Ecological differences between early- and late-flowering grassland forbs

by

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DEDICATION

To dad, for teaching me to walk in the woods.

&

To Norman, the first Naturalist I have known.

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ABSTRACT

The loss of grassland ecosystems is widespread, and the Tallgrass Prairie region of the central United States is no exception. Restoration offers a way to restore prairie plant communities, but fully restoring the diversity found in remnant prairies can be difficult. One important goal in prairie restorations is to restore diverse forb communities and this includes having forb species that flower throughout the growing season (i.e., a range of flowering phenology). Despite this goal, forbs that flower early and late in the season may be absent in restorations.

We conducted several studies to determine whether early- and late-flowering species are absent in restorations, their impact on the community, and what may limit their establishment. This included analysis of a native forb relative abundance dataset, a field experiment, and a greenhouse germination experiment.

First, we compared relative abundances of early- and late-flowering species among remnants, seed mixes, and restorations. Remnant prairies had much higher relative abundance of early-flowering forbs than mixes or restorations. Late-flowering species had higher relative abundance in remnants than mixes.

Next, we conducted a field experiment at three restorations to test whether early- and late-flowering species were seed limited or recruitment limited and whether adding them to restorations increased plant diversity. Biomass of early- and late-flowering forbs was much higher when they were added as transplants than when added as seeds. A stage transition model indicated that survivorship of target forbs was much higher in transplant plots than in seed plots. Plant diversity of these restorations was higher when forbs were added compared to controls (no forbs added to existing restorations). Diversity was higher when target forbs were transplanted than when they were seeded. Early-flowering treatments increased diversity more than late-flowering treatments, and diversity increased with the number of forb species added. Our results indicate that early- and late-flowering species are recruitment limited, not seed limited, and that diversity is lower when these species are absent in restorations. We recommend transplanting a rich mix of forbs to overcome this limitation and ensure flowering occurs throughout the growing season.

Lastly, we conducted a greenhouse experiment to investigate how early- and late-flowering forb germination may be impacted by timing of smoke cues. Given the differential response of early- and late-season species to fire season, germination cues such as smoke may play an important and possibly overlooked role in overcoming potential germination limitation and establishing more diverse restorations. We tested nine forb species of early or late flowering phenology for germination response to aerosol smoke, cold stratification, and the timing of application of those two cues (smoke before cold vs. smoke after cold). We found that species had varied response to cold and that smoke increased germination of two species. Importantly, the timing of smoke application mattered with only smoke applied prior to cold having an impact. We also found that early-flowering species had far lower germination rates than late-flowering species. The varied germination response of species to cold and importance of timing of cues for two species may be important and suggests that more targeted approaches are needed for some species in order to establish more diverse restorations.

CHAPTER 1. GENERAL INTRODUCTION

World-wide, habitat loss and degradation are contributors to widespread biodiversity loss (Butchart et al. 2010). Reductions in biodiversity have wide-ranging effects including reduced ecosystem stability, reduced services such as carbon sequestration and crop pollination, and loss of aesthetic beauty of natural systems (Kennedy et al. 2001; Briggs et al. 2005; Isbell at al. 2011; Tilman et al. 2014). Restoration is a way to restore biodiversity where it has been lost. The practice of habitat restoration has become commonplace in some regions and the value of restoring habitats has gained attention. For example, in 2002, the Convention on Biological Diversity demonstrated consensus around this issue through agreement of some world leaders to reduce biodiversity loss (Balmford et al. 2005). Further, 2021 was the first year of the Decade on Ecosystem Restoration proclaimed by the United Nations General Assembly in 2019 (Aronson et al. 2020). This is a valuable acknowledgement that biodiversity conservation is an important goal and restoration of habitats is needed world-wide.

Grasslands are one such degraded ecosystem in need of restoration. Grasslands are widespread and are present in some form on every continent except Antarctica, covering an estimated 20-25 percent of land (Wilsey 2018). The state of grasslands as an ecosystem category in general is poor and they are considered “among the most imperiled biomes in the world” (Fuhlendorf et al. 2018). The Great Plains of the United States is no exception. The Great Plains region was an expansive system that stretched from the northern to southern border, east into Indiana, and west to the Rocky Mountains. The eastern third of this range was dominated by the Tallgrass Prairie ecosystem, an area which included the state of Iowa. The vast majority of the Tallgrass Prairie has been lost, largely due to conversion of land for agriculture, leaving only remnant patches (Samson and Knopf 1994; Bock and Bock 1998; Samson et al. 2004). Although the majority of prairie has been lost in the United States, it is an ecosystem type with few protections (Aycrigg et al. 2013). In Iowa, an estimated 99.9 percent of Tallgrass Prairie has been lost, largely due to land use conversion for agriculture and other anthropogenic development (Samson and Knopf 1994). This has left only fragments of Tallgrass Prairie. Where fragments remain, prairie is often degraded due to anthropogenic activities that allow for exotic and woody plant encroachment (Bock and Bock 1998; Cully et al. 2003; Ratajczak et al. 2012).

Aside from conserving remaining fragments, restoration is the primary method to ensure the Tallgrass Prairie's continued existence (Bock and Bock 1998; Rowe 2010). Restoration is the practice of re-establishing a habitat type at a site where that habitat is now degraded or absent. This is sometimes further defined as reconstruction, the practice of seeding prairie on bare ground, or rehabilitation, the practice of working to return a degraded site (such as a heavily grazed pasture) to its former state (Packard and Mutel 1997). In Iowa, prairie restoration has become common with at least 39,000 ha of conservation plantings and approximately 685,000 ha of roadside and Conservation Reserve Program plantings (Kaul and Wilsey 2019). Thus, the amount of restored prairie land is greater than the unplowed remnants in Iowa, which necessitates a greater ecological understanding of this land use class.

Plant Diversity in Tallgrass Prairie Restorations

Prairie restorations often have quite diverse plant communities with varied forbs and grasses (Grman et al. 2021). Even with the success of many restorations, they still generally do not achieve the plant diversity levels found in remnant prairies, especially when it comes to diversity of forb species (Sluis 2002; Martin et al. 2005; Polley et al. 2005; Barak et al. 2017). The lower diversity of restorations is in part due to grasses being overrepresented, and forbs being underrepresented in restorations (Derner et al. 2004; Hillhouse and Zedler 2011). Restorations can be deficient in whole clades or groups found in remnants (Barak et al. 2017).

One aspect of forb diversity that may be incomplete in restorations is having species that flower throughout the growing season, which is an important aspect of remnant prairies (Rabinowitz and Rapp 1980; Kindscher and Wells 1995, Craine et al. 2012). Grassland forbs can be categorized by seasonality of flowering phenology with distinct groups in the spring, summer, and fall (Kindscher and Wells 1995). Having a diversity of species that flower throughout the growing season may benefit pollinators through resource provisioning (Potts et al. 2003, Hines and Hendrix 2005, Timberlake et al. 2019).

Restorations may be deficient in early-flowering and late-flowering species (Howe 1994; Carter and Blair 2012, Havens and Vitt 2016; Deever et al., Chapter 2), although this has seldom been tested. Previous publications have suggested that species could be missing due to restoration seed mix collection methods, cost of seeds, and management practices that favor mid-season species (Howe 1994; Carter and Blair 2012; Gerla et al. 2012). This suggests that while prairie restorations can be diverse, they may be missing species or groups (such as early- and late-flowering forbs) that are important for resource provisioning. With this in mind, some management plans are based on the idea that flowering throughout the growing season should be encouraged. For example, in the USA, the Conservation Reserve Program CP42 mix requires including species that flower in the early, mid, and late season (Jackson and Meissen 2019). Though such additions of early- and late-flowering species are encouraged, there are knowledge gaps in how to successfully establish them.

Increasing Diversity is Tallgrass Prairie Restoration

Understanding what limits establishment of early- and late-flowering species and at what life stage those limitations exist is important for establishment of such species in restorations. To be recruited into flowering populations, seeds must be introduced and stages of development must be attained, including germination, emergence from the soil, and recruitment into juvenile and finally flowering stages (Harper 1977). Limitation can happen at any stage, but the early stages of seed and recruitment limitation are known to be important (Harper 1977; Turnbull et al. 2000). Study of these stages has resulted in two broad categories of what may limit species establishment into the community. The first, seed limitation, suggests that establishment is limited by seeds arriving successfully from the species pool (Tilman 1997; Foster and Dickson 2004). Seed additions commonly lead to the recruitment of a few new species (Nolan et al. 2021), but results vary among studies depending on the primary productivity of the site (Foster et al. 2004). Productive sites, which are indicative of tallgrass prairie restorations, can sometimes have fewer recruits from seed additions compared to less productive sites (Foster et al. 2004). In a restoration, if a species is seed limited, then simply adding its seed should result in recruitment into the community and thus increased adult-stage abundance and ultimately overall plant diversity. The second category of what may limit establishment is recruitment limitation. If a species is recruitment limited, then adding seeds will not lead to establishment unless hazards (e.g., pathogens, granivores, competitors, and water stress) are reduced. Disturbances that reduce competition, such as grazing and fire, can be important for recruitment of species (Fuhlendorf and Engle 2004; Pywell et al. 2004; Dickson and Foster 2008). In examination of seed additions and disturbance, grazing has been found to be more important to plant species recruitment than adding seeds (Wilsey and Martin 2015), suggesting stronger recruitment limitation than seed limitation.

Mowing is a management tool sometimes used in restoration thought to function as a disturbance (Kurtz 2001; Rowe 2010). Mowing has been shown to create vegetation canopy gaps that enable seedling establishment and recruitment and increase light availability and forb seedling establishment (Williams et al. 2007). However, mowing is not always done in restorations. In a sampling of 93 tallgrass prairie restorations in Iowa, Kaul and Wilsey (2021) found that less than half of sites with records had been mowed during establishment. Though it has been demonstrated that mowing can be important, it is still largely unknown whether mowing helps at the seedling establishment or recruitment stage.

In addition to establishment limitations of added species, diversity in restorations may be influenced though a priority effect. Addition of species that are active early or late in the growing season could reduce diversity via a priority effect (Werner et al. 2016; Weidlich et al. 2021; Wilsey 2021), which would result in suppressed establishment from the restoration seed mix. Alternatively, if these species differ ecologically from other forbs, then their additions may increase diversity if their absence results from gaps in temporal niche space (Wolkovich and Cleland 2014). Adding a greater number of species may be better than adding a single target species in this case, depending on the niche space available in the community or due to a sampling effect (i.e., the increased chance of a successful species being in a higher richness species addition, Loreau and Hector 2001).

Another factor that may limit plant species establishment is failure of seeds to germinate, or germination limitation. Often, increasing diversity in restorations revolves around seed additions and diversity of restoration seed mixes. Though seed mix diversity can be a predictor of planted species diversity in restorations, certain species and groups can be absent (Sluis 2002; Barak et al. 2017; Drobney et al. 2020). Seed additions can lead to the recruitment of some species (Turnbull et al. 2000; Sluis 2002; Nolan et al. 2021). Often a sub-set of species seeded do not establish and these species can be neglected when drawing conclusions since they did not contribute to diversity (Hillhouse and Zedler 2011; Grman et al. 2015). Hillhouse and Zedler (2011) suggests that an important and overlooked factor in restorations may be germination limitation. Germination may be limited for various reasons, but chemical cues are one factor known to influence germination of varied species (Baskin and Baskin 2014; Jefferson et al. 2014). Lack of appropriate cues may inhibit germination of some species and thus community diversity. Though some species have known germination cues (e.g., light, moisture, cold, chemical compounds), many more have not been investigated for these. Germination cues may be useful as seed treatments before seeding and may be an important aspect of increasing some species establishment in restorations (Baxter and Van Staden 1994). Smoke is one such cue that is important for some species (Brown and Van Staden 1997; Van Staden et al. 2000; Jefferson et al. 2014). Historically in the Tallgrass Prairie, smoke was created from wildfires that could occur during any time of year but often occurred in the summer season (Howe 1994a; McClain et al. 2021). In prairie management today, prescribed fires are often set in the dormant season (winter or early spring), which may impact plant diversity (Howe 1995). These different fire seasons can result in distinct plant communities distinguishable by richness of early-flowering or late-flowering species (Howe 1994b; Howe 1999; Howe 2011). Specifically, summer fires resulted in higher richness of early-flowering species and spring fires resulted in increased warm-season grass species (Howe 1999; Howe 2011). Given that timing of fire (fire season) can be important, it may be that timing of application of cues such as smoke is important. Further, the impact of cues may differentially impact early- and late-flowering species. Germination cues and timing of their application may be important for increasing establishment of some species in restorations and thus overall plant diversity.

Objectives

The first objective of this research was to determine if forbs of early and late flowering phenology were less abundant in prairie restorations compared to remnant prairies, whether they were seed or recruitment limited, and if they had an effect on diversity due to a priority effect or empty niche gap. Few studies consider flowering phenology specifically when evaluating restoration diversity and no other studies have used our methods in order to compare possible seed and recruitment limitation of these species.

The second objective of this research was to determine if early- and late-flowering forb germination rate responded to cold and smoke treatments and if the timing of these cues was important. Cold and smoke are both known to influence germination in various species but timing of application of these cues in order to mimic timing of smoke from seasonal burns has not been explored in previous research.

Thesis Organization

The thesis is divided into four sections. The first chapter consists of a general introduction to the topic and study questions and discusses the main objectives of the research. The second and third chapters are papers prepared for publication. The second chapter consists of two parts. The first is a study of prairie restorations and remnants to compare relative abundances of early- and late-flowering species. The second is a forb addition experiment in restorations that compares seed and transplant success to determine if species are seed or recruitment limited, whether diversity of restorations is increased by additions of early- and late-flowering species, and whether additions of more species is preferable to additions of fewer species. The third chapter consists of a greenhouse seed germination experiment testing the effects of cold and smoke cues and their timing of application on germination rate of early- and late-flowering prairie forbs. The final chapter discusses the overall conclusions and future considerations for forb establishment in tallgrass prairie restoration.

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CHAPTER 2. RECRUITMENT LIMITATION OF EARLY- AND LATE-FLOWERING GRASSLAND FORBS CAN BE OVERCOME WITH TRANSPLANTING IN PRAIRIE RESTORATIONS

Modified from a manuscript to be submitted to *Journal of Applied Ecology*

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Abstract

One important goal in prairie restorations is to have forb species that flower throughout the growing season (i.e., a range of flowering phenology). We compared relative abundance of early- and late-flowering species among remnants, seed mixes and restorations and conducted an experiment to test whether they are seed or recruitment limited. We also tested whether adding them to restorations will increase plant diversity. The experiment was conducted at three locations (two restorations in northern Iowa and one in southern Minnesota) using a split-plot design. Main plots included mowed and unmowed treatments. Subplots tested the effect of forb additions. These were the addition of six species (three early-flowering and three late-flowering) added as seeds or transplants with varied richness levels (each species added alone, three early species added in mixture, three late species added in mixture, or the six species added in an early and late species mixture). Control plots received no forb additions. To compare establishment success, we counted seedlings and estimated target species biomass across three years (2019-

2021). Plant diversity measures (richness, Simpson’s diversity) were estimated with point-intercept techniques to determine if forb additions increased diversity. Remnant prairies had much higher relative abundance of early-flowering forbs than mixes or restorations. Late-flowering species had higher relative abundance in remnants than mixes. Biomass of early- and late-flowering forbs was much higher when they were added as transplants than when added as seeds. A stage transition model indicated that survivorship of target forbs was much higher (75%) in transplant plots than in seed plots (0.6%). Plant diversity of these restorations was higher when forbs were added compared to controls (no forbs added to existing restorations). Diversity was higher when target forbs were transplanted (8.4 species/plot and 1/D of 4.0) than when they were seeded (7.6 species/plot and 1/D of 3.8). Early-flowering treatments increased diversity more than late-flowering treatments, and diversity increased with the number of forb species added. Mowed and unmowed plots had similar levels of target species establishment and plant diversity. Our results indicate that early- and late-flowering species are recruitment limited, not seed limited, and that diversity is lower when these species are missing in restorations. We recommend transplanting a rich mix of forbs to overcome this limitation and ensure flowering occurs throughout the growing season.

Introduction

Reductions in biodiversity can reduce ecosystem stability, productivity, invasion resistance, and ecosystem services (Tilman et al. 2001; Kennedy et al. 2001; Tilman et al. 2014). Ecosystem restoration offers a way to re-establish high biodiversity, however, many prairie restorations (also known as reconstructions) fail to reach the plant diversity levels found in remnant prairies, especially when it comes to forb species (Sluis 2002; Polley et al. 2005; Barak et al. 2017). The lower diversity is in part due to grasses being overrepresented and forbs being underrepresented in restorations (Derner et al. 2004; Hillhouse and Zedler 2011). Forbs are especially important to pollinators as sources of nectar and pollen, and producing robust populations of flowering forb species is often a goal of prairie restorations.

One particular aspect of forb diversity that may be incomplete in restorations is having species that flower throughout the growing season, which is an important aspect of remnant prairies (Rabinowitz and Rapp 1980; Kindscher and Wells 1995; Craine et al. 2011). Grassland forbs can be categorized by seasonality of flowering phenology with distinct groups in the spring, summer, and fall (Kindscher and Wells 1995). Having species flower throughout the growing season may benefit pollinators (Potts et al. 2003, Hines and Hendrix 2005, Goulson et al. 2015, Timberlake et al. 2019), and many management plans are based on the idea that flowering throughout the growing season should be encouraged. For example, in the USA, the Conservation Reserve Program CP42 mix requires including species that flower in the early, mid, and late season (Jackson and Meissen 2019).

Despite such acknowledgements that a full range of flowering phenologies is important, restorations may be deficient in early flowering species (Howe 1994; Carter and Blair 2012; Havens and Vitt 2016), although this has seldom been tested. They may be deficient in late flowering species as well. Previous publications have suggested that species could be missing due to seed mix collection methods that miss early flowering species, cost of seed, and management practices that favor warm-season species (Howe 1994; Carter and Blair 2012). In particular, mechanical combine harvest of prairie seed in the fall season has been noted to miss short stature and early flowering species (Gerla et al. 2012) and could also miss species that set seed in late season after typical harvest time.

Understanding what limits establishment of early and late flowering species and at what life stage those limitations exist is important for their restoration. For species to be recruited into flowering populations, seeds must be introduced and stages of development must be attained, including germination, emergence from the soil, and development into juvenile and finally flowering stages (Harper 1977). Limitation can happen at any stage, but the early stages of seed and recruitment limitation are well known to be most critical (Harper 1977; Grime 2001). Seed limitation suggests that establishment is limited by seeds arriving successfully from the species pool (Tilman 1997; Foster and Dickson 2004). Seed additions commonly lead to the recruitment of a few new species (Nolan et al. 2021), but results vary among studies depending on the primary productivity of the site (Foster et al. 2004). Productive sites, which are typical of tallgrass prairie restorations, can sometimes have fewer recruits from seed additions compared to less productive sites due to competition (Foster et al. 2004). In a restoration, if a species is seed limited, then simply adding its seed should increase adult-stage abundance and ultimately overall plant diversity. If a species is recruitment limited, then adding seeds will not lead to establishment unless other factors (e.g., pathogens, granivores, competitors, and water stress) are reduced. Bison grazing, mowing, and other disturbances have been found to be more important to species recruitment than adding seeds (Wilsey and Martin 2015), suggesting stronger recruitment limitation than seed limitation. Here, we compare seed versus recruitment limitation between early- and late- flowering forbs in new prairie restorations.

Mowing during establishment is a common management tool in new restorations. Mowing has been suggested to reduce competition from dominant species and increase target species establishment (Kurtz 2001) and is sometimes used in the establishment stage of restorations (Rowe 2010). Mowing has been shown to create vegetation canopy gaps that enable seedling establishment and recruitment and increase light availability and forb seedling establishment (Williams et al. 2007). However, mowing is not always done in restorations. In a sampling of 93 tallgrass prairie restorations in Iowa, Kaul and Wilsey (2021) found that less than half of sites with records had been mowed during establishment. It is still largely unknown whether mowing helps at the seedling establishment or recruitment stage.

Adding forbs to ongoing restorations, especially those with very early or late flowering phenology, may affect plant diversity. Addition of species could reduce diversity via a priority effect if their arrival suppresses establishment from the restoration seed mix (Werner et al. 2015; Wilsey 2021; Weidlich et al. 2021). Alternatively, if these species differ ecologically from other forbs, then their additions may increase diversity if their absence results from gaps in temporal niche space (Wolkovich and Cleland 2014). Adding a greater number of species may be better than adding a single target species in this case, depending on the niche space available in the community or due to a sampling effect (i.e., the increased chance of a successful species being in a higher richness species addition, Loreau and Hector 2001). Here, we test whether forb additions to existing restorations will alter diversity and if effects are greater when a greater number of species is added.

We report on a comparative study and an experimental study on what determines abundance of early and late flowering species in restorations. Firstly, we compared early and late flowering forb species relative abundances in remnants, restoration seed mixes, and established restorations. Secondly, we conducted a forb addition and mowing experiment in newly established restorations to compare the effects of mowing and forb additions. Forb species with extreme flowering phenology (early or late) were the focus of our study. We tested four hypotheses: (i) early- and late-flowering species are underrepresented in restorations compared to remnants, (ii) recruitment limitation is stronger than seed limitation in these groups, especially for early-flowering species, (iii) lack of early- and late-flowering species leaves gaps in restoration diversity that are best filled with higher richness forb additions, and (iv), mowing results in increased recruitment of target early- and late-flowering forbs and in increased plant diversity.

Methods

Comparative study comparing remnants and restorations

To test if early- and late-flowering forb species are under-represented in restorations, we utilized a dataset from sampling of five prairie remnants (defined as never plowed or heavily grazed), 48 restoration seed mixes, and 93 established restorations across Iowa (Kaul and Wilsey 2021). Data on plant abundance for remnants and restorations were collected during July 2015 and 2016 using the point-intercept method and relative abundances were calculated by species (Kaul and Wilsey 2021). Species were grouped into graminoids, woody plants, or forbs, and their relative abundances were calculated by dividing abundances of each type by the total. Each forb species was assigned a designation of early-, mid-, or late-flowering and native or exotic based on mean flowering month and native status determined by a flora manual (McGregor et al. 1986; Kaul et al. 2011).

Forb addition experiment

Study Sites

We conducted the restoration experiment at three sites located in northern Iowa and southern Minnesota, USA (43.1558, -94.8841; 43.4961, -93.5589; 43.7035, -94.3260). The soils are Mollisols, and annual precipitation among sites is approximately 750 mm, 814 mm, and 864 mm (means from available data 1956-2021). Sites were planted in 2019 and followed through 2021. In 2019 sites received above average precipitation (approximately 994 mm, 1084 mm, and 1280 mm) and in 2020 sites received below average precipitation (approximately 513 mm, 660 mm, and 762 mm). In 2021 sites received approximately 835 mm, 604 mm, and 711 mm. Sites were treated as blocks in the design. Each site was a field approximately four-hectares in size that had been planted to corn (*Zea mays* L.) or soybean (*Glycine max* (L.) Merr) crops previously. Prairie seeding was done in March 2019. Each site was seeded with a separate seed mix (approximately 120-, 190-, and 70-species per site) that excluded study species. We established study plots and added study seeds and transplants in May 2019.

