HOW DOES YOUR PRAIRIE (RE)GROW?: INTERACTIONS OF SEED ADDITIONS WITH RESOURCE AVAILABILITY, HETEROGENEITY, AND DISTURBANCE ON RECRUITMENT AND DIVERSITY IN A RESTORED TALLGRASS PRAIRIE

by

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Abstract

Temperate grasslands are among the most threatened biomes in the world, with the largest historical losses due to conversion to agricultural land. While much of this biome has already been converted, there is concern the last remaining remnants in North America will be converted in response to increasing demand for crops used for ethanol production. Thus, restoring grasslands post-anthropogenic disturbance is increasingly important for conserving grassland biodiversity. Two major challenges for prairie restorations are establishing the many subdominant and rarer species found in native prairie, and offsetting the typical decline in richness and diversity over time as restorations age. Repeated seed addition of targeted species is commonly used to override low and declining plant richness and diversity. While this is generally effective early in restoration (i.e., as communities are establishing), its effectiveness in later stages (i.e., when established communities are often losing diversity) remains unknown. I investigated plant community responses to combinations of resource manipulations and disturbances coupled with a seed addition in a 15-yr old restored grassland to test the hypothesis that spatial resource heterogeneity increases the rate of colonization into established prairie restoration communities. Seeds were added to a long-term restoration experiment involving soil depth manipulations (deep, shallow) crossed with nutrient manipulations (reduced N, ambient N, enriched N). Seedling emergence was generally low and only 8 of the 14 forb species added were detected in the first growing season. I found no effect of increased resource heterogeneity on the abundance or richness of seedlings. There was a significant nutrient effect ($p < 0.1$, $\alpha = 0.1$) on seedling abundance, with higher emergence in the enriched N than the ambient N treatment. I also found unexpected nutrient effects on richness, diversity and Mean C (Mean C $= \Sigma\ CoC_i \bullet A_i$, where CoC=Coefficient of Conservatism and A=relative abundance of the ith species). All values, except Mean C, were higher in the enriched N treatment than in either the reduced or ambient N treatments. Mean C was lowest in the enriched N treatment, and highest in the whole-plot control, suggesting that the majority of species contributing to higher richness and diversity in the enriched N treatment were "weedier" species. In a separate experiment, I found no effect of small-scale disturbances (aboveground biomass removal or soil disturbance) on seedling abundance or seedling richness. I did find a marginal effect of disturbance type on

seedling richness (p=0.11, α=0.1), with higher seedling richness in the soil disturbance than the aboveground biomass removal treatment. I did not find any disturbance effects on community response variables. These results indicate that recruitment from seed additions into well-established restored communities is relatively low in the first year following a seed addition, regardless of resource availability and heterogeneity. Follow-up studies to determine recruitment rates in subsequent years are needed to elucidate whether recruitment responses are driven more by individual species differences or by environmental mechanisms.

Table of Contents

List of Figures

List of Tables

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Dedication

This thesis is dedicated to my parents, Randall and Loretta Stanton. It is their unending love, support, and encouragement that have brought me to where I am today. They shared their love of nature and reverence for the natural world with my siblings and me from the time that we were born. It is this exposure and encouragement that finally led me to the field of biology. Let us never forget the innate curiosity of children and to encourage all those we meet to question, explore and find their answers in the wonders of the natural world.

Chapter 1 - Background

Imagine riding across a vast landscape of prairie, where the only thing you can see to the horizon is a gently waving carpet of grass and wildflowers. This is a scene that I have played in my mind over and over. It is a scene that is rarely witnessed by extant human beings, especially those of us in North America. It is a sight that drew me to the very place I sit to write these words, in the heart of the Flint Hills of Kansas, the largest intact stretch of tallgrass prairie remaining in North America.

Prior to European settlement, the tallgrass prairie spanned a vast portion of North America, sprawling from south-central Canada down into southern Texas (Archibold 1995), covering over 68 million ha of land (Samson & Knopf 1994). It is estimated that a mere 4 - 13% of this historic extent remains today, with as little as one-tenth of one percent remaining in several states and provinces including much of the Great Plains (Samson & Knopf 1994, Samson *et al.* 2004). The majority of our native tallgrass prairie has been lost to cultivation and converted to agricultural croplands, largely beginning with the United States Homestead Act of 1862 (Ramankutty & Foley 1999, Samson *et al.* 2004, Eagan 2006). This Act led to the eventual plowing of millions of ha of native prairie, with the highest losses occurring in the 1920s and early 1930s (Eagan 2006). When the price of wheat sky rocketed, grasslands were ripped open, plowed under and planted with wheat. In just a few years' time, the price of wheat crashed, a terrible drought hit the Great Plains and the infamous Dust Bowl literally choked the "homesteaders" to death.

We are now faced with the potential loss of the last remaining areas of intact prairie. With advances in irrigation and drainage accompanied by a push for biofuel production backed by production subsidies and gasoline blending mandates (Fargione *et al.* 2009), another wave of grassland conversion is occurring in the northern and western Great Plains with the highest rates in the Prairie Pothole Region (Wright & Wimberly 2013, Johnston 2014). Fargione *et al.* (2009) reported that between 2005 and 2008, 4.9 million ha of land was planted to crops for ethanol production and that between September and October 2007 there was sharp decline in the amount of land enrolled in the Conservation Reserve Program (CRP). Of this 931,000 ha decline in CRP land, 850,000 ha were classified as grasslands (Fargione *et al.* 2009). Wright and Wimberly

(2013) estimated that between 2006 and 2011, grassland cover was being converted at rates of 5% - 30% on the Western edge of the Corn Belt in the Dakotas, Nebraska, Minnesota and Iowa with a net decline in grassland cover of approximately 530,000 ha. Between 1980 and 2011 the total area planted to corn or soy in North and South Dakota has more than tripled (Johnston 2014). In the Dakota Prairie Pothole Region, it is estimated that an overall increase of 27% in land area planted in corn or soy occurred between 2010-2012 alone, with much of this increase occurring at the expense of grasslands and wetlands (Johnston 2014). When considering that at most, 13% of our native, unplowed tallgrass prairie remains, conversion rates such as these are potentially devastating.

This devastation is not a mere loss of a "pretty landscape." The consequences of converting a self-sustaining ecosystem rich in biodiversity and environmental heterogeneity into an environmentally homogeneous monoculture that requires human intervention to function are extensive in size and impact. The conversion of our grasslands into row crops has resulted in widespread losses of stored soil carbon (Davidson & Ackerman 1993, Fargione *et al.* 2009, Glover *et al.* 2010, DeLuca & Zabinski 2011), excessive nitrogen (N) and phosphorus (P) inputs (Randall *et al.* 1997, Vitousek *et al.* 1997, Bennet *et al.* 2001, McKinley *et al.* 2005, Galloway *et al.* 2008, Vitousek *et al.* 2009, Gao *et al.* 2014), increased soil erosion (DeLuca & Zabinski 2011, Su *et. al.* 2004), pervasive water pollution (Foley *et al.* 2005, Bennet *et al.* 2001), unsustainable use of a limited water supply (Rosegrant *et al*. 2002), and declines in native biodiversity (Zobel *et al.* 2000, Isbell *et al.* 2011, Tillman *et al.* 2012).

Most of the tallgrass prairie was converted to agriculture primarily because of its characteristically deep fertile soils, rich in soil organic matter and carbon and its occurrence in areas with a climate conducive to crop growth. The deep A horizon of many grassland soils (i.e. topsoil) was created over thousands of years of annual growth and senescence of high volumes of above- and below-ground biomass that decomposes slowly, resulting in a large stores of organic matter with an estimated soil C storage rate in restored grasslands of 70-130 Mg (Mg= 1 x 10^6 g) C ha^{-1} in the top 20-30 cm alone (Mann 1986, Davidson & Ackerman 1993, DeLuca & Zabinsiki 2011). Several studies have documented and confirmed that cultivation of these intact systems results in the release of roughly 25-35% of the stored C inventory of uncultivated soils into the atmosphere (Detwiler 1986, Mann 1986, Post & Mann 1991, Schlesinger 1986, Davidson & Ackerman 1993) in the first 20 years following cultivation, with the majority of this

being lost within the first 5 years of cultivation (Detwiler 1986, Davidson & Ackerman 1993). Glover *et al.* (2010) reported that in Kansas, grasslands contained 43 Mg ha^{-1} more soil C than did nearby wheat fields. Alternatively, when cultivated land is taken out of production and the land is restored to perennial grassland, C sequestration rates have been estimated to be anywhere from 32.2g C m^{-2} yr^{-1} (Post & Kwon 2000) to 60.2 g m^{-2} yr^{-1} (Baer *et al.* 2002, McLauchlan *et al.* 2006). It is clear that the conversion of native tallgrass prairie, as well as short- and mixed-grass prairie, has led to an astoundingly large release of stored soil C to the atmosphere, further exacerbated by the drastic decreases in rates of soil C storage in cultivated soils and the CO_2 emissions associated with modern-day agricultural practices.

It is has also been well documented that the excessive sediments and N and P loads entering our waterways and coastal zones are primarily from agricultural runoff (Vitousek *et al.* 1997, Bennett *et al.* 2001, Foley *et al.* 2005, Galloway *et al.* 2008). These effects have recently been studied to a greater degree in light of increased demand for corn and soy, which are the primary crops that are being sown in former grasslands. Randall *et al.* (1997) reported that tile-drained crops of corn and soy lost 37 and 35 times as much, respectively, nitrate in subsurface drainage as their alfalfa and CRP counterparts. In addition, these agricultural systems were found to have increased evapotranspiration and increased discharge rates resulting in lower soil water availability and greater erosion (Randall *et al.* 1997). The impact on soil water loss, evapotranspiration, and depletion of aquifers such as the Ogallala, can be expected to be even greater in areas that are primarily irrigated, such as the state of Nebraska (Mubako & Lant 2008). Furthermore, nutrient and pesticides inputs are greatest in systems planted with corn and soy, which also have the highest rates of erosion (Mubako & Lant 2008), contributing to the rapid eutrophication of our continent's lakes, streams and oceans resulting in gulf hypoxia and the degradation of multiple ecosystems along the way (Bennett *et al.* 2001, Foley *et al.* 2005). The conversion of grassland to agricultural land also has substantial impacts on the global hydrologic cycle. Foley *et al.* (2005) reported that over 85% of the globe's water consumption is by agriculture alone. Advances in irrigation have allowed cultivation to expand into historically arid regions, leading to further declines in river and stream flow rates and over-use of ground water resulting in record low water table levels (Rosengrant *et al.* 2002, Foley *et al.* 2005).

In addition to the impacts of altered biogeochemical cycling, conversion of grasslands to agricultural fields also results in losses of native biodiversity. Several studies have demonstrated

trends in declining biodiversity with an increase in the intensity of land use and conversion (Foley *et al.* 2005, Flynn *et. al.* 2009, Isbell *et al.* 2001, Allan *et al.* 2014). To begin, in the remnant tallgrass prairie landscape at Konza Prairie Biological Station, over 600 species of plants, alone, coexist in this system (LTER c2013). This characteristically high amount of biodiversity in remnant prairies is often used as a benchmark of success in restorations. Therefore, management efforts are concentrated on maintaining and increasing levels of biodiversity through focused efforts to maintain and/or increase habitat for as many native grassland species as possible. As a result, the spatial heterogeneity of the landscape expands and subsequent increases in overall ecosystem functioning are seen. In contrast, modern agricultural practices typically focus on maximizing the productivity of a single species, often at the expense of hundreds of other species, both directly and indirectly, (Kremen *et al.* 2002, Ceballos *et al.* 2001, Geiger *et al.* 2010, Questad *et al.* 2011), and the landscape is managed for homogeneity rather than heterogeneity of resources and species.

Tallgrass prairie and other grasslands are characterized by a great deal of spatial heterogeneity at small and large scales. Maintaining this spatial heterogeneity requires periodic disturbances occurring at many scales (Howe 1994, 1999), and provides the uneven distribution of resources that allows a variety of species to successfully establish and coexist in different combinations, at different rates across a large landscape (Howe 1994, 1999, Collins & Steinauer 1998, Collins *et al.* 1998, Knapp *et al.* 1999, Rogers *et al.* 2001). These local "patches" of habit connect to create the regional matrix that promotes the high levels of biodiversity that are characteristic of grasslands (Glenn *et al.* 1990, Chesson 2000). When these matrices are fragmented and large areas of native grassland are replaced by spatially homogenous landscapes, we can expect to see loss of some species and declines in other species that are able to persist and coexist in a broken landscape (Tilman 1994, Tilman 2001 Leibold, *et al.* 2004, Kussari *et al.* 2009, Krauss *et al.* 2010). Additionally, within the homogenous environment of croplands competition for resources becomes heightened and those species often characterized as ruderal or early colonizers possess life history traits and strategies to out-compete species requiring greater resource heterogeneity, leading to an overall decline in the number of species and individuals present.

Temperate grassland ecosystems are now considered to be among the most endangered biomes in the world (Hoekstra *et. al* 2005). Nearly half (45.8%) of the world's temperate

grassland biome has been lost or converted to other uses, while an exiguous 4.6% is protected (Hoekstra *et. al* 2005). In other words, it has been estimated the world's grasslands are being converted at a rate eight times faster than the rate at which they are being preserved. It should also be noted that these figures were determined prior to the current conversion in the Prairie Pothole Region. This stark reality demands attention to and increased study of this threatened biome. Furthermore, it illustrates the reality that the majority of grasslands that our society and future societies will interact with will not be native (i.e. unplowed). Rather, they will most likely be restored or reconstructed systems.

Due to the differences in soil structure, soil resource availability, varying historical conditions, and the resultant differences in above- and belowground communities, we can expect that these restored systems are likely to function differently from one another and differently than their native counterparts. For these reasons, when practitioners speak of restoration, we do not mean that we restore a system back to its "natural state." Instead, ecological restoration is "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (SER 2004). The goal of restoration is to create an autogenic ecosystem that provides the services desired by the stakeholders investing in and supporting the restoration project. It is important to note that while ecological restoration can be and often is done with a goal of conservation and preservation, it is not synonymous with those two terms. Ecological restoration aims to restore the functioning of a system, as defined by the stakeholders involved with the project. Therefore, there are times when a restoration is undertaken not to conserve or preserve a landscape, but to create a landscape that the stakeholders desire.

Reaching the goal of restoring a self-sustaining system may require explicit attention to necessary biotic *and* abiotic factors, including the interactions among and between species and between species and their environment. To accomplish this, restoration practitioners often induce a number of biophysical manipulations, including environmental disturbances and species introductions. In short, the practice of ecological restoration is often the practice of re-assembling plant communities, especially when re-planting and restoring grasslands.

Understanding how plant communities re-assemble following disturbance is of great interest from both theoretical and practical perspectives. Disturbances are common in native grasslands, and can result from periodic fires, the activities of large ungulate grazers, the burrowing and foraging activities of small mammals, and other factors (Howe 1994, Collins &

Steinauer 1998). Understanding responses to these disturbances, and the processes leading to recovery of pre-disturbance community structure has relevance for basic population and community ecology. Because restoration can be viewed as an attempt to direct post-disturbance recovery, understanding the mechanisms underlying plant community assembly is essential for effective restoration. If we do not understand the processes involved in this type of ecosystem repair, we cannot initiate proper biophysical manipulations to facilitate the desired change from a degraded state to a sustainable system that more accurately reflects the native reference system (Clewell & Aronoson 2013), or meets the desired target state as defined by the stakeholders involved.

Through examining the effects of seed additions in conjunction with long-term manipulations of environmental heterogeneity and small-scale disturbance treatments, this study aims to add new insights into whether regional stochastic, local deterministic, or a combination of these factors constrains species diversity and species richness, henceforth referred to as diversity and richness, of restored prairie communities. With the reality that many grassland restorations have been underway for close to 50 years in some places, it is increasingly important that we understand how these factors influence community composition over longer temporal scales as well as at different stages of restoration.

Understanding community dynamics in the context of restoration ecology is important for any agency, organization or individual to successfully plan and implement a restoration plan. Knowing what outcomes are likely given the propagules available and manipulations possible will help managers to better anticipate which actions to take early on. Restoration is guided by goals, though the pathway to reach those goals can be rather blurry. There is often a great deal of uncertainty that a particular mix of species and disturbance will yield a desired result. This is particularly true when we are faced with restoring a large area in a dynamic system such as a tallgrass prairie.

The overarching goal of this thesis was to examine how propagule supply and environmental manipulations interact to affect community structure in a restored tallgrass prairie. Within this framework, I asked the following questions: (1) How have long-term manipulations of soil resource availability affected species richness and diversity in a restored tallgrass prairie? (2) How does soil resource heterogeneity affect the recruitment of new species from a seed addition into an established restored community? (3) How do small-scale disturbances that

6

increase light and microsite availability influence new species recruitment from a seed addition into an established restored community? These questions were aimed to better inform restoration practitioners on the fate of the costly seed that is added to increase the richness and diversity in prairie plantings. As my study was conducted in a 15-year old restoration with long-term soil resource manipulations, I also hoped to add insight into the responses of established communities to increased propagule supply and induced spatial heterogeneity.

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Chapter 2 - Effects of resource heterogeneity on long-term community responses and short-term seedling emergence in a restored tallgrass prairie

Abstract

In many parts of North America, only a small percentage of the historic extent of tallgrass prairie remains. With the recognition that a growing portion of the continent's tallgrass prairies will be restored ecosystems, it is imperative that we understand the mechanisms involved with maintaining this ecosystem's characteristically high levels of biodiversity. Two common challenges faced in prairie restorations are the lower levels of species diversity and richness at the onset of restoration and their subsequent declines over time. A common approach to override this decline is the addition of propagules via seed additions, though much remains unknown as to how effective this practice is, particularly in older restorations with well-established communities. One factor thought to prevent levels of species diversity and richness in restored communities from reaching levels characteristic of remnant prairies is the typically lower heterogeneity of resource availability and distribution in restored prairies. Here I tested the hypotheses that altered levels of soil resource availability will affect seedling success and enhanced heterogeneity will promote higher species diversity, richness and seedling emergence in a 15 year-old experimental prairie restoration. The original experiment was established to study the effects of soil resource availability and heterogeneity on plant species diversity and richness through manipulations to soil depth (shallow vs. deep), soil nutrient availability (reduced N, ambient N, enriched N) and the combinations of the two (shallow x reduced N, shallow x ambient N, shallow x enriched N, deep x reduced N, deep x ambient N, deep x enriched N). In this experiment I compared responses in pots that included all 6 combinations of resource availability and soil depth (maximum heterogeneity whole plot treatment) and plots that had only ambient nutrient levels and deep soils (whole-plot control treatments). In year 15 of the experiment, seventeen species not present in the existing community were added and I examined seedling responses as well as the responses of various environmental and community variables to the above treatments in the growing season immediately following the seed addition. Overall

trends in diversity and richness in the communities were different in year 15 than in earlier years of the experiment, with no detectable differences in diversity and richness with increased resource heterogeneity. Levels of nutrient availability affected several environmental and community variables, with significant differences detected between the enriched N treatment and the reduced N treatment, but no differences between the ambient N and the reduced N treatments. Overall seedling emergence was lower than expected with only 8 of the 14 forb species seeded detected, but survivorship was high (81%). Seedling abundance was higher in the enriched N treatment than the ambient N treatment, but no differences were detected between the enriched N and reduced N treatments, nor between the reduced N and ambient N treatments. No effect of resource heterogeneity was found on seedling abundance or seedling richness at the whole-plot level. The results from this study suggest that both community structure and seedling recruitment are affected by resource availability, but not by resource heterogeneity per se in well-established restored prairies. However, by comparing control treatments within the maximum heterogeneity and whole-plot controls, I did find an effect of the physical structuring and distribution of the soil depth and nutrient manipulations (a "matrix effect") on productivity and seedling abundance, suggesting that some effect of the matrix created by greater resource heterogeneity influences community structure. This study fails to support the hypothesis that greater environmental heterogeneity supports higher diversity. However, the results and conclusions of this study are limited by the short-term nature of the seedling monitoring, and further studies on the long-term effect of nutrient availability and heterogeneity in restored prairies are necessary to elucidate the mechanisms involved with reaching and maintaining desired levels of diversity and richness in restored prairies.

Introduction

The restoration of degraded ecosystems provides a unique context for examining and testing hypotheses about ecosystem functioning and processes as they relate to community assembly and anthropogenic disturbances (Camill *et al.* 2004, Temperton *et al*. 2004, Clewell & Arnonson 2012). When coupled with a robust experimental design, ecological restorations present an opportunity to explore and test various hypotheses and mechanisms related to community assembly, while still maintaining field conditions that reflect natural variation and

processes. While the use of broad spectrum restoration practices (e.g. planting times and methods, seed sources, etc.) has often meant that each restoration is in some sense a case-specific example, replicated restoration experiments can provide insights into general patterns and processes, which can be used to derive more general ecological principles about the specific system being studied.

In particular, restoration experiments provide an unprecedented opportunity to examine the mechanisms affecting biodiversity, species coexistence, and community assembly. One of the major challenges of prairie restoration is the difficulty in establishing the less common species that comprise much of the plant diversity in native prairie (Huston 1979, 1994, Camill *et al*. 2004), and the decline in species richness and diversity over time that occurs in many prairie restorations (Tilman 1993, 1997, Kindscher & Tieszen 1998, Sluis 2002, Baer *et al.* 2003, 2004, Camill *et al*. 2004, Baer *et al.*2005, McCain *et al.*2010, Middleton *et al.* 2010 Carter & Blair 2012). The most common approach to overriding this decline is to provide additional propagules through over-seeding into well-established restorations. However, little is known about how effective this approach really is, particularly in terms of its ability to establish viable populations in older restorations. In order to address this and to develop more effective means of enhancing and maintaining higher diversity in restored prairies, we need a better understanding of the mechanisms involved in promoting species coexistence and biodiversity, especially those factors and processes that prevent new species from entering and persisting in established communities (Hubbell 2001, Chase & Leibold 2003). In other words, we need to develop a better understanding of how restored communities assemble.

Community assembly can be viewed as the result of regional stochastic events and/or local deterministic events that shape both the species present and the environmental conditions that allow species to persist. Determining whether species coexistence and entry into an established prairie restoration community is a result of regional stochastic (e.g., propagule supply) or local deterministic processes (e.g. resource availability and species interactions) and whether recruitment of new species is dispersal-limited or niche-limited requires further investigation.

