and *Poa pratensis* while the less common non-natives *Cirsium arvense* and *Taraxacum officinale* were often removed as seedlings and absent from seedlings removed plots as adults. I suspect that Simpson's diversity was not affected by seedling removal because the opposing effects on richness and evenness offset each other.

Compared to their contribution to recovery in Wigan plots, seedlings did not affect community structure in Arneson plots which likely reflects site-specific environmental conditions and management legacies. Despite the absence of a spring fire in Wigan, germination was similar between sites, averaging $38 (\pm 2 \text{ SE})$ forb seedlings/m² in Wigan and $39 (\pm 2 \text{ SE})$ forb seedlings/m² in Arneson,

suggesting post-germination survival was affected. The lack of seedling establishment in Arneson raises the concern that there may have been no vegetative establishment either, but that was not the case. One month after the first clipping, more new *A. gerardii* stems were being produced in clipped than in unclipped plots in both sites, consistent with new tiller formation triggered by defoliation (Hartnett & Keeler 1995). It is likely that the weather was partly responsible for the differences between sites. Precipitation in summer 2011 was 30% above average near Wigan but average near Arneson (NOAA National Climate Data Center 2013). Also, the spring 2011 fire in Arneson removed the insulating litter layer leaving the soil surface more prone to drying (Hulbert 1969). My soil moisture measurements integrated the soil moisture from 0-11 cm and may not have detected drying in the top 1-2 cm, a depth critical to the radicals of young seedlings. These drier conditions may have decreased the survival of germinated seedlings in Arneson. After the second clipping, conditions became harsh enough to prevent the leaves of *A. gerardii* from regrowing more than a few centimeters. Due to the dry conditions, the disturbance could be considered ongoing, and the recovery in Arneson may not have begun until the following spring.

Alternately, because Arneson had higher functional group richness per plot, seedling establishment may have been decreased there by greater intra-functional group competition from the abundant forbs and legumes (Fargione & Tilman 2005). However, Arneson also had more bare ground and less *Poa pratensis* cover than Wigan (data not shown) which could have facilitated seedling establishment. Finally, some species removed in Arneson were common as adults (such as perennial *Zizia* spp.), and any seedlings may not have detectably contributed to species abundances relative to established adults.

The hypothesis that establishment from the seed bank is possible, but rare, in tallgrass prairie is prevalent in the literature (Rapp & Rabinowitz 1985; Benson & Hartnett 2006; Willand et al. 2013).

Establishment from the seed bank slightly increased diversity in a fire maintained subtropical Brazilian grassland, where seedlings comprised 10% to less than 1% of regrowing forb stems after disturbance (Fidelis 2008). Also, in an Iowa prairie, Glenn-Lewin et al. (1990) found few seedlings establishing after fires. However, they also suspected that establishment was affected by soil moisture. They had greater seedling establishment in their wetter year and I had greater establishment in the wetter of my two sites. Similarly, Bakker et al. (2003) added seeds in each of 3 years in semiarid grasslands and found that seedling establishment varied greatly by year and was positively correlated with precipitation, leading them to stress the importance of annual weather conditions for seedling survival.

It has also been suggested that due to the long lifespans of many native perennial species, their seeds may establish very rarely and still maintain their population (Benson & Hartnett 2006). Rare establishment could either take place near-continuously at low levels, perhaps even lower than the 0.5% of stems reported by Benson & Hartnett (2006), or it could occur only under limited weather and disturbance conditions. The timing of precipitation can be more influential for establishment than the amount (Ries & Svejcar 1991), and if a certain weather pattern is required, even with adequate seed supply, establishment may only be as frequent as that weather pattern, and less frequent if that weather pattern must also coincide with a disturbance. In the extreme, effective establishment from seed for some species in Australian grasslands requires excessive precipitation which may only occur on the timescale of decades (Morgan 2001; Lenz & Facelli 2005). Warner and Chesson (1985) described this as the “storage effect” model of species persistence, infrequent establishment which is then “stored” in the population by long-term adult survival. By contrast, of all the studies that found little or no effect of the seed bank on recovery from disturbance, the longest covered just four consecutive years (Rapp & Rabinowitz 1985). If establishment from the seed bank is temporally rare, it would likely be underestimated by most short duration studies.

Seedlings that do establish from the seed bank in such reconstructions are likely to be ruderal non-native species, reflecting the composition of the seed bank. Excluding the abundant seeds of the invasive cattail *Typha* sp., which requires wetland conditions, non-natives still represented more than 80% of the seedlings in both sites. While my estimates of seedling densities were lower than those reported from Illinois reconstructions (Willand et al. 2013) the greater the relative abundance of a species in the seed bank, the more likely that species will establish (Foster et al. 2004; Martin & Wilsey 2006; DiVittorio et al. 2007). The abundance of non-native seeds in reconstructions is not surprising considering that reconstructions are set within a landscape of potential sources of weedy and non-native species and that they lack a diversity of native plants to contribute seed from within. Willand et al. (2013) studied the seed banks in 16 Illinois reconstructions and also found that overall, non-native species represented 73% of germinated seeds. Collectively, these results indicate that reconstructions may be vulnerable to invasive plant establishment from the seed bank after disturbance. This would lead to increased competition with desirable native species and increased costs for invasive species control.

For reconstructions, the potential for the seed bank to contribute to recovery calls for caution in management. DiVittorio et al. (2007) found that as the supply of invasive seeds increased, the establishment of native seedlings decreased after disturbance as they were outcompeted by the invasive seedlings. Renne and Tracy (2007) found that even though a pasture's aboveground vegetation returned to its prior composition after a severe trampling disturbance, the flush of ruderal and non-native species following the disturbance left a legacy of a high density of their long-lived seeds in the seed bank. As Renne and Tracy (2007) recommend, careful consideration should be given to implementing any disturbances that may promote seedling establishment on sites containing mostly non-native seed banks. Increasing species and functional richness is another way to decrease seedling

establishment (Piper et al. 2007), however, I suggest that if managers need to add species, planting seedlings as plugs (Davies et al. 1999) may allow the use of less disturbance and thus reduce establishment from the seed bank. Species could also be added by seed, but at a much higher seeding rate to try to pre-empt establishment of the non-native seeds (Norland et al. 2013). Alternately, if the aboveground community has too much invasive species cover, it may be better to return the land to conventional agriculture to exhaust the seed bank and later replant a higher plant diversity seed mix.

Overall, in two old, low diversity reconstructed tallgrass prairies, I found that 1) the seed banks were dominated by seeds of non-native species and 2) the majority of the recovery after my disturbances occurred by vegetative growth, but the seed bank could contribute slightly. My findings concern the broader question, “How long will reconstructed prairies meet our goals?” If reconstructions’ seed banks become dominated by non-native seeds, it casts doubt on the idea that reconstructed plant communities will follow a trajectory to become more like remnant prairies through time.

Implications for Practice

- Establishment from seed can contribute to the recovery after disturbance in reconstructed tallgrass prairie.
- Seed banks in old or low diversity reconstructions are likely to be dominated by non-native and potentially invasive species. Managers should consider avoiding strategies that may increase establishment from the seed bank.
- Monthly clipping disturbances were either too frequent or too infrequent for seedling establishment. The site and that season’s precipitation and evapotranspiration will determine the effectiveness of management meant to increase or decrease seedling establishment.

CHAPTER III

DISTURBANCE FREQUENCY HAS SITE-DEPENDENT EFFECTS ON INTERSEEDING SUCCESS IN RECONSTRUCTED TALLGRASS PRAIRIES

Introduction

There is a desire to increase the plant diversity of species-poor grasslands, especially older established prairie reconstructions. Most reconstructions have lower species richness and diversity than remnant prairies (Polley et al. 2005; Ammann & Nyberg 2005) for several reasons. Reconstructions may have been sown with few species initially because seeds were expensive, unavailable, or because fall seed collection favors warm-season and later-flowering species (Carter & Blair 2012). In cases where several species were sown, their success was often limited as either few species established initially or many became locally out-competed after several years (Sluis 2002; Drayton & Primack 2012). As a result, there are thousands of acres of low diversity reconstructions in the northern tallgrass prairie (Miller 2013). Most notable are two governmental efforts to increase grassland cover on the landscape. Waterfowl Production Areas (WPAs) managed by the U.S. Fish and Wildlife Service (USFWS) were sown with few species with the intent of incorporating more later (Cami Dixon 2011, U.S. Fish and Wildlife Service, Jamestown, ND, personal communication) and even today, lands enrolled in the Conservation Reserve Program often contain five or fewer species (Symstad 2008). Several projects have attempted to improve plant diversity in these abundant grassland types, often by adding seed (interseeding) coupled with disturbance, but their success has been inconsistent (Martin & Wilsey 2006; Williams et al. 2007). I will interseed followed by different disturbance frequencies to study the mechanism that links disturbance to fine-scale resource availability to explain the success or failure of interseeded seedlings.