Experimental Design

We used a randomized block split-plot design with blocking by site. Each site had four approximately 40 m x 220 m main plots that were treated with mowing treatments (n = 2), and 20 4-m^2 subplots within each main plot that were treated with forb addition treatments. Thus, there was a total of 240 plots (3 sites x 4 main plots x 20 subplots per main plot).

Main plots tested the effect of mowing. Mowed plots were mowed 1-3 times per year to a height of 6 inches in the first two growing seasons (2019 and 2020). Photosynthetically active radiation (PAR, 400-700 nm) was measured in 10-20 locations per mowed and unmowed plot in 2019 at peak biomass (late August-early October). Measurements (μmol m^{-2} s^{-1}) were taken above the canopy and at the soil surface with a Decagon AccuPAR Ceptometer. Proportion of available PAR was calculated by dividing soil surface PAR for each location by the associated above-canopy PAR. Proportion of available PAR at soil surface was significantly higher in mowed plots (0.56 ± 0.02) than in unmowed plots (0.34 ± 0.02) after the first growing season of the restoration

Subplots tested the effects of forb additions by comparing seed vs. transplant additions, early vs. late flowering phenology, and the effect of richness (1-6 species added). In each 2 x 2 m subplot within each main plot, we applied forb addition treatments using a 2 x 10 factorial treatment arrangement, using 2 levels of addition type (as seed, or as small transplants) x 10 treatment combinations (3 early or 3 late flowering species added as monocultures, all three early or all three late flowering species added in mixture, all six species added, or nothing added as controls). The six forb species were selected based on their early or late flowering phenology; the early-flowering species were *Pedicularis canadensis*, *Pulsatilla patens,* and *Phlox pilosa* and late-flowering species were *Solidago nemoralis*, *Symphyotrichum novae-angliae,* and *Symphyotrichum sericeum*. Seed addition plots received 1080 seeds/plot (270 seeds/m^2). Seeds of appropriate ecotype (Iowa Zone 1) for the field sites were acquired from suppliers (Prairie Moon Nursery, Winona, MN; Allendan Seed Company, Winterset, IA). Transplant plots received 6 transplants/plot. Transplants were produced by growing the same seed in Iowa State University's Bessey Hall greenhouse (Ames, IA) for approximately two months before moving to the field site. Transplants were small juveniles (mean above ground mass 0.1 g; mean root mass 0.01 g) having recently developed past the seedling stage (defined by reliance on cotyledons by Fenner and Thompson 2005).

To test questions about differences in early and late flowering phenology species and how number of species added impacts the community, forbs were added as single species additions or mixture additions. Mixtures were added as the same abundance of monocultures (1080 seeds or 6 transplants per species, S = 1), either using the three early or three late flowering species (360 seeds per species, or 2 transplants per species, S = 3), or all six species combined (180 seeds per species, or 1 transplant per species, S = 6). Control plots received no forb additions.

Sampling Design

To compare establishment success, we counted target species seedlings and transplants and estimated target species aboveground biomass in each subplot in September 2019, May 2020, August 2020, June 2021, and August 2021. Biomass was measured to estimate the success of target plants in the treatments, and it is strongly correlated with flowering number and fitness (Harper 1977; Younginger et al. 2017). Individual plant biomass was estimated using basal area (calculated with the mean of two width measurements) x height measurements of one randomly selected plant of each species per plot. We then used calibrations by species to convert values to predicted biomass per plot. Calibrations were developed by making basal area x height measurements, deriving estimated biomass from these measurements, and then converting these measurements into actual biomass by drying and weighing the plant material. Plants for this process were collected in 2020 from local prairie areas outside the study locations. Predicted biomass per plot from basal area x height estimates was a strong predictor of actual biomass in all target species (R^2 values of 0.88-0.98 across species) (Supplemental table 1). Biomass per plot was calculated by multiplying calibrated biomass estimates by the number of individuals in that plot. We report here on biomass derived from these calibrations. Survivorship was calculated by dividing target species abundance by the number of planted individuals per plot. We acknowledge that some seeds may have remained viable after the three-year study period. Reported survivorship is based on this three-year period.

To determine the effect of treatments on plant diversity at the community-level, species diversity per plot was measured in late August 2020 and August 2021 using a point-intercept method. We placed a 50 cm x 50 cm quadrat in a random location in each plot and dropped five pins (one at each corner and one in the center of the quadrat), counting all hits on each pin by species. Species present in the quadrat but not touching a pin were given a value of 0.5 hits. Species diversity was quantified as the number of species per plot (S) and as species diversity based on a Simpson's diversity measure (Simpson's reciprocal index, 1/D, or $1/\sum p_i{}^2$, where p_i is the relative abundance of species i, or hits/total number of hits per plot). Relative abundance of graminoid species was also calculated by dividing the total number of hits by grasses plus sedges by the total.

Statistical Analyses

Comparative study

To compare relative abundances of early-, mid- and late-flowering forb species among restorations, remnants, and restoration seed mixes, we compared groups with a non-parametric Wilcoxon sign test (NPAR1WAY in SAS). We used only native forb relative abundances in the analysis. A Wilcoxon sign test was used because data were not normally distributed.

Forb addition experiment

Biomass, abundance, and survivorship of target forbs were analyzed with a randomized block split-plot repeated measures ANOVA. Site was a random block term. Mowing (main plot treatment), and the forb addition treatments (subplots) were fixed effects. *P. canadensis* monoculture plots were removed from the analysis due to no seedling emergence and only three individual transplants at one site (2 out of 72 plots) being observed. Repeated measures analysis used an AR(1) covariance structure and Kenward-Roger corrections. Biomass and abundance were logarithmically transformed (y + 1) to meet normality assumptions. Post-ANOVA *a priori* contrasts were used to compare means when treatment effects were significant. To test if recruitment is related to early or late flowering phenology, we used a contrast to test for differences between these two plot types. This contrast compared combined means of all early phenology treatments to combined means of all late phenology treatments. To test the effect of richness in the forb addition treatment on abundance and biomass, we used linear contrast across treatment richness levels (1, 3, and 6 species). Contrast coefficients for each richness level were estimated with Proc IML in SAS 9.4. When interactions were significant, slice tests were used to test for significant differences among treatment levels.

Simpson's diversity, richness, and graminoid relative abundance were compared among treatments with the same mixed-model analysis explained above. Richness and Simpson's diversity were logarithmically transformed (y + 1) to meet normality assumptions. Post ANOVA *a priori* contrasts were control vs. all treatment plots, early vs. late flowering treatments, and a linear contrast with the addition of control plots (0 species added, 1, 3 and 6 species). All analyses were conducted with SAS 9.4.

Results

Comparative study

Early-flowering species were more abundant in remnants compared to prairie restorations (Figure 1). Remnant prairies had more than five times higher abundance of early flowering forbs ($Z = 2.99$; $p = 0.01$, Figure 1a) and roughly two times higher abundance of mid-season forbs ($Z = 2.53$; $p = 0.03$, Figure 1b) than restorations. Late flowering forb abundance was not significantly different between remnants and restorations ($Z = 1.09$; $p = 0.52$, Figure 1c).

There were also differences in species abundances between remnants and the composition of restoration seed mixes. Early-season forbs were more abundant in remnants than in restoration mixes ($Z = 2.59$; $p = 0.03$, Figure 1a), and mid-season flowering species were about two times greater in remnants than seed mixes ($Z = 2.50$; $p = 0.03$, Figure 1b). Late flowering species were four times more abundant in remnants than mixes ($Z = 3.11$; $p < 0.01$, Figure 1c).

Forb addition experiment

Target forb biomass and abundance

Mowed and unmowed plots had similar levels of target forb biomass ($F_{1,8} = 0.4$; $p = 0.53$), abundance ($F_{1,164} = 0.1$; $p = 0.72$), and survivorship ($F_{1,8} = 0.4$; $p = 0.56$) (Table 1). Mowing did not interact with other treatments or change over time (Table 1).

Target forb biomass varied among the forb addition treatments ($F_{7,150} = 29.1$; $p < 0.001$). Biomass was almost four times higher when they were added as transplants (46.5 g/plot, ± 3.8 SE) than when added as seeds (12.9 g/plot, ± 3.8 SE) ($F_{1,156} = 177.3$; $p < 0.001$) (Figure 2a). When broken down by treatment, biomass was significantly higher in transplant than in seed plots ($F_{7,150} = 5.5$; $p < 0.001$), but the effect size varied among treatments. All treatments had 2-4 times higher biomass when introduced as transplants (Supplemental table 2) and the magnitude of this difference was larger for late flowering species (Table 1). Late-season flowering treatments produced significantly more biomass than early flowering treatments (contrast $F_{1,150} = 120.2$; $p < 0.001$). Biomass increased differentially over the course of the experiment ($F_{4,40} = 43.2$; $p < 0.001$). Biomass of each treatment fluctuated over time ($F_{28,660} = 7.8$; $p < 0.001$) as did the difference between seed vs. transplant ($F_{4,660} = 7.9$; $p < 0.001$) as biomass changed seasonally. Compared to seed plots, transplant plots of each forb addition treatment consistently had higher biomass over the course of the experiment ($F_{28,660} = 3.1$; $p < 0.001$) and differences for late flowering species were larger at the end of the growing season than at the start (Figure 3). Target forb biomass was higher with a greater number of species added (linear contrast $F_{1,156} = 7.3$; $p < 0.01$) (Table 1).

Target forb abundance varied significantly between seed vs. transplant ($F_{1,156} = 15.8$; $p < 0.001$) and varied among the forb addition treatments ($F_{7,156} = 10.1$; $p < 0.001$). Abundance was higher when forbs were added as transplants than when added as seeds (Figure 2b), even though many more individuals were added to a seed (1080) plot vs. a transplant (6) plot. Forb addition treatments and seed vs. transplant interacted ($F_{7,156} = 6.7$; $p < 0.001$). The majority of transplant plot treatments had higher abundance than seed plots (Supplemental table 2), and a slice test indicated that transplanting resulted in similar or significantly higher abundance compared to seeding for each forb addition treatment (Table 1). Early flowering treatments had significantly lower target forb abundance compared to late flowering treatments (contrast $F_{1,156} = 6.5$; $p = 0.01$). Time was important ($F_{4,41} = 22.1$; $p < 0.001$) as abundance of target species increased differentially over the course of the experiment. This rate of increase varied among forb addition treatment ($F_{28,600} = 2.5$; $p < 0.001$) and between seed and transplant ($F_{4,660} = 37.9$; $p < 0.001$). In some cases, seed plots caught up to transplant plots in seedling abundance, but this was not the case for biomass (described above) as many seeded individuals remained small. The difference in seed vs. transplant changed differentially for each forb addition treatment over time ($F_{8,660} = 1.9$; $p < 0.004$). A slice test indicated that this was because transplant plots remained fairly stable until some began to produce new seedlings in the last year of the experiment and seed plots increased in abundance until starting to level off in the last year (Figure 4). Target forb abundance was higher with a greater number of species added (linear contrast $F_{1,156} = 11.4$; $p < 0.001$) (Table 2).

Percent survivorship followed the same trends as abundance (Table 1) but suggested that the limiting step for target forbs was recruitment. Overall survivorship of seeds was extremely low compared to transplanted forbs after three years (Figure 2c). Each treatment had much higher survivorship in transplant plots (Supplemental table 2). Forb addition treatments varied significantly and the magnitude of the difference between seeded and transplanted forbs was much greater (Table 1).

Though early flowering species had lower success overall, both early and late flowering species were similarly limited at the seed-to-juvenile transition stage (Figure 7). Early flowering species had 0.4% (± 1.8% SE) survivorship when added as seeds and 51% (± 1.8% SE) when added as transplants. Late flowering species had 0.6% (± 1.9% SE) survivorship when added as seeds and 79% (± 1.9% SE) when added as transplants. The overall percent of seeds surviving to the juvenile stage was extremely low (0.6% ± 1.8% SE of 1080 seeds added). The percent of juvenile transplants surviving to the adult stage was high (75% ± 1.8% SE of 6 transplants added). The stage transition model indicated that the percent of seeds surviving to the adult stage is less than 1% in all cases (Figure 7).

Species diversity and graminoid: forb ratios

Mowed and unmowed plots had similar species richness ($p = 0.7$) with an average of 7.9 species/plot in unmowed plots and 8.0 species/plot in mowed plots. Mowing did not interact with other treatments (Table 2). Mowed and unmowed plots had similar Simpson's diversity ($p = 0.2$) with an average of value of 3.8 in unmowed plots and 4.0 in mowed plots. Mowing did not interact with other treatments (Table 2). Mowed and unmowed plots had similar graminoid abundance ($p = 0.9$) with a mean relative abundance of 0.5 in unmowed and mowed plots. Mowing did not interact with other treatments (Table 2).

Forb addition treatments increased diversity (species richness and Simpson's 1/D) of restorations (Table 2). Overall, restorations were diverse with an average of 52 seeded species recorded per site. Forb addition treatments had greater richness (contrast $F_{1,170} = 7.2$; $p < 0.008$) and Simpson's diversity (contrast $F_{1,170} = 10.0$; $p = 0.002$) than control plots (Figure 5). Diversity was higher when target forbs were transplanted (8.4 species/plot and 1/D of 4.0) than when they were seeded (7.6 species/plot and 1/D of 3.8) (Table 2). Early flowering treatments resulted in higher richness (contrast $F_{1,170} = 7.8$; $p = 0.006$) and Simpson's diversity (contrast $F_{1,170} = 76.0$; $p = 0.02$) than late flowering treatments (Figure 5). Species richness and Simpson's diversity linearly increased with the number of forb species added (linear contrast: richness $F_{1,170} = 9.2$; $p = 0.003$, diversity $F_{1,170} = 7.2$; $p = 0.01$) (Figure 6). The seed vs. transplant differences were consistent across forb addition treatments ($p = 0.6$) (Table 2).

Diversity increased between year two and three with an average of 7.1 species and 1/D of 3.6 per plot in 2020 and 8.6 species and 1/D of 4.3 in 2021 (Table 2). Treatment effects did not change significantly with time (Table 2).

Forb addition treatments also altered relative abundance of graminoid species in plots ($F_{8,170} = 3.0$; $p = 0.003$). The most abundant grasses were little bluestem (*Schizachyrium scoparium*) (pi = 0.22), Junegrass (*Koeleria macrantha*) (pi = 0.09), and Sideoats grama (*Bouteloua curtipendula*) (pi = 0.08). The most abundant forbs were giant goldenrod (*Solidago gigantea*) (pi = 0.03), Canada goldenrod (*Solidago canadensis*) (pi = 0.02), and yellow coneflower (*Ratibida pinnata*) (pi = 0.02). Treatments had lower relative abundance of graminoids compared to control plots (contrast $F_{1,170} = 6.5$; $p = 0.01$) (Figure 5c). Graminoid relative abundance was lower when target forbs were transplanted (Pi = 0.48) than when they were seeded (Pi = 0.53) ($F_{1,170} = 5.3$; $p < 0.02$). When broken down by treatment the graminoid relative abundance difference in seed vs. transplant plots was significant for two treatments (Supplemental table 3). Early flowering treatments resulted in higher graminoid relative abundance than late flowering treatments (contrast $F_{1,170} = 12.6$; $p = 0.001$) (Figure 5c). Richness level of forb additions was not related to graminoid relative abundance of plots (Table 2). Graminoid relative abundance increased between year two and three ($F_{1,196} = 59.8$ $p < 0.001$) with a mean value increasing from 0.42 in 2020 to 0.59 in 2021. Treatment effects did not change with time (Table 2).

Discussion

Prairie restorations, which are often highly diverse (Grman et al. 2013), often have lower diversity than remnants (Sluis 2002; Camill et al. 2004; Martin et al. 2005). In this study, we compared remnants and restorations to determine if early- and late-flowering forb species are less abundant in restorations and experimentally compared factors affecting success of early- and late-flowering species in restorations. We found that (i) both restoration mixes and restored prairies in our study were deficient in early-flowering species, (ii) restoration seed mixes were deficient in late-flowering species, (iii) both early- and late-flowering species were more recruitment than seed limited, (iv) higher richness forb additions increased diversity more than lower richness additions, and (v) mowing had smaller than expected effects and did not increase community-level diversity or target forb establishment.

The lower abundance of early flowering native forbs in restorations compared to remnants supports our hypothesis that these species are less common in restored sites, while the similar abundance of late flowering native forbs in restorations suggests that this is not necessarily true of this group. One likely explanation is that restorations contained an abundance of the late flowering *Solidago gigantea* and *S. canadensis*, which can come in as volunteers. Volunteer species can alter realized diversity of restorations (Barak et al. 2017). Our analysis of restoration seed mixes supports this as it shows that late-season species are not seeded at remnant abundance levels but realized late-season relative abundances were similar.

Another interesting result of this analysis is that early-season flowering forb relative abundance in seed mixes was lower than that in remnants but higher than in restorations. This means that some early flowering species were seeded, but that these species did not establish at seeded-level abundances in restorations. Havens and Vitt (2016) found that commercial seed mixes had very few or no early flowering species, but they did not report on relative abundances. In our analysis, this abundance of early flowering species in restoration seed mixes was driven by the abundance of *Zizia aurea* in the majority of mixes.

The greater recruitment limitation than seed limitation that we found here is important for prairie restoration. Our results demonstrate the efficacy of using transplants over seeds to overcome recruitment limitation and establish forb species that may be absent in restorations. Our results indicate that early and late species were recruitment limited since transplanting enabled forbs to bypass the early stages of establishment and have greater success than seeded forbs despite the much smaller number of transplants added. Early species established less successfully overall. This is likely at least in part because early flowering species tend to be shorter in stature and less competitive than later season species so are more likely to need a microsite or some other competitive edge. Previous studies have found microsites to be important for overcoming recruitment limitation (Pywell et al. 2004; Dickson and Foster 2008;

Wilsey and Martin 2015) and have found restorations to be seed limited in the short term, with seed additions increasing diversity in the first year while microsite creation alone did not (Martin and Wilsey 2006). Further study, however, demonstrated that the effect of seeding decreased over time and microsites (recruitment limitation) became more important than for long-term establishment of target species (Wilsey and Martin 2015).

Direct comparison of seeding and transplanting has not been widely explored to determine what stage limits recruitment. Middleton et al. (2010) found that a combination of seed additions and transplants increased prairie plot diversity more than seeding alone, but their experiment did not compare success of seeds alone to transplants alone. While not common in prairie restoration, transplanting has been used experimentally for the establishment of certain understory grass species (Page and Bork 2005) and transplanting small juveniles or rhizomes has been shown to be successful over seeding for establishment of some *Asclepias* and *Sisyrinchium* species into intact vegetation (Brown and Bugg 2001; Shryock 2021). While not necessary for species that readily establish from seed, this method could be especially helpful for target species such as our early- and late-flowering species. This includes species that are absent in restorations, that fail to establish from seed, and for which seed may be difficult to acquire or expensive. This may be particularly true of "conservative" species (Swink and Wilhelm 1994) which are largely found only in remnants. The rarity of both the plants and seed of these species suggests that transplanting may be an ideal option to ensure their establishment; as we demonstrated, transplanting target species can greatly increase survivorship and results in lasting increases to biomass over seeding, providing species a competitive advantage.

One might expect that over time plots with 1080 seeds would catch up to plots with six transplanted individuals, but we did not find this. Our seed plots did increase in forb density over the course of the experiment, but abundance fluctuated seasonally and even in cases where seeded abundance was relatively high (e.g., *S. novae-angliae*), survivorship was extremely low and plots did not reach total biomass levels of transplanted plots. This suggests that seeded individuals were less competitive and tended to be less robust than transplanted plants. This is important since biomass is a good indicator of herbaceous plant fitness and rates of flowering (Younginger et al. 2017). Plots that received transplants had greater flower production than seeded plots (Soley et al., unpublished data).

Transplanting can be a valuable technique in restoring diverse plant communities. Transplanted forbs increased diversity more than seeded forbs, and forb additions in general increased diversity and reduced the dominance of grasses. This increased diversity suggests that the target early- and late-flowering forbs did not have negative effects on establishing seed mix forbs due to priority effects. Seeding rare species before common ones can lead to higher diversity than when they are seeded at the same time because rare species will be able to establish with less competition (Werner et al. 2015). Transplanting our target species at the same time the general mix was seeded enabled them to be a life stage ahead and allowed them to establish with less competition than seeded target species while not suppressing community establishment. This finding is important for the restoration of diverse plant communities.

Diversity was increased more by early-flowering species than by late-flowering species and increased more when more forb species were added. All forb additions increased diversity which suggests that there was an open niche gap for early and late species in our restorations. This suggests that it is better to use more species when designing restorations in order to ensure filling of early and late season gaps, either due to niche differences with existing species or by the sampling effect. The sampling effect (Loreau and Hector 2001), or the increased chance of choosing a successful species with increased richness, can influence the mechanisms (complementarity and selection effects) that underpin the benefits of increased biodiversity making it an important consideration in restoration species mix design (Wilsey 2021). Sites vary and environmental conditions may continue to change with time, so more species rich additions will always be better for restoring productive communities since they are more likely to include species that will be successful (Wilsey 2021). Filling of early and late floral resource gaps will likely be accomplished by a rich mix of these early and late species.

In our study, one early flowering species (*Pedicularis canadensis*) had poor establishment. *Pedicularis canadensis* may have had poor establishment because it is a hemiparasite, and the open ground nature of a first-year restoration may have not provided the correct hosts. Hemiparasites can play an important role maintaining grassland plant diversity (Pywell et al. 2004) but have been noted to be absent in restorations (Barak et al. 2017). Discovering methods to establish species in this group should be an important objective of future studies.

We did not find an effect of mowing in our field study. Previous research on the effect of mowing suggests that it is beneficial in the establishment stage of restorations through suppression of more competitive species and creation of microsites that aid seedling establishment (Peltzer and Wilson 2001; Williams et al. 2007; Kaul and Wilsey 2021). Our results are contrary to this as we found no difference in target species seed or transplant success or community-level diversity between mowed and unmowed treatments. A possible explanation for these different outcomes is the frequency of mowing. Moeller (1998) and Borchardt (2001) found that more frequent mowing to continuously keep vegetation below a certain height resulted in increased richness and diversity of restoration plots. Similarly, Wilson and Clark

(2001) found more frequent and longer-term mowing (four years) reduced weed pressure and promoted native species. Our plots were mowed 1-3 times in the first and second year of the restorations which may not have been frequent enough to see the mowing effect reported in other studies. In addition, some of our transplanted species grew large enough to be physically damaged by mowing (personal observation) which may have influenced our biomass measurements in the first two years of the experiment. Mowing did have an effect on flowering rates and having flowering extended more continuously throughout the growing season (Soley et al. unpublished data).

In conclusion, our results indicate that in order to establish early and late-flowering forb species, transplanting a rich mix of forbs can be used to overcome recruitment limitation and ensure success of some species in order to increase diversity of restorations and ensure resources throughout the growing season.

Acknowledgements

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Tables and Figures

Table 1. Results from a three-year forb addition experiment in prairie restorations in northern Iowa and southern Minnesota, USA. Data was collected at five time points over the experiment. A randomized block split-plot repeated measures ANOVA was used to analyze early and late flowering target forb biomass, abundance, and survivorship. Main plots were mowed or unmowed ("Mow") and early and late flowering forbs added to subplots at varied richness levels ("Treatment") as seeds or transplanted (S vs. T). Contrasts compared the mean of all late flowering treatments to the mean of all early flowering treatments (early vs. late) and the effect of increased richness forb additions (diversity-linear). A slice test of the S vs. T*Treatment interaction was performed.