Dispersal can influence ecological processes through both population and community level dynamics by influencing species abundances and the resultant inter- and intra-specific interactions (DiVitorrio *et al*. 2007). It is no surprise then that several studies have found that

dispersal and seed availability can be a limiting factor for recruitment of new species, and may be an even larger driver than competition in the failure of native species to establish in restored prairies and grasslands (Hutchings & Booth 1996, Tilman 1997, Hamilton *et al.* 1999, Turnbull 2000, Bakker *et al.* 2003, Seabloom *et al.* 2003, Von Holle & Simberloff 2005, DiVitorrio *et al.* 2007, Kardol *et al.* 2008, Purschke *et al.* 2012). Several studies have demonstrated an increase in species richness immediately following seed additions, lending support to the 'species pool hypothesis,' which suggests that increased diversity and colonization is constrained by propagule availability, rendering community assembly dispersal-limited (Eriksson 1993, Tilman 1993, 1997; Zobel 1997, 2001; Zobel *et al.* 2000, Foster *et al.* 2007, Questad & Foster 2008). According to this hypothesis, communities are determined by regional stochastic events. If the regional species pool grows in richness, it is predicted that the local species pool will as well. Thus, it is expected that a seed addition, which manipulates the regional species pool and alleviates dispersal limitation, will result in an increase in species richness.

There is also support for the hypothesis that richness is a direct result of local deterministic factors, such as competition, disturbance and altered resource availability (Suding *et al.* 2005, Gibson *et al.* 2011). Several studies have demonstrated the role of competition for soil N, water, and light availability in influencing plant community composition, diversity and richness (Smith & Huston 1989, Wedin & Tilman 1993, Blumenthal *et al.* 2003, Gross *et al.* 2005, Lamb *et al.* 2008). Due to these documented interactions, the 'niche limitation hypothesis' posits that recruitment is limited by niche availability, rather than propagule availability (Tilman & Pacala 1993). According to this hypothesis, richness & diversity are constrained by resource availability, such as light availability and soil N availability and heterogeneity, rather than by a lack of species' presence in the regional species pool. Thus, simply alleviating dispersal limitation through a seed addition would not result in increased species richness. Rather, it is predicted that an expansion of niche space through manipulations resulting in increased soil and/or light availability and heterogeneity, will lead to an increase in species richness.

Finally, there is also support for a hypothesis predicting that community richness and diversity is a result of both stochastic and deterministic factors influencing community assembly. The 'shifting niche limitation hypothesis' proposed by Foster *et al.* (2004) predicts that richness is a product of both the regional species pool and local processes limiting niche availability. According to this hypothesis, diversity in a community is a result of the interplay between

stochastic and deterministic as well as local and regional processes (Foster 2001, Foster *et al.* 2004, Foster & Dickson 2004). If this hypothesis holds true, I would expect to observe an increase in richness when both the regional species pool and deterministic factors are manipulated, such as adding propagules to an area with a recent soil disturbance.

Through examining the effects of seed additions in conjunction with long-term environmental heterogeneity manipulations and small-scale disturbance treatments, this study will add new insights into whether regional stochastic, local deterministic, or a combination of these factors constrains diversity and richness of restored prairie communities. With the reality that many grassland restorations have been underway for close to 50 years in some places, it is increasingly important that we begin to investigate how these factors influence community composition over longer temporal scales as well as at different stages of restoration.

Furthermore, as restoration practitioners, it is imperative that we have an understanding of how direct manipulations of a system and its local, regional, stochastic and deterministic processes will influence the trajectory of recovery. If we misjudge the mechanisms involved with community assembly and ecosystem functioning, it is possible that our manipulations will facilitate a trajectory towards an undesirable state, or cause further degradation, rendering our time, labor and finances poorly spent. Thus, it is necessary that we test the above hypotheses to develop a deeper and more predictive understanding of the factors influencing and the mechanisms involved in species diversity, richness and community assembly in restored prairies.

Here I test these hypotheses in the context of a long-term experimental prairie restoration designed to investigate the role of soil resource availability (soil depth and nutrient availability) and heterogeneity in promoting diversity and richness in restorations. Knowing that the communities in this restoration have declined in richness and diversity over time (Baer *et al.* 2003, 2004), I aimed to investigate their responses to a seed addition. In this study, seventeen native prairie species, previously not documented to exist in the restored prairie community were added to the community and I tested for effects on seedling responses at three distinct levels. First, I tested for effects of the soil resource availability treatments (deep vs. shallow soil; ambient vs enriched vs reduced N) and any interactions between the soil depth and nutrient availability treatment combinations (soil x nutrient, n=6) within the whole-plot maximum heterogeneity treatment. Second, I tested for a resource heterogeneity effect, looking for differences between the control (low spatial resource heterogeneity) and the maximum

heterogeneity treatment (high spatial resource heterogeneity). Third, I tested for what will be referred to as a matrix effect. The matrix effect is the effect that the physical structuring and distribution (the matrix) of the soil depth and nutrient manipulations may have on the response variables. To do this, I compared response variables in the "control" subplots (deep x ambient N) within the whole-plot maximum heterogeneity treatment to the whole-plot control (deep x ambient N), as described in the Methods section below. I also tested for these same three effects on a variety of environmental and community variables to asses whether previous trends in community structure persist.

According to the 'shifting niche limitation hypothesis' of Foster et al. (2004), I expected to see greater emergence of seedlings in the communities with soil resource manipulations as both regional stochastic events and local deterministic events are being manipulated. Furthermore, because we know from previous studies in this restoration experiment that vegetation height is lower in the reduced N treatments (Baer *et al.* 1999), I expected that greater seedling recruitment, in response to increased light availability, would occur in the reduced N treatments compared to either the ambient or enriched N treatments.

Methods

Study site description

All research was conducted at the Konza Prairie Biological Station (KPBS), a 3,487 ha tallgrass prairie located approximately 9km south of Manhattan, Kansas, USA (30°05'N, 96°35'W). KPBS is located in the Flint Hills region of northeastern Kansas, which comprises one of the largest intact tracts of tallgrass prairie to remain in North America. The dominant vegetation at KPBS is typical of native prairie in this region, with the dominant species being C_4 grasses including: *Andropogon gerardii* (Big bluestem), *Sorghastrum nutans* (Indiangrass), *Panicum virgatum* (Switchgrass) and *Schizachyrium scoparium* (Little bluestem) (Freeman 1998). The mean annual air temperature at KPBS is 13°C, with the January temperatures ranging from -9-3°C and July temperatures ranging from 20-33°C (Nippert & Knapp 2007). Approximately 75% of the average 835 mm precipitation received annually coincides with the growing season (ca. April – August) (Hayden 1998).

Sampling and field measurements were conducted in a long-term prairie restoration experiment established in a 3.2 ha lowland agricultural field within the KPBS. The site is characterized by deep Reading silt loam soils with 0-1% slope. This area had been in agricultural production for over 50 years prior to the initial establishment of the experimental restoration in June of 1997 (Baer *et al.* 1999) and has mostly been burned on an annual basis in the spring.

Experimental design

The restoration experiment where the majority of my research was conducted was established in June of 1997. Sixteen whole plots measuring 6 x 8 m were delineated and separated by 6 m buffers. Each 6 x 8 m plot was further divided into twelve 2 x 2 m subplots for sampling ($n = 192$). Within each 2 x 2 m subplot, the center 1 m^2 was delineated and four 0.25-m^2 quadrats were established, with the southwest and the northeast quadrats reserved for non-destructive sampling, and the southeast and northwest quadrats reserved for destructive sampling. Four replicate blocks, each consisting of four 6 x 8 m plots were established. Each plot within a block was randomly assigned one of the four following whole plot treatments: untreated control (I), altered soil depth (II), altered soil N availability (III), or a combination of both altered soil depth and soil N availability (IV). See Figure 2.1 for a map of the design. The research I conducted was constricted to only those plots receiving treatment I or IV. Treatment IV consists of a factorial combination of two levels of soil depth (deep and shallow) and three levels of soil N availability (ambient, reduced and enriched). These factors were randomly assigned within a plot, with soil depth assigned to horizontal strips and soil N availability assigned to vertical strips, resulting in two 2 x 2 m replicate subplots of six different treatment combinations (shallow x ambient N, shallow x enriched N, shallow x reduced N, deep x ambient N, deep x enriched N and deep x reduced N) in each of the four treatment IV plots. For a detailed description of the experimental site description and treatments see Baer *et al.* (1999).

Seed additions

Seventeen novel species (i.e., not present in the established community and not previously added as seed) of forbs and graminoids representing a functionally diverse group were chosen to add to the restoration plots. Species were chosen based on their documented absence from the community and the availability of adequate seed supply. All seed was

purchased from Prairie Moon Nursery (Winona, Minnesota) and subjected to the recommended stratification and scarification protocols.

Seeds were added to the established restoration plots on 15 April 2013, following a prescribed spring burn. We waited for a relatively calm day with winds under 10 mph to avoid losing seeds to the wind. Seeds were mixed with damp builder's sand and hand broadcast evenly at a rate of 20 seeds m^{-2} onto each of the 16 experimental plots. Enough vegetation had emerged at the time of seeding to provide an uneven surface for the seeds to settle into without having to be raked.

Environmental variables

Soil N availability

Resin bags constructed from nylon material and filled with 20g of a 1:1 mixture of strongly acidic cation (Dowex 50 WXZ, Sigma Chemical, Stl. Loius, MO USA) and strongly basic anion (Dowex 1 x 8-50) resins preloaded with H^+ and Cl^-, respectively, were buried and used to obtain a relative index of inorganic N availability. Resin bags were placed in the surface 10 cm of soil, one in each subplot in July and harvested in November. Following removal, excess soil was rinsed from the resin bags with deionized water. The bags were then extracted with 75 mL of 2 M KCL by shaking for 1 hour at 200rpm, and filtered through 0.4-μm polycarbonate membranes. Inorganic C was analyzed calorimetrically on an Alpkem Flow Solution autoanalyzer (Clakamas, OR, USA). Nitrate (NO_3-N) was measured via diazotization with sulfanilamide after reduction through a cadmium coil (Keeney & Nelson 1982). Ammonium (NH_4-N) was measured using the phenol blue method. As the resin bags extracts had a high H^+ ion concentration, samples were neutralized prior to extraction.

Light availability

Mid-season percent light transmission through the plant canopy was quantified in all of the plots on 19 June 2013. Measurements were taken as near solar noon as possible from 11:45-13:50 Central Daylight Time in full sun conditions. In each subplot five measurements of photosynthetic photon flux density (PPFD) ($\mu mol \cdot m^{-2} \cdot s^{-1}$) were taken. One measurement was taken above the canopy and four orthogonal readings were taken at the soil surface. All measurements were taken with an AcuuPAR model LP-80 PAR/LAI ceptometer (Decagon,

Pullman, Washington) with only the top 50 cm activated. The five measurements were averaged for each location in the quadrat and then PPFD was averaged over both quadrats in each subplot. Available and intercepted PPFD were calculated for each subplot and light transmission was expressed as percentage of maximum PPFD ($n = 96$).

Community metrics

Aboveground net primary productivity (ANPP)

At the end of the growing season, aboveground biomass was harvested from one of the two quadrats reserved for destructive sampling in each of the subplots. These plots are clipped on an annual basis. Effects of repeated removal are controlled for by alternating which quadrats are clipped from year to year. Each quadrat was clipped to ground level within a frame measuring 0.1 m^2. Biomass was oven-dried at 60 ºC for a minimum of 48 hours, separated into grasses or forbs and weighed by category. The weights for each quadrat were used as an estimate of ANPP m^{-2} for each subplot. Since all plots were burned prior to the start of the growing season, annual productivity estimates reflect the biomass produced in the year of measurement.

Vegetation analyses

In early June and late August of 2013, percent cover of each plant species was visually assessed for all species rooted within each of the two 0.25-m^2 quadrats reserved for non-destructive sampling. To calculate plant species richness and diversity for each subplot, I averaged the maximum cover value for each species from the combined early and late summer sampling dates for the two quadrats within each subplot. Then each of the 12 subplot averages were averaged across each plot for whole-plot values.

Shannon's index of diversity, Simpson's index of diversity and total species richness were calculated using peak total cover as a measure of abundance for each species. Shannon's index of diversity was calculated for each subplot as $H' = - \Sigma\, p_i \ln p_i$ where p_i is equal to the proportion of total cover contributed by each species. Simpson's index of diversity was calculated as $D = \Sigma\, p_i^2$ where p_i^2 is equal to the relative cover of the ith species. As Simpson's index measures the chance that two individuals are from the same species, higher levels of

diversity are indicated by lower values of D. Finally, total richness was calculated as the mean number of species present in each subplot.

To asses the quality of the community, as defined by the presence of native species that are typically restricted to remnant prairies, I used the mean coefficients of conservatism (CoC) (Mean C). The CoC utilizes a scale from 0 to 10 to indicate species' fidelity to remnant sites, or sites that have remained relatively undisturbed by anthropogenic activity. I used CoC values determined for Kansas as available from the Kansas Biological Survey and the R.L. McGregor Herbarium at the University of Kansas. Values equal to 0 represent the "weediest" species, with values of 5 = "intermediate" and values of 10 = "most conservative" (Freeman 2012). Recent studies have suggested that Mean C explains a greater amount of variation among tallgrass prairie communities than the more conventional measures of diversity and richness (Taft *et al.* 2006, Hansen & Gibson 2014). According to Carter and Blair (2012), calculating the Mean C weighted by species' relative abundance for each sampling unit was a superior measure of variation than unweighted mean C. Thus, I choose to only calculate weighted Mean C. To calculate the weighted Mean C, the sum of each species' CoC was multiplied by its relative abundance for each subplot as follows:

$$\text{Mean C} = \Sigma \, CoC_i \bullet A_i$$

Where CoC is equal to the coefficient of conservatism for the ith species and A is equal to the relative abundance of the ith species, based on peak total cover values.

Seedling emergence and monitoring

Seedling emergence was monitored on a weekly basis, beginning in late May and continuing to the end of the growing season in mid-September, once most vegetation began to senesce. Monitoring activities decreased to a bi-weekly basis for the months of August and September, as no new relevant seedlings emerged and the communities appeared stable. Due to the difficulty in distinguishing graminoid seedlings from tillers of existing populations, I monitored only the 14 forb species.

Monitoring occurred only in those plots receiving treatment I or IV (Fig 2.1). Within these plots, each of the two 0.25-m^2 quadrats within each 2 x 2 m subplot reserved for non-destructive sampling were monitored, as described above for seedling emergence and seedling status.

When a seedling emerged that was determined to possibly be one of the 14 monitored forb species, a colored craft stick was placed on the north side of it (Fig 2.2). The color of the stick and the species name or number was recorded. As several individuals were not fully identifiable to species until near the end of the growing season, many were assigned a name using the scheme "Unknown 1, 2, 3,..." etc. Each plot was monitored for the status of the marked species as well as for any new seedlings each week. New seedlings were marked well into August, though none of these turned out to be any of the seeded species.

Statistical analyses

I examined the response of seedling emergence to treatments at three distinct levels: 1) The effect of the individual soil depth and nutrient treatments within the whole-plot maximum heterogeneity treatment (to assess effects of soil resource availability), 2) The effects of the whole-plot treatments (to assess effects of soil resource heterogeneity), and 3) The "matrix effect" of the location of the control treatments in the whole-plot treatments (to assess whether the surrounding treatments influences responses in control subplots). The overarching experimental design in this study is unique in that each block consisted of four different whole-plot treatments, each containing various dimensions and replications of experimental units (strips) assigned to the six possible treatment combinations (Fig 2.1). When an experimental design contains experimental units of differing sizes and unequal replication of treatments, differences in error structures associated with each treatment will arise (Milliken and Johnson 1992). Thus, I analyzed all data utilizing the mixed-model procedure in SAS version 9.2 (SAS Institute 2008) in order to specify the fixed and random effects.

When testing for effects of the whole-plot treatments, block was set as a random factor. Differences between the mean seedling response to each independent whole-plot treatments were examined. Block was also set as the random factor when testing for the "matrix effect" of the whole-plot maximum heterogeneity treatment. Here I tested for differences between the mean seedling response to the whole-plot control treatment and the response to *only* the deep x ambient N treatment *within* the whole-plot maximum heterogeneity treatment.

When testing for effects of the individual treatments within the whole-plot maximum heterogeneity treatment, the fixed and random effects were specified as follows: 1) Fixed effects were set for nutrient treatments, soil depth treatments and interaction between the two. 2)

Random effects were set for blocks, interaction between blocks and the soil depth and nutrient treatments, interaction between blocks, soil depth and nutrient treatments, soil depth replication, interaction between blocks and soil depth replication, and interaction between blocks, nutrient treatments and soil depth replication. Satterthwaite's method was thus used to estimate the denominator degrees of freedom associated with our different error terms (Milliken and Johnson 1992, Littell *et al.* 1996). Tukey-Kramer's adjustment for interaction contrasts was utilized to test for statistical differences between the mean responses of seedling abundance to the treatments. Due to the relatively small sample sizes of the study, the significance of all results were analyzed at α=0.10.

Relationships between community indices, seedling abundance and seedling richness were examined since a main effect of the nutrient treatments was found for seedling abundance and richness. Pearson product-moment correlation coefficients were determined from the mean response of each variable to each of the three nutrient treatment subplots, from the four whole-plot maximum heterogeneity treatments, *n*=12 (SAS Institute 2008). Due to the relatively small sample size, we analyzed the significance of all results at α=0.10.

Results

Long-term responses to restoration treatments

Responses to resource availability

Environmental variables

A significant nutrient effect was found for both resin – collected total inorganic N ($F_{2,2}$=15.02, p=0.0624) and NO_3-N ($F_{2,2}$=15.25, p=0.0615), but not for NH_4-N ($F_{2,2}$=4.65, p=0.1770). Total inorganic N was higher in the enriched N treatment than in the ambient and enriched N treatments, but no difference was detected between the ambient and enriched N treatments (l.s. mean $_{reduced}$=33.1612±575.82, l.s. mean $_{ambient}$=104.19±517.50, l.s. mean $_{enriched}$=3607.03±517.50; Tukey-adj. p $_{ambient-enriched}$= 0.0738, Tukey-adj. p $_{enriched -reduced}$ = 0.0789) (Fig 2.3). The same pattern was detected for NO_3-N (l.s. mean $_{reduced}$=5.7246±549.38, l.s. mean $_{ambient}$=53.5447±493.74, l.s. mean $_{enriched}$=3431.41±493.74; Tukey-adj. p $_{ambient-enriched}$= 0.0742, Tukey-

adj. $p_{\text{enriched -reduced}}$ = 0.0783) (Fig 2.3). There were no effects of soil depth on any of the three N availability measures, nor any significant soil depth x nutrient availability interactions.

There was a significant nutrient effect on canopy light interception ($F_{2,6}$=6.76, p=0.0290) with nearly twice the amount of light available at the soil surface in the reduced N treatments than in the enriched N treatments (l.s. mean $_{\text{reduced}}$=0.2336, l.s. mean $_{\text{enriched}}$=0.1301, SE=0.0284; Tukey-adj. p= .0242) (Fig 2.4). There were no differences in light availability between the ambient N treatment and the reduced N treatment, nor between the ambient N treatment and the enriched N treatment (Fig 2.4). There was no effect of soil depth on light availability, nor was there a significant soil depth x nutrient availability interaction.

Community variables

Surprisingly, there were no detectable differences between total, grass or forb ANPP in the three nutrient treatments. However, a significant nutrient effect and soil depth x nutrient interaction were found for both Shannon's index of diversity (H') ($F_{2,6}$=5.36, p=0.0462; $F_{2,6}$=5.68, p=0.0412) and Simpson's index of diversity (D) ($F_{2,6}$=4.01, p=0.0785; $F_{2,6}$=4.47, p=0.0647). The enriched N treatment yielded higher H' than the reduced N treatments (l.s. mean $_{\text{enriched}}$ =1.2184, l.s. mean $_{\text{reduced}}$ =0.8529, SE=0.0949; Tukey-adj. p= .0427), while no differences were detected between the ambient N treatment and either of the enriched N or reduced N treatments (Fig 2.5). Simpson's index was lower in the enriched N treatments than the reduced N treatments (l.s. mean $_{\text{enriched}}$ =0.4161, l.s. mean $_{\text{reduced}}$ =0.5903, SE=0.0474; Tukey-adj. p= .0759), while there were no differences between the ambient N treatment and either of the enriched N or reduced N treatments (Fig 2.6). The interaction of deep soil x enriched N showed greater H' than deep soil x ambient N (Tukey-adj. p= .0604) and deep soil x reduced N (Tukey-adj. p= .0604). In other words, deep soil x enriched N supported the highest H' (l.s. mean $_{\text{deep x enriched}}$ =1.3208, l.s. mean $_{\text{deep x ambient}}$=0.7556, l.s. mean$_{\text{deep x reduced}}$ =0.7389; SE=0.1264). No significant effects were detected for shallow soil x nutrient treatments. Simpson's index was lower in the deep soil x enriched N treatment than in the deep soil x ambient N treatment (Tukey-adj. p=0.0886), but only marginally lower than in the deep soil x reduced N treatment (Tukey-adj. p=0.1132) (l.s. mean $_{\text{deep x enriched}}$ =0.3700, l.s. mean$_{\text{deep x reduced}}$ =0.6399, l.s. mean $_{\text{deep x ambient}}$=0.6583; SE=0.0626). No significant effects were detected for shallow soil x nutrient treatments. No further soil depth effects were found for either diversity indices.

A significant nutrient effect was returned for seedling richness ($F_{2,6}$=4.39, p=0.0668). The enriched N treatment supported greater richness than the reduced N treatment (l.s.mean $_{enriched}$ =7.9375, l.s.mean $_{reduced}$ =6.0625, SE=0.4627; Tukey-adj. p=0 .0569), but no detectable differences were found between the ambient N treatment and either the reduced N or enriched N treatments (Fig2.7). No soil depth effect nor soil depth x nutrient interactions were found for species richness.

When testing for effects on weighted Mean C, I found evidence contrary to the other diversity measurements. A significant nutrient effect was once again detected ($F_{2,6}$=18.18, p=0.0028), but the enriched N treatment had a lower Mean C than either the ambient N (Tukey-adj. p=0.0092) or reduced N treatments (Tukey-adj. p=0.0031) (l.s. mean $_{enriched}$ =3.3250, l.s. mean$_{ambient}$ =3.9897, l.s. mean $_{reduced}$ =4.1563, SE =0.1501) (Fig 2.8). There was no detectable difference between Mean C in the reduced N and ambient N treatments. No soil depth effect and no soil depth x nutrient interactions were found.

Responses to resource heterogeneity

Environmental variables

There was again a significant difference in resin-collected total inorganic N ($F_{1,3}$=22.28, p=0.0180) and NO_3-N ($F_{1,3}$=23.68, p=0.0166) between the whole-plot maximum heterogeneity and control treatment. Both total inorganic N (l.s. mean$_{control}$=80.0634, l.s. mean$_{MH}$=1336.47, SE=191.23) and nitrate (l.s. mean$_{control}$=28.3297, l.s. mean$_{MH}$=1247.00, SE=177.38) (Fig 2.9) means were higher in the maximum heterogeneity treatment. However, there were no differences between the levels of light availability reaching the soil surface in the maximum heterogeneity and control plots, indicating that although nutrient availability affected light availability, the overall spatial heterogeneity of resources did not affect light availability.