Dispersal limitation constrains most species in fragmented prairie landscapes (Zobel et al. 2000; Foster 2001) so different methods are used to add species to a site. Species can be brought in as transplanted individuals (Davies et al. 1999), or within transplanted plugs of sod (Fraser & Kindscher 2001), but the high initial survival with these methods does not guarantee regeneration from seed which is necessary to maintain the population in the future (Godefroid et al. 2010). Alternatively, interseeding (or overseeding) is used as a more time-efficient method by adding seed through existing vegetation. Establishment success from interseeding has been highly variable (Hedberg & Kotowski 2010), with some studies reporting over 80% of sown species establishing (Hofmann & Isselstein 2004; Williams et al. 2007) and others less than 20% (Martin & Wilsey 2006; Packard & Masters 2008). When species do establish, most are represented by only a few individuals and the effect on diversity can be limited, suggesting that interseeding may not be an efficient restoration technique.

The limited success of interseeding is consistent with the competitive disadvantage seedlings face against established individuals. In remnant prairies, most recovery after disturbance is from vegetative growth rather than seedling establishment (Rapp & Rabinowitz 1985; Benson & Hartnett 2006) due to the limited resources that are stored within a seed, compared to the continuing connection vegetative growth has to its parent's resources (Hartnett 1993; He et al. 2011). It is likely vegetative growth is also dominant in reconstructions (Willand et al. 2013). Thus, interseeding is working directly against the tremendous influence that the order of species establishment has in determining community composition (Ejrnæs et al. 2006; Körner et al. 2008). Pre- or post-seeding management can be used to attempt to overcome these disadvantages, but it is often unclear what mechanism determines the success or failure of management.

Whether any individual can establish from seed within a mature community depends on three factors: the presence of seed, the presence of suitable abiotic resource levels, and compatible

interactions with neighboring species (Catford et al. 2009). Interseeding reduces propagule limitation, leaving local resource availability and neighboring species to be managed to promote establishment. This can be achieved through disturbances, such as fire, grazing, or mowing, that reduce the amount of resources taken up by neighboring plants, thus increasing the amount of resources (i.e., light, water, nutrients) available to seedlings (Davis et al. 2000). This effect of fluctuating resources has been supported experimentally at local scales (Davis & Pelsor 2001; Bartha et al. 2003; Colautti et al. 2006), but it is unclear whether disturbance regimes meant to favor seedling establishment are consistently effective.

In grasslands, seedling establishment tends to be greatest after severe or successive disturbances have abated. In an Iowa prairie, seedling establishment increased linearly with grazing intensity (Martin & Wilsey 2006). In a German grassland, seedling establishment was greater in plots clipped every one or three weeks after interseeding than in plots clipped every nine weeks (Hofmann & Isselstein 2004). After fire in an Iowa reconstructed prairie, seedling survival after two years was greater in plots mowed every week during the first growing season than in unmowed plots (Williams et al. 2007). However, no increase in survival was found by mowing every two weeks during the second growing season. These treatments are most likely successful because they remove neighbor biomass, directly increasing available light, and possibly increasing soil moisture (Owensby et al. 1970) or nitrogen availability (Hamilton & Frank 2001; but see Mikola et al. 2009).

Interseeding studies have previously compared disturbance types and frequencies, but the underlying changes in resource availability that determine seedling establishment are rarely explicitly considered. Species have specific ranges of light, soil moisture, nitrate, and temperature required for seed germination (Bewley et al. 2012; Linkies & Leubner-Metzger 2012). Following germination, seedlings need adequate light and are particularly susceptible to water stress before adventitious roots

have developed (Ries & Svejcar 1991). Separate studies have conflictingly suggested light (McCain et al. 2010), water (Martin & Wilsey 2006; Potvin 2007), or soil nitrates (Maron & Connors 1996) drive establishment. Other experiments have found that conditions and neighbors rooted within 2-8 cm of a seedling influence its survival probability (Fowler 1988; Milbau et al. 2007), and that resources can vary across similar scales (He et al. 2011). By measuring light, water, nitrate availability, and counting seedling establishment on this microsite scale, we can determine, and subsequently manage for, resources that affect seedling establishment in this system.

I added seed to low diversity reconstructions, applied different frequencies of disturbance, and measured seedling success against fine-scale resource availability in two, circa 25-year-old native, warm-season grass reconstructions. I asked: 1) Does seed addition increase diversity or richness? 2) Does disturbance frequency influence the effect of seed addition? 3) Does disturbance frequency affect resource availability? and 4) How does resource availability affect seedling abundance? Knowing how disturbances affect resource availability, and which resources in turn drive fine-scale seedling establishment in the field, can help improve management of low diversity grasslands throughout the landscape.

Methods

Study Sites

I established experimental plots in two U.S. Fish and Wildlife Service Waterfowl Production Areas, Wigan (Steele County, North Dakota; lat 47°33'12.5892"N, long -97°37'42.6678"W) and Arneson (Becker County, Minnesota; lat 46°51'13.8816"N, long -95°56'28.1934"W). Wigan was in row crop production for at least 5 years before being seeded with a 7:1 ratio of *Andropogon gerardii* to *Panicum virgatum* sometime between the late 1980s and early 1990s (Cami Dixon 2011, U.S. Fish and Wildlife

Service, Jamestown, ND, personal communication; no original records available). *A. gerardii* is now codominant with *Poa pratensis* while *P. virgatum* is rare. Wigan is a flat 3.5 ha site with a high water table and has subsequently been managed with occasional burning and broadleaf herbicide applications to control Canada thistle (*Cirsium arvense*). The soil is predominately Brantford loam, a Hapludoll (Soil Survey Staff 2011). Wigan was last burned before the study in spring 2010. Arneson was similarly in row crop production before being seeded with unknown rates of *A. gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*, and possibly *Dalea purpurea*, in the late 1980s or early 1990s (Rebecca Esser 2011, U.S. Fish and Wildlife Service, Detroit Lakes, MN, personal communication). Those species are currently common in the site, with a sparse understory of *P. pratensis*. Arneson is a 1.8 ha site on drier hilly uplands with predominantly Formdale-Langhei complex clay loam (Soil Survey Staff 2011). Arneson was last burned before the study on 10 May 2011. More than 80% of the seed bank in each site was comprised of non-native species (see Chapter 2).

Mean growing season (May-August) temperature in the region is 22.8°C (NOAA National Climate Data Center 2013). Near Wigan, mean growing season precipitation since 1900 was 29 ± 10 cm SD (Mayville, ND). The 2011 growing season was particularly wet at Wigan, 40 cm of precipitation was recorded across 43 days, with 15 days with more than 1 cm of precipitation. In 2012, 22 cm of precipitation fell across 38 days. Near Arneson, mean growing season precipitation since 1900 was 36 ± 11 cm SD (Detroit Lakes, MN). In 2011, a near-average 37 cm was recorded across 50 days, with 10 days with more than 1 cm of rain. In 2012, 32 cm fell across 44 days.

Experimental Design

A randomized, complete block design experiment with two levels of seed addition (with and without) crossed with three clipping frequencies was established at each site. Five replicate blocks were

established in each site using a randomized approach stratified by area. Each block contained six 1 x 1–m plots separated by 2 m aisles. Plots in Wigan were gently raked by hand prior to the start of the experiment to mimic the litter removing effect of fire and facilitate comparisons with the recently burned Arneson site.