	Biomass (ln)			**Abundance (ln)**			**Survivorship**		
Effect	*F*	*P*	d.f.	*F*	*p*	d.f.	*F*	*P*	d.f.
Mow	0.4	0.53	1, 8	0.1	0.72	1, 164	0.4	0.56	1, 8
Treatment	29.1	< **0.001**	7, 150	10.1	< **0.001**	7, 156	23.4	< **0.001**	7, 150
S vs. T	176.0	< **0.001**	1,150	15.8	< **0.001**	1, 156	2285.2	< **0.001**	1, 150
Treatment*S vs. T	5.5	< **0.001**	7, 150	7.0	< **0.001**	7, 156	23.0	< **0.001**	7, 150
Treatment*Mow	0.8	0.60	7, 150	0.5	0.81	7, 156	1.5	0.19	7, 150
S vs. T*Mow	0.4	0.52	1, 150	1.0	0.33	1, 156	0.9	0.35	1, 150
Treatment*S vs. T* Mow	1.1	0.36	7, 150	0.6	0.77	7, 156	1.5	0.19	7, 150
Time	43.2	< **0.001**	4, 40	22.1	< **0.001**	4, 40.6	3.5	**0.02**	4, 40
Mow*Time	1.2	0.32	4, 40	1.9	0.13	4, 40.6	2.7	**0.04**	4, 40
Treatment*Time	7.8	< **0.001**	28, 660	2.5	< **0.001**	28, 660	1.7	**0.02**	28, 660
S vs. T*Time	7.9	< **0.001**	4, 660	38.0	< **0.001**	4, 660	3.2	**0.01**	4, 660
Treatment*S vs. T*Time	3.1	< **0.001**	28, 660	1.9	**0.004**	28, 660	1.6	**0.03**	28, 660
Contrasts									
early vs. late	120.2	< **0.001**	1, 150	6.5	**0.01**	1, 156	75.1	< **0.001**	1, 150
diversity-linear	16.2	< **0.001**	1, 150	11.4	**0.001**	1, 156	11.5	**0.001**	1, 150
Slices of S vs. T within Treatment									
Forb addition treatment	*F*	*P*	d.f.	*F*	*p*	d.f.	*F*	*P*	d.f.
Early mixture	4.2	**0.04**	1, 150	3.2	0.07	1, 156	88.2	< **0.001**	1, 150
P. pilosa	19.5	< **0.001**	1, 150	0.4	0.52	1, 156	393.5	< **0.001**	1, 150
P. patens	4.5	**0.04**	1, 150	15.6	< **0.001**	1, 156	121.3	< **0.001**	1, 150
Late mixture	36.2	< **0.001**	1, 150	0.8	0.36	1, 156	408.7	< **0.001**	1, 150
S. nemoralis	82.0	< **0.001**	1, 150	35.8	< **0.001**	1, 156	455.9	< **0.001**	1, 150
S. novae-angliae	37.5	< **0.001**	1, 150	0.9	0.33	1, 156	486.9	< **0.001**	1, 150
S. sericeum	12.3	**0.001**	1, 150	5.0	**0.03**	1, 156	263.3	< **0.001**	1, 150
Early and late mixture	17.9	< **0.001**	1, 150	2.8	0.10	1, 156	228.2	< **0.001**	1, 150

Table 2. Results from a three-year forb addition experiment in prairie restorations in northern Iowa and southern Minnesota, USA. Point intercept data was collected in years two and three. A randomized block split-plot repeated measures ANOVA was used to analyze plant species diversity of plots (species richness, Simpson's 1/D, and graminoid relative abundance). Main plots were mowed or unmowed ("Mow") and early and late flowering forbs added to subplots at varied richness levels ("Treatment") as seeds or transplanted (S vs. T). Contrasts compared the mean of all late flowering treatments to the mean of all early flowering treatments (early vs. late), the effect of increased richness forb additions (diversity-linear), and control plots to other plots (control vs. others). A slice test of the S vs. T*Treatment interaction was performed when that interaction was significant.

	Richness (ln)			**Simpson's diversity (ln)**			**Graminoid pi**		
Effect	*F*	*P*	d.f.	*F*	*p*	d.f.	*F*	*P*	d.f.
Mow	0.1	0.72	1, 8	2.0	0.19	1, 8	0.02	0.88	1, 8
Treatment	2.9	**0.005**	8, 170	3.8	**< 0.001**	8, 170	3.0	**0.003**	8, 170
S vs. T	14.5	**< 0.001**	1, 170	6.2	**0.01**	1, 170	5.3	**0.02**	1, 170
Treatment*S vs. T	0.8	0.62	8, 170	0.6	0.78	8, 170	2.3	**0.02**	8, 170
Treatment*Mow	2.0	0.06	8, 170	1.3	0.24	8, 170	0.6	0.80	8, 170
S vs. T*Mow	0.02	0.89	1, 170	0.03	0.87	1, 170	0.1	0.71	1, 170
Treatment*S vs. T* Mow	0.5	0.89	8, 170	0.6	0.79	8, 170	0.5	0.84	8, 170
Time	97.2	**< 0.001**	1, 196	34.9	**< 0.001**	1, 196	59.8	**< 0.001**	1, 196
Mow*Time	2.7	0.10	1, 196	2.3	0.13	1, 196	1.7	0.20	1, 196
Treatment*Time	1.0	0.45	8, 196	1.7	0.10	8, 196	1.5	0.16	8, 196
S vs. T*Time	3.7	0.06	1, 196	1.1	0.29	1, 196	0.2	0.63	1, 196
Treatment*S vs. T*Time	0.6	0.76	8, 196	1.7	0.11	8, 196	0.2	1.0	8, 196
Treatment contrasts									
control vs. others	7.2	**0.008**	1, 170	10.0	**0.002**	1, 170	6.5	**0.01**	1, 170
early vs. late	7.8	**0.006**	1, 170	6.0	**0.02**	1, 170	12.6	**0.001**	1, 170
diversity-linear	9.2	**0.003**	1, 170	7.2	**0.008**	1, 170	1.5	0.22	1, 170
Slices of S vs. T within Treatment									
Early mixture							0.03	0.9	1, 170
P. pilosa							0.8	0.4	1, 170
P. patens							0.3	0.6	1, 170
Late mixture							5.5	**0.02**	1, 170
S. nemoralis							14.2	**< 0.001**	1, 170
S. novae-angliae							1.8	0.2	1, 170
S. sericeum							0.01	0.9	1, 170
Early and late mixture							0.01	0.9	1, 170
Control							1.4	0.2	1, 170

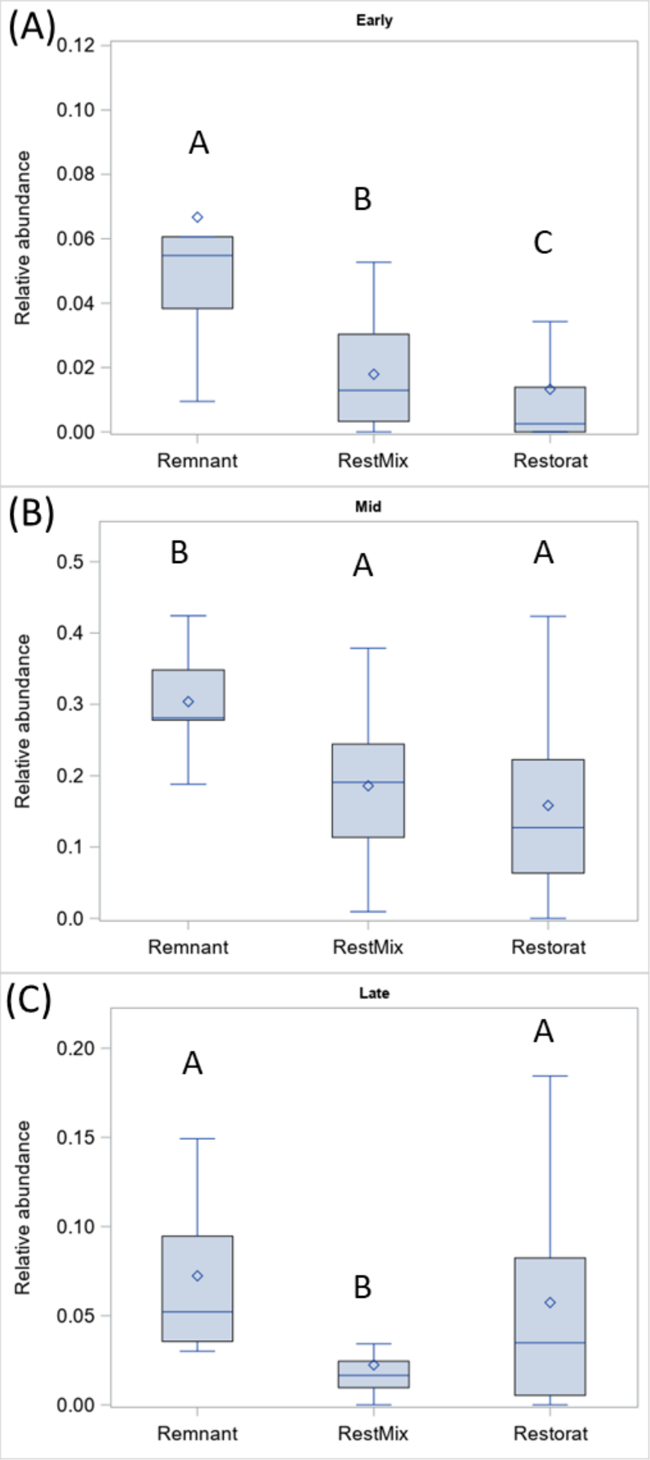


Figure 1. Relative abundances of early- (A), mid- (B), and late-season (C) flowering native forbs in 5 remnants, 94 restorations, and 48 restoration seed mixes used across Iowa, USA. Boxes show 25th and 75th percentiles, mean (triangles), and median (inner lines), and letters above boxes denote significant differences among groups.

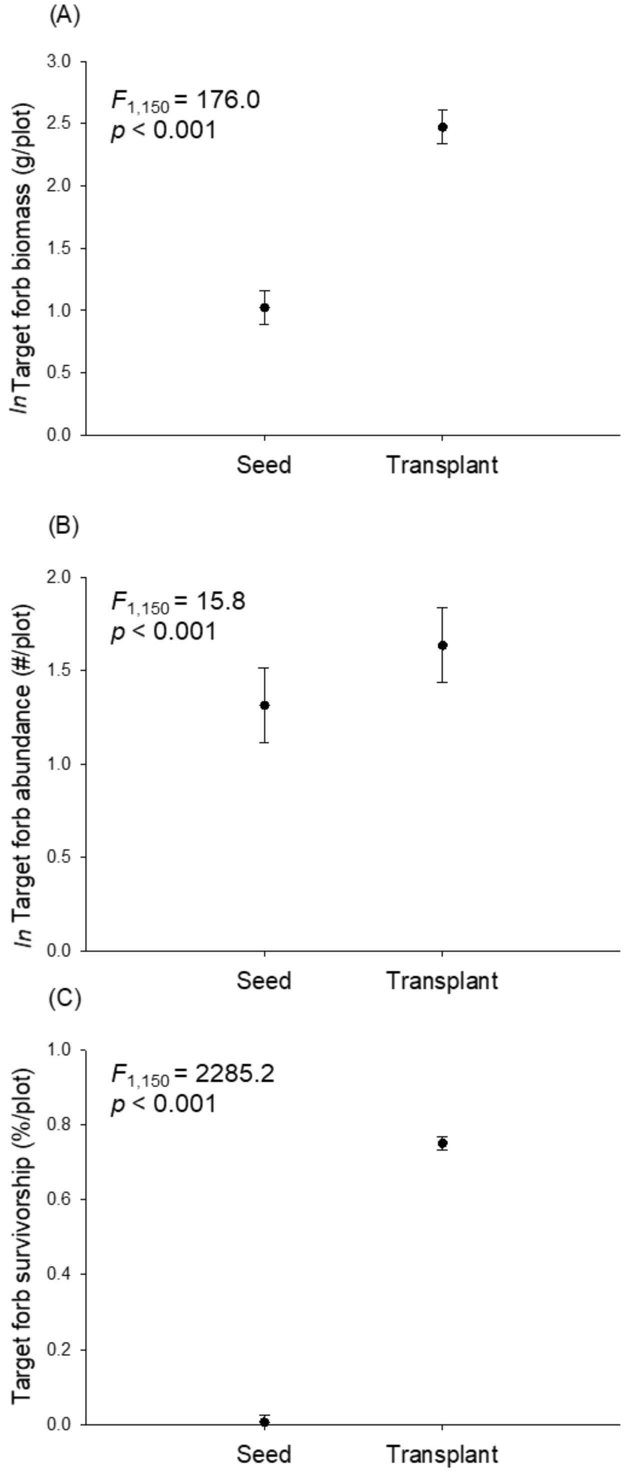


Figure 2. Comparison of seed and transplant plots in a forb addition experiment in new restorations in northern Iowa and southern Minnesota. Target forb *ln* biomass (A), *ln* abundance (B), and survivorship (C) were significantly different between transplanted and seeded plots. Plots show means ± SE.

Figure 3 (a): late-flowering treatments (biomass)

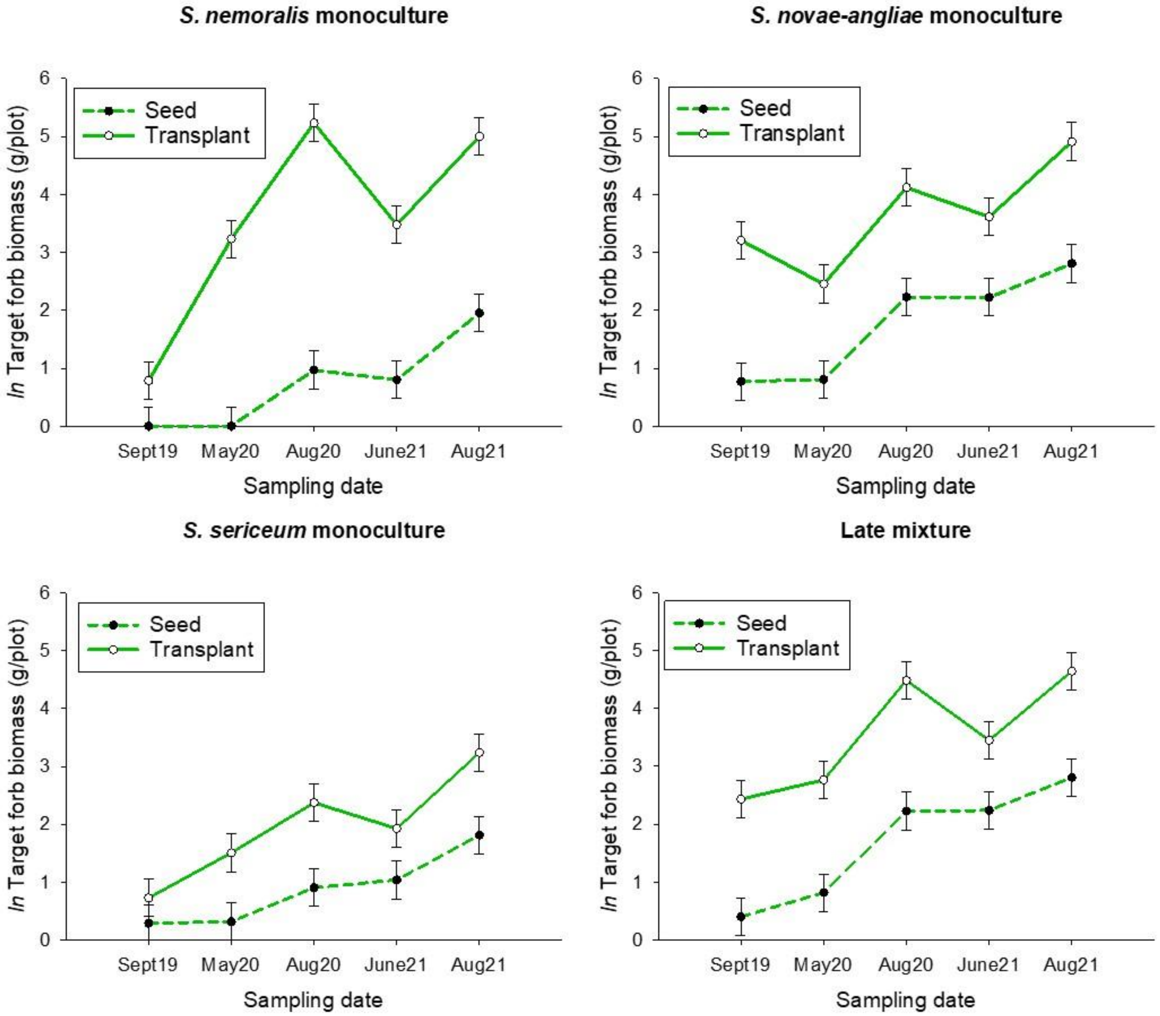
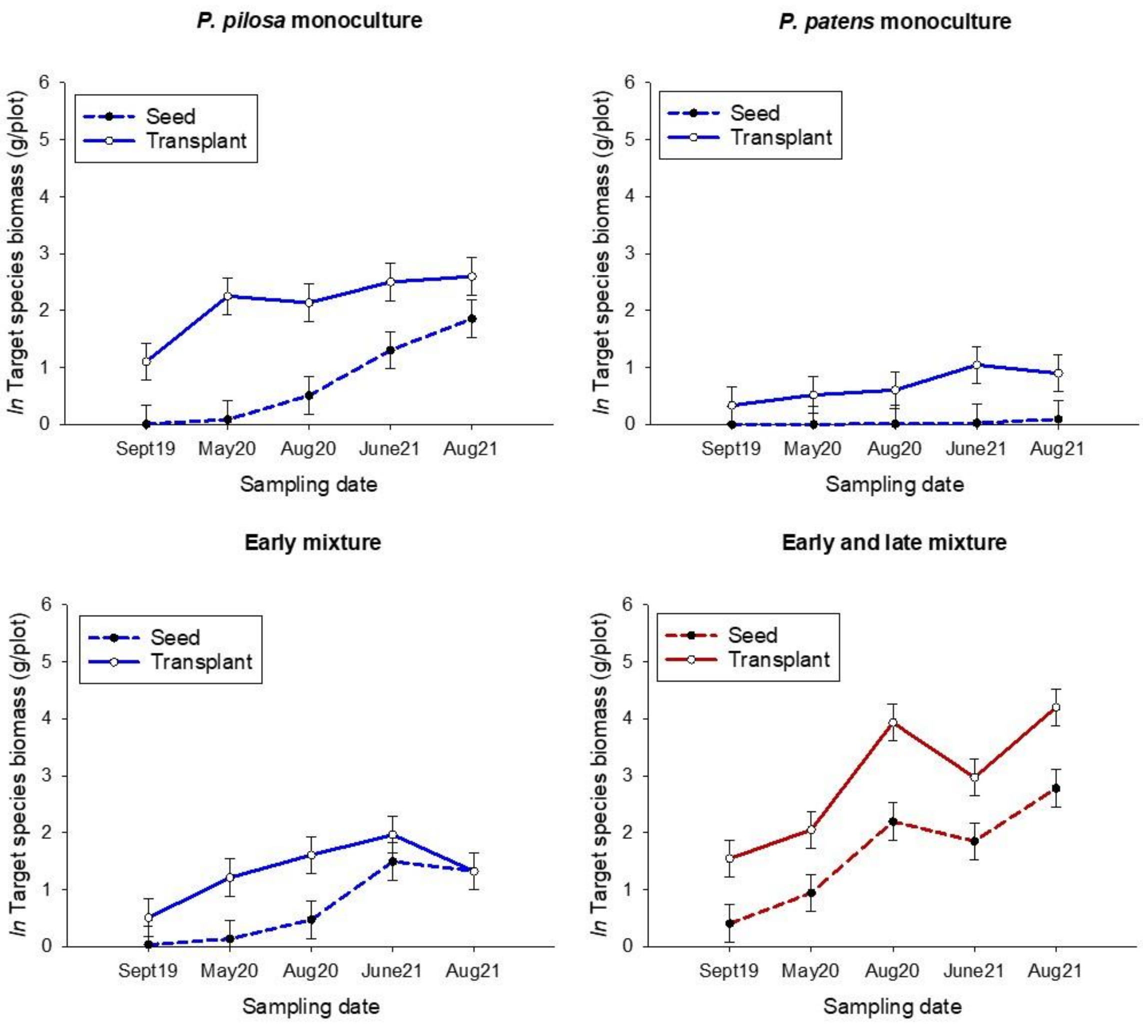


Figure 3a. Biomass (*ln* transformed) comparison of seed vs. transplant plots over five sampling dates among eight forb addition treatments in a prairie restoration experiment in northern Iowa and southern Minnesota. Error bars show standard error. Figure 3a shows late-flowering treatments. Figure 3b shows early-flowering treatments and early and late mixture treatment.

Figure 3 (b): early-flowering and six species mixture treatments (biomass)



P. pilosa monoculture

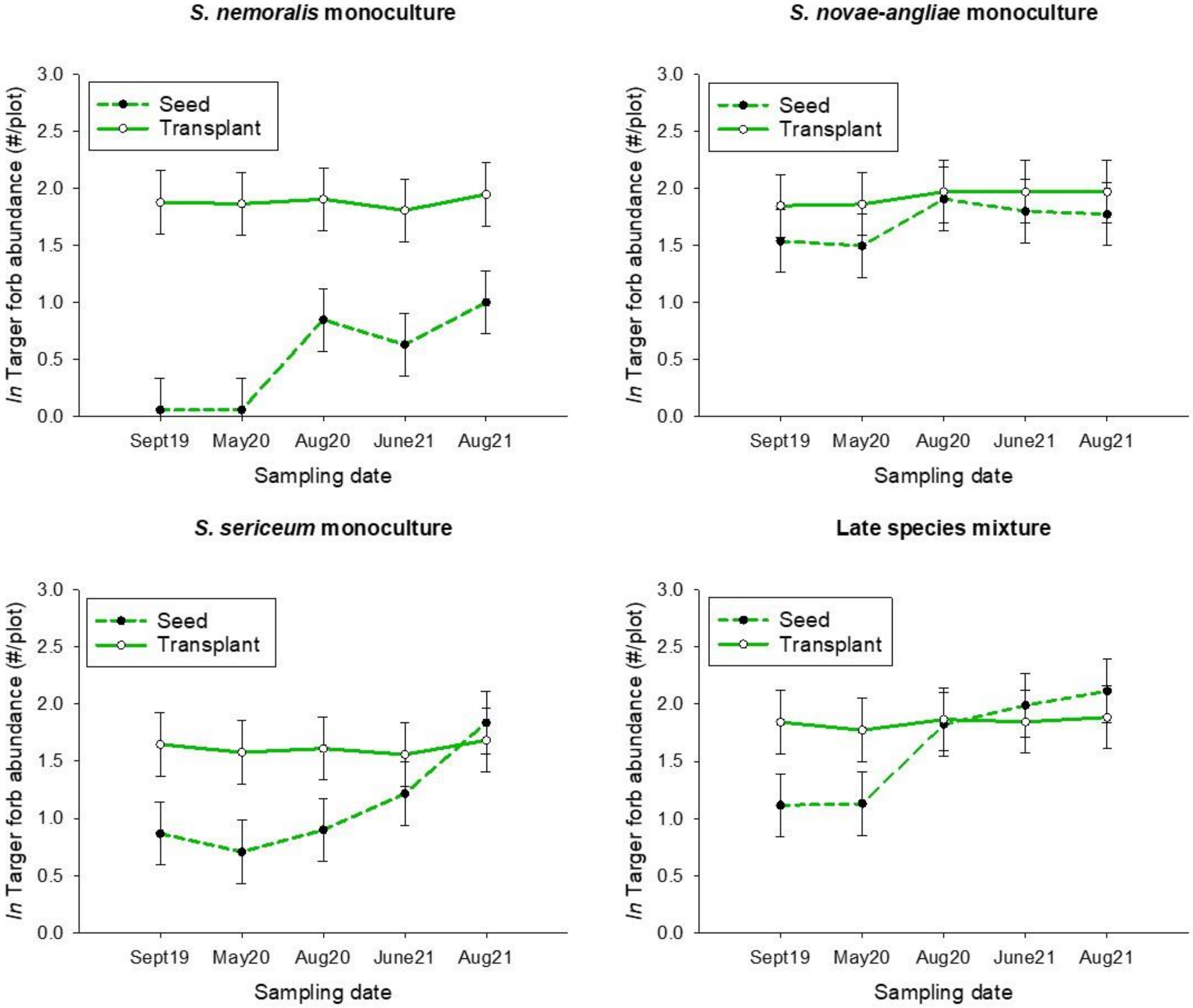
P. patens monoculture

Early mixture

Early and late mixture

Figure 3 (Continued)

Figure 4 (a): late-flowering treatments (abundance)


S. nemoralis **monoculture**

S. novae-angliae **monoculture**

S. sericeum **monoculture**

Late species mixture

Figure 4a. Abundance (*ln* transformed) comparison of seed vs. transplant plots over five sampling dates among eight forb addition treatments in a prairie restoration experiment in northern Iowa and southern Minnesota. Error bars show standard error. Figure 4a shows late-flowering treatments. 4b shows early-flowering treatments and early and late mixture treatment.

