# THE EFFECT OF SEED DISPERSAL AND RECRUITMENT ON TALLGRASS PRAIRIE COMMUNITY ASSEMBLY

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Biology — Doctor of Philosophy
Ecology, Evolution, and Behavior —Dual Major

2024

# ABSTRACT

Tallgrass prairies are among the most imperiled ecosystems globally. Since prairie landscapes and associated species are rapidly disappearing, conservationists have undertaken massive strides toward restoring and creating new prairies using seeds. Despite using diverse native seed mixes, restored prairies are often less diverse and compositionally different from remnant, never-been-plowed prairies. Understanding how seed inputs influence the formation of plant communities not only answers fundamental questions of plant community ecology but is also necessary for achieving restoration goals. In this dissertation, we investigated how vital aspects of seed sources, the seed rain and seed bank, changed during restoration (Chapter 2 and 3) and could be used to inform restoration management (Chapter 4). In an observational study, we resampled seed rain in the same remnant prairie used to conduct the first grassland seed rain study 40+ years ago and expanded to include a chronosequence of nearby restored prairies (Chapter 2). Using the same study sites, we also surveyed the aboveground flora and seed bank to determine the post-dispersal fates of seeds (Chapter 3). Lastly, we used empirical data collected from Chapter 2 to inform a field experiment testing whether species arrival based on dispersal phenology influenced community assembly outcomes (Chapter 4). Overall, we found that tallgrass prairies, especially newly restored prairies, produce record-breaking amounts of diverse seed input. Most prairie seed rain does not survive to germinate from prairie soils, and new desirable species are not recruiting in sufficient quantities for restored prairies to reach composition goals without human intervention. Lastly, when and in what order species arrived to restored communities influenced diversity, cover, and composition outcomes.

Dedicated to my mother and father – Betsy Carter and Michael Wynne – who believe that the gift of education lasts a lifetime.

# ACKNOWLEDGEMENTS

Words cannot express the immense gratitude I feel for those who have supported and inspired me throughout my journey as a scientist. Here I want to acknowledge all those who have made this dissertation possible:

My Ph.D. advisor, Dr. Lauren Sullivan, has been my chief navigator during my time first at the University of Missouri and now at Michigan State University. She has thoroughly demonstrated to me what it means to be an excellent mentor and scientist. Lauren, I will never forget your guidance and support every step of the way during these last five years. I am honored to have been your first graduate student and sincerely appreciate all you have done for me. I will always cherish our time together. My life has been made better by knowing you. Thank you.

I would also like all those who have served on my graduate committee: Dr. Lauren Sullivan, Dr. Lars Brudvig, Dr. Manuel Leal, Dr. Deborah Finke, and Dr. Elizabeth King. Your thoughtful and insightful feedback on my work has made me a better scientist and writer. I also could not have done this dissertation without Dr. John Snyder, Andrew Biggs, Keith Bennett, Judy McKinnon, Chris Newbold, Jeff Demand, Amber Edwards, Melody Kroll, Barb Sonderman, Justin Thomas, and Dana Thomas, who all contributed critical knowledge and assistance in designing and conducting my dissertation projects. I also immensely benefitted from the guidance of Dr. Alan Prather and Matthew Chansler, who taught me natural history collections skills invaluable to my career as a botanist.

Huge thank you to all past and present members of the Sullivan Lab: Dr. Lauren Sullivan, Larissa Kahan, Maya Parker-Smith, Carolyn Stephen, Dr. Zack Miller, Marissa Zaricor, Josh Klostermann, Ethan Rose, Dr. Alejandra Martínez Blancas, Dr. Laís Petri, Dr. Ashish Nerlekar, Dr. Gaurav Kandlikar, Ellie Lopez, Blake Schreck, Meredith Medley, Brandy Williams, Danielle

Gafford, Erica Eyerly, Savana Presson, Olivia DeClue, Tyler Seabold, Kelsey Jaeger, Katy McWilliams, Rachel Mickey, Shriya Deshmukh, Randal Klatt, and Sheila Vazquez-Morales. I truly believe we have the best lab and I will never forget all the joy, passion, and excitement we have shared together. Special thanks to Erica Murdock and Maya Parker-Smith, who contributed a substantial amount of time, thought, and work as research collaborators. I am particularly indebted to all those who assisted me in counting the 293,963 seeds involved in my dissertation projects and have provided me with invaluable feedback on my work. I could not have done this without you all. Thank you for taking part in this journey with me. I will miss you so much, but I look forward to seeing everything you accomplish in the future.

Although my dissertation is the direct result of the last five years of hard work, my efforts would not have been possible without all the educators who have inspired me throughout my life: Ms. Paige Whitney, who showed me the timeline of life; Ms. Monica Oliver, who taught me to love science; and Dr. E. Binney Girdler, who ignited my passion for ecology and love of plants. Thank you for setting me on the trajectory leading to this moment.

To all my friends, thank you so much for your continuous love and support throughout the years. I am incredibly grateful to have you in my life and am thankful every single day. I miss you dearly. To my family, and especially my parents Betsy Carter and Michael Wynne, I love you so much and feel like the luckiest person in the world to have you in my life. Thank you for believing in me and pushing me to achieve. I feel privileged to be your daughter. To my kitties, Tilly and Cassie, you have been my most loving companions.

I also want to acknowledge that the entirety of this work took place on the unceded lands of the Osage, Otae-Missouria, Chickasaw, Illini, Ioway, Quapaw, Shawnee, Delaware, Kickapoo, Sac & Fox, Omaha, Santee Sioux, and Anishinaabeg peoples, who were forcibly and unjustly displaced.

My work and its dissemination was graciously funded by the Michigan State University Plant Biology Department, Michigan State University Ecology, Evolution, and Behavior Program, Michigan State University College of Natural Sciences, University of Missouri Division of Biological Sciences, Prairie Fork Charitable Endowment Trust, and Long-Term Agroecosystem Research (LTAR) network (58-5070-9-016 and 58-5070-2-018).

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# CHAPTER ONE:

## Introduction

Due to extensive conversion to agricultural land after European settlement, the North American tallgrass prairie is among the most imperiled ecosystems in the world (Samson et al., 2004; Samson & Knopf, 1994). Large-scale declines in historical prairie landscapes (> 99% in some regions) and associated species have made this ecosystem a primary target of ecological restoration efforts (Rowe 2010; Samson et al. 2004; Samson & Knopf 1994). A common element of ecological restoration - the act of aiding ecosystem recovery - is using seeds to reestablish native plant biodiversity (Barak et al., 2022; Gann et al., 2019; Rowe, 2010; Wilsey, 2021). However, restored prairies are frequently divergent from old-growth, remnant prairies (e.g., Baer et al., 2002; Barak et al., 2017; Hansen & Gibson, 2014; Martin et al., 2005; Newbold et al., 2019; Polley et al., 2005; Sluis, 2002; Sluis et al., 2018), suggesting our understanding of ecological principles influencing plant community assembly is lacking.

Even when sown with diverse seed inputs, prairie restoration often produces variable, unpredictable results (Catano et al., 2023; Groves et al., 2020). Both deterministic and historically contingent processes can shape the trajectory of community assembly during restoration, leading to desirable and undesirable outcomes (Catano et al., 2023; A. T. Clark et al., 2018; Grman et al., 2015; Groves et al., 2020; Tilman, 1994; Weidlich et al., 2021; Zirbel & Brudvig, 2020). For example, seed mix designs can lead to predictable outcomes based on included species' suitability to environmental conditions (Grman et al., 2015; Zirbel & Brudvig, 2020) or competitive ability (A. T. Clark et al., 2018; Tilman, 1994), while historical contingencies including climatic differences between planting years (Catano et al., 2023; Groves et al., 2020) or variations in species arrival can result in more idiosyncratic outcomes (Weidlich et al., 2021). Since seeds are a fundamental component of tallgrass prairie restoration (Rowe, 2010), resolving how seeds influence community assembly through deterministic and historically contingent processes could provide insight into creating more predictable and favorable restoration outcomes.

Seed dispersal and recruitment are processes central to plant population and community dynamics (Beckman & Sullivan, 2023). In grassland plant communities, new species primarily arrive through the dispersing pool of seeds known as seed rain (Kalamees & Zobel, 2002; Rabinowitz & Rapp, 1980). Surviving seeds are incorporated into the soil seed bank, remaining buried until their recruitment into the aboveground flora or death (Chambers & MacMahon, 1994). As a result, the seed rain and seed bank communities are closely involved in plant community regeneration (e.g., Kalamees & Zobel, 2002; Kiss et al., 2018; Plue et al., 2021; Schott & Hamburg, 1997), succession (e.g., Huanca Nuñez et al., 2021; Sullivan et al., 2018; Tilman, 1994), novel species introduction (e.g., D'Antonio et al., 2001; DiVittorio et al., 2007; Funk et al., 2020), and regulation of genetic and species diversity (e.g., Beckman & Sullivan, 2023; Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000). Altogether, vital aspects of the seed rain and seed bank (i.e., quantity, diversity, composition, timing, etc.) and how these aspects change during assembly are important considerations for restoration efforts aiming to reestablish diverse native plant communities using seeds.

Spatial patterns of seed dispersal and recruitment can have long-term consequences on plant community diversity and composition (C. J. Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000). For example, insufficient dispersal can result in seed limitation, constraining genetic and species diversity in predictable ways (Clark et al., 2007; Foster, 2001; Grman et al., 2015; Martin & Wilsey, 2006; Myers & Harms, 2009; Turnbull et al., 2000). Seed limitation represents a common challenge to restoration efforts since restored communities are often isolated from source populations of desirable species (i.e., native species associated with remnant systems) (Grman et al., 2015; Huanca Nuñez et al., 2021; Sperry et al., 2019). Thankfully, seed limitation is easily remedied by either increasing the number of dispersing propagules through seed additions or reducing mortality factors such as seed predation (C. J. Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000). However, interpreting whether seed additions are overcoming seed limitation is difficult without knowledge of natural levels of seed dispersal (C. J. Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000).

Along with spatial patterns of seed dispersal, variations in the timing and order of species arrival can further influence the trajectory of community assembly by causing priority effects (Chase, 2003; Drake, 1991; Fukami, 2015). Priority effects occur when early-arriving species impact the establishment, growth, and reproduction of late-arriving species by capturing resources, garnering biomass, increasing population size, or modifying their environment enough to alter species interactions (e.g., Delory et al., 2019, 2021; Grainger et al., 2019; Martin & Wilsey, 2012, 2014; Vannette & Fukami, 2014; Weidlich et al., 2021; Werner et al., 2016; Young et al., 2017). Therefore, community assembly can take divergent paths depending on when and in what order species disperse to the community (Chase, 2003; Fukami, 2015; Temperton et al., 2016). As a result, priority effects have been suggested as a potential tool for guiding restoration outcomes by manipulating functional group arrival through seedings (Weidlich et al., 2021; Wilsey, 2021).

Seed rain and seed bank studies provide critical baseline knowledge needed to inform restoration and research efforts manipulating vital aspects of seed input. However, a major limitation of these efforts in grasslands is a lack of community-level seed rain and seed bank studies despite being highly desired by restoration managers (C. J. Clark et al., 2007; Funk et al., 2020; Myers & Harms, 2009; Turnbull et al., 2000). Much of our understanding of grassland community-level seed rain comes from the foundational study conducted by Rabinowitz & Rapp (1980) in a remnant tallgrass prairie. For many, Rabinowitz & Rapp's findings (1980) represent a high estimate of seed rain for not only prairies but all ecosystems (19,700 seeds $m^{-2}$ $year^{-1}$) (Kettenring & Galatowitsch, 2011; Myers & Harms, 2009). However, Rabinowitz & Rapp (1980) measured seed rain in a remnant prairie despite research and restoration efforts frequently manipulating dispersal in disturbed systems. Although it is well known that aspects of the aboveground flora change during assembly (e.g., Baer et al., 2002; Hansen & Gibson, 2014; Newbold et al., 2019), it is unclear if the seed rain and seed bank follow suit, making comparisons between the early and late stages of assembly in grasslands inappropriate as is in forests (e.g., Huanca Nuñez et al., 2021; Piotto et al., 2019; K. R. Young et al., 1987). Additionally, seed rain studies in grasslands are not replicated, making the long-term interannual variability in seed rain patterns unknown. It also remains ambiguous whether desirable species missing from restored prairie aboveground plant communities can arrive and recruit on their own or need additional human intervention (but see Sperry et al., 2019). Overall, quantifying patterns of seed dispersal and recruitment provides further insight into whether restoration efforts are producing communities on track to meet expectations while also informing new experiments testing fundamental ecological theory.

In response to these needs, we conducted observational studies investigating how patterns of seed dispersal and recruitment change during community assembly in restored and remnant tallgrass prairies (**Chapters 2 and 3**). For Chapter 2, we revisited the same remnant prairie used by Rabinowitz & Rapp (1980) to determine how vital aspects of seed rain (i.e., quantity, diversity, composition, timing) have changed in the 40+ years between studies. Furthermore, we had the unique opportunity to use a chronosequence of nearby prairie restorations (2, 5-6, and 15-years-old) to also investigate how vital seed rain aspects changed during assembly. In Chapter 2, using the same remnant prairie, we captured over two times the number of seeds and species dispersing than Rabinowitz & Rapp (1980), with considerable changes in seed rain composition 40+ years later. We also found that newly restored prairies have record-breaking numbers of seeds dispersing, over 6 times greater than those published by Rabinowitz & Rapp (1980) and 78 times greater than typical restoration seeding rates (Rowe, 2010). Lastly, we observed drastic compositional, diversity, and abundance changes in seed rain during community assembly, suggesting we must seriously reconsider our baseline for seed inputs into grassland restoration.

For Chapter 3, we returned to the same prairies used in Chapter 2 to also survey the aboveground flora and germinable soil seed bank to investigate 1) whether ambient seed sources provided missing diversity to restorations over time and 2) how plant community dynamics between the aboveground flora, seed rain, and seed bank changed during assembly. In Chapter 3, we found species missing from the aboveground flora of restorations were dispersing and recruiting from the seed rain and seed bank, respectively. However, these species were weedy, introduced, and largely undesirable to restoration efforts. Similar to our findings in Chapter 2, we observed substantial changes in compositional, diversity, and abundance metrics in the aboveground flora and seed banks of prairies across the chronosequence. While restored prairies by 15 years of age had comparable abundance and diversity metrics to the remnant prairie, the composition of the aboveground flora, seed rain, and seed bank remained divergent from the remnant. Since the aboveground flora became increasingly dissimilar to the seed rain and seed bank as prairies matured, compositional differences among these prairies will likely remain without outside intervention such as supplemental seeding or transplanting of missing species.

Based on our findings in Chapters 2 and 3, we devised an experiment investigating how priority effects based on dispersal phenology influence plant community assembly (**Chapter 4**). Prairie restorations are commonly sown with a one-time seeding event, forgoing interspecific differences in dispersal timing, which may have lasting impacts on community assembly during restoration (Myers & Harms, 2009; Rasmussen et al., 2014; Rowe, 2010; Rudolf, 2018; Zou & Rudolf, 2023). Prairies seeded in this manner are often missing early-season species, which reliably disperse before late-season species and are typically more subordinate (Deever et al., 2023; Frischie & Rowe, 2012; Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997; Sluis et al., 2018). Observed interspecific differences in dispersal timing and order, therefore, may mediate competitive interactions through priority effects; however, this remains untested in plant communities (Myers & Harms, 2009; Rasmussen et al., 2014; Rudolf, 2018; Zou & Rudolf, 2023). Longer time intervals between introductions are predicted to increase the strength of priority effects by allowing early arrivers more time to capture resources, grow, or modify their environment (Fukami, 2015; Von Gillhaussen et al., 2014; T. P. Young et al., 2017). Arrival order is also expected to result in priority effects, especially when subordinate and dominant species arrive separately (Durbecq et al., 2023; Werner et al., 2016; T. P. Young et al., 2017). Therefore, seeding early-season species long before late-season species may allow for coexistence outcomes improving the success of early-season species in prairie restorations.

In Chapter 4, we tested these hypotheses by manipulating the timing and order of arrival for 36 native prairie species classified as early-season/summer-dispersing or late-season/fall-dispersing based on our work in Chapter 2. We found that community assembly in tallgrass

prairies was contingent on immigration history, where manipulating the timing and order of species arrival resulted in divergent community assembly outcomes. Longer time intervals solely benefitted summer-dispersing species, with this guild experiencing increased richness and cover. Arrival order produced robust and asymmetric differences in richness, cover, and composition, where seeding fall-dispersing species first was strongly inhibitory to summer-dispersing species but not vice versa. Overall, seeding manipulations resulted in restored communities favorable and unfavorable to restoration efforts, supporting the role of historical contingency in producing variable restoration outcomes.

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# CHAPTER TWO:

# Quantifying seed rain patterns in a remnant and a chronosequence of restored tallgrass prairies in north central Missouri

## Abstract

Seed rain is an influential process related to plant community diversity, composition, and regeneration. However, knowledge of seed rain patterns is limited to those observed in forests and late-assembling grasslands, which might not reflect early-assembling communities such as newly restored grasslands. Resolving this gap in our understanding provides further insight into the role of seed dispersal. Here, we measured seed rain in a remnant tallgrass prairie, which was the site of the foundational grassland seed rain study in 1978, and a nearby chronosequence of tallgrass prairie restorations. We sought to determine how the quantity, seed mass traits, timing, diversity, and composition of seed rain changed (1) long-term and (2) during community assembly. To do so, we deployed artificial turf grass seed traps into 2-year-old, 5-6-year-old, and 15-year-old restored prairies and the remnant prairie, replacing traps every two weeks from May to December 2019. We captured over twice the density and richness of seed rain in the remnant prairie in 2019 compared to 1978. We also found that seed rain patterns changed as prairies aged, with each prairie possessing a distinct community of dispersing species. Significantly more seeds, seed biomass, and species were captured in the youngest restored prairie. However, seed mass traits were similar in all prairies. Except for composition, all other seed rain metrics in the oldest restoration were eventually comparable to the remnant prairie. Our results revealed that grasslands, notably young prairies, produce larger quantities of seed rain than previously known (124,806 seeds $m^{-2}$ $year^{-1}$, 97.24 g $m^{-2}$ $year^{-1}$), and seed input in all sampled prairies far exceeded restoration broadcast seeding densities. We further found that decreases in seed rain

quantity across the chronosequence did not correspond with increases in seed mass, suggesting a lack of tradeoffs between these metrics. Furthermore, tallgrass prairie restorations have not replicated the composition of seed rain seen in remnant systems. Increasing restoration seeding rates of desirable species may be needed to meet composition goals since current rates may not compete with the propagule pressure of undesirable species found in newly restored prairies.

## Introduction

Seed production and dispersal are critical processes of plant community dynamics (Beckman & Sullivan, 2023; Levine & Murrell, 2003). The dispersing pool of seeds, known as seed rain, represents an essential transition between plant generations produced via sexual reproduction (Arruda et al., 2018; Rabinowitz & Rapp, 1980). Seed rain plays a significant role in the regenerative ability (e.g., Huanca Nuñez et al., 2021; Schott & Hamburg, 1997), successional dynamics (e.g., Sullivan et al., 2018; Tilman, 1994), introductions of novel species (e.g., D'Antonio et al., 2001; DiVittorio et al., 2007), and maintenance of genetic and species diversity in plant communities (Beckman & Sullivan, 2023; Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000). Inadequate densities of dispersing propagules in the seed rain can limit plant population size and constrain local species diversity, a widespread phenomenon known as seed limitation (Clark et al., 2007; Foster, 2001; Grman et al., 2015; Martin & Wilsey, 2006; Myers & Harms, 2009; Turnbull et al., 2000). Even though seed dispersal is central to many processes related to plant community dynamics, studies on community-level seed rain remain rare (Beckman & Sullivan, 2023; Levine & Murrell, 2003).

Dispersal is key to overcoming seed limitation across ecosystems, particularly in disturbed, newly assembling communities(Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000). In many ecosystems, increasing the supply of dispersing propagules is often enough to overcome the negative effects of seed limitation and increase local species richness (Foster, 2001; Myers & Harms, 2009). For example, ecosystem restoration efforts use this principle to increase species diversity by seeding larger quantities of desirable species into restored communities (Rowe, 2010). However, pinpointing the role of seed input for population and community dynamics can be difficult when essential characteristics of ambient seed rain (e.g., quantity, diversity, and composition) are unknown (Clark et al., 2007; Myers & Harms, 2009). Despite its importance to all plant communities across community assembly, patterns of seed rain have primarily been studied in forests and mature grasslands (Arruda et al. 2018). As a result, experiments and restorations are often informed using seed rain estimates from late-assembling, mature systems, even though restoration efforts occur in early-assembling systems such as newly restored grasslands (e.g., Martin & Wilsey, 2006; Rowe, 2010). A lack of robust seed rain estimates for these systems makes it difficult to determine whether seeding densities are saturating enough to overcome seed limitation (Clark et al., 2007; Myers & Harms, 2009). Overall, resolving the existing mismatch in our understanding of seed rain at early-stages vs. late-stages of community assembly will allow for a better evaluation of the effects of seed dispersal.

Shifts in species traits relating to seed production, dispersal, and competitive ability during community assembly likely influence vital aspects of seed rain as communities age. Across ecosystems, life history and seed size are correlated with seed rain density, where fast-reproducing, short-lived, small-seeded species produce and disperse larger quantities of seeds than long-lived, large-seeded species that tend to delay reproduction (Moles et al., 2004; Moles & Westoby, 2006; Sullivan et al., 2018). The numerical advantage afforded by these smaller-seeded species is thought to be offset by large-seeded species having increased juvenile survival and competitive ability  (Moles et al., 2004; Tilman, 1994; Turnbull et al., 1999). However, tradeoffs in seed production, dispersal, and competitive ability may differ in strength between ecosystems. For example, seed rain studies using a chronosequence approach in forest ecosystems observe seed rain dispersing into mature forests is more species-rich, contains more large-seeded species, and is compositionally different compared to early-assembling forest seed rain; however, seed rain quantity may increase (Piotto et al., 2019), decrease (Young et al., 1987), or not change (Huanca Nuñez et al., 2021) with age. Limited studies in grassland ecosystems suggest declines in seed rain input but increases in large-seeded, perennial species and diversity over time, supporting tradeoffs in seed rain density and seed mass (Kettenring & Galatowitsch, 2011; West & Durham, 1991). Seed rain in grasslands is also far denser than in forests, and abiotic rather than biotic vectors play more prominent roles in grassland seed dispersal (Kettenring & Galatowitsch, 2011; Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997; West & Durham, 1991; Willand et al., 2015). Key aspects of seed rain may respond differently during community assembly in grasslands compared to forests. Therefore, measuring a suite of metrics related to seed rain is needed to critically assess how seed rain patterns change during grassland community assembly and whether these shifts are associated with changes in species traits.

To deepen our understanding of the role of dispersal during community assembly, we measured seed rain patterns in a remnant tallgrass prairie and a nearby chronosequence of restored prairies, ranging from 2 to 15 years old in Missouri, USA. Grasslands such as tallgrass prairies are ideal systems for investigating how seed rain patterns change during community assembly since they are abundant in both early and late-stages of assembly due to restoration efforts (Wilsey, 2021). We revisited the same remnant prairie (Tucker Prairie) as Rabinowitz &

Rapp (1980), who conducted the foundational work on seed rain in grasslands. Here, we had the unique opportunity to investigate how seed rain patterns have changed in the last 40+ years. We asked the following questions in our study: Do the timing, quantity, seed mass traits, diversity, and composition of seed rain patterns differ 1) between a remnant prairie measured by Rabinowitz and Rapp (1980) in 1978 versus the same prairie in 2019 and 2) between remnant and restored prairies and change during community assembly? We predicted that seed rain quantity, diversity, and composition in the remnant prairie would be similar across studies, but peaks in dispersal activity would occur earlier due to global climate change advancing plant phenology. As the restoration age increased, we predicted that the timing and quantity of dispersing seeds would become more similar to the remnant prairie since we expected the restorations to establish species dominant in the seed rain. We also predicted the total richness and quantity of dispersing seeds to be greatest in newly restored prairies and decline over time as perennial seeded vegetation displaces introduced and ruderal species from older prairies. Due to tradeoffs between seed rain density and seed mass, we expected average seed mass to also increase with as prairies aged. Lastly, we anticipated that native species diversity and overall species composition would remain divergent from the remnant prairie regardless of restoration age since strongly seed-limited species would not establish well in the prairie restorations.

## Methods

### *Study Sites*

Our study sites were at Tucker Prairie (38°56’53.6” N, 91°59’40.0” W, Callaway County, MO) and at Prairie Fork Conservation Area (38°58’29.7” N, 91°44’03.3” W, Callaway County, MO)(Figure S2.1 A). Tucker Prairie is a 59-hectare tract of unplowed North American tallgrass claypan prairie. Less than 0.5 % of intact tallgrass prairie ecosystem (i.e., never-been-plowed) remains in Missouri, and Tucker Prairie represents the last sizable claypan remnant prairie in north central Missouri (Samson & Knopf, 1994). More than 250 species of plants inhabit Tucker Prairie, with representatives from 57 families and over 150 genera (*Tropicos*, 2023). From 1958 to 2002, Tucker Prairie was burned once every four years in the late winter or early spring (Rabinowitz & Rapp, 1980). Since 2002, Tucker Prairie has been managed on a 5-year burn rotation, where units are burned once in the late winter to early spring (Jan. – Mar.) and again 2-3 years later in the late summer to early fall (Aug. – Oct.). Tucker Prairie was burned one year prior to our study and three years prior to Rabinowitz & Rapp (1980).

Prairie Fork Conservation Area (PFCA) is over 450 hectares of former agricultural land being restored to tallgrass prairie and savanna ecosystems (Newbold et al., 2019). From 2004 onwards, 16-25 hectares are newly seeded each year, with approximately 179 native prairie species collected from Tucker Prairie and other nearby remnant prairies (13.4 to 18.2 kg/ha) (Newbold et al., 2019). As a result, PFCA possesses a chronosequence of reconstructed tallgrass prairies comprised of Tucker Prairie descendants. Reconstructions are managed using a 2-4-year burn schedule (Newbold et al., 2019). For additional details on PFCA management, see Newbold et al. 2019. To capture changes in seed rain dynamics during the restoration process, we grouped our reconstructed sites into three categories, as defined by Newbold et al. (2019) as being representative of restored prairies at various stages of assembly. We measured the seed rain in an old reconstruction (seeded in 2004; burned in 2017), middle-aged reconstruction (seeded in 2013 and 2014; burned in 2017), and a young reconstruction (seeded in 2017; burned in 2018), which were all prepared using the crop method. Similar to other grassland seed rain studies (e.g., Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997), the effort required to sample seed rain at a sufficient temporal and spatial resolution and the lack of additional comparable study sites limited us to using one site per age class.

*Experimental Setup & Data Collection*

In May 2019, we deployed artificial turf grass traps (0.01 m$^{-2}$) in Tucker Prairie and in each of the focal PFCA restorations (four total sites). We used artificial turfgrass traps instead of sticky traps because of their durability and resistance to freezing, an issue encountered by Rabinowitz & Rapp (1980) (Molau & Per Mølgaard, 1996). Dispersing seeds become entangled in the blades of artificial grass and are retained until collection. At each site, we randomly established ten, 5 m long transects (Figure S2.1 A). We placed transects ~50 m apart to reduce spatial autocorrelation since most prairie species have mean predicted dispersal distances < 10 m (Sullivan et al., 2018). We then placed traps at 1 m intervals along each transect and affixed them to the ground using ground staples (five traps per transect, 50 traps per site) (Figure S2.1 B). We collected and replaced seed traps every 2 weeks from May 31st to December 12th, 2019. All traps for a transect were lumped together at each collection period. After collection, we identified seeds to the lowest taxonomic level possible using identification guides and seed reference collections similar to Rabinowitz & Rapp (1980) (Coons et al., 2019.; Martin & Barkley, 1961; details in Supporting Information). Due to identification level differences between our study and Rabinowitz & Rapp (1980), we elevated morphospecies to the same taxonomic level when comparing richness and composition between studies (e.g., *Carex bushii to Carex sp.*). Furthermore, because we sampled a slightly larger area of seed rain than Rabinowitz & Rapp (1980) (0.5 m$^2$ in 2019 vs. 0.32 m$^2$ per prairie in 1978), we compared seed rain density instead of number of seeds captured between studies. We obtained seed mass data from the Seed Information Database (SER et al., 2023) and the following sources: Barak et al. (2018), Sullivan et al. (2018), Turner & Rabinowitz (1983), and Zirbel et al. (2017). We weighed seeds for taxa lacking published data (details in Appendix A).

