WHERE SHOULD THEY COME FROM? WHERE SHOULD THEY GO? TESTING IF NON-LOCAL SEED SOURCING STRATEGIES CAN MEET RESTORATION GOALS

By

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PUBLIC ABSTRACT

The widespread loss of natural ecosystems is leading to the extinction of species across the globe. Ecosystem restoration—helping damaged ecosystems recover—can slow extinction by creating new habitat for species to survive. Recognizing restoration's importance, the United Nations declared the years 2021-2030 as "the decade on restoration" with plans to initiate restoration on 350 million hectares of land. However, whether a restoration effort will be successful can be unpredictable, with similar restoration methods producing very different outcomes, highlighting the need for additional research. A key challenge is establishing large native plant populations that can survive at restoration sites. Many desirable restoration species are rare in the areas around a restoration site, so they must be manually introduced to be part of the plant community, which is often done by planting seeds. Yet, where to obtain seed from (i.e. which seed sources) to best reestablish native plant populations remain unclear, as few studies have evaluated the impacts of seed sourcing under realistic restoration conditions. To address this gap, I developed three research projects to evaluate how seed sourcing impacts plant communities in tallgrass prairies, an imperiled grassland ecosystem that often relies on seed addition for restoration.

The first project evaluated the effectiveness of the most common seed sourcing technique, local seed sourcing. Most restoration professionals obtain seed from the nearest location possible, assuming that these populations have been exposed to environmental conditions similar to those at the restoration site and are better suited to survive and thrive in those conditions. Despite its widespread use, there is limited research testing whether local seed sources are more reliable at establishing native plant populations than seeds sourced from further away. To address this gap, I surveyed sites undergoing prairie restoration across Michigan, measuring the presence and abundance of five plant species sourced from various locations. I found that local sources were no more likely to be at a restoration site than plants sourced from further away, illustrating that local sources may not be the only suitable source of restoration material.

Next, I wanted to investigate whether mixing seeds from multiple sources (to increase the amount of genetic diversity within a plant population) could result improve restoration outcomes. While genetic diversity is known to influence plant population establishment, our understanding of how seed sourcing affects the plant community as whole, especially at restoration sites, is

limited. It is also unclear whether the effects of genetic diversity depend on factors that vary across restoration sites, such as the number of seed-eating animals, which could influence the success of multi-source seed mixes. To fill this knowledge gap, I used a restoration experiment that changed how many seed sources were used, along with other factors known to vary across restoration sites including the number of species in the seed mix and access by animals that consume plants and/or seeds (i.e consumers). I found that using a seed mix with multiple sources affected plant communities only when consumers were present. Additionally, the number of species in a seed mix had a greater influence on plant communities than the number of seed sources. These results suggest that the impact of adding sources to a seed mix depends on site conditions and is less important than other decisions, such as the number of species to include in a seed mix.

Finally, I assessed how genetic diversity and seed source location influence plant establishment under current and anticipated conditions under climate change. To do this, I established a restoration field experiment and seeded plots with three seed sourcing strategies that varied in both the number of sources and where those sources came from: (1) local (2) admixture (mixing local sources with seeds from similar climates), and (3) climate-adjusted (mixing local sources with seeds from regions expected to match future climate conditions). Some plots had chambers on them that raised the temperatures plants experienced. I found that plots seeded with the local source supported the greatest number of planted species compared to those seeded with a non-local source. However, establishment differences disappeared in high diversity seed mixes since the local source was included. Notably, climate-adjusted mixes maintained a high number of seeded species under warming, which otherwise reduced these species establishment in local and admixture plots. These findings suggest that while local seed sourcing can reliably establish plant populations, high source diversity seed mixes can too, and may enhance the community’s resilience under climate change.

My results show that the impacts of seed sourcing will likely vary across restoration efforts: while local seed sources often lead to the high establishment, this is not always the case. Also, increasing genetic diversity may increase a plant populations’ resiliency to climate change, although it may also be detrimental in some environments (i.e. those with abundant consumers). This work advances our understanding of the role seed sourcing has on restoration outcomes and helps practitioners make informed decisions on how best to restore their landscape.

ABSTRACT

The widespread loss of natural ecosystems is driving a global biodiversity crisis. Ecosystem restoration—assisting the recovery of damaged or destroyed ecosystems—combats biodiversity loss by creating landscapes that better support native species. Recognizing restoration's importance, the United Nations has declared the years 2021-2030 as "the decade on restoration" with plans to initiate restoration on 350 million hectares of land. However, restoration outcomes are highly variable, highlighting the need for additional research. A key challenge is establishing large, persistent native plant populations at restoration sites. Since many species are dispersal limited, they must be manually brought to the landscape, often through seed addition. Yet, where to obtain seed from (i.e. which seed sources) to best reestablish native plant populations remain unclear, as few studies have evaluated the impacts of seed sourcing decisions on native plant communities under realistic restoration conditions. To address this gap, I developed three research projects to evaluate how seed sourcing impacts plant communities in tallgrass prairies, an imperiled ecosystem that often relies on seed addition for restoration.

The first project evaluated the efficacy of the most common seed sourcing technique, local seed sourcing. Most practitioners source seed from the nearest location, assuming that these populations are adapted to environmental conditions similar to those at the restoration site and are best suited to establish and persist when planted. Despite its widespread use, there is limited research testing whether local seed sources are more reliable at establishing than seeds of plants sourced from further away. To address this gap, I surveyed sites undergoing prairie restoration across Michigan, measuring plant establishment for five plant species sourced from various locations. I found that local sources established no better than plants sourced from further away. Other factors, including post-seeding management and high seeding rates, were stronger predictors of plant establishment. These results illustrate that local seed may not be the only suitable source for restoration material.

Next, I wanted to investigate whether mixing multiple sources together (to increase genetic diversity) could result in greater plant establishment and plant diversity during restoration. Although genetic diversity is known to influence population-level processes such as plant establishment, our understanding of the community-level consequences of seed sourcing, especially during restoration, is limited. Moreover, it is unclear whether the effects of genetic diversity depend on site-specific factors, which could affect the success of multi-source seed

mixes in different contexts. To fill this knowledge gap, I used a restoration experiment that manipulated the number of seed sources sown, along with other factors known to vary across restoration sites including the number of species in the seed mix, proximity to the edge of a site, and vertebrate consumer access. I found that using a multi-source seed mix affected plant communities only when consumers were present. Additionally, the number of species in a seed mix had a greater influence on plant communities than the number of seed sources. These results suggest that the impact of adding sources to a seed mix is likely context-dependent and less influential than other restoration decisions, such as the number of species added to a seed mix.

Finally, I assessed how genetic diversity and seed source location influence plant establishment under current and anticipated future climate conditions. To do this, I established a restoration field experiment and seeded plots with three seed sourcing strategies that varied in both the number of sources and where those sources came from: (1) local (2) admixture (mixing local sources with seeds from similar climates), and (3) climate-adjusted (mixing local sources with seeds from regions expected to match future climate conditions). Some plots were subjected to experimental warming. I found that plots sown with the local source supported the greatest number and, often, abundance of sown species, compared to those sown with a non-local source. However, establishment differences disappeared in high diversity seed mixes when the local source was included. Notably, climate-adjusted mixes maintained high richness under warming, which otherwise reduced richness in local and admixture plots. These findings suggest that while local seed sourcing reliably meets establishment goals, high source diversity seed mixes offer similar establishment success and may enhance the community's resilience to future conditions.

Overall, my results show that seed sourcing has context dependent impacts: while local seed sources often provide high establishment, this is not consistently the case. Moreover, increasing genetic diversity may make plant populations more resilient to changing conditions, but may also be detrimental in some environments (i.e. those with abundant vertebrate consumers). This work advances our understanding of the role the role seed sourcing has on restoration outcomes and helps practitioners make informed decisions on how best to restore their landscapes.

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CHAPTER ONE: THE EFFECTS OF SEED SOURCING DECISIONS ON RESTORATION OUTCOMES

Ecosystem restoration to reverse biodiversity loss

Anthropogenic activities including urbanization, industrialization, and large-scale agriculture have transformed natural ecosystems, with estimates that up to 50% of natural landscapes have already been lost (Ellis et al., 2020; Vitousek et al., 1997). The magnitude of this loss has contributed to a global biodiversity crisis (Segan et al., 2016; Singh, 2002) and alterations in ecosystem functioning (Kardol et al., 2018). Ongoing climate change threatens additional habitat (*reviewed in* Mantyka-pringle et al., 2012) putting more species, especially those sensitive to shifting climates, at increased risk of extinction (Etterson et al., 2020). To preserve ecosystem function and biodiversity, especially for species threatened by climate change, protection of remaining natural areas remains a top priority for environmentalists and policymakers (Dinerstein et al., 2019; Teske, 2019).

For some ecosystems, though, there is not enough remaining area to retain current amounts of biodiversity (Rodrigues et al., 2004). In these situations, additional habitat must be created to prevent further biodiversity declines through a process known as ecosystem restoration, which has been in practice for almost a century (Jordan et al., 1990). Ecosystem restoration goals vary across projects, but broadly aim to reverse the impacts of habitat loss, preserve and increase biodiversity, and re-establish ecosystem functionality (Gann et al., 2019). Restoration first begins with stopping previous land degradation (e.g., logging and agriculture) followed by active interventions to re-establish native species and re-form ecosystems (Jones et al., 2018). The importance of restoration has been recognized globally, as we are at the halfway point of the United Nations “decade on restoration”, which intends to initiate restoration of over 350 million hectares of land before 2030 (UNEP, 2019). However, there are several outstanding research questions about how best to conduct restoration that must be answered for us to effectively meet this goal (Cooke et al., 2019, 2021), making emerging research on ecosystem restoration more topical than ever (Jones & Murphy, 2023).

Restoration of plant communities

A common first step in restoration is to re-establish native plant communities, which can make the environment more suitable for organisms at higher trophic levels by providing nesting habitat and food resources (Whisenant, 2002). However, many of the species that characterize these restored communities are dispersal limited due to extensive habitat loss and fragmentation, so they are unlikely to arrive at a restoration site without human assistance (Hubbell et al., 1999; Pywell et al., 2003). There are several methods for doing this including hay transfer, drill seeding, or transplanting adult plants (Kimball et al., 2015; Larson et al., 2018). However, one of the least logistically challenging and often most cost-efficient ways to establish a diverse plant community is through seed addition (Kettenring & Tarsa, 2020; Kimball et al., 2015). Restorations initiated by seed addition (i.e. "seed-based restoration") utilize seed either collected from wild populations (Broadhurst et al., 2015) or purchased from seed production farms (Nevill et al., 2016). In the latter, seed is harvested once from a wild population, planted in monoculture, and harvested and replanted for several generations to produce a bulk supply of seed (*reviewed in* Espeland et al., 2017).

Once seeds are added to a site, it is crucial that they are able to germinate and survive in order to establish large, persistent native plant populations (Gann et al., 2019). Seed-based restoration specifically introduces an important filter: seedling establishment. While many seeded seeds will germinate, survival is generally low (Larson et al., 2015; Zeiter et al., 2006). Although initial establishment is not always indicative of the relative abundance of species in mature communities, if species do not establish at all, or in relatively low abundance, they are likely to go extinct at the restoration site (Newman & Pilson, 1997; Purvis et al., 2000).

High establishment and abundance of seeded species is crucial for producing biodiverse communities at a restoration site, as these species contribute to increasing biodiversity in otherwise species-poor areas (Atkinson et al., 2022). Communities with higher biodiversity are generally more stable (Tilman & Downing, 1994; Wagg et al., 2022) and resilient to disturbance (Oliver et al., 2015) promoting ecosystem persistence over the long-term. High establishment of seeded species can also reduce the abundance of nuisance, non-seeded species (Biondini, 2007), a substantial threat to the biodiversity at a restoration site (e.g. Warren et al., 2002). High plant biodiversity has been tied to increases in biodiversity at other levels (Janz et al., 2006; Stevens & Tello, 2011), increasing the number of ecosystem services the plant community can provide (Isbell et al., 2011). Thus, information on which seeds are the most likely to establish and persist at a restoration site is imperative for meeting restoration goals.

Seed sourcing for restoration

A critical question in planning a seed-based restoration effort is determining which seed sources will promote high establishment at a restoration site. However, there is currently no consensus in the scientific community about which sources those are (*reviewed in* McKay et al., 2005; Breed et al., 2013; Bucharova et al., 2019; Gustafson et al., 2005; Jones, 2013; Prober et al., 2015). Regardless, restoration practitioners source seed from the location or producer closest to the restoration site (i.e. local seed sourcing; Gustafson et al., 2005). Practitioners assume that populations near a restoration site have experienced the same (or very similar) environment to the restoration site and therefore should be adapted to establish and persist in those conditions (Bower & Aitken, 2008; Mortlock, 2000). This is based on a wealth of past research across ecosystem types showing local adaptation in wild plant populations (Baughman et al., 2019; Bischoff et al., 2006; Hereford, 2009; Whitlock, 2015). Thus, collecting seed from the single nearest location is expected to maximize plant establishment at a restoration site compared to obtaining seeds from further away (Gustafson et al., 2005; Mortlock, 2000).

However, research testing the assumption that local seeds will establish better than seeds sourced from further away have provided mixed results: while some studies have supported the assumption (Baughman et al., 2019; Gustafson et al., 2005; Montalvo & Ellstrand, 2000) other studies show no impact of source location on establishment (Carter & Blair, 2012; Gallagher & Wagenius, 2016), and one study found that the local source had the worst establishment (Nolan et al., 2023). One explanation for these mixed results is that “local” is often arbitrarily defined in countries without clearly-defined ecoregions, or geographic areas with similar environments where plants can be exchanged without fitness decreases (i.e. the USA; Goldsmith et al., 2022). Thus, seed sources that are considered local may in fact be adapted to a different environment than the restoration site. Additionally, while conditions such as temperature and precipitation may be similar between a very local source location and the restoration site, previous land use at can modify the hydrology, soil conditions, and interspecific interactions (Koziol et al., 2012). This can create a novel environment, resulting in even locally-sourced populations not being adapted to restoration site conditions (*reviewed in* Lau et al., 2019). Without further studies on the efficacy of local seed sourcing, it is unclear whether the “local is best” approach will allow us to meet restoration goals.

Importantly, local adaptation is not the only consideration when choosing seed sources: it is also important that populations have high amounts of genetic diversity. Plant populations with high genetic diversity may better meet restoration goals than local sources by promoting high sown species establishment (Buza et al., 2000; Rius & Darling, 2014; Williams, 2001) and persistence (van Treuren et al., 1993) than low diversity populations. This may lead to a reduction in the abundance of nuisance, weedy species (Crutsinger et al., 2008) and increase ecosystem functioning (*reviewed in* Kettenring et al., 2014). Higher genetic diversity within a population can also ensure it has the phenotypic diversity necessary to undergo adaptive evolutionary change as the environment of the restoration site changes in the future (Breed et al., 2013; Burton & Burton, 2002; Davis et al., 2005), which may be lacking in a local population with low genetic diversity (Etterson et al., 2020). Given these potential benefits, increasing population genetic diversity by collecting seed from multiple locations may increase restoration success relative to using the local source alone.

Two alternatives to local seed sourcing have been proposed that increase the amount of genetic diversity in a seed mix in different ways: regional admixture (Bucharova et al., 2019) and climate-adjusted seed sourcing (Prober et al., 2015). The first proposes mixing the local source with additional sources from climatically similar environments to the restoration site to increase genetic diversity while minimizing the risk of introducing maladapted genotypes (Bucharova et al., 2019). However, if seeds from these sources are near the threshold climate they can survive in, the restoration may fail in the future as the climate changes (e.g., Etterson et al., 2020). Instead, persistence may be supported by adding several sources along a gradient of the anticipated future climate of the restoration site (Climate-adjusted seed sourcing; Prober et al., 2015). If these sources are too maladapted to survive in the current climate, though, they may not survive long enough to provide adaptive potential when the climate does change.

Importantly, practitioners have not been incentivized to move beyond local seed sourcing due to the higher costs of genetically diverse seed mixes (Barak et al., 2021; Smith et al., 2007) and without evidence supporting the use of these alternatives in restoration efforts (*but see* Höfner et al., 2021; Lindstrom et al., 2021). Most research in this field has been conducted in common gardens established in greenhouses or in common-garden fields, where environments are generally uniform or controlled. This is a problem because because a variety of other factors that differ across restoration sites that can influence variation in restored plant populations and communities including, but not limited to, the climate of the planting year (Groves et al., 2020), the interspecific diversity of the planted seed mix (Grman et al., 2013), land-use history (Carter & Blair, 2012), and management decisions (Rowe, 2010). Given that restoration outcomes can be highly variable (Brudvig, 2017), with similar restoration methods sometimes resulting in dramatically different outcomes across sites (e.g. Norland et al., 2015), it is likely that the impacts of seed sourcing decisions will depend on the context-dependencies of a given site.. Moreover, most past studies on the effects of seed sourcing locality and genetic diversity are conducted in monoculture or focus on a particular species. Given that seed based restoration efforts can include a large range of species, with one study surveying a restoration that included 221 species (Groves et al., 2020), research understanding the implications of seed sourcing decisions in a community context is needed.

In this dissertation I present a series of research projects designed to understand the relative importance of seed sourcing, empirically test assumptions of the "local is best" paradigm, and evaluate alternatives to local seed sourcing in a realistic restoration context. In chapter two, I test the assumption that locally sourced seeds will have higher establishment rates than those sourced from further away by conducting a survey of early prairie restorations under various management regimes. In chapter three, I evaluate the relative importance of seed sourcing decisions and how they are impacted by site context-dependencies by utilizing an existing restoration field experiment that manipulated the number of seed sources in a seed mix, along with many other factors known to influence restoration outcomes. In chapter four, I evaluate the relative effectiveness of three seed sourcing strategies at establishing large plant populations and diverse communities using a field experiment.

Study system

My dissertation focuses on answering these questions in tallgrass prairies. The plant communities in this ecosystem are a matrix of tall grasses and forbs, with minimal to no woody vegetation (Robertson et al., 1997). Prairies are maintained through disturbance including periodic fire and grazing by large herbivores (Collins & Wallace, 1990). Tallgrass prairies provide essential ecological benefits including habitat for birds (Bakker & Higgins, 2009) and pollinators (Werling et al., 2014), and also serve as a critical carbon sink (Soussana et al., 2010). Despite the importance of these ecosystems, up to 96% of tallgrass prairies in the North America have been replaced by agriculture and urban development (Samson et al., 2004). While conservation of in-tact prairies remains crucial, ecologists have long recognized that more prairie habitat must be created to prevent the extinction of its characteristic species, and the loss of the ecological benefits these landscapes provide (Anderson, 2009).

Given the importance of prairie restoration, these ecosystems are often considered to be the birthplace of ecological restoration (Jordan et al., 1990). For example, one of the first studies evaluating the success of a restoration effort compared to a remnant site was done in a tallgrass prairie (Cottam & Wilson, 1966). Prairies have also often been the first systems to test emerging ecological theories such as the importance of early establishment (Packard, 1994) and the relationship between plant community composition and ecosystem functioning (Hadley & Buccos, 1967). Prairies also assemble faster than most other systems (Wainwright et al., 2018) and are often restored using seed-based practices (Rowe, 2010). Applying theories about seed sourcing in this system has contributed to an ever-broadening knowledge of restoration methods in prairies and allowed me to answer several questions over a dissertation timeframe.

Chapter 2: Do local seed sources establish better than non-local seed sources?

If local seed sources are best equipped to establish at a restoration site, we would expect to see decreasing establishment rates with increasing geographic distance (*reviewed in* McKay et al., 2005). However, this assumption remains largely untested in restoration settings, and studies examining local adaptation across geographic distances in common gardens have yielded mixed results (Carter & Blair, 2012; Galloway & Fenster, 2000; Mimura & Aitken, 2007; Nolan et al., 2023). This may be because geographic distance is a poor proxy for environmental similarity (Gerst et al., 2011). Instead, direct measures of environmental differences between the source and restoration site locations may be a better predictor of plant establishment, although this hypothesis has also rarely been tested (*but see* Baughman et al., 2019). Alternatively, seed source locality may have a negligible effect compared to other factors manipulated at the restoration site including seeding rate and post-seeding management. However, I am aware of no prior studies that have tested the impacts of seed source locality under variable restoration conditions, so the importance of seed sourcing, relative to other factors, is unknown.

To test if there is a relationship between source locality and establishment in a realistic restoration context, I surveyed for the presence and abundance of six tallgrass prairie species that were seeded across 24 sites undergoing restoration. This project involved a partnership with a native seed producer that kept records of where their seed was sourced from and where it was planted. I used two measures to evaluate the importance of seed source locality: geographic distance (straight line distance between source and site) and environmental distance (dissimilarity in climate between the source and site). I did not find evidence that local seed establishes best, instead finding no relationship between establishment and either metric of locality. Other factors such as seeding rates and post-seeding management were stronger predictors of plant establishment and abundance. This chapter suggests that using exclusively local seeds may not reliably produce more successful restoration efforts than using seed from further away.

Chapter 3: What context-dependent factors influence the impacts of increased source diversity on restored plant communities?

Next, I wanted to evaluate the potential for high diversity seed mixes to produce diverse native plant communities in restoration settings (*reviewed in* Kettenring et al., 2014). Most research on the potential benefits of increasing genetic diversity have been conducted on clonal species grown in monoculture, so it is largely unknown how increasing genetic diversity can influence the entire community (*but see* Fridley et al., 2007; Lindstrom et al., 2021). Genetic diversity is not the only factor manipulated in a seed mix, though: the number of species included in a seed mix also varies, and may affect the impact of increasing genetic diversity. For example, since higher diversity plantings have been linked to decreasing abundance of weedy species (e.g. Kaul & Wilsey, 2021), increasing source diversity in species-rich seed mixes may have little effect. However, to my knowledge no studies have simultaneously manipulated genetic diversity and species diversity, so this hypothesis remain untested. Moreover, the effect increasing genetic diversity may depend on other factors at the restoration site, such as the amount of edge habitat in a site (Kennedy et al., 2002), and consumer pressure (Drescher & Nolan, 2023). Understanding how these factors interact in a realistic restoration context is crucial for predicting restoration outcomes (Brudvig, 2017). However, because few studies have examined the effects of increased source diversity across restoration sites, these hypotheses largely remain untested.