Community variables

Very few effects of nutrient availability, scaled up to the larger whole-plot level, were found, indicating that overall there is little effect of the spatial heterogeneity of resource availability on the community structure. I detected no differences between total ANPP, grass ANPP or forb ANPP in the whole-plot maximum heterogeneity and control treatments in 2013. Likewise, there were no detectable differences in diversity measures (H' and D) and richness

26

between the maximum heterogeneity and control plots. However, there was a significant whole-plot treatment effect on Mean C values ($F_{1,3}$=8.29, p=0.0636), with higher Mean C in the whole-plot control than in the whole-plot maximum heterogeneity treatment (l.s.mean$_{control}$=4.1005, l.s.mean$_{MH}$=3.8237, SE =0.08949) (Fig 2.10).

Responses to the matrix of resource availability and heterogeneity

Environmental variables

Overall, no differences in environmental responses were detected between the whole-plot control treatment (deep soil x ambient N) and the control subplots (deep soil x ambient N) within the whole-plot maximum heterogeneity treatment. No differences in the level of resin-collected total inorganic N, NO_3-N, or NH_4-N were detected nor were any in light availability at the soil surface.

Community variables

Akin to the environmental variables, there were no matrix effects on community variables, aside from one. I did find statistically significant differences in total ANPP ($F_{1,3}$=5.59, p=0.0990), forb ANPP ($F_{1,3}$=6.82, p=0.0796) and a marginal difference in grass ANPP ($F_{1,3}$=5.01, p=0.1111) between the maximum heterogeneity control subplots (deep x ambient N) and the whole-plot controls (deep x ambient N), with higher productivity in the maximum heterogeneity control plots (Fig 2.11). However, no differences were detected for H', D, species richness, or Mean C.

Seedling emergence

Of the 14 forb species seeded, I recorded emergence for only 8 species (Table2.1). A total of 85 individuals emerged with all but 14 of these surviving to the end of the growing season (Table2.1). Despite the low species emergence, overall seedling survival was quite high at 81%, which is typical of other seedling emergence field studies (Kitajima & Tilman 1996, Hillhouse & Zedler 2001, Isselstein *et al.* 2002). The rank-abundance curve for the species that emerged clearly demonstrates that the majority of species detected had only a few individuals emerge, while one species, *Mirabilis nyctaginea,* accounted for over 71% of the total seedling abundances (Fig 2.12).

Responses to resource availability

Mixed-model ANOVAs indicated a significant nutrient effect on seedling abundance ($F_{2/6}$=4.61, p=.0613), with greater emergence in the enriched N treatment than in the ambient N treatment (l.s. mean $_{enriched}$ =1.3125, l.s. mean$_{ambient}$ =0.3750, SE=0.2262; Tukey adj. p=.0593) (Fig 2.13).   There were no significant differences in seedling abundance between ambient N and reduced N treatments or reduced N and enriched N treatments. Similarly, significant differences in seedling richness were found ($F_{2/6}$=3.95, p=0.0805), with higher richness in the enriched N treatment than the ambient N treatment (l.s. mean $_{enriched}$ =1.0625, l.s. mean$_{ambient}$ =0.3125, SE=0.1989; Tukey adj. p=.0700) (Fig 2.14). No differences were found between the ambient N and reduced N treatments or between the reduced N and enriched N treatments.

Responses to resource heterogeneity

There were no detectable differences in the number of seedlings to emerge between the whole-plot maximum heterogeneity treatment and the control, indicating that the observed nutrient availability effects did not "scale-up" to the whole-plot level (Fig 2.15). Likewise, no detectable differences in seedling richness were found (Fig 2.16).

Responses to the matrix of resource availability and heterogeneity

When I compared seedling abundance in the control (deep x ambient N) subplots within the maximum heterogeneity plots to the whole-plot controls (deep x ambient N), I did find a significant matrix effect ($F_{1,3}$=5.67, p=0.0975), whereby nearly three times as many seedlings emerged in the whole-plot control compared to the maximum heterogeneity control subplots (Fig 2.17). Interestingly, there was not a statistical difference in seedling richness between the two (Fig 2.17), indicating that there was an effect of the matrix on the actual number of seedlings to emerge, but not on the number of species to emerge. In other words, there was not a matrix effect on species, but rather on actual individuals.

Relationships between environmental, community and seedling responses

I also examined relationships between seedling emergence and the environmental and community variables in relationship to the nutrient effect found through the ANOVA, but found very few correlations. Aside from a strong, significant correlation between seedling abundance and seedling richness (Fig 2.18), the only relationships I found were between the indices of

diversity (H' and D) and both seedling abundance and richness (Fig 2.19). Seedling abundance and seedling richness were both positively correlated with H' while negatively correlated with D.

Discussion

Long-term responses to restoration treatments

Overall I did not find evidence for the expected differences in community composition and structure between the whole-plot control and the whole-plot maximum heterogeneity treatments. I found no differences between ANPP, light availability, diversity, or richness in the whole-plot control versus the whole-plot maximum heterogeneity treatment. The lack of difference in diversity is not surprising, as long-term data from these plots has shown that diversity is historically the same between the two (Baer *et al.* 2003, Baer *et al.* 2004). However, I did find a difference in the Mean C, with the whole-plot control having a higher value, and thus hosting a community more reflective of remnant prairie than the whole-plot maximum heterogeneity treatment. This warrants a re-examination of the long-term data using the measure of weighted Mean C to determine whether this difference has been a consistent trend or has changed over time.

A recent examination of the long-term community composition trends showed that while there have been no differences in diversity (Fig 2.20), there have been differences in total richness (Fig 2.21). An overall decline in total richness across time was observed for both the whole-plot maximum heterogeneity and the whole-plot control treatments (Fig 2.20). No differences in richness were found between the two whole-plot treatments for the first 8 years of the experiment (1998-2005) (Fig 2.21). However, following the seed addition in 2005, there was a slight increase in richness for both the maximum heterogeneity treatment and the control, indicating that recruitment did occur. This finding offers evidence that richness was limited by propagule supply in *both* the maximum heterogeneity and the control plots. In subsequent years, richness in the maximum heterogeneity plots surpassed that of the control plots, indicating that perhaps niche limitation may be preventing the establishment of new species in the control plots. In 2013, though, I again observed no difference in richness between the maximum heterogeneity treatment and the control (Fig 2.20). These long-term changes suggest that there may still be a

relatively high degree of species turnover in these plots, most likely as result of environmental filtering due to changes in soil resource availability over time.

Soil resource availability in the whole-plot maximum heterogeneity treatments has been shown to differ across time in response to manipulations, and the total extractable NO_3-N levels in the control plots have decreased to levels more similar to those found in native prairie (Baer & Blair 2008). Furthermore, the levels of NO_3-N remain different between the two whole-plot treatments, presumably driven by the enriched N treatment. Within the maximum heterogeneity plots, there was no difference in NO_3-N between the ambient and the reduced N treatments, while the enriched N treatments had significantly higher levels of NO_3-N than other treatments. Moreover, there was no difference between the deep x ambient N treatments within the whole-plot maximum heterogeneity treatments and the control plots, indicating that there was no matrix effect on NO_3-N availability. This provides further evidence that the differences in total extractable NO_3-N levels between the control and the maximum heterogeneity plots are driven by the enriched N treatment.

Although I did not find significant differences in the community variables between the whole-plot treatments, I did find a nutrient effect within the whole-plot maximum heterogeneity treatment. Former studies examining the effects of the whole-plot and within-plot treatments found nutrient effects on various aspects of the community (Baer *et al.* 2003,2004), some of which are similar to my findings for 2013, and some of which differ. Within the first three years of this restoration experiment (1998-2001), there was a nutrient effect on ANPP, with the highest production occurring in the enriched N treatment driven by grass productivity, which accounted for 90% of all biomass produced within the first three years (Baer *et al.* 2003). There was no nutrient effect on forb productivity. However, in year 15 (2013) of the experiment, I found no nutrient effect on ANPP, grass, or forb production.

There was also a nutrient effect on diversity and richness within the first 3 years of the experiment, with a decline in richness over time in the ambient N and enriched N treatments, but an increase from year 2 to year 3 in the reduced N treatment (Baer *et al.* 2003, 2004). By year 3 of the study, richness and diversity were highest in the reduced N treatment and lowest in the enriched N treatments (Baer *et al.* 2003, 2004). By the 15th year, this radically changed. In 2013, diversity and richness were higher in the enriched N than the reduced N treatment, with no differences between the ambient N treatment and the enriched or reduced N treatments.

Additionally, diversity was inversely related to both N availability and total ANPP in year 3 of the study, while neither of these relationships were found in 2013. In fact, I found that richness was positively related to ANPP and grass productivity in 2013 while diversity was not statistically related to ANPP at all. Furthermore, richness was inversely related to Mean C while positively related to diversity, indicating again that there is a substantial difference between Shannon's index of diversity and Mean C. Due to the method of weighting Mean C by species abundances that I used, this finding indicates that the species that are colonizing and successfully establishing in the maximum heterogeneity plots are primarily those with low CoC values, or are species that are more characteristic of recently disturbed areas. However, it must also be recognized that the average CoC value for the seed addition in 2013 was lower than that of 2005, which was lower than that of the original seed mixture in 1998. Thus, the finding the Mean C is lower in the maximum heterogeneity plots than in the control plots may also suggest that greater recruitment of species from the seed additions has occurred.

It is also worth noting that these plots are burned most every year and are not grazed. In the highly productive tallgrass prairie of Kansas, frequent burning coupled with a lack of grazing results in communities dominated by C_4 grasses with overall lower diversity and richness, reduced temporal variability and decreased community heterogeneity than communities subjected to grazing and less frequent burn intervals (Collins & Steinauer 1998). The communities present in both the whole-plot maximum heterogeneity treatment and control plots support this assertion, especially when considering the decline of diversity and richness over time. However, recent analyses of long-term species composition data suggest that the communities in the whole-plot maximum heterogeneity treatment exhibit greater community heterogeneity over time, and thus greater temporal variability than the communities in the control plots (Sara Baer, *personal communication*). This offers evidence that the soil resource manipulations do indeed provide some level of spatial and/or temporal heterogeneity exceeding that of the control plots, allowing for a greater amount of species turnover over the long-term. It is likely that these trends are driven by higher rates of both colonization and extirpation in the maximum heterogeneity plots, and may indicate that these communities are less stable than those in the control plots.

31

Seedling emergence and community responses in 2013

When considering the role of niche availability and propagule supply in increasing diversity and richness of established restored prairie communities, my study suggests that this process is largely limited by propagule supply and not spatial heterogeneity or niche limitations. I found no effect of the whole-plot maximum heterogeneity treatment on the overall community indices, aside from Mean C. Likewise, no whole-plot treatment effects were found for the abundance or richness of seedlings. This demonstrates that the presence of subdominant forbs that contribute the most to richness and diversity in native tallgrass prairie (Freeman 1998) is largely limited by propagule supply in restored prairie rather than lack of spatial heterogeneity or open niches. As this particular restoration experiment is now in its 15th year, these findings also suggest that spatial heterogeneity to promote open niches may develop adequately on its own over the long-term. If the findings hold that there are no differences in richness or recruitment from seed additions in the years following this study, it would suggest that managers are indeed using scientifically sound practices through over-seeding without inducing manipulations to increase resource heterogeneity. Simply adding additional propagules may be the simplest and most effective method of increasing richness in even older restorations.

This assertion may be further supported by the findings concerning the matrix effect on both community variables and seedling abundance. Total ANPP, grass, and forb production, were all marginally higher in the whole-plot maximum heterogeneity control (deep x ambient N) than in the whole-plot control (deep x ambient N). Conversely, seedling abundance was twice as high in the whole-plot control (deep x ambient N) than in the whole-plot maximum heterogeneity control (deep x ambient N). There were no differences in any of the diversity, richness or Mean C measures. This suggests that there is no effect of spatial resource heterogeneity on the species present, but that there is an effect on actual biomass production and the ability of novel species to establish. The increased levels of biomass production coupled with the reduced abundance of seedlings in the control treatment (deep x ambient N) within the matrix suggest that seedling emergence is somehow impacted by the matrix of surrounding vegetation. It may be that the enriched N treatment is driving competitive relationships within this matrix that are prohibitive to seedling emergence. Alternatively, it may be that the higher total richness in the heterogeneity matrix is an indication that the communities are indeed more saturated than the whole-plot controls, and thus can not support the same number of seedlings. The lower abundance of

seedlings found in the deep x ambient N treatments within the matrix of increased spatial resource heterogeneity offers further evidence that diversity and richness are more limited by propagule supply than niche availability in our restoration. In addition, as there was no difference in the richness of seedlings between these two treatments, only between actual abundance, it appears that the communities within the matrix of greater resource heterogeneity may be more saturated and unable to support the same numbers of novel individuals as those that are in the whole-plot control treatments. In short, by increasing spatial and resource heterogeneity, we may have decreased the relative ability of these communities to successfully recruit and support novel species arriving through seed additions.

Furthermore, the finding that Mean C was higher in the control than in the heterogeneity treatment may imply that there is a negative effect of induced resource manipulations on the quality of the community. Within the whole-plot maximum heterogeneity treatments, Mean C was lowest in the enriched N treatment, with no difference between the ambient and reduced N. These findings together suggest that the lower value of Mean C in the whole-plot heterogeneity treatments is driven primarily by the enriched N treatment. Moreover, the enriched N treatment had higher diversity, total richness, and seedling abundance than the reduced N treatments. This suggests that there are a larger number of ruderal species that contribute to both richness and diversity while lowering Mean C within the enriched N treatment. This is a clear example of the need for more widespread use of measures such as Mean C when assessing restored communities. If the goal is to restore communities that are more reflective of native communities, then our measurements must weight species abundances by their fidelity to remnant habitats rather than treating all species alike. Moreover, when conducting long-term monitoring, Mean C can offer insight into the trends that restored communities follow in assembling communities that are more reflective of remnant habitats.

The other interesting finding is that seedling abundance was three times higher in the enriched N than the ambient N treatments, suggesting that within the heterogeneity matrix, the enriched N treatments are more suitable for the species that were seeded than the ambient N treatments. This is contrary to our hypothesis that seedling emergence would be highest in reduced N treatments as a result of increased light availability. Studies investigating resource heterogeneity in grasslands have overwhelmingly found decreased richness and diversity with increased N availability (Baer *et al.* 2003,2004, 2005; Camill *et al.* 2004, Gendron & Wilson

2007, Liira *et al.* 2012). This is generally believed to be a result of increased biomass production of a few dominant species (Baer *et al.* 2003, Gendron & Wilson 2007, Wilson & Tilman 2002), leading to decreased light availability and thus lowering the competitive ability of subdominant species. However, Wilson and Tilman (2012) noted that in the presence of increased nutrient availability, a shift from below-ground competition for resources to above-ground resource competition occurs, resulting in the dominant species not preventing colonization from occurring, but rather leading to increased extirpation. In the context of this study, and the finding that within the resource manipulation treatments, the highest abundance of seedlings occurred in the enriched N treatment, Wilson and Tilman's argument that the observed trends in diversity and richness as a response to increased N availability is a result of increased extirpation rather than reduced colonization, appears to be supported.

Higher seedling abundance in the enriched N treatment as well as higher diversity and richness may be an effect of increased water availability in conjunction with increased soil N availability. When considering the physiological needs of seedlings, water availability may be more of a limiting resource than light availability in older tallgrass prairie restorations. My personal observations combined with other studies (Rossiter 2013, Stevens *et al.* 2006), suggest that there is a balance between too much and too little light at the soil surface. Too little light and the seeds never germinate or seedlings die from inability to produce enough food. Too much light and the seedlings die from exposure in a stressful microclimate. The early summer of 2013 was a fairly cool and wet season at KPBS, and the enriched N treatment developed a fairly full canopy early on (this also explains the discrepancy in nutrient effects on PPFD but not ANPP). I also observed a lot of fungi growing in many of the restoration plots. I noted that they appeared to be more abundant and much larger in size in the enriched N treatments than in any other treatment. This observation may suggest that moisture levels were higher in these treatments. The increased canopy cover would lower the rate of evaporation from the soil surface, and increase the soil water availability near the surface of the soil profile, allowing seeds to imbibe, germinate and the emergent seedlings to have access to adequate water while also protecting them from over-exposure and desiccation. I suspect that these are the mechanisms involved with finding that higher numbers of seedlings emerged in the enriched N treatments, in addition to the fact that the there is an abundance of N, a known limiting resource in tallgrass prairie systems (Risser & Parton 1982, Ojima *et al.*1994, Blair 1997). This could also explain the higher levels

34

of diversity and richness, as many of the species that contribute to the higher richness are more ruderal and often have less extensive root systems than dominant grasses and clonal forbs. With less extensive root systems, these species would experience higher fecundity in a microhabitat with higher soil water and soil N availability. In addition, according to Wilson and Tilman's (2012) findings, the communities in these fertilized treatments would have lower belowground competition than the ambient N treatments, thus allowing species with lower competitive abilities to experience less competitive pressure for those belowground resources, and more readily establish in those sites.

Conclusions

Overall, this study provided no support for the diversity-heterogeneity hypothesis, nor for the niche-limitation hypothesis. What these data do suggest is that over-seeding is effective, to a degree, in increasing richness and diversity in well-established restored tallgrass prairies. Whether it is effective in over-riding the observed decline in richness and diversity across time remains unclear and warrants further study. Additionally, this study offers evidence that utilizing weighted Mean C as a measure of community diversity may be more informative, particularly for restorations, than the more conventional Shannon's or Simpson's indices of diversity. Finally, there is still a lack of understanding of the mechanisms regulating seedling emergence, survival and establishment in established prairie communities. This study neither supported nor refuted any real mechanisms. Studies spanning spatial and temporal scales larger than this one are needed in order to provide enough data to really assess whether species' individual variability or environmental mechanisms drive seedling emergence and establishment in tallgrass prairie restorations.

Figures and Tables
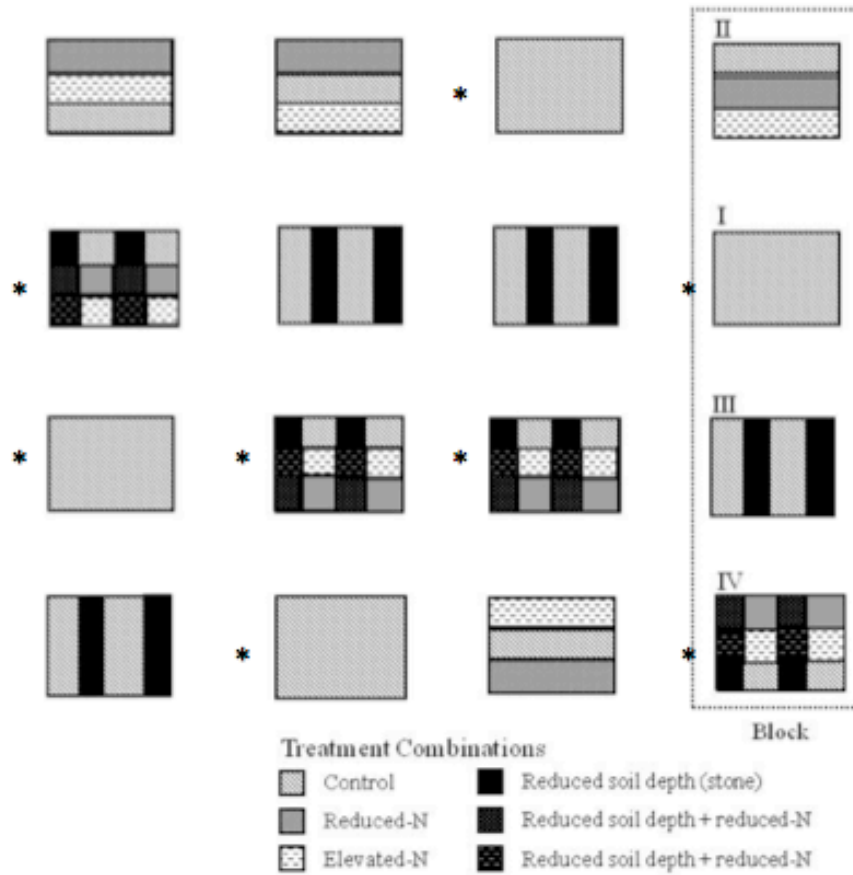
Figure 2.1 Experimental design illustrating whole-plot heterogeneity treatments and individual subplot treatments, within each whole plot. Each plot contains twelve 2 m x 2 m subplots. Whole-plot designations are: I=control, II=nutrient heterogeneity, III=soil depth heterogeneity, IV=maximum heterogeneity. This particular study utilized only those plots designated by an asterisk (whole-plot treatments I and IV).