Figure 4 (b): early-flowering and six species mixture treatments (abundance)

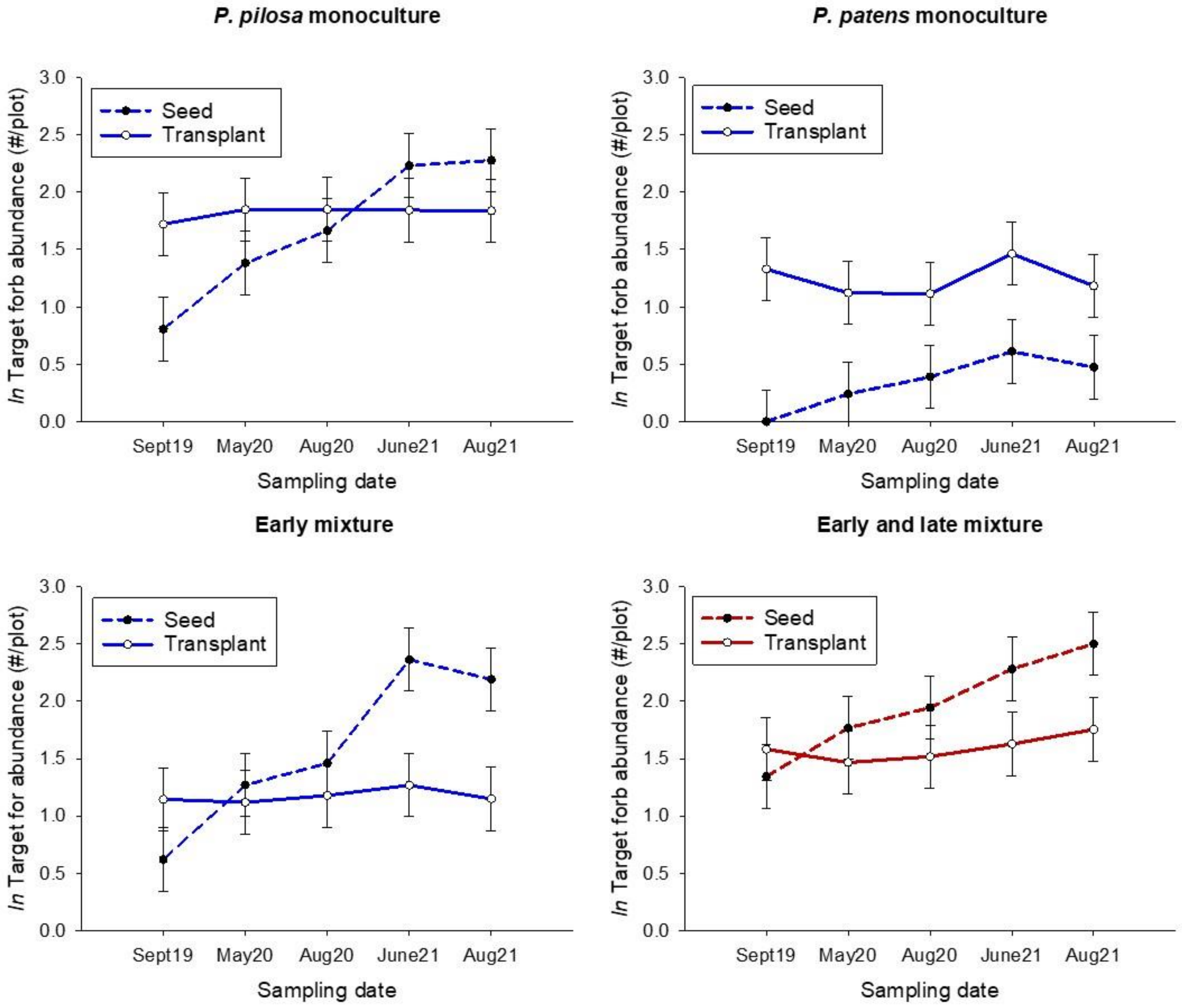


Figure 4 (Continued)

Figure 5 (a): diversity measures (richness)

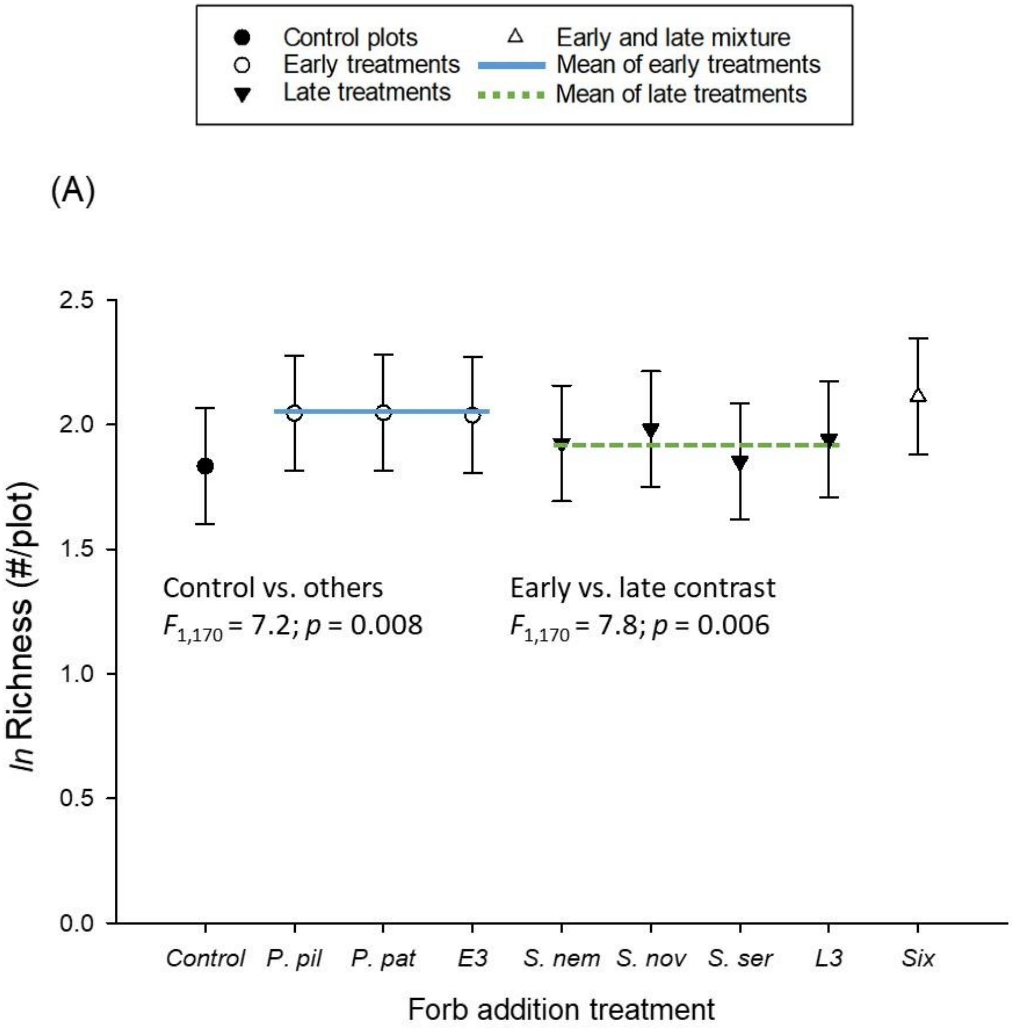


Figure 5a. Diversity (*ln* richness, (5A), *ln* Simpson's diversity, (5B), and graminoid relative abundance, (5C)) among forb addition treatment in a prairie restoration experiment in southern Minnesota and northern Iowa. Treatments increased diversity compared to control plots. A contrast of early and late phenology treatments indicated that on average early flowering species (means shown by blue lines) increased diversity more than late flowering treatments (green dashed lines), though there was variation within those groups. Plots show LS means by treatment ± SE. Dotted line indicates minimum possible value for Simpson's diversity (B).

Figure 5 (b): diversity measures (Simpson's reciprocal index)

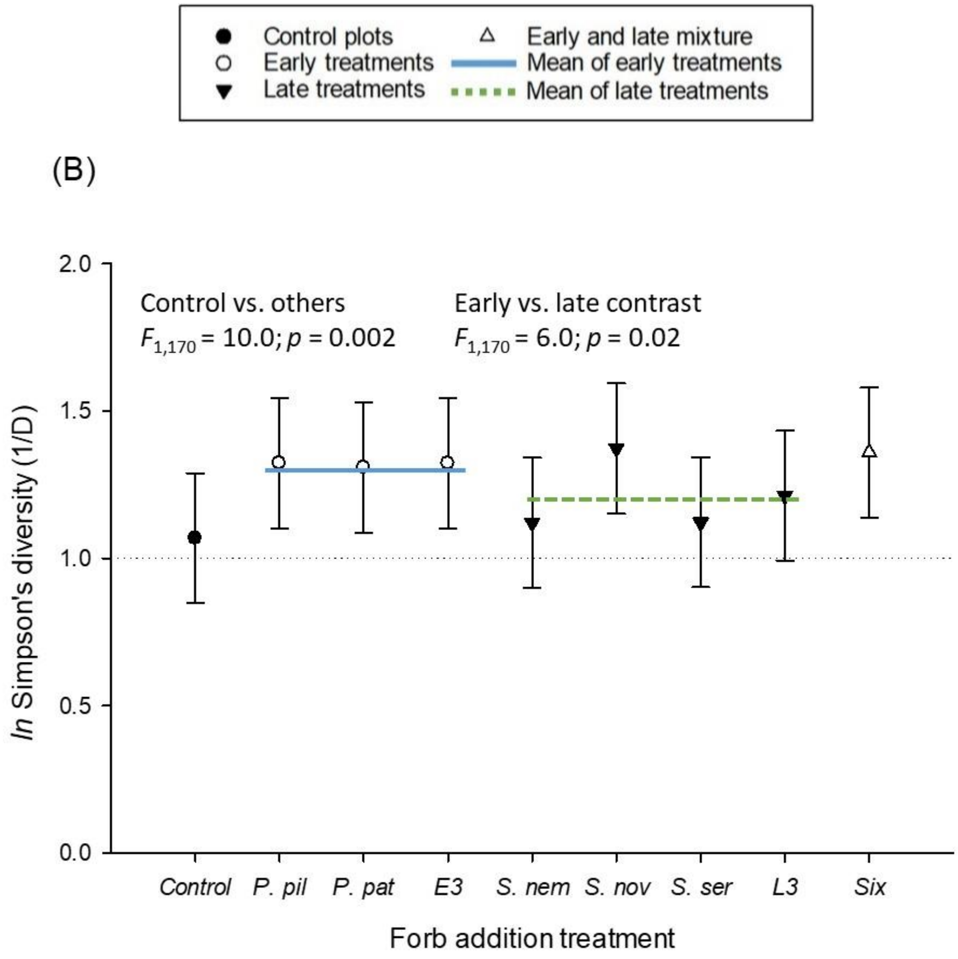


Figure 5 (Continued)

Figure 5 (c): diversity measures (graminoid Pi)

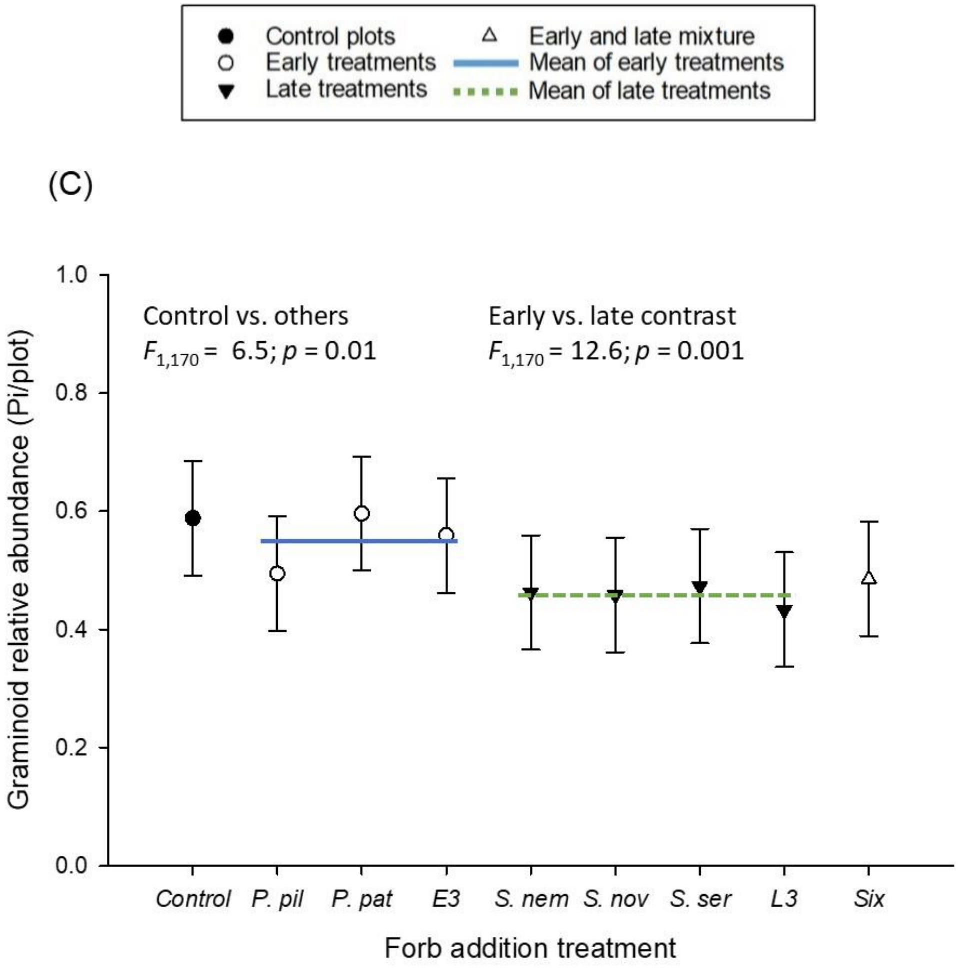


Figure 5 (Continued)

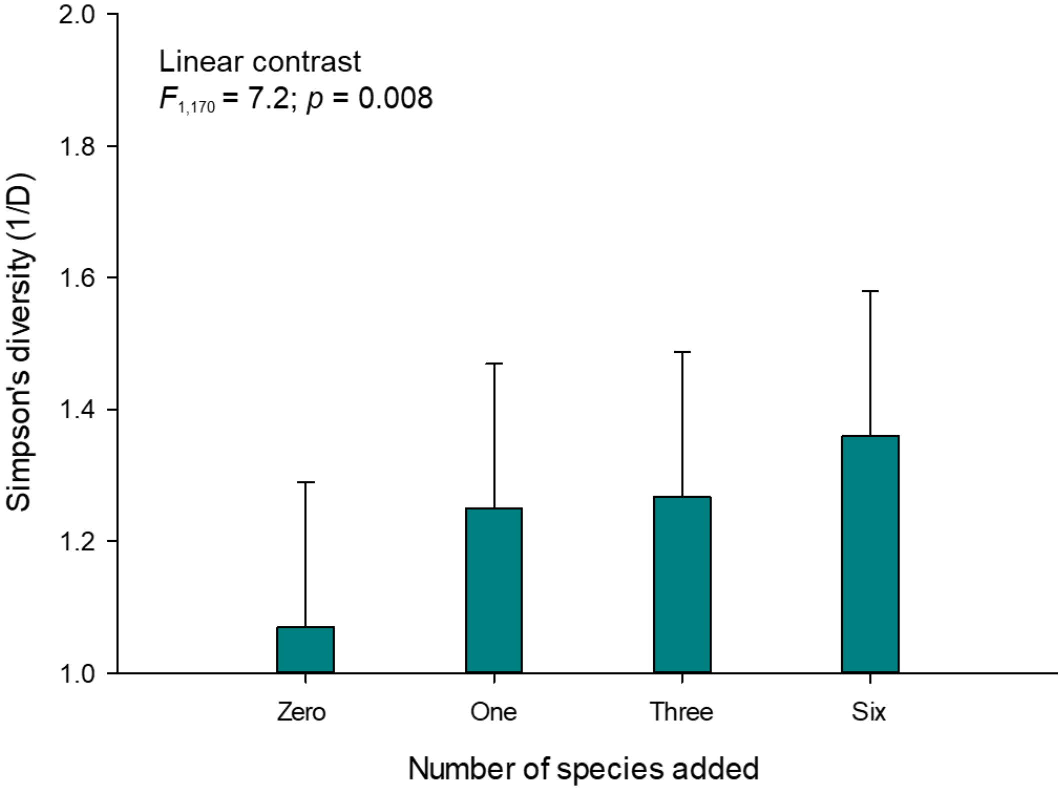


Figure 6. Mean Simpson's diversity of each forb addition richness level planted into newly seeded restorations. Species were added as monocultures (one), three early or three late species mixtures (three), or six species early and late mixtures (six), or controls (zero species added). A linear contrast indicated that higher richness forb additions increased diversity more than lower richness additions. Error bars show standard error.

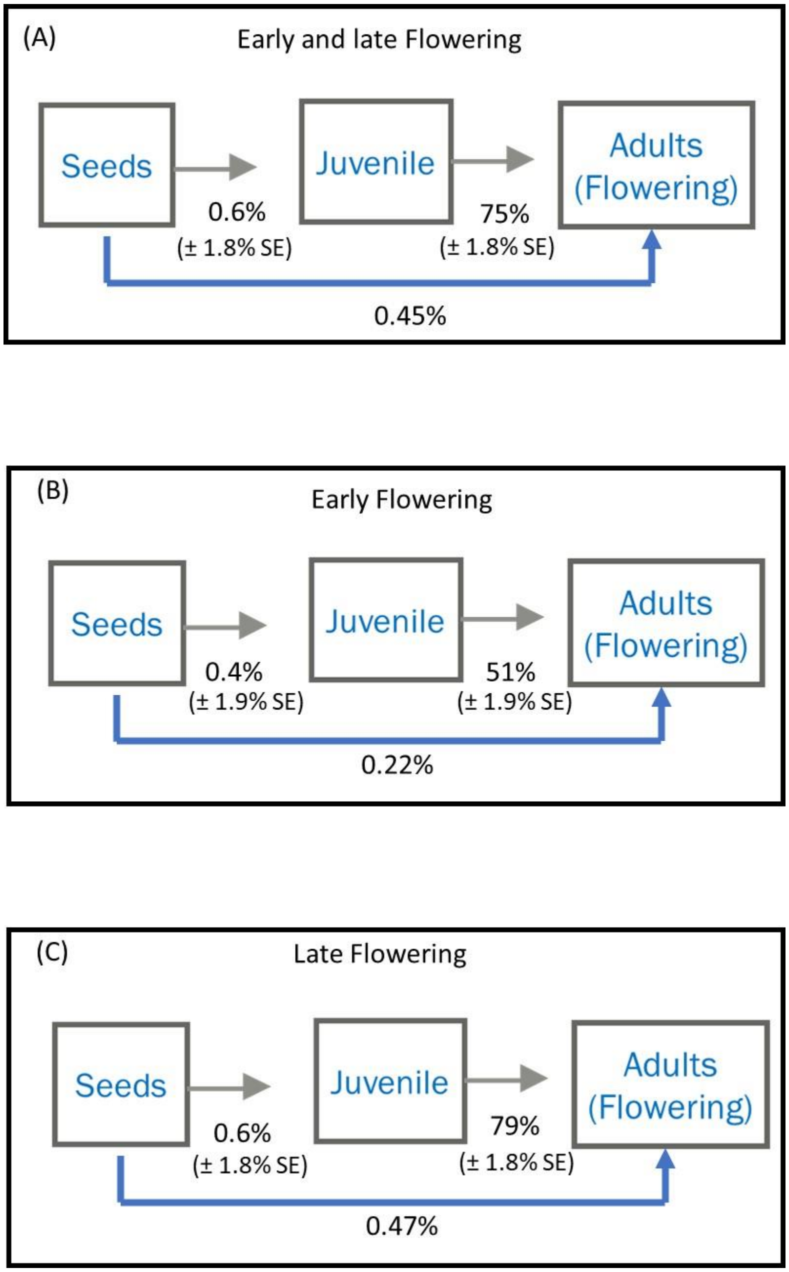


Figure 7. Life stage transition model based on survivorship of seeds and transplants (juveniles) in a restoration forb addition experiment. Target forbs had much higher survivorship when they were started as transplants and able to bypass the seed-juvenile stage (box A). This was consistent between early flowering (box B) and late flowering (box C) species.