To address these gaps, I worked within an existing prairie restoration experiment that manipulated the number of species and sources of those species (to increase within-species genetic diversity) in seed mixes across 12 fields. This experiment also included vertebrate consumer exclusion at the center and edge of each site. I used data collected during the first growing season to examine how decisions made during the seed mix design process can impact initial plant establishment, and surveys of the same communities five years later to see whether these effects persisted over time. I found that the effects of seed source diversity were always contingent on consumer access and had largely disappeared by the fifth growing season. Additionally, there was only an interactive effect between species diversity and source diversity when individual species identities were considered. This chapter demonstrates that the impacts of increased source diversity will likely be inconsistent across restoration efforts, so practitioners should carefully consider the context of each restoration site before designing a seed mix for restoration.

Chapter 4: How do three different seed sourcing strategies impact plant establishment relative to one another?

Finally, I wanted to understand the relative benefits of three different seed sourcing strategies commonly discussed in the literature. Local seed sourcing is the most commonly used practice amongst restoration practitioners, based on the assumption that nearby sources are the most likely to establish (Mortlock, 2000). However, there is limited research testing this assumption, and findings have been mixed (*reviewed in* McKay et al., 2005). Two alternatives to local seed sourcing, regional admixture (Bucharova et al., 2019) and climate-adjusted (Prober et al., 2015) seed sourcing have been proposed, which both aim to increase genetic diversity but do so in different ways. Admixture seed sourcing combines local and climatically similar sources to reduce the risk of introducing maladapted genotypes, while climate-adjusted sourcing includes seeds from anticipated future climates to enhance long term persistence, especially under climate change. However, few studies have evaluated the efficacy of these sourcing alternatives in a realistic restoration context (*but see* Höfner et al., 2021; Lindstrom et al., 2021) so it is unclear whether they are able to reliably meet establish goals or how their performance compares to local seed sourcing.

To understand the relative benefits of these three seed sourcing strategies (local, admixture, and climate-adjusted), I created 11 tallgrass prairie seed mixes that modified the number of seed sources and where those seed sources were sourced from. I also assembled open-top chambers on some plots to increase daytime temperatures and test how different sourcing strategies would perform under experimental warming. I found evidence supporting the “local is best” paradigm, since most (but not all) of the seeded species established better when sourced locally. When local and non-local sources were combined, though, this mitigated decreases in establishment in non-local sources, and these multi-source mixes had more flowering individuals than the local source alone. Finally, there was some evidence that climate-adjusted mixes were better able to withstand warming by retaining high seeded richness under experimental warming, which was reduced in plots sown with local or admixture sources. Overall, this chapter suggests that local seed sourcing alone produces high establishment success. However, in unpredictable or changing environments, especially those expected to experience changes in climate, alternative seed sourcing strategies may provide benefits such as increased flowering and adaptation to warmer climates.

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CHAPTER TWO: SEVERAL MEASURES OF SEED SOURCE LOCALITY FAIL TO PREDICT PLANT ESTABLISHMENT IN EARLY PRAIRIE RESTORATIONS

The work presented in this chapter is part of the final publication:

Pizza, R. B., Foster, J., & Brudvig, L. A. (2023). Where should they come from? Where should they go? Several measures of seed source locality fail to predict plant establishment in early prairie restorations. *Ecological Solutions and Evidence*, 4(2), e12223.

Abstract

During the “decade on restoration,” we must understand how to reliably re-establish native plant populations. When establishing populations through seed addition, practitioners often prioritize obtaining seed from locations geographically near the restoration site (i.e. “local seed sourcing”). They are assumed to be under similar environmental conditions to the restoration site and should establish more robust plant populations and preserve local biotic interactions better than seeds sourced from further away. However, this assumption remains virtually untested in realistic restoration settings and the importance of seed sourcing, relative to other factors such as seeding rate and management regimes, is unclear. To determine if seed sourcing decisions impact plant establishment, abundance, and phenology, we developed a partnership between university-researchers and a native seed producer that kept records on where their seed was sourced from and where it was planted. At each site, we recorded the abundance and phenological stage of five commonly used tallgrass prairie restoration species seeded at 24 sites undergoing restoration across Michigan. We considered two measures of seed source locality: geographic distance (seeds were sourced from locations 6-750km away from their respective restoration sites) and climate distance. We also obtained data on the seeding rate and post-seeding management at each site. We found that no measure of seed source locality predicted the likelihood of plant establishment or abundance at restoration sites. However, sites sown with seed from further away, or from cooler and wetter climates, had a greater proportion of flowering individuals earlier in the season. Finally, sites with higher seeding rates had greater plant abundance, and post-seeding management of the restoration site increased the likelihood a species would establish by 36%. Overall, these results support that seed sourcing decisions did not impact plant establishment or abundance in our system. However, using less-local seed sources may alter flowering phenology. Our results suggest that tallgrass prairie restoration efforts should prioritize higher seeding rates, post-seeding management, and might expand the

region seed sources are considered “local”, though this may impact flowering phenology. Future research leveraging native seed producer records can help answer critical questions about restoration seed sourcing.

Introduction

Almost 50% of the area once covered by natural ecosystems has been lost due to conversion of this land to cities, roadways, large-scale agriculture, and other forms of human land use (Vitousek et al., 1997; Ellis et al., 2020). In response, the United Nations has declared the years 2021-2030 as “the decade on restoration,” with intentions to restore over 350 million hectares of land (UNEP, 2019). To meet these goals, we must develop strategies to increase the success of restoration outcomes (which are notably unpredictable; Suding 2011). This includes ensuring that native plant populations establish and persist, even under the degraded site conditions at the onset of restoration efforts (SER International Science & Policy working group, 2004; Benayas et al., 2009). Although some target plant species may arrive at a restoration site on their own, species with limited dispersal capabilities may not (e.g., Hubbel et al., 1999; Pywell et al., 2002; *reviewed in* Holl & Aide, 2011), producing a community primarily composed of nuisance species that persist in the seedbank (e.g., Pyke et al., 2013). The re-establishment of these dispersal-limited species is primarily driven by active reintroduction, most commonly through seed addition (Kimball et al., 2015; Kettenring & Tarsa, 2020). Despite the importance of seed sowing, it is unclear which seed sources will best establish self-sustaining native plant populations (Hufford & Mazer, 2003; *reviewed in* Jones 2013; McKay 2005; Prober et al., 2015; Bucharova et al., 2019).

Current best practices for seed-based restoration support obtaining seed from one or more locations geographically near the restoration site, also known as local seed sourcing (Montalvo & Ellestrand, 2000; Gustafson et al., 2005; McKay et al., 2005). Since there are numerous studies identifying differentiation in plant populations caused by specific adaptations to their environment (i.e., local adaptation; Turesson 1922; Hereford et al., 2009; Whitlock, 2015), practitioners assume local seed sources are under the same (or very similar) environmental conditions as the restoration site and will be better adapted to thrive under those conditions (Lessica & Allendorf, 1999; Mortlock 2000; Bower & Aitken, 2008) compared to less local seed sources (Gustafson et al., 2005; *reviewed in* vander Mijnsbrugge et al., 2010; Gallagher &

Wagenius, 2016). For many species, systems, and geographic regions, though, this assumption remains untested.

Seed sourcing decisions may impact restoration outcomes by affecting initial plant establishment or by introducing individuals with phenological traits that decrease population persistence or alter interactions with other species. Maladapted seed sources may have long-term consequences for focal population development: although initial establishment is not always indicative of the relative abundance of species in mature ecosystems, low initial establishment can increase population extinction risk at the restoration site due to low genetic diversity (Newman & Pilson, 1997) or demographic stochasticity (Purvis et al., 2000; Shirver et al., 2019). Low initial establishment can also cause the restoration to be dominated by non-sown species (Warren et al., 2002), especially at the onset of restoration after clearing the land of pre-existing vegetation (SER International Science & Policy working group, 2004). If sown seed establishment is low, non-sown species can reestablish in their place.

Additionally, seed sources from more distant locations may be adapted to different local climates, which may impact traits important for persistence at a restoration site (such as flowering time; Neil & Wu, 2006; Anderson et al., 2012). If populations established from less local seed sources flower at different times than local populations would, this could result in a pollinator mismatch: a reduction in the interactions between plants and their pollinators (*reviewed in* Bucharova 2017). Low pollination rates can substantially reduce reproduction in primarily outcrossing species (e.g., Rafferty & Ives, 2012; Thomson 2019), which could decrease long-term persistence for that species at a restoration site. A mismatch could also reduce resources available to local pollinators, affecting their population demographics (e.g., Kudo & Ida, 2013) and, beyond that, could decrease the biodiversity of the entire ecosystem (Ramos-Jilberto et al., 2020).

Despite fears that less local seed will have reduced establishment and persistence at a restoration site, experimental results are mixed. While some studies have shown lower establishment of seed sourced from further away in single-species common-garden approaches (Montalvo & Ellstrand, 2000; Raabová et al., 2007) others see no difference in establishment (Galloway & Fenster, 2000; Smith et al., 2005) or find results differ by species (Carter & Blair, 2012). Further, studies identifying shifts in flowering phenology have largely been done in the context of climate change (e.g., Kudo & Ida, 2013; Høye et al., 2013; Ogilvie et al., 2017). Few field studies have quantified the phenology of less local plant populations in current climates, nor how that may impact local pollinators. Of those few, one study found no difference in flowering phenology between the local population (Ohio) and plants from populations as far away as Texas (Selbo & Snow, 2005) while another did observe earlier flowering phenology in the most distant seed source, but this resulted in two times more interactions with pollinators than the local source (Bucharova et al., 2022). Given the mixed results, there is not strong evidence that local seed sources consistently establish better, nor better retain biotic interactions, than more distant seed sources under realistic restoration site conditions. Moreover, since there are substantial concerns that we will be unable to collect enough seed from local populations (Broadhurst et al., 2015; Nevill et al., 2016) and native seed producers (Ladoucer et al., 2018) to sustainably meet the high demand for restoration seed, using less local seed sources may become necessary; in some regions, it is even advocated for (Hancock et al., 2022).

One explanation for these mixed results is that predicting whether a seed source will be adapted to the climatic conditions at the restoration site is difficult. Several studies have shown that plants can be adapted to a wide variety of climatic variables ranging from annual temperatures (Baughman et al., 2019) to season-specific precipitation (Blumenthal et al., 2020). However, since the home environments of seed sources are rarely quantified, practitioners instead often use geographic distance (straight line distance between a seed source and a restoration site location) as a proxy for climate similarity (following the first law of geography, Tobler 1997; *reviewed in* McKay et al., 2005). However, environmental conditions rarely change linearly over geographic space (Gerst et al., 2011; Tang et al., 2012). Therefore, geographic distance may be a poor proxy for environmental similarity and whether a seed source will result in high plant establishment and persistence at a restoration site (e.g., Wright et al., 2017). Instead, it may be worthwhile to quantify the differences in climates between source and site locations (i.e., “environmental distance”) to predict a seed source’s suitability to a restoration site. Not only are measures of environmental distance more direct than geographic distance, but they also have the utility of including directionality. For example, although two plant populations may be equidistant from the restoration site, the population from drier conditions than the restoration site may outperform plants from wetter conditions (Midolo & Wellstein, 2020). While tools designed to describe the climate space of a particular location are increasingly available (e.g., PRISM climate group), and using them has become a focus in recent local adaptation literature (*reviewed in* Lortie et al., 2022) their utility in the context of restoration seed sourcing is largely unknown.

Finally, few studies have measured the effects of seed sourcing on plant populations under realistic restoration field settings (*reviewed in* Gibson et al., 2016). This is a problem, as the results from common gardens may not translate to the complex nature of restorations (*reviewed in* Lau et al., 2019). At a restoration site, there are many other factors influencing the establishment of target populations aside from the source of the seed, including the age of the planting (Applestein et al., 2018), the seeding rate (Maron & Sims 1997; Barr et al., 2017), post-seeding management decisions (Maron & Jefferies, 1999; Flory 2010; Dowhower et al., 2020), the soil conditions of the restoration site (Bassett et al., 2005; Haan et al., 2012), and others (including biotic factors such as herbivory; Appelstein et al., 2018). Given the many factors affecting population establishment and persistence, alongside the mixed results from controlled common-garden studies, it is unclear how important seed sourcing decisions are relative to other factors that affect restoration outcomes.

To understand the impacts of seed sourcing decisions on the establishment and phenology of native species in realistic restoration settings, we developed a collaborative research project between university researchers, a native seed producer, and 22 land stewards. Using the data kept by this native seed producer on where they purchased seeds from and where they were planted, we located 24 recently installed tallgrass prairie restoration efforts across Michigan, which sourced seed from locations 6 -750km away from the restoration site. At each site, we surveyed plant establishment, abundance, and phenology of five commonly planted tallgrass prairie restoration species. Using these data, we answered the following research questions: (1) Do measures of seed source locality (either geographic or climatic) explain the variation in plant establishment, abundance, and phenology? (2) How important are other factors, such as seeding rate, post-seeding management, and soil conditions at the site, for predicting the establishment, abundance, and phenology of restored plant populations relative to measures of seed source locality? We predict that if local seed sourcing is important for plant populations, we would observe reduced establishment and abundance, and greater changes in phenology at sites where less-local seed is sown. We also expect that direct measures of the environmental distance between the seed source and the restoration site will be better predictors of our response variables than geographic distance. Finally, we expect environmental and management factors to influence plant populations across restoration sites, although we make no predictions as to their importance relative to seed source locality.

Methods

Sites

We selected 24 newly installed prairies from across Michigan, ranging from 0.0003-0.03 km^2 to survey from mid-July to mid-August of 2021 (Fig. 2.1a). Sites were seeded between 2017-2020 and differed in the land use immediately prior to restoration initiation, planting methods, land management, and site preparation (Table A2.1). The amount of seed per species added to the site varied (0.002 – 3.36 g/m^2) and half of the sites had no post-seeding management while the other half had some form of management (e.g., burning, mowing, weed removal, or a combination of these). Since management approaches varied substantially between sites, and there were not many site replicates for each type of approach, post-seeding management actions were coarsely grouped into a yes or no variable. The seed mix planted at each site varied in geographic origin and each mix contained at least two of the five focal species in this study. All sites were either on public land or on private land surveyed with the owner's permission.

Study species

The seed mixes for the restoration sites in this study were supplied by Native Connections seed farm (Kalamazoo, MI, USA), a native seed company that produces some prairie species locally and acquires others from other Midwest seed producers for prairie restorations throughout Michigan. For a set of these restorations, they have detailed records of where seeds were obtained from, as well as where those seeds were planted. We only surveyed sites where a single source of each species was planted (excluding any sites where overseeding of our focal species was done). Using those records, we selected five focal species that were commonly sown into prairie restorations in Michigan and obtained from multiple source locations: *Schizachyrium scoparium* (sown at 20 sites), *Ratibida pinnata* (21 sites), *Rudbeckia triloba* (10 sites), *Symphyotrichum laeve* (14 sites), and *Solidago rigida* (21 sites). Overall, we sourced seed from five seed producers, nine different states, and 18 different locations (Fig. 2.1b). All seeds were produced on native seed farms, originally established with seed from wild populations. Since seeds were not always sourced from areas near the production farm (although they often were), we considered the source location of the seed to be the wild population where the seed lot originated from. While most seed producers could provide a single county or township where their seed lot was sourced from (12 seed lots), three seed lots were composed of seed from multiple counties, and another three could only be described generally (e.g., “from the southern half of Michigan"). In these cases, the most centroid point (between counties, or the center of a state) was recorded as the source location.

Establishment surveys

To quantify species establishment and abundance, we established a 25m transect in a random direction at the approximate center of each site. If a site could not fit a 25m transect, the transect was laid out along the longest portion of the site and rounded down to the nearest 5m. Five 5x5m subplots, or as many as could fit given the length of the transect, were marked on either side of the transect (up to 10 subplots total, per transect) and we counted the number of individuals of each species in each subplot to quantify plant abundance. To expedite field surveys, once 20 individuals of any focal species were found in a subplot, we recorded the distance along the transect where the 20th individual was recorded and counting for that species ceased; this smaller area then comprised our subsample of the subplot and was used to estimate the number of individuals of that species at the 25m^2 scale. Since several of these species are clonal, we assumed that clusters of stems at least 15.24cm (0.5ft) away from other clusters of stems were separate individuals. Occasionally, one or several focal species were not present in any of the subplots. Then, we constructed another 10x25m plot directly adjacent to the first; if a species was found in this plot, it was recorded as “present” in the site, but not included in abundance measurements. If it was not found, it was recorded as “absent”. We used this presence/absence data to quantify the likelihood that a species would establish at a site.

Flowering Phenology

To gather data on differences in flowering phenology, we measured the abundance of flowering or seed-setting individuals (individuals with open flowers and/or dispersing seed) relative to the number of total individuals of each species in each 25m^2 subplot. Since we conducted surveys once during the growing season due to time constraints, we assumed that sites with a greater proportion of flowering individuals during the survey had populations with an earlier flowering phenology (Lisi & Schindler, 2011).

Site environmental factors

We also collected data on the environmental conditions of the sites. We used soil water holding capacity to characterize site soils, as it is known to affect the abundance of sown prairie species (Zirbel & Brudvig 2020; Grman et al., 2021). We collected 10cm soil cores at 15 points along the entire 25m^2 subplot and pooled these together for each site. We dried soils in the lab and conducted soil water holding capacity measurements, as the proportionate difference between saturated wet weight and oven dry weight, following the same methods as Brudvig & Damschen (2011).

Data analysis

Source and Site Climates

All analyses were performed in R studio using R version 4.2.0 (R Core Team, 2020). We took a broad approach to defining the climate of our source and site locations, since plants may be adapted to a wide variety of correlated climatic variables ranging from annual temperatures (e.g., Baughman et al., 2019) to season-specific precipitation (Blumenthal et al., 2020). We generated 19 bioclimatic variables (Hijmans et al., 2005) from 800m resolution PRISM (PRISM climate group) monthly climate data averaged across the years 2017-2021 ("dismo" package; version 1.3-5; Hijmans et al., 2021). These variables included temperature and precipitation metrics both annually and seasonally, which have been shown to influence the abundance of sown species in prairie restorations (Groves et al., 2020). Since many of these variables were highly correlated, we utilized a Principal Components Analysis (PCA) to summarize them (see Fig. S1). The three highest loading axes explained 84% of the total variation (see Table S2 for the top nine loadings of each variable). We recorded the value along each PCA axis for each source and site location and subtracted the value for each source location from the value of each site where a given species was planted. This value was used in analyses to account for differences in climate between the source and site locations (named PC1 difference, PC2 difference, and PC3 difference).

Plant Establishment and Abundance

To test how seed sourcing and other management factors influenced the plant populations of our focal species, we focused on two metrics: whether a species established at a site (yes/no) and the number of individuals of that species per 25m^2 area. We analyzed the likelihood of any given species establishing at a site using a generalized linear mixed model with a binomial distribution and a logit link with the following standardized predictor variables: geographic distance (km), PC1 difference, PC2 difference, PC3 difference, site age (years), seeding rate (oz/acre) for each species, whether a site was managed, and soil water holding capacity (%). Species identity was used as a random factor. Of all the variables used in analyses, only geographic distance and PC1 difference were marginally correlated (Pearson's R = 0.67; Fig. A2.2). However, the VIF's of the model including all variables were < 2 indicating multicollinearity was low, so all variables were included. Rather than choosing the best-fitting model with only one or two predictors, excluding less powerful predictors despite their biologically relevant contributions, we used a model averaging approach using all subsetted model combinations (128 total models; Hoeting et al., 1999; Hooten & Hobbs, 2015). We estimated regression coefficients of each predictor by averaging the coefficients of each subset model weighted by each model's AIC ("MuMIn" package; version 1.4.6; Bartoń 2022). Since each variable was used in the same number of models, the relative importance of each variable was quantified through adding the AIC weight of each variable in all model combinations (the sum of weights [SW]). Since there is no cut-off value for SW to indicate significance (Galipaud et al., 2014), we rank the relative importance of each variable by its SW (Burnham & Anderson, 2004). Using the best-fitting model (including any biologically relevant covariates) we visualized the conditional effects of the two most important variables ("ggeffects" package; Lüdecke 2018) to understand their effect on each response variable. As the model averaging approach intentionally selects higher-fitting models, no p-values are reported in this manuscript.

The average (or estimated) number of individuals was averaged across every subplot at a given site, to provide a single value for the average individuals in a $25m^2$ area at each site. The count data were overdispersed but not zero-inflated (ratio of expected to observed zeroes 1.01:1, p = 1), so we analyzed this variable with a negative binomial generalized mixed model ("glmmTMB" package version 1.1.3; Brooks et al., 2013) using the same predictors and model averaging strategy as above (128 total models; all other model assumptions were met).

Additionally, we wanted to test the sensitivity of our choice to use a centroid point as the source location for the seed lots we could not track to a single county (n = 9), as well our inclusion of sources that were composed of seed from multiple counties (n = 3). We tested the former by creating new source coordinate variables for those datapoints: source coordinates that are the furthest away from the planted site, and coordinates that are the closest to the planted site, given the collection region provided by the seed producer. We tested the latter by removing those composite points and re-running our analyses. In both cases, we found no qualitative changes in the relative importance of variables for the likelihood of establishment nor average count analyses, but there were changes in flowering phenology (Figs. S3 & S4). Therefore, we used the centroid point as the estimated source location in further analyses, as we think this minimizes the possible incorrectness of the source location we chose, but present the results of these alternative analyses for transparency. We also chose to retain the three composite sources in our analysis, as all of our source populations could have differing levels of genetic diversity caused by a multitude of factors including the size of the original source population (Newman & Pilson, 1997), cultivation practices (Dyer et al., 2016), years a source has been cultivated (Pizza et al., 2021), and many others. Since we lack information on these factors from all the sources, and make no attempts to quantify the effect of genetic diversity on our results, we do not feel excluding these datapoints is appropriate, but do present the results of removing these points in the supplementary materials for transparency.

Flowering Phenology

To test if seed sourcing, or other management decisions, affected flowering phenology across the sites, we considered the number of flowering individuals relative to the total number of individuals of each species in each 25m^2 subplot. Since we conducted surveys once during the growing season due to time constraints, we assumed that sites with a greater proportion of flowering individuals during the survey had populations with an earlier flowering phenology (Lisi & Schindler, 2011) while accounting for differences in site latitude, survey date, and site age. Since it is unknown whether vegetative individuals would flower that year or not, we created two flowering variables: one including those vegetative individuals in the total, and one not including vegetative individuals. Both were fit with a negative binomial mixed model, which was the only model of three (untransformed linear, square root transformed linear, and negative binomial) that met model assumptions given the high number of zeroes in the dataset. This model included the same predictors as the establishment models (geographic distance (km), PC1 difference, PC2 difference, PC3 difference, site age (yrs), seeding rate (oz/acre) for each species, whether a site was managed, and soil water holding capacity), and additionally included restoration site latitude and the Julian survey date as covariates, the former to control for further north sites flowering later than further south sites, and the latter to control for sites surveyed later in the year being further along phenologically (see table A2.2 for regression coefficients for these covariates). Since there was no qualitative difference in the statistical output of our two models, we report only the first flowering variable (including vegetative individuals in the total), but report the results of our alternative analysis in the supplemental materials (Fig. A2.5). All predictor regression coefficient values were averaged across 512 subset models.