Figure 2.2 Typical seedling *(L.aspera)* **marked with purple craft stick on the North side to monitor survival throughout the growing season.**
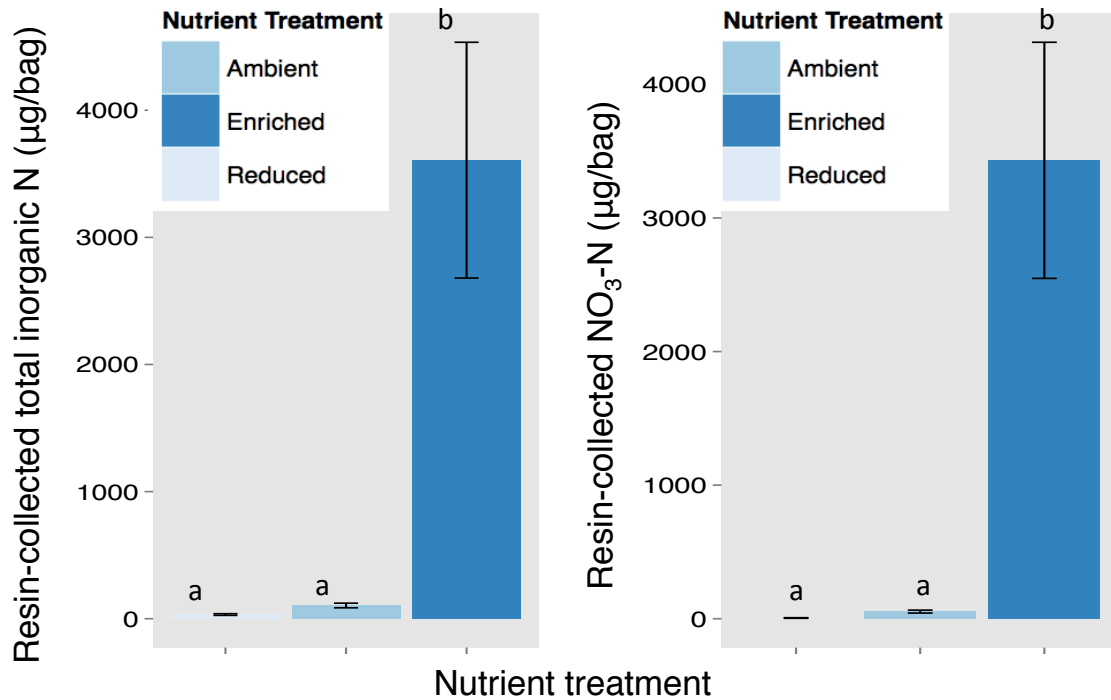
Figure 2.3 Significantly higher levels of resin-collected total inorganic N and resin-collected NO$_3$-N were found in the enriched N treatment than in the reduced N or ambient N treatments, indicating that the enriched N treatment was effective in creating differences in soil resource availability within the maximum heterogeneity treatment. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. All statistics, as reported in the text, are derived from Proc Mixed ANOVAs, with significance tested for at α=0.1 and generated by SAS version 9.2 (SAS Institute 2008).
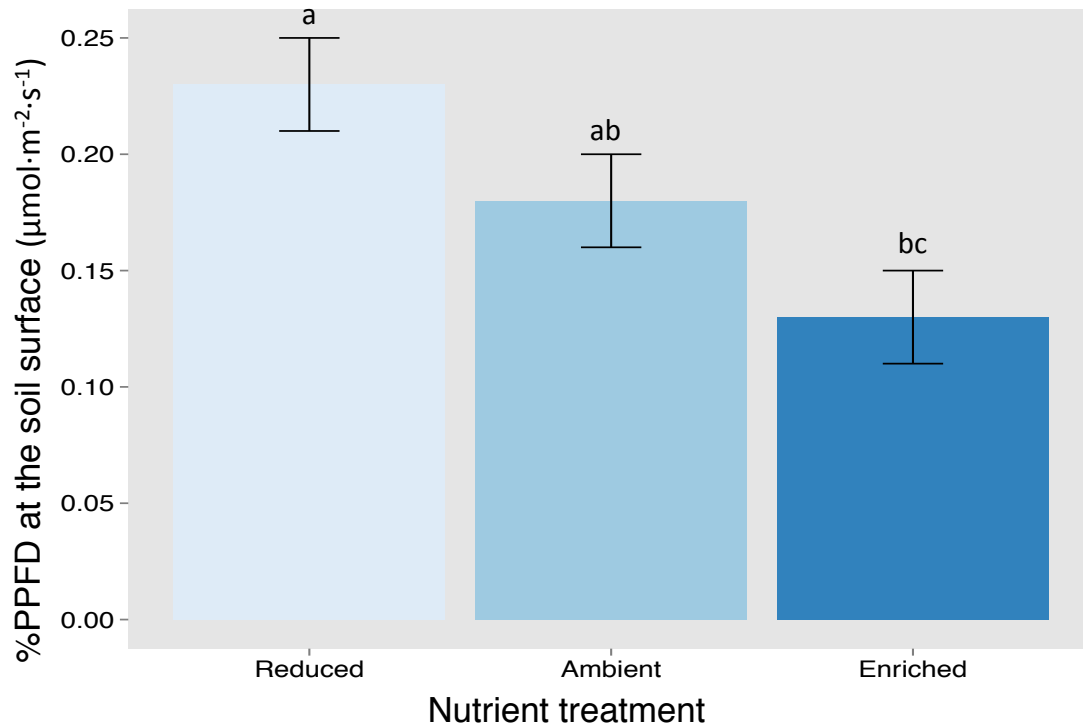
Figure 2.4 Differences in light availability at the soil surface between the three nutrient treatments within the whole-plot maximum heterogeneity treatments. Greater light availability was found in the reduced N treatment than in the enriched N treatment, though no differences were detected between the ambient N treatment and either the reduced N or enriched N treatments. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
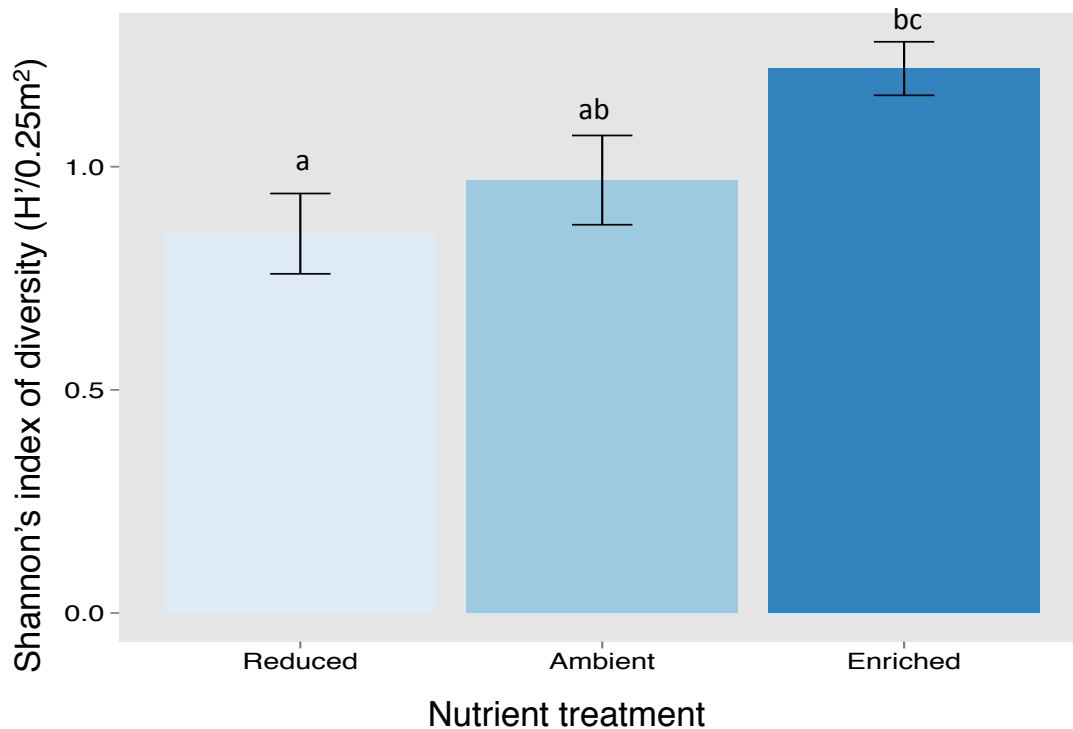
Figure 2.5 Higher diversity, as measured by Shannon's index of diversity (H'), was found in the enriched N treatment than in the reduced N treatment, though no differences were detected between the ambient N treatment and either the reduced N or enriched N treatments. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
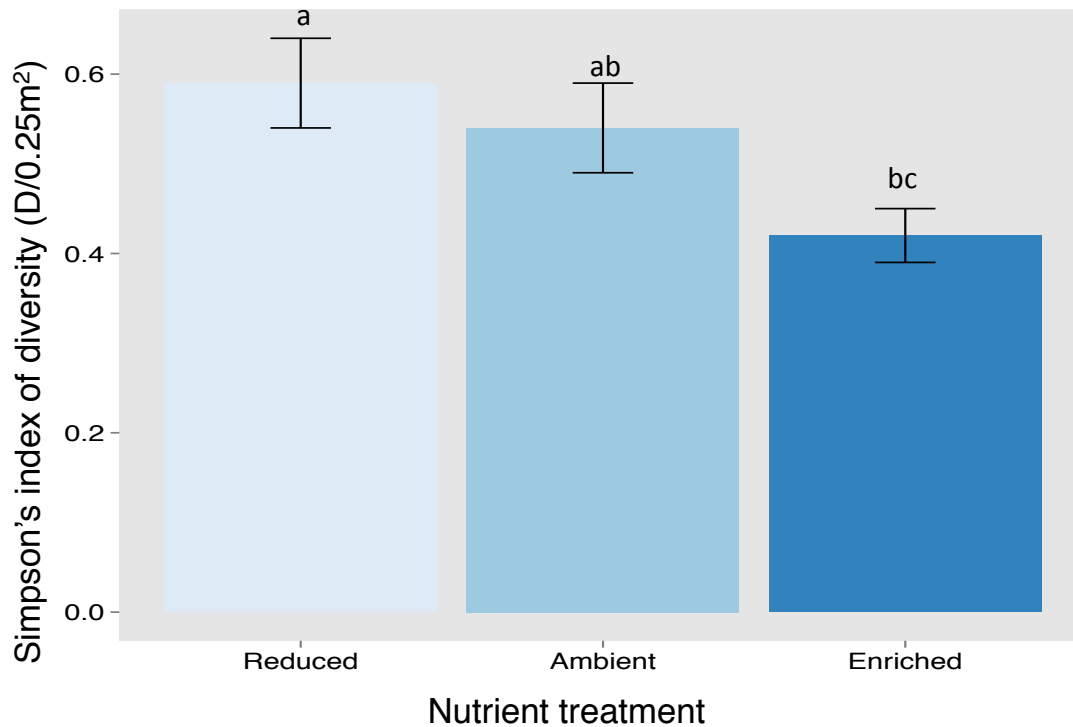
Figure 2.6 According to the values of Simpson's index of diversity (D), there is a higher chance of finding two individuals from the same species in the reduced N treatment than in the enriched N treatment. Thus, higher diversity was again found in the enriched N treatment than in the reduced N treatment, as higher values of D indicate lower levels of diversity. No differences were detected between the ambient N treatment and either the reduced N or enriched N treatments. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
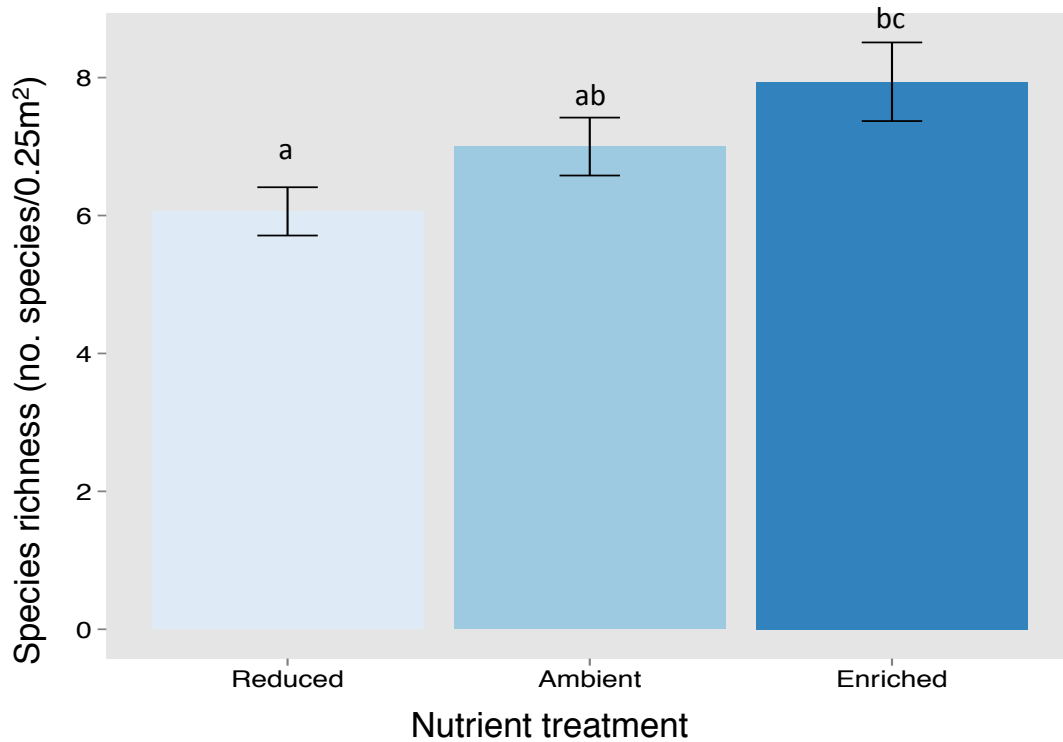
Figure 2.7 Higher species richness was found in the enriched N treatment than in the reduced N treatment, though no differences were detected between the ambient N treatment and either the reduced N or enriched N treatments. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
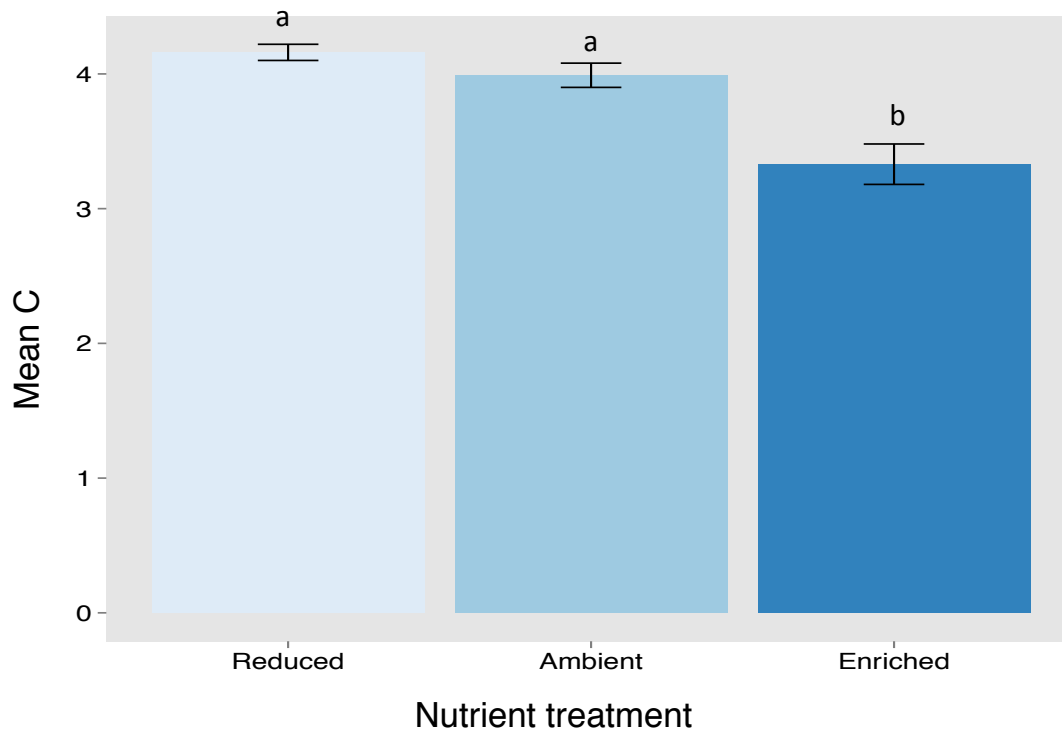
Figure 2.8 Lower Mean C was found in the enriched N treatment than in the ambient N and reduced N treatments, though no differences were detected between the ambient N treatment and the reduced N. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
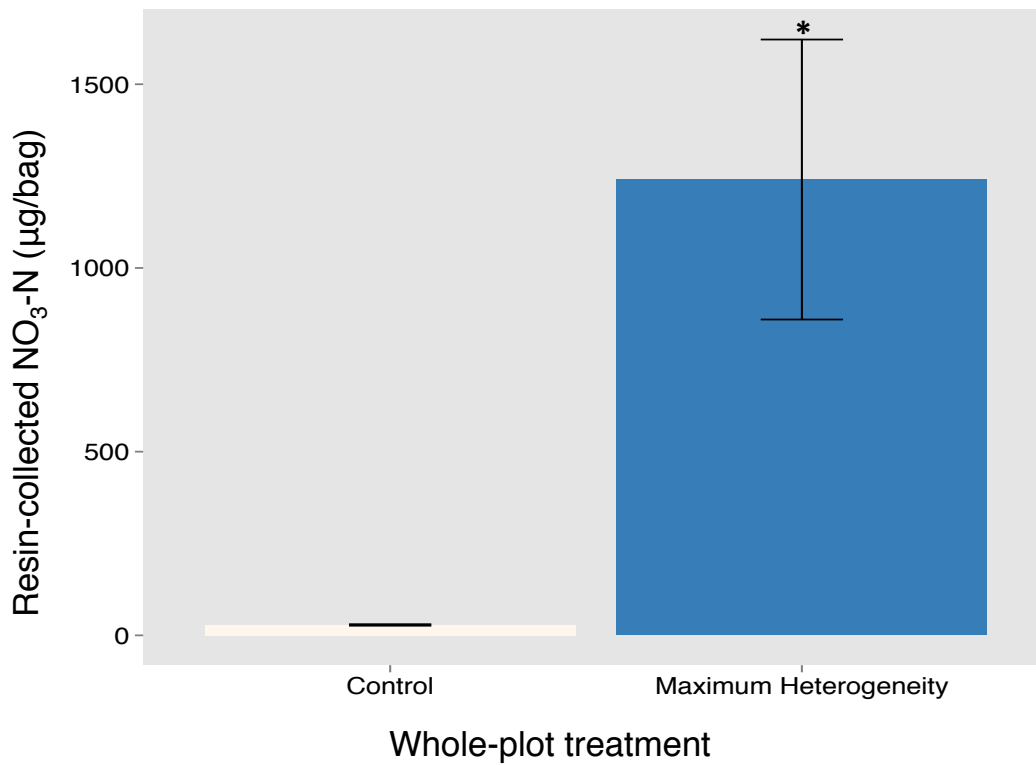
Figure 2.9 Significantly higher levels of resin-collected NO₃-N were found in the whole-plot maximum heterogeneity treatment than in the whole-plot control, indicating that the nutrient availability treatment is indeed effective in creating differences in soil resource availability between the maximum heterogeneity treatment and the control. Asterisk indicates statistically significant differences between the whole-plot treatment means. Statistics are as described in Figure 2.3.
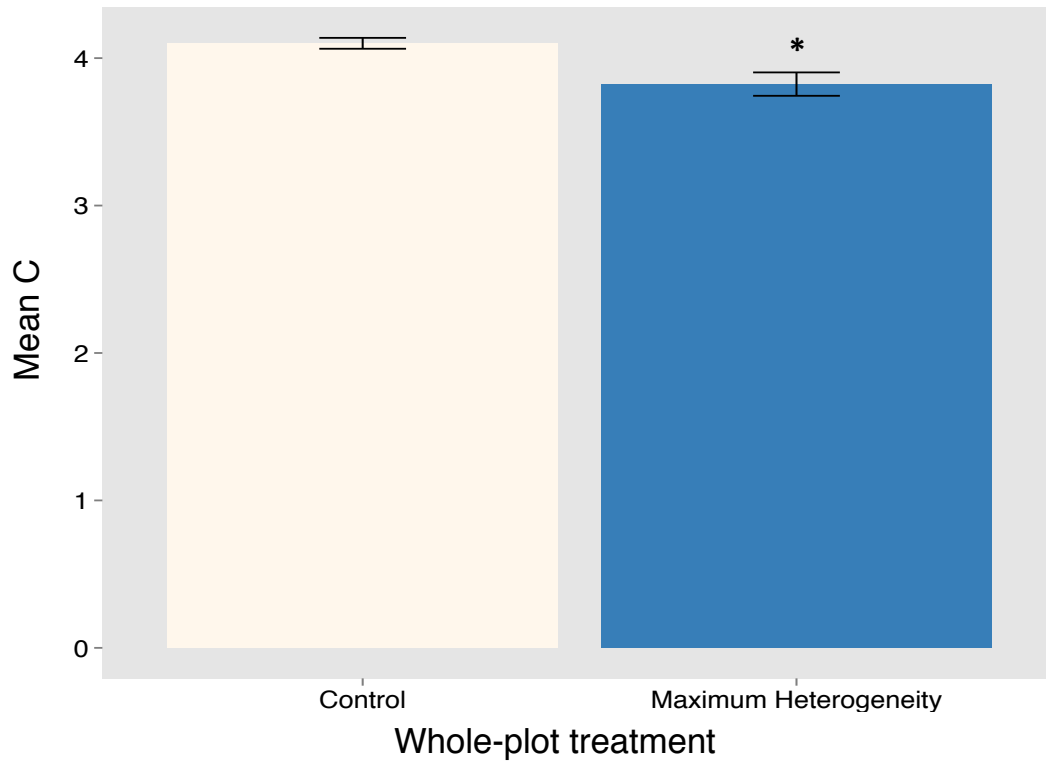
Figure 2.10 The maximum heterogeneity treatment hosts a slightly lower Mean C than the whole-plot control, indicating that the control communities have a slightly higher abundance of species that are more characteristic of native, undisturbed prairie. Asterisk indicates statistically significant differences between the whole-plot treatment means. Statistics are as described in Figure 2.3.
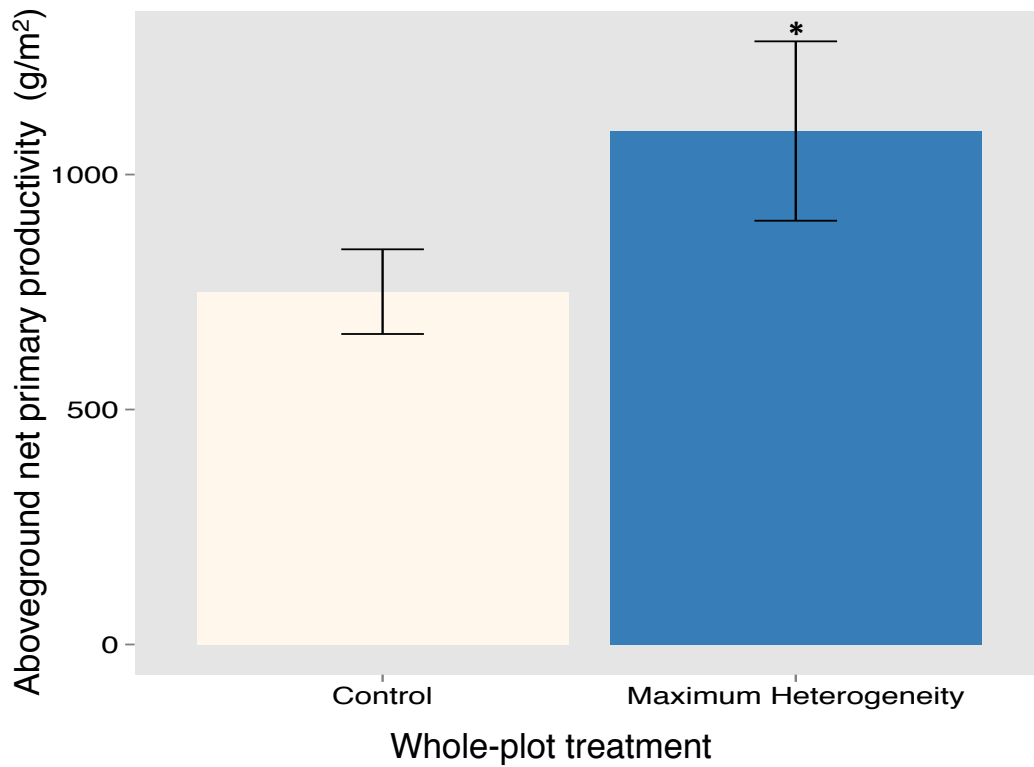
Figure 2.11 The control subplots (deep soil x ambient N) within the whole-plot maximum heterogeneity treatments yielded higher ANPP than the whole-plot controls (deep soil x ambient N), indicating that there is some effect of the physical matrix of soil resource heterogeneity on plant growth. Asterisk indicates statistically significant differences between the whole-plot treatment means for the deep soil x ambient N treatment only. Statistics are as described in Figure 2.3.