Plots designated for seed addition received 800 seeds/m² of each of 10 species native to the region (Bergman 1912; Shunk 1917; Cami Dixon 2011, U.S. Fish and Wildlife Service, Jamestown, ND, personal communication). My seed rate is at the upper end of the range applied by similar studies (Foster et al. 2004; Martin and Wilsey 2006; Williams et al. 2007). It was chosen to make sure seed supply was not limiting, thus ensuring I was testing the effects of microsite conditions. The species pool was limited to one species per genus to simplify seedling identification. Seeds harvested within the previous 2 years were obtained from regional suppliers (Prairie Restorations Inc., Moorhead, MN; Rick Jasken, Becker County, MN). *Liatris* seeds were stratified in a dry, unheated building over the preceding winter. Seeds were mixed with sand and uniformly broadcast by hand in mid-May 2011. The densely tomentose seeds of *A. cylindrica* were separately pushed onto the ground by hand which limited wind dispersal out of the plots. Concurrent with seeding, a germination test of each added species was conducted by spreading 20 seeds on potting soil in the greenhouse and keeping them moist over two months (germination percentage in parentheses). The species were: *Anemone cylindrica* (20%), *Amorpha canescens* (0%), *Dalea purpurea* (5%), *Monarda fistulosa* (35%), *Liatris pycnostachya* for Arneson (20%) and *Liatris punctata* for Wigan (70%), *Rudbeckia hirta* (45%), *Solidago rigida* (60%), *Elymus candensis* (40%), *Koeleria macrantha* (40%), and *Zigadenus elegans* (5%).

Plots were either unclipped (0x), clipped once by hand (1x), or clipped multiple times (2-3x). The first clipping was done when less than 50% of the photosynthetically active radiation (PAR) was transmitted to the soil surface (approximately mid-June). Plots were clipped for a second time at both

sites about four weeks later. By mid-September, vegetation had regrown (< 50% soil surface PAR) in Wigan, but not Arneson, to necessitate a third clipping. Vegetation was initially clipped at 5-10 cm above the soil surface because that is near the lower limit of a mower that would be used on a large-scale project. Clipping height was subsequently increased to 10 cm, then 20 cm, to reduce damage to growing seedlings (Williams et al. 2007). All vegetation was clipped within the plot and 50 cm beyond the plot edge to avoid edge effects. Cut vegetation was gently raked away.

Sampling

Each seed added plot was divided into four quadrants and a circular (20 cm diameter) “microsite” was defined in the center of each quadrant. Available light, soil moisture, and soil nitrate were measured in each microsite in 2011. The percent of photosynthetically active radiation (PAR or light) reaching the soil surface was measured with an AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA). The ceptometer recorded PAR along a 20 cm long segment which was inserted along a north-south axis through the center of each microsite. Measurements were taken on clear days between 10:30 and 2:30 CST. PAR was measured at the start of the growing season, immediately before and after each clipping, and at the peak of biomass in August 2011. Soil nitrate (ppm) was measured by collecting a 10 cm soil core (1.9 cm diameter) from the center of each microsite. One core was taken at the start of the growing season and another at peak biomass at least 6 cm away from the previous core location. Soil samples were kept at -2 °C for 6 months before they were sent to Kansas State Agronomy Soil Testing Lab (Manhattan, KS, USA) for analysis of nitrate concentration by KCl extraction. Volumetric soil water content (%) of the top 11 cm of soil was measured with a FieldScout 100 time domain reflectometry probe (Spectrum Technologies; Plainfield, IL, USA) inserted flush with the soil surface. Measurements were taken at least 6 cm away from soil core locations at the start of the growing

season, at each clipping, and at peak biomass. In each microsite, the number and identity of all seedlings were recorded in September of 2011 and 2012, along with the number of ramets of all species rooted in the microsite. Other covariates recorded in each microsite were the percentage of bare ground, the litter depth (mm), and in Arneson, the percent area covered by *Schizachyrium scoparium* tussocks.

To determine plot-scale diversity, species abundances in each plot were quantified using the point intercept method (Jonasson 1988) at peak warm-season biomass in August 2011 and 2012, as well as at the peak of cool-season biomass in June 2012. A frame was used to position 2 mm diameter pins vertically through the vegetation and the number of leaves touched by pins were recorded by species. In 2011, 20 pins per plot were arranged with four pins at the corners of a 10 x 10-cm square centered in each quadrant, and at the corners of a 14 x 14-cm square at the plot center. In 2012, 40 pins per plot were used to better sample establishing seedlings, distributed uniformly across each plot at the intersections of perpendicular lines spaced 14 cm apart (Fig. 1, pg. 16). Simpson's diversity ($1/D$) was calculated for each plot as $1/D = 1 / \sum (p_i^2)$ where p_i is the proportion of total pin touches in the plot made by species i . Species richness (S) was counted and used to calculate evenness (E) as $E = 1/D/S$.

Data Analysis

Sites were analyzed separately due to differences in site history and characteristics. Repeated measures ANOVAs (proc GLM, SAS Version 9.3, SAS Institute Inc.) with model terms block (fixed), seed, disturbance, seed x disturbance and each term's interaction with time were used to analyze the community indices: diversity, richness, and evenness, and the resources: light, soil moisture, and soil nitrate. Residual plots were examined for each ANOVA and they confirmed that the assumptions of normality, linearity, and homoscedasticity were satisfied. No transformations were needed. Significant

terms were decomposed with a post-hoc Tukey's test for each time period to test for differences among levels.

ANCOVAs were used to test each year's seedling abundance by disturbance and resources (proc GLM). The sum of seedlings of all species was used as the response unless any species was more abundant than the others combined, overwhelming their variability. Abundant species were analyzed individually. Each summed-species response still contained several zero values producing severely heterogeneous variances that transformation did not resolve. Those responses were analyzed with a Poisson error distribution (proc GENMOD). A dispersion parameter scaled to deviance was always included. All assumptions were met. If disturbance frequency was significant in a Poisson model, comparisons of frequencies were made using group specific standard errors.

The ANCOVA models for both sites were simplified by using plot average values for resources and plot sums for seedling abundances. Initial models attempted to incorporate the spatial structure of the four microsites per plot by using plot as a random effect. However, the fixed block and random plot factors co-varied and so variation was not meaningfully partitioned. Due to the small sample size of the plot averaged dataset, covariates were included in the model one at a time. For light, soil moisture, and nitrate, season averages in 2011 were used for covariates. Covariates that did not improve model fit were removed. Light availability was correlated with disturbance level in both sites and so each value was adjusted by subtracting the mean light availability of its disturbance level (Milliken and Johnson 2002, p543).

Results

Seed addition affected plot richness and evenness, but not diversity. By August 2012, seed addition had increased average plot richness by about two species in both sites (Table 3). In Arneson,

seed addition plots contained 7.6 ± 0.65 species (mean \pm standard error hereafter) while controls contained 5.4 ± 0.42 species. In Wigan, seed addition plots contained 6.4 ± 0.64 species while controls contained 4.5 ± 0.54 species. Of the 10 species added, a total of seven were found across Wigan and six across Arneson. *Koeleria macrantha*, *Amorpha canescens*, and *Zigadenus elegans* never germinated in the field while numerous *Monarda fistulosa* and *Dalea purpurea* seedlings had established by September 2012. The *Liatris* species in both sites germinated well, but few survived into 2012. By the end of the second growing season, seed addition plots were less even in both sites (Table 3; Arneson: 0.34 ± 0.03 vs. 0.45 ± 0.05 ; Wigan: 0.36 ± 0.02 vs. 0.47 ± 0.05). Richness and evenness were never affected by disturbance frequency. Independent of seed treatment, disturbance initially affected diversity in both sites, but this effect disappeared by 2012 (Fig. 4).

Table 3. F-values from repeated measures ANOVAs of Simpson's diversity, evenness, and species richness for each site. Seed was added to half of the plots and plots were clipped (disturbance) 0, 1, or 2 (Arneson) or 3 (Wigan) times. Measurements were repeated in August 2011, June 2012, and August 2012.