Results

Climate data

The first PC axis explained 54% of the variation in our climate variables and was negatively associated with the mean temperature of the driest month, the minimum temperature of the coldest month, and the temperature annual range (Fig. A2.1a.; see table A2.2 for loadings of the top 9 variables for each axis). We interpreted higher positive PC1 differences to represent when seeds were sourced from environments with warmer and wetter winters than the site. PC axis 2 explained 18% of the variation in our climate variables and had negative associations with the mean temperature of the warmest quarter, maximum temperature of the warmest month, and precipitation in the wettest quarter. Higher positive PC2 differences represent when seeds were sourced from environments with warmer and wetter summers than the site. Finally, PC axis 3 explained 14% of the variation in our climate variables, with higher positive PC3 differences representing when seeds were sourced from hotter and drier environments than the site. Geographic distance was not significantly correlated with any of the climate variables (VIF < 2; Fig. A2.1).

Plant Establishment and Abundance

The two most important predictors for whether a species would establish at a site were whether the site was managed post-seeding and the seeding rate of that species (Fig. 2.2). Sites under any form of post-seeding management were 36% more likely to have any given species present than unmanaged sites (Fig. 2.3; $\beta = 1.01 \pm 0.65$, sw = 0.85). Sites with higher seeding rates also experienced more reliable establishment than sites with lower seeding rates (Fig 2.4; $\beta = 0.39 \pm 0.36$, sw = 0.66). The most important predictor for plant abundance was seeding rate: adding 3x more seed than average to a site increased plant abundance by 10 individuals per 25m^2 (Fig. 2.5; $\beta = 0.22 \pm 0.12$, sw = 0.72). Importantly, all predictors of seed source locality (geographic and environmental) had low importance in our models for plant establishment and abundance (Fig. 2.2; table A2.3). Site-specific factors, including soil water holding capacity and site age were also not important predictors (Fig. 2.2; table A2.3).

Plant Phenology

The two most important predictors of plant flowering phenology were PC3 difference and geographic distance (Fig. 2.2). There appears to be no effect on flowering phenology when sourcing seeds from warmer and drier locations than the restoration site (when PC3 difference < 0), but there were more flowering individuals when seeds were sourced from cooler and wetter locations than the restoration site (when PC3 difference > 0; Fig. 2.6; β = 0.57 ± 0.47, sw = 0.73). Restoration sites sown with seed from locations geographically far from the restoration site tended to have more advanced flowering phenology (β = 0.65 ± 0.55, sw = 0.71), although this relationship is not linear (Fig. 2.7). Other analyzed factors including soil water holding capacity, site age, management, and seeding rate were not important predictors (Fig. 2.2; table A2.3).

Discussion

Although it is widely believed that local seed sources will result in the highest initial establishment at restoration plantings (*reviewed in* McKay et al., 2005; *but see* Hancock et al., 2022), our results do not substantiate that claim. No metrics of seed source locality were good predictors of plant establishment or abundance at our newly installed restoration sites. Instead, seeding rate and post-seeding management were the only measured factors that reliably increased plant establishment and abundance. However, plants from less local seed sources did flower earlier than local seed sources, which could affect population dynamics and biotic interactions at restoration sites.

Plant establishment & abundance

A key finding in our study was that neither the geographic distance nor climate similarity between seed sources and restoration plantings predicted initial plant establishment or abundance. Our results add to a small, but growing, body of evidence that the importance of seed source locality for plant establishment in restoration efforts is outweighed by other factors. Research on the early establishment of four *Andropogon gerardii* ecotypes in a multispecies community study showed no difference in percent cover for that species (a proxy for plant abundance) during the first two years of establishment (Galliart et al., 2018), although differences did emerge after that time. Another study did observe differences in establishment success between different ecotypes of four plant species, but these differences were not explained by geographic or environmental distance (Bischoff et al., 2010). Importantly, both studies were done under natural field conditions and are most comparable with our study design, emphasizing that seed sourcing may have a measurable impact on plant establishment in less natural conditions (e.g., Montalvo & Ellstrand, 2000) but its impact may disappear when establishment is influenced by multiple other factors. Additionally, in our study differences in the environments between each source and the sites at which it was planted did not appear to be geographically structured, indicating that geographic distance is a poor proxy for climate similarity. This could be explained by seed lots adapting to the conditions on the farm, especially those that were grown on the farm for several generations (Pizza et al., 2021), if those conditions differ substantially from the source environment. However, since the number of generations each seed lot was grown on the farm was not available, we are unable to test that hypothesis. It is also important to acknowledge that our metrics of locality may have been too coarse to capture environmental variation that causes local adaptation (which can occur at very fine scales, Knight & Miller, 2004). Although other measures of environmental similarity such as soil composition (Macel et al., 2007) may have captured that variation, they may not be as readily available to restoration practitioners or native seed producers. Given currently available metrics, our results support that selecting the nearest seed source does not seem to impact sown species establishment. Instead, it provides evidence that there are likely multiple suitable sources of restoration seed available for each project, and that using seed sources < 700km away from the restoration site is likely to reduce the probability of introducing maladapted seed, as no differences in establishment or abundance were seen in that range in our study.

The most important predictors for whether a plant would establish or not were post-seeding management and seeding rate. Since few sites performed identical management strategies, our management variable encompassed any form of post-seeding intervention to promote sown species establishment. However, all managed sites experienced some amount of mowing. This is consistent with other literature, showing that mowing can increase the abundance of sown forbs by reducing shade competition from larger established grasses (e.g., Maron & Jefferies, 2001; Williams et al., 2007; Dowhower et al., 2020). This was further supported in our study, as unmanaged sites were 36% less likely to have our studied species establish than managed sites. Although the importance of mowing restoration sites is widely understood by restoration practitioners as a method to increase the density of sown forbs (Rowe 2010), half of our sites were unmanaged. This was often due to financial constraints which prevented land stewards from monitoring the site for management needs (R. Pizza pers. obs.; Phillips-Mao et al., 2015; Barak et al., 2021). Thus, the positive effects of any form of management on plant establishment demonstrated in our study can be used by land stewards to justify funding these post-seeding interventions, especially mowing.

The other factor that increased the likelihood of establishment is increased seeding rate: mirroring other studies in prairie systems (Applestein et al., 2017; Barr et al., 2017). Although the increase in establishment (~15%), and abundance (~10 individuals per 25m^2) after tripling the amount of seed sown observed in our study may seem small, it could have long-term demographic consequences: populations with more individuals tend to have greater genetic diversity (Ellegren & Galtier, 2016), and are less likely to go extinct (Newman & Pilson, 1997; Purvis et al., 2000). Thus, increasing the number of individuals present in the early establishment stage may increase the likelihood that a population will persist into the future. Higher seeding rates can also decrease the establishment of nuisance species at the restoration site (Pyke et al., 2013). Therefore, although seeds can be the most expensive part of a restoration project (Phillips-Mao et al., 2015), sowing seeds at greater densities may reduce the necessity of costly invasive species removal later in the restoration process.

Importantly, while our data do not show an overall trend of local seed sources establishing best, the relative importance of seed sourcing likely varies between species due to the range of environmental conditions it can inhabit (e.g., Macel et al., 2012). Species that have populations in a wide range of climates (e.g., *Andropogon gerardii*) may have more differentiated phenotypes than species that persist in a narrower range of climates (Galliart et al., 2018). Additionally, in more extreme environments such as the arid Great Basin, local adaptation may be more important for plant establishment (Baughman et al., 2012) than in less climatically extreme environments like the Midwest (*reviewed in* Hereford 2009). Additionally, it is possible that planting year weather may be more important for plant establishment than overall climate averages (e.g. Groves et al., 2020). Finally, our study only focused on the initial establishment stage, so it is possible that seed sourcing may impact restored populations at later successional stages (e.g., Galliart et al., 2018). Since the biotic context of a site changes as the community develops, traits that allow persistence during early establishment may not confer persistence at later successional stages. Future studies focusing on the importance of seed sourcing for individual species, especially those commonly used in restoration, and at later successional stages, should be conducted to understand the species-specific and long-term consequences of seed sourcing decisions.

Plant phenology

An important finding in this study was that less local seed sources, both geographically and environmentally, flowered earlier than more local sources. This was also the only analysis where a measure of locality was an important predictor, indicating that even coarse measures of locality may account for changes in phenology. This is likely caused by flowering phenology being strongly determined by temperature (Hülber et al., 2010) and latitude (Debieu et al., 2013; Rushing et al., 2021) whereas establishment is influenced by many other factors (e.g., Zirbel & Brudvig, 2020). Our results contrasted with previous studies on the impacts of seed sourcing decisions on plant phenology (Selbo & Snow, 2005; Bucharova et al., 2022). Selbo & Snow (2005) observed no difference in flowering phenology between ecotypes across a large geographic gradient in an experimental setup that more closely resembles ours, whereas Bucharova and colleagues (2022) observed significant earlier phenology, but in ecotypes < 400km away from one another when plants were grown in pots in a common garden. Given these conflicting results, we conclude that seed sourcing decisions can impact plant flowering phenology, but the mechanisms behind that shift are not consistent. Importantly, this analysis appears to be sensitive to changes in individual datapoints (see Figs. A2.4 & A2.5). Since we did not observe this sensitivity in the establishment or average count analyses, future studies including a larger number of sites to survey should be done to confirm the trends observed in this study. Future research to understand the mechanisms behind these shifts in flowering phenology may help practitioners understand when using less local seed sources could affect biotic interactions at a restoration site.

In our study, the only measure of environmental distance that had a positive relationship with flowering plant abundance (PC3; sourcing plants from cooler and wetter locations and planting them in warmer and drier ones) is not correlated with geographic distance. This further solidifies that geographic distance may be a poor proxy for climate similarity, and we suggest considering the difference in temperature and precipitation, especially during the winter and summer season (the primary variables that categorize this PC axis) to predict whether plants from sources further away will have a different flowering time than more locally sourced individuals. While plants from cooler and wetter environments may be adapted to shorter growing seasons, and therefore flower earlier than local sources (Haggerty & Galloway, 2011; *but see* Bradley St. Clair et al., 2013), the mechanism explaining the relationship between geographic distance and flowering time is unclear. Although there is evidence that flowering phenology can be driven by differences in temperatures at different latitudes (Debieu et al., 2013) and precipitation at different longitudes (Samis et al., 2012) neither source latitude or longitude were good predictors of flowering phenology (Fig. A2.3). Another hypothesis is that since most seeds sown to the sites in our study originated from native seed production farms, cultivation practices including supplemental watering or early harvesting times could have unintentionally selected for early flowering plants (e.g., Dyer et al., 2016). Future work comparing restorations sown with seed from different producers in similar climates could parse out this relationship.

This shift in phenology may indicate that there could be a mismatch between plant pollen resources and pollinator abundance if pollinators emerge later in the season, which could affect both the plant populations through decreased reproduction, and the pollinator communities through decreased floral resources. Additionally, since earlier flowering phenology can be related to earlier phenology in other developmental stages (such as green-up time; Delbart et al., 2015), less local seed sources could have altered relationships with competitor plants, altering community composition at these sites (Wilsey et al., 2011). Since our study does not measure the duration of flowering, early flowering plants may have continued flowering throughout the season, providing even more resources to pollinators (e.g., Bucharova et al., 2022). Finally, since we did not measure seed set, it is unclear if, or how, an earlier flowering time can affect plant reproductive potential, or consequences for the timing and amount of fruits and seeds available to consumers. Given these questions, the mixed results of studies like this in the past, and the sensitivity of our analysis to individual datapoints, our results point to the need for further research on how seed sourcing decisions affect plant phenology.

Importance of co-designed research projects

This study exemplifies the utility of co-designed research projects, both for data availability and research potential. Due to the increased interest in using local genotypes for restoration, Native Connections seed farm had kept detailed records about where their seed for all species was procured from, and where it was planted, for the last five years. Without these detailed records, it would be difficult to parse where seed for each project was sourced from, making a large-scale project like this either impossible, or incredibly costly. Importantly, Native Connections was not the only seed producer with detailed records: despite no seed certification programs in the United States requiring that native seed producers report the source of the seeds they sell (Pedrini & Dixon, 2020), five of the six native seed producers could confirm the county that their seed was collected from for some (if not all) of their species, and all could report at least what state the seed came from. We suspect that records like these are available more broadly, both geographically and across ecosystems. If so, utilizing them can generate countless more sites to use to further understand the implications of seed sourcing decisions in different ecosystems across the world. Given the importance of their contributions, seed producers' participation in these co-designed research projects should be formally recognized.

Conclusions

Our results suggest that seed source locality, at the geographic and environmental scales measured, does not predict whether plants will establish, nor in what abundance, at restoration sites. This contributes to growing evidence that, under realistic restoration settings, local seed sources do not always establish better than less local sources. The results of this research may help expand the region(s) which practitioners might consider local, and further emphasize the importance of pre- and post-seeding management on ensuring population establishment. Future studies should test these same ideas at restoration sites in a larger geographic range, and in systems other than tallgrass prairies, to see if these trends translate to other geographic areas and ecosystems. Although much research still needs to be done, this project exemplifies the importance of collaborative research and challenging paradigms.

Figures

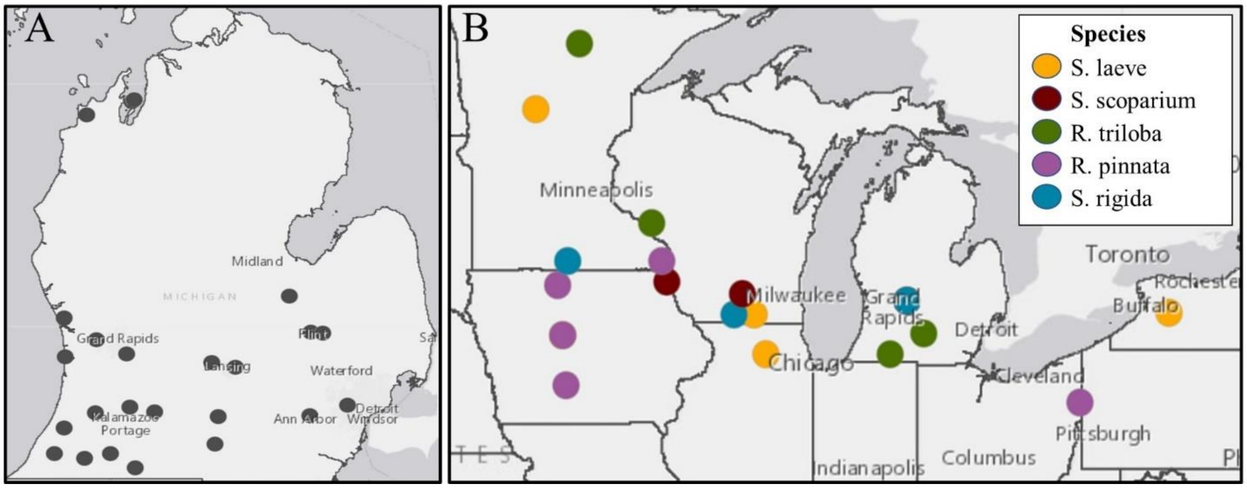

Figure 2.1: Map of Michigan indicating the locations of the 24 restored prairies where surveys took place (a) and the 18 source locations for the five prairie species surveyed (b). Not all species were obtained from each source, nor planted at each site.

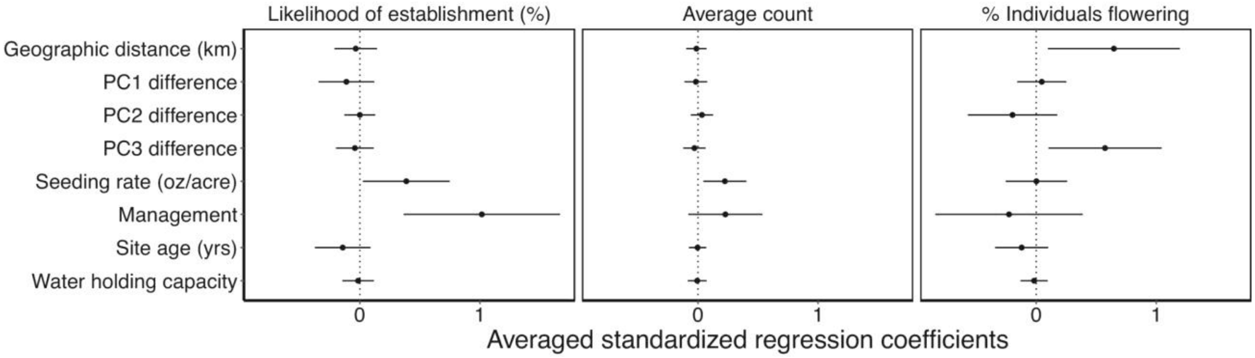

Figure 2.2: Standardized regression coefficients (± standard error) estimated through model averaging for three response variables to quantify the effects of seeding restorations with seed sourced from various degrees of locality. See table S2 for all model statistics and sum of weights (SW).

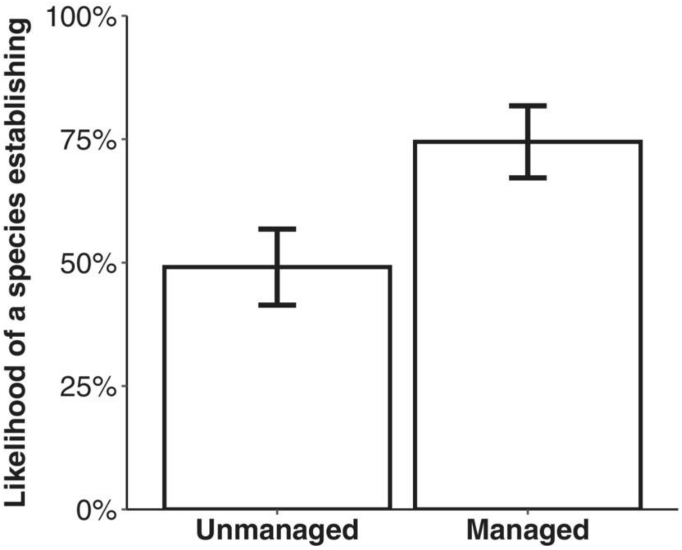

Figure 2.3: Conditional effects of management on the likelihood a given species would arrive at a site, taking into account different seeding rates (n = 84). Bars indicate mean values calculated in emmeans and error bars show standard error. Estimated means were calculated using the best fit model in the model averaging output.

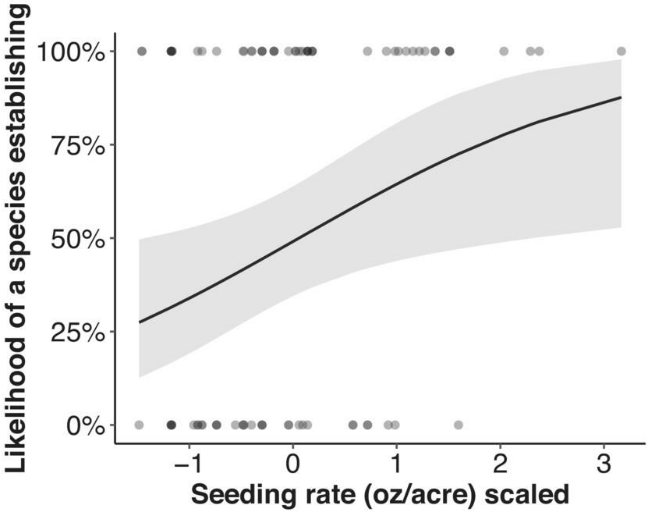

Figure 2.4: Conditional effects of seeding rate (scaled [mean / standard deviation] for easier visualization) on the likelihood a given species would arrive at a site, taking into account different management regimes (n = 84). Dark line indicates regression of two variables, and shaded area shows standard error of the regression line. Regressions were created using the best fit model in the model averaging output.

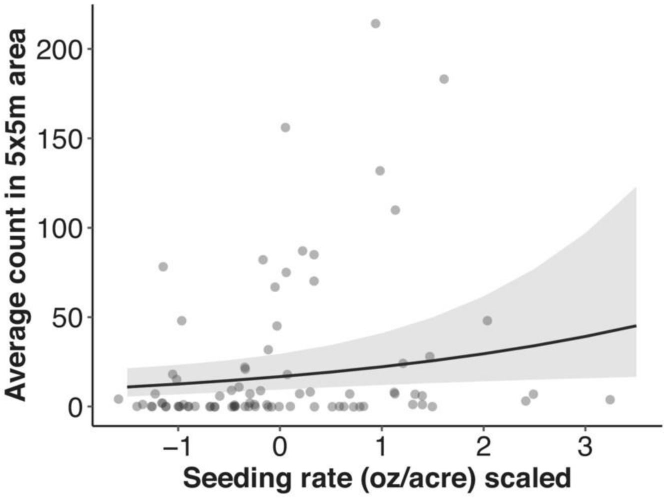

Figure 2.5: Conditional effects of increased seed addition (scaled [mean / standard deviation] for easier visualization) on the number of individuals of a given species in a 25m^2 area, while accounting for differences in management (n = 84). Dark line indicates regression of two variables, and shaded area shows standard error of the regression line. Regressions were created using the best fit model in the model averaging output.

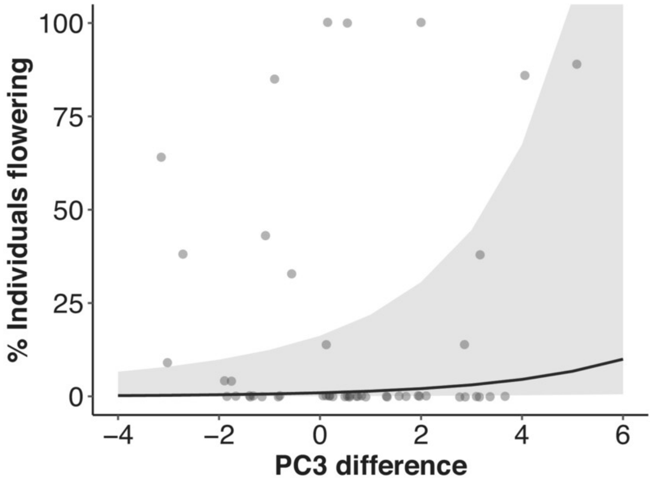

Figure 2.6: Conditional effects of increasing environmental distance (PC3) on the percentage of individuals of a given species at a site that are flowering taking into account any differences attributed to geographic distance, survey date, and site latitude (n = 84). PC3 difference values < 0 indicate when seed was sown from locations cooler and wetter than the site, and values > 0 indicate when seed was sourced from locations warmer and drier than the site. Dark line indicates regression of two variables, and shaded area shows standard error of the regression line. Regressions were created using the best fit model in the model averaging output, with the addition of relevant covariates.