### *Data Analysis*

#### *Quantity and seed mass traits of seed rain*

We fit a generalized linear model (GLM) predicting the total number of seeds for the whole year as a function of site (remnant, young reconstruction, middle-aged reconstruction, and old reconstruction) to detect differences in seed rain quantity between prairies. We used a negative binomial distribution (log link) ("Mass" package; Venables & Ripley, 2002) to account for overdispersion in the data. We then conducted a post hoc analysis using Dunnett-style contrasts ("emmeans" package; Lenth et al., 2023) with a multiple comparison adjustment for three tests to determine whether reconstructed sites significantly differed in the number of seeds dispersing compared to the remnant prairie. Similarly, we compared seed biomass (mg) between sites with a GLM using a gamma distribution (log link) followed by a Dunnett-style contrasts post hoc analysis.

We also calculated the community weight mean (CWM) for seed mass (mg) at each site to determine whether seed mass traits changed during assembly. We accomplished this by adjusting seed mass for every species by weighting the number of seeds captured per species at each transect. Then, we used those values to calculate CWMs representative of the "typical" seed mass encountered at transects. Afterward, we fit a linear model predicting the CWM of seed mass (mg) as a function of site. We omitted all unidentified taxa from analyses involving seed mass (mg).

*Diversity and composition of seed rain*

To determine whether annual seed rain from remnant and reconstructed prairies differed in richness, we fit a GLM using a Poisson distribution (log link) predicting total richness as a function of site as before. We then conducted another post hoc analysis using Dunnett-style contrasts to compare reconstruction ages to the remnant, correcting for three tests. We used a similar model and post hoc test to analyze differences in native morphospecies richness between remnant and reconstructed prairies. We also fit a linear model for predicting Shannon diversity index as a function of site. We did not use unidentified taxa in our analysis of richness or Shannon diversity index.

To quantify annual seed rain compositional differences between sites, we first created a community distance matrix using Bray-Curtis dissimilarity, which considers standardized changes in species abundance between sites. Before calculating Bray-Curtis dissimilarity, we relativized species abundance by site total (across rows). We then visualized compositional differences with non-metric multidimensional scaling (NMDS) ordination using two dimensions. We used the envfit function (permutations = 5000) in the “vegan” package (Oksanen et al., 2020) to identify species associated with the compositional differences among sites and plotted them as vectors ($p < 0.001$).

Using permutational multivariate analysis of variance (PERMANOVA, permutations = 999) and post hoc pairwise comparison tests ("pairwiseAdonis" package; Martinez Arbizu, 2020), we examined potential differences in species composition between remnant and reconstructed prairies. To account for multiple comparisons, we adjusted p-values using a Bonferroni correction. We used the “vegan” package (Oksanen et al., 2020) to conduct all multivariate analyses. We conducted all analyses and visualizations using R (version 4.2.2) and RStudio (version 2023.06.1+524).

## Results

### *Quantity, seed mass traits, and timing of seed rain*

We captured 121,163 seeds representing 129 morphospecies and 34 plant families across all prairies sampled between May 31st and December 12th, 2019. Estimated seed rain density for every prairie in 2019 far exceeded the estimate reported by Rabinowitz and Rapp (1980) for a remnant tallgrass prairie in 1978 (Table 2.1). At the same remnant prairie, we captured over two times the density of seed rain in 2019 than in 1978. The quantity and biomass of seed rain in reconstructions decreased with age (Figure 2.1 A, B). By at least 5-6 years post-initial seeding, the number and biomass of seeds dispersing in reconstructions was comparable to the remnant prairie in 2019. Only the youngest reconstruction had significantly more seeds and biomass dispersing than the remnant prairie sampled in the same year (Figure 2.1 A, B; Table S2.1). We found no significant change in CWM seed mass between sites (Figure 2.1 C, $F_{3,36} = 1.29$, $R^2 = 0.097$, $p = 0.29$).

**Table 2.1.** Attributes of seed rain captured in a young (2-year-old), middle-aged (5-6-year-old), and old (15-year-old) prairie reconstruction and the same remnant prairie in 1978 and 2019. Characteristics of seed rain in 1978 were obtained from Rabinowitz and Rapp (1980). Numbers outside parentheses indicate the number of morphospecies we successfully identified. Numbers inside parentheses reflect the number of morphospecies observed using the same taxonomic level of identification as Rabinowitz and Rapp (1980).

| Site | Total seeds | Seed rain density (Seeds $m^{-2}$ $year^{-1}$) | Total morphospecies richness | Seeds identified (%) |
|---|---|---|---|---|
| **Young** | 62,403 | 124,806 | 87 (83) | 99.99 |
| **Middle** | 24,077 | 48,154 | 82 (77) | 99.95 |
| **Old** | 13,876 | 27,752 | 68 (64) | 99.97 |
| **Remnant 2019** | 20,807 | 41,614 | 76 (72) | 99.94 |
| **Remnant 1978 *** | 6,597 | 20,740 | -- (32) | 99.51 |

*Trapped seeds in 9 cm diameter sticky traps (n = 50) covered in tanglefoot (Rabinowitz & Rapp, 1980). Reported arithmetic mean for seed rain density.

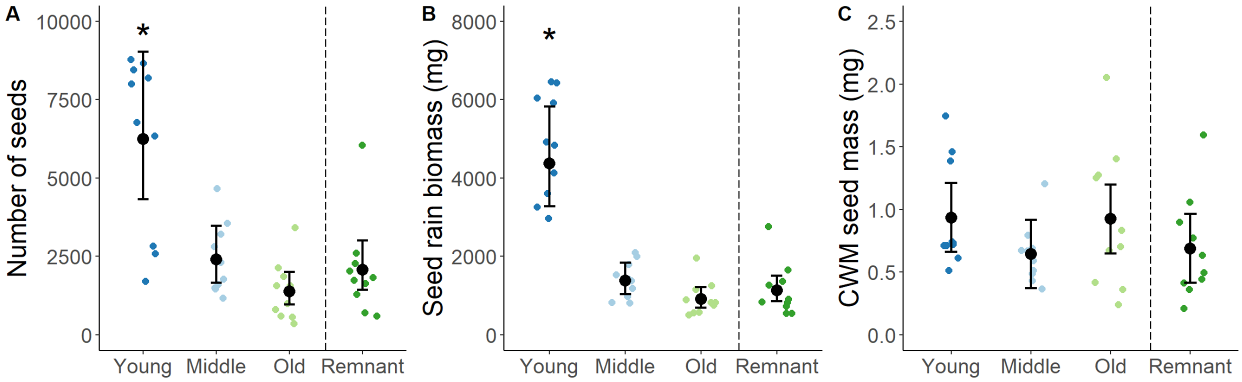

**Figure 2.1. A)** The number of seeds, **B)** seed rain biomass (mg), and **C)** CWM seed mass (mg) captured per transect in the remnant prairie in 2019 (dark green), young (2-year-old, dark blue), middle-aged (5-6-year-old, light blue), and old (15-year-old, light green) reconstructed prairies. Error bars represent 95% confidence intervals around model estimates (black). Asterisks indicate sites with significant differences from the remnant prairie ($p < 0.05$).

The timing of seed rain in all prairies exhibited a bimodal pattern of dispersal, where seed rain density peaked once in the early summer and again in the fall (Figure 2.2). However, compared to 1978, the timing of peak dispersal activity occurred earlier in 2019. The timing of the seed rain converged with the remnant prairie (2019) as the prairie reconstructions matured. As expected, the youngest reconstructed prairie was the most divergent, where the timing of the first peak in seed rain was delayed by a month compared to the other prairies sampled in 2019. Overall, the timing of the seed rain captured in the middle-aged and old reconstructed prairie closely resembled the timing of the remnant prairie in 2019.

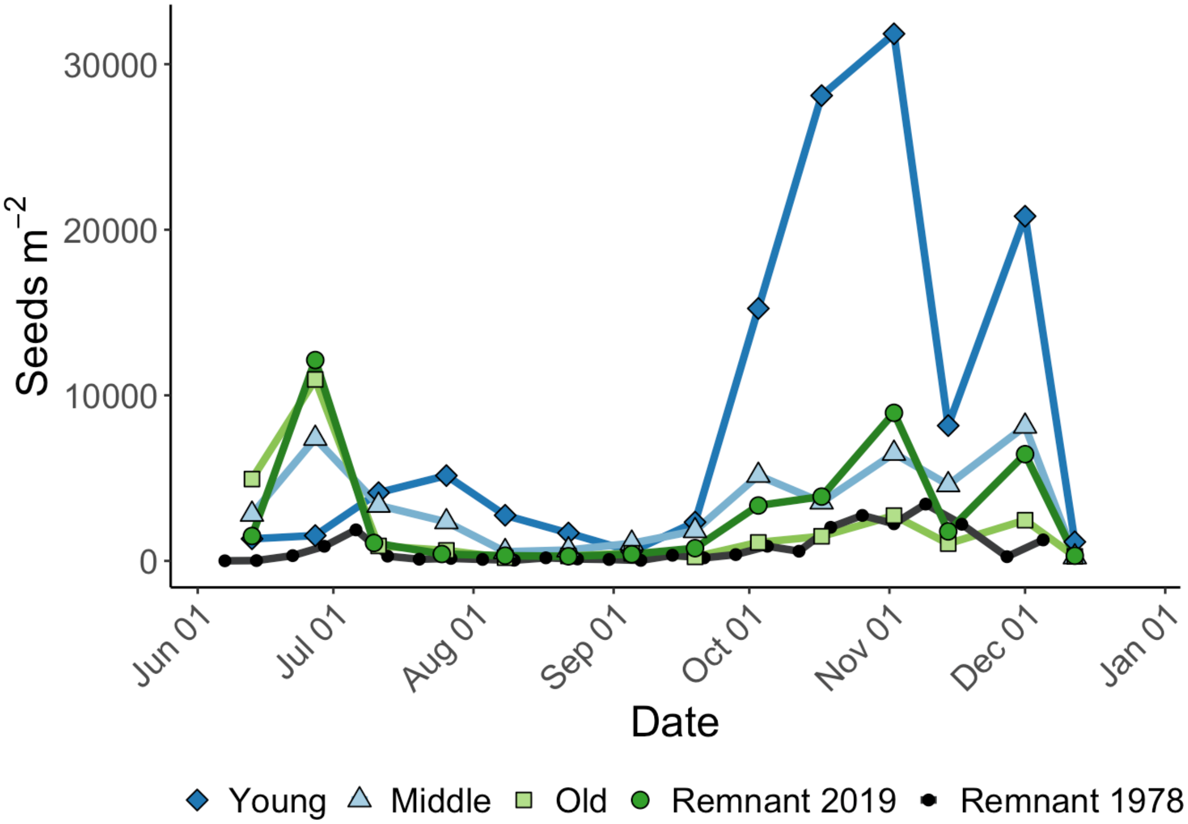

**Figure 2.2.** Timing of seed rain in reconstructed prairies of various ages and a remnant prairie. Data for the remnant in 1978 was obtained from Rabinowitz and Rapp (1980).

*Diversity and composition of seed rain*

Mean total richness was significantly greater in the young and middle-aged reconstructed prairie compared to the remnant (2019) (Figure 2.3 A, Table S2.1). However, all reconstructed prairies had comparable native morphospecies richness to the remnant prairie in 2019 (Figure 2.3

B, Table S2.1). Total and native richness also trended upward during the study period, with more morphospecies captured in the fall than in the summer (Figure 2.3 C, D). All prairies sampled in 2019 had far greater morphospecies captured than the remnant prairie in 1978 (Table 2.1). In fact, the remnant in 2019 had over twice the number of morphospecies present. All sites shared similar mean Shannon diversity index values ranging from 1.81 ± 0.44 in the youngest reconstruction to 2.28 ± 0.28 in the middle-aged reconstruction ($F_{3,36} = 2.637$, $R^2 = 0.18$, $p = 0.064$).

Species composition of seed rain significantly differed between all sites (Figure 2.4, Table S2.2). Reconstructed prairies of comparable ages shared the most similarities regarding species composition. Except for some marginal overlap with the oldest reconstruction, the remnant prairie contained a distinct community of dispersing seeds compared to reconstructed sites. Based on the species vectors (Figure 2.4), life history played an important role in compositional changes across the reconstructed prairies. Influential annual/biannual species in the seed rain aligned with younger reconstructions and perennial species with older reconstructions and the remnant prairie.

Most of the seeds captured at each site consisted of a handful of dominant species, with many rare species contributing few seeds (Figure S2.2). For example, the top three most frequently captured species represented 63.1% of the total seed rain for the remnant prairie in 2019*,* 76.9% for the remnant prairie in 1978, 63.9% for the old reconstruction, 42.0% for the middle-aged reconstruction, and 63.7% for the young reconstruction. Dominant species in the youngest reconstruction were fecund, disturbance-tolerant species, including smooth crabgrass *Digitaria ischaemum* and boneset *Eupatorium sp.* However, 15 years post-initial seeding, many of these species were almost absent in the oldest reconstruction and similarly rare in the remnant prairie. Instead, native perennial graminoids (e.g., rushes *Juncus sp.*, yellow prairie grass *Sorghastrum nutans*) and forbs (e.g., mountain mint *Pycnanthemum tenuifolium*, foxglove beardtongue *Penstemon digitalis*) dominated the seed rain in the older reconstructions and the remnant prairie (2019 and 1978). Dominant species in the seed rain did not necessarily reflect the dominant species in the vegetation. For example, rushes Juncus sp. were abundant in the seed rain of older prairies but rare in the vegetation (see Appendix A, Figure S2.4).

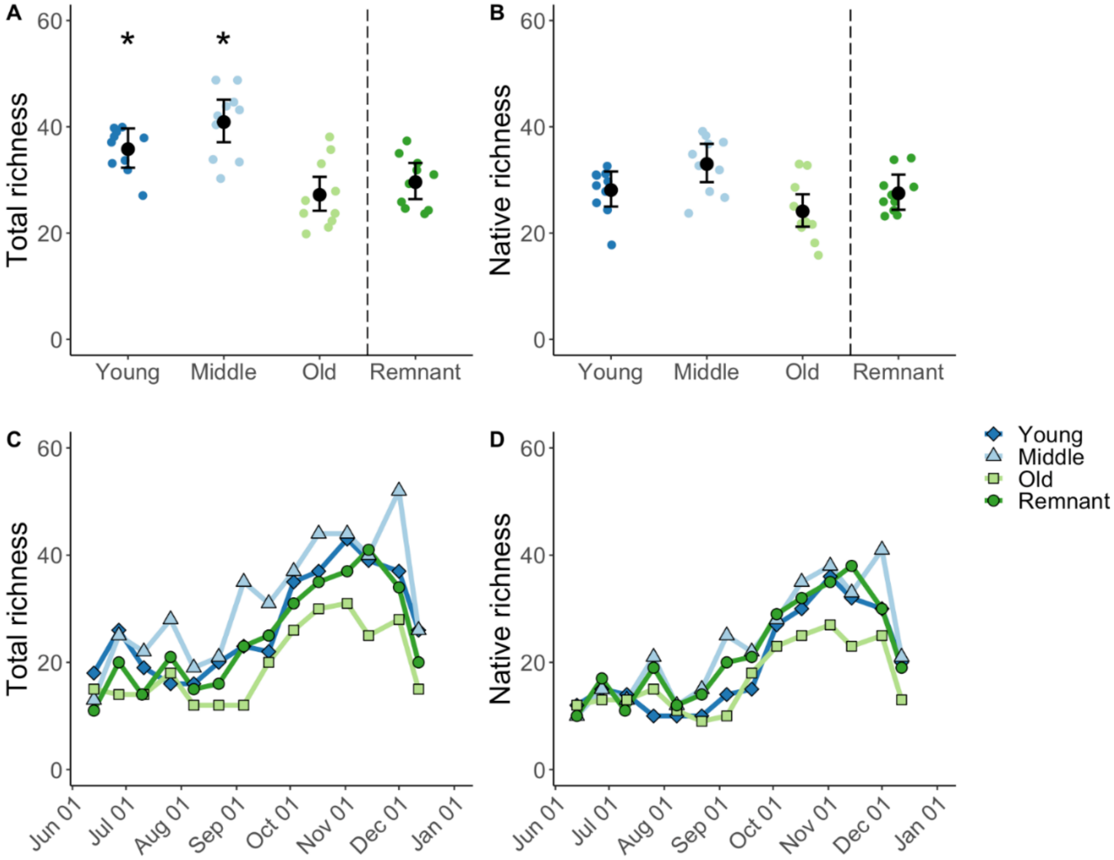

**Figure 2.3.** The total (**A**, **C**) and native (**B**, **D**) morphospecies richness of seed rain captured per transect in the remnant prairie in 2019 (dark green) and the young (2-year-old, dark blue), middle-aged (5-6-year-old, light blue), and old (15-year-old, light green) reconstructed prairies. Error bars represent 95% confidence intervals around model estimates (black). Asterisks indicate sites with significant differences from the remnant prairie ($p < 0.05$).

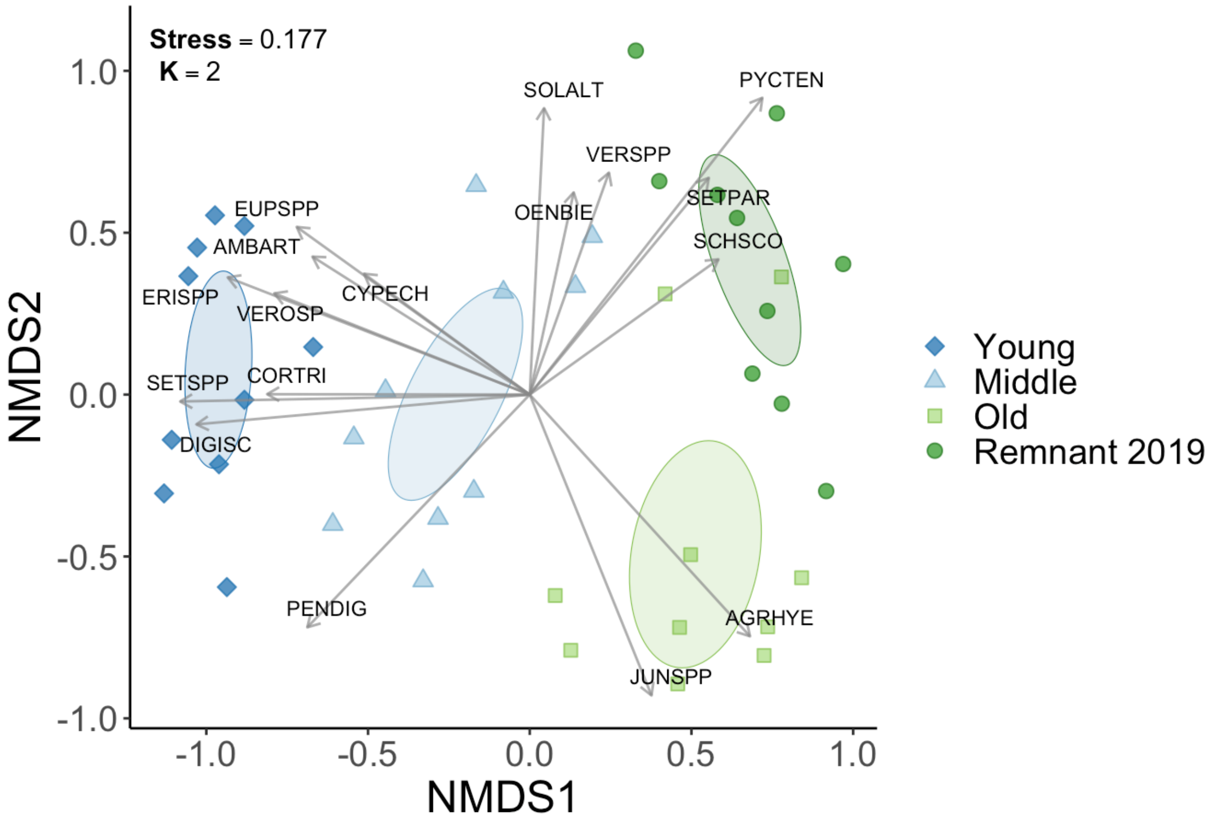

**Figure 2.4.** Non-metric multidimensional scaling analysis (NMDS) ordination using Bray-Curtis dissimilarity visualizing seed rain species composition at a remnant prairie in 2019 and prairie reconstructions of various ages. Ellipses represent 95% confidence intervals calculated from standard error. Plot vectors represent species significantly associated with seed rain composition ($p \leq 0.001$) Species names are as follows: AGRHYE = *Agrostis hyemalis*, AMBART = *Ambrosia artemissiifolia*, CORTRI = *Coreopsis tripteris,* CYPECH = *Cyperus echinatus*, DIGISC = *Digitaria ischaemum*, ERISPP = *Erigeron sp.,* EUPSPP = *Eupatorium sp.,* JUNSPP = *Juncus sp.*, OENBIE = *Oenothera biennis*, PENDIG = *Penstemon digitalis*, PYCTEN = *Pycnanthemum tenuifolium*, SCHSCHO = *Schizachyrium scoparium*, SETPAR = *Setaria parviflora*, SETSPP = *Setaria sp.*, SOLALT = *Solidago altissima,* VEROSP = *Veronica sp.,* VERSPP = *Vernonia sp*.

## Discussion

Our work demonstrated that seed rain patterns are dynamic in grassland ecosystems and change during community assembly. We report a record amount of seed rain in a grassland ecosystem, where we caught 124,806 seeds $m^{-2}$ year $^{-1}$ (97.24 g $m^{-2}$ year $^{-1}$) in the 2-year-old prairie, a number far exceeding most seeding densities of tallgrass prairie restorations, grassland seed addition experiments, and previously published grassland seed rain studies (Myers & Harms, 2009; Rowe, 2010; Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997; Willand et al., 2015; West & Durham, 1996). Despite observing declines in seed rain quantity and biomass over the chronosequence, we did not observe a corresponding increase in CWM seed mass, suggesting that small and large-seeded species are arriving at the same microsites in Missouri prairies of all ages. Although the timing, quantity, and diversity of seed rain in older restored prairies were comparable to the remnant prairie, we found seed rain composition of restored prairies remained divergent from the remnant community even 15 years after planting. Each prairie contained a distinct community of dispersing species despite being initially seeded with species collected from the remnant prairie. Because seed limitation is a common occurrence in grasslands, species missing from the seed rain will likely remain absent due to insufficient dispersal from source populations (Clark et al., 2007; Myers & Harms, 2009; Turnbull et al., 2000).

### *Differences in seed rain at the remnant prairie*

We found three major differences in seed rain patterns at the same remnant prairie used by Rabinowitz & Rapp (1980). First, peak dispersal activity shifted to occur earlier over the 41 years between studies. Second, the abundance and diversity of seed rain considerably increased since 1978. Lastly, we observed major shifts in seed rain composition between 1978 and 2019.

Overall, these findings provide evidence that seed rain patterns are highly variable even within the same mature community.

As expected, we observed an advancement in dispersal phenology for both peaks in dispersal activity in 2019 compared to 1978, where the first and second peaks occurred two weeks and one week earlier, respectively. Plant phenology is sensitive to global climate change, particularly for early-season species (Sherry et al., 2007; Zettlemoyer et al., 2021). Because timing of dispersal activity in conjecture with climatic conditions is likely pivotal for plant recruitment and coexistence outcomes, changes in dispersal phenology could have widespread consequences (DiVittorio et al., 2007; Forrest & Miller-Rushing, 2010). Additional community-level seed rain studies will help reveal whether our findings represent typical interannual variation in peak dispersal activity or long-lasting phenological change.

Secondly, our work revealed significant differences in seed rain quantity and diversity at the same remnant prairie 41 years after Rabinowitz & Rapp's (1980) foundational study, despite sampling the same number of slightly larger seed traps (100 $cm^2$ – 2019, 63.6 $cm^2$ – 1978). Against our initial predictions, we captured twice the amount of seed input and morphospecies in 2019 than in 1978. Increases in seed rain quantity and richness between studies were not a result of corresponding long-term increases in aboveground flora richness (see Supplemental Information), but could reflect a change in overall species composition (Figure S2.4). Additionally, differences in prescribed fire timing (burned 1 year prior to 2019, 3 years prior to 1978) and precipitation during months critical for seed production (above average in 2019, below average in 1978) presumably contributed to the substantial changes in seed rain density and richness observed between studies since these factors can stimulate the flowering and reproductive output of prairie species (Table S2.3) (Daubenmire, 1968; Rabinowitz et al. 1989;

Wagenius et al. 2020). Due to the large interannual variation observed between studies, we encourage future work to explore how climatic conditions and fire influence community-level seed production.

Lastly, we observed shifts in the dominant species composition of seed rain captured between 1978 and 2019, suggesting major compositional changes have occurred between studies (Rabinowitz & Rapp, 1980) (Figure S2.3). For example, mountain mint *Pycnanthemum tenuifolium*, a species rare in the aboveground flora and absent from the seed rain in 1978 (Rabinowitz & Rapp, 1980; Rabinowitz et al. 1981), was the most frequently captured species in 2019 at the same prairie. Despite catching twice the number of morphospecies, we were still unable to recapture 28.1% of the species caught in the 1978 study (Rabinowitz & Rapp, 1980). Because we used artificial turfgrass traps instead of sticky traps, trap design could have contributed to the observed differences in seed rain patterns between years. However, concurrent changes in the aboveground flora over a 38-year period at the remnant prairie provides additional support that differences in seed rain composition between studies does not represent typical interannual variation or methodology differences but rather long-term change (see Supplemental Information, Figure S2.4). Since species establishment and richness are dependent on intra- and interspecific propagule supply, changes in annual seed input can have major implications for the future community composition of the aboveground flora (DiVittorio et al., 2007; Myers & Harms, 2009).

Many studies have cited Rabinowitz & Rapp's (1980) findings as a high estimate for seed rain, not only in grasslands but for all ecosystems (e.g., Jochems et al., 2022; Kettenring & Galatowitsch, 2011; Martin & Wilsey, 2006; Myers & Harms, 2009). Although it is unclear whether 2019 represents a typical or extreme year, our results show that grasslands can produce far more abundant and diverse seed rain than previously thought. Seed production in remnant prairies is likely even greater than our findings suggest due to trap obstruction from vegetation (Brown & Cahill Jr., 2020), post-dispersal seed predation (Johnson & Zettlemoyer, 2022), and secondary dispersion of seeds by wind, water, and animals (Chambers & MacMahon, 1994). Because our trapping design did not exclude animals, animal behavior may have influenced species representation in our estimates. For example, species favored by animal predators (e.g., prairie dropseed *Sporobolus heterolepis*) or reliant on animal dispersers (e.g., wild strawberry *Fragaria virginica*) were likely underrepresented in our traps as evidence by their high presence in the aboveground flora and low to no presence in our seed rain samples (Figure S2.4) (Parker-Smith, 2022). Regardless, we captured over 20 times the amount of seed biomass, including unassisted, wind, and animal-dispersed species, in the remnant in 2019 (22.92 g $m^{-2}$) compared to typical restoration seeding rates (Rowe, 2010). Long-lasting differences from remnant aboveground vegetational communities in restored prairies may result from continued seed limitation.

### *Differences in seed rain across a restoration chronosequence*

We found considerable differences in seed rain patterns across a time-since-restoration gradient of tallgrass prairie restorations. Seed rain captured in the newly restored prairie was significantly more abundant and diverse compared to older restorations; however, increases in richness were solely due to introduced species.  Each restored prairie along the chronosequence had a distinct community of dispersing seeds with notable changes in dominant species over time. Consequently, the timing of dispersal activity reflected the phenology of dominant species, which differed most in the youngest prairie restoration compared to the older restorations. Our results indicate that key aspects of the seed rain change substantially as restored communities age.

Observed changes in seed rain diversity and composition across a restoration gradient closely mirrored well-established patterns in the vegetative community, where prairie restorations become increasingly dominated by perennial grasses over time. Seed rain in young prairie restorations was dominated by introduced, annual, and disturbance-tolerant species that were eventually displaced by native perennial species 15 years after restoration efforts began, resulting in the oldest restoration losing total richness. Unlike other studies that saw a reduction in native richness in the aboveground plant community of older restorations (e.g., Hansen & Gibson, 2014; Sluis, 2002), we captured comparable native richness of dispersing seeds in all restored prairies. However, the identities of these native species changed as the prairies aged. Overall, a combination of native species turnover and displacement of introduced species resulted in distinct seed rain compositions across the chronosequence.

Although many studies observe a negative relationship between seed rain density and seed size at the species level (Moles et al., 2004; Moles & Westoby, 2006; Turnbull et al., 1999), we found that this relationship does not extend to the community level. Against our expectations, small and large-seeded species were similarly present in the seed rain of restored communities of all ages despite the incredible quantity of seeds dispersing in early-successional prairies, indicating a lack of a numeric competitive advantage to small-seeded species.  Instead, changes in seed rain quantity occurred because of compositional shifts toward long-lived perennial species. Given the well-documented evidence that large-seeded species tend to be seed limited (Clark et al., 2007), our results support the role of post-dispersal processes that limit recruitment of large-seeded species (e.g., consumers, disease, etc.) (Chambers & MacMahon, 1994).