Source	df	Wigan			Arneson		
		SimpD	Even	Rich	SimpD	Even	Rich
Block	4,20	1.43	3.32**	7.54**	0.73	3.59*	6.98**
Seed (S)	1,20	3.54†	1.36	7.96*	2.46	1.41	10.73**
Disturbance (D)	2,20	0.04	1.21	1.85	0.11	2.61	1.46
S x D	2,20	0.37	0.30	0.29	1.25	1.04	0.61
Time	2,40	3.68*	33.31**	30.02**	33.87**	3.40*	18.23**
Time x Block	8,40	3.78**	1.36	0.93	1.83	2.01†	4.32**
Time x S	2,40	1.70	6.06**	15.77**	0.34	4.57*	8.40**
Time x D	4,40	4.40**	0.10	2.10	3.93*	0.98	0.98
Time x S x D	4,40	0.75	1.06	0.71	0.52	1.97	0.98

† $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

As expected, increasing disturbance frequency increased light availability (Table 4). Season average soil surface PAR was greater in the most frequently clipped plots (Arneson: $67 \pm 2.3\%$; Wigan:

55 ± 3.7%) than in unclipped plots (Arneson: 47 ± 2.3%; W: 38 ± 3.7%). However, disturbance did not affect soil moisture or nitrate in either site. Disturbance, but not available resources, affected the number of seedlings. *Monarda fistulosa* (in Wigan in 2011 and 2012 and in Arneson in 2012) and *Liatris punctata* (in Wigan in 2011) were abundant enough to be analyzed separately from the total of seedlings of other species. In Wigan, disturbance frequency did not affect the number of seedlings of *Monarda fistulosa* (6.5 ± 1.6; total across four microsites, 0.126m²), *Liatris punctata* (8.7 ± 2.2) or seedlings of the other less abundant species (5.0 ± 1.1) by September of the first growing season. However, after the second season, seedlings of the less abundant (non-*M. fistulosa*) species were more numerous in plots clipped three times than in unclipped plots (Table 5, Fig 5A). Conversely, in Arneson in the first year, non-*M. fistulosa* species were more numerous in unclipped and once clipped plots than in plots clipped two times (Table 5, Fig 5B). However, during the second year, the number of seedlings had increased such that seedlings of both *M. fistulosa* (11.2 ± 1.5) and other species (3.5 ± 0.7) were equally numerous across disturbance treatments.

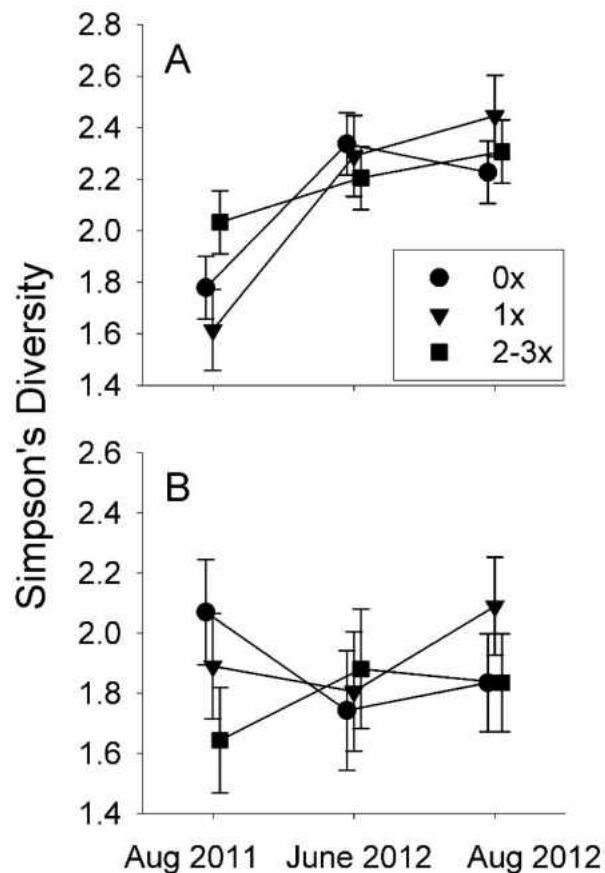


Figure 4. Disturbance affected Simpson's diversity (mean \pm SE) differently across time in A) Arneson and B) Wigan, regardless of seed addition. Disturbance frequencies are 0x (zero), 1x (one), or 2-3x (two, Arneson, or three, Wigan) clippings at four to six week intervals during summer 2011.

Table 4. F-values from a repeated measures ANOVA of plot averages of photosynthetically active radiation (PAR) transmission to the soil surface, recorded seven times during the first growing season (2011) in each site. Plots were clipped (disturbance) zero, one, or two (Arneson) or three (Wigan) times.

Source	<i>df</i>	Wigan PAR	Arneson PAR
Block	4,8	3.15	1.65
Disturbance	2,8	11.74**	24.10**
Date	9,72	224.8**	388.6**
Date x Block	36,72	4.31**	3.45**
Date x Disturbance	18,72	5.68**	11.00**

† $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

Table 5. F-values from ANOVAs of seedling abundance per plot. No resource or microsite neighborhood covariates were retained in the models. Plots were clipped (disturbance) zero, one, or two (Arneson) or three (Wigan) times. *Monarda fistulosa* (MF) and *Liatris punctata* (LP) were exceptionally abundant and analyzed separately from the other species in some years. Responses with severely non-normal distributions were better fitted using a Poisson distribution (indicated by ‡) rather than a linear model.

Source	df	2011			2012	
		MF	LP	All others‡	MF	All others‡
Wigan						
Block	4,8	0.19	1.78	5.04*	12.60**	8.21**
Disturbance	2,8	0.13	0.23	1.07	1.48	6.95*
Arneson						
Block	4,8			4.94*	2.87†	2.08
Disturbance	2,8			5.71*	1.41	0.31

† p < 0.10, * p < 0.05, ** p < 0.01

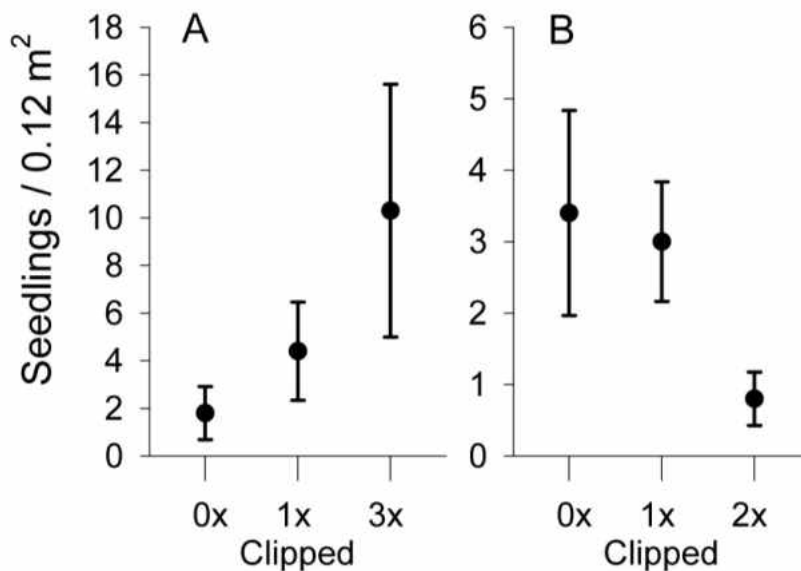


Figure 5. Number of seedlings (mean \pm SE) within the four microsites in a plot (0.126m²) of A) less-frequent (non-*Monarda fistulosa*) species in Wigan in September 2012 and B) of all species in Arneson in September 2011.

Discussion

My interseeding project was mostly unsuccessful at establishing new species in old, low diversity reconstructions. Adding seeds increased plot richness by a few species after two growing seasons, but these species did not substantially affect Simpson's diversity at the plot-scale, even with increasing frequencies of clipping disturbance. Increased clipping frequency did proportionally increase light available to seedlings, but soil moisture and nitrate were not detectably affected. Seven of the ten sown species were present, but most were represented by only a few individuals despite an unusually high seeding rate. For those seven species, the percentage of seeds sown that established ranged from 0.2-3.0%. This is less than the 4-8% seed to seedling efficiency reported for all species combined after weekly mowing in Iowa (Williams et al. 2007). After two growing seasons, the tallest individuals were mostly 20-30 cm, still below the canopy of grass leaves. After three growing seasons, only *Monarda fistulosa* (in Wigan and Arneson) and *Dalea purpurea* (in Wigan) had grown into the canopy.