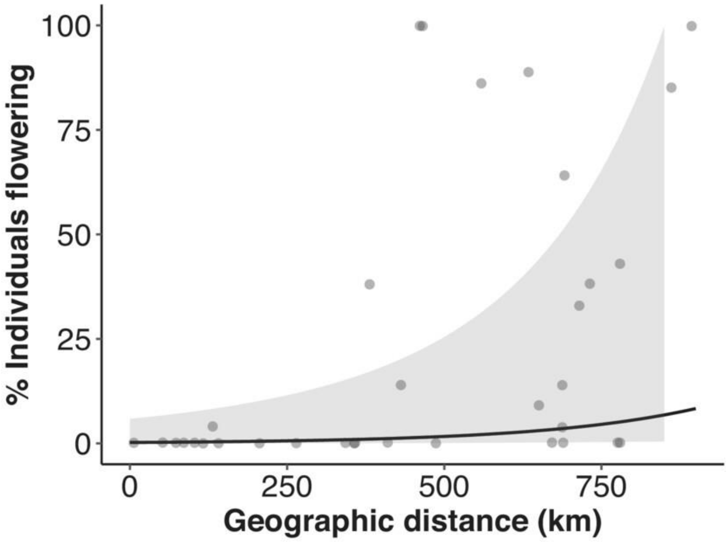

Figure 2.7: Conditional effects of increasing geographic distance on the percentage of individuals of a given species at a site that are flowering, taking into account any differences attributed to differences in climate, survey date, and site latitude (n = 84). Dark line indicates regression of two variables, and shaded area shows standard error of the regression line. Regressions were created using the best fit model in the model averaging output, with the addition of relevant covariates.

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APPENDIX

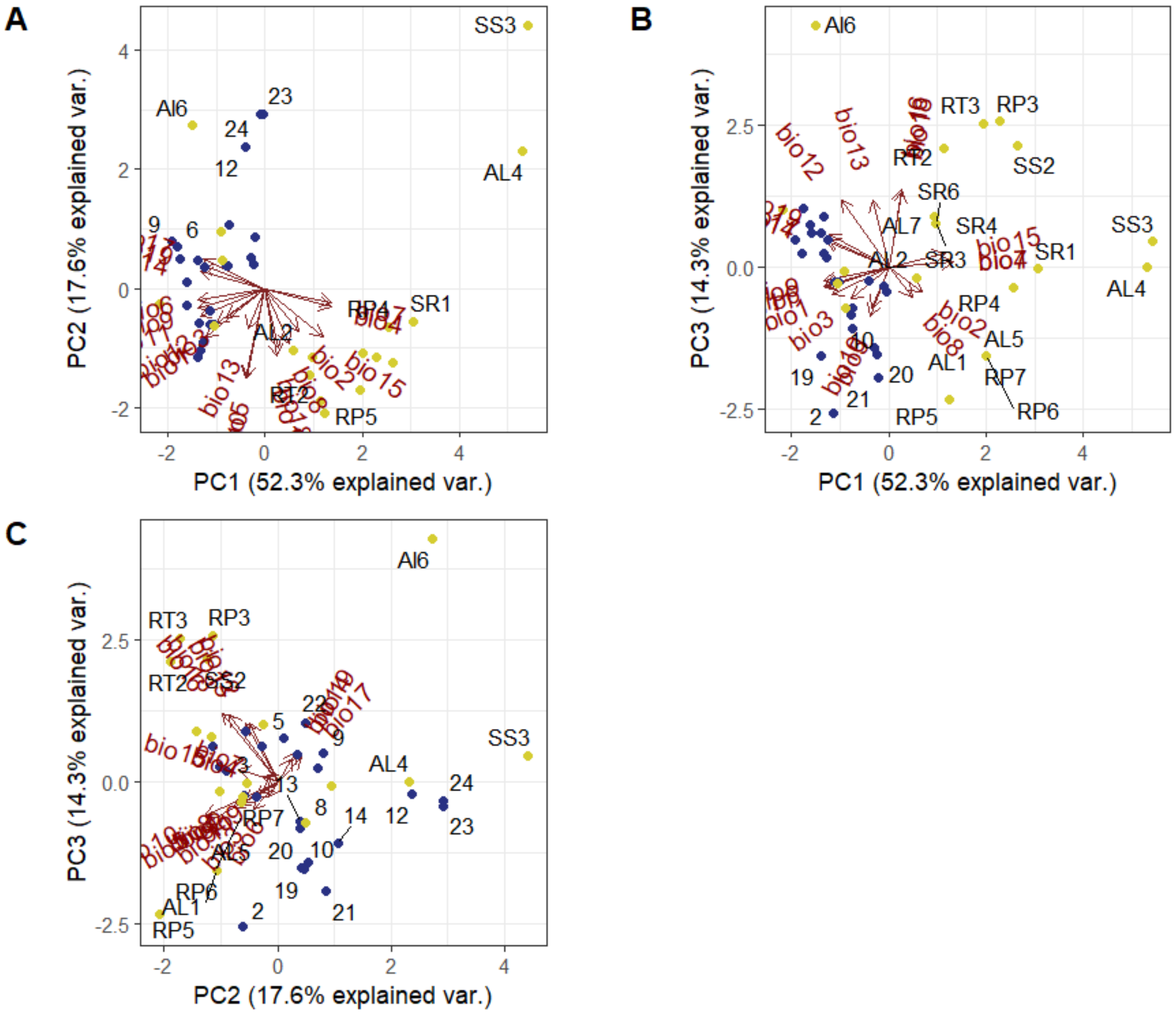

Figure A2.1: PC biplots of all three PC axes**.** Blue dots indicate sites and gold dots indicate sources. See table S1 for descriptions of bioclimatic variables.

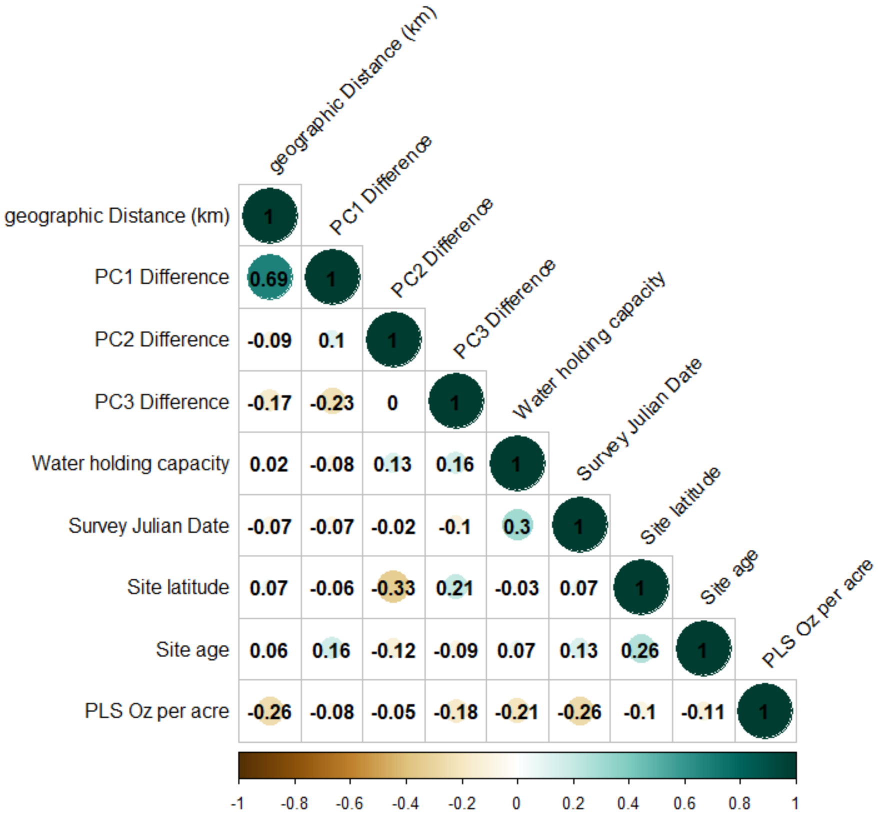

Figure A2.2. Correlation coefficients between all variables used in analyses. Dot sizes indicate the magnitude of the relationship.

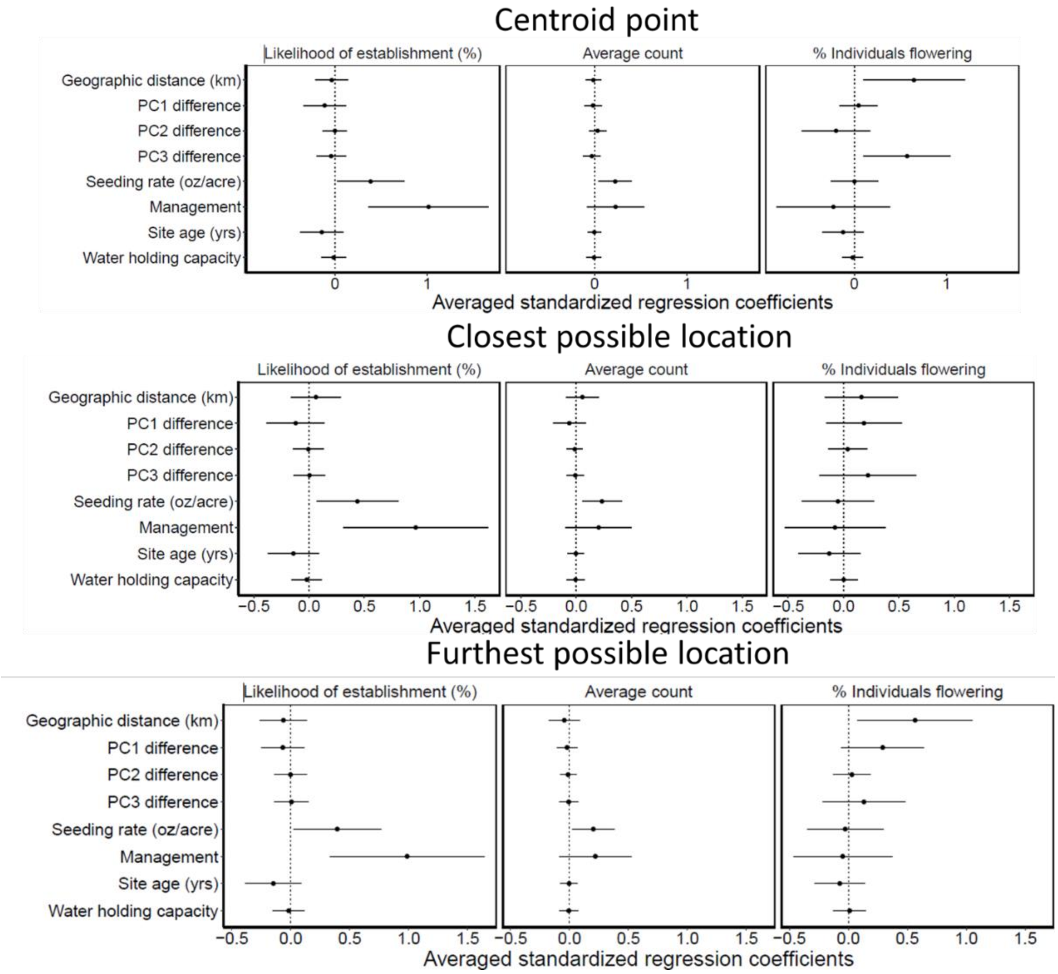

Figure A2.3: Standardized regression coefficients after changing the geographic location of 9 points where exact source location was unknown**.** Standardized regression coefficients (± standard error) estimated through model averaging for three response variables to quantify the effects of seeding restorations with seed sourced from various degrees of locality. For datapoints where the exact location of the source was not known (n = 9), we used the most central point as the estimated source location. We compared these to when we used the closest possible place the source could be relative to the planting site (center panel) and the furthest possible location of the source relative to the planting site (bottom panel).

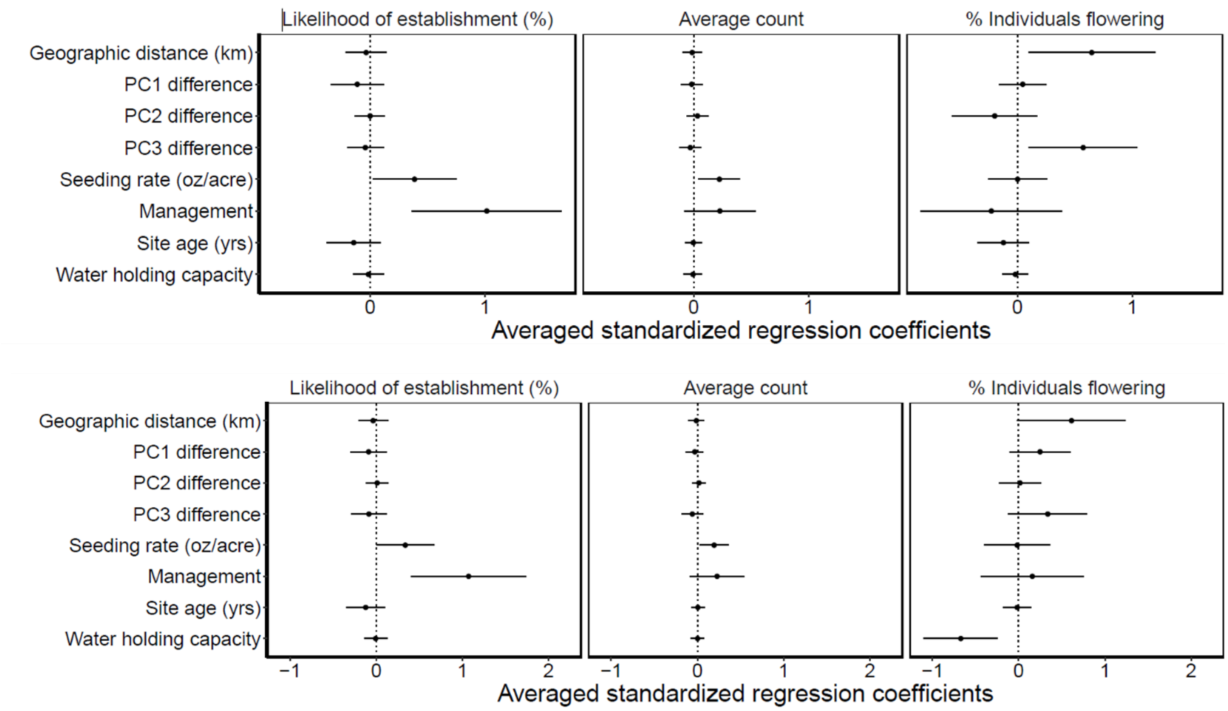

Figure A2.4: Standardized regression coefficients before and after removing datapoints where the source location was composed of more than one population. Standardized regression coefficients (± standard error) for all datapoints (n = 84, as presented in the manuscript; top) and for all the datapoints except the admixture populations (n = 79; bottom) estimated through model averaging for three response variables to quantify the effects of seeding restorations with seed sourced from various degrees of locality.

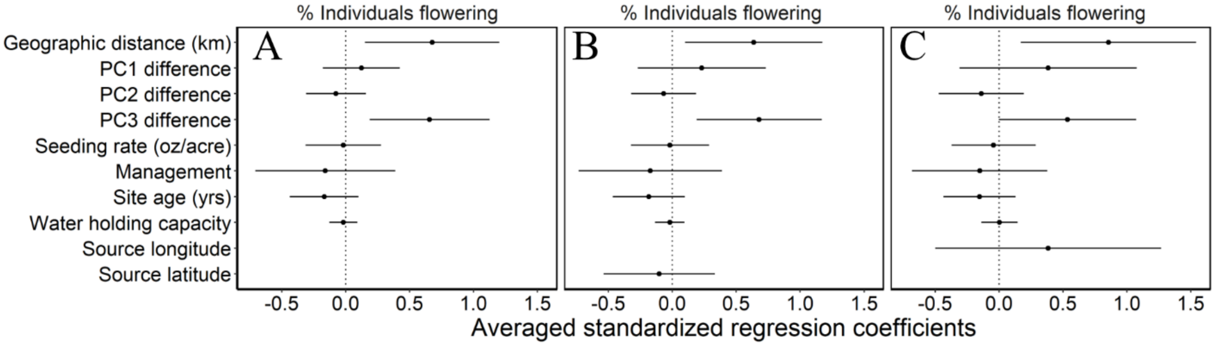

Figure A2.5: Standardized regression coefficients for % flowering individuals including source geographic covariates. Standardized regression coefficients (± standard error) determined through model averaging. Each model included either no description of site geographic location (A), the sources latitude (B) or the sources longitude (C) to determine if source latitude or longitude could provide a mechanism for the large effect size of geographic distance in our model.

Table A2.1: Details of each of the 24 prairie restoration sites surveyed. Details about the location, land preparation, management for each of the restored prairie sites surveyed, as well as which of the five focal species were planted at each site and at what seeding densities. For sites where specific information was unknown or unavailable, the cell is filled with “UNK”.

Site ID	**Site Latitude**	**Seeding density of planted species (g/m^2)**				
		R. pinnata	***R. triloba***	***S. laeve***	***S. rigida***	***S. scoparium***
1	42.37678	3.46	---	1.08	2.16	0.72
2	42.39430	6.12	5.71	---	2.45	19.57
3	41.98220	14.75	13.96	---	6.03	39.65
4	41.94282	9.51	3.57	1.18	1.18	57.09
5	42.20323	17.12	---	5.71	12.13	249.81
6	43.14087	6.32	4.75	1.59	1.58	---
7	41.98284	4.07	4.07	2.03	---	57.09
8	42.30046	5.62	2.11	0.70	0.70	22.50
9	42.80488	3.84	---	---	3.84	38.34
10	43.01428	---	---	1.42	4.28	---
11	42.05913	2.43	---	---	1.07	28.65
12	44.82885	15.13	---	---	6.57	---
13	42.75627	17.84	---	4.46	4.46	160.59
14	42.72123	71.37	71.37	---	---	1427.48
15	42.33675	10.47	---	1.23	1.23	53.53
16	42.83042	3.42	---	1.07	2.85	49.96
17	42.33065	6.47	---	0.81	3.23	29.12
18	41.86200	3.24	---	---	3.57	199.85
19	42.30792	15.83	15.05	---	6.49	42.82
20	43.32234	0.99	---	---	0.41	19.14
21	43.01303	0.10	---	---	0.04	1.91
22	42.95311	2.89	0.90	0.34	0.34	---
23	44.93877	---	---	---	2.63	36.11
24	44.95201	---	---	---	1.38	19.03

Table A2.1 (cont'd)

Site ID	Site Latitude	Planting season	Planting year	Previous land type	Managed (yes/no)
1	42.37678	UNK	UNK	Oldfield	Y
2	42.39430	Fall	2019	Lawn	Y
3	41.98220	Spring	2018	Lawn	N
4	41.94282	Spring	2018	Forest	Y
5	42.20323	Fall	2017	Oldfield	N
6	43.14087	Summer	2017	Forest	Y
7	41.98284	Spring	2019	grassland	N
8	42.30046	Spring	2019	Farmland	Y
9	42.80488	UNK	UNK	UNK	N
10	43.01428	Spring	2018	Pavement	Y
11	42.05913	Spring	2019	Grassland	Y
12	44.82885	Spring	2017	Forest	Y
13	42.75627	Fall	2018	Oldfield	Y
14	42.72123	Spring	2018	Lawn	N
15	42.33675	Spring	2018	Oldfield	Y
16	42.83042	Spring	2020	Pavement	Y
17	42.33065	Spring	2018	Farmland	Y
18	41.86200	Spring	2018	Forest	N
19	42.30792	Fall	2018	Grassland	Y
20	43.32234	Fall	2017	Forest	N
21	43.01303	Fall	2019	Lawn	N
22	42.95311	Fall	2017	Lawn	Y
23	44.93877	Spring	2018	UNK	Y
24	44.95201	Spring	2018	UNK	Y

Table A2.2: PC axis variables interpretation. PCA output List of the top 9 bioclimate variables generated from the R package ‘dismo’ from the top 3 PCA axis variables (combined explained 84% of the variation in all 19 climate variables) along with the loadings of each variable along each axis, and the biological interpretation taking into consideration the variable and the sign of the loading variable (positive or negative). In our data, the warmest quarter was June-Aug., coldest quarter was Dec-Feb, wettest quarter was Aug.-Oct., driest quarter was Nov.-Jan. (data not shown).

PC1: 52% of the variation

Climate Variable	Variable number	PC Loading	Interpretation
Mean temp. driest quarter	BIO 9	-31	Cold winter
Min. temp. coldest month	BIO 6	-31	Cold winter
Temp. annual range	BIO 7	31	High temp. variation
Temp. seasonality	BIO 4	30	High temp variation
Mean temp. coldest quarter	BIO 11	-29	Cold winter
Precip. driest month	BIO 14	-29	Dry winter
Precip. driest quarter	BIO 17	-28	Dry winter
Mean annual temp.	BIO 1	-26	Cold
Precip. seasonality	BIO 15	-26	Consistent precipitation

PC2: 18% of the variation

Climate Variable	Variable number	PC Loading	Interpretation
Mean temp warmest quarter	BIO 10	-44	Cool summer
Max temp. warmest month	BIO 5	-44	Cool summer
Precip. wettest quarter	BIO 16	-33	Dry summer
Precip. warmest quarter	BIO 18	-29	Dry summer
Mean temp wettest quarter	BIO 8	-27	Cool summer
Mean annual temp.	BIO 1	-24	Cool
Precip. seasonality	BIO 15	-21	Consistent precip.
Mean diurnal range	BIO 2	-20	Consistent temp.
Precip. wettest month	BIO 13	-20	Dry summer

PC3: 14% of the variation

Climate Variable	Variable number	PC Loading	Interpretation
Precip. Wettest quarter	BIO 16	44	Wet winter
Precip. Warmest quarter	BIO 18	42	Wet summer
Annual precip.	BIO 12	37	Wet
Max temp. warmest month	BIO 5	-27	Cool summer
Mean annual temp.	BIO 10	-22	Cool
Isothermality	BIO 3	-18	Cool days, warm nights
Precip. coldest quarter	BIO 19	18	Wet winter
Precip. driest quarter	BIO 17	15	Wet summer
Precip. driest month	BIO 14	14	Wet winter

Table A2.3: Complete statistics for the three response variables used to quantify plant establishment at various prairie restorations. Beta values and standard errors were calculated through a model averaging approach, and the relative importance of each variable, or the sum of its weight (SW) in all of the averaged models is reported as well.