Additionally, introduced species were also prevalent in the younger restorations, which are often more fecund than native congeners and may explain differences in seed input across the chronosequence of restorations (Pyšek & Richardson, 2007). Other grassland seed rain studies similarly report denser seed input in young communities compared to older restored or remnant communities (Kettenring & Galatowitsch, 2011; West & Durham, 1991), suggesting that larger quantities of seed input may be characteristic of early-assembling grassland communities.

### *Implications for Research and Management*

We demonstrated that tallgrass prairie seed rain is far more diverse and abundant than previously estimated. Furthermore, we showed that seed dispersal patterns change during community assembly, with young communities having significantly greater propagule pressure than older communities. Together, our research supports that restoration efforts have yet to fully replicate the community composition seen at remnant prairies (Barak et al., 2017; Newbold et al., 2019; Sluis et al., 2018). Species only present in the remnant prairie seed rain were often understory species like yellow star grass *Hypoxis hirsuta and* arrow-leaf violet *Viola sagittata*, which are difficult to establish and highly desirable in restorations (Barak et al., 2022). Because these species rely on seed dispersal to arrive in new communities, they will likely remain absent without human assistance (Sperry et al., 2019). Restoration efforts aim to overcome seed limitation by broadcast seeding at rates based on studies of remnant prairie seed rain such as Rabinowitz & Rapp (1980) (Rowe, 2010). However, we captured over twice the amount of seed input in the same remnant and over six times the amount in the newly restored prairie as Rabinowitz & Rapp (1980). New species establishment from seed dispersal is an inherently probabilistic process, where increasing propagule supply correspondingly increases the chances of successfully entering the community (D’Antonio et al., 2001; DiVittorio et al., 2007).

Therefore, increasing restoration seeding rates may be needed to meet restoration composition and diversity goals since current rates may not compete with the propagule pressure of undesirable species found in newly restored prairies.

## Acknowledgments

We thank Prairie Fork Conservation Area for allowing us to use their property and Chris Newbold, Jeff Demand, Amber Edwards, and Melody Kroll for their help with site selection and information. We thank Larissa Kahan, Savana Presson, Kelsey Jaeger, Danielle Gafford, and Christian Perez-Martinez for assistance with data collection, John Snyder for providing valuable data analysis input, and Deborah Finke, Elizabeth King, Manuel Leal, Lars Brudvig, Gaurav Kandlikar, the Sullivan Lab, Pieter De Frenne and two anonymous reviewers for providing excellent feedback on our manuscript. We also thank James Carrel for graciously providing us with floristic survey data of Tucker Prairie in 1981. The Prairie Fork Charitable Endowment Trust and Long-Term Agroecosystem Research (LTAR) network (58-5070-9-016 and 58-5070-2-018) provided funding that supported our research. LTAR is supported by the United States Department of Agriculture.

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# APPENDIX A: QUANTIFYING SEED RAIN PATTERNS IN A REMNANT AND A CHRONOSEQUENCE OF RESTORED TALLGRASS PRAIRIES IN NORTH CENTRAL MISSOURI

## *Supplemental Information on Methods*

### *Processing of seed traps*

After collection, all traps from a transect and sampling period were grouped together and sieved through a series of soil sieves (1 mm, 500 µm, 250 µm mesh). We then counted and identified captured seeds in sieved layers. Because of their extremely small size, we estimated the number of rush seeds (*Juncus sp.*) when there were over 200 seeds present in a sample. We first sieved the samples through 180 µm and 150 µm mesh soil sieves. Then we subsampled each sieved layer by calculating the average number of rush seeds per 1 $cm^2$ area (n = 3) and multiplying that average by the total area covered by the sample layer. Lastly, we summed the number of estimated rush seeds per layer to calculate the total number of rush seeds in a sample.

### *Seed Mass Data*

We also individually weighed seeds (21 per species) to obtain the mean one seed mass (mg) for taxa lacking accessible data. We were unable to obtain seed mass for two taxa because of their rarity and lack of published data in the literature. For species with multiple reported masses, we converted all weights to one seed mass (mg) and used the average value for analysis. When we could only identify taxa to genus level, we calculated the average seed mass for all members of that genus known to inhabit our study sites.

### *Comparison of vegetation in the remnant prairie between 1981 and 2019*

To determine whether the remnant prairie also experienced long-term changes in aboveground flora diversity and composition, we compared floristic survey data collected in

1981 to data we collected in 2019. In 1981 (Jul. – Aug.), the aboveground flora was assessed by counting the number of stems per species in 1 $m^2$ frame quadrats regularly spaced at 50 to 150 m intervals across the entire prairie. For our analysis, we only used data collected in 1981 from the northern portion of the remnant prairie where our seed rain transects were located, representing a sampling area of 23 $m^2$ ($n = 23$ quadrats). In 2019 (Aug. – Sep.), we sampled the vegetational community in a 1 $m^2$ area around each seed trap at transects. Since one transect was dropped from the analysis due to incomplete data, our sampling area represents 45 $m^2$ of vegetation ($n = 45$ quadrats).  Because we measured the percent aerial cover of all vascular species rooted within sampling areas instead of the number of stems in 2019, we standardized all floristic data by presence/absence. Additionally, we elevated certain taxa to genus level due to differences in taxonomic identification level between surveys (e.g., *Carex sp*., *Jucus sp.*, etc.). Unidentified taxa were removed from all further analyses.

We assessed whether there were differences in aboveground flora morphospecies richness between 1981 and 2019 by predicting richness as a function of the survey year using a generalized linear model with a Poisson distribution followed by an analysis of deviance test. Furthermore, we visualized long-term changes in species composition using non-metric multidimensional scaling (NMDS) ordination ($k = 3$, stress = 0.16) followed by a permutational multivariate analysis of variance (PERMANOVA) test using Jaccard distance. We also identified influential species using the envfit() function (permutations = 5000) in the “vegan” package and plotted them as vectors in our NMDS ordination ($p < 0.0002$).

## *Supplemental Information on Results*

### *Comparison of vegetation in the remnant prairie between 1981 and 2019*

We found that the average number of morphospecies did not differ between 1981 (18.9 ± 3.29 species per $m^2$) and 2019 (18.2 ± 2.96 species per $m^2$) in the same remnant prairie flora ($X^2_1 = 0.34$, $p = 0.56$). However, the species composition of the aboveground flora changed in the 38 years between surveys (pseudo-F = 12.57, $R^2 = 0.16$, $p < 0.001$).

*Supplemental Figures & Tables*

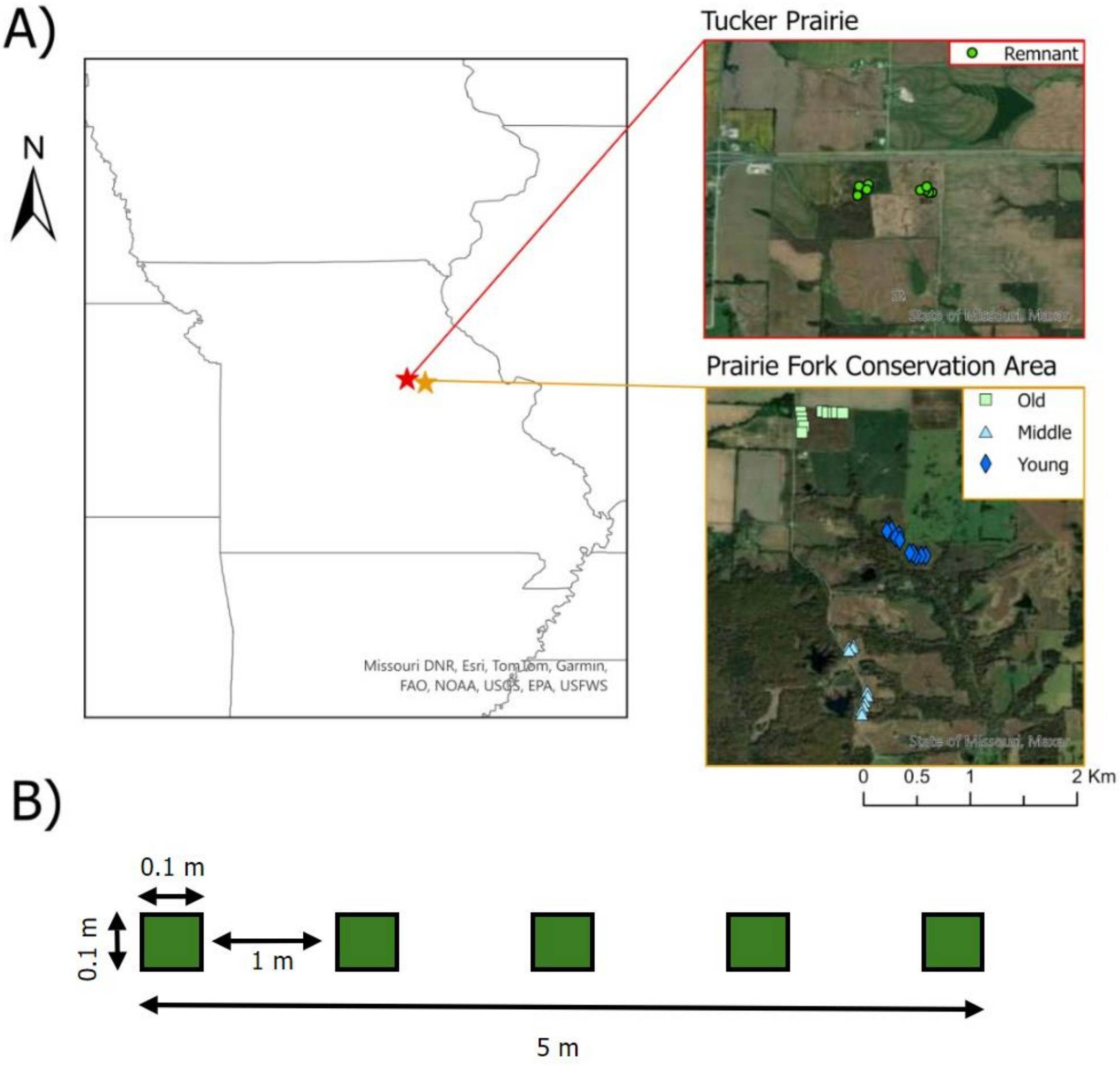

**Figure S2.1. A)** Map of study sites, Tucker Prairie and Prairie Fork Conservation Area, and transect locations in the old, middle-aged, and young restored and the remnant prairie (n = 10 transects per site; 40 transects total). **B)** Experimental design of transects used to sample seed rain in prairies. Green squares represent artificial turfgrass seed trap locations within a transect (n = 5 traps per transect; 50 traps per site; 200 traps total). Traps were replaced every 2 weeks from May to Dec. 2019.

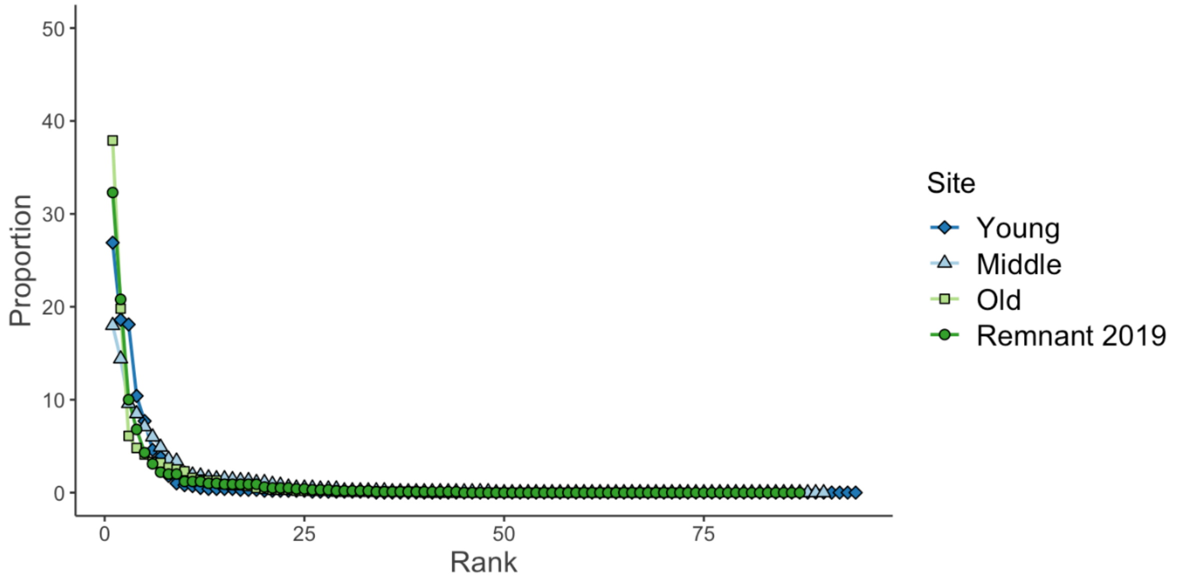

**Figure S2.2.** Rank abundance curves for a chronosequence of prairie restorations (young = 2-year-old, middle = 5-6-year, and old = 15-year-old) and a remnant prairie in 2019.

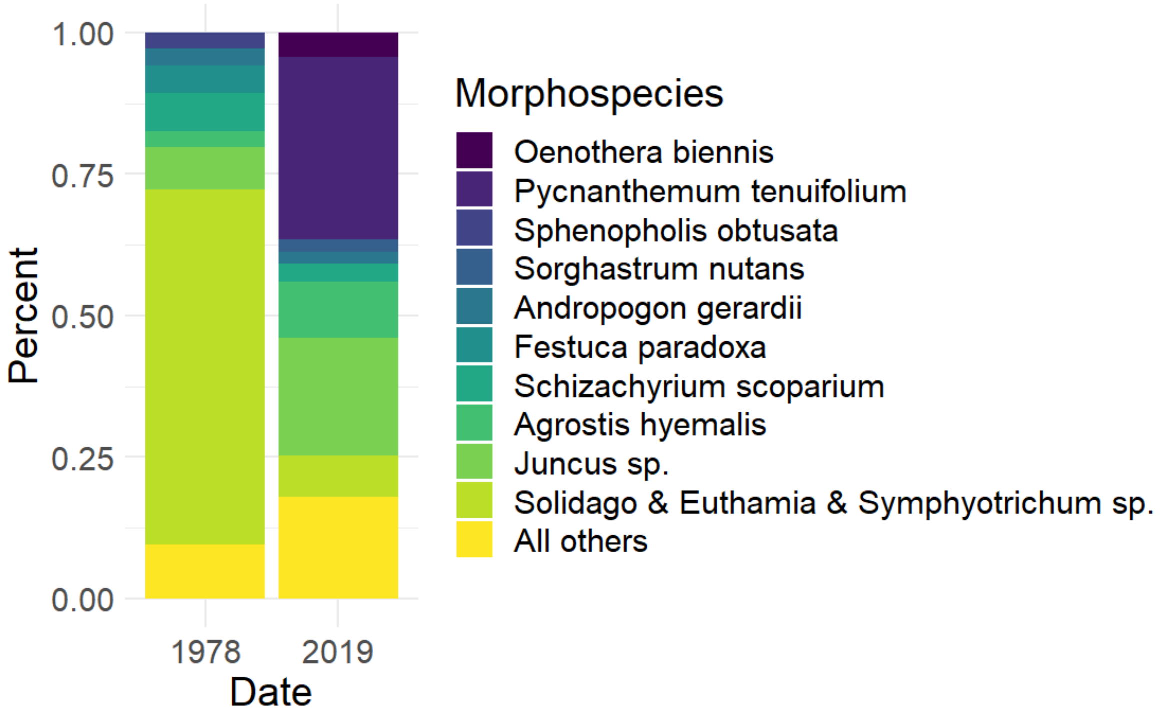

**Figure S2.3.** Comparison of dominant morphospecies in the seed rain between 1978 (Rabinowitz & Rapp 1980) and 2019 at the same remnant tallgrass prairie. Species that individually contributed less than 2% of the total seed rain during a sampling year were grouped together in the “all others” category.

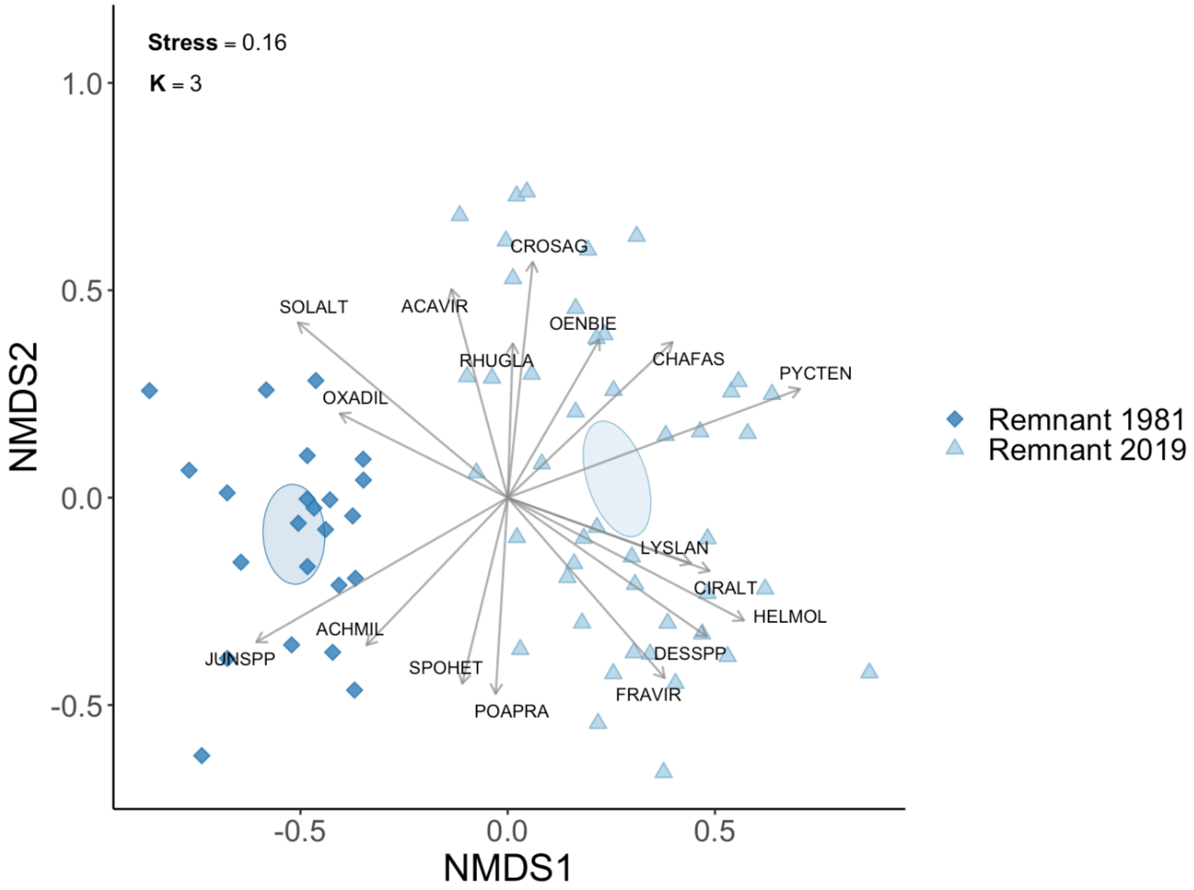

**Figure S2.4.** Non-metric multidimensional scaling (NMDS) ordination visualizing the vegetational composition of the remnant prairie in 1981 (dark blue diamonds) and 2019 (light blue triangles) in three dimensions. Ellipses represent 95% confidence intervals calculated from standard error. Plot vectors represent species significantly associated with the composition of the aboveground flora ($p < 0.0002$): ACAVIR = *Acalypha virginica*, ACHMIL = *Achillea millefolium*, CHAFAS = *Chamaecrista fasciculata*, CIRALT = *Cirsium altissimum*, CROSAG = *Crotalaria sagittalis*, DESSPP = *Desmodium sp*., FRAVIR = *Fragaria virginiana*, HELMOL = *Helianthus mollis*, JUNSPP = *Juncus sp*., LYSLAN = *Lysimachia lanceolata*, OENBIE = *Oenothera biennis*, OXADIL = *Oxalis dillenii*, POAPRA = *Poa pratensis*, PYCTEN = *Pycnanthemum tenuifolium*, RHUGLA = *Rhus glabra*, SOLALT = *Solidago altissima*, SPOHET = *Sporobolus heterolepis*.

**Table S2.1.** Summary results for pairwise contrast tests comparing the mean number of seeds (Z, (P)), mean seed biomass (mg) (T,(P)), mean total morphospecies richness (Z, (P)), and mean native morphospecies richness (Z, (P)) between reconstructed prairies of various ages and the remnant prairie in 2019. Bolded values indicate significance at the α 0.05 level.

| **Variable** | **Young – Remnant** | **Middle – Remnant** | **Old – Remnant** |
|---|---|---|---|
| Number of seeds | 4.13 **(< 0.001)** | 0.55 (0.548) | -1.52 (0.300) |
| Seed biomass (mg) | **7.48 (<0.001)** | 1.054 (0.58) | -1.06 (0.58) |
| Total richness | 2.46 **(< 0.05)** | 4.28 **(< 0.001)** | - 0.97 (0.634) |
| Native richness | 0.30 (0.964) | 2.28 (0.062) | - 1.45 (0.335) |

**Table S2.2.** PERMANOVA and pairwise comparison results from comparing seed rain composition between reconstructed prairies of various ages and the remnant prairie in 2019.

| **Source** | **Df** | **SS** | **$R^2$** | **F (P)** |
|---|---|---|---|---|
| Site | 3 | 5.16 | 0.41 | 8.22 **(< 0.001)** |
| Residual | 36 | 7.54 | 0.59 | - |
| *Pairwise comparison* | | | | |
| Young – Middle | 1 | 1.19 | 0.25 | 6.06 **(< 0.001)** |
| Young – Old | 1 | 2.43 | 0.39 | 11.34 **(< 0.001)** |
| Young – Remnant | 1 | 2.640 | 0.43 | 13.79 **(< 0.001)** |
| Middle – Old | 1 | 1.21 | 0.23 | 5.32 **(< 0.001)** |
| Middle – Remnant | 1 | 1.71 | 0.32 | 8.39 **(< 0.001)** |
| Old – Remnant | 1 | 1.14 | 0.22 | 5.15 **(< 0.01)** |

**Table S2.3.** Monthly precipitation (mm) and average air temperature (°C) in 1978 and 2019 in Callaway County, MO (station USC00234271). Mean values represent the mean and standard deviation (SD) of these factors during the 41-year period between studies. Dashes represent missing values. Data obtained from the National Centers for Environmental Information.

| **Month** | **Precipitation (mm)** | | | **Average Air Temperature (ºC)** | | |
|---|---|---|---|---|---|---|
| | **1978** | **2019** | **Mean ± SD** | **1978** | **2019** | **Mean ± SD** |
| Jan. | 19.81 | 81.02 | 52.98 ± 39.76 | -6.89 | -1.17 | -1.21 ± 2.81 |
| Feb. | 25.65 | 69.09 | 52.53 ± 36.55 | -5.89 | -0.17 | 0.85 ± 3.30 |
| Mar. | 112.78 | 118.62 | 80.44 ± 42.50 | 2.89 | 4.67 | 6.72 ± 2.39 |
| Apr. | 114.05 | 72.64 | 112.71 ± 60.97 | 14.06 | 13.72 | 12.92 ± 1.73 |
| May | 111.76 | 222.00 | 131.12 ± 62.35 | 17.00 | 18.50 | 18.15 ± 1.70 |
| Jun. | 59.18 | 104.65 | 102.27 ± 65.04 | 23.22 | 22.78 | 23.28 ± 1.17 |
| Jul. | 106.42 | 42.16 | 104.7 ± 67.11 | 26.22 | 26.28 | 25.74 ± 1.39 |
| Aug. | 89.66 | 199.14 | 100.91 ± 62.77 | 24.83 | 24.72 | 24.89 ± 1.69 |
| Sep. | 47.24 | - | 94.75 ± 81.99 | 23.00 | 12.78 | 20.36 ± 1.61 |
| Oct. | 56.64 | 188.21 | 92.85 ± 68.14 | 12.89 | 4.94 | 13.58 ± 1.70 |
| Nov. | 121.15 | 54.61 | 83.95 ± 63.56 | 12.89 | 4.11 | 7.04 ± 2.37 |
| Dec. | - | 29.72 | 57.03 ± 40.25 | 8.00 | 0.94 | 0.98 ± 2.61 |
| Total | 864.36 | 1,341.88 | 972.86 ± 321.83 | | | |

# CHAPTER THREE:

## Seed rain, seed bank, and vegetational dynamics of remnant and restored tallgrass prairies

### Abstract

Seed-based restoration has been increasingly recognized as a viable method for alleviating the loss of tallgrass prairies by creating new assemblages of prairie flora using seeds. Despite using diverse inputs of native seeds, restoration efforts frequently fall short of plant diversity and composition goals. Ambient seed sources, such as the seed rain and seed bank, could provide this missing diversity to restorations but our understanding of their relationship to the flora remains unclear. To understand how these sources influence vital aspects of prairie communities during restoration, we conducted a holistic survey of the aboveground flora, seed rain, and seed bank in a remnant and nearby chronosequence of restored Missouri prairies. We found that while tallgrass prairies produce extraordinary amounts of diverse seed inputs, most seeds and species are not found in seed banks, especially in older restored and remnant prairies. Although the native diversity of the aboveground flora, seed rain, and seed bank in restorations was eventually comparable to those in the remnant prairie, the composition of these community types in the oldest restoration remained divergent from the remnant. Furthermore, the composition of the aboveground flora became increasingly dissimilar to the composition of the seed rain and seed bank over time, suggesting that seed inputs are most influential at early stages of restoration. Species missing from the aboveground flora but present in the seed rain and seed bank tended to be undesirable to restoration efforts. Restoring populations of chronically missing and rare species will likely require further human intervention.

## Introduction

Seeding native species is central to tallgrass prairie restoration efforts in reestablishing diverse and compositionally similar native plant communities to those found in remnant prairies (Barak et al., 2022; Rowe, 2010; Ruiz-Jaen & Aide, 2005). Restoration success is most frequently assessed using floristic surveys to determine whether restored plant communities are on track to meet restoration goals (Ruiz-Jaen & Aide, 2005). Commonly, these surveys find that even when sown with diverse seed inputs, restored prairies are less diverse and compositionally differ from reference remnant prairies (Barak et al., 2017; Martin et al., 2005; Sluis et al., 2018). Additionally, restored prairies often become grass-dominated and lose forb diversity over time (Baer et al., 2002; Hansen & Gibson, 2014; Newbold et al., 2019). While floristic surveys adequately assess the diversity and composition of recruited plant communities, they do not evaluate the pools of species that could potentially recruit into the aboveground flora from ambient seed sources (i.e., not deliberately seeded by humans). Whether ambient seed sources could act as a source of missing diversity or help buffer against diversity losses from the aboveground flora is unclear, given the rarity of studies assessing multiple plant species pools simultaneously (i.e., seed rain, seed bank, and aboveground flora).

Ambient seed sources that can promote new species recruitment include the seed rain (pool of dispersing seeds) or the germinable seed bank (viable seeds in the soil) (Figure 3.1). These seed sources could act as stores of diversity absent from the aboveground flora, that could one day establish in the focal community (Pärtel et al., 2011). Quantifying the contents of the seed rain and germinable seed bank, hereafter referred to as seed bank, is critical for predicting if new desirable species, or native species representative of mature prairies, can recruit in restored communities on their own or whether management intervention is needed. Prairies produce the densest and most diverse seed inputs globally (Kettenring & Galatowitsch, 2011; Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997) and thus have the potential to have high recruitment from seed. Seeds can arrive from both local and non-local sources, both of which can substantially influence plant community and population dynamics (Beckman & Sullivan, 2023; Nathan, 2006). Still, dispersal as seed rain is only one part of the journey seeds undertake to become established plants. Seeds that survive dispersal are incorporated into the seed bank where they remain dormant until conditions favor germination (J. C. Chambers & MacMahon, 1994). Seed banks can act as reservoirs for species and genetic diversity no longer present in the aboveground flora (Funk et al., 2020; Schulz et al., 2018; Templeton & Levin, 1979). Species in the persistent seed bank can remain viable in the soil for decades or even over a hundred years (Fleming et al., 2023); however, long-dormant species in prairies tend to be undesirable (i.e., annual, ruderal, and/or introduced species) to restoration practitioners (Rabinowitz, 1981; Zylka et al., 2016) (but see Johnson & Anderson 1986). Although seed rain and seed banks have clear connections to the aboveground flora, studies that examine all three communities are rare in grasslands (Arruda et al., 2018; Beckman & Sullivan, 2023). Consideration of the connections between the aboveground flora, seed rain, and soil seed bank over a time-since-restoration gradient can provide insight into whether ambient seed sources can provide missing diversity to restorations over time.