The plot-scale evenness and diversity results reflect this limited establishment. In seeds added plots, newly established individuals were represented by a few pin touches, thus exaggerating the differences in relative abundance between these and the co-dominant grasses and reducing evenness. The lack of a diversity response to seed addition is consistent with the counteracting effects of the slight increase in richness and decrease in evenness. The initial effect of disturbance on diversity occurred because vegetation of the dominant grasses in the 2-3x plots had not fully recovered by the measurements in August 2011, changing the ratio of pin touches between the dominant grasses and rarer forbs.

There are multiple explanations for the poor establishment of most sown species. While the species that never appeared (*Amorpha canescens* and *Zigadenus elegans*) were also non-viable in my germination test, germination of the other species may have been limited by poor seed-soil contact.

Seed-soil contact is important for germination (Bewley et al. 2012) because greater soil contact increases water availability to the seed during imbibition and growth (Fenner 1985). After broadcasting, I did not take additional steps to increase seed-soil contact. Other interseeding projects, though not all (Martin & Wilsey 2006; Williams et al. 2007), have increased seed-soil contact with seed drills, rollers, cultipackers, raking (Packard & Masters 2008), or via fall seeding (snow compression). Alternately, seeds may have germinated, but then perished, if treatments did not provide enough available resources, or if the sequence of conditions was unsuitable (Eddleman & Meinhardt 1978). Ultimately, the high abundance of only *Monarda fistulosa* is consistent with a review that found that only a few interseeded species ever become common, and those that do are usually strongly competitive generalists with high seedling growth rates (Pywell et al. 2003).

I was able to affect overall seedling establishment by varying clipping frequencies, but this effect was not consistent between sites. This could be due to the substantial differences in abiotic conditions between the two sites. Wigan likely provided a moister environment for seeds and seedlings because it received above average precipitation and because of its low-lying topography. With adequate water, finding more seedlings in more frequently clipped plots is consistent with light limitation of establishment, as has been found in other grassland systems (Milbau et al. 2007; McCain et al. 2010). Arneson received average precipitation, has more drained hilly topography and had a blackened soil surface following the spring fire. This could lead the top few centimeters of more frequently clipped plots to dry more quickly. My water availability measurements did not differ between clipping frequencies, but they may not accurately represent site conditions for two reasons. First, my measurements integrated water content of the top 11 cm of soil, but the water availability near the soil surface (0-2 cm) may be more important for seed germination and immediate survival (Ries & Svejcar

1991). Secondly, measurements were not taken at consistent intervals after rainfall events and may not have captured the influence of variation in soil moisture that occurred after these events.

Reduced seedling establishment with increasing disturbance in Arneson is consistent with effects of water limitation caused by direct sunlight reaching the soil surface, heating and drying it. Too much light can harm seedlings as evidenced by reports of desiccation mortality (Gutterman & Gozlan 1998). Smith et al. (2004) found that removing 50% of the tillers of the dominant grass reduced colonization by the non-native legume *Melilotus officinalis*, and surmised that this occurred due to increased stress caused by increased light transmission. Prevention of moisture stress may partly explain the benefits to seedlings sometimes provided by leaf litter (MacDougall & Turkington 2005; Eckstein & Donath 2005), cover crops (Packard & Mutel 1997), or nurse plants (Hofmann & Isselstein 2004). Stevens et al. (2006) suggest seedlings can better cope with a surplus of water, rather than a shortage. They found that with increasing soil moisture, seedlings face increased competition from neighboring plants, but that this is outweighed by greater reductions in seedling mortality.

The contrasting results between my two sites support the stochastic influence of annual weather conditions on seedling establishment. Another interseeding study suspected that an unusually dry month following a mowing caused high seedling mortality (Snyder et al. 2008). A study of replicate interseeding across three years found more seedling establishment in wet than dry years (Bakker et al. 2003). Also, a manipulative interseeding experiment found that irrigation, interacting with a site's fertility and productivity, affected establishment of colonizing seedlings (Dickson & Foster 2008). So although managers cannot foresee the weather after sowing, adapting management and disturbance frequency to the conditions could help establishment. Furthermore, finding a way to irrigate may be worth considering. Alternatively, interseeding over the course of multiple years would increase the

likelihood of seeds experiencing favorable weather conditions for their establishment (Pywell et al. 2003), as occurred in Arneson with greater precipitation in the second year.

Interseeding can increase richness, but only slightly, and this depends on stochastic site conditions. Given the low establishment and the cost of seed, interseeding may not be a highly effective practice. Further research into increasing establishment from interseeding and alternatives to interseeding, like seedling transplants and sod transfers (salvaged from prairie about to be lost; Fraser & Kindscher 2001), is warranted. For sites with invasive species problems or sites lacking any sensitive native species, tilling the site and later replanting a high diversity seed mix may be most efficient. If interseeding is chosen to enhance a site containing valuable elements, such as conservative species or good soil structure, clipping can increase seedling establishment if managers can allow flexibility in applying the number and timing of disturbances relative to that site and that season's conditions.

CHAPTER IV

CONCLUSION

In both sites, establishment from seed was low, whether from the seed bank or added seed. A possible explanation for this is that there was not enough disturbance in Wigan and too much disturbance in Arneson. More precisely, due to dry conditions in 2011 in Arneson, the disturbance could be considered to be ongoing until 2012, while in Wigan, the most seedlings were in the most frequently clipped plots, leaving the possibility that more disturbance would have produced yet more seedlings. In either case, there may not have been enough variation in available resources to provide optimal conditions for seedlings. However, the low establishment I observed is consistent with the hypothesis that recruitment from seed by most prairie perennial species is a rare process. All prairie plants are capable of producing seeds, and therefore, we could assume that seeds must be an important form of regeneration for all species. However, if individuals can survive for decades (Harper 1977), then their population could persist even if establishment from seed was very rare ("storage effect"; Warner & Chesson 1985). Establishment could either be so spatially rare as to never appear as more than one seedling per study or so temporally rare (e.g. dependent on a certain weather pattern; sensu Lenz & Facelli 2005) that most short-duration studies would never coincide with periods of establishment.

Seedling abundance was influenced by clipping frequency, which was correlated to light availability. However, I cannot say that light was the most influential resource, because the responses of soil moisture and nitrate were inconclusive. The lack of significance of microsite resource levels in the models of seedling abundance was due to low variability of soil moisture and nitrate values throughout

each site. The lack of change in the availability of water and nitrate following clipping was surprising, suggesting either that the clippings actually had little to no effect, perhaps if they were not severe enough, or that my measurement protocols were not precise enough. For nitrogen, the timing of my measurements was planned to look for season-long changes in nitrate availability, rather than levels during peak nitrogen uptake during periods of rapid growth. For soil moisture, although nearby weather stations suggest that precipitation, and thus soil moisture, was a driving force on seedling establishment, my soil moisture measurements cannot directly confirm this. My measurements averaged the moisture in the top 11 cm of soil, rather than the top 0-2 cm of soil that must sustain the radicals of recently germinated seedlings. I would also advise taking moisture measurements more frequently than I did, or at least taking them at a set interval after rainfall events, which would detect if moisture was being taken up from saturated soils at different rates between treatments before evening out at similar, lower moisture levels. In the future, it would also be better to include more sites given the drastic difference in the results between my two. The considerable variation within just one site suggests a great range of conditions in the landscape. In Wigan, block four was wetter, with much more bare ground, and no *Poa pratensis* compared to the other blocks. Block four had far more seedling establishment and could almost be considered a different site.

As an aside, I would like to state my suspicion that the non-native *Poa pratensis* may have a huge effect on seedling establishment. It is a sod-forming grass, it is a cool-season species, it is very prevalent across the landscape, and it can dominate a site. For all those reasons, *P. pratensis* could easily be preventing seedling establishment by occupying almost all “gaps” that would otherwise exist in a community. In the absence of fire, it can also produce a thick litter layer that reduces germination and establishment (Bosy & Reader 1995). More attention should be paid to the effects of this species.