	Likelihood of establishment			**Average abundance in 25m^2 area**			**% Flowering**		
	SW	**β**	**SE**	**SW**	**β**	**SE**	**SW**	**β**	**SE**
PLS (oz/acre)	0.66	0.39	0.36	0.72	0.22	0.12	0.21	0.01	0.25
Geographic distance (km)	0.27	-0.03	0.18	0.25	-0.01	0.08	0.71	0.65	0.55
PC1 difference	0.36	-0.11	0.23	0.25	-0.02	0.09	0.25	0.05	0.21
PC2 difference	0.24	-0.01	0.13	0.30	0.03	0.09	0.37	-0.12	0.36
PC3 difference	0.27	-0.04	0.16	0.29	-0.03	0.09	0.73	0.57	0.47
Site age (yrs)	0.44	-0.14	0.23	0.24	0.01	0.07	0.39	-0.12	0.22
Management	0.85	1.01	0.65	0.51	0.23	0.31	0.30	-0.22	0.61
WHC	0.25	-0.01	0.13	0.24	-0.01	0.07	0.22	-0.02	0.11
Survey julian date	---	---	---	---	---	---	0.81	0.53	0.37
Sqrt (Site latitude)	---	---	---	---	---	---	0.25	0.66	4.83

CHAPTER 3: RELATIVE EFFECTS OF SEED MIX DESIGN, CONSUMER PRESSURE, AND EDGE PROXIMITY ON COMMUNITY STRUCTURE IN RESTORED PRAIRIES

The work presented in this chapter is part of the final publication:

Pizza, R. B., Turley, N. E., & Brudvig, L. A. (2025). Relative effects of seed mix design, consumer pressure, and edge proximity on community structure in restored prairies. *Ecological Applications*, *35*(1), e3083.

Abstract

A central goal of ecosystem restoration is to promote diverse, native-dominated plant communities. However, restoration outcomes can be highly variable. One cause of this variation may be the decisions made during the seed mix design process, such as choosing the number of species to include (sown diversity), or the number of locations each species should be sourced from (source diversity, manipulated to affect genetic diversity). The effects that seed mixes have on plant communities may be further modified by other factors at the restoration site, including edge proximity and consumer pressure. However, few studies have evaluated both these seed mix attributes together, and none have done so while accounting for realistic restoration site attributes. To address this research need, we conducted a prairie restoration experiment where two aspects of a seed mix (sown diversity and source diversity), edge proximity, and vertebrate consumer access were manipulated across 12 replicate fields. We found that when seed mix design impacted plant community structure, these effects were dependent on consumer access or edge proximity and were more prominent after one vs. five growing seasons. Low seed source diversity plots had more sown species than high source diversity ones, but only when consumers had access. Similarly, low species diversity plots had higher richness and cover of species included in both the low and high species diversity mixes, but this effect weakened over time. Additionally, plots with high species-diversity were buffered from the typically detrimental effects of edges and consumers, although this did not always result in greater sown species abundance. Unexpectedly, plots with the most sown species were those sown with either low source diversity or low species diversity seed mixes, perhaps due to lower seeding rates of reliably establishing species. Our results illustrate how the influences of seed mix design on restored plant communities can be highly contingent on factors like edges, consumers, and time.

Introduction

Ecological restoration is a key tool to reverse widespread habitat loss (UNEP, 2019) and increase global biodiversity (Benayas et al. 2009). Commonly, the step in these projects is to reestablish a native-dominated plant community, often by adding seeds to ensure that target species arrive (Kimball et al. 2015). When developing a seed mix to use in a restoration effort, managers must decide how many species to include (Barak et al. 2021), and where each species is sourced from (Bucharova et al. 2019; Meissen et al. 2020). Yet, how these two axes of seed mix design together influence plant community structure remains largely unknown, and their effects may depend on conditions at restoration sites.

Manipulating both aspects of seed mix diversity together at a restoration site could influence plant communities in additive or interactive ways. Increasing the number of species in a seed mix can increase the number of target species that establish (Barr et al., 2017; Larson et al. 2011; Minor et al., 2021) and the diversity of the restored plant community (Kaul & Wilsey, 2021). Similar effects could be seen by increasing the number of places each species is sourced from to increase genetic diversity. For example, if species from different sources differs phenotypically, and each species can occupy a wider niche width (Roughgarden, 1972), species that would otherwise competitively exclude one another can instead coexist (Fridley et al., 2007; Vellend & Geber 2005; Whittaker, 1970). Higher source diversity seed mixes can also increase the probability that at least some of the seeds will be adapted to restoration site conditions (Kettenring et al., 2014). The number of species and sources in a seed mix could interactively affect plant communities. For example, adding additional sources may broaden the niche width of an already dominant species, making it even more dominant (Vellend & Geber 2005). Thus, additional species included in a seed mix may initially establish, but ultimately be outcompeted by other over-dominant species (Grman et al., 2021). However, few studies have simultaneously manipulated species and source diversity in plant communities (*but see* Fridley et al., 2007), and none have done this in a realistic restoration context, so it is unclear whether these metrics of seed mix design will interact, nor how this could affect the plant community at a restoration site.

Additionally, the effects seed mix design has on community structure may be contingent on context-dependencies at the restoration site. Context-dependencies can result in restoration efforts performed with identical methods producing very different outcomes (e.g. Norland et al. 2015). Thus, understanding how restoration methods are influenced by context-dependencies can inform restoration practitioners on the efficacy of certain methods under the realistic restoration conditions. While there are many abiotic and biotic factors that can influence restoration outcomes (Brudvig 2017), two important factors duringseed-based restoration in this system are edge effects and consumer pressure (granivory and/or herbivory). At a restoration site, edges are primary areas of invasion of non-sown species (Vila & Ibanez, 2011) and high levels of invasion can reduce sown species abundance, especially in prairie systems (Warren et al., 2002). If increasing either species or source diversity in a seed mix expands the niche space that sown species occupy, this can reduce invasion at the edges of sites (Kennedy et al., 2002) subsequently increasing restoration success. Consumer pressure can also influence plant species abundances and persistence during restoration (Rebollo et al., 2013): high herbivore pressure has been shown to decrease plant species richness by as much as 85% (Xu et al., 2023) and granivory has been shown to significantly decrease plant establishment in prairie restorations (Pellish et al. 2018). The effects that these consumers have on restored plant communities may be modified by seed mix design. For example, if there are phenotypic differences in seed morphology resulting in only some seed sources being desirable by consumers (Howe & Brown, 1999), high source diversity plantings may be buffered from the detrimental impacts of consumers. Alternatively, if herbivory is higher when more species or sources are sown in a seed mix (Drescher & Nolan, 2023), high diversity seed mixes may have low sown species establishment at restoration sites with strong consumer pressure. However, few studies have empirically tested how seed mix design effects are mitigated by these context-dependencies at the restoration site (Barr et al., 2017; Cook-Patton et al., 2011).

Finally, the ways seed mix decisions impact plant communities may vary temporally. For example, increasing the number of species in a seed mix may initially result in a community with greater sown diversity, but these effects may fade with time as species diversity declines due to competitive exclusion (Barber et al., 2017; 2019). Source diversity may show the opposite pattern: processes such as niche partitioning may take time to play out (Kettenring et al. 2014). Thus, restorations with low or high source diversity may appear similar at the onset of restoration, but high source diversity plantings may end up with greater sown diversity. Moreover, since previous research has demonstrated that both edge proximity (Debinski & Holt, 2000; Porensky et al., 2012) and consumer effects (Barber et al., 2017) vary temporally, the ways in which these factors modify the effects of seed mix diversity are unlikely to be consistent over time. However, previous research on the effects of seed mix design has typically surveyed communities at only one timepoint, and studies that surveyed communities over time (Galliart et al., 2019; Larson et al., 2011; 2013) did not account for context-dependencies. Understanding the long-term effects of manipulating species diversity and source diversity in a seed mix, while also considering how the effects of these decisions can be modified by external factors, will help us better predict how seed mix designs can influence restoration outcomes.

To test how manipulating two aspects of seed mix design, species and source diversity, can influence plant communities undergoing restoration, we asked: (1) how does increasing seed mix species diversity and source diversity, both separately and interactively, affect the number of species and their abundances in a plant community. Further, to understand the interactions between seed mix design decisions and context-dependencies, we asked: how are the effects of these seed mix aspects contingent on edge proximity, consumer pressure, and temporal dynamics? To address these questions, we conducted a prairie restoration experiment by factorially manipulating the number of species and seed sources in seed mixes sown across twelve fields. We coupled this large-scale experiment with experimental vertebrate exclusion at both the center and edge of each site. We surveyed plant communities twice: once during the first growing season to examine how decisions made during the seed mix design process can impact initial plant establishment, and again five years later to see whether these effects persisted over time.

Methods

Experimental design

Our experiment used a split-plot design, manipulating seed source diversity at the field level and species diversity at the half-field level. During the 2015 growing season, we selected twelve fields (ranging from 0.2 to 3.5ha) around Kellogg Biological Station in southwest Michigan, USA (42.4059, -85.4022) to undergo prairie restoration (Fig. 3.1). All fields were previously cultivated and the soil tilled, and were old fields dominated by non-native species before planting. We prepared the fields by mowing the existing vegetation and applying glyphosate herbicide twice before planting, which was successful at killing aboveground plant structures. To initiate restoration, we drilled prairie seeds (~330 seeds/m^2; see table A3.1 for individual species contributions) into each field following the 2015 growing season with a modified Truax seed drill pulled behind a tractor. We tested the impacts of source diversity with twelve focal species, each of which was purchased from three different locations in the central USA: locally (sourced from the geographically nearest location possible), from more distant, but climatically similar locations (Wisconsin, Minnesota, Illinois, or Iowa), or from southern locations (Missouri or Iowa; table A3.1). We verified with the seed producers that they sourced their seed locally. We randomly assigned sites to be planted either with seed from only one of those sources (low source diversity; n = 6; 2 fields of each source), or a seed mix that combined all three sources (n = 6). Source diversity was manipulated at the field level to reduce gene flow between high and low source diversity treatments. To test the effects of species diversity, we manipulated the number of species that were added to each half of the field (n = 12 in each treatment). We randomly assigned one half of the field to be seeded with only the 12 focal species (low species diversity). To the other, we added those 12 focal species plus an additional 58 species (high species diversity; table A3.1; Fig. 3.1). The additional species, due to limitations on where each species could be purchased from, were sourced from various locations across the Midwest, with each species sourced from a single location. Since the source location of most species differed, we did not consider source identity as a factor in our analyses. All seed mixes contained the same total density of seeds (g/m^2)

Two weeks after planting, we assembled 2.25m^2 vertebrate consumer exclosures at the center (at the center point of the half-field) and edge (1.5m away from the edge) of each half-field (n = 48; Fig. 3.1). Due to differences in field size, the distance between center and edge plots differed across fields. The plant communities surrounding the fields differed (either a forest edge, a crop field, or a grass lawn) and our sown species were rare or absent from all adjoining habitats. Exclosures consisted of 110-120cm tall walls of 6.35mm wire mesh, buried 10-20cm underground in an attempt to impede entrance by digging mammals. They also had 10cm of metal flashing on the tops of the walls to prevent small mammals from climbing in. Each exclosure had a paired pseudo-exclosure (hereafter referred to as “control”) with only three walls with holes in the bottom to allow entry for vertebrates (Fig. 3.1). We randomized which plot had the exclosure and which one had the pseudo-exclosure for each pairing. We placed bird netting on top of the exclosures after planting and removed it after the first growing season. While we did not conduct consumer surveys, previous work has shown that granivores (meadow voles, various mouse species, and arthropods) and herbivores (white tailed deer, meadow voles, rabbits, and woodchucks) are important in this system (Anderson et al. 2001; Howe et al. 2006; Linabury et al., 2019).

Plant Community Surveys

We surveyed plant communities in Sept. 2016 and July 2021 by placing a 1×1m quadrat in the center of each exclosure and control exclosure. We recorded the identity and visually estimated percent cover of each species. In 2021, to account for variation caused by site-specific environmental differences, we pooled 10cm deep soil cores from nine points just outside each exclosure and control exclosure and processed them for soil water holding capacity (the proportionate difference between saturated wet and oven dry weights (*following* Brudvig and Damschen 2011). We chose this method to characterize the soils as it has been shown to correlate with the abundance of prairie species in our region (Grman et al. 2021; Zirbel and Brudvig 2020) and with other soil attributes such as soil organic matter and Nitrogen content (Bordoloi et al. 2019).

Data analysis

We performed all analyses in R studio using R version 4.2.3 (R Core Team, 2023). We used the inverse Simpson's diversity to measure species diversity (Clarke et al., 2014) and its corresponding evenness metric (Smith & Wilson, 1996) since these metrics are commonly used when species dominance is predicted to be important in community assembly (Morris et al., 2014). Since we expected communities to shift tremendously due to succession in between the first and fifth growing season regardless of our measured factors, we did not include year as a fixed effect in our models. Instead, we ran each model twice, once for each year data was collected.

Univariate analyses

We analyzed species richness, evenness, Simpson's diversity, sown species richness and focal species richness (species that were manipulated in the seed sourcing treatment) using separate mixed effects linear models for each response variable (Bates et al., 2014). All models included the following predictors: seed source diversity, species diversity, edge proximity, consumer access, as well as every 2-way interaction between these factors. We also included soil water holding capacity as a covariate. To account for the split-split-plot design, we included a random factor that nested exclosure status, edge proximity, and seed mix diversity. After running the analyses, we removed the interaction between edge proximity and exclosure status, as this interaction was never significant in any analyses, nor did it directly relate to our research questions. All corresponding statistical results are reported in the supplement (table A3.2).

To establish if seed mix design influenced dominant species, we also analyzed the cover data for the most common species in our plots. We defined a species as "common" if it was found in at least half (45) of our plots in a given year. In 2016, common species were *Daucus carota*, *Elymus repens*, *Plantago lanceolata*, and *Solidago canadensis*. In 2021, common species were two weedy non-native species, *Elymus repens* and *Poa pratensis*, and one weedy native species *Solidago canadensis*. Although these species were not locally abundant in every plot, they often were: the cover of these species in each plot often exceeded 40%, and cover reached up to 95% in some plots. Since no sown species were ever common by our definition, we also analyzed the most abundant sown species five years after establishment (when they were most prevalent): *Andropogon gerardii* (found in 24 plots) and *Echinacea purpurea* (31 plots). All species-level models were over dispersed but not zero-inflated (all zero-inflation tests $p > 0.6$; DHARMa package version 0.4.6; Hartig & Hartig, 2017), so we used a negative binomial mixed model (Brooks et al., 2017). Due to limitations of this model, the coefficients are not standardized. All corresponding statistical results are reported in the supplement (table A3.3).

Multivariate analyses

To test the effects of seed mix design and other factors on community composition, we conducted a PERMANOVA analysis using the adonis2 function (Vegan package version 2.6-4; Okansen, 2010). Because of our split-split-plot design (the source diversity treatment being applied to the entire field) and limitations of the adonis2 function, we could not analyze the effects of species diversity, source diversity, edge proximity, and consumer pressure in one model. Thus, we split this analysis into two components: one to test the effects of source diversity and species diversity on community composition, and another to test the effects of species diversity, edge proximity, and consumer access on species covers. We ran the first model using only source diversity, species diversity, and their interaction as fixed effects. We also included field ID as a fixed effect nested within half-field ID to account for field differences and interpreted it as a random factor, to account for limitations with fitting random effects in the adonis2 function. If any factors were significant in analyses, we ran tests of dispersion on any significant terms using the betadisper function (Vegan package version 2.6-4;Okansen, 2010). We ran the second model with the same factors as all the univariate models, except field ID nested within half-field ID was used as a fixed effect to correct for the degrees of freedom not accounted for without using a nested random effect (owing to limitations with fitting random effects in the adonis2 function). In this model, all permutations were conducted within fields. Both models had 10,000 permutations. To visualize differences in plant communities based on the interaction between source diversity and species diversity, we used a canonical analysis of principal coordinates (Anderson & Willis, 2003) of bray-curtis dissimilarity matrices using the function “capscale” (Vegan package version 2.6-4; Okansen, 2010). All corresponding statistical results are reported in the supplement (table A3.4).

Results

Relative importance of factors

Overall, our measured factors (Source diversity, species diversity, edge proximity and consumer pressure) had the greatest impact during the first year of establishment, with 83% of models having at least one significant factor, compared to only 43% of models conducted on 2021 data (tables A3.2-A3.4, Figs. S3.1-S3.3). Additionally, the amount of variance explained for factors in our models was considerably higher in 2016, especially those related to the species sown into the experiment (Fig. 3.2). Across both years, seed source diversity was a significant factor in 28% of all univariate analyses, but only through interactions with other factors, especially the exclusion of consumers (Figs. A3.1-A3.3; table A3.2). Seeded species diversity was a significant factor in 58% of analyses, both as a main effect and as interaction terms with consumer exclusion or edge proximity (Figs. A3.1-A3.3; table A3.2). Edge proximity was a significant predictor in 31% and consumer pressure in 27% of analyses (Figs. A3.1-A3.3; tables A3.2 & A3.3).

Main effects of seed mix design

Seed source diversity was never a significant main effect in our community level models (table A3.2). Sown species diversity was a significant main effect, but only for sown species responses. Focal species richness (sown species where seed source was manipulated) was 38% higher in low sown species diversity plots during the first year of growth (Fig. 3.3a.) Five years later, there was marginal evidence that there were more focal species in low species diversity plots compared to high species diversity plots, although focal species richness was lower than it was during establishment (Fig. 3.3a). There was a more pronounced difference in focal species cover: it was 92% higher (Fig. 3.3b) in low species diversity plots compared to high species-diversity plots. Five years later, though, this trend disappeared. The interaction term between source diversity and species diversity was never significant in any of our community measures (table A3.2).

Interactions between seed mix design, edge proximity, and consumer pressure

The effects that seed mix design had on plant communities were often dependent on edge proximity and consumer pressure. In the first growing season, low seed source diversity plots had on average, one additional focal species (sown species where seed source was manipulated), but only in plots that allowed consumer access (Fig. 3.4a). This relationship persisted 5 years later, although the effect was weaker. We observed a similar trend for focal species cover (~1.5% greater cover in low source diversity plots; Fig. 3.4b) and sown species richness (~1 species; table A3.2), but these trends did not persist through 2021. At the center of fields, species evenness was 25% higher (Fig. 3.5a), community diversity 36% higher (Fig. 3.5b), and sown species cover 75% higher (Fig. 3.5c) than at the edge of fields during the first growing season, but only in low species diversity plots. All three of these interactive effects disappeared when plots were surveyed again five years later (Fig. 3.5).

There was a more complex relationship between species diversity and consumer pressure: five years after establishment, community diversity was 27% higher in plots where consumers were excluded, but only in low species-diversity halves (Fig. 3.6a). This trend was not observed during the first growing season. We observed a different trend for sown species: sown species richness was 41% higher (Fig. 3.6b) and focal species richness was 51% higher (Fig. 3.6c) when consumer access was prevented, but now only in high species diversity plots and only during the first growing season.

There was never an interaction between seed source diversity and species diversity for any of our univariate metrics of community structure. However, there was weak evidence that evenness was higher in high seed source diversity fields, but only in low diversity halves during the first growing season (table A3.2). We did, however, observe an interaction between species diversity and source diversity in multivariate analyses of community structure both during initial establishment (Fig. 3.7a) and five years later (Fig. 3.7b; table A3.4), although the effect was small ($R^2 = 0.02$). There was no difference in multivariate dispersion among treatments in either year (all $p > 0.15$).

Main effects of context-dependencies

There were (~1.25) more sown species and marginally greater focal species cover at the center of fields than at the edges during the first year of establishment, and ~1 more species at the center of fields than at the edges five years after establishment (table A3.2). Consumer pressure was never significant as a main effect in our models (table A3.2). Finally, water holding capacity was only significant in one analysis, with drier fields having greater community diversity (table A3.2). The field random effect (the 12 locations) explained, on average, 42% (±0.12 sd) of the variation in models.

Individual species responses

Of the five non-sown species and two sown species we investigated, three were impacted by our factors: *Elymus repens*, *Plantago lanceolata*, and *Echinacea purpurea*. The other four species were not impacted by any of our measured factors, although *Solidago canadensis* was marginally more abundant at the center of sites than at the edges during the first growing season (table A3.3). During the first growing season, the non-sown species *Elymus repens* cover tended to be higher in the low source diversity plots, but not significantly. Five years later, though, *E. repens* cover was 30% higher in low source diversity plots when consumers had access, but 22% lower in high source diversity plots (Fig. 3.8a). The cover of *Plantago lanceolata* during the first growing season was 65% higher at the center of fields than at the edges in low source diversity plots, but edge proximity did not affect cover in high species-diversity plots (Fig. 3.8b). *Elymus repens* cover was 30% higher in low source diversity plots than high source diversity plots, but only when consumers were present (Fig 3.9a) and only during the first growing season. Excluding consumers in plots with high seed source diversity increased *Echinacea purpurea* cover by 60%, whereas in plots with low source diversity it decreased cover by 53% (Fig 3.9b).

Two species' covers were impacted by the interaction between source diversity and species diversity, but in different ways. In 2021, in low source diversity fields there was 31% greater cover of *Elymus repens* in low species diversity plots compared to high species diversity plots, but there is (Fig. 3.10a). Of note, *Elymus repens* cover was qualitatively higher in the low source diversity plots during the first growing season regardless of sown species diversity. The other species, *Echinacea purpurea*, had 87% greater cover in high source diversity plots, but only when species diversity was low (Fig. 3.10b).

Discussion

To understand the consequences of seed mix design decisions on restored plant communities in a realistic restoration context, we conducted a large-scale experiment that manipulated both source and species diversity in a seed mix and accounted for the influences of edge proximity, consumer pressure, and time. Overall, when we found evidence of seed mix design decisions affecting plant community structure, the effects were dependent on consumer access or edge proximity and were most prominent during the first growing season. Additionally, high species-diversity plots were buffered from the effects of edges and consumer access, which decreased sown species abundance in low species diversity plots. However, these plots did not always have the greatest sown species abundance. Thus, our results suggest that increasing the number of sown species can reduce the effects of site-specific contingencies, whereas increasing sown source diversity may result in reduced sown species establishment.