All plots rank abundance curve

Maximum heterogeneity rank abundance curve

Control Plots Rank Abundance curve
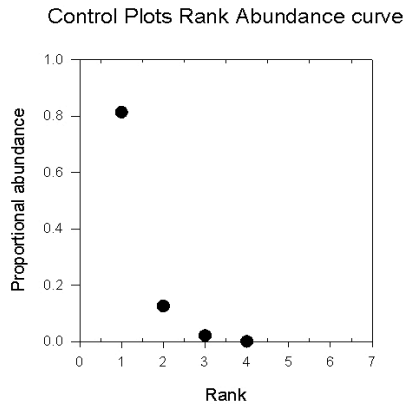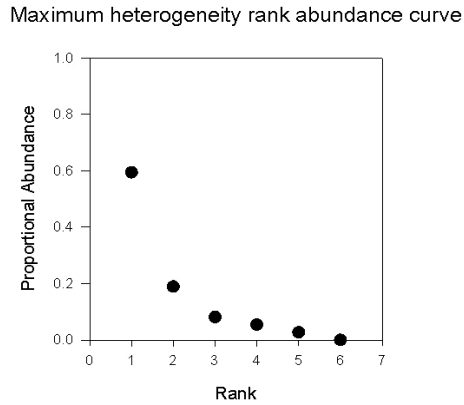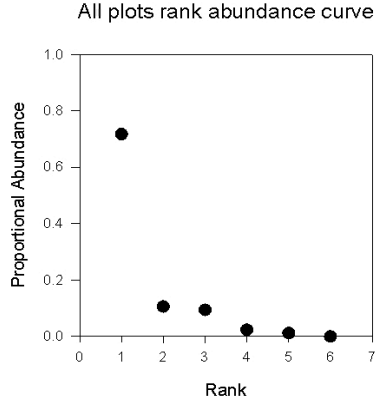
Figure 2.12 Rank-abundance curves for seedling abundance (total number of species/0.25 m^2) by species in both the control and maximum heterogeneity whole-plot treatments, the whole-plot maximum heterogeneity treatment only, and whole plot control treatment only, respectively. The overall trend in a disproportionately high abundance of *M. nyctaginea* exists for both whole-plot treatments, demonstrating this species' effective colonization tendencies.
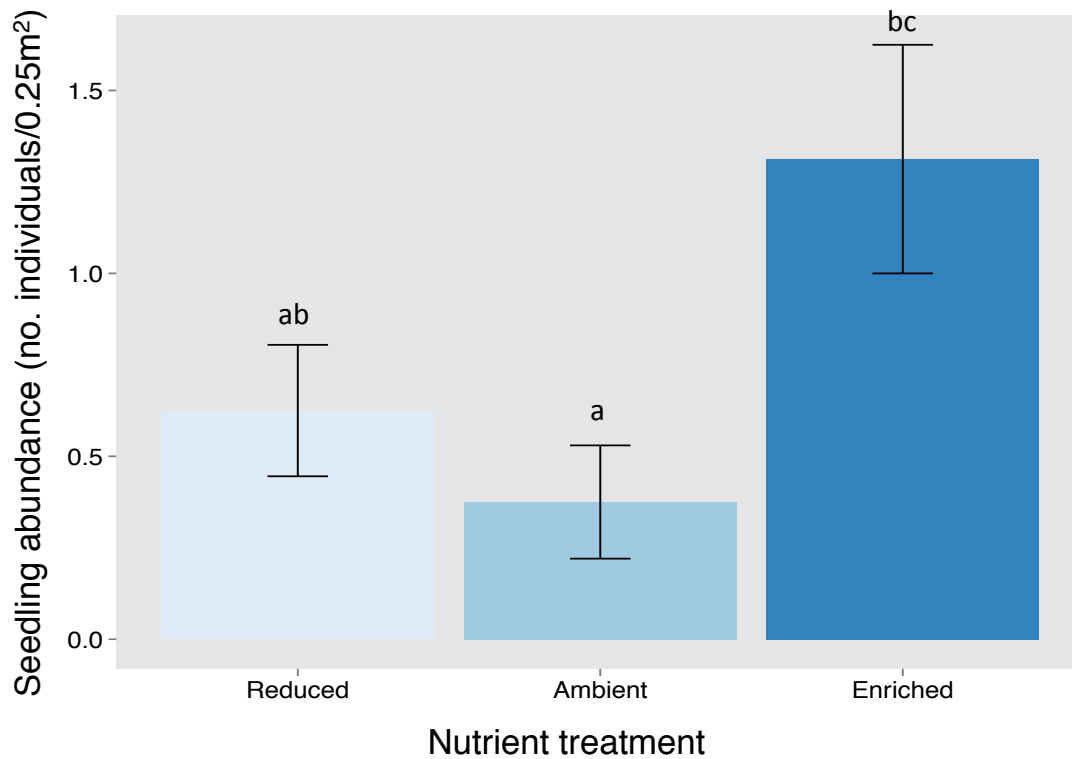
Figure 2.13 Seedling abundance was higher in the enriched N treatment than the ambient N treatment, though no differences were detected between the enriched N treatment and the reduced N treatment, nor between the ambient N treatment and the reduced N treatment. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
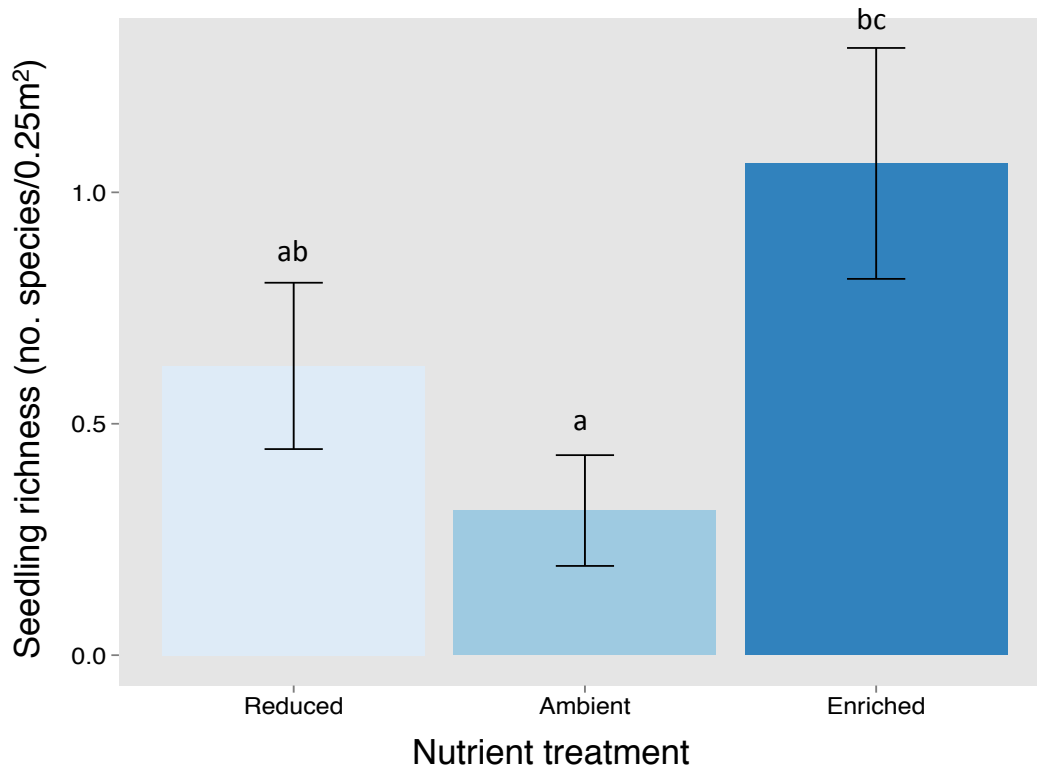
Figure 2.14 Seedling richness was also higher in the enriched N treatment than the ambient N treatment, though no differences were detected between the enriched N treatment and the reduced N treatment, nor between the ambient N treatment and the reduced N treatment. Letters indicate statistically significant differences between treatment means, as averaged across treatments within a plot. Statistics are as described in Figure 2.3.
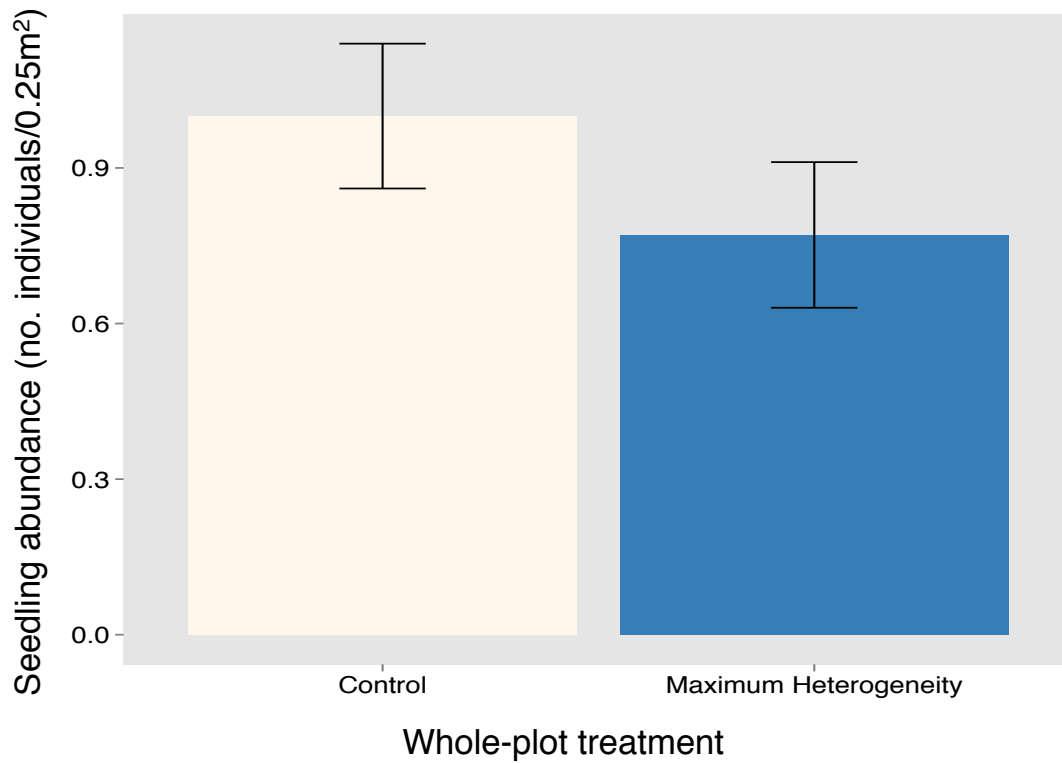
Figure 2.15 No difference in seedling abundance was found between the whole-plot maximum heterogeneity treatment and the control, indicating that while there is evidence that resource availability effects seedling abundance, there is no evidence that resource heterogeneity does. Statistics are as described in Figure 2.3.
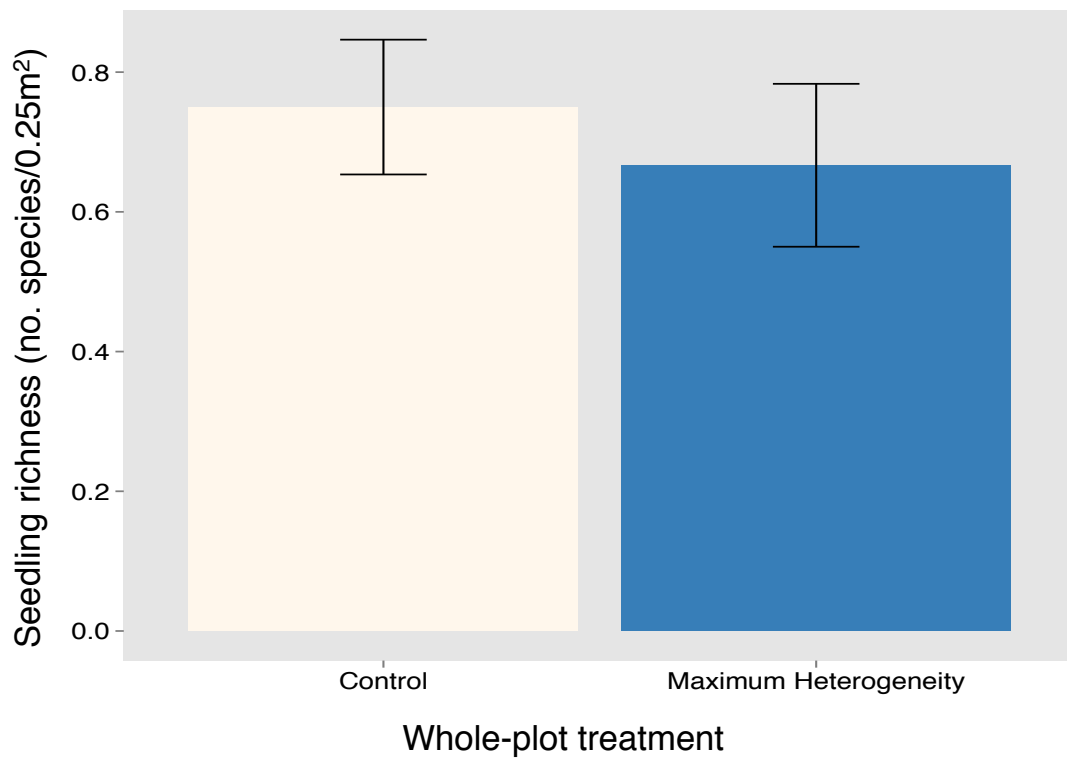
Figure 2.16 No difference in seedling richness was found between the whole-plot maximum heterogeneity treatment and the control, indicating that while there is evidence that resource availability effects seedling richness, there is no evidence that resource heterogeneity does. Statistics are as described in Figure 2.3.
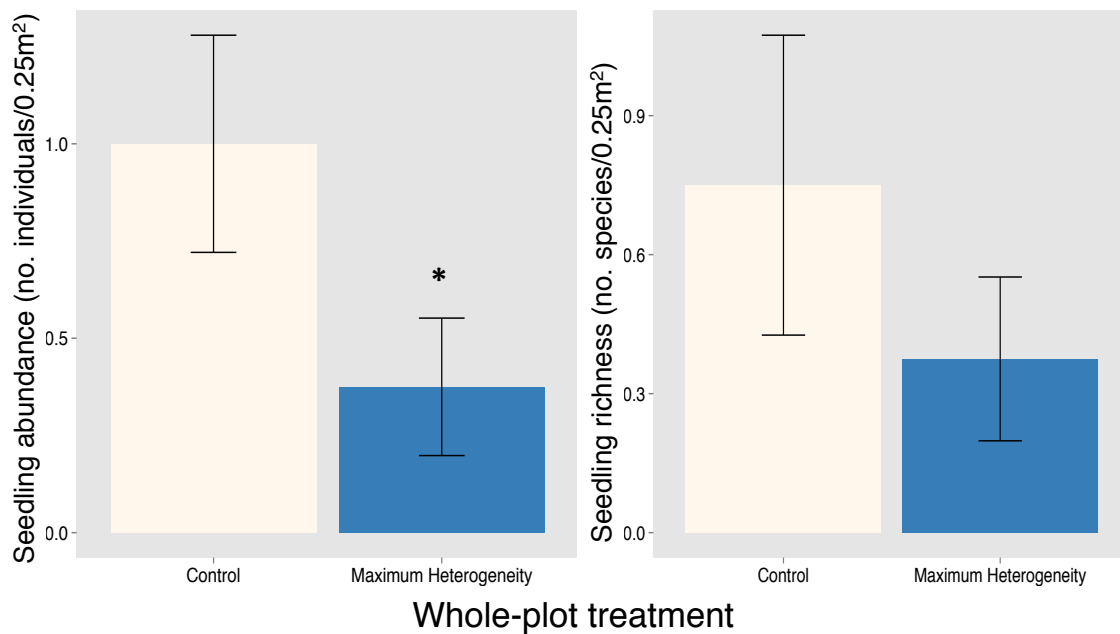
Figure 2.17 The whole-plot controls (deep soil x ambient N) had higher seedling abundance than the control subplots (deep soil x ambient N) within the whole-plot maximum heterogeneity, but no detectable difference was found with seedling richness. This indicates that there is some effect of the physical matrix of soil resource heterogeneity on the actual number of seedlings to emerge, but not on the number of species to emerge. Asterisk indicates statistically significant differences between the whole-plot treatment means for the deep soil x ambient N treatment only. Statistics are as described in Figure 2.5.
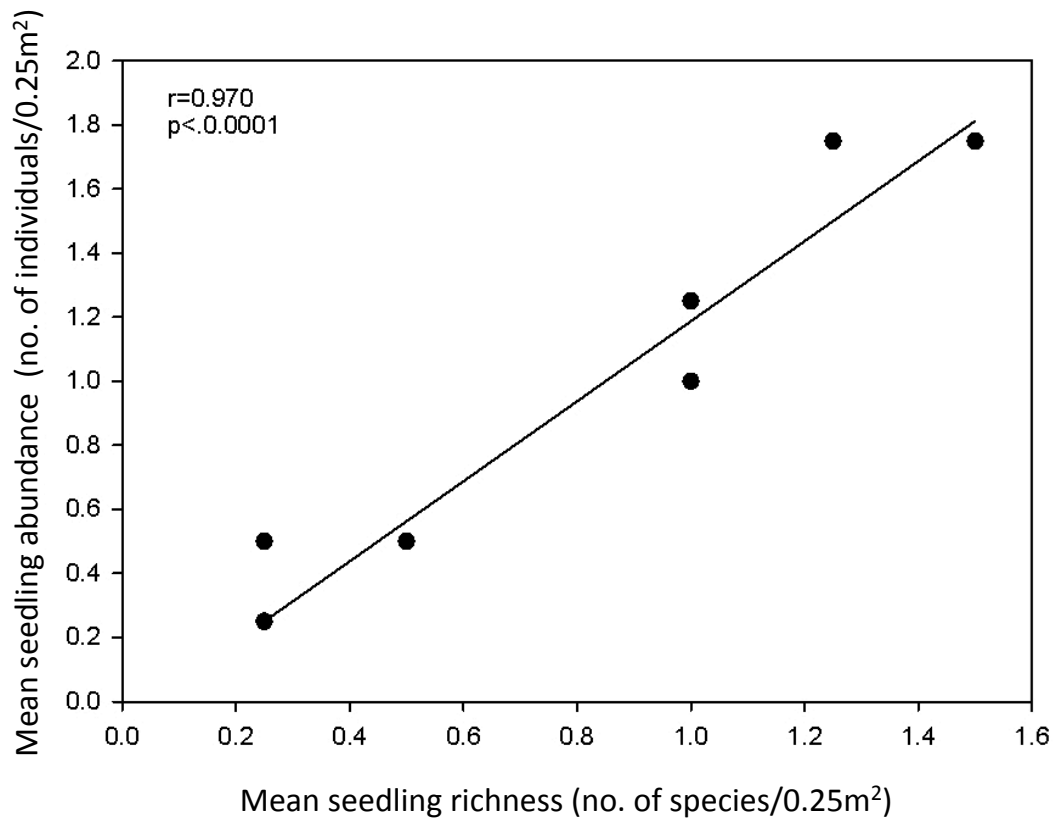
Figure 2.18 Relationship between mean seedling abundance and mean seedling richness.
Correlation was performed on the mean response of variables to the nutrient treatments in
each of the whole-plot maximum heterogeneity treatments (*n*=12). Significant relationships
were determined from Pearson's correlation coefficients at α=0.1 derived using SAS (SAS
Institute 2008).
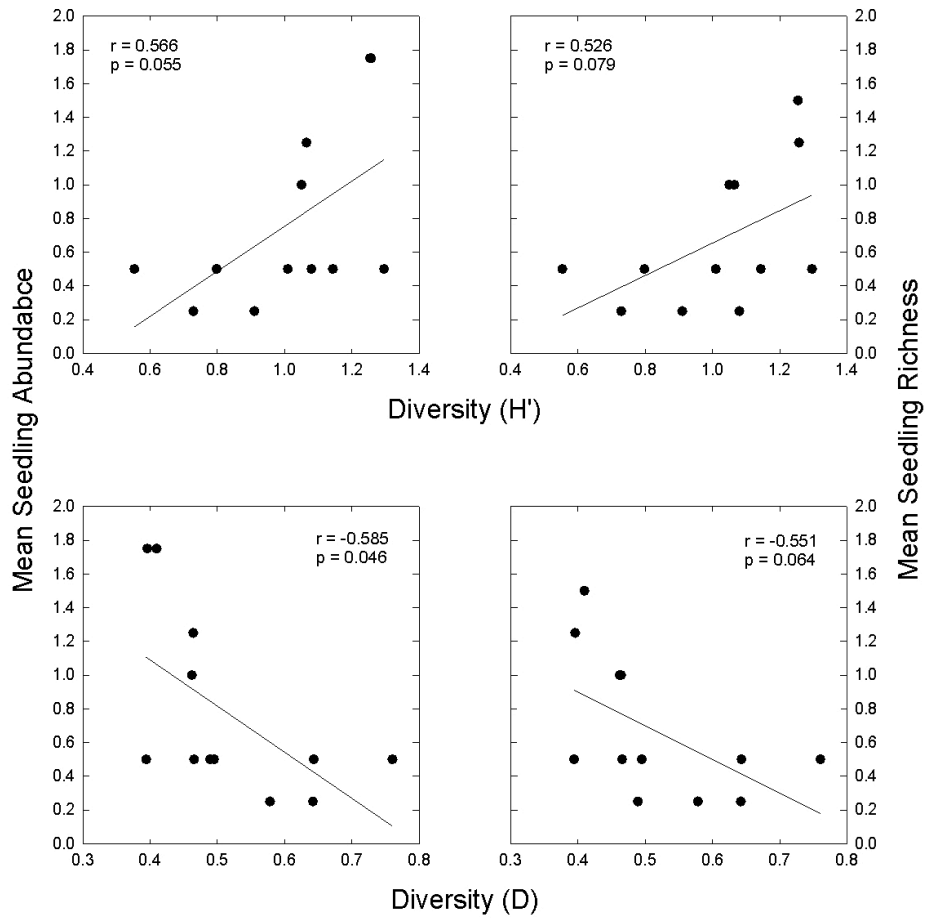
Figure 2.19 Relationships between diversity of the surrounding community, as measured by Shannon's index of diversity (H') (top) and Simpson's index of diversity (D) (bottom), and mean seedling abundance (left) and mean seedling richness (right). Correlations were performed on the mean response of variables to the nutrient treatments in each of the whole-plot maximum heterogeneity treatments (*n*=12). Significant relationships were determined from Pearson's correlation coefficients at α=0.1 derived using SAS (SAS Institute 2008).
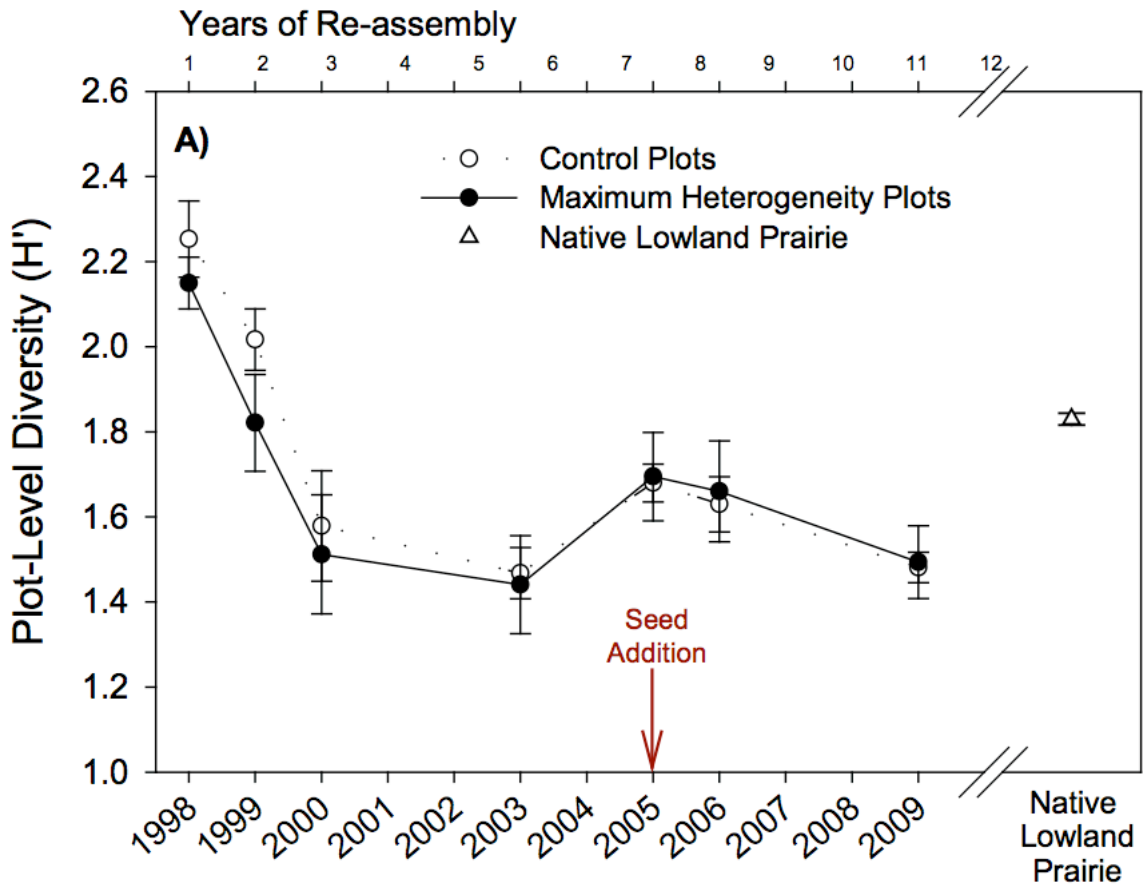
Figure 2.20 Plot level plant diversity, as measured by Shannon's index of diversity (H'), in the whole-plot maximum heterogeneity and control treatments from 1998 to 2009, as well as the mean diversity for a native lowland reference prairie. No statistically significant differences exist between the two whole-plot treatments at any year of community assembly. (Unpublished data, Sara Baer, *personal communication*).
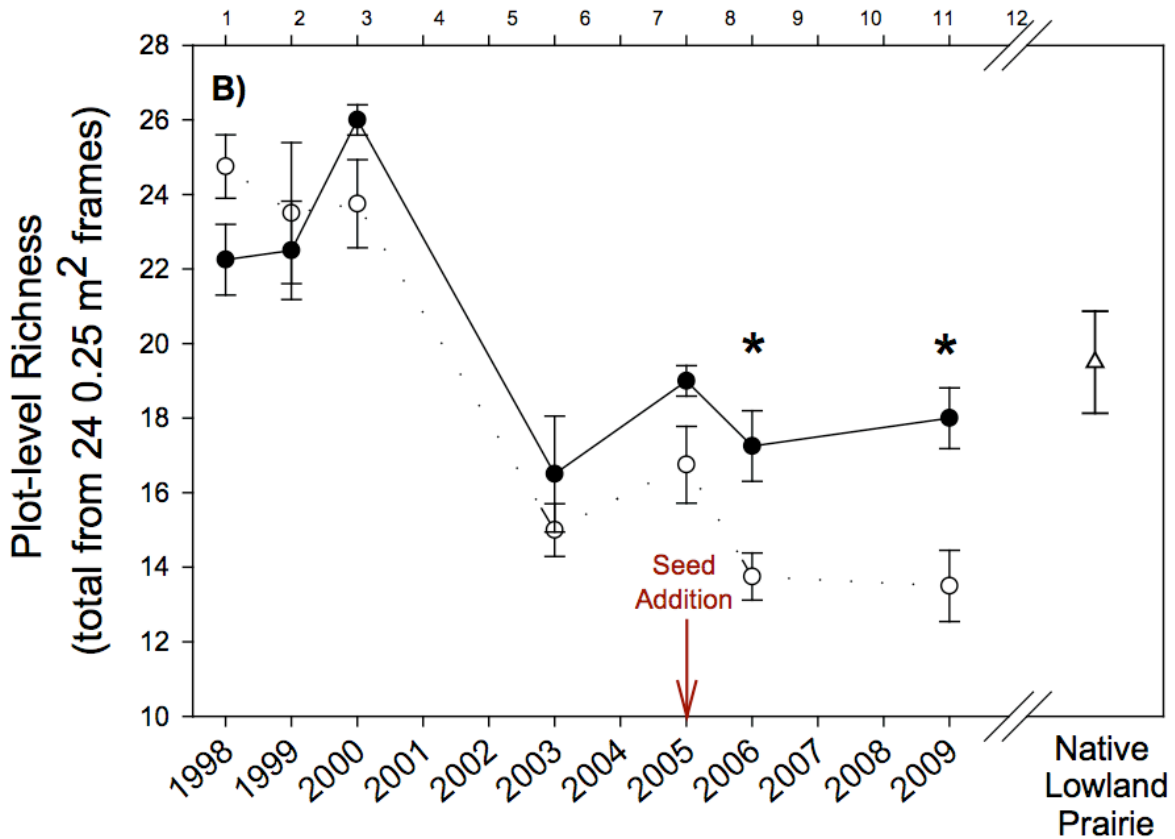
Figure 2.21 Plot level richness, as measured by the total number of species present/0.25m^2, in the whole-plot maximum heterogeneity and control treatments from 1998 to 2009, as well as the mean species richness for the native lowland reference prairie. Statistically significant differences are indicated by asterisks and exist between the two whole-plot treatments in year 9 and year 12 of community assembly. This appears to be a product of the seed addition in year 8 (2005). (Unpublished data, Sara Baer, *personal communication*).