While we have decades of evidence about how the aboveground flora changes compositionally as restorations age (e.g., Baer et al., 2002; Hansen & Gibson, 2014; Newbold et al., 2019), we do not have clear predictions for how restoration influences seed rain and seed banks. For instance, seed rain may further reinforce the composition of the pre-existing vegetational community over succession, as in tropical forests (Huanca Nuñez et al., 2021). Seed banks have also been shown to buffer against diversity and compositional change in the aboveground flora in a variety of systems across succession by hosting diverse assemblages of species also found in the flora (Plue et al., 2021). However, this might be limited to systems that form persistent seed banks (Kiss et al., 2018). In contrast, ambient seed sources may become less influential to the composition of the aboveground flora over time as vegetative reproduction becomes dominant, resulting in seed banks unrepresentative of the current flora (Benson & Hartnett, 2006). Additionally, established vegetation can act as barrier to the seed rain, preventing the transient seed bank from being replenished by the previous year's seed rain (Brown & Cahill Jr., 2020). Looking at the transitional trajectory of community composition across the aboveground flora, seed rain, and seed bank would reveal whether ambient seed sources remain influential to the aboveground flora as prairies age and if prairie restorations are on track to one day replicate the composition observed at remnant prairies.

Here, we report a holistic study investigating three types of plant communities (i.e., aboveground flora, seed rain, and seed bank) in a north central Missouri remnant tallgrass prairie and a nearby chronosequence of restored prairies. Our first objective (1) was to determine whether desirable (i.e., native mid to late-successional prairie) species missing from the aboveground flora were found in the seed rain and seed bank. We expected to capture diversity missing from the aboveground flora in the seed rain and seed bank, but these species may be undesirable to restoration efforts. Additionally, we identified species indicative of remnant aboveground prairie flora that were absent from the restorations. We also (2) sought to quantify how vital aspects (e.g., quantity, diversity, and composition) of the aboveground flora, seed rain, and seed bank changed across the time-since-restoration gradient and compare to a nearby remnant prairie to provide a more comprehensive perspective on whether restoration alters or replicates these aspects. We expected the number of germinable seeds in the seed bank to decrease over time as perennial vegetation establishes with similar quantities of seedlings germinating from prairie soils in the old restoration and the remnant. Since prairie restorations often lose diversity over time and most seeds arrive from the local flora, we also expected to observe reductions in the number of species in the seed bank over the time-since-restoration gradient and fewer native species in the oldest restoration compared to the remnant prairie. Furthermore, we predicted that if restorations were on track to meet their goals, seed bank communities would become more similar in composition to the remnant community seed bank over the time-since-restoration gradient. However, if seed bank communities remain highly divergent 15+ years later, intervention is likely needed to fulfill goals. We also anticipated that (3) if changes in vital aspects of the seed bank (i.e., diversity and composition) reflect changes in the aboveground flora and seed rain over time, then seed banks have a high potential to buffer against compositional change and diversity loss in the flora. In contrast, increasing dissimilarity in aspects between the aboveground flora, seed rain, and seed bank as prairies age suggests that ambient seed sources have a diminished capacity to maintain the aboveground flora long-term.

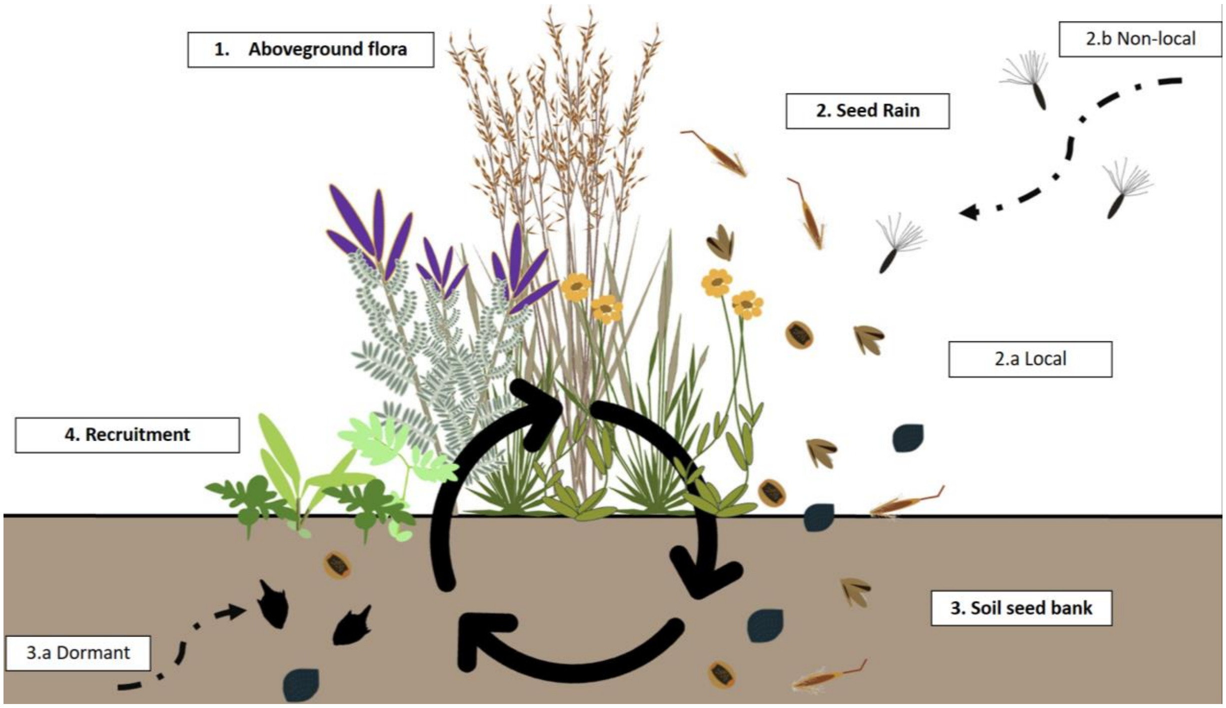

**Figure 3.1.** Each growing season the aboveground flora community (**1**) produces the pool of dispersing seeds known as seed rain (**2**). Seed rain either arrives from local (**2.a**) or non-local (**2.b**) sources. Seeds that survive dispersal as seed rain become incorporated into the soil seed bank (**3**). Seeds remain dormant in the seed bank until conditions are favorable for their germination (**3.a**). Recruitment (**4**) occurs when individuals successfully join the aboveground community.

## Methods

### *Study Sites*

To assess community dynamics during restoration, we conducted floristic, seed rain, and seed bank surveys in a remnant prairie (Tucker Prairie; 38°56’53.6” N, 91°59’40.0” W) and chronosequence of restored prairies (Prairie Fork Conservation Area; 38°58’29.7” N, 91°44’03.3” W) in Callaway County, MO. Tucker Prairie is one of the largest tracts of unplowed claypan tallgrass prairie left in north central Missouri (~59 hectares). Typical for tallgrass prairie, Tucker Prairie is dominated by warm-season grasses including big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), prairie dropseed (*Sporobolus heterolepis*), and yellow prairie grass (*Sorghastrum nutans*). Over 250 species of plants representing 57 families and over 150 genera have been documented at Tucker Prairie (Tropicos 2023). Starting in 2004, nearby Prairie Fork Conservation Area (PFCA) (~18 miles from Tucker Prairie) began converting agricultural land into claypan tallgrass prairie using seeds collected from Tucker Prairie and other local prairies (Newbold et al., 2019). Around 16-25 hectares were seeded annually, creating a mosaic of variously aged prairies (Newbold et al., 2019). As defined by Newbold et al. (2019), we used prairies seeded in 2004, 2013-2014, and 2017 to represent an old, middle-aged, and young restored prairie, respectively. The effort required to capture a holistic assessment of plant community type (i.e., aboveground flora, seed rain, and seed bank) in restored and remnant grasslands limited us to using one site per age class. For additional details on PFCA and Tucker Prairie management, see Newbold et al. (2019), Chapter 2, and Appendix B (Table S3.1).

### *Experimental Setup:*

At each focal restored and remnant prairie, we established ten 5 m-long transects to survey the aboveground flora, seed rain, and seed bank communities. We also placed transects ~50 m apart to ensure we collected independent samples of each community type (i.e., aboveground flora, seed rain, seed bank).

### *Seed rain sampling:*

We captured seed rain using artificial turfgrass traps (0.01 $m^{-2}$) at the transects we established in each restored and remnant prairie (four sites total, ten transects per site). Each transect contained five traps, which we exchanged every two weeks from May 31st to December 12th, 2019. We then combined all traps at each transect for a collection period before quantifying and identifying seeds to the lowest taxonomic level possible. For more information regarding seed rain collection and identification, see Chapter 2.

*Aboveground flora sampling:*

In 2019, we sampled species vegetative cover twice at the same transects used to capture seed rain, once in the early summer (June - July) and again at peak biomass (August - September) to capture species with early and late-season phenology. During the first sampling period, we only recorded species presence in 1 $m^2$ areas surrounding each seed trap. At peak biomass, we returned and sampled the percent aerial cover of all vascular plant species rooted in the same 1 $m^2$ areas. Since most early-season species were resurveyed during peak biomass or rare we considered each species present in the first survey to have 1% cover and added their cover to the late-season cover for analysis. One transect from the remnant prairie was dropped from our analysis due to incomplete data. We identified species according to Yatskievych (1999, 2006, 2013).

*Seed bank sampling:*

In March 2020, before the growing season, we collected soil samples at each focal prairie to quantify the seed rain that successfully transitioned into the seed bank. To compare the seed bank and the previous year's seed rain, we collected 5 (10 x 10 x 10 $cm^3$; 1000 $cm^3$) soil cores approximately 0.5 m away from where we captured seed rain at each transect in 2019. We allowed the soil samples to dry before removing all non-seed plant material (e.g., roots and rhizomes) to ensure seedlings only germinated from seeds. We then homogenized all samples within a transect and subsampled ~1500 $cm^3$ of soil to spread ~1 cm deep over ~ 2 cm sterile potting soil in plastic germination trays. Starting in July 2020, we randomly placed the trays (n = 40) in a greenhouse and watered them when dry. Amongst the 40 trays containing soil samples, we also put an additional ten trays containing only sterile potting soil to assess whether any contamination occurred from external sources. In control trays, we found only 19 seedlings representing 7 species (< 0.5 % of all seedlings), suggesting minimal contamination. We periodically randomized trays to reduce variation caused by tray location. We checked trays daily for germination and removed seedlings from trays once identifiable. After germination ceased in July 2021, we placed all trays into a cold room for ~4 months to replicate conditions necessary to break dormancy for any remaining dormant seeds. Post-vernalization, we returned the trays to the greenhouse, stirred the soil samples, and monitored for additional germination. We ended the study one year post-vernalization.

### *Data Analysis:*

### *Determining whether the seed rain and seed bank act as sources of diversity missing from the aboveground flora*

To determine whether the seed bank and seed rain are acting as sources of desirable diversity missing from the aboveground flora at the site level, we classified species based on their relative abundance in the aboveground flora, seed rain, and seed bank communities in each study prairie using ternary diagrams ("ggtern" package; Hamilton & Ferry, 2018). Ternary diagrams use normalized data to visualize the ratios of three variables, which in our case are species abundance in the aboveground flora, seed rain, and seed bank. For example, data points in the center of the plot indicated species that are equally proportionate in all community types and germinate reliably well from seed, while points at the vertices are species captured in only one community type. Based on these classifications, we identified which species at the site level were consistently found in the seed rain and seed bank and whether they were also present in the aboveground flora.

To determine whether new species were arriving from outside the local flora sampled at transects, we calculated the proportion of new species found in the seed rain but absent from the local aboveground flora at each transect for each prairie age. Similarly, we calculated the proportion of seeds of new species to the aboveground flora supplied by the seed rain at each transect. To determine whether the supply of new species or individuals from non-localized dispersal changed during restoration, we used linear regression models predicting either the proportion of new species or individuals as a function of prairie age. We then used a post hoc analysis using Dunnet-style contrasts ("emmeans" package; Lenth et al., 2023) with an adjustment for three tests to identify whether non-local seed rain differed between restored prairies of various ages and the remnant prairie.  We did a similar analysis to quantify the seed bank's potential contribution of new species and individuals missing from the aboveground flora and the previous year's seed rain.

We also conducted a species indicator analysis to identify species closely associated with each community type (i.e., aboveground flora, seed rain, seed bank) for each prairie. We used the *multipatt()* function found in the 'indicspecies' package to first calculate Indicator Values, which measured species associations with community types and prairie age, and then conducted permutational tests assessing the statistical significance of these associations (De Cáceres & Legendre, 2009). We restricted our analysis to singleton prairie age and community type combinations. Species identified as being strongly affiliated with the remnant aboveground flora and absent from restorations of all ages and community types we defined as potential targets for restoration efforts.

### *Quantifying how vital aspects and relationships of the aboveground flora, seed rain, and seed bank changed during restoration*

#### *Quantity of germinable seeds*

To assess whether there were differences in the number of germinable seeds in seed banks over succession, we used a generalized linear model with a negative binomial distribution to predict the number of seedlings germinated as a function of prairie age ("MASS" package; Venables & Ripley, 2002).  We then used Dunnett-style contrasts ("emmeans" package; Lenth et al. 2023) adjusted for three tests to determine whether a young, middle-aged, and old restored prairie differed in the number of germinable seeds compared to a remnant prairie. The quantity of seeds in the seed rain was quantified in Chapter 2.

#### *Community diversity*

We quantified differences in aboveground flora and seed bank diversity (total richness, native richness, Shannon diversity index) across the time-since-restoration gradient using generalized linear models with either a Poisson distribution to predict species richness or a Normal distribution to predict Shannon diversity index as a function of prairie age for each community. We used Ladd & Thomas (2015) to determine the provenance of each species. We again used Dunnett-style contrasts ("emmeans" package; Lenth et al., 2023) to assess whether restored prairies of various ages differed in their total richness, native richness, and Shannon diversity index compared to a remnant prairie. Differences in the diversity of the seed rain were previously assessed in Chapter 2.

#### *Community Composition*

To quantify compositional differences among community types (aboveground flora, seed rain, and seed bank) during restoration, we first constructed a community distance matrix using

Bray-Curtis dissimilarity and the relativized species abundances (Hellinger transformation) for all community types at each transect by prairie age combination ("vegan" package; Oksanen et al., 2020). Because certain taxa were identifiable to species in some community types but not others (e.g., *Carex sp.*), we had to elevate these taxa to genus level during our analysis. We then quantified statistical differences among prairie ages and community types using permutational multivariate analysis of variance (PERMANOVA) predicting composition as a function of prairie age, community type, and their interaction ("vegan" package; Oksanen et al., 2020). Afterward, we assessed pairwise differences in composition between prairie age, communities, and their interaction with a Bonferroni correction to adjust for multiple comparisons ("pairwiseAdonis" package; Martinez Arbizu, 2020). To reduce the number of pairwise comparisons, we only compared the old restoration to the remnant prairie to assess whether restoration efforts were successful in replicating the aboveground flora, seed rain, and seed bank composition observed at the remnant. We visualized compositional differences using principal coordinates analysis (PCoA) ordination ("ecodist" package; Goslee & Urban, 2007).

### *Community type turnover*

We used Bray-Curtis dissimilarity to assess the turnover between the aboveground flora, seed rain, and seed bank in a time-since-restoration gradient of prairies and a remnant prairie. To quantify compositional changes between community types, we constructed additional community matrices containing relativized species abundances (Hellinger transformation) for each pair of community types (aboveground vs. seed rain, seed rain vs. seed bank, and aboveground vs. seed bank) ("vegan" package; Oksanen et al., 2020). Similar to Eskelinen et al. (2023), we used these matrices to calculate Bray-Curtis dissimilarity for each community type comparison. To determine whether community types became more compositionally similar during restoration, we used linear regression models to predict dissimilarity for each pair of community types as a function of prairie age. We further assessed whether restored prairies significantly differed in their relationships between community types compared to the remnant prairie with post hoc analyses using Dunnet-style contrasts ("emmeans" package; Lenth et al., 2023) and a multiple comparison adjustment for three tests.

### *Data reproducibility and accessibility*

We used R (version 4.3.2) and RStudio (version 2023.06.1+524 "Mountain Hydrangea") to conduct all analyses and create data visualizations (R Core Team, 2022). We also used the "tidyverse" package (Wickham et al., 2019) to manipulate data and create visualizations.

## Results

### *Determining whether the seed rain and seed bank act as sources of diversity missing from the aboveground flora*

In total, we germinated 5,469 seedlings representing 87 morphospecies from soils collected from our sites. At the site level, most species that successfully germinated from seed banks were also found in the aboveground flora (Figure 3.2, Table S3.2, Table S3.3, Table S3.4, Table S3.5). Many of these species were abundant in the cover and seed rain but rare in the seed banks of restored and remnant prairies alike. However, in all sampled prairies, most species found in the aboveground flora and/or seed rain never germinated from the seed bank (49 to 65% lost). Species that tended to be proportionally represented in all three communities were ruderal and undesirable to restoration efforts (e.g., wood sorrel *Oxalis dillenii*).

To determine what community types non-local species arrive from, we explored which new species arrived at the transect level (Figure 3.3). Species missing from the aboveground flora but present in the seed rain (Figure 3.3 A) and seed bank (Figure 3.3 B) were found in similar proportions at transects when comparing the restored prairies to the remnant (Table S3.6). Only when comparing the previous year's seed rain to the seed bank were there differences in the proportion of new species germinating from the seed bank unfound in the seed rain between restored and remnant prairies. In specific, significantly greater proportions of new species absent from the seed rain were germinating from soils collected from the oldest restoration compared to the remnant. Notably, unlike at the site level, a large proportion of species present in old restored (0.57 novel to transect, 0.22 novel to site) and remnant (0.47 novel to transect, 0.10 novel to site) seed banks were novel to the aboveground flora at the transect level. When also considering new species abundance, the proportion of novel seeds of species missing from the aboveground flora and seed rain but germinated from seed banks increased over a time-since-restoration gradient at transects and were comparable to the remnant 5-6+ years after seeding (Figure 3.3 E, F, Table S3.6). The novel proportion of novel seeds of species absent from the flora but captured in the seed rain trended towards increasing with prairie age, where there were fewer novel seeds dispersing in the youngest restoration compared to the remnant (Figure 3.3 D). However, for all prairies, the seed bank (5% to 56% new to flora) acted as a larger source than the seed rain (1.4% to 7.9% new to flora) for providing individuals of new species to the aboveground flora.

While the aboveground flora in restored prairies transitioned towards being associated with desirable native species over time, the seed banks of all prairies harbored species undesirable to restoration efforts (Table S3.7). Many prairie seed bank indicator species included introduced, annual, and/or weedy species (e.g., yellow rocketcress *Barbarea vulgaris*, fall panicgrass *Panicum dichotomiflorum*, and tall waterhemp *Amaranthus tuberculatus*). Although desirable species (e.g., Junegrass *Koeleria macrantha*, prairie gentians *Gentiana sp.*) were indicator species in the seed rain of middle-aged and old restored prairies, we did not find evidence of their concomitant survival in the seed bank. Desirable species affiliated with the aboveground flora of the remnant prairie but missing from restorations of all ages were often diminutive, native, perennial species (e.g., wild strawberry *Fragaria virginiana*, arrowleaf violet *Viola sagittata*, and lance-leaved loosestrife *Lysimachia lanceolata*).

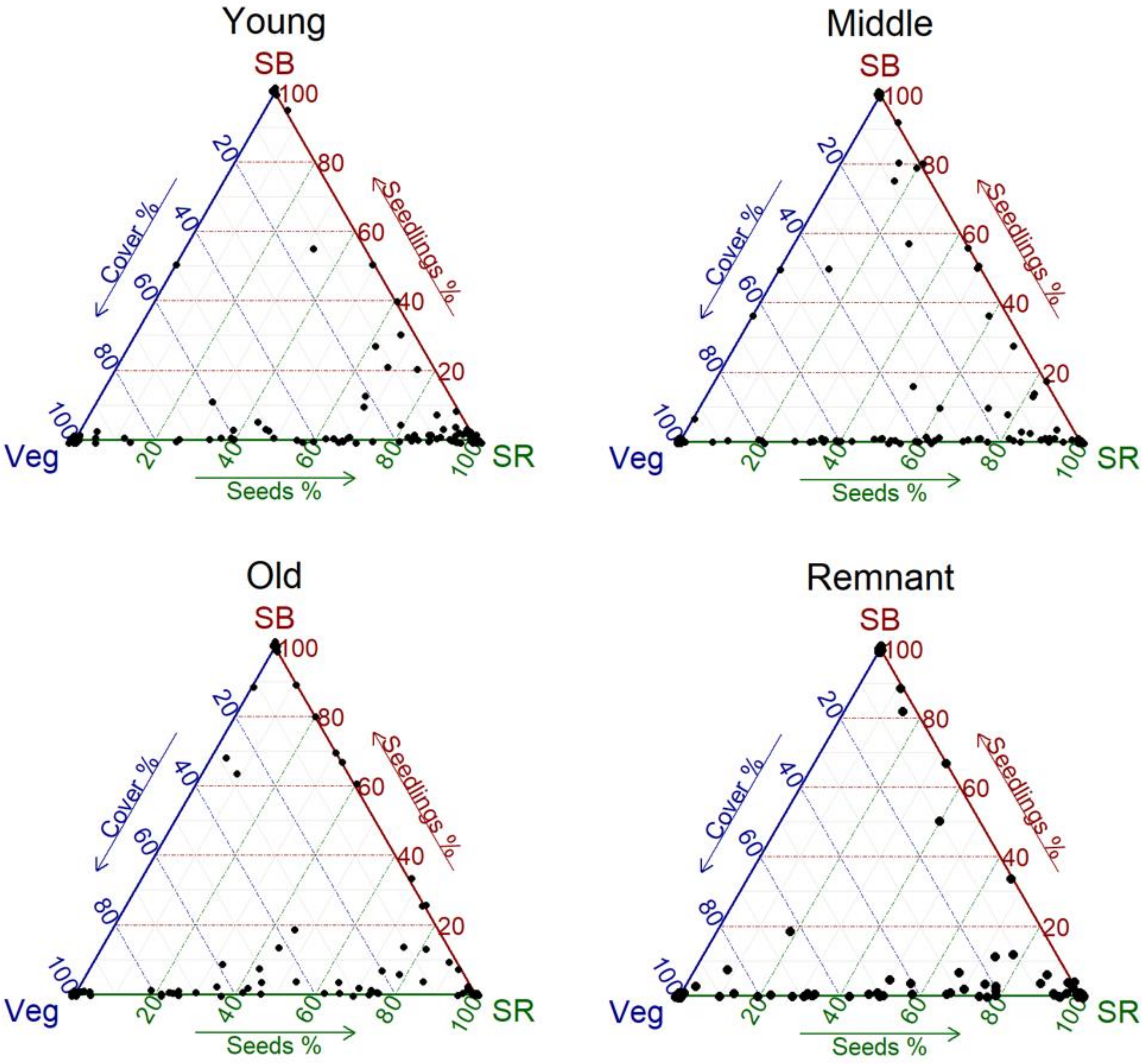

**Figure 3.2.** Ternary plots showing the relative mean percentages that species (black points) are found in the aboveground vegetation (Veg), seed rain (SR), and soil seed bank (SB) across a time-since-restoration gradient of young (seeded in 2017), middle-aged (seeded in 2013-2014), and old (seeded in 2004) restored prairies and a remnant prairie.

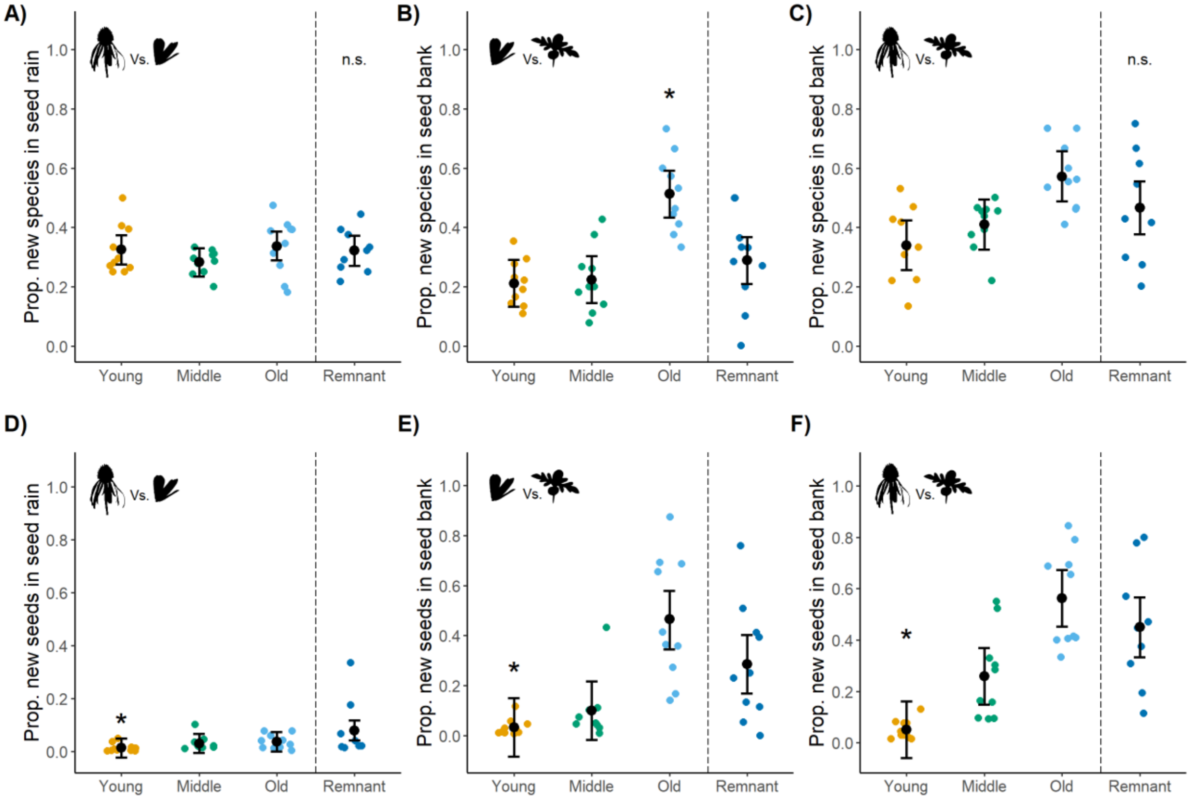

**Figure 3.3.** Proportion of novel propagules and species present in the **A)**, **D)** seed rain absent in the local aboveground cover or present in the soil seed bank absent in the **B)**, **E)** seed rain or **C)**, **F)** aboveground cover in restored (young = seeded 2017, middle = seeded 2013-2014, old = seeded 2004) and remnant prairies. Error bars represent 95% confidence intervals around model estimates. Asterisks indicate prairies with significant differences in immigration compared to the remnant prairie ($p < 0.05$).

### *Quantifying how vital aspects of the aboveground flora, seed rain, and seed bank change during restoration*

#### *Quantity of germinable seeds*

The number of germinable seeds diminished as prairies aged ($X^2_3 = 63.57$, $p < 0.001$), with young ($Z = 6.44$, $p < 0.001$) and middle-aged ($Z = 2.76$, $p < 0.05$) restored prairies having significantly more germinable seeds in their soils compared to the remnant prairie (Figure S3.1). But, fifteen years after restoration efforts began, the old restored prairie had similar amounts of seedlings germinating from the soil seed bank as the remnant ($Z = -0.31$, $p = 0.96$). However, prairies of all ages experienced considerable losses in the number of viable seeds when comparing the density of seeds captured in the seed rain compared to those we germinated from the seed bank (Table 3.1).

**Table 3.1.** Density of seeds in the seed rain and seed bank in a young (seeded in 2017), middle-aged (seeded in 2013-2014), old (seeded in 2004), and remnant prairie. Loss refers to the percent loss in the number of seeds dispersing as seed rain compared to the number of viable seeds germinated from the seed bank. Seed rain data obtained from Chapter 2. Total sampling surface area for the seed rain and seed bank was 0.5 $m^2$ and 0.15 $m^2$ per prairie, respectively.

| | Seeds $m^{-2}$ | Seedlings $m^{-2}$ | Loss (%) |
|---|---|---|---|
| Young | 124,806 | 21,400 | 82.85 |
| Middle | 48,154 | 7,900 | 83.60 |
| Old | 27,752 | 3,427 | 87.65 |
| Remnant | 41,614 | 3733 | 91.03 |

*Community diversity*

The total species richness of the aboveground flora significantly differed over the time-since-restoration gradient ($X^2_3 = 25.43$, $p < 0.001$) (Figure S3.2 A). Aboveground flora native species richness also differed over time, suggesting that increases in total species richness were not solely due to an increased presence of introduced species ($X^2_3 = 18.70$, $p < 0.001$) (Figure S3.2 B). Despite never having a legacy of farming, the remnant prairie did not possess the greatest total or native richness in the aboveground flora (Table S3.8). Instead, species richness peaked in the middle-aged restored prairie. However, by fifteen years, the restored prairie flora was no longer more species rich than the remnant. While Shannon diversity of the aboveground flora varied among the restored prairies ($F_{3,35} = 3.33$, $p < 0.05$), there were no differences in flora diversity when comparing the restored prairies to the remnant (Figure S3.2 C, Table S3.8).