Increasing source or species diversity reduced sown species establishment

Generally, our results did not support our predictions for the ways seed source diversity could influence plant community structure. For example, we expected to see higher focal species richness and cover when plots were seeded with multiple sources, due to increased niche width and reduced inter-specific competition (Vellend & Geber, 2005). Instead, focal species richness was lowest in these plots. One of our focal species, *Echinacea purpurea* did have greater cover in the high source diversity plots, providing some support that competition could be reduced with higher genetic diversity. Our other prediction was that the number of sown species present in the community could decrease if increased source diversity allowed one of our focal species to become overdominant (Vellend & Geber, 2005). However, since none of our sown species were ever common, our study did not provide a strong test of this hypothesis. Due to the abundance of non-sown species observed in this study, the sown cover we measured may not have been sufficient to cause some of the hypothesized effects of seed source diversity.

The effects of source diversity were often modified by consumer access. Our twelve focal species were least abundant in high source diversity plantings, but only when consumers had access. This indicates that the effects that source diversity has on restoration outcomes may be due to trophic interactions with other species, rather than inherent properties of the plants themselves. Some possible explanations for this trend are that granivores preferred certain sources over others (Lundgren & Rosentrater, 2007; Perkins et al., 2007), or that high source diversity communities attracted more consumers through either increases in traits desirable to generalist consumers or by increasing the variety of traits to attract a greater diversity of consumers (Castagneyrol et al., 2012; Koricheva & Hayes, 2018; Orrock & Witter, 2010). Interestingly, these trends only persisted during the first growing season: five years later, focal species richness and cover were no different between the low and high-source diversity fields. This suggests that our exclosures had the strongest effects on granivory, which we expect to be important for plant community establishment from seed at the onset of restoration, rather than herbivory, which may become a more important process once the community is established.

Sown species diversity more commonly influenced community structure in our experiment, although it did not always result in more sown species in each plot. In high species diversity plots, we observed lower richness and cover of our sown species, especially the twelve focal species. However, this was contingent on consumer access and edges. In part, this may be a sampling effect: measuring at the plot-level, we were inherently less-likely to come across our focal species that were diluted in the high-diversity seed mix (Šímová et al., 2013). However, since both plantings had the same seeding density, we would still expect to see equal numbers of sown species. Instead, there is evidence that adding additional species, at the cost of decreasing the amount of seed from reliably establishing ones, ultimately reduced the number of sown species in each plot. For example, *Andropogon gerardii* and *Echinacea purpurea*, our most common sown species, were both in the low species diversity mix. Given that adding additional species can be expensive (Schaub et al., 2021), practitioners should carefully consider the benefits of adding additional species if they are unable to do so without reducing the overall seeding rate of the species already in the mix.

Sown species diversity effects were often contingent on edge proximity and consumer pressure

Although seeding higher diversity seed mixes reduced sown species cover, we also found evidence that these seed mixes can minimize the often-detrimental effects of edge proximity and consumer pressure. For example, community diversity was highest at the center and lowest at the edges of fields that were sown with fewer species during the first growing season. However, fields sown with higher species diversity seed mixes had consistent levels of community diversity across the center and edges of fields. It is possible that despite high species diversity plots having fewer sown species, the sown species that are present are more functionally diverse, or have traits similar to invading species, allowing for greater niche coverage and less opportunity for non-sown species to invade and establish (Kennedy et al., 2002). We saw similar patterns when consumer access was manipulated: plots sown with fewer species exhibited high community diversity only when consumers were excluded, but plots sown with more species retained high levels of diversity, even when consumers had access. We expect that, since some species were likely consumed more readily than others (Herrera & Pellmyr, 2009), some species in the low-diversity mixtures are being lost and not replaced by other seeded species. In the high-species mixtures, those species are more likely replaced due to functional redundancy in the seed mix (Petchey & Gaston, 2002) maintaining high levels of diversity (Palmer et al., 1997; Tilman & Downing, 1994). Given that edge effects and consumer pressure can lead to unpredictable restoration outcomes, increasing species diversity in a seed mix at sites where these factors are strong, without decreasing the seeding rate of well-establishing species, could result in more diverse restoration plantings.

Manipulating both source and species diversity had species-specific effects

The interaction between source diversity and species diversity was rarely an important factor in any of our plant community measures. However, it was significant in analyses that accounted for species identity: our multivariate analysis suggested that communities were structured by the combination of the number of sources of our focal species and the number of additional species in the seed mix. Although this interaction did not explain a large amount of variation in community composition, it suggests that species responded differently to the source and species diversity treatment.

There was also some evidence for an interaction between source diversity and seeded species diversity when considering individual species, but only five years after planting. *Elymus repens* cover was lowest when either source or species diversity was increased, suggesting that increasing diversity in the seed mix in any way makes it more difficult for this exotic, invasive species to establish. Since *E. repens* is a long-lived perennial grass, it took time to become established in the fields, explaining why its cover was not significantly affected by our seed mix in the first year. Future research designed to understand why *E. repens*, unlike any of the other dominant non-sown species found in our plots, was impacted by seed mix design may illuminate other ways seed mix decisions can influence restoration outcomes.

Of the two sown species that were most dominant in our plots, only one was affected by seed mix diversity (*Echinacea purpurea)*, a forb commonly sown in prairie restorations. This species had higher cover in high-source diversity fields. Although this increase in cover was not present in fields with high species diversity, we suspect this is caused by this species being sown at a lower rate in this seed mix and would have otherwise had comparable levels of cover. This indicates that outside of the preferential consumption of some seed sources, there may be some species-specific advantages to existing in populations with higher source diversity While the mechanism driving this increase in cover requires further study, we hypothesize that increased source diversity of *E. purpurea* resulted in greater niche width, reducing intraspecific competition and increasing cover, as has been observed in other studies (e.g., Crutsinger et al., 2008).

Seed mix design effects were strongest during initial establishment

One of the clearest effects in this study is that seed mix decisions have their greatest effect during the first year of establishment, with those effects dissipating over time. During the first growing season, the species that arrived in each plot were clearly determined by the seed mix added to the site. By the fifth year, though, non-sown species established and competitive interactions between those species were likely the primary drivers of community structure. While it may appear that seed mix decisions do not have long-term consequences, there are alternatives that should be considered. First, since the effects of seed mix diversity are scale dependent, with clearer patterns emerging at larger scales (Catano et al. 2021). Thus, some of the effects that we observed at the plot scale in the first year may have still been present five years later, but only apparent at larger scales. Additionally, sown species were often rare in our plots, especially those sown with high species diversity seed mixes. Had there been a greater abundance of sown species in our experiment, the effects of seed mix design may have been more apparent.

Moreover, since we only measured plant communities at two timepoints, we may have missed patterns related to inter-annual variation, especially those driven by climate. Previous work in grassland systems has demonstrated that precipitation can have a large effect on annual turnover in plant communities, especially for rare species (Cleland et al., 2013; Groves et al., 2020). Our sites (Michigan, USA) experienced higher than average precipitation during many of the survey years, especially during the planting year (PRISM climate group, 2018; Appendix 1: Figure S3). This wetter climate may have bolstered the cover of non-sown species (Groves et al., 2020), suppressing the abundance of sown species. Thus, the sown species that were present when we surveyed may not represent the communities we will observe in the future. Given the call to better understand the long-term dynamics of restoration decisions (Kaul and Wilsey 2021), our experiment will provide invaluable opportunities to continue to monitor these plant communities into the future.

Conclusions

Overall, our results exemplify the importance of accounting for context-dependencies in restoration research. Had we not accounted for edge effects and consumer access in our experiment, our data would suggest that modifying sown species diversity largely had no effect on restored plant communities. Instead, our results indicate that there are some conditions in which seed mix diversity is likely to influence restoration outcomes. For example, if planting in an area with large amounts of edge or high consumer pressure, increasing the number of species in a seed mix may reduce those effects. However, this will only be true if the seed mix retains high seeding rates of reliably establishing species. Increasing source diversity may have neutral effects in fields with low consumer pressure (and perhaps benefit some species such as *E. purpurea),* but detrimental impacts in fields with more consumers. Our study indicates that restoration outcomes may be driven both by the decisions that managers make as well as the environmental conditions the communities establish in and provides potential drivers for variation in restoration outcomes.

Figures

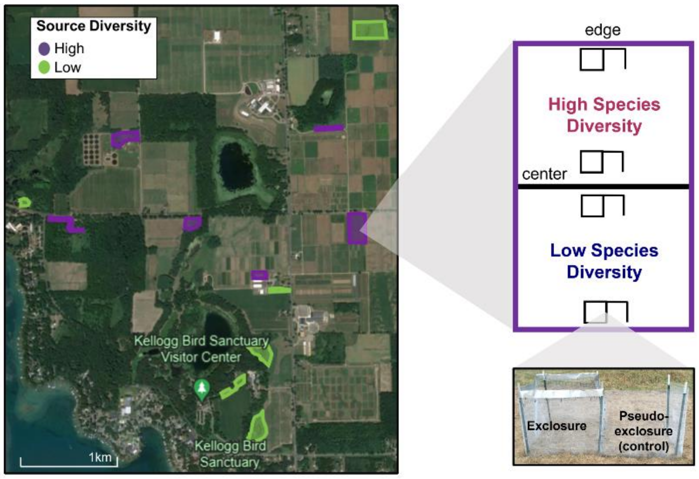

Figure 3.1: Illustration of the 12 tallgrass prairies undergoing restoration in this study. Fields were seeded with either one or three sources for 12 focal species. Half of each field was seeded with only the twelve focal species (low species diversity) and the other half with the twelve focal species plus 57 more (high species diversity). At the center and edge of each half-field, 1.5m × 1.5m exclosures were built to exclude vertebrate consumers, and each exclosure was paired with a pseudo-exclosure which was enclosed on only three walls. Plots were surveyed within each of these exclosures and pseudo-exclosures. The map was created using Google Earth, and the image of the exclosures was taken by Nash Turley.

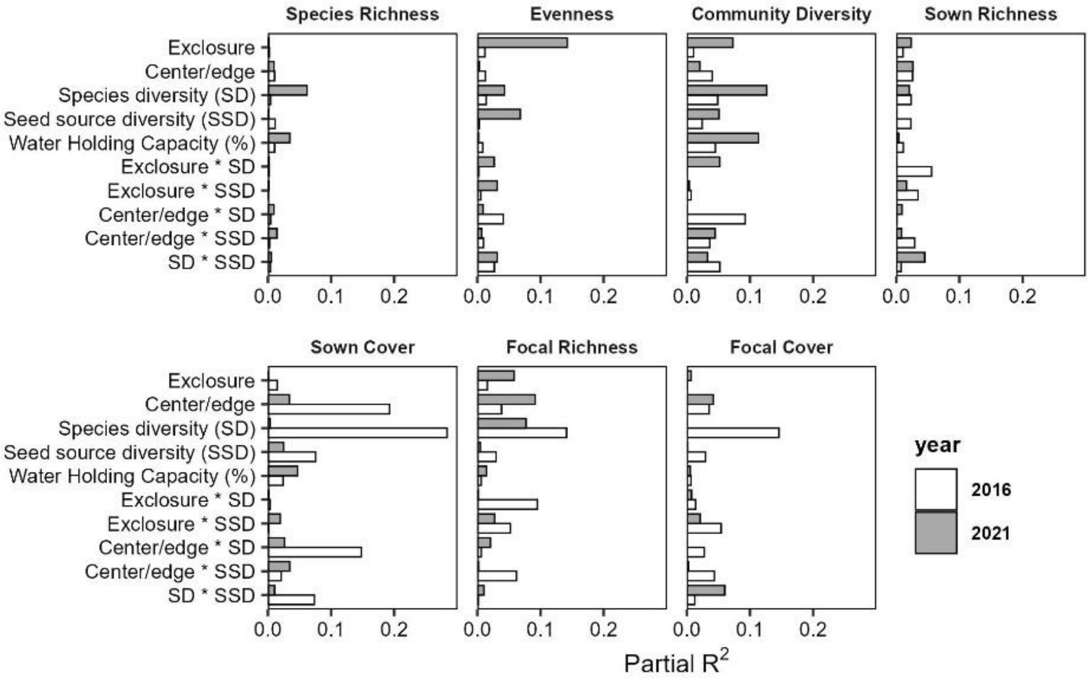

Figure 3.2: Partial R^2 values for seven community response variables measured in 2016 (white bars) and 2021 (grey bars) to quantify the effects of seed mix design, edge effects, consumer pressure, and their interactions on the presence and abundance of species sown into 12 tallgrass prairie fields undergoing restoration. “Sown” refers to any species that were included in either the low or high species diversity seed mix, and “Focal” refers to the 12 species included in both seed mixes where seed source diversity was manipulated.

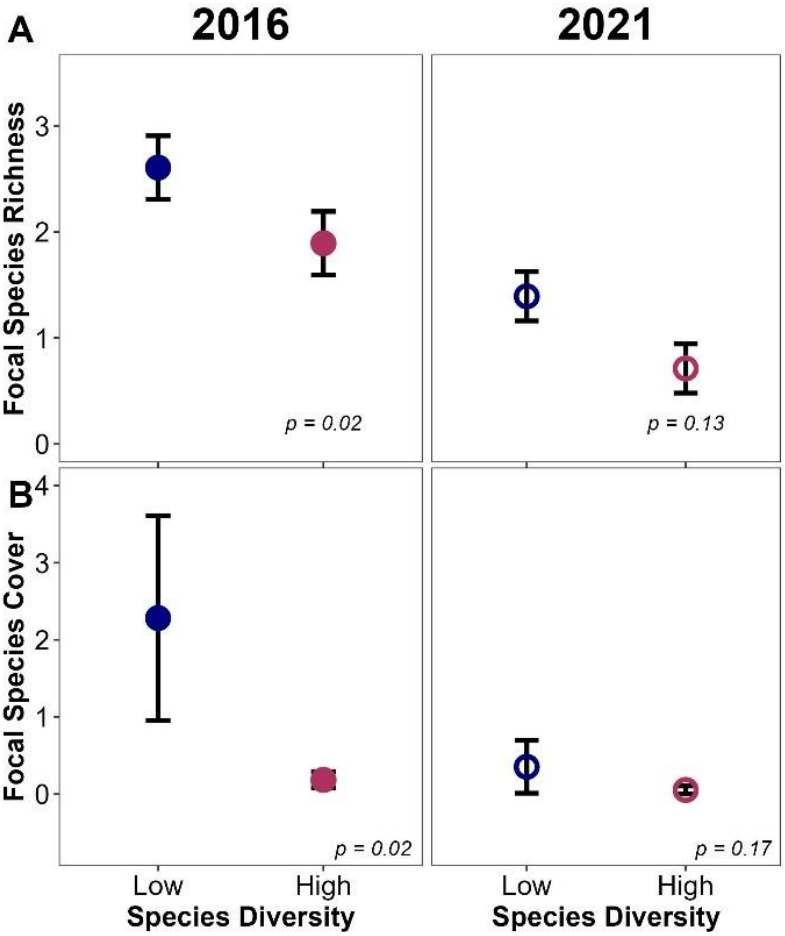

Figure 3.3: Conditional effects, accounting for other model factors, of the number of species used in a seed mix on cover of (A) focal species richness and (B) focal species cover both in the first growing season (closed circles) and 5 years after establishment (open circles; n = 96) in 12 tallgrass prairies undergoing restoration. “Focal” refers to the 12 species included in both the high and low diversity seed mixes where seed source diversity was manipulated. Dots indicate mean values calculated with emmeans() and error bars show standard error. P-values on each panel reflect the species diversity factor significance in each model.

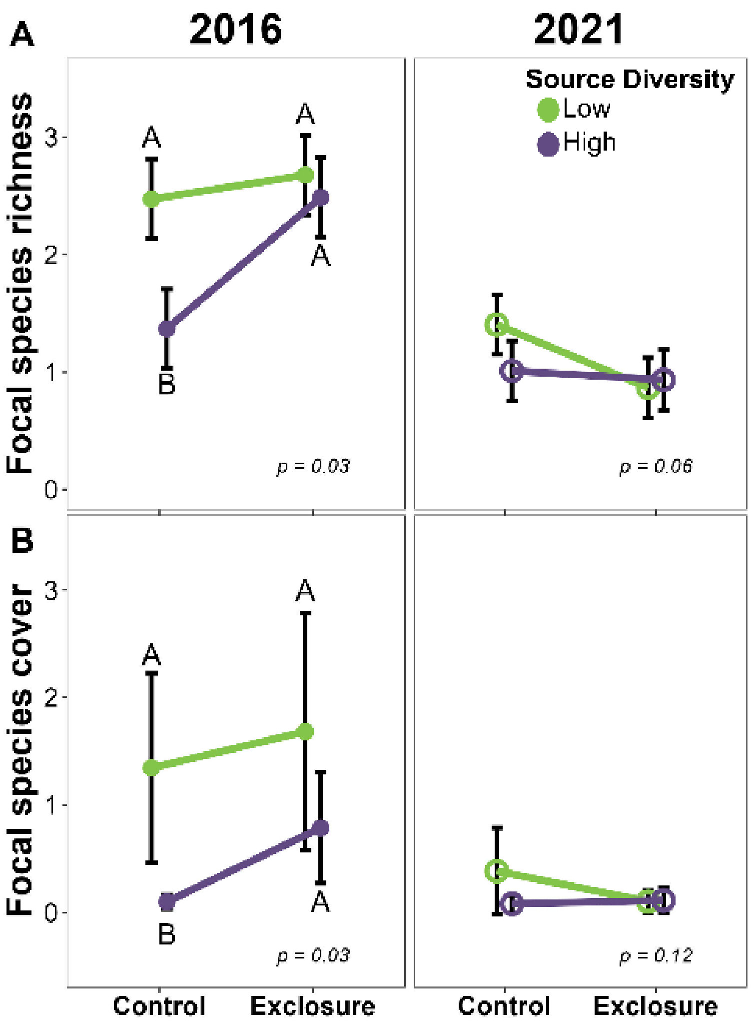

Figure 3.4: Conditional effects, accounting for other model factors, of the interaction between the number of sources used in a seed mix and whether a plot was excluded from consumers on (A) the number of focal species in a plot, (B) the cover of those focal species both in the first growing season (closed circles) and 5 years after establishment (open circles; n = 96) in 12 tallgrass prairie fields undergoing restoration. "Focal" refers to the 12 species included in both the high and low diversity seed mixes where seed source diversity was manipulated. Dots indicate mean values calculated with emmeans(), error bars show standard error, letters indicate differences between groups based on post-hoc tests, and p-values on plots are for the interaction effect. Plots with no letters indicate either significant effects of the interaction between source diversity and exclosure with no differences in conditional means, or an insignificant interaction effect (refer to model effect p-value on each panel).

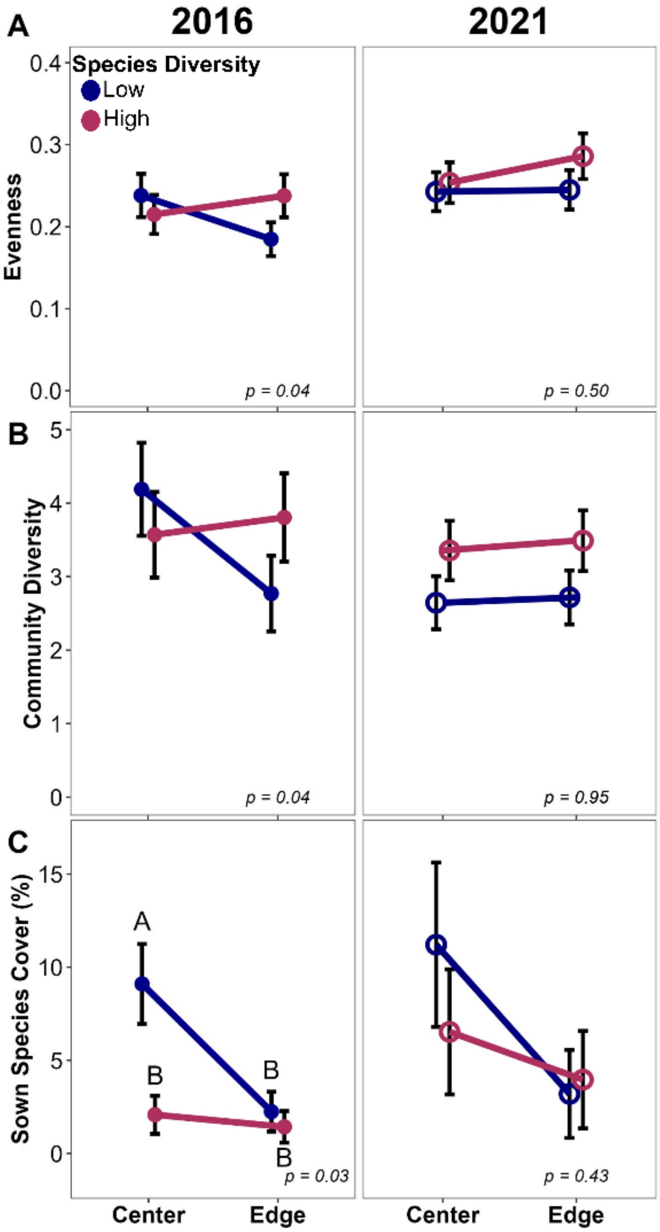

Figure 3.5: Conditional effects, accounting for other model factors, of the interaction between the number of species used in a seed mix and whether a plot was at the center or edge of a field on the (A) Evenness of species, (B) community diversity and (C) sown species cover both in the first growing season (closed circles) and 5 years after establishment (open circles; n = 96) in 12 tallgrass prairies undergoing restoration. Dots indicate mean values calculated with emmeans(), error bars show standard error, and letters above bars indicate significance groups. Plots with no letters indicate either significant effects of the interaction between source diversity and exclosure with no differences in conditional means, or an insignificant interaction effect (refer to model effect p-value on each panel).