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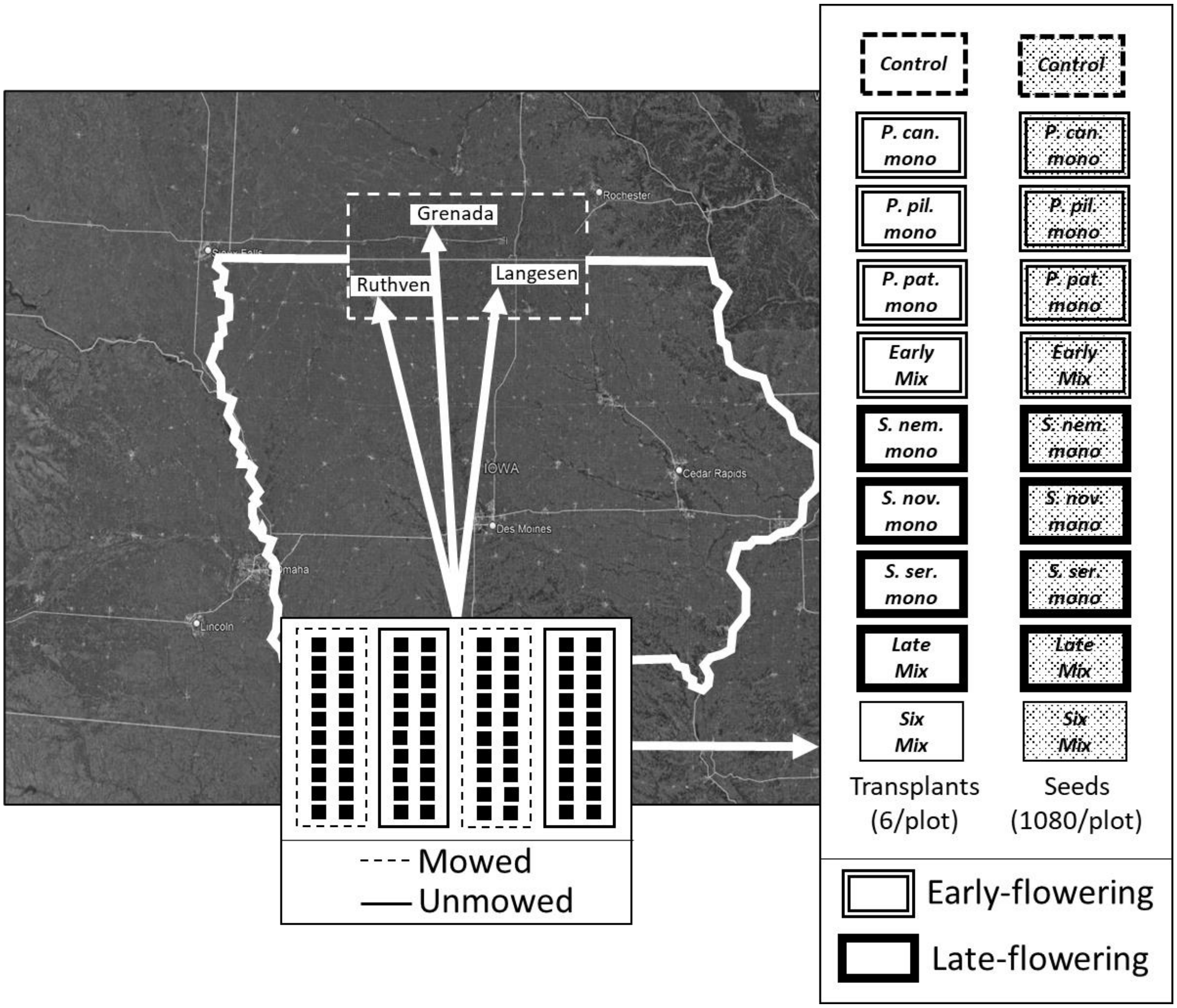
Appendix A. Forb Addition Study Experimental Design

Diagram showing three study locations (blocks) with whole plots and sub-plots. Each site contained a mowed or unmowed whole plot in replicate. Each whole plot contained twenty sub-plots. Sub-plots consisted of early-flowering monocultures of each study species, late-flowering monocultures of each study species, early-flowering mixtures, late-flowering mixtures, early- and late-flowering mixtures (six species mixture), and unaltered control plots. Each of these forb additions were added as seeds or transplants.



Appendix B. Supplemental Tables

Supplemental table 1. Biomass estimate calibration for early and late target forbs used in a prairie restoration forb addition experiment. Regression of biomass estimates (basal area x height) (X axis) and actual dry biomass of species (Y axis).

Species	N	R^2	Slope	Intercept	*p*	*F*	d.f.
P. pilosa	5	0.95	0.00024966	0.005	0.001	143.7	1, 3
P. patens	6	0.98	0.00069919	-0.04	0.001	80.4	1, 4
S. nemoralis	8	0.88	0.00045246	0.137	0.001	45.9	1, 6
S. novae-angliae	9	0.94	0.00045988	0.1	<0.001	111.5	1, 7
S. sericeum	10	0.98	0.00121	-0.06	<0.001	353.2	1, 8

Supplemental table 2. Least square mean estimates of biomass, abundance, and survivorship for seed vs. transplant plots among forb addition treatments in a three-year forb addition experiment in Iowa and Minnesota, USA.

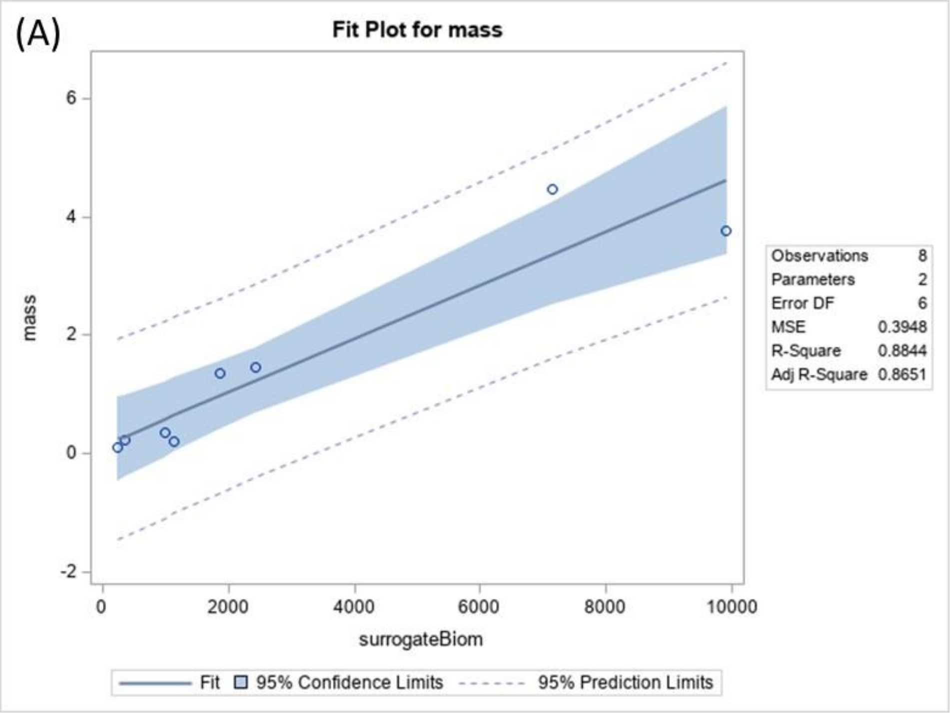
	***ln* Biomass (g/plot)**			***ln* Abundance (#/plot)**			**Survivorship (%/plot)**		
Forb addition treatment	Seed plot	Transplant plot	SE	Seed mean	Transplant mean	SE	Seed mean	Transplant mean	SE
Early mixture	0.69	1.32	0.24	1.58	1.17	0.25	0.006	0.42	0.03
P. pilosa	0.75	2.12	0.24	1.67	1.82	0.25	0.007	0.88	0.03
P. patens	0.02	0.68	0.24	0.34	1.24	0.25	0.001	0.49	0.03
Late mixture	1.70	3.56	0.24	1.64	1.84	0.25	0.009	0.90	0.03
S. nemoralis	0.75	3.55	0.24	0.52	1.88	0.25	0.001	0.94	0.03
S. novae-angliae	1.77	3.66	0.24	1.70	1.92	0.25	0.009	0.98	0.03
S. sericeum	0.87	1.95	0.24	1.11	1.62	0.25	0.005	0.72	0.03
Early and late mixture	1.63	2.94	0.24	1.97	1.59	0.25	0.01	0.68	0.03

Supplemental Table 3. Least square mean estimates of graminoid relative abundance for seed vs. transplant plots among forb addition treatments in a three-year forb addition experiment in Iowa and Minnesota, USA.

	Graminoid Pi		
Forb addition treatment	Seed mean	Transplant mean	SE
Early mixture	0.57	0.55	0.10
P. pilosa	0.53	0.46	0.10
P. patens	0.58	0.61	0.10
Late mixture	0.51	0.35	0.10
S. nemoralis	0.59	0.33	0.10
S. novae-angliae	0.50	0.41	0.10
S. sericeum	0.48	0.47	0.10
Early and late mixture	0.49	0.48	0.10

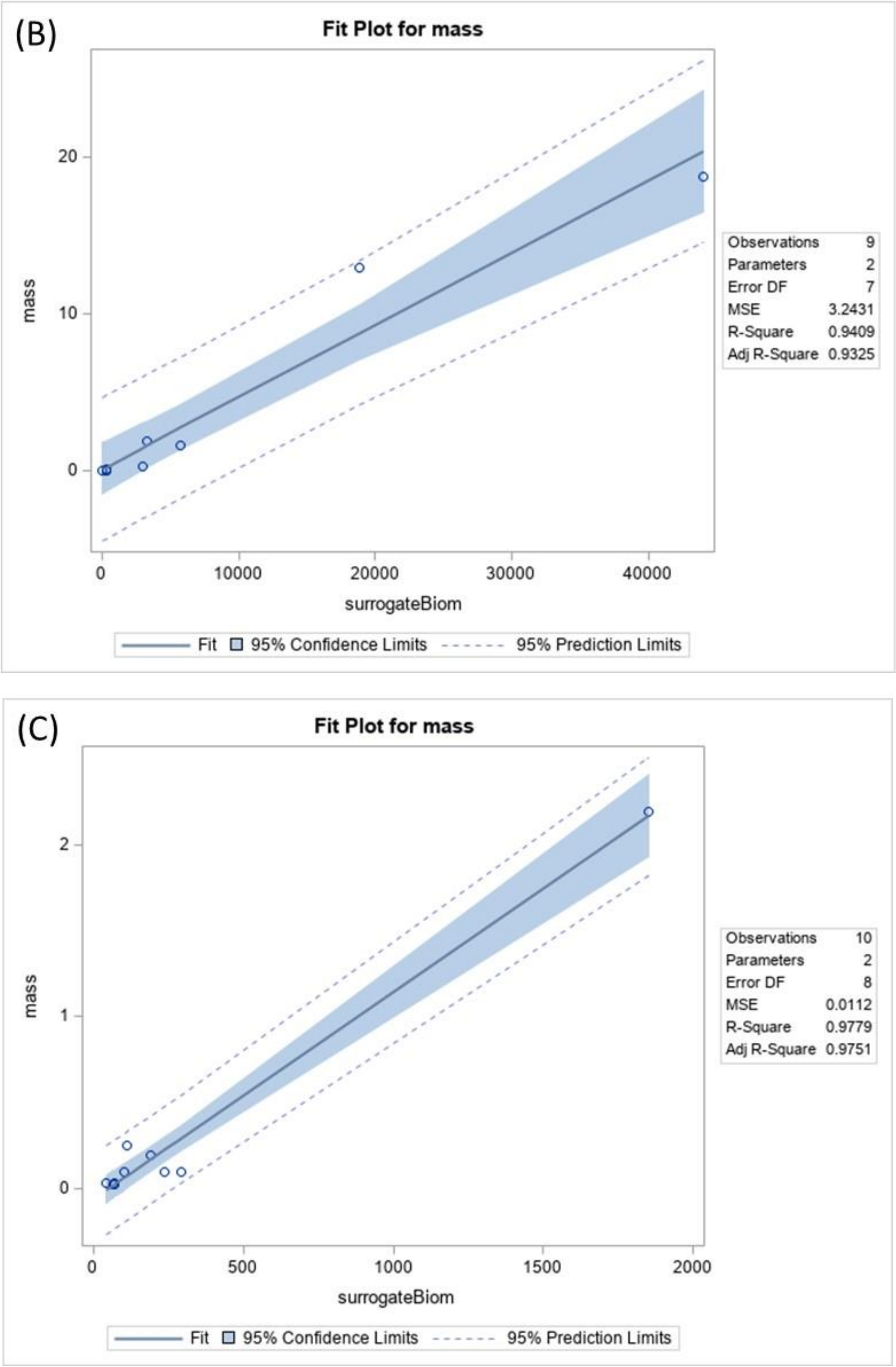
Appendix C. Supplemental Figures

Supplemental figure 1 (a): Regression plot for biomass calibration



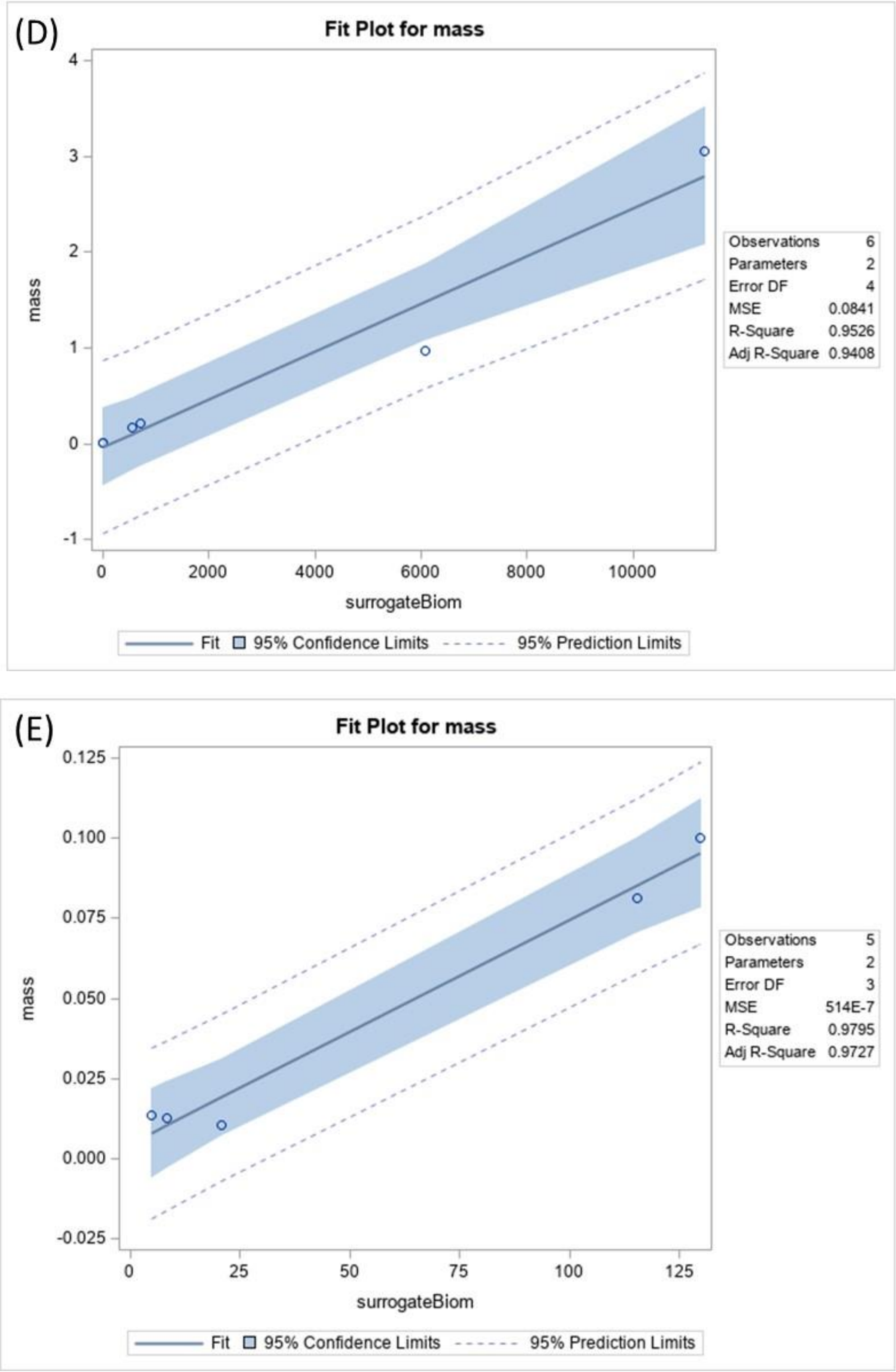
Supplemental figure 1. Plots of observed mass by predicted mass from our non-destructive measure in five species of forbs. *Solidago nemoralis* (A), *Symphyotrichum novae-angliae* (B), *Symphyotrichum sericeum* (C), *Phlox pilosa* (D), and *Pulsatilla patens* (E).

Supplemental figure 1 (b) and (c): Regression plots for biomass calibration



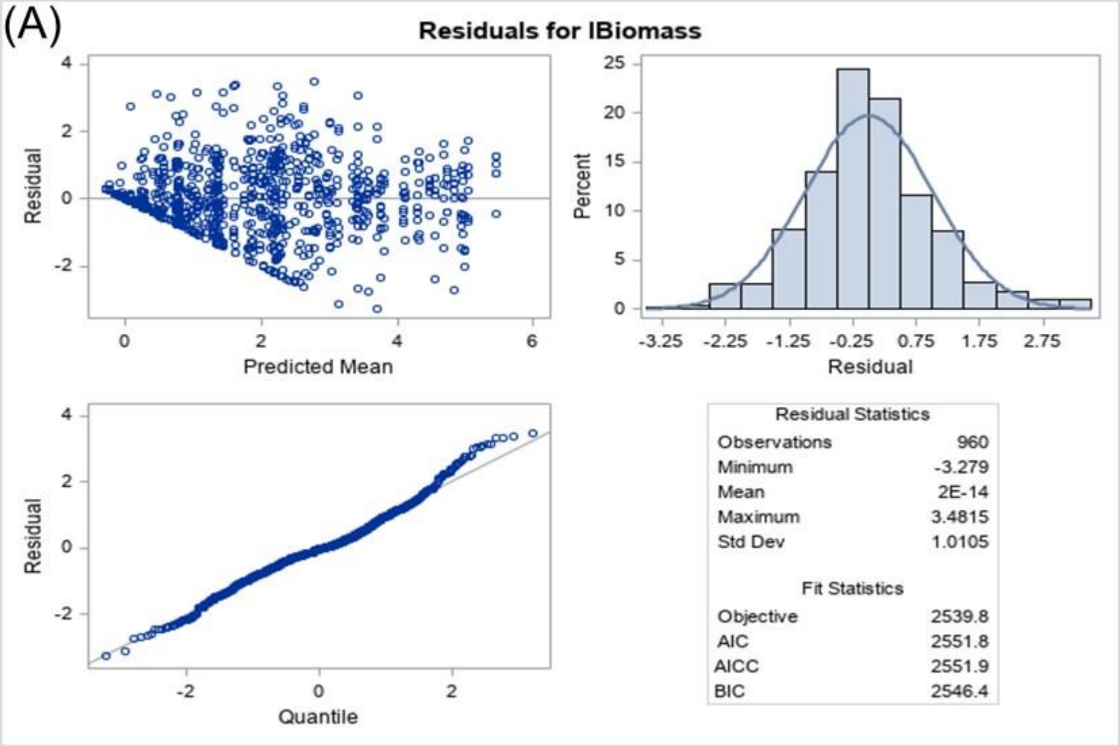
Supplemental figure 1 (Continued)

Supplemental figure 1 (D) and (E): Regression plots for biomass calibration



Supplemental figure 1 (Continued)

Supplemental figure 2 (a): Residuals for *ln* transformed biomass

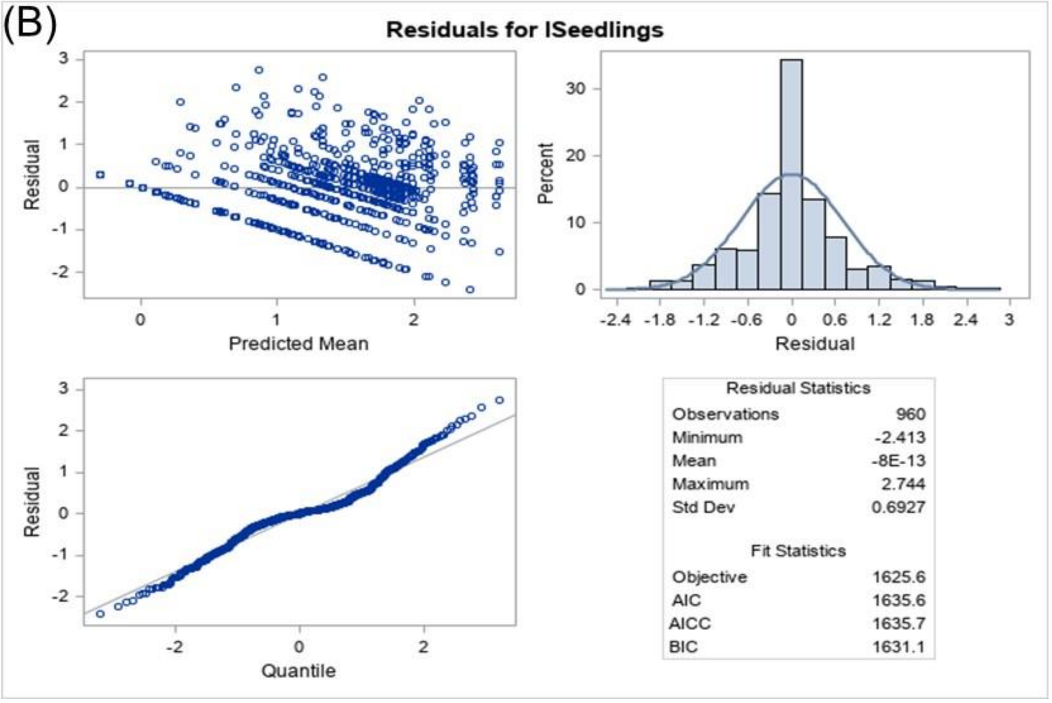


Residual Statistics	
Observations	960
Minimum	-3.279
Mean	2E-14
Maximum	3.4815
Std Dev	1.0105

Fit Statistics	
Objective	2539.8
AIC	2551.8
AICC	2551.9
BIC	2546.4

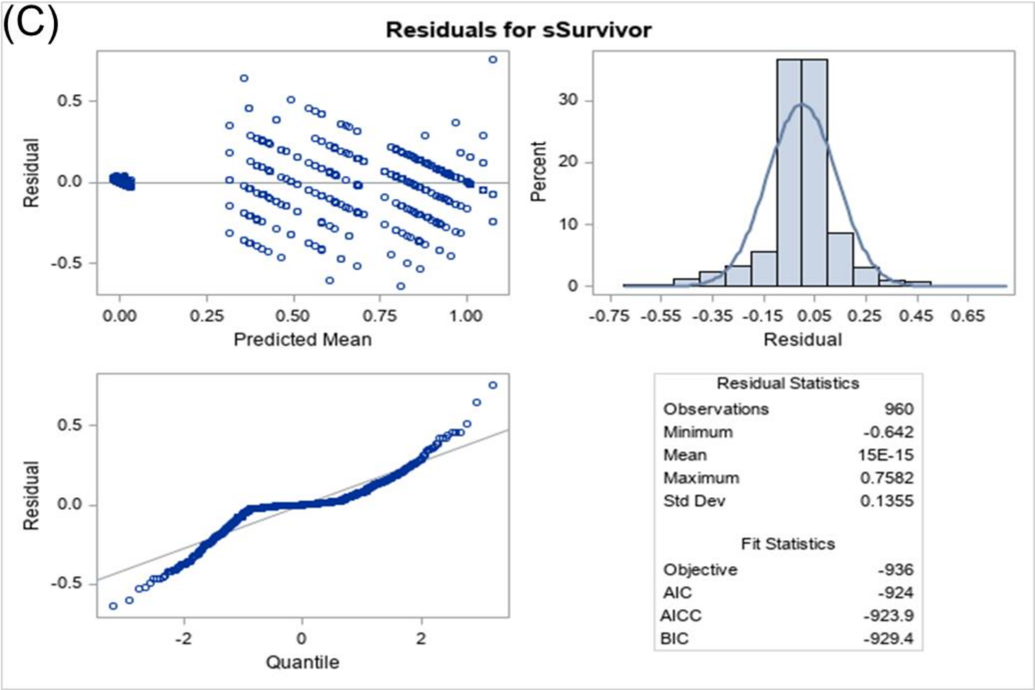
Supplemental Figure 2. Residual plots from a randomized block split-plot repeated measures ANOVA analyzing the response of target forbs in a three-year prairie restoration forb addition experiment. Responses were *ln* biomass (A), *ln* abundance (B), and survivorship (C).