Species Name	Total no. individuals to emerge	Total no. individuals to surive to end of growing season	% Surviorship
Ageratina altissima (L.) R.M. King & H. Rob.	1	1	100
Bouteloua gracilis (Wild. Ex Kunth) Lag. Ex Griffiths	NA	NA	NA
Carex brevior (Dewey) Mack.	NA	NA	NA
Elymus canadensis L.	NA	NA	NA
Grindelia squarrosa (Pursh) Dunal	8	5	62.5
Helianthus pauciflorus Nutt.	1	0	0
Liatris aspera Michx.	1	1	100
Linum sulcatum Riddell	0	0	NA
Lobelia siphilitica L.	0	0	NA
Mirabilis nyctaginea (Michx.) MacMill.	61	52	85.25
Oligoneuron rigidum (L.) Small var. *rigidum*	2	2	100
Onosmodium bejariense DC. ex A. DC. var *occidentale* (Mack.) B.L. Turner	0	0	NA
Potentilla arguta Pursh	0	0	NA
Rumex altissimus Alph. Wood	2	2	100
Silene stellata (L.) W.T. Aiton	0	0	NA
Silphium integrifolium Michx.	9	6	66.67
Symphyotrichum drummondii (Lindl.) G.L. Nesom	0	0	NA
TOTAL	85	69	81

Table 2.1 Total emergence and percent survivorship for each of the 17 species added to the experimental plots in 2013. Nomenclature follows USDA Plants Database.

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Chapter 3 - Effects of small-scale disturbances on seedling emergence

Abstract

Reaching and maintaining levels of plant diversity and richness that parallel those found in remnant prairie remains a challenge for prairie restoration. The absence of species from the local species pool and the absence of frequent small-scale disturbances are thought to be two factors in preventing the maintenance of higher levels of diversity in restored prairies. Thus, seed additions are a common practice to both introduce and maintain species to the local species pool. While it is well documented that tallgrass prairie requires both large-scale and small-scale disturbances to maintain its characteristically high levels of plant diversity, the role of small-scale disturbances in promoting the establishment of subdominant species in restored prairies is less understood. Thus, the goal of this study was to examine whether two levels of small-scale disturbance, a soil disturbance and aboveground biomass removal, enhanced seedling emergence in the growing season immediately following a seed addition in a well-established restored prairie. No differences were found in the abundance or in the richness of seedlings to emerge in either the soil disturbance or the aboveground biomass removal treatment from the control. However, overall seedling emergence quite low, with emergence of only 4 of the 17 species detected and a total of 14 individuals detected. Low emergence precludes any strong conclusions about the effect of the disturbance treatments. Additional studies examining the interplay between seed additions and small-scale disturbances carried out over the long-term may be required to determine whether such disturbances increase colonization rates of species added via seed additions over a longer time scale.

Introduction

Tallgrass prairie is a system that requires frequent small-scale disturbances in conjunction with infrequent large-scale disturbances to maintain plant diversity and ecosystem functioning over space and time (Collins & Glenn 1990, Collins & Steinauer 1998, Collins *et al.* 1998, Knapp *et al.* 1999 , Fuhlendorf & Engel 2004, Veen *et al.* 2008). Large-scale disturbances such

as fire and grazing, and the effects of their frequency, intensity and interactions have been relatively well-studied and are well-understood for native prairies (Collins & Steinauer 1998, Collins *et al*. 1998, Knapp *et al*. 1999), with slightly less robustness for restored prairies (Tilman 1993, Copeland *et al*. 2002, Pywell *et al*. 2002, Martin *et al*. 2005). What remains less understood are the effects of smaller-scale disturbances on structuring the communities in native, as well as restored prairies, and the role they play in facilitating colonization by subdominant species (Rodgers & Hartnett 2001,Williams, *et al*. 2007, Questad & Foster 2008), which account for the majority of diversity in tallgrass prairie plant communities (Freeman 1998).

It is believed that species coexistence and co-occurrence, which leads to high diversity and richness, is reliant on spatial heterogeneity of abiotic and biotic resources and structure (Hutchinson 1959, Tilman & Pacala 1993, Tilman 1994, Reynolds *et al.* 2007). One theory of coexistence predicts that species-environment sorting acts to increase species coexistence, and thus richness, when resources affecting fitness are spatially heterogeneous within a system (Hutchinson 1959, Tilman & Pacala 1993). In addition, the intermediate disturbance hypothesis posits that species diversity (and thus species coexistence) will be highest in a system subjected to moderate levels of disturbance (Hobbs & Huenneke 1992). While these hypotheses have been well-studied and documented in tallgrass prairie for large-scale disturbances of fire and grazing, they remain less-studied for small-scale disturbances.

Many of the studies that examine the role of disturbance in species establishment in grasslands have looked at small-scale disturbances similar to those caused by small mammals and grazing by larger ungulates (Rogers *et al*. 2001, Rogers & Hartnett 2001a, 2001b, Gendron & Wilson 2002, Wilson & Tilman 2002, Bakker *et al*. 2003, DiVitorrio *et al*. 2007, Williams *et al*. 2007, Gendron & Wilson 2002, Martin and Wilsey 2012, Rogers & Hartnett 2001a, 2001b, Rogers *et al*. 2001, Wilson & Tilman 2002, Williams *et al*. 2007). The most profound effect of these types of disturbances is the reduction, if not complete eradication of aboveground biomass, which in turn increases light availability and creates microsites open for colonization (Bakker *et al.* 2003, Gendron & Wilson 2007). It has been documented that light availability is one of the major limiting resources affecting species richness and the emergence and establishment of seedlings in established prairie communities (Tilman 1993,Collins *et al.* 1998, Foster *et al.* 2002, Baer *et al.* 2003, 2004, Williams *et al.* 2007). Several studies have also demonstrated that dispersal and seed limitation can be a driving factor in the failure of additional native species to

establish in restored prairies (Bakker *et al.* 2003, DiVittorio *et al.* 2007, Hamilton *et al.* 1999, Hutchings & Booth 1996, Kardol *et al.* 2008, Purscheke *et al.* 2012, Seabloom *et al.* 2003, Tilman 1997, Turnbull *et al.* 2000, Von Holle & Simberloff 2005).

The overall approach to investigating this problem of depauperate communities in established prairie restorations has been to examine the interplay between resource availability and propagule supply, leaving us with multiple variations on the theme of disturbance and seed additions. The challenge with this approach is that the seemingly small differences in methods, sites, community compositions, etc. add up to make one very unclear picture of the role that small-scale disturbance and propagule supply play in influencing species richness in restored prairie communities. When faced with the challenge of reaching and maintaining diversity in restored communities that is comparable to remnant prairie communities (Huston 1979, 1994, Camill *et al*. 2004), we must determine what this relationship is in well-established restorations and consider how management approaches include and/or affect these small-scale disturbances.

Thus, I examined the effect of two levels of disturbance combined with a seed addition in an older restored prairie with levels of diversity and richness below those of neighboring remnant prairie. My first treatment was a small-scale soil disturbance designed to increase bare soil patches while also creating gaps in the canopy. My second treatment was a simple clipping treatment to remove aboveground biomass and open the canopy relatively early in the growing season. I hypothesized that greater numbers of seedlings would emerge in the disturbance treatments than the control, with the greatest numbers emerging in the plots receiving the soil disturbance treatment, as these plots should provide both open ground for colonization, as well as increased light availability for germination and successful seedling growth.

Methods

Study site description

For a description of the study site, refer to the Methods section in Chapter 2.

Experimental design

Adjacent to the sixteen soil resource heterogeneity plots, an area measuring 10 x 50 m was delineated in June of 1997 as an additional restoration area to conduct research. Like the

heterogeneity plots in the former chapter, the adjacent restoration area was initially seeded with 42 species representative of native lowland prairie in the Flint Hills. See Appendix A for a list of species. Prior to this study, the 10 x 50 m restoration area had only been seeded with the original 42 species. This site is also subject to dispersal from nearby native prairie as well as ex-arable lands that host exotic species, reflecting conditions that are often present in prairie restorations.

Twelve 2 m x 2 m were established in March 2013. The inner 1 m^2 of each plot was delineated and four 0.25-m^2 quadrats were delimited. The southwest and the northeast quadrats were reserved for non-destructive sampling, and the southeast and northwest quadrats were reserved for destructive sampling. Each plot was randomly assigned one of three treatments, (A) control, (B) aboveground biomass removal, or (C) soil disturbance. The treatments were applied to only the center 1 m^2 of the plots. Four replicates of each treatment were assigned. See Fig 3.1 for a map of the experimental design.

Disturbance treatments

The aboveground biomass removal treatment was applied once in the season, on 10 June 2013 during peak biomass growth. All biomass was clipped with hedge shears or scissors to a height of 10 cm to mimic effects of natural grazing, or management activities such as mowing/haying that increase light availability. The soil disturbance treatment was applied on 26 April 2013 just prior to the seed addition. A hand-held cultivator (Garden Weasel®) was run through these plots in two 0.25-m strips, alternating with uncultivated strips to mimic the effects of patchy soil disturbance or management activities such as disk harrowing. The cultivator was passed through each strip several times to break up the top 5cm of soil and loosen standing clumps of *Andropogon gerardii*, increasing light availability at the soil surface at the beginning of the growing season and creating microsite availability on the soil surface.

Seed additions

Seventeen species of forbs and graminoids, not documented as present in the established community and representing a functionally diverse group, were chosen to add to the existing community. Species were chosen based on their absence from the community and the availability of seed. All seed was purchased from Prairie Moon Nursery (Winona, Minnesota) and subjected to the recommended stratification and scarification protocols.

Seeds were added to the 12 disturbance plots on 29 April 2013, following a prescribed spring burn. We waited for a relatively calm day with winds under 10 mph to avoid losing seeds to the wind. Seeds were mixed with damp builder's sand and hand broadcast evenly at a rate of 20 seeds m^{-2} onto each of the 12 plots. Enough vegetation had emerged at the time of seeding to provide an uneven surface for the seeds to settle into without having to be raked.

Seedling emergence and monitoring

Seedling emergence was monitored on a weekly basis, beginning in late May and continued to the end of the growing season in mid-September, once most vegetation began to senesce. Monitoring activities decreased to bi-weekly for the months of August and September, as no new relevant seedlings emerged and the communities appeared stable. Due to the difficulty in distinguishing graminoid seedlings from tillers of existing populations, I choose to monitor only the 14 forb species.

Within each plot the two 0.25-m^2 quadrats reserved for non-destructive sampling were monitored on a weekly basis for seedling emergence and seedling status. When a seedling emerged that was determined to possibly be one of the 14 monitored forb species, a colored craft stick was placed on the north side of it. The color of the stick and the species name or number was recorded. As several individuals were not fully identifiable to species until near the end of the growing season, many were assigned a name using the scheme "Unknown 1, 2, 3,..." etc. Each plot was monitored for the status of the marked species as well as for any new seedlings each week. New seedlings were marked well into August, though none of these turned out to be any of the seeded species.

Light availability

Mid-season percent light transmission through the plant canopy was quantified in all of the plots on 14 June 2013. Measurements were taken as near solar noon as possible in full sun conditions. In each subplot five measurements of photosynthetic photon flux density (PPFD) (μmol·m^{-2}·s^{-1}) were taken. One measurement was taken above the canopy and four orthogonal readings were taken at the soil surface. All measurements were taken with an AcuuPAR model LP-80 PAR/LAI ceptometer (Decagon, Pullman, Washington) with only the top 50 cm activated. The five measurements were averaged for each location in the quadrat and then PPFD was

averaged over both quadrats in each plot. Available and intercepted PPFD were calculated for each subplot and light transmission was expressed as percentage of maximum PPFD ($n = 24$).

Community indices

Shannon's index of diversity, Simpson's index of diversity and total richness were calculated using peak total cover as a measure of abundance for each species. Shannon's index of diversity was calculated for each subplot as $H' = -\Sigma\, p_i \ln p_i$, where p_i is equal to the proportion of total cover contributed by each of the ith species. Simpson's index of diversity was calculated as $D = \Sigma\, p_i^2$ where p_i^2 is equal to the relative cover of the ith species. Total richness was calculated as the mean number of species present in each subplot, as averaged across the 2 subplots for each plot.

To asses the quality of the community, as defined by the presence of native species that are typically restricted to undisturbed pre-settlement /native prairies, I used the mean coefficients of conservatism (CoC) (Mean C). CoC utilizes a scale from 0 to 10 to indicate species' fidelity to remnant sites, or sites that have remained relatively undisturbed by anthropogenic activity. I used CoC values determined for Kansas as available from the Kansas Biological Survey and the R.L. McGregor Herbarium at the University of Kansas. Values equal to 0 represent the "weediest" species, with values of 5 = "intermediate" and values of 10 = "most conservative." Recent studies have suggested that Mean C explains a greater amount of variation among tallgrass prairie communities than the more conventional measures of diversity and richness (Taft *et al.* 2006, Hansen & Gibson 2014). According to Carter and Blair (2012), calculating the Mean C weighted by species' relative abundance for each sampling unit was a superior measure of variation than unweighted mean C. Thus, I choose to only calculate weighted Mean C. To calculate the weighted Mean C, we took the sum of each species' CoC multiplied by its relative abundance for each subplot as follows:

$$\text{Mean C} = \Sigma\, \text{CoC}_i \bullet \text{A}_i$$

Where CoC is equal to the coefficient of conservatism for the ith species and A is equal to the relative abundance of the ith species, based on peak total cover values.

Seedling emergence and monitoring

Seedling emergence was monitored on a weekly basis, beginning in late May and continued to the end of the growing season in mid-September, once most vegetation began to

senesce. Monitoring activities decreased to bi-weekly for the months of August and September, as no new relevant seedlings emerged and the communities appeared stable. Due to the difficulty in distinguishing graminoid seedlings from tillers of existing populations, I choose to monitor only the fourteen forb species.

Within each plot the two 0.25-m^2 quadrats reserved for non-destructive sampling were monitored on a weekly basis for seedling emergence and seedling status. When a seedling emerged that was determined to possibly be one of the 14 monitored forb species, a colored craft stick was placed on the north side of it. The color of the stick and the species name or number was recorded. As several individuals were not fully identifiable to species until near the end of the growing season, many were assigned a name using the scheme "Unknown 1, 2, 3,..." etc. Each plot was monitored for the status of the marked species as well as for any new seedlings each week. New seedlings were marked well into August, though none of these turned out to be any of the seeded species.

Statistical analyses

I examined the response of seedling emergence to both the overall treatment of disturbance (soil disturbance and aboveground biomass removal treatments combined) versus the control, as well as the separate responses to the two disturbance treatments. As the experiment was established in a completely randomized design, a one-way ANOVA utilizing PROC GLM in SAS (SAS Institute 2008) was used to test for treatment effects. When testing for plot treatment effects, the differences between the mean seedling responses to each independent whole-plot treatment were examined (*n*=12) and plot treatment was set as a random factor. When testing for individual disturbance treatment effects, the differences between the mean seedling responses to each independent disturbance treatment were examined (*n*=8) and disturbance treatment was set as a random factor. We utilized the same model to test for differences in community variables between the control and disturbance treatment, as well as between the two disturbance treatments (clip and soil). Due to the relatively small sample size, I analyzed the significance of all results at α=0.10.

Relationships between community indices, seedling abundance and seedling richness were examined utilizing Spearman rank correlation coefficients determined from the mean

response of each variable in each independent plot, *n*=4 (SAS Institute 2008). Due to the relatively small sample size, I analyzed the significance of all results utilizing an α=0.10.