Soil seed banks trended towards possessing varying levels of total species richness over time ($X^2_3$ = 6.48, p = 0.09) (Figure S3.2 D). Again, middle-aged restored prairies had the most diverse germinable seed banks (Table S3.8). In contrast to the aboveground flora, native species richness in seed banks was comparable across the chronosequence of restored prairies and the remnant ($X^2_3$ = 0.32, p = 0.96) (Figure S3.2 E, Table S3.8). Therefore, differences in total species richness were a result of additional introduced species in the soils of the middle-aged restored prairie. Shannon diversity of seeds banks increased over the time-since-restoration gradient, with the youngest restoration having the least diverse pool of germinable seeds compared to the remnant (Figure S3.2 F, Table S3.8). In our previous study, we also found that the total species richness of seed rain decreased in older prairies, but native species richness and Shannon diversity remained constant across the same time-since-restoration gradient (Chapter 2).

### *Community composition*

Prairies of all ages were compositionally distinct (Figure 3.4, Table S3.9). Even though prairies of similar ages shared the most compositional similarities, plant communities in the oldest restoration had yet to converge with those in the remnant prairie. Additionally, plant communities types hosted different assemblages of species. In general, the composition of the aboveground flora and seed rain resembled each other more than the aboveground flora and seed bank. When considering the interaction between prairie age and community type, we found that the old restored prairie had distinct communities of the aboveground flora, seed rain, and germinable seed bank to the remnant.

### *Community Type Turnover*

Dissimilarity in community composition between community types increased during restoration, where the seed rain and seed bank did not reinforce the composition of the aboveground flora as restored prairies matured (*Aboveground vs. Seed rain:* $F_{3,35}$ = 14.29, p <

0.001; *Seed rain vs. Seed bank:* $F_{3,36} = 11.12$, $p < 0.001$; *Aboveground vs. Seed Bank:* $F_{3,35} = 22.99$, $p < 0.001$) (Figure 3.5). Younger restored prairies had seed rain compositions that more closely resembled the aboveground flora compared to the remnant prairie (Figure 3.5 A, Table S3.10). Furthermore, the seed bank was more similar in composition to the seed rain and aboveground community at the young restored prairie than at the remnant prairie (Figure 3.5 B, C, Table S3.10).

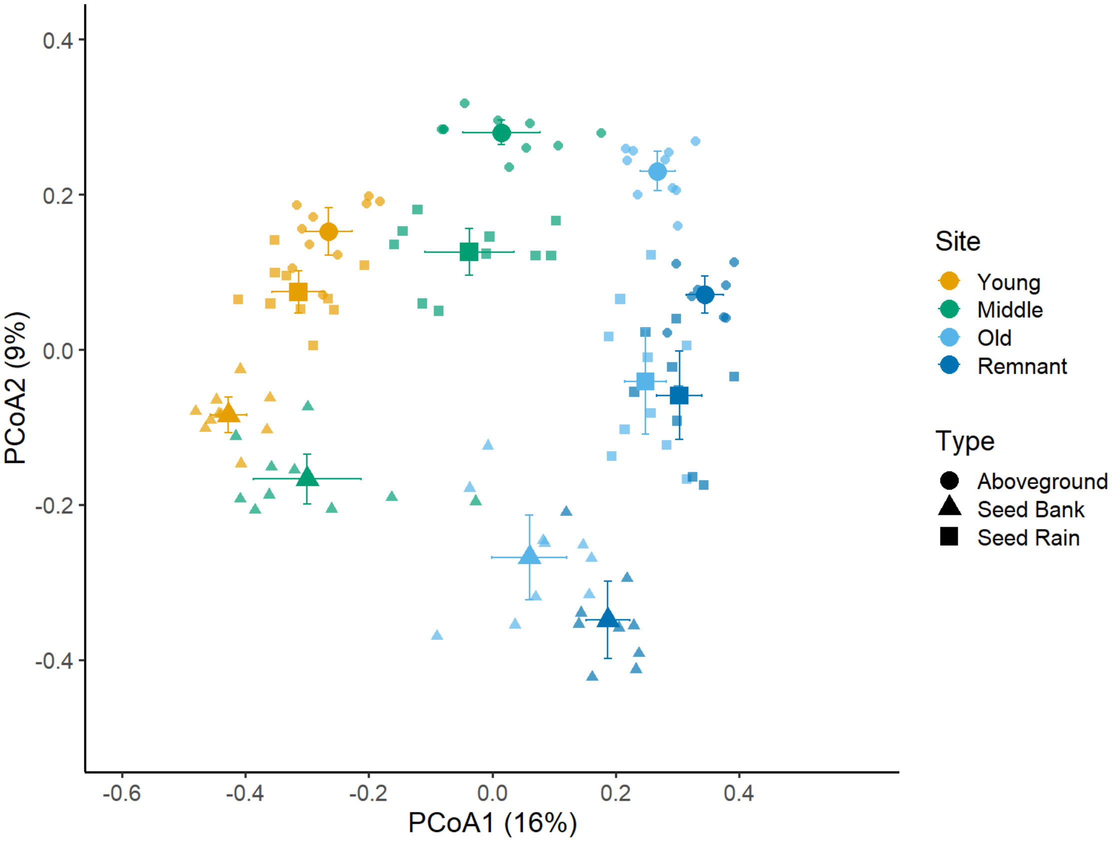

**Figure 3.4.** Principal coordinates analysis ordination using Bray-Curtis dissimilarity visualizing compositional differences in the aboveground, seed rain, and seed bank communities found in a chronosequence of restored prairies (young = seeded 2017, middle = seeded 2013-2014, old = seeded 2004) and a remnant prairie. Large symbols represent community centroids. Error bars represent 95% confidence intervals.

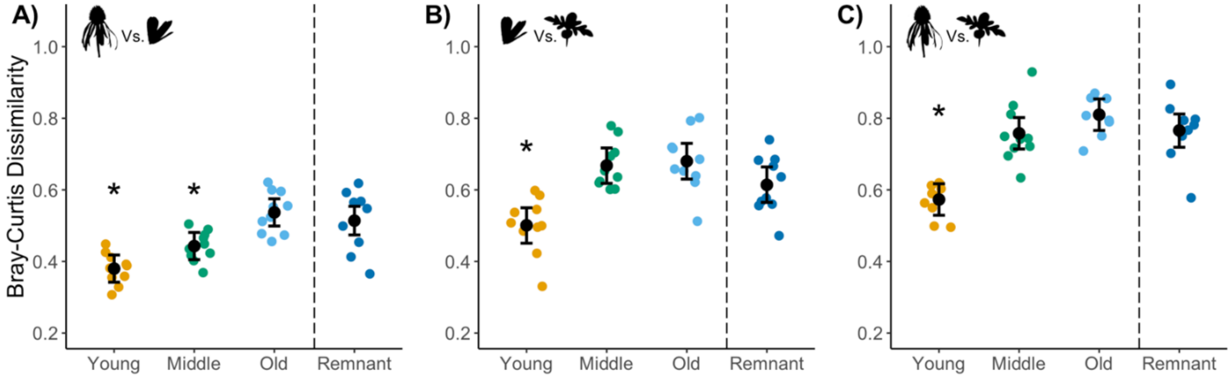

**Figure 3.5.** Turnover of the **A)** aboveground flora and seed rain, **B)** seed rain and germinable seed bank and **C)** aboveground flora and germinable seed bank over a time-since-restoration gradient of restored prairies and a remnant prairie. Error bars represent 95% confidence intervals around model estimates. Asterisks indicate prairies with significant differences in Bray-Curtis dissimilarity compared to the remnant prairie ($p < 0.05$).

## Discussion

Our study provides unique insight into the fates of seeds during restoration across three community types in tallgrass prairies. In general, we discovered ambient seed sources did not act as stores of missing desirable diversity. Although we found that non-local dispersal and the persistent seed bank could supply new species missing from the aboveground flora, these species were often undesirable (i.e., weedy, ruderal, or introduced) to restoration efforts. Old restored and remnant prairie seed banks, in particular, contained a sizable amount of weedy species. We also observed that tallgrass prairies were characterized by high rates of seed mortality, where far fewer seeds germinated from prairie soils compared to the previous years' seed rain. Vital aspects of prairie flora, seed rain, and seed banks also changed during restoration, where the quantity, diversity, but not composition of these communities were comparable to the remnant prairie 15+ years after restoration began. Additionally, the composition of the seed rain and seed bank became increasingly dissimilar to the aboveground flora over time. Overall, we found that ambient seed sources had little potential to rectify compositional differences between remnant and restored prairies. Chronically missing desirable species will likely require further human intervention to establish in restored communities.

Even though the seed rain and seed banks of restored prairies contained species missing from the aboveground flora, these species were frequently undesirable. In general, we found seed banks contained more missing diversity from the flora than the seed rain, especially in old restored and remnant prairies. Seed banks, in particular, had the potential to contribute a large proportion of novel species and individuals to the local flora at small scales (i.e., transect-level). However, this potential diminished at the site level, where most species germinating from prairie soils were present in the flora to some degree at sites. Regardless, the missing diversity we captured in all prairies at both scales contained numerous annual, introduced, and/or weedy species not cited by managers as desirable for restoration (Barak et al., 2022). Aligned with the findings of others, we found that native species missing from our restored prairies but found in our remnant prairie were often understory species with an early-season phenology (e.g., arrowleaf violet, *Viola sagittata*) (Deever et al., 2023; Frischie & Rowe, 2012; Sluis et al., 2018). Additionally, many of the species common to our remnant prairie were not commercially available or readily recommended in prairie seed mixes (e.g., *Galium obtusum, Setaria parviflora, Lysimachia lanceolata, Potentilla simplex, Cirsium altissimum)* (Kaul et al., 2023; W. Sluis, 2020). It is unclear exactly why these species are missing from restorations, but continued seed limitation could be a potential culprit since we found non-local dispersal events were rare. As a result, these species likely have limited ability to disperse in sufficient quantities to recruit into restorations on their own.

Despite restored and remnant prairies producing extraordinary amounts of annual seed input, we germinated only a small fraction of seeds from the seed bank when compared to what was captured the previous year as seed rain. Similar to the seed rain (Chapter 2), we found that the number of germinable seeds decreased as restorations aged and was eventually comparable to the remnant prairie 15+ years after restoration. Differences in germinable seed input across the chronosequence may have reflected communities shifting from being annual to perennial-dominated. Overall, seed mortality was high, ranging from a 83 to 93% reduction in seeds from the seed rain to the seed bank. Other paired seed rain and seed bank studies have also reported high rates of mortality when seeds transition into the soil (Rabinowitz, 1981; Schott & Hamburg, 1997). Seeds face many challenges to surviving dispersal (J. C. Chambers & MacMahon, 1994). For instance, mammals represent a substantial threat to seed survival in prairies (S. E. Johnson & Zettlemoyer, 2022), and their exclusion increases seedling germination from grassland seed banks (Eskelinen et al., 2023). Plants themselves can also act as a physical barrier to dispersing seeds, preventing seed banks from being replenished by seed rain (Brown & Cahill Jr., 2020). Altogether, our results suggest that desirable seeded species absent from restorations may need additional management interventions beyond business-as-usual practices to combat the high rates of seed mortality observed in prairies.

After fifteen years of restoration, the oldest restored prairie had comparable levels of diversity to the remnant prairie across all community types. Although many studies observe differences in native diversity among remnant and restored prairies (Barak et al., 2017; Martin et al., 2005; Sluis et al., 2018), we found that aboveground flora native richness peaked in the middle-aged restored prairie with no discernable differences in seed rain (Chapter 2) or seed bank native species richness between the old restored or remnant prairie (similar to Zylka et al. 2016). Still, we observed declines in native richness between the middle-aged and old restored prairies for the aboveground flora and seed rain (Chapter 2), but not the seed bank. Since many restored prairies lose forb diversity over time, perhaps differences in richness will become more apparent as the old prairie continues to mature (Baer et al., 2002; Hansen & Gibson, 2014). Furthermore, diversity losses in the aboveground flora and seed rain were not being buffered by increased diversity in the soils of the oldest restoration, suggesting that extinction might outweigh establishment events from ambient seed sources. In general, seed banks were depauperate, a trend observed in other prairie seed bank studies (Rabinowitz, 1981; Schott & Hamburg, 1997; Zylka et al., 2016). While the oldest restoration and remnant were similarly diverse, the identities of species inhabiting those prairies differed, as evidenced by considerable compositional differences in all community types across the restoration gradient.

All restored and remnant prairies possessed distinct communities of plants in the aboveground flora, seed rain, and seed bank. Similar to other prairie studies, sharp reductions in diversity when moving from the aboveground flora to the seed rain to the seed bank likely contributed to the large differences in composition among community types (Rabinowitz, 1981; Schott & Hamburg, 1997; Zylka et al., 2016). Restored communities of the same type also moved closer but did not converge with the composition of communities inhabiting the remnant prairie, a result also reported for the aboveground flora by Newbold et al. (2019). However, when looking at the community trajectory of seed sources, the composition of viable seeds was increasingly directed away from ”future” states of aboveground flora. In fact, the composition of seed rain and seed banks became increasingly dissimilar to the aboveground flora over time. Unlike other systems, seed sources in prairies do not seem to reinforce the composition of the aboveground flora or buffer against compositional change (Huanca Nuñez et al., 2021; Kiss et al., 2018; Plue et al., 2021). Instead, prairie seed banks appear to act as stores of early-successional and weedy species displaced from the aboveground community (Rabinowitz, 1981;

Schott & Hamburg, 1997; Zylka et al., 2016). Therefore, vegetative reproduction in late-successional communities is likely more influential in reinforcing the composition of the aboveground vegetation (Benson & Hartnett, 2006). This is not to say that only early-successional species recruited from seed banks or seeds are unimportant at all successional stages. We also observed desirable native perennial species germinating from prairie soils of all ages, just in reduced amounts compared to their relative abundance in the aboveground flora and seed rain.

Restoration seeding densities are not replicating observed tallgrass prairie seed input (Rabinowitz & Rapp, 1980; Rowe, 2010). Prairie plants produce tremendous amounts of seeds in hopes that a few survive. As we and others observed, most seeds and species are filtered out before they can recruit (Rabinowitz, 1981; Schott & Hamburg, 1997). Bolstering seed supply, especially for species we identified as associated with remnant prairies but missing from restorations, should be a high priority for restoration efforts. Multi-year seedings or multiple seedings per year early in restoration may also increase the representation of historically excluded species (Frischie & Rowe, 2012; Sluis et al., 2018). Alternatively, transplanting plugs or rhizomes of species that do not recruit well from seed may increase their representation in restorations (Deever et al., 2023; Sluis, 2020). However, this is more time-intensive, expensive, and laborious than seed additions. Overall, we suggest that restoration practitioners do not rely on ambient seed sources to provide missing diversity and instead seed missing desirable species at increased rates early on during restoration.

## Acknowledgments

We thank Prairie Fork Conservation Area for providing access to their property and Chris Newbold, Jeff Demand, Amber Edwards, and Melody Kroll for their help with site selection and information. We also thank Justin and Dana Thomas for their assistance with plant identification and Matthew Chandler for his help developing voucher specimens for the MSU Herbarium. We are grateful for the assistance Maya Parker-Smith, Erica Murdock, Larissa Kahan, Savana Presson, Kelsey Jaeger, Danielle Gafford, and Christian Perez-Martinez provided us in the field and lab to quantify seed rain. Lastly, Deborah Finke, Elizabeth King, Manuel Leal, Lars Brudvig, and the Sullivan Lab gave invaluable feedback on our manuscript. The Prairie Fork Charitable Endowment Trust and Long-Term Agroecosystem Research (LTAR) network (58-5070-9-016 and 58-5070-2-018) provided funding that supported our research. LTAR is supported by the United States Department of Agriculture. This is KBS contribution # ____.

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# APPENDIX B: SEED RAIN, SEED BANK, AND VEGETATIONAL DYNAMICS OF REMNANT AND RESTORED TALLGRASS PRAIRIES

*Supplemental Figures & Tables*

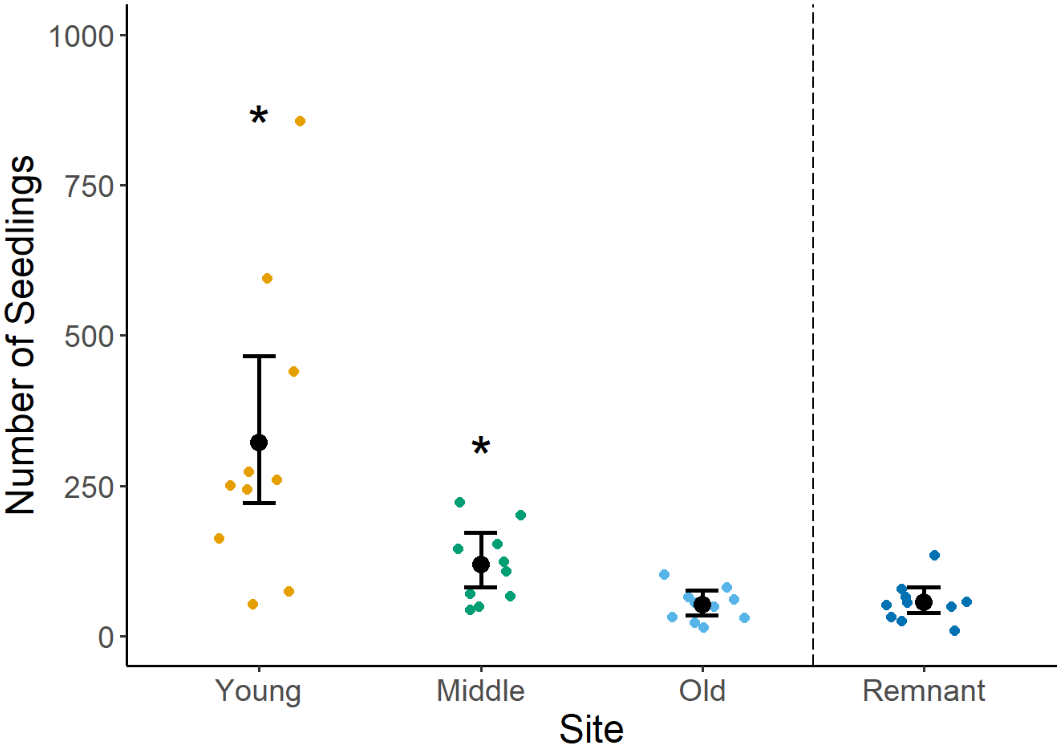

**Figure S3.1.** The number of seedlings germinated from seed banks decreased as prairie restorations (Young = seeded 2017, Middle = seeded 2013-2014, and old = seeded 2004) matured and were comparable to the remnant fifteen years after restoration efforts began. Error bars represent 95% confidence intervals around model estimates. Asterisks indicate restored prairies with significant differences in richness compared to the remnant prairie ($p < 0.05$).

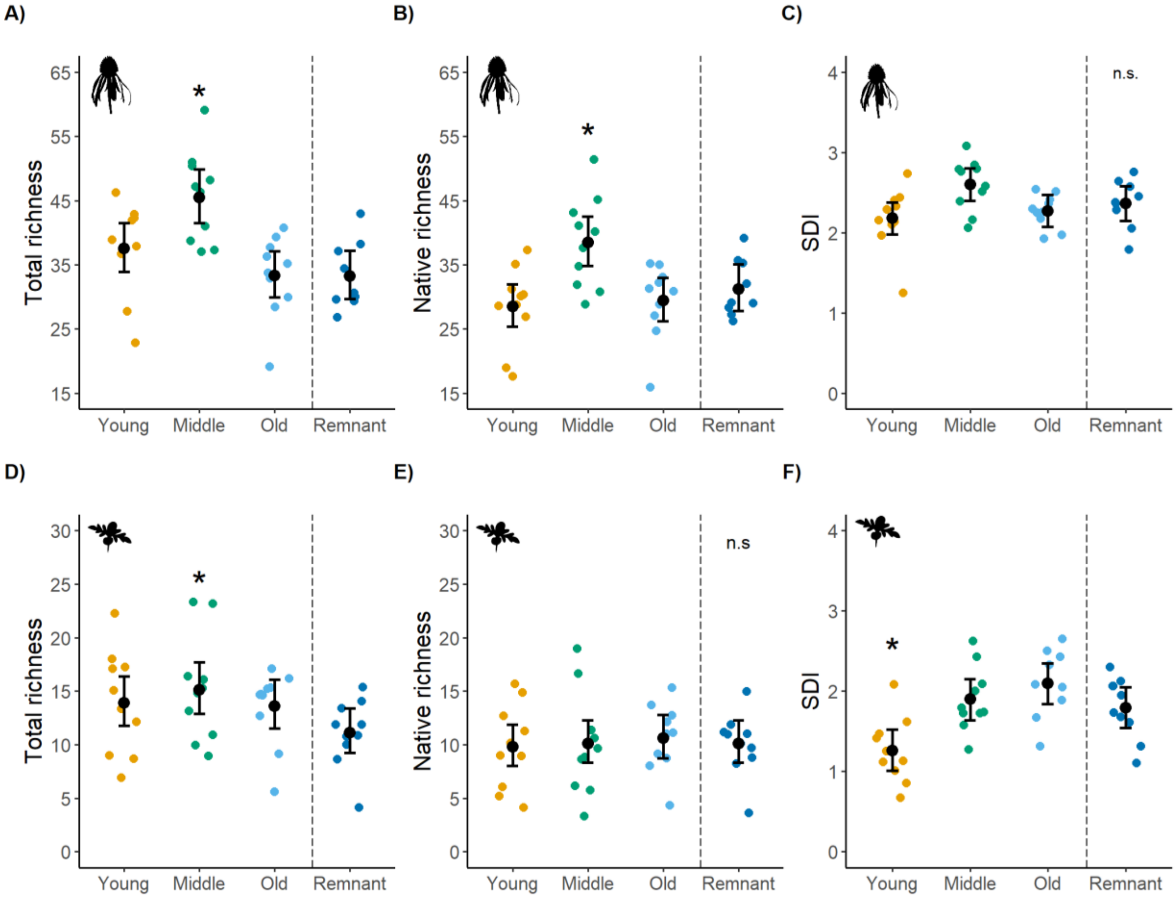

**Figure S3.2.** Total and native morphospecies richness and Shannon diversity index (SDI) of the **A), B), C)** aboveground flora and **C), D), E)** soil seed bank community across a time-since-restoration gradient of young (seeded in 2017), middle-aged (seeded in 2013-2014), and old (seeded in 2004) restored prairies and a remnant prairie. Error bars represent 95% confidence intervals around model estimates. Asterisks indicate prairies with significant differences in richness compared to the remnant prairie ($p < 0.05$).

**Table S3.1.** Year of last prescribed burn at the restored (young = seeded in 2017, middle-aged = seeded in 2013-2014, and old = seeded in 2004) and remnant prairies.

| Site | Prairie Age | Year of last burn |
|---|---|---|
| Tucker Prairie | Remnant | 2018 |
| Prairie Fork Conservation Area | Young | 2018 |
| | Middle | 2020 |
| | Old | 2017 |

**Table S3.2.** Species are categorized based on which community types they were found in at the young restored prairie seeded in 2017 (n =  116 morphospecies).

| **Category** | **Species Code** | **Scientific Name** |
|---|---|---|
| Aboveground flora only (n = 25) | AMOCAN | *Amorpha canescens* |
| | ASCSYR | *Asclepias syriaca* |
| | BAPALB | *Baptisia alba var. macrophylla* |
| | DALPUR | *Dalea purpurea* |
| | DESILL | *Desmanthus illinoensis* |
| | ECHPAL | *Echinacea pallida* |
| | ELYCAN | *Elymus canadensis* |
| | EUPCOR | *Euphorbia corollata* |
| | EUTGYM | *Euthamia gymnospermoides* |
| | HELMOL | *Helianthus mollis* |
| | JUNVIR | *Juniperus virginiana* |
| | LACSER | *Lactuca serriola* |
| | MUHSPP | *Muhlenbergia sp.* |
| | PARINT | *Parthenium integrifolium* |
| | PLAMAJ | *Plantago major* |
| | RANABO | *Ranunculus abortivus* |
| | RUMCRI | *Rumex crispus* |
| | SILLAC | *Silphium laciniatum* |
| | SOLCAR | *Solanum carolinense* |
| | SOLNEM | *Solidago nemoralis* |
| | SOLSPE | *Solidago speciosa* |
| | SYMORB | *Symphoricarpos orbiculatus* |
| | TRICAM | *Trifolium campestre* |
| | TRIREP | *Trifolium repens* |
| | ULMPUM | *Ulmus pumila* |
| Seed rain only (n = 17) | AGASPP | *Agalinis sp.* |
| | ANDGER | *Andropogon gerardii* |
| | BOLAST | *Boltonia asteroides* |
| | CARSPP | *Carex sp.* |
| | CHEALB | *Chenopodium album* |
| | CIRALT | *Cirsium altissimum* |
| | DAUCAR | *Daucus carota* |
| | DICLAN | *Dichanthelium lanuginosum* |
| | EREHIE | *Erechtites hieraciifolius* |
| | ERYYUC | *Eryngium yuccifolium* |
| | FESARU | *Festuca arundinacea* |
| | KOEMAC | *Koeleria macrantha* |
| | LEUVUL | *Leucanthemum vulgare* |
| | PLAOCC | *Plantanus occidentalis* |
| | SCLTRI | *Scleria triglomerata* |
| | TRIFLA | *Tridens flavus* |
| | VULOCT | *Vulpia octoflora* |
| Seed bank only (n = 6) | ABUTHE | *Abutilon thapsus* |
| | CARDSP | *Cardamine sp.* |
| | EUPHUM | *Euphorbia humistrata* |
| | PLAPUS | *Plantago pusilla* |
| | POACHA | *Poa chapmaniana* |
| | POPDEL | *Populus deltoides* |
| Aboveground flora and seed rain (n = 33) | ACAVIR | *Acalypha virginica* |
| | ANAMIN | *Anagallis minima* |
| | BIDARI | *Bidens aristosa* |
| | BROJAP | *Bromus japonicus* |

**Table S3.2 (cont'd)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora and seed rain (n = 33) | CAPBUR | *Capsella bursa-pastoris* |
| | CORLAN | *Coreopsis lanceolata* |
| | CORTRI | *Coreopsis tripteris* |
| | DESSPP | *Desmodium sp.* |
| | ECHCRU | *Echinochloa crus-galli* |
| | ERASPE | *Eragrostis spectabilis* |
| | ERIVIL | *Eriochloa villosa* |
| | FESPAR | *Festuca paradoxa* |
| | GALAPA | *Galium aparine* |
| | HYPPUN | *Hypericum punctatum* |
| | LESCAP | *Lespedeza capitata* |
| | LESVIR | *Lespedeza virginica* |
| | LIAPYC | *Liatris pycnostachya* |
| | MONFIS | *Monarda fistulosa var. fistulosa* |
| | MYOVER | *Myosotis verna* |
| | POAPRA | *Poa pratensis* |
| | PYCPIL | *Pycnanthemum pilosum* |
| | PYCTEN | *Pycnanthemum tenuifolium* |
| | RUDSUB | *Rudbeckia subtomentosa* |
| | SCHSCO | *Schizachyrium scoparium* |
| | SCISPP | *Scirpus sp.* |
| | SILINT | *Silphium integrifolium* |
| | SOLRIG | *Solidago rigida* |
| | SORNUT | *Sorghastrum nutans* |
| | SYMNOV | *Symphyotrichum novae-angliae* |
| | TAROFF | *Taraxacum officinale* |
| | THLARV | *Thlaspi arvense* |
| | TRIPER | *Triodanis perfoliata* |
| | VERSPP | *Vernonia sp.* |
| Aboveground flora and seed bank (n = 1) | AGRHYE | *Agrostis hyemalis* |
| Seed rain and seed bank (n = 5) | AMATUB | *Amaranthus tuberculatus* |
| | MOLVER | *Mollugo verticillata* |
| | PANCAP | *Panicum capillare* |
| | SPHOBT | *Sphenopholis obtusata* |
| | VERHAS | *Verbena hastata* |
| Aboveground flora, seed rain, and seed bank (n = 29) | ACHMIL | *Achillea millefolium* |
| | ALOCAR | *Alopecurus carolinianus* |
| | AMBART | *Ambrosia artemisiifolia* |
| | BARVUL | *Barbarea vulgaris* |
| | CERSPP | *Cerastium sp.* |
| | CHAFAS | *Chamaecrista fasciculata* |
| | CONCAN | *Conyza canadensis* |
| | CYPSPP | *Cyperus sp.* |
| | DIGISC | *Digitaria ischaemum* |
| | ERISPP | *Erigeron sp.* |
| | EUPSPP | *Eupatorium sp.* |
| | GERCAR | *Geranium carolinianum* |
| | JUNSPP | *Juncus sp.* |
| | KUMSPP | *Kummerowia sp.* |
| | LEPVIR | *Lespedeza virginica* |
| | LESCUN | *Lespedeza cuneata* |
| | MEDLUP | *Medicago lupulina* |
| | MELSPP | *Melilotus sp.* |

**Table S3.2 (cont’d)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora, seed rain, and seed bank (n = 29) | OENSPP | *Oenothera sp.* |
| | OXADIL | *Oxalis dillenii* |
| | PENDIG | *Penstemon digitalis* |
| | PLAVIR | *Plantago virginica* |
| | RATPIN | *Ratibida pinnata* |
| | RUDHIR | *Rudbeckia hirta var. pulcherrima* |
| | SETSPP | *Setaria sp.* |
| | SOLALT | *Solidago altissima* |
| | STRLEI | *Strophostyles leiosperma* |
| | SYMSPP | *Symphyotrichum sp.* |
| | VEROSP | *Veronica sp.* |

**Table S3.3.** Species are categorized based on which community types they were found in at the middle-aged restored prairie seeded in 2013-2014 (n = 117 morphospecies).