Supplemental figure 2 (b) and (c): Residuals for *ln* transformed abundance and survivorship



Residual Statistics	
Observations	960
Minimum	-2.413
Mean	-8E-13
Maximum	2.744
Std Dev	0.6927

Fit Statistics	
Objective	1625.6
AIC	1635.6
AICC	1635.7
BIC	1631.1

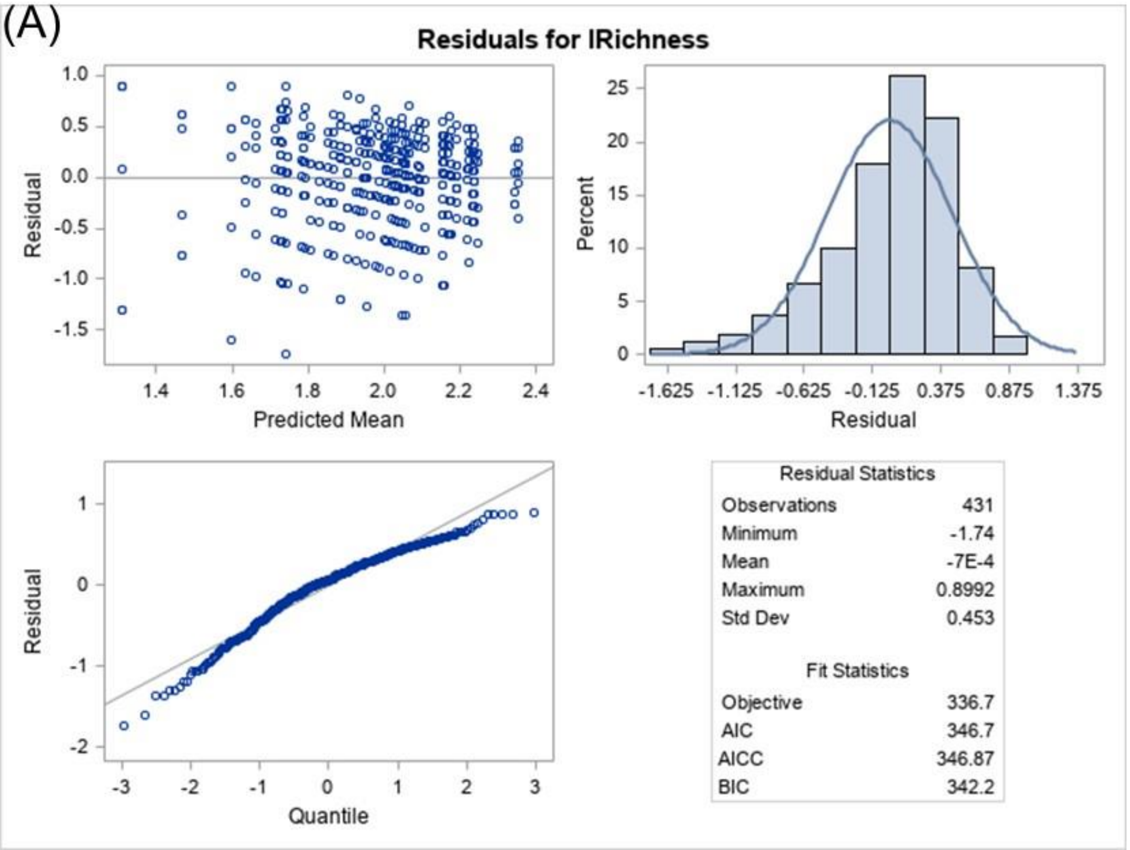


Residual Statistics	
Observations	960
Minimum	-0.642
Mean	15E-15
Maximum	0.7582
Std Dev	0.1355

Fit Statistics	
Objective	-936
AIC	-924
AICC	-923.9
BIC	-929.4

Supplemental figure 2 (Continued)

Supplemental figure 3 (a): Residuals for *ln* transformed richness

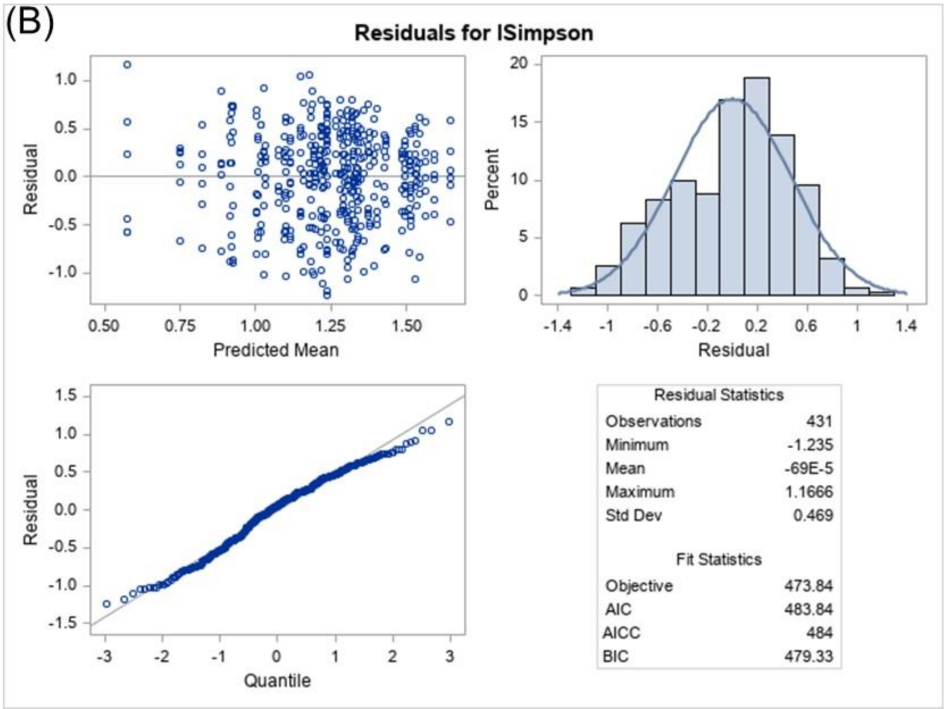


Residual Statistics	
Observations	431
Minimum	-1.74
Mean	-7E-4
Maximum	0.8992
Std Dev	0.453

Fit Statistics	
Objective	336.7
AIC	346.7
AICC	346.87
BIC	342.2

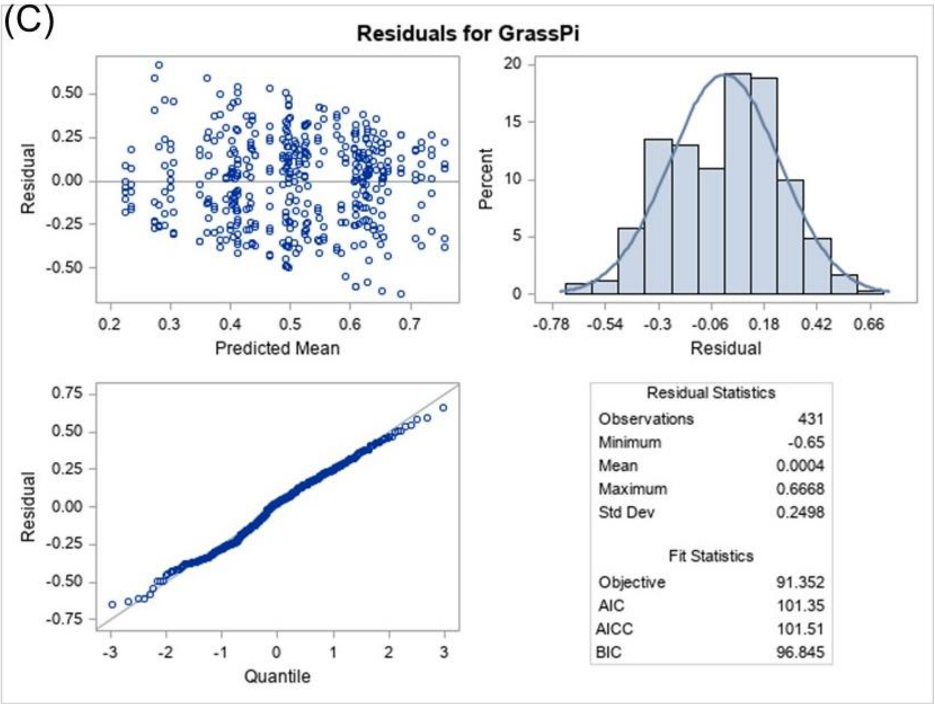
Supplemental Figure 3. Residual plots from a randomized block split-plot repeated measures ANOVA analyzing the response of diversity measures in a three-year prairie restoration forb addition experiment. Plant diversity responses were *ln* Richness (A), *ln* Simpson’s diversity (B), and graminoid species relative abundance (C).

Supplemental figure 3 (b) and (c): Residuals for *ln* Simpson’s 1/D and graminoid Pi



Residual Statistics	
Observations	431
Minimum	-1.235
Mean	-69E-5
Maximum	1.1666
Std Dev	0.469

Fit Statistics	
Objective	473.84
AIC	483.84
AICC	484
BIC	479.33

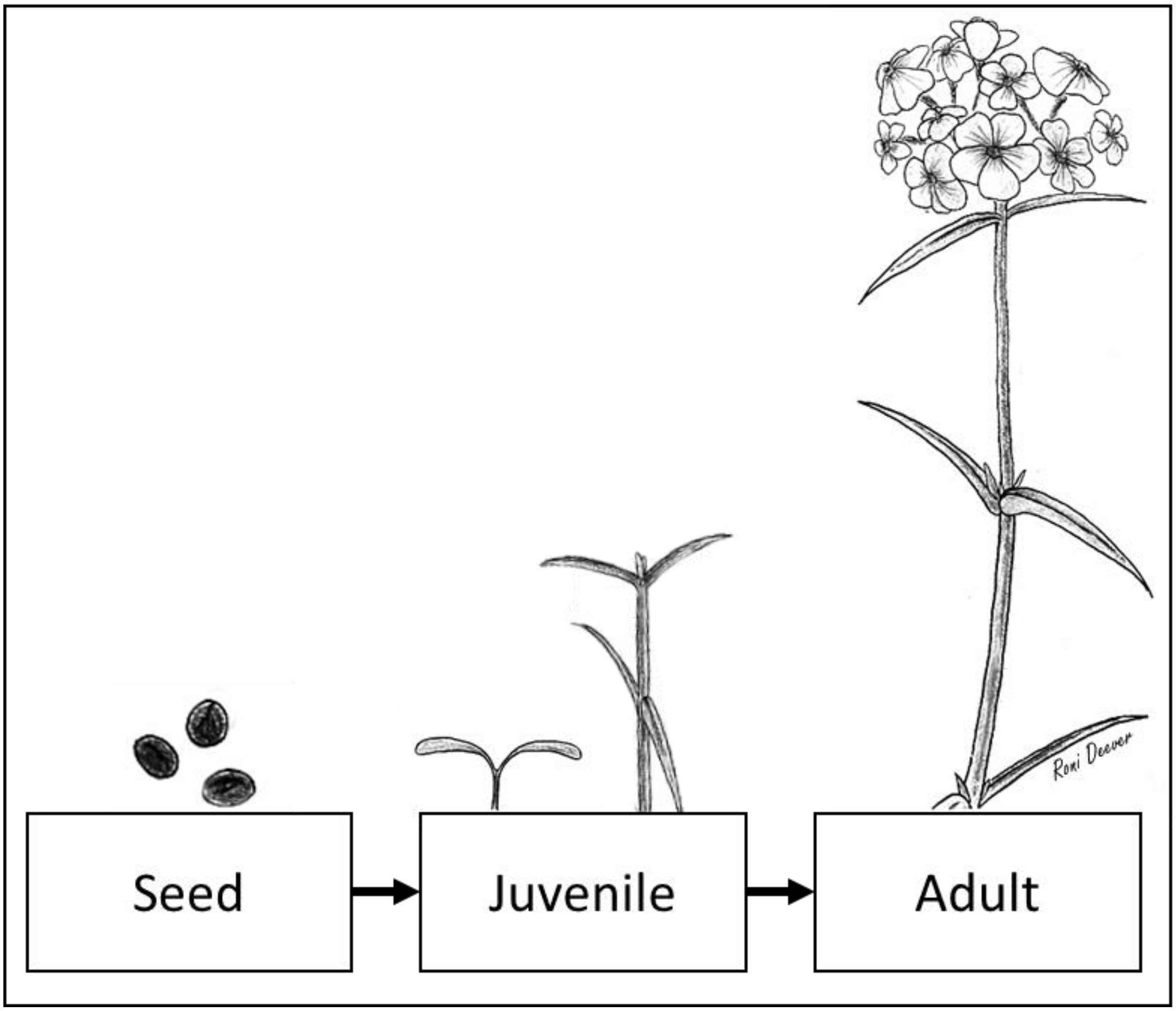


Residual Statistics	
Observations	431
Minimum	-0.65
Mean	0.0004
Maximum	0.6668
Std Dev	0.2498

Fit Statistics	
Objective	91.352
AIC	101.35
AICC	101.51
BIC	96.845

Supplemental figure 3 (Continued)

Appendix D. Life Stage Transition Model of a Prairie Forb



Life stage transition model of a prairie forb (*Phlox pilosa*) with illustrated primary features of stages. Forbs in the forb addition experiment were limited at the seed-juvenile transition stage. Images not to scale.

Appendix E. Point-intercept Species list

Appendix table 1. All species identified in point-intercept quadrats in the forb addition experiment in years two and three. Presence at one of the three restoration sites indicated with an (X).

Scientific name	**Common name**	**Ruthven**	**Granada**	**Langesen**
Forb species				
Achillea millefolium	Yarrow	X	X	X
Amaranthus tuberculatus	Waterhemp			X
Ambrosia artemisiifolia	Annual ragweed	X	X	X
Ambrosia psilostachya	Perennial ragweed		X	
Anemone canadensis	Canada anemone	X	X	
Anemone cylindrica	Thimbleweed	X		
Anemone virginiana	Tall thimbleweed		X	
Apocynum cannabinum	Indian hemp	X		
Artemisia ludoviciana	White prairie sage	X	X	
Asclepias incarnata	Swamp milkweed	X		X
Asclepias syriaca	Common milkweed	X		X
Asclepias tuberosa	Butterfly milkweed	X	X	X
Asclepias verticillata	Whorled milkweed		X	X
Astragalus canadensis	Canadian milkvetch	X	X	
Baptisia bracteata	Cream false indigo	X	X	
Brickellia eupatorioides	False boneset		X	
Chamaecrista fasciculate	Partridge pea	X	X	X
Chenopodium album	Lambsquarters		X	X
Cirsium arvense	Canada thistle	X	X	X
Conyza canadensis	Horseweed	X	X	X
Coreopsis palmata	Prairie coreopsis	X	X	X
Dalea candida	White prairie clover	X	X	X
Dalea purpurea	Purple prairie clover	X	X	X
Daucus carota	Wild carrot	X		X
Desmodium canadense	Showy tick trefoil	X	X	X
Echinacea pallida	Pale purple coneflower	X	X	
Erigeron sp.	Fleabane sp.		X	
Eryngium yuccifolium	Rattlesnake master	X	X	X
Eupatorium perfoliatum	Common boneset			X
Euphorbia prostrata	Prostrate euphorbia			X
Euthamia graminifolia	Grass-leaved goldenrod		X	
Galium boreale	Bedstraw			X
Helianthus grosseserratus	Sawtooth sunflower	X	X	
Helianthus maximiliani	Maximilian sunflower		X	
Helianthus pauciflorus	Stiff sunflower	X	X	
heliopsis helianthoides	Oxeye sunflower	X	X	X
Heuchera richardsonii	Prairie alumroot	X		

Appendix table 1. Continued

Scientific name	**Common name**	**Ruthven**	**Granada**	**Langesen**
Krigia biflora	Two-flowered cynthia	X	X	X
Lactuca canadensis	Canada wild lettuce			X
Lactuca serriola	Prickly lettuce		X	X
Lespedeza capitata	Round-headed bush clover		X	X
Liatris aspera	Rough blazing star		X	
Liatris ligulistylis	Meadow blazing star		X	
Liatris pycnostachya	Prairie blazing star		X	X
Lysimachia quadrifolia	Whorled loosestrife			X
Melilotus alba	White sweet clover		X	
Monarda fistulosa	Bee balm	X	X	X
Oenothera biennis	Common evening primrose	X		X
Oenothera sp.	Primrose sp	X		
Oxalis stricta	Wood sorrel	X	X	X
Pedicularis canadense	Wood betony			X
Penstemon grandiflorus	Large beardtongue		X	
Phlox pilosa	Prairie phlox	X	X	X
Physostegia virginiana	Obedient plant		X	
Polygonum convolvulus	Black bindweed			X
Polygonum pensylvanicum	Smartweed			X
Potentilla arguda	Prairie cinquefoil	X	X	X
Pulsatilla patens	Pasque flower	X	X	X
Pycnanthemum virginianum	Virginia mountain mint	X	X	X
Ratibida pinnata	Greyhead coneflower	X	X	X
Rudbeckia hirta	Black-eyed susan	X	X	X
Rudbeckia laciniata	Cutleaf coneflower		X	
Rumex crispus	Curly dock		X	
Silene vulgaris	Maidenstears	X		X
Silphium integrifolium	Rosinweed		X	
Silphium laciniatum	Compass plant	X	X	X
Silphium perfoliatum	Cup plant	X		
Solidago canadensis	Canada goldenrod	X	X	
Solidago gigantea	Giant goldenrod	X	X	X
Solidago nemoralis	Old field goldenrod	X	X	X
Solidago rigida	Stiff goldenrod	X	X	X
Solidago sp.	Goldenrod sp.			X
Solidago speciosa	Showy goldenrod	X		
Sonchus arvensis	Sowthistle	X	X	
Symphyotrichum ericoides	Heath aster	X	X	
Symphyotrichum laeve	Smooth blue aster	X	X	X
Symphyotrichum lanceolatum	Lance-leaved aster	X		X

Appendix table 1. Continued

Scientific name	**Common name**	**Ruthven**	**Granada**	**Langesen**
Symphyotrichum novae-angliae	New England aster	X	X	X
Symphyotrichum pilosum	Hairy aster	X	X	X
Symphyotrichum sericeum	Silky aster	X	X	X
Symphyotrichum sp.	Aster sp.		X	
Taraxacum officinale	Dandelion	X	X	X
Verbena stricta	Hoary vervain	X	X	
Vernonia fasciculata	Prairie ironweed			X
Vicia americana	American vetch	X	X	X
Zizia aptera	Heart-leaf Golden Alexanders	X	X	
Zizia aurea	Golden Alexanders	X	X	X
Graminoid species				
Agrostis gigantea	Redtop		X	
Andropogon gerardii	Big bluestem	X	X	X
Bouteloua curtipendula	Sideoats grama	X	X	X
Bromus inermis	Smooth brome	X	X	X
Bromus tectorum	Cheatgrass			X
Carex annectens	yellow-fruit sedge		X	
Carex brevior	Plains oval sedge	X	X	X
Carex molesta	Troublesome sedge		X	
Carex sp.	Sedge sp.	X	X	
Carex vulpinoidea	Fox sedge		X	X
Cyperus esculentus	Yellow nutsedge			X
Dichanthelium scribnerianum	Scribner's panic grass		X	
Digitaria ischaemum	Smooth crabgrass			X
Digitaria sanguinalis	Hairy crabgrass			X
Echinochloa crus-galli	Barnyard grass			X
Elymus canadensis	Canada wild rye	X		X
Elymus repens	Quackgrass		X	
Elymus trachycaulus	Slender wheatgrass	X	X	
Elymus virginicus	Virginia wild rye	X	X	X
Festuca arundinacea	tall fescue			X
Juncus dudleyi	Dudley's rush			X
Juncus effusus	Common rush		X	
Koeleria macrantha	Prairie Junegrass	X	X	X
Muhlenbergia mexicana	Mexican muhly		X	
Muhlenbergia racemosa	Marsh muhly		X	
Panicum dichotomiflorum	Fall panicgrass			X
Panicum virgatum	Switchgrass	X	X	X
Phalaris arundinacea	Reed canary grass			X
Phleum pratense	Timothy grass	X		

Appendix table 1. Continued

Scientific name	**Common name**	**Ruthven**	**Granada**	**Langesen**
Poa pratensis	Kentucky bluegrass	X	X	X
Schizachyrium scoparium	Little bluestem	X	X	X
Setaria pumila	Yellow foxtail		X	X
Setaria viridis	Green foxtail			X
Sorghastrum nutans	Indiangrass	X	X	X
Spartina pectinata	Prairie cordgrass	X	X	X
Sporobolus compositus	Rough dropseed		X	X
Sporobolus heterolepis	Prairie dropseed		X	X
Woody species				
Acer negundo	Boxelder		X	
Ceanothus americanus	New Jersey tea	X		
Fraxinus sp.	Ash sp.		X	
Morus alba	White mulberry		X	
Populus deltoides	Eastern cottonwood		X	
Rubus sp.	Bramble		X	

Appendix F. Restoration Seed Mix Species

Appendix table 2. Seed lists species lists provided by Department of Natural Resources for three restoration sites. An (X) indicates a species was seeded at that site. Species denoted with an (*) are species seeded into areas of the Ruthven (Eaton Wildlife Management Area) restoration site designated by the Department of Natural Resources as hydric (*) or xeric (**) that largely were located outside of the study area.