Results

Overall, seedling emergence was very low in the disturbance plots. Only one of the four control plots had seeded species emerge while two of the clipping treatment plots and three of the soil disturbance treatment plots had seeded species emerge. Of the fourteen forb species seeded, only four species were detected to emerge and eleven failed to emerge (Table 3.1). A total of fourteen individuals emerged with seven surviving to the end of the growing season and seven dying before the end of the growing season (Table 3.1). Overall seedling survival was much lower than in the environmental heterogeneity plots, with an even 50% survival and 50% death rate (Table 3.1).

One-way ANOVA returned no treatment effects on community response variables, except for % PPFD reaching the soil surface. A statistically significant difference in light availability was found between the control and the treatment (F_1=12.62, p=.0052), with the lower levels occurring in the control (l.s. mean $_{control}$=0.2142, l.s. mean $_{disturbance}$=0.4829, SE=0.0153). Furthermore, when broken down by treatment level, statistically significant differences (F_2=31.98, p <.0001) were found between the control, the soil disturbance treatment and the biomass removal treatments (l.s. mean $_{control}$=0.2142, l.s. mean $_{soil}$=0.3657, l.s. mean $_{biomass}$=0.6002, SE=0.0047; Tukey-adj. p $_{control - soil}$ = 0.0303, Tukey-adj. p $_{control - biomass}$ < 0.0001, Tukey-adj. p $_{soil - biomass}$ = 0.0024) (Fig 3.2). Thus, the objective to increase light availability by removing aboveground biomass and applying a soil disturbance treatment did indeed work. Furthermore, these results suggest that the treatments did not affect the overall community structure, which was another objective, but neither did they appear to have an effect on seedling abundance or richness. I found no statistical support for my hypothesis that seedling abundance would be higher in the disturbance treatments than the control (Fig 3.3). Likewise, no detectable difference in seedling richness was found between the control and the disturbance treatment (Fig 3.4).

When testing the hypothesis that seedling abundance would be higher in the soil disturbance than in the aboveground biomass removal treatment or control, I again found no

statistically significant difference (Fig 3.5). However, on average, seedling abundance was four times higher in the soil disturbance treatment than in the aboveground biomass removal treatment (l.s.mean $_{soil}$=2.0, l.s.mean $_{biomass}$=0.5; p=0.1682) and two times higher than in the control (l.s.mean $_{control}$=1.0, l.s.mean $_{soil}$=2.0; p=0.5783). Likewise, no difference in seedling richness was detected (Fig 3.5). When looking for differences between the soil disturbance and the biomass removal treatments, a very marginal difference was found between seedling richness (F_1=3.43, p=0.1135), with richness being five times higher in the soil disturbance treatment. However, these two means were so low that they are not biologically significant (Fig 3.6). Considering that the samples sizes were relatively small, these results suggest that if this experiment were to be scaled up, it is probable that statistically significant differences would be found in seedling abundance and richness between the soil disturbance and aboveground biomass removal treatments. However, the actual emergence numbers were so low that it is difficult to make any concrete conclusions.

Additionally, when examining relationships between community variables and seedling emergence responses, I found very few statistically significant relationships. Total seedling abundance was negatively related to Mean C, as was seedling richness (Fig 3.7). Otherwise, there were no significant relationships, including no relationship between light availability and seedling abundance or richness. As stated previously, emergence was extremely low in the control treatment, which most likely accounts for the lack of relationships when examining data from the control and treatment together.

Discussion

It has been well documented that smaller-scale disturbance is not only a driving factor in community assembly, but particularly when coupled with propagule supply, disturbance often results in an increase in species richness within a site (Foster 2001, Foster *et. al* 2004, Gross *et al.* 2005, Myers & Harms 2009). In addition, experiments involving small-scale disturbances that increase light and microsite availability, without seed additions, have demonstrated that seedling recruitment from the local species pool is often enhanced by these disturbances, given that the timing of the disturbance aligns with the timing of seed rain (Hobbs & Mooney 1985). Based on these findings, I hypothesized that greater numbers of seedlings would emerge in the

soil disturbance treatment than either the aboveground biomass removal or control treatments, as this treatment was expected to provide both increased light availability and microsite availability.

The results from this study do not support the hypothesis that seedling recruitment is higher when propagules are added in conjunction with a recent soil disturbance. While seedling abundance was highest in the soil disturbance treatments, it was not statistically different from the control or clipping treatment. A marginally significant difference between seedling richness in the soil disturbance and aboveground biomass removal treatments was found, with greater richness in the soil disturbance treatment. This suggests that disturbing the soil surface increases the chances for successful seedling recruitment by a greater number of species than simply removing aboveground biomass.

However, this study is not alone in finding little or no support for the hypothesis that disturbances to the canopy and soil surface structure increase rates of seedling establishment. Other studies have also provided alternate evidence, suggesting that there are other mechanisms governing the process of seedling recruitment in native (Rogers *et al.* 2001, Rogers & Hartnett 2001b) and restored tallgrass prairie (Reader & Buck 1991, Dickson & Foster 2008, Wilsey & Polley 2003). In particular, studies that have assessed interactions between fire and disturbance (Rogers & Hartnett 2001a), or water availability and disturbance (Dickson & Foster 2008, Wilsey & Polley 2003), have found that there are often significant interactions that affect the germination and survival rates of species in the face of various biomass removal and soil disturbances.

Reader & Buck's (1991) study investigating seedling establishment on soil mounds in old-fields suggest that seed mass may be more influential in the successful establishment of seeded species than interspecies variation in either potential seedling establishment or natural seedling establishment. Other studies have also supported the assertion that seed mass may be an important mechanism regulating seedling establishment, as larger seeds experience lower rates of drying out (Harper *et al.* 1965, Harper & Benton 1966) while also having the advantage of establishing a larger root system in a shorter amount of time then smaller seeds (Baker 1972, Platt & Weiss 1977, Stebbins, 1976, Peart 1989, Coomes & Grubb 2003). Oster & Eriksson (2012) also found evidence of a positive seed size - recruitment effect, with higher seedling recruitment from the largest-seeded species added to an established grassland community. Considering that the aboveground biomass removal treatment resulted in twice the %PPFD

reaching the soil surface as in the soil disturbance treatment, the lower seedling richness may be a result of increased seed desiccation due to greater exposure and drier soil microclimate. An examination of the seed mass for the 14 forb seeds added may offer further insight into both the patterns found in the disturbance treatments, as well as the overall question of whether seed mass is a predictor of a species' ability to establish by seed in disturbed tallgrass prairie, as suggested by others (Gross & Werner 1982, Reader & Buck 1991, Coomes & Grubb 2003).

While my data do not offer support for the hypothesis that seedling recruitment is higher in areas recently disturbed, they may offer insight into a subject that I originally did not intend. Studies in native tallgrass prairie at KPBS have demonstrated that recruitment of subdominant forbs and thereby species richness did not increase in the presence of simulated pocket gopher burrows (Rogers & Hartnett 2001, 2001b, Rogers *et al.* 2001). Rather than the expected forb recruitment occurring on mounds, graminoid recruitment via clonal colonization was most prominent (Rogers & Hartnett 2001b, Rogers *et al.* 2001). Additionally, over larger temporal and spatial scales, these types of disturbances have the potential to influence the dominance structure of tallgrass prairie communities (Rogers & Hartnett 2001a). As this study took place in a restoration that was established 15 years ago, my findings may suggest that while the community is much less diverse with lower richness than nearby native communities, it may function similarly when disturbed in a similar way.

There are several differences between this study and those conducted by Rogers *et al.* (2001), and I do not claim to draw parallels. However, when considering the basic effects of small mammal activity, particularly pocket gophers, in providing bare soil open for colonization and gaps in canopy cover, the soil disturbance and aboveground biomass treatments in this study had similar effects. Measurements of light availability indicated that microsite availability was successfully created through the reduction of canopy cover in a community dominated by highly productive C_4 grasses. This gives evidence that the disturbances did create at least a partially similar effect on the physical structure of the communities to those in Rogers *et al.* (2001) and Rogers & Hartnett (2001b).

This study took place over a single year, and thus I cannot draw any conclusions about the changes in the species composition of the community as a result of the soil disturbance, nor about the effects of these treatments on larger temporal and spatial scales. It would be interesting to expand the spatial and temporal breadth of this study to assess whether the shifts in

forb and graminoid richness that Rogers *et al.* 2001 witnessed also occurred in our restored communities.

Lastly, the finding that seedling abundance and seedling richness were negatively correlated with Mean C was surprising, especially considering that this was the only statistically significant correlation between seedling responses and environmental and community variables. The fact that the CoC of the two most abundant seedlings is 0 may be driving this relationship, though their relative abundance is so low that it is arguably negligible. Another likely explanation is simply that my seedling data are so few that a strong statistical relationship was found but there is no biological significance to it. I am hesitant to draw any more conclusions on this finding due to the insufficient data, but it would be interesting to see if this relationship continued with greater sample sizes over a longer period. If this trend were found to continue, it could signify that species are successfully recruited from seed via a trait-based mechanism of the existing community.

Given the inconclusive results of this study, in combination with the overall cacophony of results in disturbance and seedling recruitment studies (Hobbs & Mooney 1985, Wilson & Tilman 2002, Forbis *eta al.* 2004, Gross *et al.* 2005, Martin & Wilsey 2006, Gendron & Wilson 2007, Williams *et al.* 2007, Questad & Foster 2008, Dickson & Busby 2009), there is a need for additional studies on how seedling recruitment in restored prairie communities responds to small-scale disturbances over the long-term. These types of studies will also allow us to better understand the differences and/or similarities between functioning in less diverse, species-poor restored prairie communities and their more diverse and species-rich native counterparts.

Figures and Tables
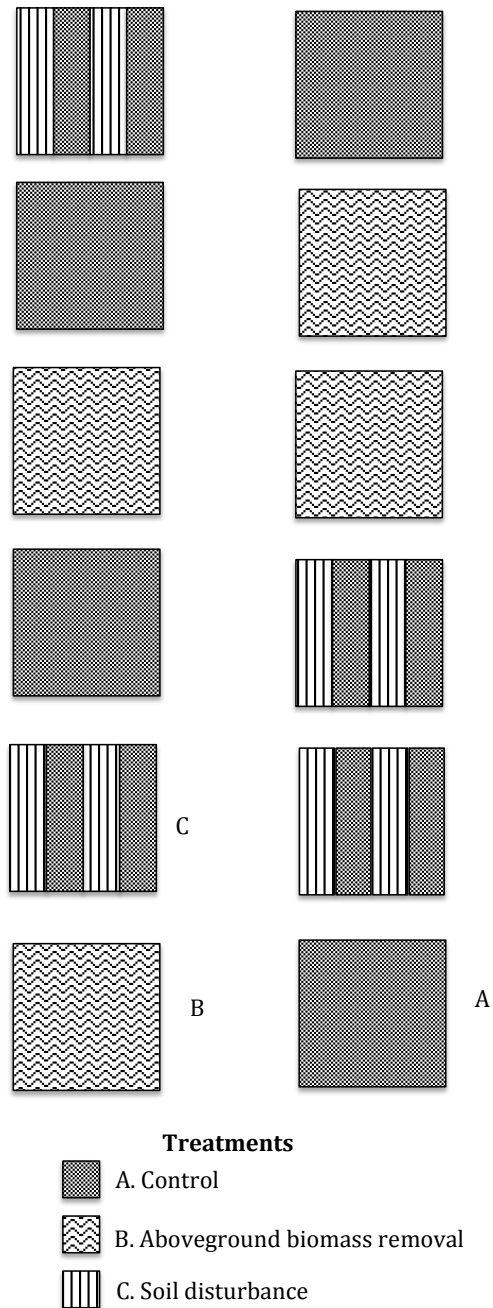
Figure 3.1 Plot map for disturbance treatments. Control, aboveground biomass removal (clipping to 10 cm.), and soil disturbance (cultivator in alternating 25cm strips) were randomly assigned to the center 1m^2 of 2 x 2 m delineated plots. Each treatment was replicated four times (n=4).
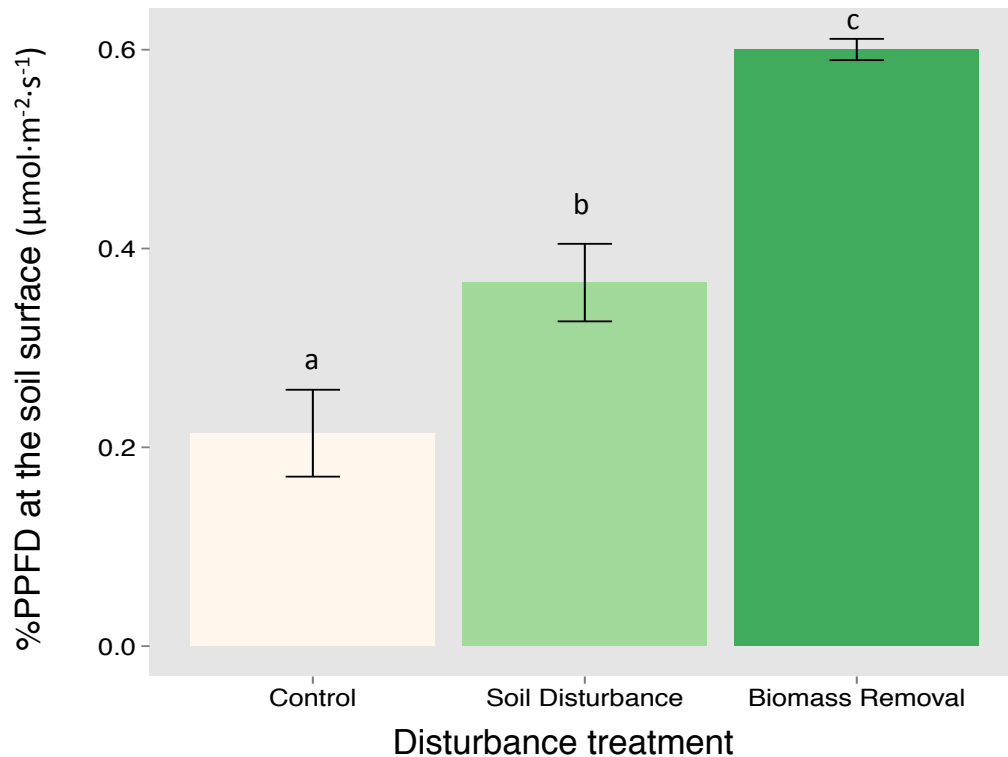
Figure 3.2 Light availability, as measured as % PPFD at the soil surface, was significantly affected by disturbance treatments. The soil disturbance resulted in twice the level of availability as in the control while the biomass removal resulted in nearly three times the level as in the control. Letters indicate statistically significant differences between treatment means. All statistics, as reported in the text, are derived from Proc GLM ANOVAs, with significance tested at α = 0.1 and generated by SAS version 9.2 (SAS Institute 2008).
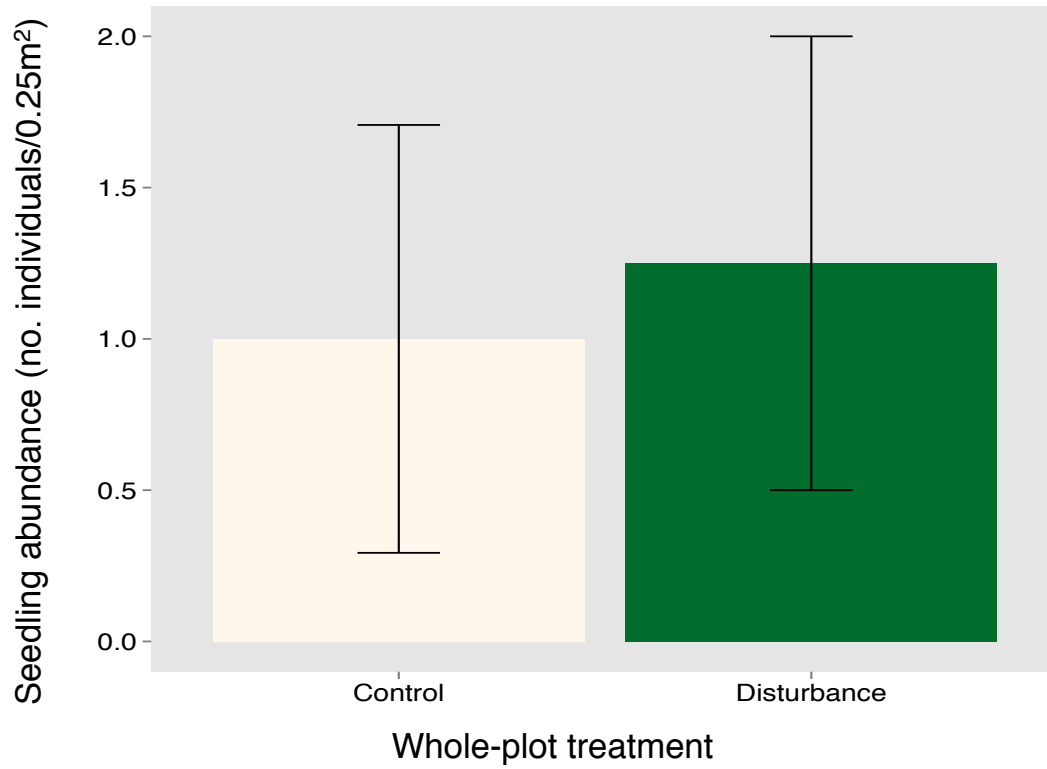
Figure 3.3 Seedling abundance did not differ between the control and the disturbance treatment combined, indicating no effect of increased light availability or microsite availability on seedling abundance. Statistics are as described in Figure 3.2.
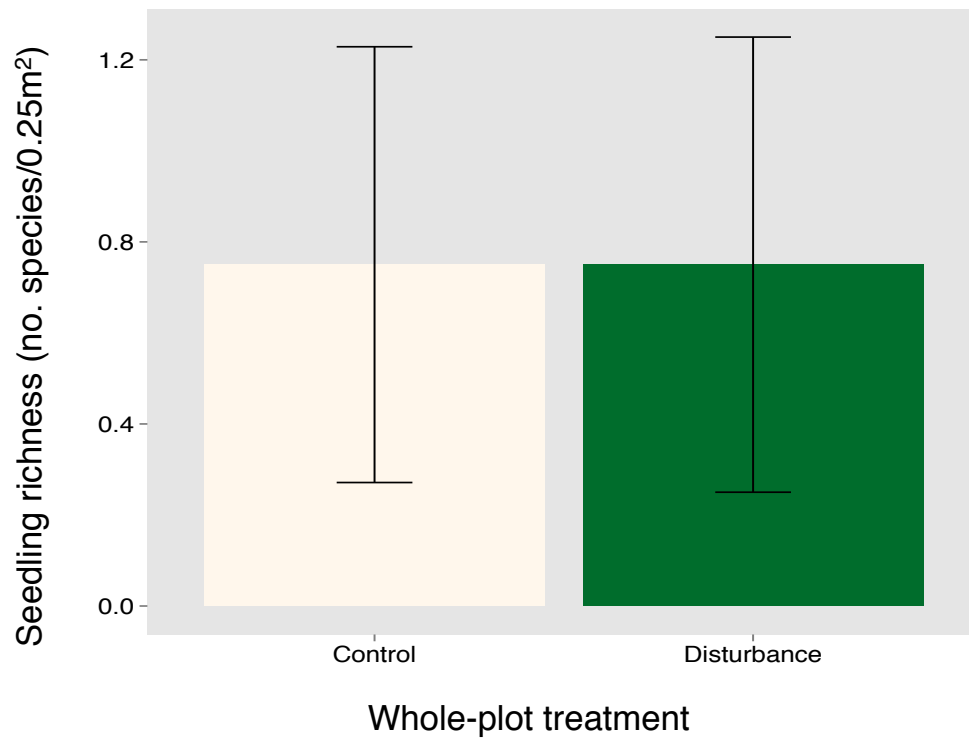
Figure 3.4 Seedling richness did not differ between the control and the disturbance treatments combined, indicating no effect of increased light availability or microsite availability on seedling richness. Statistics are as described in Figure 3.2.
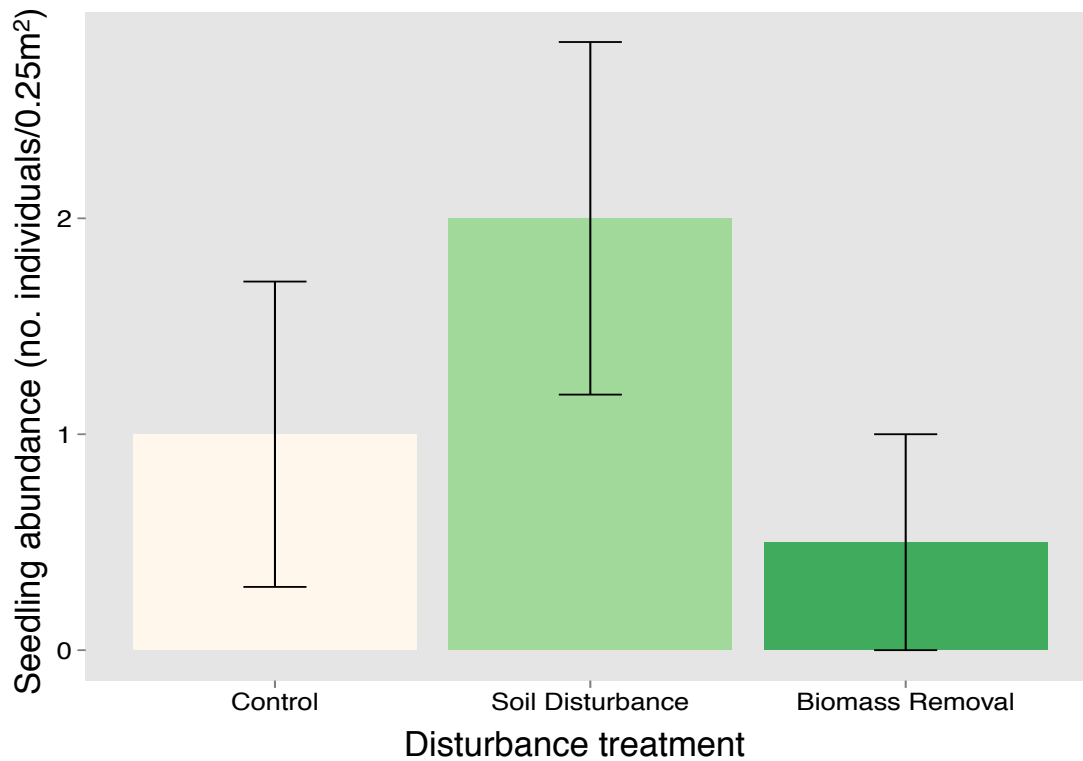
Figure 3.5 Overall seedling abundance was quite low in this experiment. No statistically significant differences in seedling abundance were detected between the control and the two disturbance treatments, indicating no effect of increased light availability or microsite availability on the number of seedlings to emerge. While no statistically significant differences were found, it is clear that arithmetically, the mean abundance of seedlings in the soil disturbance treatment was twice as high as in the control, and four times as high in the biomass removal treatment. It is possible that statistically significant differences would be found with larger samples sizes and over additional years of study. Statistics are as described in Figure 3.2.
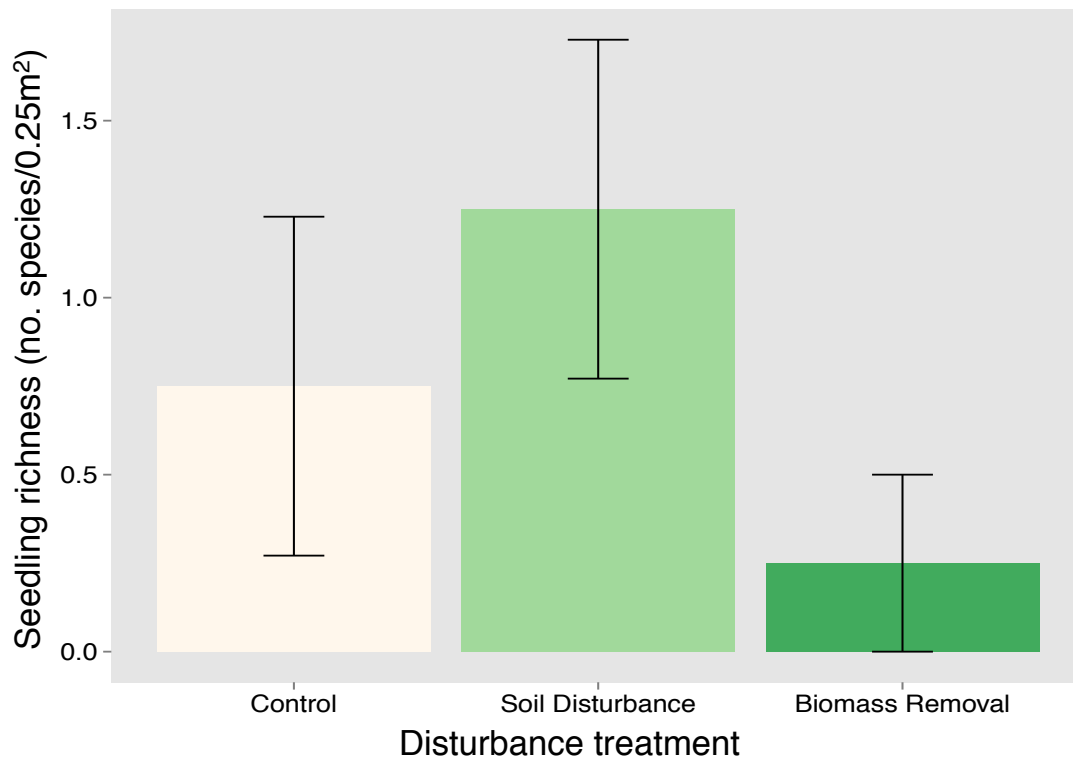
Figure 3.6 Seedling richness overall was quite low in this experiment and did not differ statistically between the control, the soil disturbance treatment and the biomass removal treatment, indicating no effect of increased light availability or microsite availability on the number of species to emerge. Statistics are as described in Figure 3.2.
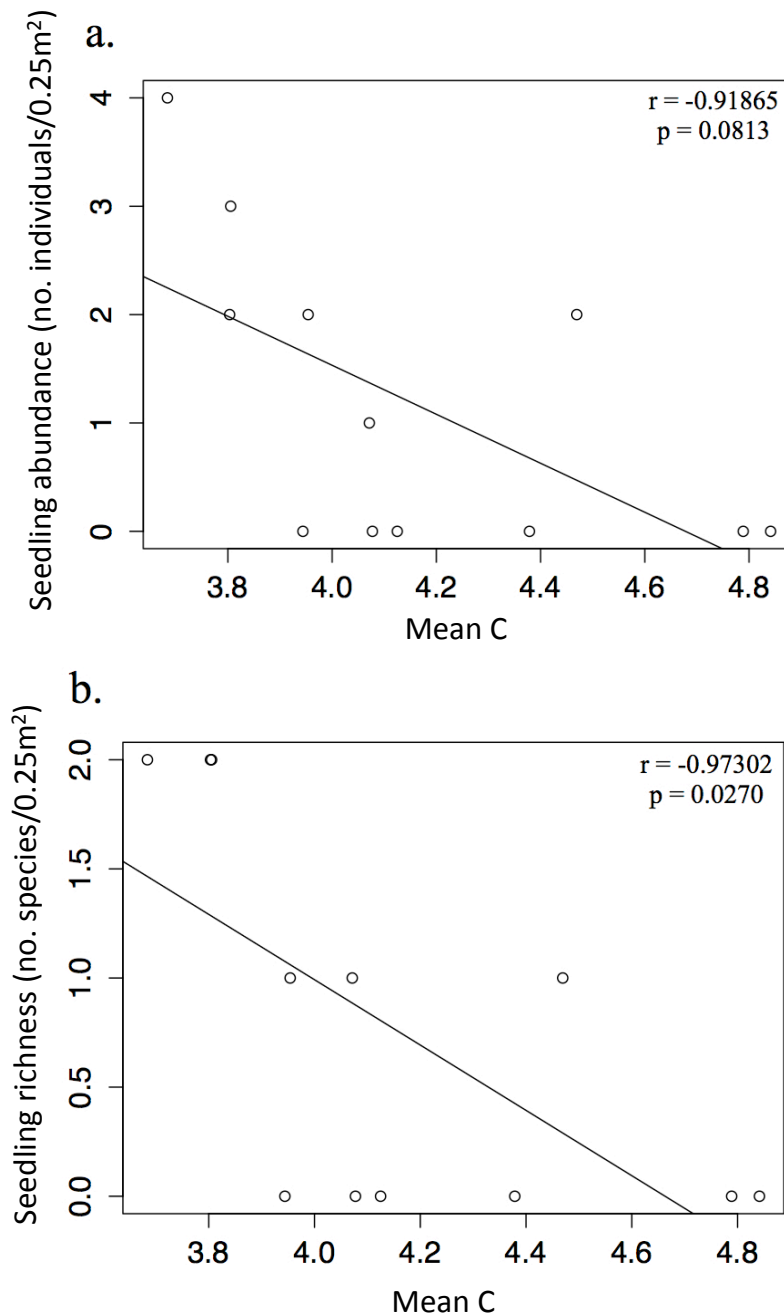
Figure 3.7 Relationships between Mean C, calculated as explained in the Methods section, and seedling abundance (a) and seedling richness (b) in disturbance and control plots. Correlations were performed on the mean response of variables, as averaged across each of the 2 subplot in each of the individual disturbance and control plots (*n*=12). Significant relationships were determined from Pearson's correlation coefficients at *α*=0.1 derived using SAS (SAS Institute 2008).