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora only (n = 25) | BAPALB | *Baptisia alba var. macrophylla* |
| | BAPRA | *Baptisia bracteata* |
| | CIRALT | *Cirsium altissimum* |
| | EUTGYM | *Euthamia gymnospermoides* |
| | GENSPP | *Gentiana sp.* |
| | HELFLE | *Helenium flexuosum* |
| | HELSPP | *Helianthus sp.* |
| | JUNVIR | *Juniperus virginiana* |
| | LACSER | *Lactuca serriola* |
| | LIASPP | *Liatris sp.* |
| | LIASQU | *Liatris squarrosa* |
| | LOBSPI | *Lobelia spicata* |
| | MUHSPP | *Muhlenbergia sp.* |
| | PANVIR | *Panicum virgatum* |
| | PARINT | *Parthenium integrifolium* |
| | RANABO | *Ranunculus abortivus* |
| | SENMAR | *Senna marilandica* |
| | SILLAC | *Silphium laciniatum* |
| | SISPP | *Sisyrinchium sp.* |
| | SOLSPE | *Solidago speciosa* |
| | SPILAC | *Spiranthes lacera* |
| | TRICAM | *Trifolium campestre* |
| | VERHEL | *Verbesina helianthoides* |
| | VERSPP | *Vernonia sp.* |
| | VIOSPP | *Viola sp.* |
| Seed rain only (n = 9) | ANAMIN | *Anagallis minima* |
| | DIAARM | *Dianthus armeria* |
| | DICLAN | *Dichanthelium lanuginosum* |
| | ECHCRU | *Echinochloa crus-galli* |
| | ELESPP | *Eleocharis sp.* |
| | GALAPA | *Galium aparine* |
| | GERCAR | *Geranium carolinianum* |
| | LIAPYC | *Liatris pycnostachya* |
| | LINSUL | *Linum sulcatum* |
| Seed bank only (n = 9) | CARDSP | *Cardamine sp.* |
| | DIPLAC | *Dipsacus laciniatus* |
| | MOLVER | *Mollugo verticillata* |
| | PANDIC | *Panicum dichotomiflorum* |
| | POPDEL | *Populus deltoides* |
| | POROLE | *Portulaca oleracea* |
| | SOLCAR | *Solanum carolinense* |
| | TAROFF | *Taraxacum officinale* |
| | VERTHA | *Verbascum thapsus* |
| Aboveground flora and seed rain (n = 35) | ACAVIR | *Acalypha virginica* |
| | AGASPP | *Agalinis sp.* |
| | AMBART | *Ambrosia artemisiifolia* |
| | ANDGER | *Andropogon gerardii* |
| | BIDARI | *Bidens aristosa* |
| | BLECIL | *Blephilia ciliata* |
| | BOLAST | *Boltonia asteroides* |
| | BROJAP | *Bromus japonicus* |
| | CARSPP | *Carex sp.* |

**Table S3.3 (cont'd)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora and seed rain (n = 35) | CHAFAS | *Chamaecrista fasciculata* |
| | CORPAL | *Coreopsis palmata* |
| | CORTRI | *Coreopsis tripteris* |
| | DESSPP | *Desmodium sp.* |
| | ECHPAL | *Echinacea pallida* |
| | ERYYUC | *Eryngium yuccifolium* |
| | FESARU | *Festuca arundinacea* |
| | FESPAR | *Festuca paradoxa* |
| | HELMOL | *Helianthus mollis* |
| | KOEMAC | *Koeleria macrantha* |
| | KUMSPP | *Kummerowia sp.* |
| | LESCAP | *Lespedeza capitata* |
| | LESCUN | *Lespedeza cuneata* |
| | LESVIR | *Lespedeza virginica* |
| | LYTALA | *Lythrum alatum* |
| | MONFIS | *Monarda fistulosa var. fistulosa* |
| | MYOVER | *Myosotis verna* |
| | RUDSUB | *Rudbeckia subtomentosa* |
| | SCISPP | *Scirpus sp.* |
| | SILINT | *Silphium integrifolium* |
| | SOLNEM | *Solidago nemoralis* |
| | SOLRIG | *Solidago rigida* |
| | SPOCOM | *Sporobolus compositus* |
| | SPOHET | *Sporobolus heterolepis* |
| | SYMNOV | *Symphyotrichum novae-angliae* |
| | TRIFLA | *Tridens flavus* |
| Aboveground flora and seed bank (n = 3) | ACHMIL | *Achillea millefolium* |
| | AGRHYE | *Agrostis hyemalis* |
| | ALOCAR | *Alopecurus carolinianus* |
| Seed rain and seed bank (n = 6) | AMATUB | *Amaranthus tuberculatus* |
| | HORPUS | *Hordeum pusillum* |
| | LEPVIR | *Lepidium virginicum* |
| | PANCAP | *Panicum capillare* |
| | PERLON | *Persicaria longiseta* |
| | THLARV | *Thlaspi arvense* |
| Aboveground flora, seed rain, and seed bank (n = 30) | BARVUL | *Barbarea vulgaris* |
| | CERSPP | *Cerastium sp.* |
| | CONCAN | *Conyza canadensis* |
| | CORLAN | *Coreopsis lanceolata* |
| | CYPSPP | *Cyperus sp.* |
| | DIGISC | *Digitaria ischaemum* |
| | ERASPE | *Eragrostis spectabilis* |
| | ERISPP | *Erigeron sp.* |
| | EUPSPP | *Eupatorium sp.* |
| | HYPPUN | *Hypericum punctatum* |
| | JUNSPP | *Juncus sp.* |
| | MEDLUP | *Medicago lupulina* |
| | MELSPP | *Melilotus sp.* |
| | OENSPP | *Oenothera sp.* |
| | OXADIL | *Oxalis dillenii* |
| | PENDIG | *Penstemon digitalis* |
| | PLAVIR | *Plantago virginica* |
| | POAPRA | *Poa pratensis* |

**Table S3.3 (cont'd)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora, seed rain, and seed bank (n = 30) | PYCPIL | *Pycnanthemum pilosum* |
| | PYCTEN | *Pcynanthemum tenuifolium* |
| | RATPIN | *Ratibida pinnata* |
| | RUDHIR | *Rudbeckia hirta var. pulcherrima* |
| | RUMCRI | *Rumex cripsus* |
| | SCHSCO | *Schizachyrium scoparium* |
| | SETSPP | *Setaria sp.* |
| | SOLALT | *Solidago altissimum* |
| | SORNUT | *Sorghastrum nutans* |
| | SPHOBT | *Sphenopholis obtusata* |
| | SYMPSPP | *Symphyotrichum sp.* |
| | VEROSP | *Veronica sp.* |

**Table S3.4.** Species are categorized based on which community types they were found in at the old prairie restoration seeded in 2004 (n = 100 morphospecies).

| **Category** | **Species Code** | **Scientific Name** |
|---|---|---|
| Aboveground flora only (n = 23) | ACERUB | *Acer rubrum* |
| | AGRGIG | *Agrostis gigantea* |
| | AMBART | *Ambrosia artemisiifolia* |
| | AMBPSI | *Ambrosia psilostachya* |
| | BAPAUS | *Baptisia australis* |
| | BAPBRA | *Baptisia bracteata* |
| | BROJAP | *Bromus japonicus* |
| | CAMRAD | *Campsis radicans* |
| | ECHPAL | *Echinacea pallida* |
| | ELYVIR | *Elymus virginicus* |
| | FRAVIR | *Fragaria virginiana* |
| | HYPPUN | *Hypericum punctatum* |
| | LESPRO | *Lespedeza procumbens* |
| | LIAPYC | *Liatris pycnostachya* |
| | PARINT | *Parthenium integrifolium* |
| | ROSCAR | *Rosa carolina* |
| | RUBSPP | *Rubus sp.* |
| | SILLAC | *Silphium laciniatum* |
| | SOLRIG | *Solidago rigida* |
| | SPILAC | *Spiranthes lacera* |
| | SPOHET | *Sporobolus heterolepis* |
| | ULMPUM | *Ulmus pumila* |
| | ZIZAUR | *Zizia aurea* |
| Seed rain only (n = 6) | BOLAST | *Boltonia asteroides* |
| | CAPBUR | *Capsella bursa-pastoris* |
| | ECHCRU | *Echinochloa crus-galli* |
| | FESPAR | *Festuca paradoxa* |
| | SOLNEM | *Solidago nemoralis* |
| | SPOCOM | *Sporobolus compositus* |
| Seed bank only (n = 12) | AMATUB | *Amaranthus tuberculatus* |
| | BARVUL | *Barbarea vulgaris* |
| | CARDSP | *Cardamine sp.* |
| | DIGISC | *Digitaria ischaemum* |
| | DIPLAC | *Dipsacus laciniatus* |
| | HORPUS | *Hordeum pusillum* |
| | PANDIC | *Panicum dichotomiflorum* |
| | POACHA | *Poa chapmaniana* |
| | POPDEL | *Populus deltoides* |
| | TAROFF | *Taraxacum officinale* |
| | THLARV | *Thlaspi arvense* |
| | VERHAS | *Verbena hastata* |
| Aboveground flora and seed rain (n = 20) | AMOCAN | *Amorpha canescens* |
| | ANAMIN | *Anagallis minima* |
| | BAPALB | *Baptisia alba var. macrophylla* |
| | CHAFAS | *Chamaecrista fasciculata* |
| | CORTRI | *Coreopsis tripteris* |
| | ERYYUC | *Eryngium yuccifolium* |
| | GENSPP | *Gentiana sp.* |
| | HELHEL | *Heliopsis helianthoides* |
| | HELMOL | *Helianthus mollis* |
| | KUMSPP | *Kummerowia sp.* |
| | LESVIR | *Lespedeza virginica* |

**Table S3.4 (cont'd)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora and seed rain (n = 20) | MONFIS | *Monarda fistulosa var. fistulosa* |
| | MYOVER | *Myosotis verna* |
| | PYCTEN | *Pycnanthemum tenuifolium* |
| | RUDSUB | *Rudbeckia subtomentosa* |
| | SALAZU | *Salvia azurea var. grandiflora* |
| | SILINT | *Silphium integrifolium* |
| | STRLEI | *Strophostyles leiosperma* |
| | VERSPP | *Vernonia sp.* |
| | VULOCT | *Vulpia octoflora* |
| Aboveground flora and seed bank (n = 1) | LEPDEN | *Lepidium densiflorum* |
| Seed rain and seed bank (n = 10) | ALOCAR | *Alopecurus carolinianus* |
| | CONCAN | *Conyza canadensis* |
| | EUPSPP | *Eupatorium sp.* |
| | IPOHED | *Ipomoea hederacea* |
| | LEPVIR | *Lepidium virginicum* |
| | MEDLUP | *Medicago lupulina* |
| | MOLVER | *Mollugo verticillata* |
| | PLAVIR | *Plantago virginica* |
| | SETSPP | *Setaria sp.* |
| | TRIFLA | *Tridens flavus* |
| Aboveground, seed rain, and seed bank (n = 28) | ACAVIR | *Acalypha virginica* |
| | ACHMIL | *Achillea millefolium* |
| | AGRHYE | *Agrostis hyemalis* |
| | ANDGER | *Andropogon gerardii* |
| | CARSPP | *Carex sp.* |
| | CERSPP | *Cerastium sp.* |
| | CYPSPP | *Cyperus sp.* |
| | DESSPP | *Desmodium sp.* |
| | ERASPE | *Eragrostis spectabilis* |
| | ERISPP | *Erigeron sp.* |
| | JUNSPP | *Juncus sp.* |
| | LESCAP | *Lespedeza capitata* |
| | LESCUN | *Lespedeza cuneata* |
| | MELSPP | *Melilotus sp.* |
| | OENSPP | *Oenothera sp.* |
| | OXADIL | *Oxalis dillenii* |
| | PENDIG | *Penstemon digitalis* |
| | POAPRA | *Poa pratensis* |
| | PYCPIL | *Pcynanthemum pilosum* |
| | RATPIN | *Ratibida pinnata* |
| | RUDHIR | *Rudbeckia hirta var. pulcherrima* |
| | SCHSCO | *Schizachyrium scoparium* |
| | SOLALT | *Solidago altissima* |
| | SORNUT | *Sorghastrum nutans* |
| | SPHOBT | *Sphenopholis obtusata* |
| | SYMSPP | *Symphyotrichum sp.* |
| | TRAOHI | *Tradescantia ohiensis* |
| | VEROSP | *Veronica sp.* |

**Table S3.5.** Species are categorized based on which community types they were found in at the remnant prairie (n = 96 morphospecies).

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora only (n = 24) | AGRGIG | *Agrostis gigantea* |
| | ANTNEG | *Antennaria neglecta* |
| | BAPALB | *Baptisia alba var. macrophylla* |
| | BAPBRA | *Baptisia bracteata* |
| | COMUMB | *Comandra umbellata* |
| | FRAVIR | *Fragaria virginiana* |
| | GENSPP | *Gentiana sp.* |
| | LACSER | *Lactuca serriola* |
| | LESVIR | *Lespedeza virginica* |
| | MEDLUP | *Medicago lupulina* |
| | MUHGLA | *Muhlenbergia glabrifloris* |
| | POTSIM | *Potentilla simplex* |
| | RHUCOP | *Rhus copallinum* |
| | RHUGLA | *Rhus glabra* |
| | ROSCAR | *Rosa carolina* |
| | ROSSPP | *Rosa sp.* |
| | SCLTRI | *Scleria triglomerata* |
| | SOLCAR | *Solanum carolinense* |
| | SOLMIS | *Solidago missouriensis* |
| | STRLEI | *Strophostyles leiosperma* |
| | SYMORB | *Symphoricarpus orbiculatus* |
| | TRICAM | *Trifolium campestre* |
| | TRISPP | *Trifolium sp.* |
| | ULMSPP | *Ulmus sp.* |
| Seed rain only (n = 14) | AMOCAN | *Amorpha canescens* |
| | BARVUL | *Barbarea vulgaris* |
| | CERSPP | *Cerastium sp.* |
| | CHEALB | *Chenopodium album* |
| | CORTRI | *Coreopsis tripteris* |
| | ERASPE | *Eragrostis spectabilis* |
| | LESCUN | *Lespedeza cuneata* |
| | MELSPP | *Melilotus sp.* |
| | MONFIS | *Monarda fistulosa var. fistulosa* |
| | PENDIG | *Penstemon digitalis* |
| | RATPIN | *Ratibida pinnata* |
| | RUDSUB | *Rudbeckia subtomentosa* |
| | SCISPP | *Scirpus sp.* |
| | TRIFLA | *Tridens flavus* |
| Seed bank only (n = 6) | DIGSAN | *Digitaria sanguinalis* |
| | KUMSPP | *Kummerowia sp.* |
| | PLAOCC | *Plantanus occidentalis* |
| | POPDEL | *Populus deltoides* |
| | TOXRAD | *Toxicodendron radicans* |
| | TRIREP | *Trifolium repens* |
| Aboveground flora and seed rain (n = 23) | ACAVIR | *Acalypha virginica* |
| | AGASPP | *Agalinis sp.* |
| | BIDARI | *Bidens aristosa* |
| | CARSPP | *Carex sp.* |
| | ELESPP | *Eleocharis sp.* |
| | ERYYUC | *Eryngium yuccifolium* |
| | EUPCOR | *Euphorbia corollata* |
| | FESPAR | *Festuca paradoxa* |

**Table S3.5 (cont'd)**

| Category | Species Code | Scientific Name |
|---|---|---|
| Aboveground flora and seed rain (n = 23) | GALOBT | *Galium obtusum* |
| | HELMOL | *Helianthus mollis* |
| | HYPPUN | *Hypericum punctatum* |
| | LESCAP | *Lespedeza capitata* |
| | LINSUL | *Linum sulcatum* |
| | LOBSPI | *Lobelia spicata* |
| | MYOVER | *Myosotis verna* |
| | POLSPP | *Polygala sp.* |
| | RUBSPP | *Rubus sp.* |
| | SETPAR | *Setaria parviflora* |
| | SPOHET | *Sporobolus heterolepis* |
| | TRIPER | *Triodanis perfoliata* |
| | VERSPP | *Vernonia sp.* |
| | VIOSAG | *Viola sagittata* |
| | VULOCT | *Vulpia octoflora* |
| Aboveground flora and seed bank (n = 0) | None | |
| Seed rain and seed bank (n = 4) | CARDSP | *Cardamine sp.* |
| | DIGISC | *Digitaria ischaemum* |
| | ERISPP | *Erigeron sp.* |
| | VERHAS | *Verbena hastata* |
| Aboveground flora, seed rain, and seed bank (n = 25) | ACHMIL | *Achillea millefolium* |
| | AGRHYE | *Agrostis hyemalis* |
| | AMBART | *Ambrosia artemisiifolia* |
| | ANDGER | *Andropogon gerardii* |
| | CHAFAS | *Chamaecrista fasciculata* |
| | CIRALT | *Cirsium altissimum* |
| | CONCAN | *Conyza canadensis* |
| | CROSAG | *Crotalaria sagittalis* |
| | DESSPP | *Desmodium sp.* |
| | DICLAN | *Dichanthelium lanuginosum* |
| | EUPSPP | *Eupatorium sp.* |
| | EUTGYM | *Euthamia gymnospermoides* |
| | JUNSPP | *Juncus sp.* |
| | LYSLAN | *Lysimachia lanceolata* |
| | OENSPP | *Oenothera sp.* |
| | OXADIL | *Oxalis dillenii* |
| | PLAVIR | *Plantago virginica* |
| | POAPRA | *Poa pratensis* |
| | PYCTEN | *Pycnanthemum tenuifolium* |
| | RUDHIR | *Rudbeckia hirta var. pulcherrima* |
| | SCHSCO | *Schizachyrium scoparium* |
| | SOLALT | *Solidago altissima* |
| | SORNUT | *Sorghastrum nutans* |
| | SPHOBT | *Sphenopholis obtusata* |
| | SYMSPP | *Symphyotrichum sp.* |

**Table S3.6.** Summary results for ANOVA and pairwise contrast tests comparing the proportion of new seeds and species between paired community types over a time-since-restoration gradient of restored and remnant prairies. Bolded values indicate significance at the α 0.05 level.

| **Proportion of new** | **Community type comparison** | **Df** | **F (P)** | **Young – Remnant** | **Middle – Remnant** | **Old - Remnant** |
|---|---|---|---|---|---|---|
| Seeds | Aboveground vs. Seed rain | 3, 35 | 2.29 (0.09) | **-2.539 (< 0.05)** | -1.884 (0.17) | -1.647 (0.26) |
| | Seed rain vs. Seed bank | 3, 36 | **11.28 (< 0.001)** | **-3.091 (< 0.05)** | -2.281 (0.075) | 2.172 (0.10) |
| | Aboveground vs. Seed bank | 3, 35 | **16.73 (< 0.001)** | **-5.022 (< 0.001)** | -2.404 (0.06) | 1.420 (0.37) |
| Species | Aboveground vs. Seed rain | 3, 35 | 0.96 (0.42) | -1.11 (0.55) | 0.450 (0.92) | 0.092 (1.00) |
| | Seed rain vs. Seed bank | 3, 36 | **12.89 (< 0.001)** | -1.393 (0.38) | -1.172 (0.51) | **4.07 (< 0.001)** |
| | Aboveground vs. Seed bank | 3, 35 | **5.62 (< 0.01)** | -2.099 (0.11) | -0.938 (0.66) | 1.765 (0.21) |

**Table S3.7.** Indicator species for each community type (aboveground flora, seed rain, and seed bank) and site combination (young = seeded 2017, middle = seeded 2013-2014, old = seeded 2004, remnant = intact prairie). We only reported species with a test statistic > 0.5 and p-value < 0.01.

| | Aboveground | Seed Rain | Seed Bank |
|---|---|---|---|
| Young | *Kummerowia sp.*<br>*Coreopsis tripteris*<br>*Ranunculus abortivus*<br>*Ambrosia artemisiifolia*<br>*Strophostyles leiosperma*<br>*Chamaecrista fasciculata*<br>*Acalypha virginica* | *Eupatorium sp.*<br>*Myosotis verna*<br>*Erigeron sp.* | *Digitaria ischaemum*<br>*Setaria sp.* |
| Middle | *Solidago rigida*<br>*Symphyotrichum novae-angliae*<br>*Ratibida pinnata*<br>*Monarda fistulosa var. fistulosa*<br>*Verbesina helianthoides*<br>*Pycnanthemum pilosum*<br>*Rudbeckia subtomentosa*<br>*Bromus japonicus*<br>*Bidens aristosa* | *Sphenopholis obtusata*<br>*Boltonia asteroides*<br>*Koeleria macrantha*<br>*Penstemon digitalis* | *Barbarea vulgaris*<br>*Oxalis dillenii* |
| Old | *Baptisia australis*<br>*Salvia azurea*<br>*Silphium integrifolium*<br>*Parthenium integrifolium*<br>*Silphium laciniatum*<br>*Elymus virginicus*<br>*Eryngium yuccifolium*<br>*Sporobolus heterolepis*<br>*Heliopsis helianthoides* | *Tradescantia ohiensis*<br>*Lepidium virginica*<br>*Juncus sp.*<br>*Gentiana sp.* | *Panicum dichotomiflorum*<br>*Amaranthus tuberculatus*<br>*Conyza canadensis*<br>*Cardamine sp.*<br>*Ipomoea hederacea* |
| Remnant | *Rosa carolina*<br>*Potentilla simplex*<br>*Fragaria virginiana*<br>*Lysimachia lanceolata*<br>*Vernonia sp.*<br>*Galium obtusum*<br>*Setaria parviflora*<br>*Euthamia gymnospermoides*<br>*Viola sagittata*<br>*Helianthus mollis*<br>*Desmodium sp.*<br>*Cirsium altissimum*<br>*Androgon gerardii*<br>*Euphorbia corollata*<br>*Rubus sp.*<br>*Schizachyrium scoparium*<br>*Dichanthelium lanuginosum* | *Pycnanthemum tenuifolium* | *Agrostis hyemalis* |

**Table S3.8**. Summary results for pairwise contrast tests comparing total and native species richness (Z, (P)) and Shannon Diversity Index (SDI) (T, (P)) across a time-since-restoration gradient of restored and remnant prairies. Bolded values indicate significance at the α 0.05 level.

| Community type | Response | Young – Remnant | Middle – Remnant | Old - Remnant |
|---|---|---|---|---|
| Aboveground flora | Total | 1.562 (0.28) | **4.224 (< 0.001)** | 0.029 (1.0) |
| | Native | -1.085 (0.56) | **2.670 (< 0.05)** | -0.721 (0.78) |
| | SDI | -1.292 (0.44) | 1.629 (0.27) | -0.663 (0.82) |
| Seed bank | Total | 1.767 (0.19) | **2.461 (< 0.05)** | 1.588 (0.27) |
| | Native | -0.213 (0.98) | 0.00 (1.00) | 0.347 (0.95) |
| | SDI | **-2.995 (< 0.05)** | 0.582 (0.86) | 1.716 (0.23) |

**Table S3.9**. Summary results for permutational multivariate analysis of variance and post hoc pairwise comparison tests predicting community composition as a function of prairie age, community type (aboveground, seed rain, seed bank), and their interaction. Multiple comparisons were adjusted using a Bonferroni correction. Bolded values indicate significance at the α 0.05 level.

| | Source | Df | SS | $R^2$ | F (P) |
|---|---|---|---|---|---|
| | Site | 3 | 10.84 | 0.31 | **26.56 (< 0.001)** |
| | Type | 2 | 5.65 | 0.16 | **20.76 (< 0.001)** |
| | Site:Type | 6 | 3.78 | 0.11 | **4.63 (< 0.001)** |
| | Residual | 105 | 14.30 | 0.41 | - |
| *Pairwise comparison* | | | | | |
| Old – Remnant | Site | 1 | 2.11 | 0.14 | **13.56 (< 0.001)** |
| | Type | 2 | 4.05 | 0.27 | **13.03 (< 0.001)** |
| | Site:Type | 2 | 1.20 | 0.08 | **3.85 (< 0.001)** |
| | Residual | 51 | 7.93 | 0.52 | |

**Table S3.10.** Summary results for pairwise contrast tests comparing Bray-Curtis dissimilarity (T, (P)) between paired community types over a successional gradient of restored and remnant prairies. Bolded values indicate significance at the α 0.05 level.

| Community type comparison | Young – Remnant | Middle – Remnant | Old - Remnant |
|---|---|---|---|
| Aboveground vs. Seed rain | **-4.925 (< 0.001)** | **-2.598 (< 0.05)** | 0.849 (0.71) |
| Seed rain vs. Seed bank | **-3.281 (< 0.01)** | 1.535 (0.31) | 1.887 (0.17) |
| Aboveground vs. Seed bank | **-6.074 (< 0.001)** | -0.249 (0.98) | 1.394 (0.38) |

# CHAPTER FOUR:

## Priority effects based on dispersal phenology alter plant community assembly

### Abstract

Variations in the timing and order of species arrival can result in priority effects, which influence community assembly outcomes. However, priority effects have primarily been tested by manipulating functional guilds that do not always vary in their arrival, making it difficult to predict how priority effects operate during assembly in natural systems. Whether traits that promote natural variation in arrival, such as dispersal phenology, result in alternative assembly outcomes is unclear. To test how arrival differences based on dispersal phenology interact with timing (i.e., length in time between species arrival) and order (i.e., sequence of species arrival) effects, we initiated an experiment varying the arrival of 36 tallgrass prairie species categorized with either summer or fall-dispersing phenology via seed additions. Altering arrival based on dispersal phenology resulted in diversity, cover, and composition differences. Longer time intervals between seedings resulted in stronger effects that benefited summer more than fall-dispersing species. We also observed asymmetric priority effects, where seeding order mattered, aiding subordinate summer more than dominant fall species. Thus, traits that allow subordinate species to arrive long before dominant species may help to promote coexistence. More broadly, traits related to plant species' arrival can result in historical contingencies with potential application for ecological restoration.

### Introduction

Priority effects, or when variations in the timing and order of species arrival impact community assembly, have been increasingly recognized as a key mechanism for influencing the structure and function of animal (Alford & Wilbur, 1985; Chase, 2003; Drake, 1991), plant

(Temperton et al., 2016; Weidlich et al., 2021), and microbial (Drake, 1991; Grainger et al., 2019; Vannette & Fukami, 2014) communities (Drake, 1991). For priority effects to occur, early-arriving species must either facilitate or inhibit the establishment, growth, and reproduction of late-arriving species (Delory et al., 2019; Weidlich et al., 2021). Arriving early allows species to garner biomass, increase their population size, capture limiting resources, and modify their environment before late-arriving species enter the community, altering competitive and coexistence outcomes (Delory et al., 2021; Fukami, 2015; Grainger et al., 2019; Vannette & Fukami, 2014; Werner et al., 2016; Wilsey, 2021; Zou & Rudolf, 2023). Significant long-term consequences of priority effects arise when variations in assembly history produce multiple compositionally dissimilar communities even when species pools and environmental conditions are shared (i.e., alternative states) (Chase, 2003; Drake, 1991; Fukami, 2015; L. M. Martin & Wilsey, 2012, 2014; Temperton et al., 2016). Therefore, the timing and order of species arrival from the regional species pool can cause considerable, possibly long-term, effects on composition among communities.