The non-native dominated composition of the seed bank reflects the many pockets of ruderal and non-native species in the landscape, especially nearby stands of *Typha* sp. and *Cirsium arvense*. This is similar to the findings of studies in tallgrass prairie remnants that the composition of the seed bank can be quite different from the established vegetation (Rabinowitz & Rapp 1980; Johnson & Anderson 1986; Abrams 1988). Those findings suggest that the seed bank may be more important for providing a way for species to colonize during changing conditions than for maintaining the current community composition. My results add a cautionary note to this, because if a disturbance coincides with weather conditions favorable for establishment from the seed bank, the majority of the seeds are non-native species.

In cases where seedling establishment is desirable, my study adds another poor showing for interseeding, with effectively only two of the ten added species established by the third growing season. Due to interseeding's limited effectiveness, it is worth considering other methods that might help us increase the diversity in species-poor grasslands. Here I discuss five alternatives:

I found that post-seeding defoliation disturbances can increase establishment, so further adaptations may be worth considering. Providing enough disturbance to maximize light without drying out seedlings should work if the weather can be successfully predicted or at least closely followed. Alternately, mowing in a heterogeneous pattern to produce random overlapping patterns of disturbance over the course of a growing season, or more simply, grazing, would increase the likelihood of suitable conditions for seedling establishment in at least some areas of a site.

If establishment from seed was very rare in historical grassland systems, occurring over long timescales, then it may be unreasonable to expect much establishment after a single sowing. It is conceivable that a species may best establish from seed after a rare sequence of weather conditions, such as after a drought that has killed many of the dominant grass ramets and then one or more wet

years, perhaps with annual species facilitating their establishment (Weaver & Mueller 1942). Under a less extreme scenario, if the different weather conditions of different years favor establishment of different species (Eddleman & Meinhardt 1978), then interseeding lower densities of seed over multiple years may eventually favor establishment of more species.

I wonder if more research on species-specific conditions for establishment (regeneration niche; Grubb 1977) for species that are considered conservative or specialist would point to tailored actions to increase their establishment (Godefroid et al. 2010). What sort of niche did they use historically? Do they have traits that are adapted to certain establishment scenarios? Did they benefit from relationships with bison or insects which are not available in reconstructions?

Rather than require species to establish from seed at our convenience, young individuals could be added in soil plugs (Davies et al. 1999) or as part of a sod transplant (Fraser & Kindscher 2001). This would work well for species that have strong clonal growth and rarely can establish from seed. The downside is that if added species do not reproduce, they may die out after several years (Godefroid et al. 2010), and this may not be noticed if monitoring of the site is minimal.

Finally, some sites may have become so degraded due to invasion or extirpation of native species that drastic action (disc-ing, tilling, herbiciding) is warranted. For an extreme example, if a site is so invaded that the likelihood of successful control is low or the cost would be too high, then it might be more efficient to return the land to agriculture. The site could be replanted later once the seed bank has been depleted, or the site could be sold and the proceeds used to start a new reconstruction in a more advantageous place in the landscape.

My overall finding was that recovery from seed in old, low diversity reconstructed tallgrass prairies does occur, but is limited, whether from the seed bank or added seed. When and where seed establishment occurs may be predicted based on soil moisture and light availability, the availability of

these two resources being determined by the interaction of weather (precipitation and evapotranspiration) and management that defoliates neighboring plants. Because of this, post-interseeding management should be responsive to the developing weather of the season to balance light and moisture availability for growing seedlings.

REFERENCES

- Abrams, M. D. 1988. Effects of burning regime on buried seed banks and canopy coverage in a Kansas tallgrass prairie. *The Southwestern Naturalist* **33**:65–70.
- Ammann, R. L., and D. W. Nyberg. 2005. Vegetation height and quality of original and reconstructed tallgrass prairies. *The American Midland Naturalist* **154**:55–66.
- Augustine, D. J., and D. A. Frank. 2001. Effects of migratory grazers on spatial heterogeneity of soil nitrogen properties in a grassland ecosystem. *Ecology* **82**:3149–3162.
- Bakker, J., S. Wilson, and J. Christian. 2003. Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications* **13**:137–153.
- Bartha, S., S. Meiners, S. T. A. Pickett, and M. L. Cadenasso. 2003. Plant colonization windows in a mesic old field succession. *Applied Vegetation Science* **6**:205–212.
- Baskin, C. C., and J. M. Baskin. 2001. *Seeds: ecology, biogeography, and evolution of dormancy and germination*, 1st edition. Academic Press, San Diego.
- Benson, E. J., and D. C. Hartnett. 2006. The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology* **187**:163–178.
- Benson, E. J., D. C. Hartnett, and K. H. Mann. 2004. Belowground bud banks and meristem limitation in tallgrass prairie plant populations. *American Journal of Botany* **91**:416–421.
- Bergman, H.F. 1912. A report on the plant surveys of Barnes County, North Dakota. Pages 121-150 in N.D. Soil and Geological Survey 6th Biennial Report. Bismarck, ND.
- Bewley, D. J., K. J. Bradford, H. W. M. Hilhorst, and H. Nonogaki. 2012. *Seeds: physiology of development, germination and dormancy*, 3rd edition. Springer, New York.
- Biondini, M. 2007. Plant diversity, production, stability, and susceptibility to invasion in restored northern tall grass prairies (United States). *Restoration Ecology* **15**:77–87.
- Biswell, H., and J. Weaver. 1933. Effect of frequent clipping on the development of roots and tops of grasses in prairie sod. *Ecology* **14**:368–390.

- Blair, J. M. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. *Ecology* **78**:2359–2368.
- Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. *Ecological Applications* **13**:605–615.
- Bosy, J. L., and R. J. Reader. 1995. Mechanisms underlying the suppression of forb seedling emergence by grass (*Poa pratensis*) litter. *Functional Ecology* **9**:635–639.
- Broadhurst, L. M., A. Lowe, D. J. Coates, S. A. Cunningham, M. McDonald, P. A. Vesk, and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* **1**:587–597.
- Brown, C. S., and K. J. Rice. 2010. Effects of belowground resource use complementarity on invasion of constructed grassland plant communities. *Biological Invasions* **12**:1319–1334.
- Carter, D. L., and J. M. Blair. 2012. Recovery of native plant community characteristics on a chronosequence of restored prairies seeded into pastures in West-Central Iowa. *Restoration Ecology* **20**:170–179.
- Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions* **15**:22–40.
- Cattellino, P. J., I. R. Noble, R. O. Slatyer, and S. R. Kessell. 1979. Predicting the multiple pathways of plant succession. *Environmental Management* **3**:41–50.
- Colautti, R. I., I. A. Grigorovich, and H. J. MacIsaac. 2006. Propagule pressure: a null model for biological invasions. *Biological Invasions* **8**:1023–1037.
- Crawley, M., S. Brown, M. Heard, and G. Edwards. 1999. Invasion-resistance in experimental grassland communities: species richness or species identity? *Ecology Letters* **2**:140–148.
- Davies, A., N. P. Dunnett, and T. Kendle. 1999. The importance of transplant size and gap width in the botanical enrichment of species-poor grasslands in Britain. *Restoration Ecology* **7**:271–280.
- Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* **88**:528–534.
- Davis, M. A., and M. Pelsor. 2001. Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters* **4**:421–428.
- Davis, M. A., K. Thompson, and J. P. Grime. 2001. Charles S. Elton and the dissociation of invasion ecology from the rest of ecology. *Diversity and Distributions* **7**:97–102.