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Forb species				
Achillea millefolium	Yarrow	X	X	X
*Acorus calamus**	Sweet flag	X		X
*Alisma plantago-aquatica**	Water plantain	X		X
Allium canadense	Wild garlic		X	X
Allium stellatum	Wild prairie onion	X		X
Anemone canadensis	Canada anemone	X		
Anemone cylindrica	Thimbleweed	X	X	
Anemone virginiana	Tall thimbleweed	X	X	X
Apocynum cannabinum	Hemp dogbane	X		X
Artemisia ludoviciana	White prairie sage	X	X	X
*Asclepias incarnata**	Swamp milkweed	X		X
Asclepias sullivantii	Prairie milkweed	X		X
Asclepias syriaca	Common milkweed	X	X	X
Asclepias tuberosa	Butterfly milkweed	X	X	X
Asclepias verticillata	Whorled milkweed	X	X	X

Appendix table 2. Continued

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Astragalus canadensis	Canada milk vetch	X	X	X
Baptisia bracteata	Cream false indigo	X	X	X
Baptisia lactea	White wild indigo	X		X
Bidens connata	Purple-stemmed tickseed			X
*Brickellia eupatorioides***	False boneset	X	X	
*Cacalia plantaginea***	Prairie indian plantain	X		X
Caltha palustris	Marsh marigold			X
Campanula aparinoides	Marsh bellflower			X
Chamaecrista fasciculata	Partridge pea	X	X	X
*Cicuta maculata**	Water Hemlock	X		X
*Cirsium altissimum***	Tall thistle	X		X
Cirsium discolor	Field thistle	X	X	
Cirsium flodmanii	Flodman’s thistle			X
Comandra umbellata	Bastard toadflax			X
Coreopsis palmata	Prairie coreopsis	X	X	X
Dalea candida	White prairie clover	X	X	X
Dalea purpurea	Purple prairie clover	X	X	X
Delphinium virescens	Prairie larkspur			X
Desmodium canadense	Showy tick-trefoil	X	X	X
Desmodium illinoense	Illinois tick-trefoil			X
Echinacea angustifolia	Narrow-leaved purple coneflower	X		
Echinacea pallida	Pale purple coneflower	X	X	X
Erigeron philadelphicus	Philadelphia fleabane			X
Eryngium yuccifolium	Rattlesnake master	X	X	X
Eupatorium altissimum	Tall boneset	X		X
Eupatorium perfoliatum	Boneset	X		X
Eupatorium purpureum	Sweet joe pye weed	X		X
Euphorbia corollata	Flowering Spurge			X
Euthamia graminifolia	Grass-leaved goldenrod	X	X	X
Fragaria virginiana	Wild Strawberry	X		X
Galium boreale	Bedstraw			X
Galium obtusum	Wild madder			X
*Gentiana andrewsii**	Bottle gentian	X		
Gentiana flavida	Cream gentian	X		
Gentiana puberulenta	Prairie gentian			X
Geranium maculatum	Wild geranium	X		X
*Glycyrrhiza lepidota***	Wild licorice	X		X
*Helenium autumnale**	Sneezeweed	X		X
Helianthus giganteus	Tall sunflower			X

Appendix table 2. Continued

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Helianthus grosseserratus	Sawtooth sunflower	X		X
Helianthus maximiliani	Maximilian sunflower		X	X
Helianthus pauciflorus	Stiff sunflower		X	
*Helianthus rigidus***	Prairie sunflower	X		X
Heliopsis helianthoides	Ox-eye Sunflower	X	X	X
Heuchera richardsonii	Alum Root	X	X	X
Hieracium umbellatum	Hawkweed	X		X
Hypericum pyramidatum	Giant st. john's wort	X		X
Hypoxis hirsuta	Yellow star grass			X
*Iris shrevei**	Blue flag iris	X		X
Lathyrus palustris	Marsh vetchling			X
Lathyrus venosus	Veiny pea			X
Lepidium draba	Whitetop			X
Lespedeza capitata	Round-headed bushclover	X	X	X
Liatris aspera	Rough blazing Star	X		X
Liatris ligulistylis	Meadow blazingstar		X	X
Liatris punctata	Dotted blazing star			X
Liatris pycnostachya	Prairie blazing star	X		X
Liatris spp.	Blazingstar spp.		X	
Lilium michiganense	Michigan lily			X
Lilium philadelphicum	Prairie cup lily			X
Linum sulcatum	Wild flax			X
Lithospermum canescens	Hoary puccoon			X
Lithospermum incisum	Fringed puccoon			X
*Lobelia siphilitica**	Great blue lobelia	X		
Lobelia spicata	Pale spiked lobelia	X	X	X
Lycopus americanus	Water horehound			X
Lysimachia ciliata	Fringed loosestrife			X
*Lysimachia quadriflora**	Prairie loosestrife	X		X
Lysimachia thyrsiflora	Tufted loosestrife			X
*Mentha arvensis**	Wild mint	X		X
*Mimulus ringens**	Monkey Flower	X		X
Mirabilis albida	Pale four-o’clock			X
Mirabilis hirsuta	Hairy four-o’clock			X
Mirabilis nyctaginea	Wild four-o'clock	X		X
Monarda fistulosa	Wild bergamot	X	X	X
Nothocalais cuspidata	Prairie false dandelion			X
Oenothera biennis	Evening Primrose	X	X	
Oenothera serrulata	Toothed evening primrose			X
Onosmodium molle	False gromwell			X

Appendix table 2. Continued

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Oxalis violacea	Violet wood sorrel			X
Parthenium integrifolium	Wild quinine			X
Pedicularis canadensis	Prairie lousewort	X		
*Pedicularis lanceolata**	Swamp lousewort	X		X
Pediomelum argophyllum	Silvery scurf pea			X
*Penstemon grandiflorus***	Large-flowered beard tongue	X	X	X
*Phlox maculata**	Marsh phlox	X		X
Phlox pilosa	Prairie Phlox	X	X	
Physalis heterophylla	Clammy ground cherry			X
Physalis virginiana	Virginia ground cherry			X
*Physostegia virginiana**	Obedient plant	X		X
Polygala verticillata	Whorled milkwort			X
Potentilla arguta	Prairie cinquefoil	X	X	X
Potentilla paradoxa	Bushy cinquefoil			X
Prenanthes racemosa	Glaucous white lettuce		X	X
Prunella vulgaris	Self-heal			X
Pycnanthemum virginianum	Virginia mountain mint	X	X	X
Ratibida pinnata	Grey-headed coneflower	X	X	X
Rosa arkansana	Prairie wild rose	X	X	X
Rosa blanda	Smooth wild rose	X	X	X
Rudbeckia hirta	Black-eyed susan	X	X	X
Ruellia humilis	Hairy wild pentunia	X		X
*Rumex orbiculatus**	Great water dock	X		X
*Sagittaria latifolia**	Common arrowhead	X		
Scutellaria parvula	Smaller skullcap			X
Senecio pauperculus	Ragwort			X
Senecio plattensis	Prairie ragwort	X		X
Silphium laciniatum	Compass plant	X	X	X
Silphium perfoliatum	Cup plant	X		X
Solidago canadensis	Canada goldenrod			X
Solidago gigantea	Smooth goldenrod			X
Solidago juncea	Early goldenrod			X
*Solidago riddellii**	Riddles goldenrod	X		X
Solidago rigida	Stiff goldenrod	X	X	X
Spolidago speciosa	Showy goldenrod	X		X
Stachys palustris	Woundwort			X
Symphyotrichum ericoides	Heath aster	X	X	X
Symphyotrichum laeve	Smooth blue aster	X	X	X
Symphyotrichum lanceolatum	Lance-leaved aster	X		X

Appendix table 2. Continued

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Symphyotrichum novae-angliae	New England aster	X		
Symphyotrichum oolentangiense	Sky blue aster			X
Symphyotrichum sericeum	Silky aster			X
Symphyotrichum spp.	Aster spp.		X	
Symphyotrichum umbellatus	Flat-topped aster	X		X
Teucrium canadense	American germander	X		
Thalictrum dasycarpum	Purple meadow rue	X	X	X
Tradescantia bracteata	Prairie spiderwort	X	X	X
Verbena hastata	Blue vervain	X		
Verbena stricta	Hoary vervain	X	X	X
Vernonia baldwinii	Baldwin’s ironweed			X
Vernonia fasciculata	Ironweed	X		X
Veronicastrum virginicum	Culvers root	X	X	X
Vicia americana	American vetch	X	X	X
Zigadenus elegans	White camas		X	X
Zizia aptera	Heart-leaved golden alexanders	X	X	X
Zizia aurea	Golden Alexanders	X	X	X
Graminoid species				
Agropyron trachycaulum	Slender Wheatgrass	X		
Andropogon gerardii	Big Bluestem	X		X
Bouteloua curtipendula	Sideoats Grama	X	X	X
Bromus kalmii	Prairie brome			X
*Calamagrostis canadensis**	Blue joint grass	X		X
Calamagrostis inexpansa	Northern reed grass			X
Carex annectens	Yellow fox sedge		X	
*Carex atherodes**	Wheat sedge	X		X
Carex bebbii	Bebb's oval sedge			X
Carex bicknellii	Bicknell's sedge		X	X
Carex brevior	Plains oval sedge	X	X	X
Carex comosa	Bristly sedge			X
*Carex cristatella**	Crested Oval Sedge	X		X
Carex gravida	Heavy sedge		X	
*Carex hystericina**	Porcupine sedge	X		X
*Carex lacustris**	Commonlake sedge	X		X
Carex molesta	Field oval sedge	X	X	X
Carex normalis	Spreading oval sedge	X		X
Carex pellita	Woolly sedge			X
Carex praegracilis	Expressway sedge			X
*Carex prairea**	Prairie sedge	X		X

Appendix table 2. Continued

Scientific Name	**Common Name**	**Eaton**	**Granada**	**Langesen**
Carex sartwellii	Sartwell’s sedge			X
Carex stipata	Awlfruit sedge			X
*Carex stricta**	Upright sedge	X		X
Carex tetanica	Common stiff sedge			X
*Carex vulpinoidea**	Fox sedge	X		X
Eleocharis erythropoda	Bald spikerush			X
Elymus canadensis	Canada wild rye	X		X
Elymus virginicus	Virginia wild rye	X		X
*Glyceria grandis**	Reed manna grass	X		
Glyceria striata	Fowl manna grass	X		X
Hierochloe odorata	Holy grass			X
Juncus dudleyi	Dudley's rush	X		X
*Juncus interior**	Inland rush	X		X
Juncus tenuis	Path rush			X
Juncus torreyi	Torrey's rush			X
Koeleria macrantha	June grass	X	X	X
Leersia oryzoides	Rice cut grass	X		X
Muhlenbergia mexicana	Leafy satin grass		X	
Muhlenbergia racemosa	Upland wild timothy		X	
Panicum scribnerianum	Scribner’s panic grass			X
Panicum virgatum	Switchgrass	X		X
Schizachyrium scoparium	Little bluesteam	X	X	X
Sisyrinchium campestre	Blue-eyed grass			X
Sorghastrum nutans	Indian grass	X	X	X
*Sparganium eurycarpum**	Giant bur-reed	X		X
*Spartina pectinata**	Prairie cordgrass	X		X
Sporobolus compositus	Tall dropseed	X	X	X
Sporobolus heterolepis	Prairie dropseed	X	X	X
Sprghastrum nutans	Indian grass	X		
Stipa spartea	Porcupine grass	X	X	X
*Carex atherodes**	Wheat sedge	X		X
Woody species				
Amorpha canescens	Lead plant	X	X	X
Ceanothus americanus	New Jersey tea	X		X

CHAPTER 3. TIMING OF APPLICATION OF AERSOSOL SMOKE AND COLD TREATMENTS IS IMPORTANT FOR GERMINATION OF GRASSLAND FORB SPECIES

Modified from a manuscript to be submitted to *Plant Ecology*

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Abstract

The diversity of forb species in tallgrass prairie restorations often fails to match the diversity of forbs found in remnant prairies, particularly when it comes to those with early flowering phenology. Given the role of fire in modulating prairie plant diversity, germination cues such as smoke may play an important and possibly overlooked role in overcoming potential germination limitation and establishing more diverse restorations. We tested nine forb species of early or late flowering phenology for germination response to aerosol smoke, cold stratification, and the timing of application of those two cues (smoke before cold vs. smoke after cold). We found that species had varied response to cold and that smoke increased germination of two species. Importantly, the timing of smoke application mattered with only smoke applied prior to cold having an impact. We also found that early-flowering species had far lower germination rates than late-flowering species. The varied germination response of species to treatments and importance of timing of cues for two species may be important and suggests that more targeted approaches are needed for some species in order to establish more diverse restorations.

Introduction

Forbs are an important component of prairie diversity but establishment of some groups into restorations is poorly understood. Understanding seed germination requirements of these species may be important for restoring prairie diversity. Plant diversity in prairies largely comes from forbs, which make up the majority of species compared to grasses (Collins et al. 1998; Polley et al. 2007). One aspect of forb diversity is variation in phenology, the timing of biological events. Forbs can be categorized by season of flowering phenology, and these can be broadly described as early-, mid-, and late-season flowering (Kindscher and Wells 1995; Craine et al. 2012). There is still much to be learned about these groups and establishing prairie diversity.

In the Midwestern United States, much of the historic prairie ecosystem has been lost (Samson and Knopf 1994). Restoration is a way to restore prairies where they have been lost, but it can be difficult to fully replicate the plant diversity found in remnant prairie fragments largely due to the failure to establish a diversity of forbs (Sluis 2002; Polley et al. 2005). In terms of phenological diversity, early species in particular can be missing in restorations compared to remnants (Howe 1994a; Carter and Blair 2012; Deever et al., Chapter 2). Studies on increasing diversity in restorations are often focused on successful establishment of target species and sometimes note that a subset of species fails to establish from seed (Grman et al. 2015; Drobney et al. 2020; Nolan et al. 2021). Often, little attention is given to why some species fail to establish successfully (Hillhouse and Zedler 2011).

Establishment of some forbs is poorly understood but one possible reason may be that they are limited by low germination (Qu et al. 2004; Hillhouse and Zedler 2011) and may have unique ecologies that require specific dormancy-breaking cues. Abiotic, chemical, and physical cues have all been shown to be involved in breaking or inhibiting germination in some species (Baskin and Baskin 2014), though there are still many gaps in our understanding. It has been suggested that germination limitation may play an important and overlooked role in explaining low restoration diversity (Hillhouse and Zedler 2011). If this is the case, understanding cues that inhibit or encourage dormancy may be important components of increasing germination of forbs seeded into restorations.

Temperature is one such abiotic factor important for breaking seed dormancy and increasing germination. Cold stratification is a commonly used method for breaking dormancy for temperate species as it is thought to replicate freeze-thaw cycles of the winter months (Packard and Mutel 1997; Baskin and Baskin 2014). Tallgrass prairie species can have varied responses to cold (Greene and Curtis 1950). Cold does not always act as a trigger, however, and some species are known to have multiple dormancy triggers (Qu et al. 2004; Schwilk and Zavala 2012; Baskin and Baskin 2014) while others likely have triggers that have not been determined.

Smoke, independent of fires and the heat they produce, is another abiotic factor that can have an effect on forb germination rate. Though extreme heat is associated with smoke from fires, smoke has been shown to influence germination independently (Long et al. 2011; Schwilk and Zavala 2012; Jefferson et al. 2014). Various forms of smoke (aerosol, liquid, chemical derivatives) have been used to demonstrate its effects (Jefferson et al. 2014). Smoke can inhibit or promote seed germination (Dixon et al. 1995; Schwilk and Zavala 2012). This varied response suggests there is more to learn about the role smoke plays in germination and prairie diversity. At this point, only a small fraction of species has been studied.

In prairies, smoke is produced by fires that are important in modulating prairie plant communities historically (Howe 1994a; McClain et al. 2021) and in managing prairies today

(Knapp et al. 2009). The impact of fire on plant diversity can depend on the season in which the fire occurred (Knapp et al. 2009; Dickson et al. 2019). Fire season can differentially impact early- and late-season species (Howe 1994b; Howe 2011). Howe (1999) demonstrated that early-flowering *Zizia aurea* abundance greatly increased with late season compared to early season burns. This varied response of the community to timing of fire may be important, especially for early- and late-flowering species.

In a previous study (Deever et al., Chapter 2), we conducted a forb addition experiment testing what limits the establishment of early- and late-flowering forbs and how their introductions to restorations influence plant diversity. We found that early-flowering species had lower success overall, and both early- and late-flowering species were recruitment limited and had very low survivorship from seed. Further, additions of both these groups increased plant diversity, though additions of early-flowering species increased diversity more than additions of late-flowering species (Deever et al., Chapter 2). This suggests that early-flowering species in particular are difficult to establish and further study is needed to determine methods for their successful establishment. Here, we build on this in a greenhouse germination experiment by examining how timing of smoke and cold cues influences germination of early- and late-flowering forb species.

Given that fire season can be important in modulating the prairie plant community and that smoke in particular can play a role in germination, the timing (season) of smoke application as a germination cue may be important. We hypothesized that if fire can differentially impact plant diversity depending on fire season, perhaps smoke cues can differentially impact species depending on timing (season) of smoke's application. Since timing around the winter season is what largely distinguishes late and early season burns, this may be an important component of the role of fire and smoke. Given the varied response of early and late season prairie species to burn timing, it is possible that they have evolved germination cues around this seasonal variation, i.e., they have different responses to smoke depending on the time of year. Even though burn season varies and smoke is a known cue, previous research has not examined the timing of smoke application.

Given their low abundance in restorations (Deever et al., Chapter 2), we hypothesized that early species would have lower germination than late flowering species in our experiment. Further, the timing of smoke application relative to cold stratification may be important and affect early- and late-flowering species differently. Specifically, our hypotheses were (i) early flowering forbs would have reduced germination compared to late flowering forbs, (ii) forb germination would be increased by cold and smoke stratification, and (iii) early and late flowering prairie forbs would respond differently to smoke applied before cold stratification compared to smoke applied after cold stratification. Here, we report on the response of nine prairie forb species to cold, aerosol smoke, and the timing of these two cues.

Methods

We used nine prairie forb species based on early and late flowering phenology (Table 1) to test germination response to cold stratification, smoke exposure, and timing of smoke exposure relative to cold stratification. Seeds were purchased from a seed merchant (Prairie Moon Nursery, Winona, MN). Cold stratification treatments (cold and no cold control) and smoke treatments (pre-cold (fall) smoke, post-cold (spring) smoke, and no smoke control) were applied in a 9 x 2 x 3 factorial arrangement. Three replicates of 50 seeds per treatment per species were used for a total of 162 pots each containing 50 seeds.

Cold stratification treatment consisted of a placing seeds and a small amount of moistened sand into plastic bags with several small holes for gas exchange. These were left in a 6.5° C cooler for several months (October 19-April 20). Smoke treatment consisted of producing aerosol smoke with a beehive smoker and pumping it through a 1 m tube into a sealed chamber containing suspended tulle fabric bundles of seed. Smoke was produced by burning dried material from two common prairie species (*Andropogon gerardi* and *Heliopsis helianthoides*). Smoke production and pumping was maintained for five minutes, after which the tube was sealed, and chamber left for an additional 10 minutes. This resulted in a total smoke time of fifteen minutes which is within the smoke period used in previous studies (Jefferson et al. 2008; Schwilk and Zavala 2012). Internal temperature of the chamber was monitored throughout and did not rise more than 6.5 °C above ambient. Smoke treatments were applied before cold treatment in October of 2019 and after cold treatment in April 2020. No cold and no smoke treatment seeds were stored at room temperature until use. After treatments were applied, seeds were planted in pots Iowa State University's Bessey greenhouse (Ames, IA, USA) and germinant number/50 recorded every other day. Germinants were removed from pots immediately after recording. The experiment was continued until 7 days had passed with no new seedling emergence.

Statistical analysis

We analyzed germination rate (emergent seedling #/50) among treatments using a generalized linear model (proc GLIMMIX in SAS 9.4) with a Poisson distribution. Replicate was a random term, and all other others were fixed. We used an *a priori* contrast of early and late species to test for a difference between these two groups. This contrast compared the means of all early phenology species to the means of all late phenology species. When interactions were significant, we used slice tests to compare treatments to their respective controls. In addition, for smoke treatments we used a Tukey's test (Tukey-Kramer adjustment for multiple comparisons) for comparisons between each of the three treatments.

Results

Overall mean germination varied significantly among species ($F_{8,124} = 141.6$; $p < 0.001$) and was highly variable with percent germination ranging from approximately 4% to 70% (Figure 1). Spring flowering species had lower germination, on average, than fall flowering species (contrast $F_{1,124} = 193.9$; $p < 0.001$). Mean spring flowering species germination was 7.2% and mean fall flowering species germination was 35% (Figure 1). The main effects of smoke ($p = 0.07$) and cold ($p = 0.1$) and were not statistically significant, but species did interact with these treatments differentially (Table 1).

Species had varied responses to smoke ($F_{16,124} = 2.5$; $p = 0.002$) (Table 2). Of the nine species tested, *Anemone canadensis* ($F_{2,124} = 4.5$; $p = 0.01$) and *Symphyotrichum sericeum* ($F_{2,124} = 9.1$; $p < 0.001$) responded to smoke. For both these species, response depended on timing of smoke application (Figure 2). *Anemone* produced similar numbers of germinants after spring smoke (3.6% ± 1.8% SE) and in the control (4.0% ± 1.5% SE) ($p = 0.8$) while fall smoke (9.0% ± 2.0% SE) increased germination compared to control ($t = 2.3$; $p = 0.02$) and spring smoke ($t = 2.5$; $p = 0.01$). The response of *S. sericeum* to spring smoke (22.6% ± 10.6% SE) was similar to controls (29% ± 8.4% SE) ($p = 0.1$) but fall smoke (42% ± 9.4 SE) increased germination compared to control ($t = 2.7$; $p = 0.009$) and spring smoke ($t = 4.1$; $p < 0.001$).

Species also had varied response to cold ($F_{16,124} = 10.9$; $p < 0.001$). Of the species tested, five had decreased germination and two had increased germination after cold treatment (Figure 3) while two showed no difference between treatment and control (Table 2). Cold increased the germination of *Sisyrinchium* (5.8% ± 1.6% SE) compared to control (2.6% ± 0.8% SE) ($F_{1,124} =$

4.9; $p = 0.03$). Similarly, *Viola* germination increased from 0.44% (± 0.44% SE) under control conditions to 7.2% (± 1.7% SE) under cold treatment ($F_{1,124} = 14.5$; $p < 0.001$). Germination of three other early-flowering species was reduced by cold compared to control. These included *Anemone* (cold 4.0% ± 1.2% SE vs. control 7.2% ± 1.9% SE) ($F_{1,124} = 3.8$; $p = 0.05$), *Antennaria* (cold 2.0% ± 0.82% SE vs. control 5.4% ± 1.4% SE) ($F_{1,124} = 6.3$; $p = 0.01$), and *Pulsatilla* (cold 6.2% ± 2.0% SE vs. control 12.8% ± 2.2% SE) ($F_{1,124} = 10.0$; $p = 0.002$). Similarly, two late-flowering species had reduced germination. These were *Solidago* (cold 2.6% ± 0.6% SE vs. control 10.2% ± 1.6% SE) ($F_{1,124} = 17.2$; $p < 0.001$) and *S. sericeum* (cold 14.6% ± 6.6 SE vs. control 47.8% ± 4.0% SE) ($F_{1,124} = 70.4$; $p < 0.001$). *S. novae-angliae* had the highest germination rate of all species but had similar germination in control and cold treatments (control 71.6% ± 2.6% SE) (p = 0.1). Similarly, *Zizia* had relatively high germination for a spring flowering species but showed similar germination between cold treatments (control 18.0% ± 2.2% SE) (Supplemental table 1).

Discussion

Smoke treatments can sometimes lead to increased germination of prairie forbs (Jefferson et al. 2014). Jefferson et al. (2008) found aerosol smoke to have varied effects on 37 Tallgrass Prairie species, promoting germination in roughly one third and having no effect or decreasing germination in others. We found that smoke treatments increased germination in two species and for both of these species, the timing of smoke application was important, with increases in germination only when smoke was added in the fall prior to cold treatment.