Although the timing and order of species arrival can each have different contributions to priority effects (Von Gillhaussen et al., 2014), experiments often conflate the two. Timing relates to the interval between species introductions. While short intervals (i.e., days to weeks) between arrival can result in priority effects (Blackford et al., 2020; Grman & Suding, 2010; Körner et al., 2008), generally longer intervals tend to produce stronger, more persistent priority effects by allowing early-arriving species additional time to grow and modify their environment (Von Gillhaussen et al., 2014; T. P. Young et al., 2017). On the other hand, order relates to the sequential pattern in which species arrive. Experimental studies manipulating the arrival order of broad functional groups tend to show competitive outcomes are influenced by the identity of early arrivers (Delory et al., 2019; Durbecq et al., 2023; Grman & Suding, 2010; Körner et al., 2008; Temperton et al., 2016; Weidlich et al., 2017, 2018; Werner et al., 2016; Wohlwend et al., 2019; T. P. Young et al., 2017). For example, less competitively dominant species can be maintained in a system if they arrive first (Durbecq et al., 2023; Werner et al., 2016; T. P. Young et al., 2017). Arrival order often results in asymmetric priority effects, where the impact of having an early arriver advantage is not equivalent between functional groups when compared to arriving simultaneously with a competing guild (Werner et al., 2016; T. P. Young et al., 2017). For instance, sowing grasses first can result in inhibitory priority effects, while adding legumes first generally leads to facilitative effects compared to simultaneous seeding (Delory et al., 2019; Von Gillhaussen et al., 2014; Weidlich et al., 2017, 2018; Wilsey, 2021). Timing and order effects have largely been demonstrated by manipulating the arrival of plant functional groups (grasses vs. legumes vs. forbs) or provenance (native vs. introduced) (Weidlich et al., 2021). However, entire plant functional groups do not disperse simultaneously or before one another without human assistance. Testing timing and order effects based on species traits relevant to arrival would provide novel insight into how priority effects alter community assembly.

Species traits that promote variation in timing and order of species arrival have clear connections to historical contingency since differences in arrival are necessary to induce priority effects (Chase, 2003; Fukami, 2015; Zou & Rudolf, 2023). For example, dispersal phenology, or the seasonal activity of organismal movement, is a trait explicitly linked to species arrival and can create priority effects depending on when and in what order species are introduced (Rasmussen et al., 2014; Rudolf, 2018; Zou & Rudolf, 2023). Many plant communities exhibit seasonal timing and order patterns in dispersal activity, where early-season species disperse before late-season species in predictable ways (Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997; Chapter 2). Within seasons, species have small-scale differences in dispersal timing that may also influence competitive interactions (Blackford et al., 2020; Rabinowitz & Rapp, 1980; Chapter 2). Temporal partitioning of dispersal phenology may allow for coexistence through priority effects, especially when subordinate species disperse before dominant ones (Durbecq et al., 2023; Eddy & Van Auken, 2019; Myers & Harms, 2009; Schofield et al., 2018). Despite being relevant to species arrival, the impact of dispersal phenology on community assembly remains untested in plant communities.

Ecosystems undergoing ecological restoration, such as tallgrass prairies, are ideal systems for testing priority effects since restoration practitioners frequently make decisions about the timing and order of species arrival  (Catano et al., 2023; Weidlich et al., 2021; Zirbel & Brudvig, 2020). Typically, practitioners add their desired species pool in a single simultaneous seeding event that eliminates opportunities for priority effects to support diversity (Rowe, 2010). However, restored prairies seeded in this manner are commonly missing early-season species, suggesting dispersal phenology is a relevant trait to these communities (Frischie & Rowe, 2012; Sluis et al., 2018). Seeding early-season species first with longer periods of time between arrival could allow this guild to grow large enough to shift competitive interactions, resulting in co-occurrence with late-season species  (Durbecq et al., 2023; Rasmussen et al., 2014; Wilsey, 2021). Therefore, manipulating arrival based on dispersal phenology to favor early-season species could increase their representation in restored grasslands while simultaneously testing how timing and order effects influence community assembly.

In 2021, we initiated a multi-year field experiment to determine whether manipulating assembly history, timing, and order based on dispersal phenological guild (early-season/summer-dispersing vs. late-season/fall-dispersing) resulted in persistent priority effects in restored tallgrass prairie plant communities. To test the role of assembly history (1), we manipulated whether species were introduced with variation in their arrival or simultaneously (no timing or order). We expected that if assembly history mattered, varying arrival would produce plant communities differing in diversity, seeded species cover, and/or species composition despite having a shared species pool and environment.  In addition to testing assembly history effects, we also varied the (2) timing interval between species arrival (short-term vs long-term) in the same phenological guild order and (3) order of dispersal phenological guild arrival using the same timing interval between additions. We predicted that longer intervals between arrival would produce stronger and more persistent priority effects by providing early arrivers more time to establish and grow before late arrivers dispersed into the community. Lastly, we expected priority effects to be asymmetric, where providing a temporal advantage to early-season species would increase their presence and cover more than late-season species since late-season species are considered strong competitors in temperate grasslands (A. T. Clark et al., 2018).

## Methods

Our experiment utilized a former agricultural field (~1 acre) at the University of Missouri’s Bradford Research Center in Columbia, MO (38.893604, -92.201154, Boone County, MO). Soils at our site were similar to those in tallgrass claypan prairies, which are characterized by an upper layer of silt loam followed by a hard claypan underneath. Before our experiment, the field we used grew herbicide-resistant soybeans for at least three years, reflecting conditions similar to most prairie restorations before seeding (Newbold et al., 2019; Rowe, 2010). In March 2021, we tilled our study site and hand-removed rhizome clumps to create a smooth surface before our first seeding.

We used seed additions to manipulate the arrival of 36 native tallgrass prairie plant species (Table S4.1). Since prairies experience two peaks in dispersal activity (Rabinowitz & Rapp, 1980; Schott & Hamburg, 1997; Chapter 2), we classified species into two dispersal guilds: summer-dispersing species (first peak in dispersal activity before September 1st, hereafter referred to as summer species) and fall-dispersing species (hereafter referred to as fall species). For our study, dispersal guild is more informative than flowering guild because dispersal does not always immediately follow flowering (e.g., *Penstemon digitalis* flowers April - June but seeds in late September - December). We based our classifications on expert opinion (see Acknowledgments), the literature (Rabinowitz & Rapp, 1980), and our work on seed rain patterns in Missouri grasslands (Chapter 2). Species used in our study consisted of 29 native species captured in our previous study on seed rain and seven native summer species that regional managers (see Acknowledgements) cited as having minimal success in prairie restorations (e.g., *Viola pedatifida)* (Table S4.1). When possible, we obtained seeds from local ecotype commercial sellers. However, several summer species were not grown commercially in Missouri and were sourced elsewhere. All seeds for a species were sourced from the same supplier to eliminate variation caused by sourcing differences. We stored the seeds in a refrigerator (2.78 °C) until seeding.

We used a randomized block design to test the effects of seeding timing and order (Figure S4.1). Each block (n = 6) contained four plots (each 4 $m^2$) that were randomly assigned one of the following four arrival treatments (Figure 4.1):

(i) The simultaneous addition of all 36 species on March 22nd, 2021 (Simultaneous; no timing or order treatment).

(ii) The addition of species in order of their first peak in dispersal activity from May 24th to November 19th, 2021 (see Table S4.1 for specific dates) (Natural; timing treatment).

(iii) The lumped addition of 18 summer species on March 22nd, 2021 followed by a lumped addition of 18 fall species approximately five months later on September 5th, 2021 (Summer first; timing and order treatment).

(iv) The lumped addition of 18 fall species on March 22nd, 2021 followed by a lumped addition of 18 summer species approximately five months later on September 5th, 2021 (Fall first; order treatment).

To test timing effects, we compared the Summer first and Natural treatments, which added species in the same dispersal phenological guild order but varied the time interval between additions (5 months vs. ~2 weeks). We used the Summer first and Fall first treatments to test order effects since these treatments varied the order of dispersal phenological guild arrival at the same time interval. We also seeded white clover (*Trifolium repens*) in the aisles between the experimental plots to prevent erosion. Starting in the fall of 2022, we removed invading white clover and woody species annually from plots. Other unseeded species were not weeded to better reflect realistic assembly processes.

We started seeding at the end of the dormant season (March 22nd, 2021) and continued until late fall (November 19th, 2021) (Figure 4.1, Table S4.1). We hand-seeded species into experimental plots at a density of 50 seeds $m^{-2}$ per species using sand as a broadcasting agent. For treatments requiring multiple seedings (Natural, Summer first, Fall first), we incorporated M-Binder tackifier (Ecology Controls, Carpinteria, CA), a natural adhesive often used in hydroseeding, into the seeding mixes to increase soil-seed contact without disturbing the existing vegetation. After seeding, we lightly watered plots to activate the tackifier. All plots, including the Simultaneous and No seeding treatment, received equal amounts of sand, tackifier, and water during every seeding to eliminate potential differences caused by these additions.

Starting in 2022, we conducted floristic surveys for two years to assess plant community diversity and composition in experimental plots. To determine species abundance, we measured the percent aerial cover of all vascular plant species rooted within a 1 $m^2$ subplot in the center of the experimental plot. We measured vegetative cover twice yearly, once in the early summer (late June) and again at peak biomass (August – September), and used the maximum percent cover value for species present in both surveys. We identified species according to Yatskievych (1999, 2006, 2013).

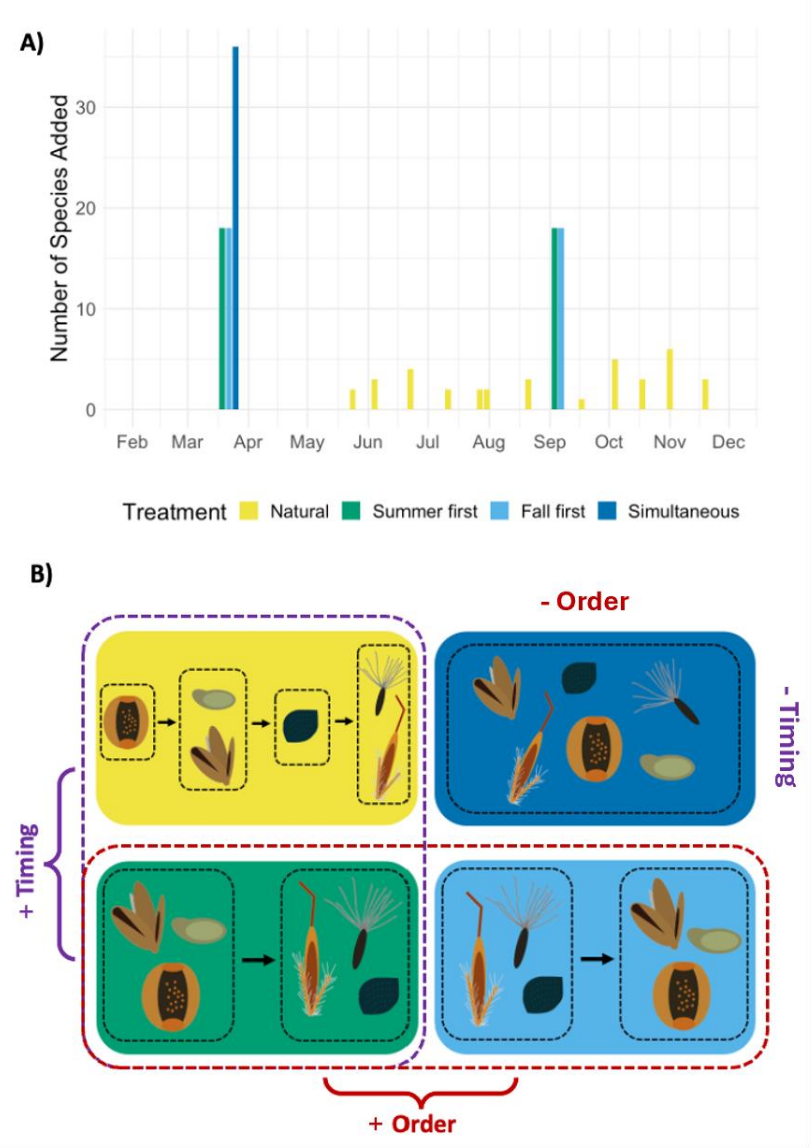

**Figure 4.1. A)** Timeline of seed additions (Mar. 22nd – Nov. 19th, 2021). All treatments incorporated the same 18 summer and 18 fall species but manipulated the timing and order of arrival. **B)** Experimental schematic of the following seeding treatments testing whether varying the species arrival influences community assembly: simultaneous addition of all 36 species (Simultaneous), the addition of species according to their dispersal phenology (Natural), the addition of 18 summer species followed by the later addition of 18 fall species (Summer first), and the addition of 18 fall species followed by the later addition of 18 summer species (Fall first). We tested whether seeding with or without timing or arrival produced divergent community outcomes by comparing the Simultaneous treatment (no timing or order) to all other treatments. We tested timing effects using the Natural and Summer first treatments that varied the timing interval (short-term vs. long-term) between species additions in the same phenological guild order. We tested order effects using the Summer first and Fall first treatments that manipulated guild arrival order at the same time intervals.

### *Data Analysis*

#### *Diversity differences*

To determine the effects of species arrival on diversity, we fit mixed-effects linear models with block as a random effect to assess whether the predictor variables of year, arrival treatment, and their interaction influenced total species richness, sown summer species richness, and sown fall species richness in assembling tallgrass prairie plant communities ("lme4" package; Bates et al., 2015). In cases where random effect variance was estimated as near zero and prevented model convergence, we dropped the random effect and fit a simpler linear model instead. Furthermore, we fit an additive model when the interaction between arrival treatment and year was not significant. For each model, we conducted a type III analysis of variance/deviance (ANOVA) ("car" package; Fox & Weisberg, 2019) followed by a post hoc Tukey test to identify significant pairwise differences to further evaluate arrival, order, timing, and year effects while correcting for multiple comparisons ("emmeans" package; Lenth et al., 2023).

#### *Seeded cover differences*

To test whether manipulating species arrival resulted in differences in seeded cover, we fit binomial regression models predicting the proportion of total, summer, and fall seeded cover as a function of year, arrival treatment, and their interaction ("stats" package; R Core Team, 2022). We did not use block as a random effect since its inclusion did not explain additional variance and impeded model convergence for all models predicting seeded cover. In cases where the interaction term was not significant, we fit additive effects models instead. Again, we conducted type III analysis of deviance tests (“car” package; Fox & Weisberg, 2019) for each fitted model to ascertain whether the arrival treatment and year influenced the cover of seeded species. We further analyzed arrival, order, timing, and years effects using pairwise comparisons of estimated marginal means, correcting for multiple comparisons using the Tukey method (“emmeans” package; Lenth et al., 2023).

### *Species-level early arriver advantage*

To determine whether individual species benefitted from being given an early arriver advantage, we calculated the difference in cover for each species when seeded first vs. second. For this analysis, we only used species cover from seeding treatments that added all 18 summer or all 18 fall species first (Summer and Fall first), followed by the rest of the species pool five months later since these treatments varied the order of arrival using the same time interval. We conducted a one-sample t-test to determine whether the difference in species cover was significantly greater or less than zero, indicating arrival order influenced species cover.

### *Compositional differences*

To determine whether the timing of species arrival influenced the composition of tallgrass prairie plant communities, we created community distance matrixes using Bray-Curtis dissimilarity and relativized species percent vegetative cover using a Hellinger transformation for each year. Afterward, we visualized yearly compositional differences between seed addition treatments using non-metric multidimensional scaling (NMDS). We further analyzed compositional differences by conducting a permutational analysis of variance test (PERMANOVA) to assess whether seeding treatments influenced community assembly (permutations = 999). Due to differences in composition among treatments, we did additional post hoc pairwise comparison tests using the pairwise.adonis2() function found in the “pairwiseAdonis” package to evaluate arrival, timing, and order effects (Martinez Arbizu, 2017). Using the envfit function (permutations = 999) (“vegan” package; Oksanen et al., 2020), we also identified species significantly associated with compositional differences among seeding treatments ($p < 0.01$). We used the "vegan" package to conduct all other multivariate analyses (Oksanen et al., 2020).

*Data reproducibility and accessibility*

We used R (version 4.2.2) and RStudio (version 2023.03.0+386) to conduct all analyses and create data visualizations (R Core Team, 2022). Data manipulation and visualizations were accomplished using the "tidyverse" package (Wickham et al., 2019).

## Results

We encountered 25 of the 36 species (69%) seeded into our experiment (Table S4.1). Dispersal guild affected species recruitment. Overall, more fall species were successfully recruited than summer species, with 15 out of 18 fall (83%) and five out of 18 (28%) summer species persisting in experimental plots two years post-seeding.  In 2023, we were unable to relocate five species, including white prairie clover (*Dalea candida*), rattlebox (*Crotalaria saggitalis*), grooved yellow flax (*Linum sulcatum*), little bluestem (*Schizachryium scoparium*), and prairie coreopsis (*Coreopsis palmata*) that were previously seen prior to 2023.

*Diversity: Does assembly history matter?*

Manipulation of species arrival history via seeding treatments significantly influenced community diversity, including total species richness (Figure 4.2 A; $X^2_3 = 27.11$, $P < 0.001$), sown summer species richness (Figure 4.2 B; $C^2_3 = 43.66$, $P < 0.001$), and sown fall species richness (Figure 4.2 C; $F_{3,43} = 32.09$ , $P < 0.001$). Total species richness decreased ($X^2_1 = 5.64$, $P < 0.05$) while summer and fall species richness remained unchanged between study years (*Summer species:* $X^2_1 = 0.81$, $P = 0.37$, $P < 0.05$; *Fall species:* $F_{1,43} = 0.32$, $P = 0.58$). Declines in total species richness between study years were thus attributed to losses in unsown species richness.

Seeding the entire species pool without order or timing (Simultaneous) resulted in communities with significantly greater total species richness compared to seeding all summer species first (Summer First), all fall species first (Fall first), and species according to their observed peak dispersal phenology (Natural) (Figure 4.2, Table S4.2). Simultaneous seeding also resulted in significantly greater summer species richness than treatments seeding fall species first (Fall first) or following natural dispersal phenology (Natural). However, simultaneous seeding produced communities with comparable summer species richness to when summer species were seeded first (Summer first). Simultaneous seeding also significantly increased fall species richness compared to when summer species were seeded first (Summer first) or according to their natural dispersal phenology (Natural), but not when fall species were seeded first (Fall first).

### *Diversity: Does timing matter?*

Timing interval only significantly influenced sown summer species diversity, where having a 5-month interval between additions (Summer First) increased summer species richness compared to seeding every two weeks (Natural) (Figure 4.2, Table S4.2). Total and fall species richness were comparable between timing treatments.

### *Diversity: Does order matter?*

Order of dispersal phenological guild arrival (Summer first vs. Fall first) did not result in differences in total species richness (Figure 4.2, Table S4.2). However, arrival order significantly influenced the richness of sown summer and fall species. Treatments that seeded a particular guild first had significantly more members of that guild compared to treatments that seeded them second.

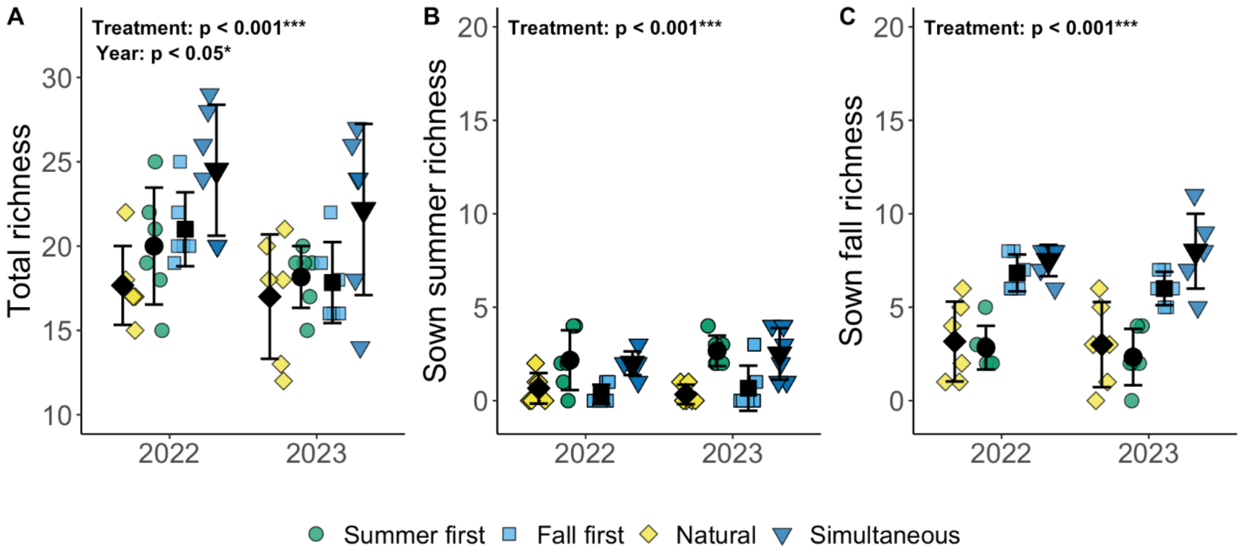

**Figure 4.2. A)** Total, **B)** sown summer, and **C)** sown fall species richness for communities that manipulated species arrival via four seeding treatments: at first peak in dispersal activity (Natural, yellow diamond), all summer species followed by all fall species (Summer first, green circles), all fall species followed by all summer species (Fall first, light blue squares), and all species at the same time (Simultaneous, dark blue upside-down triangles). Black symbols and error bars represent the sample mean ± SD. Multiple comparisons were adjusted using the Tukey method.

*Cover: Does assembly history matter?*

Arrival treatments also significantly influenced the proportion of total, summer, and fall seeded cover in experimental communities (*Total cover*: $X^2_3 = 272.44$, $P < 0.001$, Figure 4.3 A; *Summer species cover*: $X^2_3 = 373.50$, $P < 0.001$, Figure 4.3 B; *Fall species cover*: $X^2_3 = 566.51$, $P < 0.001$, Figure 4.3 C). In general, the proportion of seeded cover increased between 2022 and 2023 (*Total cover:* $X^2_1 = 32.04$, $P < 0.001$*; Summer cover:* $X^2_1 = 106.69$, $P < 0.001$; *Fall cover:* $X^2_1 = 7.57$, $P < 0.01$). We only found a significant interaction between year and arrival treatment for total seeded cover, which was caused by the Summer first treatment having accelerated gains in summer cover between 2022 and 2023 ($X^2_3 = 10.31$, $P < 0.05$).

Seeding without timing or order (Simultaneous) produced communities with similar total and fall seeded cover compared to when fall species were seeded first (Fall first) (Figure 4.3 A, C, Table S4.3). However, simultaneous seeding had significantly more total and fall seeded cover than communities sown with summer species before fall species (Natural and Summer first). In contrast, simultaneous seeding had significantly less summer seeded cover than sowing all summer species first (Summer first), but greater cover than when fall species were seeded with priority (Fall first) or when species were added in two-week intervals at peak dispersal (Natural) (Figure 4.3 B).

### *Cover: Does timing matter?*

Timing interval between species additions influenced the proportion of seeded cover, where sowing all summer-dispersing species in five-month intervals versus two-week intervals (Summer first vs. Natural) resulted in significant increases in total and summer seeded cover by 2023 (Figure 4.3 A, B, Table S4.3). Fall seeded cover was comparable between both timing treatments (Summer first and Natural) (Figure 4.3 C).

### *Cover: Does order matter?*

Order of phenological guild arrival (Summer first vs. Fall first) resulted in differences in the proportion of total, fall, and summer seeded cover (Figure 4.3, Table S4.3). When examining the proportion of seeded cover separately by phenological dispersal guild, most of the total seeded cover consisted of sown fall species. Both dispersal guilds had significantly greater seeded cover when sown first. Communities sown with all summer species first (Summer first) had a significantly greater proportion of summer species cover than when their arrival was delayed (Fall first) (Figure 4.3 B). In a similar fashion, treatments seeding fall species with priority (Fall first) had far greater sown fall cover than when fall species were seeded second (Summer first) (Figure 4.3 C).

At the species level, mean cover was greatest when species were seeded with an early-arriver advantage (Figure 4.4, $t_{89} = 5.49$, $P < 0.001$). Delaying arrival by five months reduced species cover for both fall and summer species alike. We found that the cover of five species greatly benefitted from being seeded first, including grey-headed coneflower (*Ratibida pinnata*), yarrow (*Achillea millefolium*), yellow prairie grass (*Sorghastrum nutans*), tall tickseed (*Coreopsis tripteris*), and Junegrass (*Koeleria macrantha*). Only two species, foxglove beardtongue (*Penstemon digitalis*) and beebalm (*Monarda fistulosa*), showed a slight but not significant trend towards having greater cover when seeded second.

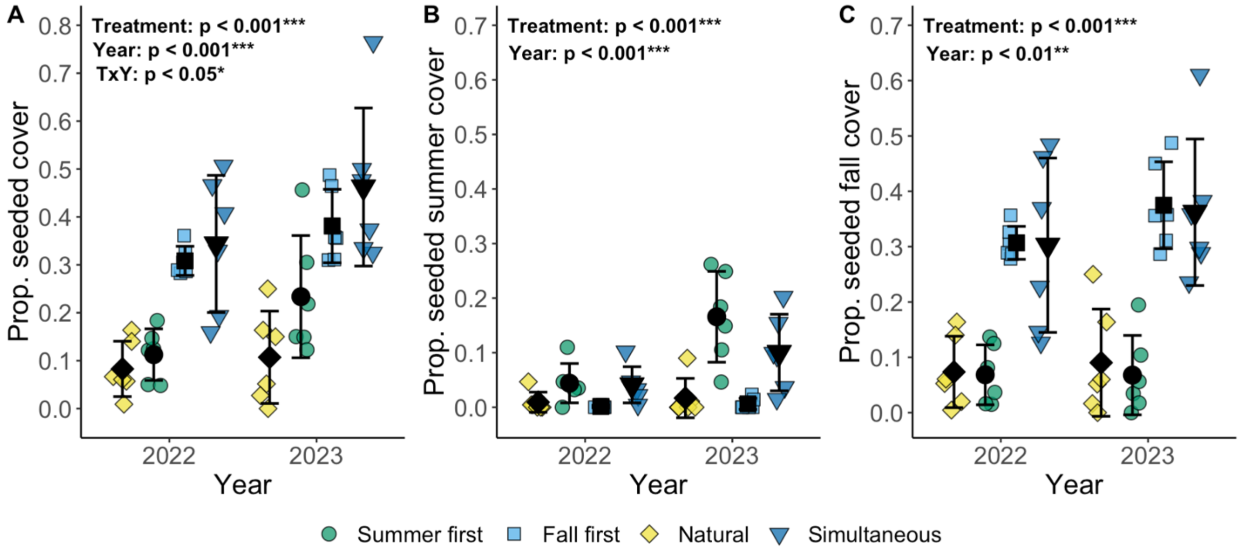

**Figure 4.3.** The mean proportion of **A)** total, **B)** summer, and **C)** fall seeded vegetative cover in communities that manipulated species arrival via four seeding treatments: at first peak in dispersal activity (Natural, yellow), all summer species followed by all fall species (Summer first, green), all fall g species followed by all summer species (Fall first, light blue), and all species at the same time (Simultaneous, dark blue) in 2022 and 2023. Black symbols and error bars represent the sample mean ± SD.