- Dickson, T. L., and B. L. Foster. 2008. The relative importance of the species pool, productivity and disturbance in regulating grassland plant species richness: a field experiment. *Journal of Ecology* **96**:937–946.
- DiVittorio, C. T., J. D. Corbin, and C. M. D’Antonio. 2007. Spatial and temporal patterns of seed dispersal: an important determinant of grassland invasion. *Ecological Applications* **17**:311–316.
- Domek, T. 1998. Last call for tallgrass in North Dakota. *North Dakota Outdoors* **60**:14–19.
- Drayton, B., and R. B. Primack. 2012. Success rates for reintroductions of eight perennial plant species after 15 years. *Restoration Ecology* **20**:299–303.
- Dreber, N., and K. Esler. 2011. Spatio-temporal variation in soil seed banks under contrasting grazing regimes following low and high seasonal rainfall in arid Namibia. *Journal of Arid Environments* **75**:174–184.
- Dukes, J. S. 2001. Biodiversity and invasibility in grassland microcosms. *Oecologia* **126**:563–568.
- Eckstein, R. L., and T. W. Donath. 2005. Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *Journal of Ecology* **93**:807–816.
- Eddleman, L. E., and P. L. Meinhardt. 1978. Seed viability and seedling vigor in selected prairie plants. Pages 12–17 *Proceedings of the sixth North American prairie conference*.
- Eisenhauer, N., and S. Scheu. 2008. Earthworms as drivers of the competition between grasses and legumes. *Soil Biology and Biochemistry* **40**:2650–2659.
- Ejrnæs, R., H. Bruun, and B. J. Graae. 2006. Community assembly in experimental grasslands: suitable environment or timely arrival? *Ecology* **87**:1225–1233.
- Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America* **100**:8916–20.
- Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters* **8**:604–611.
- Fenner, M. 1985. *Seed ecology*. Chapman and Hall, London.
- Fidelis, A. 2008. Fire in subtropical grasslands in Southern Brazil: effects on plant strategies and vegetation dynamics. Thesis. Technische Universität München, Munich.
- Foster, B. L. 2001. Constraints on colonization and species richness along a grassland productivity gradient: the role of propagule availability. *Ecology Letters* **4**:530–535.

- Foster, B. L., T. L. Dickson, C. A. Murphy, I. S. Karel, and V. H. Smith. 2004. Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *Journal of Ecology* **92**:435–449.
- Fraser, A., and K. Kindscher. 2001. Tree spade transplanting of *Spartina pectinata* and *Eleocharis macrostachya* (Britt.) in a prairie wetland restoration site. *Aquatic Botany* **71**:297–304.
- Galatowitsch, S. M., and A. G. van Der Valk. 1996. The vegetation of restored and natural prairie wetlands. *Ecological Applications* **6**:102–112.
- Gibson, D. J. 2009. *Grasses and grassland ecology*. Oxford University Press Inc., New York, USA.
- Glenn-Lewin, D. C., L. A. Johnson, T. W. Jurik, A. Akey, M. Leoschke, and T. Rosburg. 1990. Fire in central North American grasslands: vegetative reproduction, seed germination, and seedling establishment. Pages 28–45 in S. C. and L. Wallace, editor. *Fire in North American tallgrass prairies*. University of Oklahoma Press, Norman, OK.
- Godefroid, S., C. Piazza, G. Rossi, S. Buord, A.-D. Stevens, R. Aguraiuja, C. Cowell, C. W. Weekley, G. Vogg, and J. M. Iriondo. 2010. How successful are plant species reintroductions? *Biological Conservation* **144**:672–682.
- Graham, P. H. 2005. Practices and issues in the inoculation of prairie legumes used in revegetation and restoration. *Ecological Restoration* **23**:187–195.
- Gross, K. 1980. Colonization by *Verbascum thapsus* (Mullein) of an old-field in Michigan: experiments on the effects of vegetation. *Journal of Ecology* **68**:919–927.
- Grubb, P. J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* **52**:107–145.
- Gutterman, Y., and S. Gozlan. 1998. Amounts of winter or summer rain triggering germination and “the point of no return” of seedling desiccation tolerance, of some *Hordeum spontaneum* local ecotypes in Israel. *Plant and Soil* **204**:223–234.
- Hamilton, E., and D. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* **82**:2397–2402.
- Harper, J. L. 1977. *Population biology of plants*. Academic Press, London.
- Hartnett, D. C. 1993. Regulation of clonal growth and dynamics of *Panicum virgatum* (Poaceae) in tallgrass prairie: effects of neighbor removal and nutrient addition. *American Journal of Botany* **80**:1114–1120.

- Hartnett, D. C., and K. H. Keeler. 1995. Population processes. Pages 82–99 in A. Joern and K. H. Keeler, editors. *The changing prairie : North American grasslands*. Oxford University Press Inc., New York, USA.
- Hayes, G. F., and K. D. Holl. 2003. Site-specific responses of native and exotic species to disturbances in a mesic grassland community. *Applied Vegetation Science* **6**:235–244.
- He, W.-M., P. Alpert, F.-H. Yu, L.-L. Zhang, and M. Dong. 2011. Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants. *Journal of Ecology* **99**:1202–1210.
- Hedberg, P., and W. Kotowski. 2010. New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. *Journal for Nature Conservation* **18**:304–308.
- Helzer, C. 2013. The prairie ecologist: why there is no cookbook for restoring and managing prairies. URL <http://prairieecologist.com/2013/08/12/why-there-is-no-cookbook-for-restoring-and-managing-prairies/> [accessed 15 August 2013].
- Hofmann, M., and J. Isselstein. 2004. Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science* **7**:193–200.
- Hulbert, L. C. 1969. Fire and litter effects in undisturbed bluestem prairie in Kansas. *Ecology* **50**:874–877.
- Hutchings, M. J., and K. D. Booth. 1996. Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land. I. The potential roles of the seed bank and the seed rain. *Journal of Applied Ecology* **33**:1171–1181.
- Ilmarinen, K., J. Mikola, K. Nissinen, and M. Vestberg. 2009. Role of soil organisms in the maintenance of species-rich seminatural grasslands through mowing. *Restoration Ecology* **17**:78–88.
- Jacquemyn, H., C. V. Mechelen, R. Brys, and O. Honnay. 2011. Management effects on the vegetation and soil seed bank of calcareous grasslands: An 11-year experiment. *Biological Conservation* **144**:416–422.
- James, J. J., M. A. Caird, R. E. Drenovsky, and R. L. Sheley. 2006. Influence of resource pulses and perennial neighbors on the establishment of an invasive annual grass in the Mojave Desert. *Journal of Arid Environments* **67**:528–534.
- Johnson, L. C., and J. R. Matchett. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. *Ecology* **82**:3377–3389.
- Johnson, R. G., and R. C. Anderson. 1986. The seed bank of a tallgrass prairie in Illinois. *American Midland Naturalist* **115**:123–130.

- Johnstone, I. 1986. Plant invasion windows: a time-based classification of invasion potential. *Biological Reviews* **61**:369–394.
- Jonasson, S. 1988. Evaluation of the point intercept method for the estimation of plant biomass. *Oikos* **52**:101–106.
- Kedzierski, G. 2013. Native seed production for grassland restoration — a grower’s perspective. *Ecological Restoration* **31**:130–134.
- Kenworthy, J. B. 1963. Temperatures in heather burning. *Nature* **200**:1226.
- Körner, C., J. Stöcklin, L. Reuther-Thiébaud, and S. Pelaez-Riedl. 2008. Small differences in arrival time influence composition and productivity of plant communities. *The New Phytologist* **177**:698–705.
- Lenz, T. I., and J. M. Facelli. 2005. The role of seed limitation and resource availability in the recruitment of native perennial grasses and exotics in a South Australian grassland. *Austral Ecology* **30**:684–694.
- Linkies, A., and G. Leubner-Metzger. 2012. Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. *Plant Cell Reports* **31**:253–270.
- MacDougall, A., and R. Turkington. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* **86**:42–55.
- Maron, J., and P. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia* **105**:302–312.
- Martin, L. M., and B. J. Wilsey. 2006. Assessing grassland restoration success: relative roles of seed additions and native ungulate activities. *Journal of Applied Ecology* **43**:1098–1109.
- McCain, K. N. S., S. G. Baer, J. M. Blair, and G. W. T. Wilson. 2010. Dominant grasses suppress local diversity in restored tallgrass prairie. *Restoration Ecology* **18**:40–49.
- McKendrick, J. D., C. E. Owensby, and R. M. Hyde. 1975. Big bluestem and indiagrass vegetative reproduction and annual reserve carbohydrate and nitrogen cycles. *Agro-Ecosystems* **2**:75–93.
- Mikola, J., H. Setälä, P. Virkajärvi, K. Saarijärvi, K. Ilmarinen, W. Voigt, and M. Vestberg. 2009. Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. *Ecological Monographs* **79**:221–244.
- Milbau, a., I. Nijs, F. De Raedemaeker, D. Reheul, and B. De Cauwer. 2005. Invasion in grassland gaps: the role of neighbourhood richness, light availability and species complementarity during two successive years. *Functional Ecology* **19**:27–37.