We found prairie forbs to have a wide range of germination rates. Fall flowering species, on average, had far higher germination rates than spring flowering species. Highly variable germination rates among prairie forbs has been reported (Green and Curtis 1950; Sorensen and

Holden 1974; Voigt 1977), but the much lower germination rate of early flowering species has not been specifically addressed and may be important for restoration. Early species can be under-represented or missing in restored prairie (Carter and Blair 2012; Deever et al., Chapter 2), and this may in part be because some have limited germination. Hillhouse and Zedler (2011) suggest that once identified, germination limited species could be increased in restorations by increasing the number of seeds planted. This may not be practical for rare target species or for species that may have multiple germination cues or where the timing of these cues matters. For such species the ability to increase germination rate through seed treatments may be important for increasing their abundance in restored communities. Application of seed treatments such as smoke may be a useful management tool in prairie restoration (Cox et al. 2017). For seed treatments to be successful, determining responses of specific species of interest is important.

Forbs in our study showed varied responses to cold treatment. We are among studies that have noted varied response to cold among prairie species (Greene and Curtis 1950). Cold increased germination in two species in our study which aligns with common recommendations for cold stratification and its demonstrated effects on many prairie species (Greene and Curtis 1950; Packard and Mutel 1997). *Viola pedata* was one species that required cold to germinate. This is important since this species tends to be difficult to establish in restored communities and is of special interest due to its role as the host plant for the Regal Fritillary butterfly (Debinski and Kelly 1998). These are the types of species that will benefit from understanding species-specific germination requirements.

Cold treatment also decreased germination rate of several forbs in our study. This is reportedly less common than cold increasing germination but is not unheard of (Greene and Curtis 1950). Responses to cues may also vary between field and glass house conditions, though this is not always the case (Hillhouse and Zedler 2011; Cox et al. 2017). In our study, *Solidago nemoralis* had reduced germination after cold treatment, but Deever et al. (Chapter 2, unpublished data) found that seedlings of this species were not present in experimental plots until the first winter (cold period) after seeding had passed, though they were present at extremely low numbers (< 1% survivorship) . This suggests that blanket recommendations for seed treatment may not be ideal for establishing target species given their potentially varied response. Further, cold may be important in its relation to timing of application of other cues for some species.

Approximately 22% of species had increased germination after smoke exposure. Importantly, we found for the first time that the timing of smoke application relative to cold mattered. It is not uncommon to find only a subset of species tested responding to cues. In Australian and South African species, positive germination response after smoke exposure can be near 50% and higher (Brown et al. 1993; Dixon et al. 1995). Among species from the Great Plains region of the United States, positive response to smoke is often lower, around 10-25% (Jefferson et al. 2008; Schwilk and Zavala 2012; Bennett and Perkins 2017). While others have found similar percentages of species that respond to smoke, to our knowledge previous studies have not investigated the role timing of cues may play. In two *Salvia* species for example, aerosol smoke can act as a replacement for wet cold treatment (Schwilk and Zavala 2012). Some species, such as *Echinacea*, may also have multiple cues that interact to stimulate germination (Qu et al. 2004). For this species, in our case, there was increased germination after multiple cues but only if smoke was applied before cold stratification.

We were not able to test possible mechanisms for the timing to smoke response, but we can suggest some possibilities. It is possible that once the smoke volatiles coat a seed, the cold wet period weakens the seed coat and moisture carries the smoke as the seed is imbibed. Water can act as a vehicle to deliver smoke chemicals. In Australia, rains can help move smoke chemicals into the soil, stimulating germination in some seedbank species (Roche et al. 1997). It is possible that cold season after smoke exposure acts in a similar way. In contrast, when applying smoke after the cold period, the penetration of the seed coat may have already taken place and the smoke chemicals are not readily transported. Further research is needed to test the mechanisms behind the timing response.

The timing of cues may be related to the evolution of these species with fire timing. Wildfires can occur at any time of year (Knapp et al. 2009; McClain et al. 2021). If the timing of fires and associated cues such as smoke can differentially impact species, then cues at different times may differentially impact the plant community. This may be important if historic wildfire timing varies from prescribed fire season used in management. Historically, fires frequently occurred in the growing season prior to the onset of winter (Howe 1994a; McClain et al. 2021). Current management practices of prairie tend to prescribe fire in the dormant season during or shortly after the winter cold (Howe 1994a; Dickson et al. 2019). This likely favors certain plant species over others (Howe 1994b; Howe 2011). Our results suggest that for some species, the role of smoke as a germination trigger may depend on fires being in the summer or early fall season.

In addition to prescribed fire season, knowledge of how timing of treatments influences germination may be important if cues such as smoke treatment are used as a management tool. Smoke has been shown to influence germination when applied before seeding (Cox et al. 2017) or when applied to field plots after seeding (Dixon 1995). Here, we suggest that smoke should be applied in the fall before the dormant season occurs for the two prairie species in question. This could likely be done with aerosol or liquid smoke application to seed supplies. Alternatively, smoke could be applied to seeded sites through spraying of a liquid smoke solution or through aerosol smoke application to small target areas (Dixon et al. 1995; Abella 2009).

Varied responses to cues may be important for establishing diverse plant communities. If species are seeded but lack the appropriate cue(s) to germinate or a cue is not applied at the correct time, they may not establish into the community. This could be because they are germination limited without the appropriate cue, or cues come too late to aid in successful establishment in the long term. In the latter case, if a cue comes too late after seeding (smoke from a fire after some of the seeded community has established for example) it may be too late for that plant to become established due to a priority effect. Exotic species may exhibit a priority effect over natives by germinating more quickly and at higher percentages (Wilsey et al. 2015). In addition, germination cues such as smoke may increase the germination of weedy species (discussed in Cox et al. 2017) which could result in reduced establishment of target species. Timing of cues may be an important consideration for establishing diverse target species.

The variable germination rates and varied response of forb germination rate to treatments suggests that a diversity of cues or treatments may be needed to successfully establish diversity of target species. Future work should focus on germination requirements of species of interest and how to apply this knowledge when planning a restoration. If all species do not establish and respond to treatments in the same way, then not all seed should be handled the same way when applying seed mixes for restoration.

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Tables and Figures

Table 1. Type III tests of fixed effects and contrast of fall and spring flowering species for germination rate response of nine prairie forb species to smoke and cold treatments. Main effects of cold and smoke were not significant, but species had varied germination and differential responses to cold and smoke. A contrast indicated spring and fall flowering species had significantly different germination.

Source	d.f.	*F*	*p*
Smoke	2, 124	2.8	0.07
Species	8, 124	141.6	< **0.001**
Species*Smoke	16, 124	2.5	**0.002**
Cold	1, 124	2.4	0.1
Species*Cold	8, 124	10.9	< **0.001**
Contrast			
Spring vs. fall flowering	1, 124	193.9	< **0.001**

Table 2. Germination rate response of nine prairie forb species after cold and smoke treatments. Compared to controls (no cold and no smoke), responses (R) were positive (+), negative (-), or not significant (0). p-values from slice tests of species*treatment interactions.

Scientific name	Common name	R cold	*p*	$F_{1,124}$	R smoke	*p*	$F_{1,124}$
Spring flowering phenology							
Anemone canadensis	Canada Anemone	-	0.05	3.8	+	**0.01**	4.5
Antennaria plantaginifolia	Pussytoes	-	**0.01**	6.3	ns	0.2	1.4
Pulsatilla patens	Pasque Flower	-	**0.002**	10.0	ns	0.2	1.4
Sisyrinchium albidum	Blue-eyed Grass	+	**0.03**	4.9	ns	0.3	1.1
Viola pedata	Birdfoot Violet	+	< **0.001**	14.5	ns	0.06	2.9
Zizia aptera	Heart-leaf Alexanders	Ns	0.1	2.2	ns	0.5	0.8
Fall flowering phenology							
Solidago nemoralis	Old Field Goldenrod	-	< **0.001**	17.2	ns	0.6	0.5
Symphyotrichum novae-angliae	New England Aster	Ns	0.1	2.1	ns	0.3	1.2
Symphyotrichum sericeum	Silky Aster	-	< **0.001**	70.4	+	< **0.001**	9.1

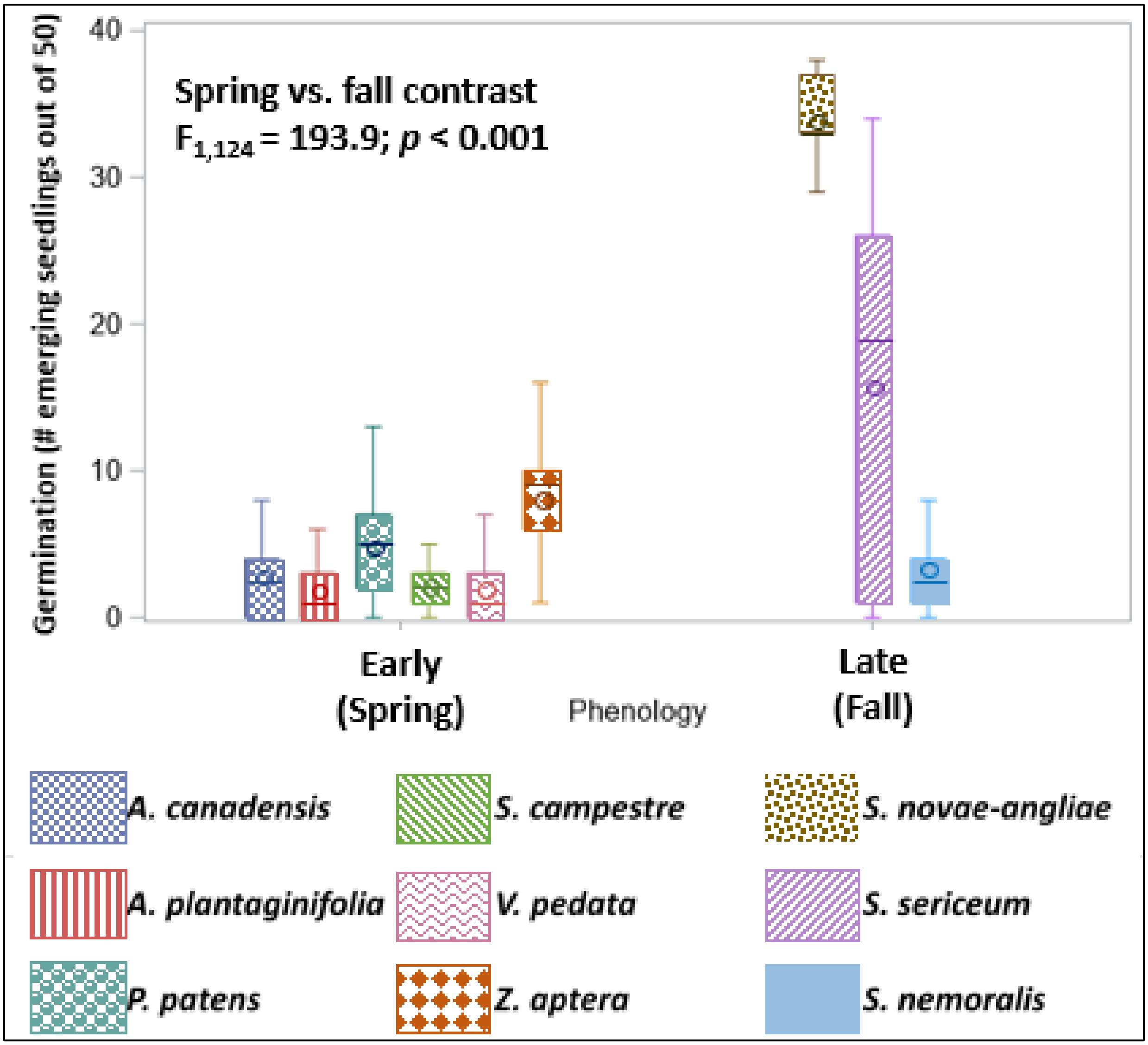

Figure 1. Overall germination rate of species across cold, smoke, and control treatments in a prairie forb germination study. The main effect of species was significant ($F_{8,124} = 141.6$; $p < 0.001$) with species showing varied germination rates. A contrast of spring (early) and fall (late) flowering species indicated a significant difference in germination rate of these two groups. Mean of spring flowering species is 3.6/50 and of fall flowering is 17.5/50. Boxes show means (circles), medians (inner lines), 25th and 75th percentiles, and bars show maximum and minimum observations.

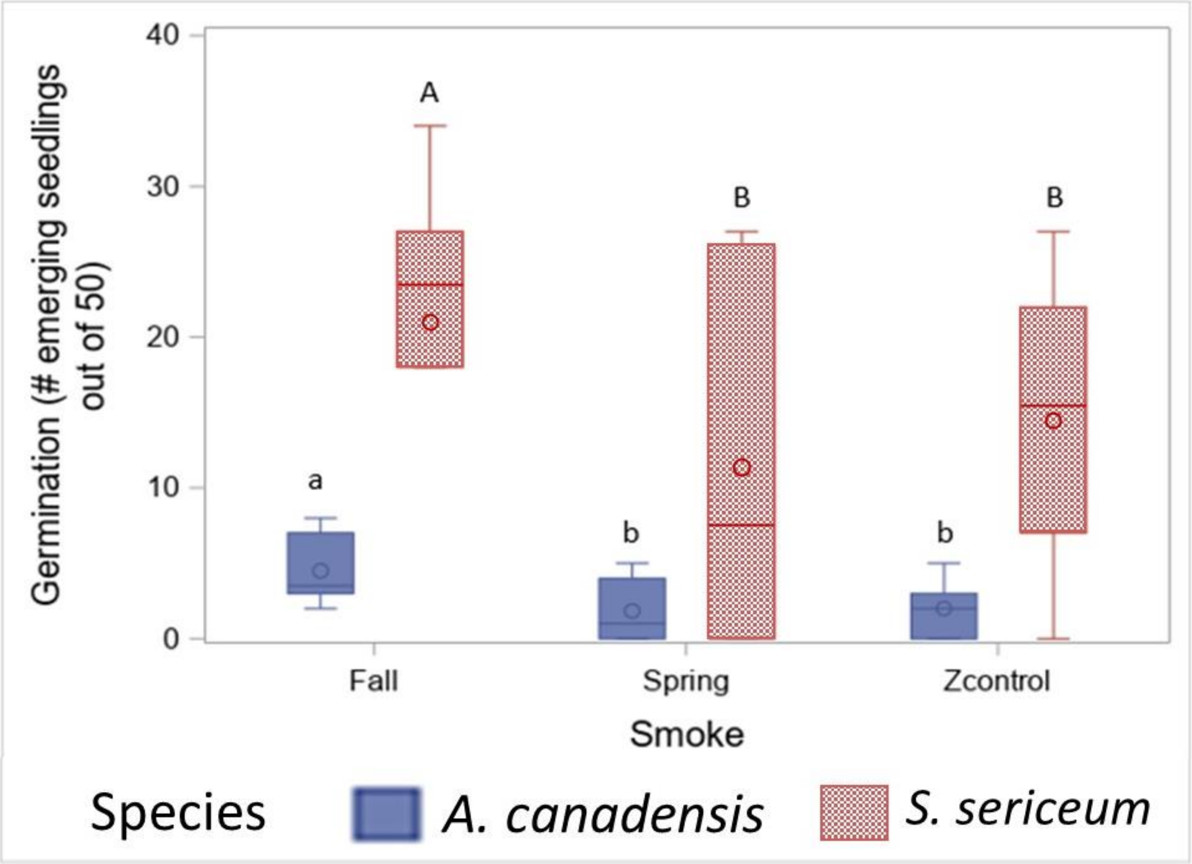

Figure 2. Germination rate of *Anemone canadensis* and *Symphyotrichum sericeum* after smoke treatments applied before cold treatment (Fall smoke) or after cold treatment (Spring smoke), and no smoke (zcontrol). Both species had increased germination following application of fall smoke but similar germination to controls following spring smoke. Boxes with the same letters are not significantly different. Boxes show means (circles), medians (inner lines), 25th and 75th percentiles, and bars show maximum and minimum observations.

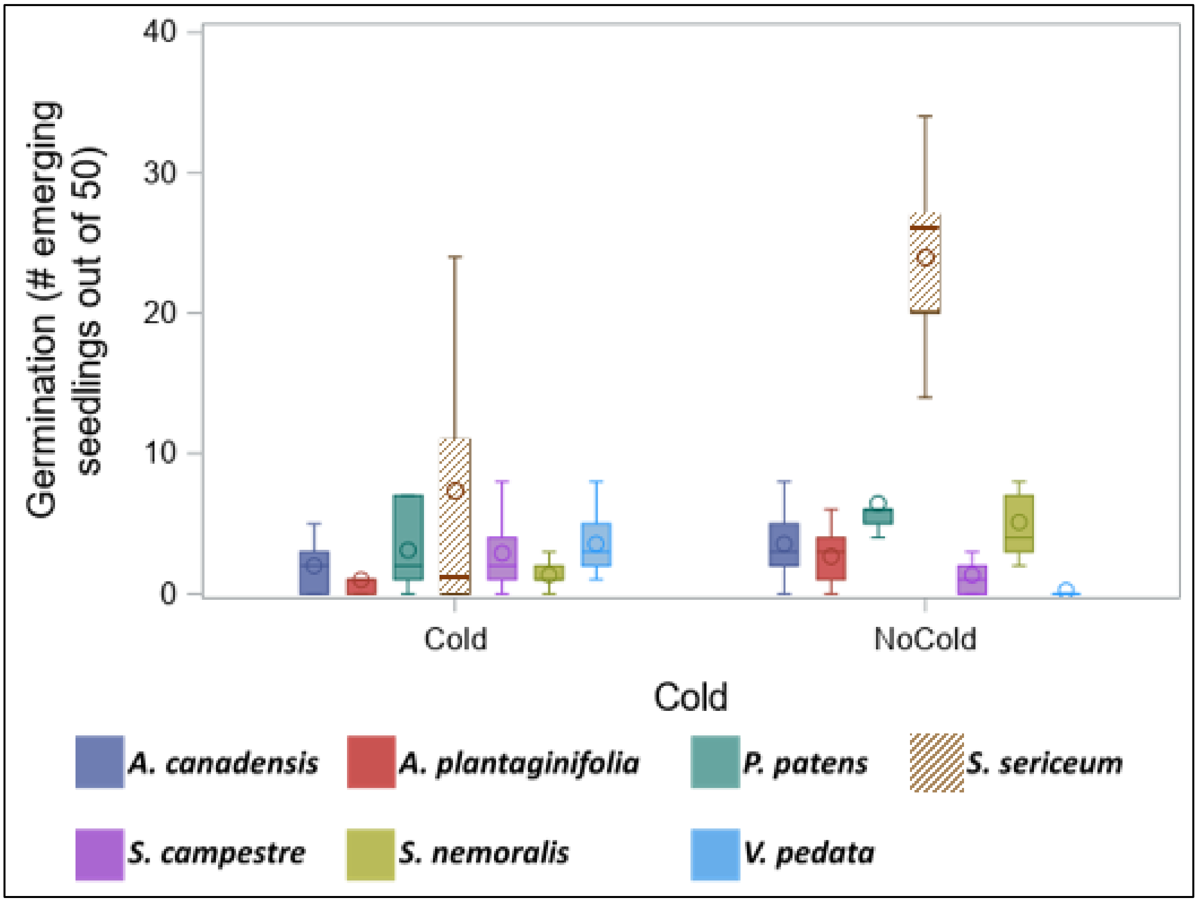

Figure 3. Germination rate of forb species after cold treatments compared to no cold. Compared to control (NoCold), two species (*Sisyrinchium* and *Viola*) had increased germination after cold exposure while five species (*Anemone*, *Antennaria*, *Pulsatilla*, *Solidago*, *S. sericeum*) had decreased germination.

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Appendix A. Supplemental Table

Supplemental table 1. Mean germination rate (# emerging seedlings out of 50) of nine prairie forbs exposed to smoke and cold treatments. *No smoke* and *No cold* are controls for their respective treatment.

Species	**Fall smoke**		**Spring smoke**		**No smoke**		**Cold**		**No cold**	
Spring flowering	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Anemone canadensis	4.5	2.43	1.83	2.23	2.00	1.90	2.00	1.80	3.56	2.79
Antennaria plantaginifolia	1.17	1.60	1.83	1.33	2.5	2.59	1.00	1.23	2.67	2.12
Pulsatilla patens	6.00	4.29	4.00	2.61	4.33	3.98	3.11	3.06	6.44	3.43
Sisyrinchium albidum	1.66	1.21	1.83	1.47	2.83	3.13	2.89	2.47	1.33	1.22
Viola pedata	1.50	1.87	1.17	1.33	3.00	3.69	2.56	2.55	0.22	0.67
Zizia aptera	9.00	5.51	8.00	2.10	7.00	2.45	7.00	3.61	9.00	3.43
Fall flowering										
Solidago nemoralis	3.67	3.20	2.67	2.80	3.33	2.16	1.33	1.00	5.11	2.37
Symphyotrichum novae-angliae	30.83	7.25	35.67	4.63	34.83	2.23	31.78	5.93	35.78	3.90
Symphyotrichum sericeum	21.0	11.56	11.33	12.89	14.50	10.21	7.33	9.95	23.89	5.99

Appendix B. Seed Smoking Apparatus

CHAPTER 4. GENERAL CONCLUSIONS

Restoration is important for continued existence of the tallgrass prairie. It is successful at building diverse plant communities, but there are gaps in knowledge and effectiveness that require further study. My research focused on whether early-flowering and late-flowering forbs were less abundant in restorations compared to remnants and what ecological processes underpin their establishment success or failure. My results provide practical insight into what limits establishment of these forbs and how to successfully establish them.

The first part of my research found that early-flowering species were less abundant in prairie restorations compared to prairie remnants. The forb addition experiment found that additions of early- and late-flowering species increased plant community diversity and that early-flowering forb additions increased diversity more than late-flowering forb additions. These results suggest an empty temporal niche in restorations for early-flowering and late-flowering species that can be filled with higher richness additions of these species.

Further, I found that early-flowering and late-flowering forbs were recruitment limited and not seed limited and that the seed-juvenile stage of establishment was limiting. The success of transplants over seeds in this experiment is important for restoration. This suggests that transplanting can be used to establish species of interest that do not establish readily from seed in the field. Transplanting rich mix of target forbs will ensure success of groups like our early-flowering and late-season species.

The second part of my research found that early-flowering species had much lower germination rate than late-flowering species. Further, forb species had varied germination response to cold and two species responded to smoke treatments. Most notably, timing of application of a smoke cue was important for increasing germination of those two species. This is important since timing of cues has not been specifically addressed and may be important for establishment of some species.

Taken together these results suggest several important considerations for prairie restoration. These include that early-flowering species are more difficult to establish than late-flowering due to both lower germination rate and lower survivorship in the field. Transplanting is a viable method to establish these and other missing or hard to establish species in restorations. Further, to realize goals such as extending flowering phenology of restorations, higher richness additions should be used to ensure filling of available niche space.

Increasing germination likely requires species-specific approaches. Species respond differently to cues, so blanket approaches (such as treating and spreading all seed in the same way) are not ideal when attempting to establish target species and thus increase plant diversity in restorations. For example, if smoke treatments are used, it is likely that only a subset of species will respond, and this response may depend on timing of application. This means that planning is needed if smoke treatments are to be applied prior to a cold season and seeding.

Future Considerations

These studies identified a gap in restoration success that demonstrates the need for further study. We tested three early-flowering and three late-flowering species but there are many more species in these groups that are absent in restorations. Future research should examine more of these species, such as hemiparasite species. Further, the impact of these early and late floral resources on pollinators should be examined. In addition, a more targeted approach to establishing target species is important and this should involve study of germination cues for species as well as timing of these cues. Mechanisms of these cues should be explored to increase germination and successfully establish target forb species.