Species Name	Total no. individuals to emerge	Total no. individuals to surive to end of growing season	% Surviorship
Ageratina altissima (L.) R.M. King & H. Rob.	0	0	NA
Bouteloua gracilis (Wild. Ex Kunth) Lag. Ex Griffiths	NA	NA	NA
Carex brevior (Dewey) Mack.	NA	NA	NA
Elymus canadensis L.	NA	NA	NA
Grindelia squarrosa (Pursh) Dunal	8	5	62.5
Helianthus pauciflorus Nutt.	0	0	NA
Liatris aspera Michx.	1	0	100
Linum sulcatum Riddell	0	0	NA
Lobelia siphilitica L.	0	0	NA
Mirabilis nyctaginea (Michx.) MacMill.	4	1	25
Oligoneuron rigidum (L.) Small var. *rigidum*	1	1	100
Onosmodium bejariense DC. ex A. DC. var *occidentale* (Mack.) B.L. Turner	0	0	NA
Potentilla arguta Pursh	0	0	NA
Rumex altissimus Alph. Wood	0	0	NA
Silene stellata (L.) W.T. Aiton	0	0	NA
Silphium integrifolium Michx.	0	0	NA
Symphyotrichum drummondii (Lindl.) G.L. Nesom	0	0	NA
TOTAL	14	7	50

Table 3.1 Total emergence and percent survivorship for each of the 17 species added to the disturbance plots. Nomenclature follows USDA Plants Database.

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Chapter 4 - Conclusion

In summary, these studies combined suggest that attempts to override the decline in species diversity and richness observed in well-established restored prairies through seed additions may not be enhanced by manipulations to increase spatial heterogeneity. Neither the resource availability manipulations nor the small-scale disturbances supported a greater number of seedlings than did the controls. However, there were two main patterns that did emerge across both studies: 1) Seedling emergence was relatively low and 2) Great variability existed in species' emergence. Neither of these were a surprise. I expected to see a greater number of species emerge than actually did, but the variation in species' responses was as expected. Data from both of these studies do indeed suggest that colonization into a well-established community varies greatly from species to species, with ruderal species being more successful at colonizing in the first growing season than more conservative species. Again, this was expected as several other studies report great variation in interspecies germination and survival rates under both controlled and field environments (Harper *et al.* 1965, Harper & Benton 1966, Baker 1972, Platt & Weiss 1977, Stebbins, 1976, Peart 1989, Kitajima & Tilman 1996 , Hillhouse & Zedler 2001, Isselstein *et al.* 2002, Coomes & Grubb 2003, Öster & Erikson 2012). My results add to these studies' evidence that species responses are to a degree species-specific.

I did not, however, expect to see such high abundances of a single species, as was found with *Mirabilis nyctaginea* in the long-term resource heterogeneity experimental plots. This finding can be simply explained, as *M. nyctaginea* is listed as a Class A noxious weed in some states in the USA, such as Washington, it has a Coefficient of conservatism (CoC) of 0 in Kansas (Freeman 2012), and several authors have described it as being "weedy or invasive"(USDA 2014). It is logical that this ruderal species would successfully colonize an area where it has been seeded. When I examined the same plots early in the second growing season, I not only found several *M. nyctaginea* individuals re-emerging from the prior year, but I also noted several new individuals emerging from the second seed addition conducted in the winter of 2013, following the initial growing season. The second most abundant species was *Grindelia squarrosa*, which is also considered to be "weedy or invasive" in several areas of the USA (USDA 2014) and again has CoC of 0 in Kansas (Freeman 2012). I also found several of these individuals re-emerging as well as new seedlings emerging at the start of the second growing

season. Simply stated, species with traits that allow them to colonize and quickly establish reproductive population (i.e. ruderal) were the species most successful at emerging and establishing in the first growing season following a seed addition, regardless of treatment.

It has been recommended by other studies to seed more aggressive and dominant species at lower seeding densities at the onset of restorations in order to allow subdominant species to establish viable populations early on (Bakker & Wilson 2004, Baer *et al.* 2005, Dickson & Busby 2009, Rowe 2010). The findings from both experiments in this study support this conclusion and suggest that characteristically ruderal species can be introduced at much later stages of restoration and still successfully establish viable populations. Thus, it may be wise to wait until a desired representation of subdominant species is established prior to introducing species that are known to have "weedy or invasive" tendencies, as it appears that they will be successful in establishing populations within a well-established community.

Conducting additional years of monitoring on the emergence and fate of seedlings in both the resource availability and heterogeneity and disturbance treatment plots would offer greater insight into both the inter-species variation and mechanistic influences on seedling emergence and establishment in well-established restored prairie communities. Due to the high variability in seedling emergence, I would suggest any similar studies to consider sampling larger areas and to continue monitoring over several growing seasons. Data collected from a larger areas over several years will most likely yield results that are better able to detect inter and intra species variations in seedling emergence and establishment responses to manipulations of both soil resource heterogeneity and small-scale disturbances. Due to the relatively small sample sizes that I took over only a single growing season, it is quite likely that this study had limited ability to detect any predictable patterns in seedling emergence that could better inform management practices.

Furthermore, I conducted an additional study to examine the compositional differences between soil seed banks in the restored prairie and native prairie. I extracted cores and conducted greenhouse emergence trials on two separate occasions, both with high failure rates of emergence. I am unsure of the reasons behind the dual failure, as I followed standard methods utilized by several others for several decades. Whatever the reasons, I did have successful emergence of well over a dozen different species of forbs, including *Potentilla arguta*, a species seeded in the spring of 2013, but that I never detected to emerge. Two *P. arguta* individuals

emerged in the greenhouse from two different soil cores taken from two different subplots in the monitored restoration plots in early November 2013. This finding suggests that the seed was indeed viable, and that there were biotic or abiotic factors preventing the species from emerging in the field that were not present in the greenhouse. It may be that some of the species needed an additional stratification, as all of the soil cores taken in November were subjected to a second 8-week stratification. Thus, it is possible that more species will be detected to emerge two or more seasons after the initial seed addition.

In addition, the results of the study in the resources availability and heterogeneity experiment suggest that there may be a balance between the levels of aboveground and belowground resource availability that influence the ability of species to germinate and survive as seedlings. There is a current focus in the restoration ecology literature on the role of nurse plants in aiding the successful establishment of desired species in a number of ecosystems. It is possible that in tallgrass prairie systems the dominant grasses function as nurse plants for some subdominant species by allowing adequate light availability at the soil surface early in the growing season for germination, but then reducing light availability at the soil surface later in the season to prevent seedling desiccation from overexposure. This reduction in light reaching the soil surface may also create a soil microclimate that is slightly cooler and moister than areas with less canopy coverage, again promoting higher rates of germination and lower rates of seedling desiccation. It could be that the growth patterns of the dominant grasses may alter microclimates in such a way as to actually promote germination and seedling survival. Studies investigating the hypothesis that dominant grasses act as nurse plants in tallgrass prairie restorations are recommended to add insight into this possible balance of light availability and soil microclimate in seedling emergence.

An additional challenge that I expected was difficulty in positively identifying seedlings. I did not, however, anticipate such an extensive lack of seedling identification resources as I found. Not only did I find few published guides, but I also found a lack of human resources. While there are a handful of identification resources for many of the more "popular" forbs and grasses used in prairie plantings and sold in many commercial seed mixes, there are simply no reliable publications for identifying a broad array of prairie forbs and grasses at either the seedling or juvenile stage. Furthermore there are few individuals available to aid in seedling identification, as taxonomic work is based almost solely on mature reproductive individuals, for

obvious reasons. I thus argue that there needs to be a greater attention paid to the characteristics of prairie forbs and grasses at seedling, juvenile and non-flowering stages.

Considering that managers often have targeted species they are introducing to an area, identifying seedlings in the first few growing seasons in order to protect or enhance their continued survival can be invaluable. However, if the immature plants are not positively identified, then measures to promote their survival cannot be taken. Likewise, when seedlings and juveniles of undesirable species, especially invasive species, can be positively identified then early actions for removal can be taken and further spreading of the species can be prevented. Thus, there is a great need for the field of ecological restoration to have adequate resources for positive identification of species at *all* stages of life, from seedling to juvenile to reproductive to fruiting. Aside from aiding managers and land owners in identifying young plants to take early action, such resources would also allow field scientists to properly identifying species at various life stages, and to collect more accurate data on species composition and changes over time. Developing such resources could also help advance the fields of taxonomy and systematics by providing additional specimens at various life stages to utilize for both morphological and genetic identification purposes.

In closing, there is still much to be learned about the efficacy of over-seeding into well-established restored prairie communities to increase species richness and diversity. The studies reported in this thesis fail to offer any substantial evidence that increased soil resource availability and heterogeneity or small-scale disturbances increase the likelihood of seedling emergence and survival in the growing season immediately following a seed addition. However, the findings may offer evidence that the restored community is functioning in a similar way to native prairie communities, in that recruitment of new individuals into the community does not primarily occur from seed, but rather vegetative reproduction (Rogers & Hartnett 2011, Benson & Hartnett 2006). Thus, I would recommend future studies that not only explore the role of dominant grasses as nurse plants for seedlings, but that also investigate belowground dynamics to better isolate mechanisms regulating species diversity and richness in restored prairie communities. It is also possible that many of the mechanisms we are searching for and fail to detect exist at the evolutionary and genetic levels. I again encourage the partnering of ecologists and systematists to explore the relationships between ecology and evolution in a more applied setting, and suspect that the mechanisms both fields are struggling to detect are in fact

intertwined with one another. It would be interesting and possibly very informative to determine whether the phylogenetic relatedness among the species in the established community and between the community and the introduced species plays a role in determining which species are able to successfully establish viable populations. Considering that these communities evolved over extended periods of time, it is possible that we are overlooking the role of evolutionary relationships when we attempt to re-assemble communities based on the few remnant communities that we have today. Through exploring questions that encompass both the ecological and evolutionary aspects of plant community assembly in restorations, we may be better able to elucidate the mechanisms that are involved with reaching and maintaining plant species diversity and richness in not only tallgrass prairie, but other ecosystems as well.

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Appendix A - Species addition list

Species	Family	CoC	Year Added
Andropogon gerardii Vitman	Poaceae	4	1998
Bouteloua curtipendula (Michx.) Torr.	Poaceae	5	1998
Koeleria macrantha (Ledeb.) Schult.	Poaceae	6	1998
Panicum virgatum L. var *virgatum*	Poaceae	4	1998
Schizachyrium scoparium (Michx.) Nash	Poaceae	5	1998
Sorghastrum nutans (L.) Nash	Poaceae	5	1998
Sporobolus compositus (Poir.) Merr.	Poaceae	3	1998
Sporobolus heterolepis (A. Gray) A. Gray	Poaceae	8	1998
Amorpha canescens Pursh	Fabaceae	7	1998
Artemisia ludoviciana Nutt.	Asteraceae	2	1998
Asclepias verticillata L.	Apocynaceae	1	1998
Asclepias viridis Walter	Apocynaceae	1	1998
Baptisia australis (L.) R. Br.	Fabaceae	6	1998
Baptisia bracteata Muhl. ex Elliott	Fabaceae	6	1998
Brickellia eupatorioides (L.) Shinners	Asteraceae	2	1998
Callirhoe involucrata (Torr. & A. Gray) A. Gray	Malvaceae	1	1998
Ceanothus herbaceus Raf.	Rhamnaceae	8	1998
Dalea candida Michx. Ex Wild.	Fabaceae	7	1998
Dalea purpurea Vent.	Fabaceae	7	1998
Desmanthus illinoensis (Michx.) MacMill. ex B.L. Rob. & Fernald	Fabaceae	2	1998
Echinacea angustifolia DC.	Asteraceae	6	1998
Lepidium densiflorum Schrad.	Brassicaeae	0	1998
Lespedeza capitata Michx.	Fabaceae	6	1998
Liatris punctata Hook.	Asteraceae	5	1998
Mimosa nuttallii (DC ex. Britton & Rose) B.L. Turner	Fabaceae	6	1998
Oenothera macrocarpa Nutt.	Onagraceae	5	1998
Packera plattensis (Nutt.) W.A. Weber & Á. Löve	Asteraceae	5	1998
Penstemon cobae Nutt.	Plantaginacea	5	1998
Penstemon grandiflorus Nutt.	Plantaginacea	6	1998
Psoralidium tenuiflorum (Pursh) Rydb.	Fabaceae	3	1998
Ratibida columnifera (Nutt.) Woot. & Standl.	Asteraceae	4	1998
Rosa arkansana Porter	Rosaceae	4	1998
Ruellia humilis Nutt.	Acanthaceae	3	1998
Salvia azurea Michx. ex Lam.	Lamiaceae	4	1998
Sisyrinchium campestre E.P. Bicknell	Iridaceae	6	1998
Solidago canadensis L.	Asteraceae	2	1998
Solidago missouriensis Nutt.	Asteraceae	5	1998
Symphyotrichum ericoides (L.) G. L. Nesom	Asteraceae	5	1998
Symphyotrichum oblongifolium (Nutt.) G. L. Nesom	Asteraceae	5	1998
Symphyotrichum sericeum (Vent.) G.L. Nesom	Asteraceae	8	1998
Triodanis perfoliata (L.) Nieuwl.	Campanulaceae	2	1998
Vernonia baldwinii Torr.	Asteraceae	2	1998

Achillea millefolium L.	Asteraceae	1	2005
Asclepias tuberosa L.	Apocynaceae	6	2005
Delphinium carolinianum Walter ssp. *vimineum* (Nutt.) R.E. Brooks	Ranunculaceae	6	2005
Desmodium illinoense A. Gray	Fabaceae	5	2005
Eupatorium altissimum L.	Asteraceae	2	2005
Heliopsis helianthoides (L.) Sweet	Asteraceae	5	2005
Monarda fistulosa L.	Lamiaceae	3	2005
Oenothera biennis L.	Onagraceae	0	2005
Penstemon tubaeflorus Nutt.	Plantaginacea	3	2005
Rudbeckia hirta L.	Asteraceae	2	2005
Silphium laciniatum L.	Asteraceae	4	2005
Solidago speciosa Nutt.	Asteraceae	7	2005
Symphyotrichum laeve (L.) Á. Löve & D. Löve	Asteraceae	7	2005
Teucrium canadense L.	Lamiaceae	1	2005
Tradescantia bracteata Small	Commelinaceae	5	2005
Bouteloua gracilis (Wild. Ex Kunth) Lag. Ex Griffiths	Poaceae	5	2013
Carex brevior (Dewey) Mack.	Poaceae	5	2013
Elymus canadensis L.	Poaceae	5	2013
Ageratina altissimaII (L.) R.M. King & H. Rob.	Asteraceae	1	2013
Grindelia squarrosa (Pursh) Dunal	Asteraceae	0	2013
Helianthus pauciflorus Nutt.	Asteraceae	5	2013
Liatris aspera Michx.	Asteraceae	6	2013
Linum sulcatum Riddell	Linaceae	6	2013
Lobelia siphilitica L.	Campanulaceae	4	2013
Mirabilis nyctaginea (Michx.) MacMill.	Nyctaginaceae	0	2013
Oligoneuron rigidum (L.) Small var. *rigidum*	Asteraceae	3	2013
Onosmodium bejariense DC. Ex A. DC. var *occidentale* (Mack.) B.L. Turner	Boraginaceae	4	2013
Potentilla arguta Pursh	Rosaceae	6	2013
Rumex altissimus Alph. Wood	Polygonaceae	0	2013
Silene stellata (L.) W.T. Aiton	Caryophyllaceae	5	2013
Silphium integrifolium Michx.	Asteraceae	3	2013
Symphyotrichum drummondii (Lindl.) G.L. Nesom	Asteraceae	2	2013

Table A.1 Comprehensive species addition list for experiments in Chapters 2 &3, listed chronologically. The small-scale disturbance experiment (Ch. 3) did not receive the seed addition in 2005, but did receive the seed additions in 1998 and 2013. Nomenclature follows USDA Plants Database with family assignments following APG III (Stevens 2013). Coefficients of conservatism (CoC) listed follow those determined for the state of Kansas by the Kansas Biological Survey and the R.L. McGregor Herbarium (Freeman 2012).

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