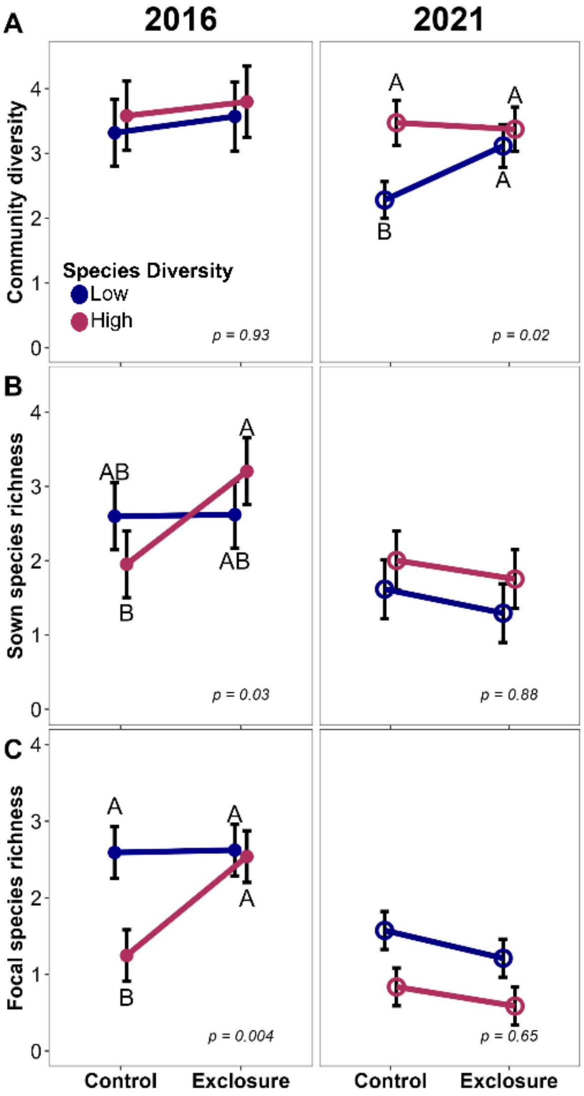

Figure 3.6: Conditional effects, accounting for other model factors, of the interaction between the number of species used in a seed mix and whether a plot allowed consumer access on (A) Community diversity (B) Sown species richness and (C) focal species richness both in the first growing season (closed circles) and 5 years after establishment (open circles; n = 96) in 12 tallgrass prairies undergoing restoration. “Sown” refers to any species that were included in either the low or high species diversity seed mix, and “Focal” refers to the 12 species included in both seed mixes where seed source diversity was manipulated. Dots indicate mean values calculated with emmeans(), error bars show standard error, and letters above bars indicate significance groups. Plots with no letters indicate either significant effects of the interaction between source diversity and exclosure with no differences in conditional means, or an insignificant interaction effect (refer to model effect p-value on each panel).

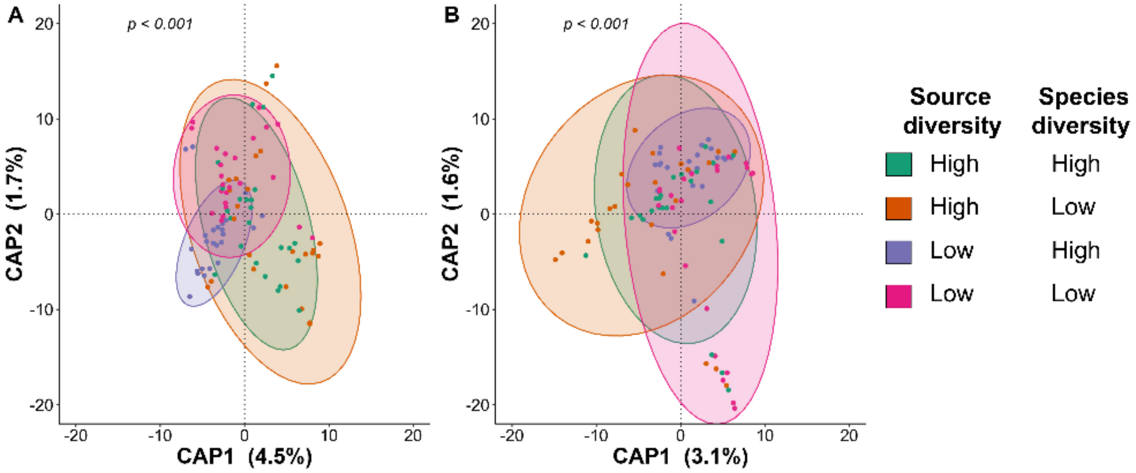

Figure 3.7: Canonical analysis of principal coordinates (CAP plots) for the interactive effect of source diversity and species diversity in a seed mix on plant community composition during the first growing season (A; PERMANOVA $R^2 = 0.02$) and 5 years following establishment (B; PERMANOVA $R^2 = 0.02$) in 12 tallgrass prairies undergoing restoration. For full PERMANOVA statistics, see table S3.5.

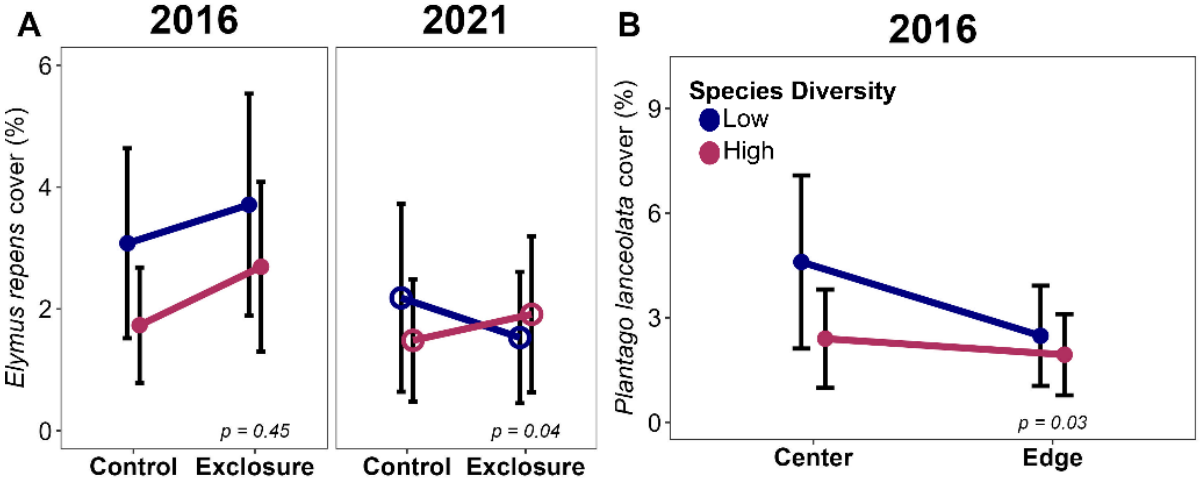

Figure 3.8: Conditional effects, accounting for other model factors, of the interaction between (A) the number of species used in a seed mix and whether a plot allowed consumer access on the percent cover of *Elymus repens* during the first growing season (closed circles) and five years after establishment (open circles) and (B) the number of species used in a seed mix and whether a plot was at the center or edge of a field on the percent cover of *Plantago lanceolata* during the first growing season(open circles; n = 96) in 12 tallgrass prairies undergoing restoration. Dots indicate mean values calculated with emmeans(), and error bars show standard error.

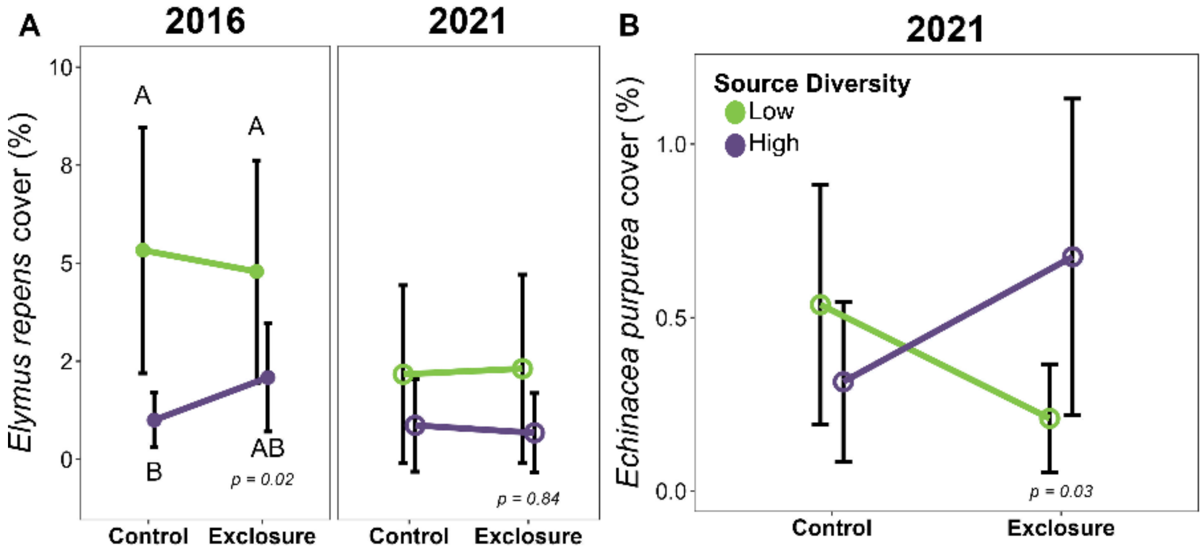

Figure 3.9: Conditional effects, accounting for other model factors, of the interaction between the number of species and seed sources used in a seed mix and whether plots allowed consumer access on the cover of (A) *Elymus repens* during the first growing season (closed circles) and five years after establishment (open circles) and (B) *Echinacea purpurea* five years after establishment (n = 96) in 12 tallgrass prairies undergoing restoration. Dots indicate estimated mean values calculated with emmeans(), error bars show standard error, and letters above bars indicate significance groups. Plots with no letters indicate either significant effects of the interaction between source diversity and exclosure with no differences in conditional means, or an insignificant interaction effect (refer to model effect p-value on each panel).

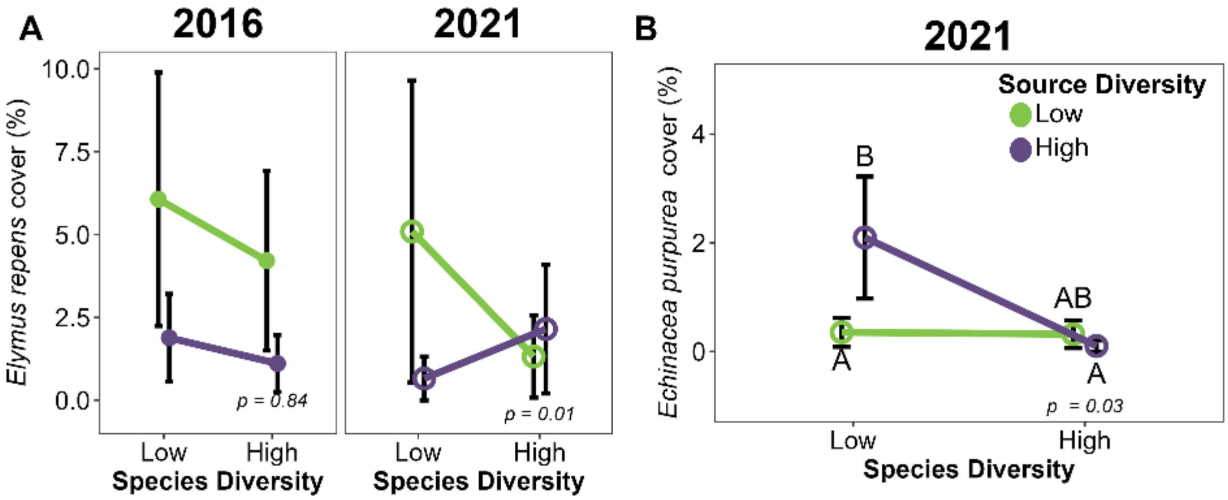

Figure 3.10: Conditional effects, accounting for other model factors, of the interaction between the number of species and seed sources used in a seed mix on the cover of (A) *Elymus repens* during establishment during the first growing season (closed circles) and five years later (open circles) and (B) *Echinacea purpurea* five years after establishment (n = 96) in 12 tallgrass prairies undergoing restoration. Dots indicate estimated mean values calculated with emmeans(), error bars show standard error, and letters above bars indicate significance groups. Plots with no letters indicate either significant effects of the interaction between source diversity and exclosure with no differences in conditional means, or an insignificant interaction effect (refer to model effect p-value on each panel).

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APPENDIX

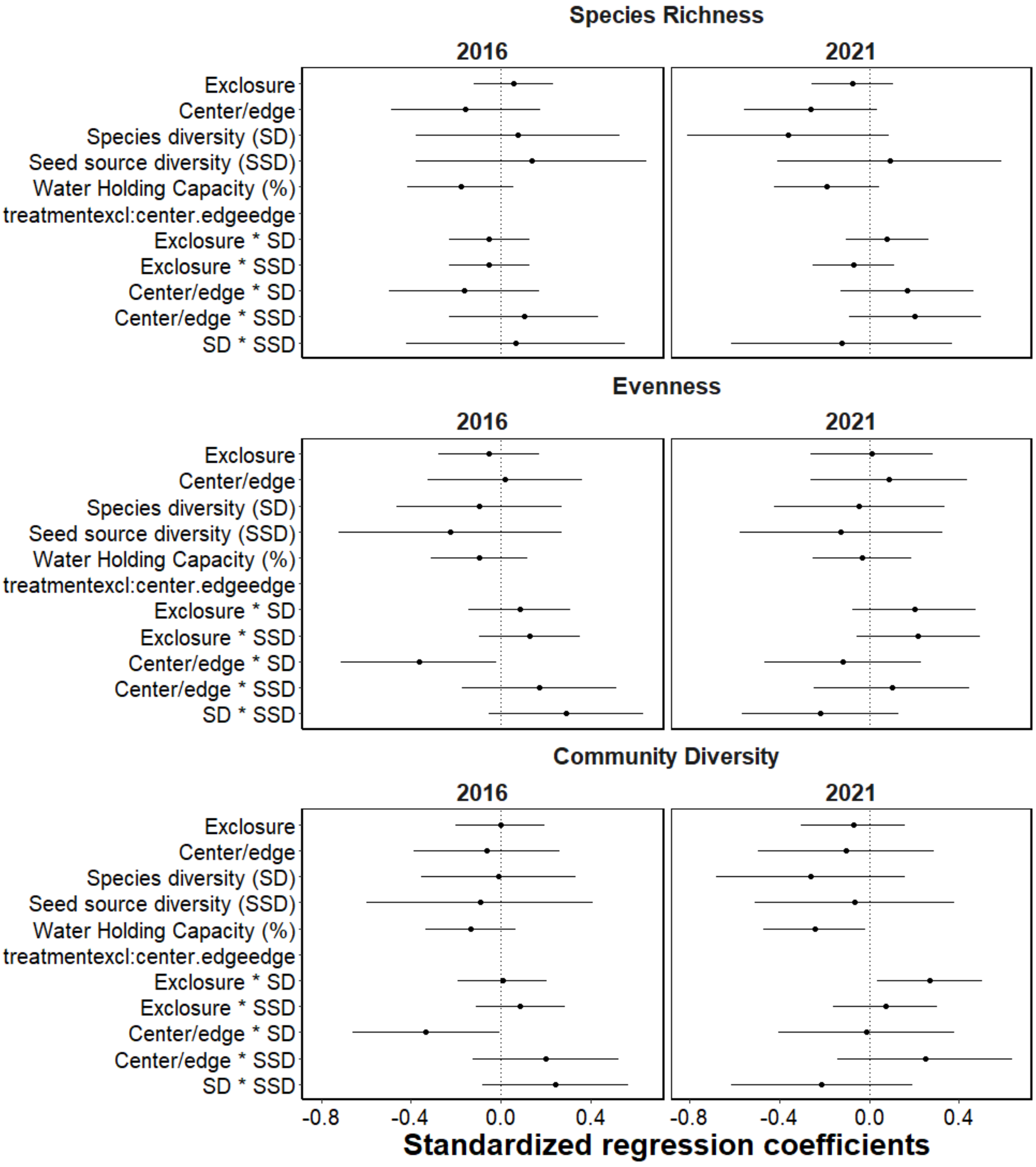

Figure A3.1: Standardized regression coefficients and 95% confidence intervals for three response variables measured in 2016 and 2021 to quantify the effects of seed mix design, edge effects, consumer pressure, and their interactions, on community composition of tallgrass prairie sites undergoing restoration.

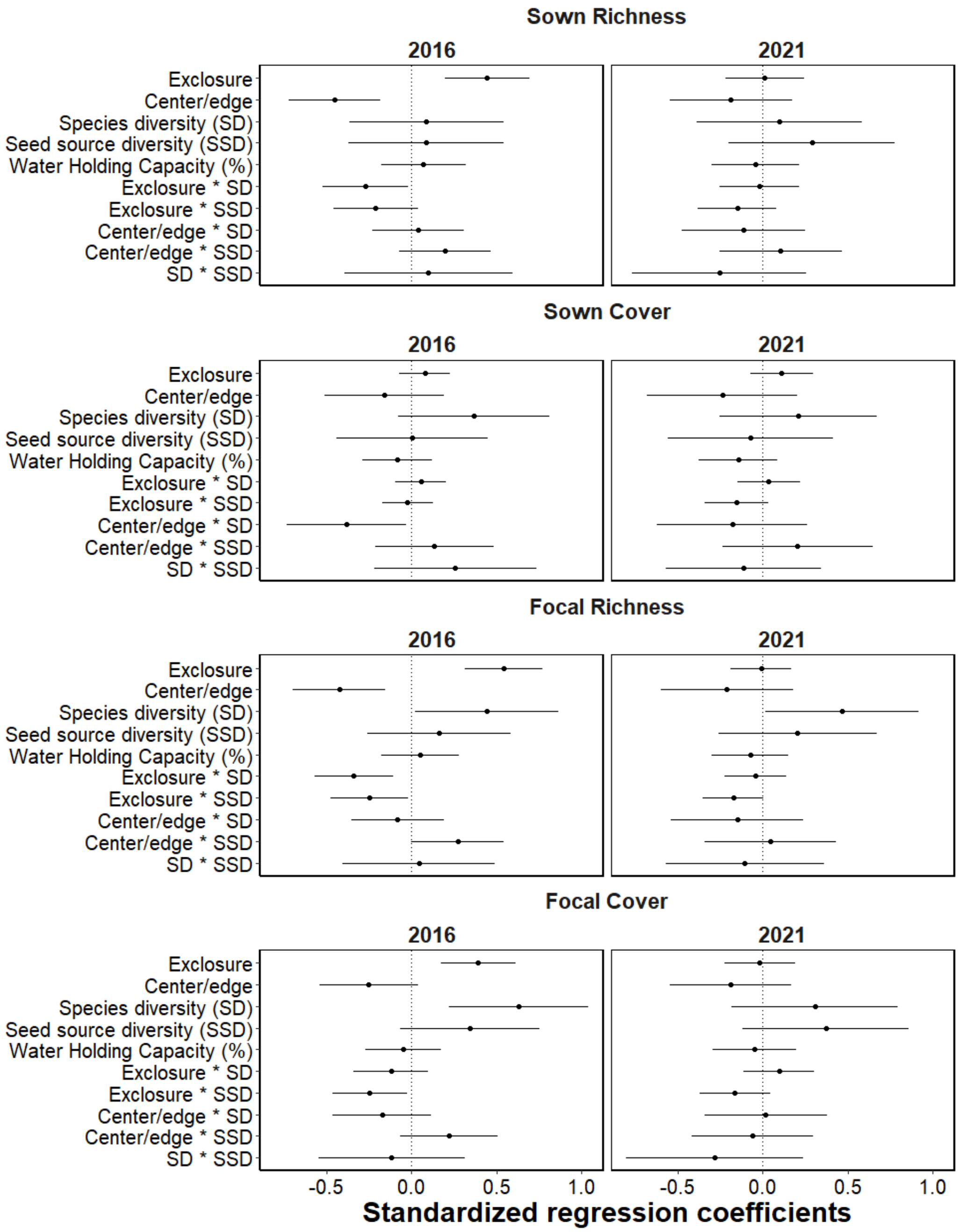

Figure A3.2: Standardized regression coefficients and 95% confidence intervals for four sown species response variables measured in 2016 and 2021 to quantify the effects of seed mix design, edge effects, consumer pressure, and their interactions on the presence and abundance of species sown into tallgrass prairie sites undergoing restoration “Sown” refers to any species that were included in either the low or high species diversity seed mix, and “Focal” refers to the 12 species included in both seed mixes where seed source diversity was manipulated.

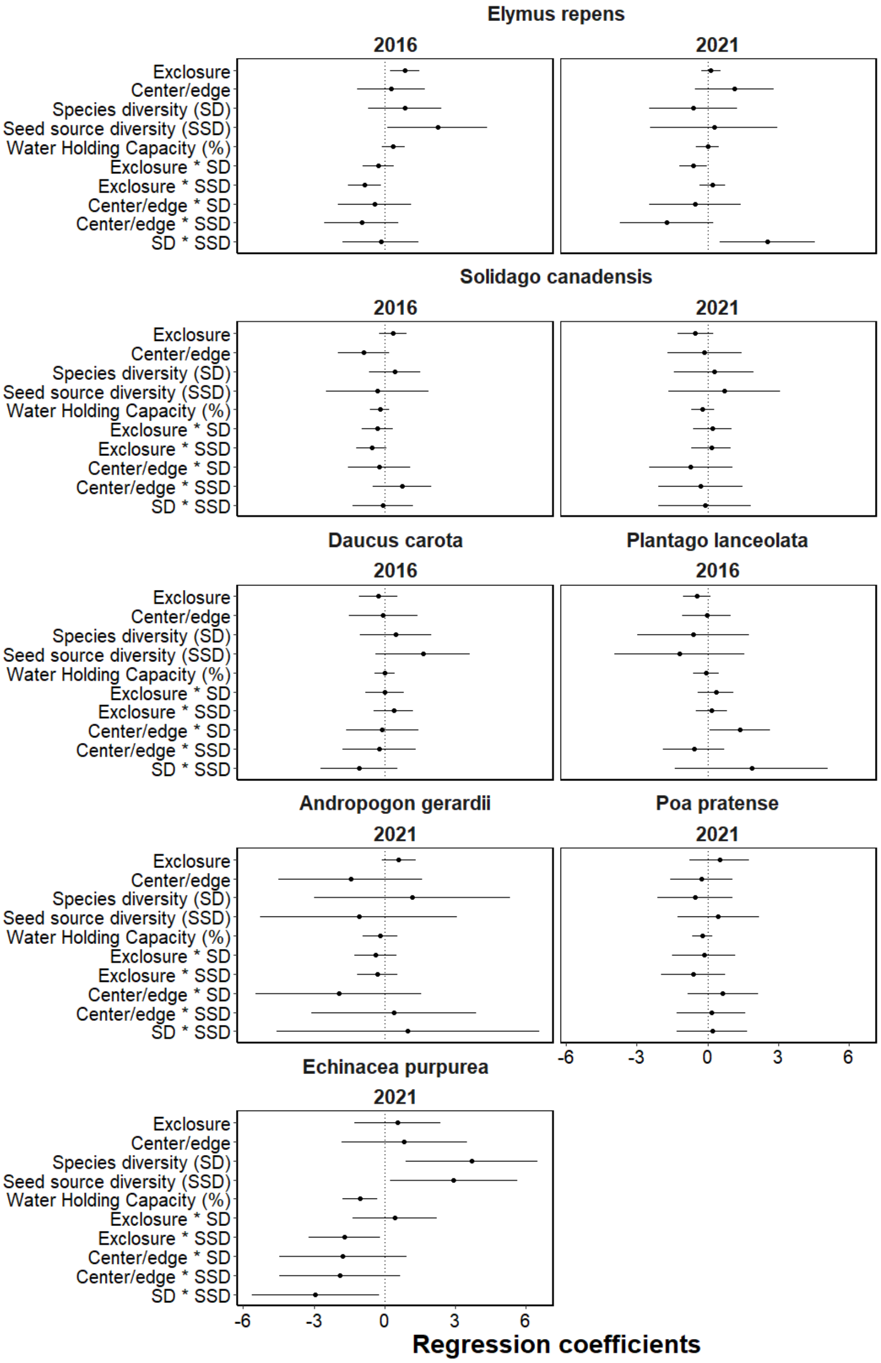

Figure A3.3: Regression coefficients and 95% confidence intervals for the % cover of 6 common species (found in at least 45 of our plots) measured in 2016 and 2021 to quantify the effects of seed mix design, edge effects, consumer pressure, and their interactions on the presence and abundance of common species into tallgrass prairie sites undergoing restoration. Some species that were common in 2016 were not common in 2021, and visa-versa. Due to limitations of this modeling package, coefficients are not standardized.