- Milbau, A., D. Reheul, B. De Cauwer, and I. Nijs. 2007. Factors determining plant–neighbour interactions on different spatial scales in young species-rich grassland communities. *Ecological Research* **22**:242–247.
- Miller, C. F. 2013. The evolving understanding of grassland restoration seeding protocols. *Ecological Restoration* **31**:127–130.
- Milliken, G. A., and D. E. Johnson. 2002. Analysis of messy data. Volume III: analysis of covariance. CRC Press, Boca Raton, FL.
- Morgan, J. 2001. Seedling recruitment patterns over 4 years in an Australian perennial grassland community with different fire histories. *Journal of Ecology* **89**:908–919.
- Mwangi, P. N., M. Schmitz, C. Scherber, C. Roscher, J. Schumacher, M. Scherer-Lorenzen, W. W. Weisser, and B. Schmid. 2007. Niche pre-emption increases with species richness in experimental plant communities. *Journal of Ecology* **95**:65–78.
- Naeem, S., D. E. Bunker, A. Hector, M. Loreau, and C. Perrings (Eds.). 2009. Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective. Oxford University Press Inc., New York, USA.
- National Oceanic and Atmospheric Administration, National Climatic Data Center, 2011-2012. URL <http://www.ncdc.noaa.gov> [accessed on 2 February 2013]
- Norland, J., S. Fasching, C. Dixon, K. Askerooth, K. Kelsey, and G. Wang. 2013. Reduced establishment of Canada thistle (*Cirsium arvense*) using functionally similar native forbs. *Ecological Restoration* **31**:143–147.
- Owensby, C. E., R. M. Hyde, and K. L. Anderson. 1970. Effects of clipping and supplemental nitrogen and water on loamy upland bluestem range. *Journal of Range Management* **23**:341–346.
- Packard, S., and L. Masters. 2008. Interseeding with and without raking (Illinois). *Ecological Restoration* **26**:113–114.
- Packard, S., and C. Mutel (Eds.). 1997. The tallgrass restoration handbook. Island Press, Washington, D.C.
- Piper, J. K., E. S. Schmidt, and A. J. Janzen. 2007. Effects of species richness on resident and target species components in a prairie restoration. *Restoration Ecology* **15**:189–198.
- Polley, H., J. Derner, and B. Wilsey. 2005. Patterns of plant species diversity in remnant and restored tallgrass prairies. *Restoration Ecology* **13**:480–487.
- Potvin, M. A. 2007. Establishment of native grass seedlings along a topographic/moisture gradient in the Nebraska Sandhills. *American Midland Naturalist* **130**:248–261.

- Pywell, R. F., J. M. Bullock, D. B. Roy, L. Warman, K. J. Walker, and P. Rothery. 2003. Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology* **40**:65–77.
- Rabinowitz, D., and J. Rapp. 1980. Seed rain in a North American tall grass prairie. *Journal of Applied Ecology* **17**:793–802.
- Rapp, J., and D. Rabinowitz. 1985. Colonization and establishment of Missouri prairie plants on artificial soil disturbances. I. Dynamics of forb and graminoid seedlings and shoots. *American Journal of Botany* **72**:1618–1628.
- Rayburn, A., and E. Laca. 2013. Strip-seeding for grassland restoration: past successes and future potential. *Ecological Restoration* **31**:147–154.
- Renne, I. J., and B. F. Tracy. 2007. Disturbance persistence in managed grasslands: shifts in aboveground community structure and the weed seed bank. *Plant Ecology* **190**:71–80.
- Ries, R. E., and T. J. Svejcar. 1991. The grass seedling: when is it established? *Journal of Range Management* **44**:574–576.
- Sher, A. A., and L. A. Hyatt. 1999. The disturbed resource-flux invasion matrix: a new framework for patterns of plant invasion. *Biological Invasions* **1**:107–114.
- Shunk, R.A. 1917. Plant associations of Shenford and Owego Townships, Ransom County, North Dakota. Thesis. University of North Dakota, Grand Forks, ND.
- Sluis, W. J. 2002. Patterns of species richness and composition in re-created grassland. *Restoration Ecology* **10**:677–684.
- Šmilauerová, M., and P. Šmilauer. 2010. First come, first served: grasses have a head start on forbs with prompt nutrient patch occupation. *Plant and Soil* **328**:327–336.
- Smith, M. D., J. C. Wilcox, T. Kelly, and A. K. Knapp. 2004. Dominance not richness determines invasibility of tallgrass prairie. *Oikos* **106**:253–262.
- Snyder, L. J., P. E. Rothrock, R. T. Reber, and N. D. Simmons. 2008. Interseeding forbs in a tallgrass prairie restoration: effects of three disturbance regimes - first growing season. Thesis. Taylor University, Upland, IN.
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. URL <http://websoilsurvey.nrcs.usda.gov/> [accessed 2 February 2011].
- Stevens, M. H. H., R. Shirk, and C. E. Steiner. 2006. Water and fertilizer have opposite effects on plant species richness in a mesic early successional habitat. *Plant Ecology* **183**:27–34.

- Symstad, A. J. 2008. Does biodiversity-ecosystem function science apply to prairie restoration? *Ecological Restoration* **26**:100–102.
- Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* **78**:81–92.
- Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. *Science* **294**:843–845.
- Wang, N., J.-Y. Jiao, Y.-F. Jia, W.-J. Bai, and Z.-G. Zhang. 2010. Germinable soil seed banks and the restoration potential of abandoned cropland on the Chinese hilly-gullied loess plateau. *Environmental Management* **46**:367–77.
- Warner, R., and P. Chesson. 1985. Coexistence mediated by recruitment fluctuations: a field guide to the storage effect. *American Naturalist* **125**:769–787.
- Watkinson, A. R., and J. C. Powell. 1993. Seedling recruitment and the maintenance of clonal diversity in plant populations – a computer simulation of *Ranunculus repens*. *Journal of Ecology* **81**:707–717.
- Weaver, J. E., and I. M. Mueller. 1942. Role of seedlings in recovery of Midwestern ranges from drought. *Ecology* **23**:275–294.
- Weber, S. 1999. Designing seed mixes for prairie restoration: revisiting the formula. *Ecological Restoration* **17**:196–201.
- Wedin, D., and D. Tilman. 1993. Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. *Ecological Monographs* **63**:199–229.
- Willand, J. E., S. G. Baer, D. J. Gibson, and R. P. Klopff. 2013. Temporal dynamics of plant community regeneration sources during tallgrass prairie restoration. *Plant Ecology* **214**:1169–1180.
- Williams, D. W., L. L. Jackson, and D. D. Smith. 2007. Effects of frequent mowing on survival and persistence of forbs seeded into a species-poor grassland. *Restoration Ecology* **15**:24–33.
- Wilson, J. B., and W. G. Lee. 2000. C - S - R triangle theory: community-level predictions, tests, evaluation of criticisms, and relation to other theories. *Oikos* **91**:77–96.
- Yurkonis, K. A. 2013. Can we reconstruct grasslands to better resist invasion? *Ecological Restoration* **31**:120–124.
- Yurkonis, K. A., and S. J. Meiners. 2006. Drought impacts and recovery are driven by local variation in species turnover. *Plant Ecology* **184**:325–336.
- Yurkonis, K. A., B. J. Wilsey, K. A. Moloney, and A. G. Van Der Valk. 2008. The impact of seeding method on diversity and plant distribution in two restored grasslands. *Restoration Ecology* **18**:311–321.

Zobel, M., M. Otsus, J. Liira, M. Moora, and T. Möls. 2000. Is small-scale species richness limited by seed availability or microsite availability? *Ecology* **81**:3274–3282.