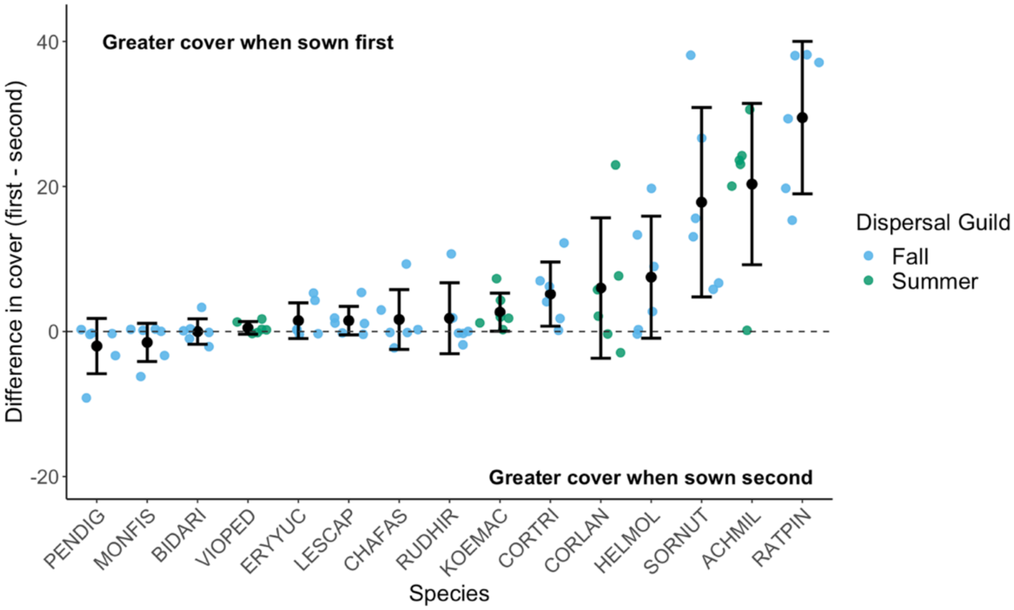

**Figure 4.4.** Difference in vegetative cover when species were added with priority compared to without. The dashed line (y = 0) indicates no difference in cover for a species when sown first vs. second. Values above the dashed line indicate species with greater cover when seeded with an early arriver advantage and values below have greater cover when seeding was delayed. Error bars represent 95% confidence intervals around the mean cover for each species (black circle; see Table S4.1 for names).

*Composition: Does assembly history matter?*

Manipulating species arrival via seeding treatments strongly altered community composition in 2022 (Figure 4.5 A; pseudo-$F_{3,23} = 2.32$, $R^2 = 0.26$, $P < 0.01$) and 2023 (Figure 4.5 B; pseudo-$F_{3,23} = 3.19$, $R^2 = 0.32$, $P < 0.01$). In 2022, communities sown without timing or order (Simultaneous) had significant differences in composition compared to those that varied assembly history (Summer first, Fall first, and Natural) (Table S4.4). Differences in composition remained the following year.

*Composition: Does timing matter?*

Varying the time interval of species arrival by sowing summer species first (Summer first) or at natural dispersal phenology (Natural) resulted in communities with similar aboveground compositions in 2022 and 2023 (Figure 4.5, Table S4.4).

*Composition: Does order matter?*

Manipulating dispersal guild arrival order (Summer first vs. Fall first) resulted in robust differences in community composition in 2022 and 2023 (Figure 4.5, Table S4.4). Based on species vectors, fall species were strongly associated with communities that seeded this guild first. Unsown species were more associated with communities that delayed the seeding of fall species. By 2023, no summer sown species were identified as significant to the $\alpha$ 0.01 level.

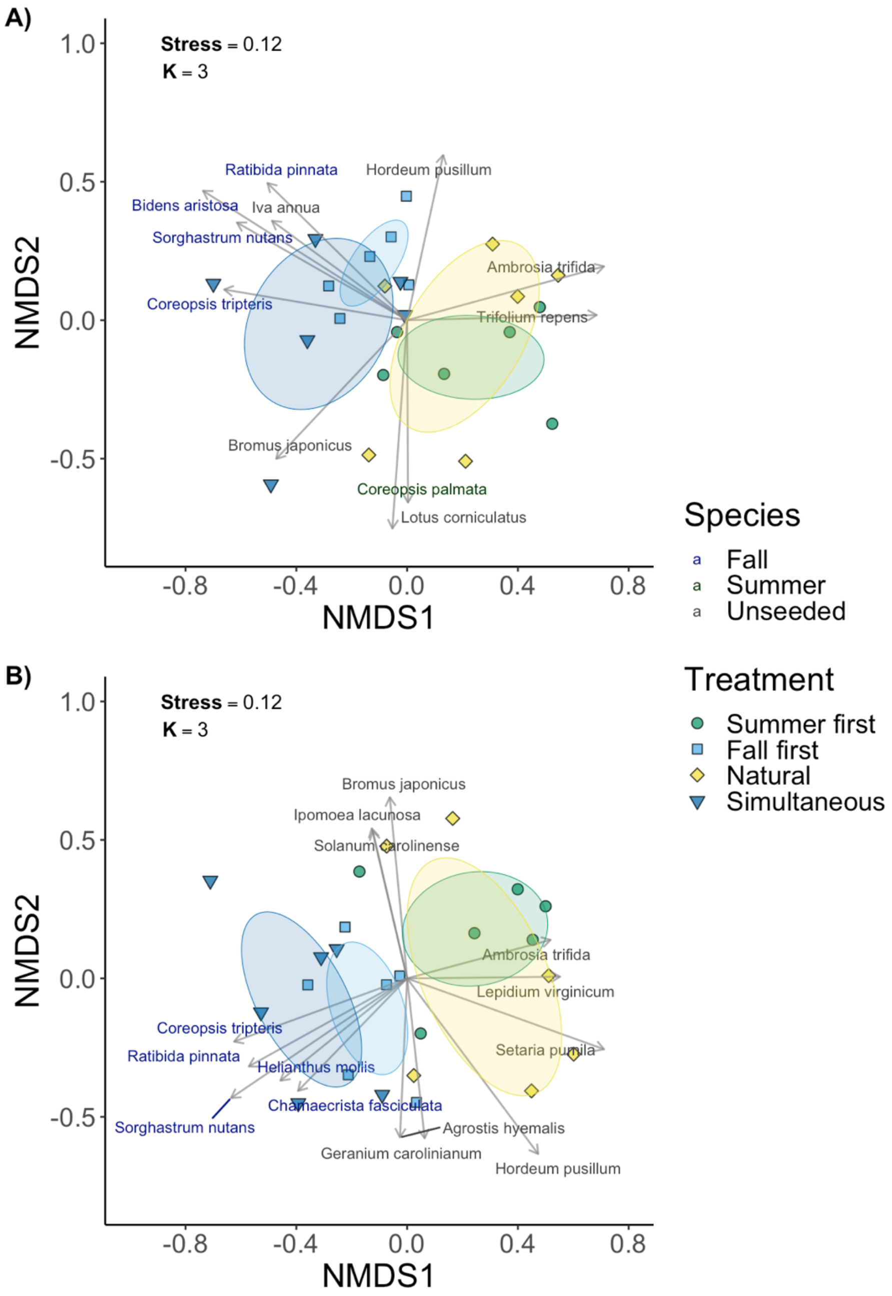

**Figure 4.5.** Non-metric multidimensional (NMDS) ordinations using Bray-Curtis dissimilarity for **A)** 2022 and **B)** 2023 to visualize difference in aboveground composition between communities that manipulated species arrival via four seeding treatments: at first peak in dispersal activity (Natural, yellow diamond), all summer species followed by all fall species (Summer first, green circles), all fall species followed by all summer species (Fall first, light blue squares), and all species at the same time (Simultaneous, dark blue upside-down triangles). Ellipses represent 95% confidence intervals calculated from standard error around treatment centroids. Plot vectors represent species (dark green = sown summer species, dark blue = sown fall species, grey = unsown species) significantly associated with community composition ($P \leq 0.01$).

## Discussion

Our work demonstrated that priority effects, resulting from variations in the timing and order of species arrival based on dispersal phenology, influenced the community assembly of tallgrass prairie plant communities. Manipulating only the assembly history of a shared species pool in the same environment was enough to produce considerable differences in diversity, cover, and composition between treatments seeded with or without timing and order. Timing of arrival also mattered, where even short intervals between arrival altered summer species richness and cover, specifically. However, we observed that dispersal guild arrival order produced even stronger priority effects. Both summer and fall species benefitted from increased richness and cover from being seeded first and were negatively impacted by having their arrival delayed. Differences in arrival order also produced strong compositional differences among seeding treatments lasting for at least two years. We found that having an early-arriver advantage was asymmetric and more beneficial to summer species than fall species, as evidenced by the fact that summer but not fall species achieved higher cover when seeded first than with each other. Importantly, the early inclusion of summer species did not prevent the establishment of at least some late-arriving fall species, unlike vice versa. Traits that allow subordinate species to arrive long before dominant species are likely important to maintaining coexistence in prairie ecosystems. Overall, our results highlight the importance of considering how functional traits relevant to the timing and order of species arrival can influence the trajectory of community assembly.

### *Does assembly history matter?*

Whether the outcomes of community assembly in similar environments are deterministic or sensitive to variations in species arrival has been debated in ecology for nearly 100 years (see

Clements, 1916, Gleason, 1927). Our results support a growing body of literature that history matters to plant communities, where we found that seeding species with or without priority produced communities differing in species diversity, cover, and composition despite sharing a species pool and abiotic conditions – a phenomenon termed alternative states (Durbecq et al., 2023; L. M. Martin & Wilsey, 2012, 2014; Temperton et al., 2016; Weidlich et al., 2017; Werner et al., 2016; Wohlwend et al., 2019). Similar to Martin & Wilsey (2012, 2014), seeding strong competitors and subordinate species at equal rates did not homogenize community composition as expected under deterministic models such as competition/colonization (Tilman, 1994; Turnbull et al., 2000). Instead, variations in arrival history were enough to disrupt competitive hierarchies, allowing for alternative states to occur. Unlike some studies that report priority effects fade over time (Collinge & Ray, 2009; Weidlich et al., 2017; T. P. Young et al., 2017), we observed differences in composition throughout our study. Even though our experiment only lasted for two years, studies in comparable grassland systems (see Martin & Wilsey, 2012, 2014; Wohlwend et al., 2019) have reported evidence of priority effects lasting over 7 years after manipulating species arrival.

### *Does timing matter?*

We found that varying the time interval between species introduction resulted in differences in diversity and cover, but not composition. Increasing the time between arrival provides more opportunities for early-arriving species to modify their environment and garner biomass before new species arrive, thus we expected longer intervals to produce stronger priority effects (Fukami, 2015; Von Gillhaussen et al., 2014; T. P. Young et al., 2017). Aligned with our predictions, we observed that longer intervals (Summer first) increased summer seeded cover and richness compared to short intervals in arrival (Natural). We also found that dispersal guild differed in sensitivity to arrival timing since fall species richness and cover were not influenced by sowing interval. Even though our short-term priority treatment (Natural) better reflected natural dispersal patterns, it also provided additional opportunities for unsown species to recruit from the preexisting soil seed bank, preempting the arrival of seeded species and resulting in more variable community outcomes. However, despite increased variability in composition, variations in timing interval did not result in compositionally distinct communities. By seeding species according to their natural dispersal phenology, we simulated passive reassembly of prairie communities without seed limitation. Therefore, removing barriers to dispersal alone without consideration of arrival timing is insufficient for recreating native communities. Given our results and others (L. M. Martin & Wilsey, 2012, 2014; Wohlwend et al., 2019; T. P. Young et al., 2017), increasing the amount of time between species arrival is more likely to result in alternative states (Wilsey, 2021).

### *Does order matter?*

Order of dispersal phenological guild arrival altered competitive outcomes resulting in sown richness, cover, and compositional differences that lasted at least two years. In temperate grasslands, many fall species, including warm-season $C_4$ grasses, are strong competitors (A. T. Clark et al., 2018). Previous priority effect studies have also observed that the early arrival of grasses is more inhibitory than other functional groups (Delory et al., 2019; Weidlich et al., 2017, 2018) and that priority effects are often asymmetric (Durbecq et al., 2023; Werner et al., 2016; T. P. Young et al., 2017). Therefore, we expected seeding fall species first to result in stronger inhibitory effects than sowing summer species with priority. As predicted, we found that manipulating guild arrival order resulted in asymmetric priority effects, where summer species benefitted more from being seeded first than fall species. Both guilds benefitted from being sowed first. For example, fall species were inhibitory, and sowing this guild five months before summer species (Fall first) almost completely prevented summer species establishment. While seeding summer species with priority (Summer first) was also inhibitory, sowing this guild first allowed for the co-occurrence of fall forbs and legumes, but not $C_4$ grasses. Although cover is not explicitly a measure of size, summer species achieved greater cover when sown first rather than with fall species, which may have mediated competitive interactions allowing for coexistence (Rasmussen et al., 2014; Wilsey, 2021). However, delaying the arrival of fall species increased the proportion of unsown species cover, suggesting that seeding fall species early can confer increased invasion resistance. Arrival order also had a strong species-specific response. Some species were relatively unaffected by arriving later, while others benefitted immensely, suggesting the importance of context dependence. Altogether, arrival order can strongly influence community structure and composition, especially when subordinate and dominant species arrive separately.

### *Practical implications for ecological restoration*

Our experiment further demonstrated that priority effects can be manipulated to guide restoration outcomes (Weidlich et al., 2021). Most prairie restorations are seeded with a one-time simultaneous addition of the target species pool to achieve diversity and composition goals (Rowe, 2010). However, restoration efforts often produce variable results, with historical contingencies likely playing a central role in creating disparate outcomes (Catano et al., 2023; Groves et al., 2020). In support of historical contingency, we found that only altering the intraannual arrival history of a shared species pool was enough to produce divergent community assembly trajectories, resulting in both desirable and undesirable restoration outcomes. Simultaneous seeding produced the most diverse communities by both establishing fall and summer species whilst preempting the recruitment of species in the seed bank. In contrast, seeding species at their observed natural dispersal timing allowed unsown species to recruit, causing an unfavorable priority effect that excluded sown summer species. We found that summer species, an underrepresented guild in prairie restorations, greatly benefitted cover-wise when sown earlier than fall species (Deever et al., 2023; Frischie & Rowe, 2012; Sluis et al., 2018). Adding subordinate before dominant species, either from transplanting or seeding, might increase their long-term success in restored communities through size-asymmetric competition (Deever et al., 2023; Durbecq et al., 2023; Wilsey, 2021; T. P. Young et al., 2017).

### *Conclusions*

Our study showed, for the first time, that manipulating species arrival based on dispersal phenology can induce priority effects under field conditions in plant communities. Other traits related to species arrival, such as dispersal ability or germination phenology, are likely also relevant to historical contingency and should be further explored for use in ecological restoration (Blackford et al., 2020; Fukami, 2015; Zou & Rudolf, 2023). Additionally, our study revealed that summer species are more sensitive to arrival timing and order than fall species. Given that global climate change is altering plant phenology, especially for early-season species, shifting environmental conditions may further influence competitive outcomes through priority effects (Blackford et al., 2020; Sherry et al., 2007; Zettlemoyer et al., 2021; Zou & Rudolf, 2023). Although we based species arrival using dispersal phenology, there are likely other correlated traits (e.g., size, rarity, etc.) that may further explain why summer species were more sensitive to arrival timing and order. Continued efforts investigating how species traits and environmental conditions interact with priority effects will benefit our understanding of community assembly rules that can simultaneously benefit restoration efforts.

## Acknowledgments

We thank Andrew Biggs for his help with site selection, preparation, and maintenance. Additionally, we would like to thank Keith Bennett and Judy McKinnon for their assistance in selecting native species desirable to restoration practitioners and providing expertise on when these species disperse.  We also thank Savana Presson, Danielle Gafford, Erica Eyerly, Kelsey Jaeger, Meredith Medley, Tyler Seabold, Olivia DeClue, Katy McWilliams, Maya Parker-Smith, Carolyn Stephen, Larissa Kahan, Gaurav Kandlikar, Varsha Kandlikar, Shawn Thomas, and Alejandro Chavez Treviño for their assistance in preparing seed mixes, site maintenance, and data collection. We thank Deborah Finke, Elizabeth King, Manuel Leal, Lars Brudvig, and the Sullivan Lab for giving invaluable feedback on our manuscript. The Prairie Fork Charitable Endowment Trust, the University of Missouri Division of Biological Sciences, the Michigan State University Department of Plant Biology, and the Long-Term Agroecosystem Research (LTAR) network (58-5070-9-016 and 58-5070-2-018) provided funding that supported our research. LTAR is supported by the United States Department of Agriculture.

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# APPENDIX C: PRIORITY EFFECTS BASED ON DISPERSAL PHENOLOGY ALTER PLANT COMMUNITY ASSEMBLY

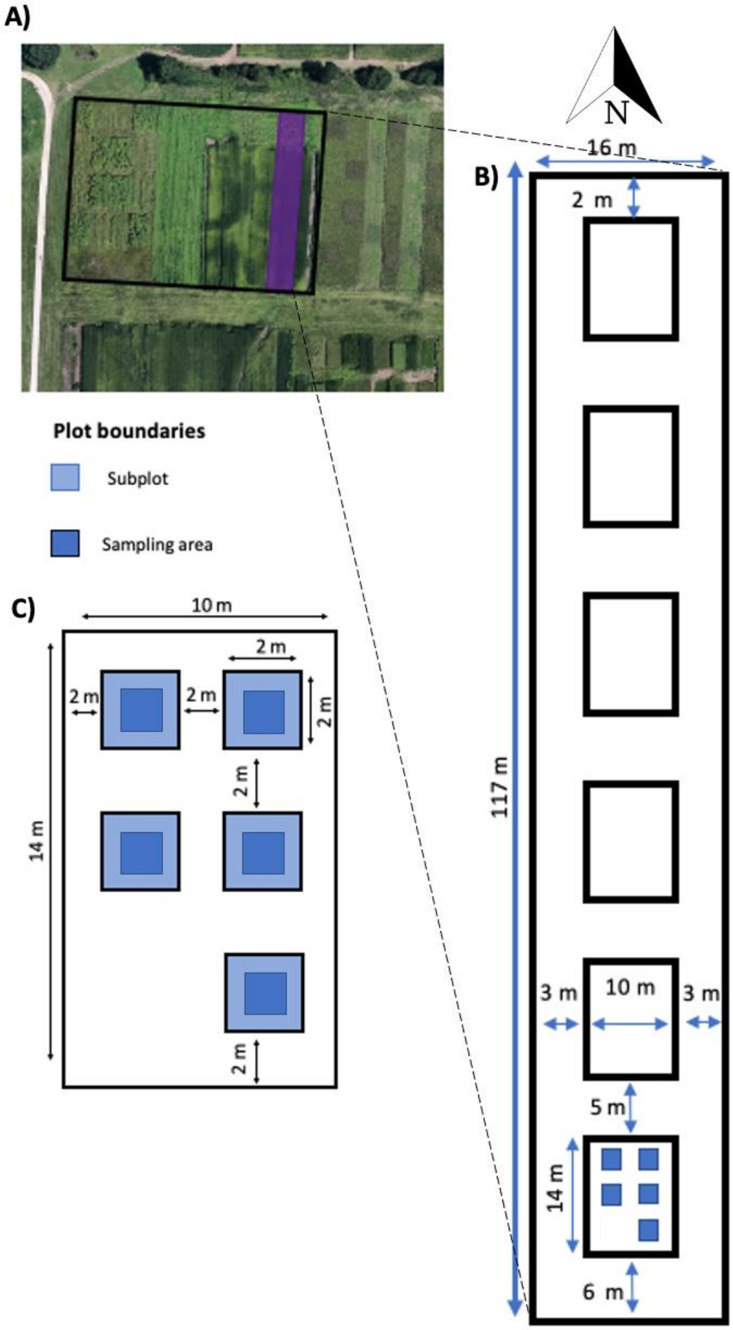

**Figure S4.1**. Schematic of experimental setup. **A)** Aerial overview of experimental area (indicated in purple) at Bradford Research Center (Boone County, MO). **B)** Experimental area showing block locations and dimensions. C) An example block with subplot (light blue) and sampling area locations and dimensions (dark blue).

**Table S4.1.** Species seeded in experimental plots (50 seeds $m^{-2}$). Phenology dispersal guild refers to whether species experienced their first peak in dispersal before September 1st (Summer) or after (Fall). Date sown indicates date species were added in the natural dispersal phenology seeding treatment. Species seen at any point during the experiment are denoted with an X.

| Dispersal Guild | Scientific Name | Common Name | Date Sown | Seen | Functional group |
|---|---|---|---|---|---|
| Summer | *Achillea millefolium* | Yarrow | Aug. 1st | X | Forb |
| | *Coreopsis lanceolata* | Lanceleaf coreopsis | Jun. 4th | X | |
| | *Coreopsis palmata* | Prairie coreopsis | Jul. 11th | X | |
| | *Dodecatheon meadia* | Shooting star | Jul. 11th | | |
| | *Heuchera richardsonii* | Prairie alumroot | Jun. 22nd | | |
| | *Linum sulcatum* | Grooved yellow flax | Aug. 2nd | X | |
| | *Lobelia spicata* | Palespike lobelia | Jun. 22nd | | |
| | *Melanthium virginicum* | Bunchflower | Aug. 2nd | | |
| | *Packera plattensis* | Prairie ragwort | May 24th | | |
| | *Sisrynchium campestre* | Prairie blue-eyed grass | Jun. 4th | | |
| | *Tradescantia ohiensis* | Ohio spiderwort | Jun. 22nd | | |
| | *Viola pedatifida* | Prairie violet | May 24th | X | |
| | *Amorpha canescens* | Lead plant | Jul. 25th | | Legume |
| | *Dalea candida* | White prairie clover | Aug. 21st | X | |
| | *Dalea purpurea* | Purple prairie clover | Aug. 21st | | |
| | *Carex bushii* | Bush's sedge | Jun. 22nd | | Graminoid |
| | *Koeleria macrantha* | Junegrass | Jul. 25th | X | |
| | *Sphenopholis obtusata* | Wedge grass | Jun. 4th | X | |
| Fall | *Bidens aristosa* | Tickseed sunflower | Oct. 18th | X | Forb |
| | *Coreopsis tripteris* | Tall coreopsis | Nov. 19th | X | |
| | *Eryngium yuccifolium* | Rattlesnake master | Nov. 1st | X | |
| | *Helianthus mollis* | Ashy sunflower | Oct. 18th | X | |
| | *Hypericum punctatum* | Dotted St. John's wort | Nov. 1st | | |
| | *Liatris pycnostachya* | Prairie blazing star | Nov. 1st | X | |
| | *Monarda fistulosa* | Wild bergamot | Oct. 4th | X | |
| | *Penstemon digitalis* | White beardtongue | Nov. 1st | X | |
| | *Pycnanthemum tenuifolium* | Slender mountain mint | Nov. 19th | X | |
| | *Ratibida pinnata* | Grayheaded coneflower | Oct. 4th | X | |
| | *Rudbeckia hirta* | Black eyed Susan | Oct. 4th | X | |
| | *Solidago rigida* | Stiff goldenrod | Nov. 19th | X | |
| | *Chamaecrista fasciculata* | Partridge pea | Oct. 4th | X | Legume |
| | *Crotalaria sagittalis* | Rattlebox | Sep. 17th | X | |
| | *Lespedeza capitata* | Roundhead bushclover | Oct. 18th | X | |
| | *Schizachyrium scoparium* | Little bluestem | Nov. 1st | X | Graminoid |
| | *Sorghastrum nutans* | Yellow prairie grass | Nov. 1st | X | |
| | *Sporobolus heterolepis* | Prairie dropseed | Oct. 4th | X | |

**Table S4.2.** Summary results for pairwise contrast tests comparing the total species richness (t, (P)), sown summer species richness (t, (P)), and sown fall species richness (t, (P)) in communities that manipulated species arrival. Bolded values indicate significance at the α 0.05 level. Multiple comparisons were adjusted using the Tukey method.

| **Contrast** | **Species Richness** | | |
|---|---|---|---|
| | Total | Summer | Fall |
| Summer first – Fall first | -0.27 (0.99) | 4.89 (< **0.001**) | -6.14 (< **0.001**) |
| Summer first – Natural | -1.39 (0.51) | 4.89 (< **0.001**) | -0.80 (0.85) |
| Summer first – Simultaneous | -3.37 (< **0.01**) | 0.43 (0.97) | -8.27 (< **0.001**) |
| Fall first – Natural | -1.65 (0.36) | 0.00 (1.00) | 5.34 (< **0.001**) |
| Fall first – Simultaneous | -3.11 (< **0.05**) | -4.46 (< **0.001**) | -2.14 (0.16) |
| Natural – Simultaneous | -4.76 (< **0.001**) | -4.46 (< **0.001**) | -7.47 (< **0.001**) |

**Table S4.3.** Summary results for pairwise contrast tests comparing the proportion of total (Z, (P)), summer-dispersing (Z, (P)), and fall-dispersing (Z, (P)) seeded aboveground cover in communities that manipulated species arrival. Bolded values indicate significance at the α 0.05 level. Multiple comparisons were adjusted using the Tukey method.

| **Contrast** | **Seeded Cover** | | |
|---|---|---|---|
| | **Total** | **Summer** | **Fall** |
| *Seeding treatment* | | | |
| Summer first – Fall first | -10.77 (< **0.001**) | 9.84 (< **0.001**) | -17.55 (< **0.001**) |
| Summer first – Natural | 6.20 (< **0.001**) | 10.52 (< **0.001**) | -1.52 (0.43) |
| Summer first – Simultaneous | -12.79 (< **0.001**) | 3.39 (< **0.001**) | -16.88 (< **0.001**) |
| Fall first – Natural | 16.04 (< **0.001**) | -3.28 (< **0.001**) | 16.73 (< **0.001**) |
| Fall first – Simultaneous | -2.51 (0.06) | -8.71 (< **0.001**) | 0.73 (0.88) |
| Natural – Simultaneous | -17.75 (< **0.001**) | -8.50 (< **0.001**) | -16.03 (< **0.001**) |
| *Year* | | | |
| 2022 – 2023 | -6.39 (< **0.001**) | -9.78 (< **0.001**) | -2.751 (< **0.01**) |
| *Seeding Treatment x Year* | | | |
| Summer first 22 - Fall first 22 | -9.06 (< **0.001**) | n.s. | n.s |
| Summer first 22 – Natural 22 | 2.76 (0.1) | | |
| Summer first 22 – Simultaneous 22 | -10.22 (< **0.001**) | | |
| Summer first 22 - Summer first 23 | -5.57 (< **0.001**) | | |
| Summer first 22 - Fall first 23 | -11.08 (< **0.001**) | | |
| Summer first 22 – Natural 23 | 0.91 (0.99) | | |
| Summer first 22 – Simultaneous 23 | -12.57 (< **0.001**) | | |
| Fall first 22 – Natural 22 | 11.15 (< **0.001**) | | |
| Fall first 22 – Simultaneous 22 | -1.56 (0.78) | | |
| Fall first 22 – Summer first 23 | 3.57 (< **0.01**) | | |
| Fall first 22 – Fall first 23 | -2.77 (0.10) | | |
| Fall first 22 – Natural 23 | 9.65 (< **0.001**) | | |
| Fall first 22 – Simultaneous 23 | -4.78 (< **0.001**) | | |
| Natural 22 – Simultaneous 22 | -12.17 (< **0.001**) | | |
| Natural 22 – Summer first 23 | -7.98 (< **0.001**) | | |
| Natural 22 – Fall first 23 | -12.92 (< **0.001**) | | |
| Natural 22 – Natural 23 | -1.84 (0.60) | | |

**Table S4.3 (cont’d)**

| Contrast | Seeded Cover | | |
|---|---|---|---|
| | **Total** | **Summer** | **Fall** |
| *Seeding Treatment x Year* | | | |
| Natural 22 – Simultaneous 23 | -14.23 (< **0.001**) | n.s. | n.s. |
| Simultaneous 22 – Summer first 23 | 4.94 (< **0.001**) | | |
| Simultaneous 22 – Fall first 23 | -1.23 (0.92) | | |
| Simultaneous 22 – Natural 23 | 10.75 (< **0.001**) | | |
| Simultaneous 22 – Simultaneous 23 | -3.25 (< **0.5**) | | |
| Summer first 23 – Fall first 23 | -5.97 (< **0.001**) | | |
| Summer first 23 – Natural 23 | 6.31 (< **0.001**) | | |
| Summer first 23 – Simultaneous 23 | -7.73 (< **0.001**) | | |
| Fall first 23 – Natural 23 | 11.56 (< **0.001**) | | |
| Fall first 23 – Simultaneous 23 | -1.99 (0.49) | | |
| Natural 23 – Simultaneous 23 | -12.98 (< **0.001**) | | |

**Table S4.4.** Pairwise comparison results of seeding treatments after permutational analysis of variance tests of aboveground community composition in 2022 and 2023. Multiple comparisons were adjusted using a Bonferroni correction.

| Contrast | Composition | |
|---|---|---|
| | **2022** | **2023** |
| Summer first – Fall first | 3.28 (< **0.01**) | 5.18 (< **0.01**) |
| Summer first – Natural | 0.67 (0.81) | 1.58 (0.09) |
| Summer first – Simultaneous | 2.67 (< **0.01**) | 3.46 (< **0.01**) |
| Fall first – Natural | 2.47 (< **0.01**) | 2.77 (< **0.01**) |
| Fall first – Simultaneous | 1.91 (< **0.05**) | 2.53 (< **0.01**) |
| Natural – Simultaneous | 3.1 (< **0.01**) | 3.94 (< **0.01**) |