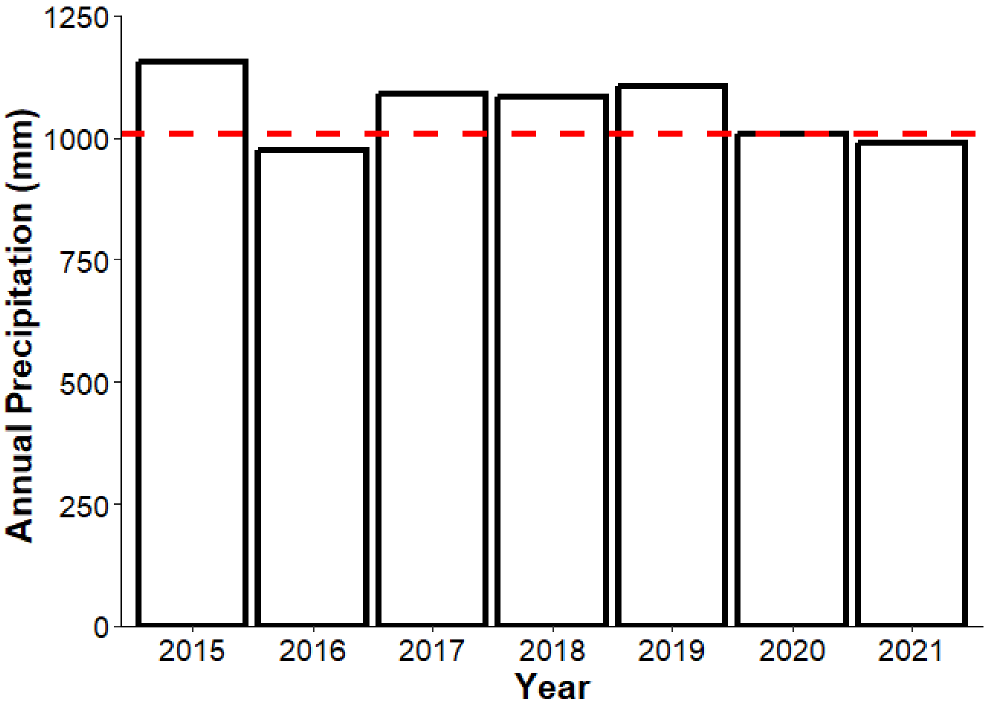

Figure A3.4: Annual precipitation values for the years our experiment ran: 2015 was the year of the first survey and 2021 was the year of the second. The red dashed line represents the 30-year average precipitation values from the PRISM climate database. Climate data were obtained from a weather station at the NSF Long-term Ecological Research Program at the Kellogg Biological Station and by Michigan State University AgBioResearch.

Table A3.1: Species lists and seeding rates (seeds/m^2) for both the low and high species diversity seed mixes. Source information was only available for the focal species where source was manipulated in the experiment. For sites where source diversity was high, each source represents ~1/3 of the total number of seeds sown.

Species	**Low species diversity mix seeds/m^2**	**High species diversity mix seeds/m^2**	**Local source**	**Midwest Source**	**Southern Source**
Andropogon gerardii	48.87	22.28	Wexford Co., MI	Kenosha Co., WI	Macon Co. MO
Bouteloua curtipendua	44.45	22.28	IL	Green Co., WI	Livingston Co., MO
Elymus canadensis	44.45	24.43	Newton Co., IN	Waushara Co., WI	IA
Koeleria macrantha	44.45	24.76	Wexford Co., MI	Columbia Co., WI	IA
Schizachyrium scoparium	49.41	22.28	St. Joseph Co., MI	Columbia Co., WI	MO zone 1 ecotype
Chamecrista fasciculata	16.36	2.15	Lucas Co., OH	Grant Co., WI	Cole Co., MO
Coreopsis lanceloata	13.89	3.23	Newton Co.,IN	Wi	Joplin Co., MO
Dalea purpurea	11.41	2.69	Ontario, Canada	Dakota Co., MN	MO
Echinacea purpurea	13.35	3.23	Lucas Co., OH	Madison, IA	Putnam Co., MO
Lespedeza capitata	16.36	2.80	OH	Whiteside Co., IL	Miller Co., MO
Ratibida pinnata	16.36	3.23	OH	Madison, IA	Greene & Hickory Co., MO
Rudbeckia hirta	10.66	2.26	OH	Kenosha Co., WI	Barton Co., MO
Bromus kalmii		15.82			
Carex bicknellii		4.20			
Carex brevior		7.21			
Carex granularis		1.94			
Carex molesta		6.14			
Carex muhlenbergii		1.51			
Carex normalis		3.12			
Carex vulpinoidea		24.76			

Table A3.1 (cont'd)

Species	**Low species diversity mix seeds/m^2**	**High species diversity mix seeds/m^2**
Panicum virgatum		13.89
Sorghastrum nutans		41.55
Spartina pectinata		4.84
Sporobolus cryptandrus		49.41
Sprobolus heterolepis		7.86
Agastache nepetoides		5.60
Allium cernuum		1.83
Aquilegia canadensis		2.37
Arnoglossum atriplicifolium		1.51
Asclepias tuberosa		1.08
Asclepias verticillata		1.40
Astragalus canadensis		4.20
Baptisia lactea		0.86
Coreopsis tripteris		1.72
Desmanthus illinoensis		0.54
Desmodium canadense		0.86
Desmodium illinoense		0.54
Potentila arguta		14.21
Eryngium yuccifolium		3.66
Gaura biennis		1.29
Helianthus occidentalis		1.72
Heliopsis helianthoides		1.51
Liatris aspera		1.94
Liatris cylindracea		1.72
Lupinus perennis		0.54
Monarda punctata		5.60
Parthenium integrifolium		0.86

Table A3.1 (cont'd)

Species	Low species diversity mix seeds/m^2	High species diversity mix seeds/m^2
Pycnanthemum tenuifolium		23.36
Rudbeckia triloba		4.20
Senna hebecarpa		0.65
Silphium integrifolium		0.65
Silphium laciniatum		0.32
Silphium terebinthinaceum		0.22
Solidago rigida		2.58
Solidago speciosa		5.92
Symphyotrichum ericoides		12.38
Symphyotrichum laeve		13.56
Symphyotrichum novae-angliae		4.09
Symphyotrichum oolentangiense		9.90
Tephrosia virginiana		0.65
Thaspium trifoliatum		2.26
Tradescantia ohiensis		0.97
Verbena stricta		3.44
Vernonia fasciculata		3.01
Veronicastrum virginicum		49.41
Zizia aurea		1.40

Table A3.2: Beta values and standard errors for the response variables used to quantify the effects of seed mix diversity, edge effects, and consumer pressure on restored prairie plant communities. Experimental plots were measured during early establishment (2016) and five years later (2021) to identify persistence. †p < 0.10. *p < 0.05.; **p < 0.01.; ***p < 0.001.

	Species richness				**Evenness**				**Community diversity**				**Sown Richness**			
	2016		*2021*		*2016*		*2021*		*2016*		*2021*		*2016*		*2021*	
	β	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**
Exclosure	0.05	0.09	-0.08	0.09	-0.05	0.12	0.01	0.14	0.01	0.10	-0.07	0.11	**0.45 *****	0.13	0.01	0.12
Center/edge	-0.15	0.17	**-0.26†**	0.15	0.02	0.18	0.09	0.18	-0.06	0.17	-0.10	0.20	**-0.45 ****	0.14	-0.18	0.18
Species diversity (SD)	0.07	0.23	-0.36	0.23	-0.10	0.19	-0.04	0.19	-0.01	0.18	-0.26	0.21	0.09	0.23	0.10	0.25
Seed source Diversity (SSD)	0.14	0.26	0.09	0.25	-0.22	0.25	-0.13	0.23	-0.09	0.26	-0.06	0.22	0.09	0.23	0.29	0.25
Water holding capacity	-0.18	0.12	-0.19	0.12	-0.09	0.11	-0.03	0.11	-0.13	0.10	**-0.24***	0.12	0.07	0.13	-0.04	0.13
Exclosure*SD	-0.05	0.09	0.08	0.09	0.08	0.12	0.20	0.14	0.01	0.10	**0.27***	0.12	**-0.27***	0.13	-0.02	0.12
Exclosure*SSD	-0.05	0.09	-0.07	0.09	0.13	0.12	0.22	0.14	0.08	0.10	0.07	0.12	**-0.21†**	0.13	-0.15	0.12
Center/edge* SD	-0.16	0.18	0.17	0.15	**-0.37***	0.18	-0.11	0.18	**-0.33***	0.17	-0.01	0.20	0.04	0.14	-0.11	0.19
Center/edge *SSD	0.10	0.17	0.20	0.15	0.17	0.18	0.10	0.18	0.20	0.16	0.25	0.20	0.19	0.14	0.11	0.18
SD* SSD	0.07	0.25	-0.12	0.25	**0.29†**	0.18	-0.22	0.18	0.24	0.17	-0.21	0.21	0.10	0.25	-0.25	0.26

Table A3.2 (cont’d)

	Sown Cover				**Focal Richness**				**Focal cover**			
	2016		*2021*		*2016*		*2016*		*2021*		*2016*	
	β	**SE**	**β**	**β**	**SE**	**β**	**β**	**SE**	**β**	**β**	**SE**	**β**
Exclosure	0.08	0.08	0.11	0.08	0.08	0.11	0.08	0.08	0.11	0.08	0.08	0.11
Center/edge	-0.16	0.18	-0.24	-0.16	0.18	-0.24	-0.16	0.18	-0.24	-0.16	0.18	-0.24
Species diversity (SD)	0.37	0.22	0.21	0.37	0.22	0.21	0.37	0.22	0.21	0.37	0.22	0.21
Seed source Diversity (SSD)	0.01	0.23	-0.07	0.01	0.23	-0.07	0.01	0.23	-0.07	0.01	0.23	-0.07
Water holding capacity	-0.08	0.11	-0.14	-0.08	0.11	-0.14	-0.08	0.11	-0.14	-0.08	0.11	-0.14
Exclosure*SD	0.05	0.08	0.03	0.05	0.08	0.03	0.05	0.08	0.03	0.05	0.08	0.03
Exclosure*SSD	-0.02	0.08	-0.15	-0.02	0.08	-0.15	-0.02	0.08	-0.15	-0.02	0.08	-0.15
Center/edge* SD	**-0.38***	0.18	-0.18	**-0.38***	0.18	-0.18	**-0.38***	0.18	-0.18	**-0.38***	0.18	-0.18
Center/edge *SSD	0.13	0.18	0.21	0.13	0.18	0.21	0.13	0.18	0.21	0.13	0.18	0.21
SD* SSD	0.26	0.24	-0.11	0.26	0.24	-0.11	0.26	0.24	-0.11	0.26	0.24	-0.11

Table A3.3: Beta values and standard errors for the cover of 6 common species (found in at least half of our plots) used to quantify the effects of seed mix diversity, edge effects, and consumer pressure on restored prairie plant communities. Experimental plots were measured during early establishment (2016) and five years later (2021) to identify persistence; some species that were common in 2016 were not common in 2021, and visa-versa. †p < 0.10. *p < 0.05.; **p < 0.01.; ***p < 0.001.

	Elymus Repens				***Solidago Canadensis***				***Daucus Carota***		***Plantago Lanceolata***	
	2016		*2021*		*2016*		*2021*		*2016*		*2016*	
	β	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**
Exclosure	**0.87***	0.32	0.15	0.21	0.34	0.30	-0.52	0.38	-0.27	0.41	-0.45	0.30
Center/edge	0.28	0.73	1.13	0.85	-0.89	0.55	-0.13	0.80	0.05	0.74	-0.04	0.53
Species diversity (SD)	0.86	0.78	-0.61	0.95	0.45	0.55	0.28	0.86	0.47	0.77	-0.62	1.21
Seed source Diversity (SSD)	**2.25***	1.08	0.28	1.37	-0.32	1.11	0.71	1.20	1.63	1.02	-1.19	1.41
Water holding capacity	0.37	0.25	0.01	0.25	-0.21	0.21	-0.21	0.24	0.02	0.22	-0.06	0.28
Exclosure*SD	-0.25	0.34	**-0.61***	0.29	-0.30	0.33	0.22	0.42	0.01	0.42	0.35	0.38
Exclosure*SSD	**-0.84***	0.35	0.21	0.28	**-0.55†**	0.32	0.15	0.42	0.38	0.42	0.19	0.34
Center/edge* SD	-0.41	0.79	-0.51	0.98	-0.22	0.66	-0.71	0.90	-0.09	0.79	**1.38***	0.65
Center/edge *SSD	-0.98	0.81	**-1.73†**	1.01	0.74	0.64	-0.29	0.91	-0.23	0.79	-0.58	0.66
SD* SSD	-0.16	0.82	**2.53***	1.03	-0.07	0.65	-0.11	1.01	-1.07	0.84	1.87	1.65

Table A3.3 (cont’d)

	Andropogon gerardii *2021*		***Poa pratense*** *2021*		***Echinacea purpurea*** *2021*	
	β	**SE**	**β**	**β**	**SE**	**β**
Exclosure	0.60	0.37	0.52	0.60	0.37	0.52
Center/edge	-1.44	1.55	-0.27	-1.44	1.55	-0.27
Species diversity (SD)	1.16	2.12	-0.52	1.16	2.12	-0.52
Seed source Diversity (SSD)	-1.10	2.13	0.46	-1.10	2.13	0.46
Water holding capacity	-0.18	0.37	-0.22	-0.18	0.37	-0.22
Exclosure*SD	-0.39	0.46	-0.15	-0.39	0.46	-0.15
Exclosure*SSD	-0.30	0.44	-0.60	-0.30	0.44	-0.60
Center/edge* SD	-1.94	1.79	0.66	-1.94	1.79	0.66
Center/edge *SSD	0.39	1.78	0.15	0.39	1.78	0.15
SD* SSD	1.00	2.84	0.21	1.00	2.84	0.21

Table A3.4: Partial regression coefficients and F statistics for two PERMANOVA analyses used to quantify the effects of seed mix diversity, edge effects, and consumer pressure on the community composition of restored prairie plant communities. Model 1 was used to understand the effects of seed mix diversity on community composition, and model 2 was used to understand the impact of edge effects and consumer pressure, as well as their interaction with species diversity of a seed mix. Plots were measured during early establishment (2016) and five years later (2021) to identify persistence; *p < 0.05.; **p < 0.01.; ***p < 0.001.

	Model 1				**Model 2**			
	2016		***2021***		***2016***		***2021***	
	R^2	***F***	R^2	***F***	R^2	***F***	R^2	***F***
Seed source diversity (SSD)	0.04	**8.49*****	0.02	**4.01****	---	---	---	---
Species diversity (SD	0.02	**4.42*****	0.02	**3.00*****	0.02	**4.54*****	0.02	**3.06*****
SSD*SD	0.02	**3.65*****	0.02	**3.53*****	---	---	---	---
Water holding capacity (%)	0.02	**5.12*****	0.04	**7.18*****	0.02	**5.13****	0.04	**7.66*****
Site/half	0.19	**3.98*****	0.17	**3.21*****	0.21	**4.01*****	0.19	**3.27*****
Exclosure	---	---	---	---	0.01	0.19	0.01	1.27
Center/edge	---	---	---	---	0.02	**3.74*****	0.01	**2.02***
Exclosure*SD	---	---	---	---	0.01	0.47	0.01	0.64
Center/edge* SD	---	---	---	---	0.01	**2.11***	0.01	**1.76***

CHAPTER 4: LOCAL SEED SOURCING ENHANCES PLANT ESTABLISHMENT DURING EXPERIMENTAL RESTORATION, BUT THE ADDITION OF NON-LOCAL SOURCES MAY PROVIDE FUTURE ADVANTAGES

Abstract

A key decision in restoration planning is where to obtain seeds from to establish persistent native plant populations. The most widespread practice, local seed sourcing, assumes seeds from nearby locations will result in higher rates of plant establishment and persistence than seeds sourced from further away, although the limited research testing this assumption shows mixed results. Two alternative seed sourcing strategies have been proposed to boost plant establishment by adding non-local sources to the local one (to increase genetic diversity and overall adaptation to varied environmental factors). Admixture seed sourcing combines seeds from multiple nearby sources, which is presumed to increase genetic diversity while reducing the risk of introducing maladapted genotypes. Climate-adjusted seed sourcing selects seeds from regions matching future climate projections, increasing genetic diversity through intentional inclusion of non-local genotypes, which may increase the probability of future population persistence, albeit with a higher potential for introducing maladapted genotypes. Since admixture and climate-adjusted sourcing techniques have rarely been tested in the field, and have never been compared to one another, it is unclear how they compare in terms of sown species establishment or climate resilience. To address this gap, we designed a prairie restoration field experiment testing how sown species richness, abundance, and flowering phenology are influenced by (1) locality of each seed source (2) the number of seed sources used, (3) the multi-source sourcing strategy (local vs. admixture vs. climate-adjusted) used. We also investigated how these influences differ between experimentally warmed and ambient conditions. We found that, in single-source plots, local sources produced the highest establishment and abundance of 62% of our sown species. However, once local and non-local sources were combined, high diversity mixes had more flowering individuals than the local source alone. Finally, there was some evidence that climate-adjusted mixes maintained high sown richness despite experimental warming. These results provide some support for the use of local seed sourcing, although adding non-local sources can extend flowering durations and potentially increase resilience to higher temperatures. While maintaining local seed sources should remain a priority, increasing source diversity could also improve restoration outcomes in an uncertain future.

Introduction

Habitat destruction, climate change, and other anthropogenic activity have resulted in widespread habitat degradation and global declines of biodiversity, threatening ecosystem functionality (Ellis et al., 2020; Kardol et al., 2018; Mantyka-pringle et al., 2012; Vitousek et al., 1997). In an attempt to prevent further losses in biodiversity, ecosystem restoration has become an international priority (UNEP, 2019). A common first step in restoration is the re-establishment of native plant populations, often by the addition of seeds to overcome dispersal limitations (Holl & Aide, 2011). A key decision in this process is determining where to obtain seeds from to maximize restoration success: choosing sources that establish large, persistent plant populations can have cascading consequences including the development of high diversity plant communities (Maynard et al., 2017), resulting in more biodiversity at higher trophic levels (Nicholls & Altieri, 2013) and improvements to ecosystem functionality (Tilman et al., 2014). As we approach the halfway point of the decade on restoration (UNEP, 2019), research is necessary to identify which seed sourcing methods produce these desirable restoration outcomes.

The most common restoration practice is local seed sourcing (*reviewed in* McKay et al., 2005), but there is conflicting evidence that this seed sourcing strategy is reliably better than non-local sourcing. Practitioners prioritize sourcing seeds from a geographically nearby source to a restoration site based on the theory of local adaptation (Hereford, 2009; Turesson, 1922), assuming that nearby sources are the most likely to be adapted to environmental conditions similar to those at a restoration site (Lesica & Allendorf, 1999; Mortlock, 2000). While some studies have confirmed this assumption through high performance of local seed sources (Baughman et al., 2019; Gustafson et al., 2005; Montalvo & Ellstrand, 2000), other studies showed no effect of source location (Carter & Blair, 2012; Gallagher & Wagenius, 2016; Pizza et al., 2023), and one study found that the local source performed worse than sources from further away (Nolan et al., 2023). Among the explanations for these mixed results is that past land use may significantly alter the biotic and abiotic environment at the restoration site (*reviewed in* Lau et al., 2019), so even highly local populations may not be exposed to the same conditions as the restoration site. Additionally, since the strength of local adaptation varies between species due to differences in gene flow, dispersal, or life-history strategy (*reviewed in* Kawecki & Ebert, 2018; Macel et al., 2007) and can even be variable within populations (Raabová et al., 2007), the benefits of local seed sourcing are unlikely to be uniform across species. Finally, there are concerns that prioritizing a single seed source may result in restoration plantings that are missing crucial genetic diversity to adapt when conditions at the restoration site change (i.e., adaptive potential; Breed et al., 2013; Davis et al., 2005).

Given these concerns, alternative seed sourcing strategies have been proposed to mix additional sources with the local source, prioritizing genetic diversity rather than trying to match a single seed source to the conditions of a restoration site (Hughes et al., 2008; St. Clair et al., 2020). Combining sources should increase intraspecific phenotypic variation, which may in turn increase establishment (Crawford & Whitney, 2010) through reduced intraspecific competition due to niche complementarity (Fridley et al., 2007; *reviewed in* Vellend & Geber, 2005), increased floral resources for pollinators due to a wider variation in flowering time (Bucharova et al., 2022), and increase adaptive potential. Each of these mechanisms could promote population persistence (*reviewed in* Kettenring et al., 2014). Seeding multiple sources can also be a bet-hedging strategy, decreasing the likelihood of a poorly-establishing, maladapted plant population, especially if local sources are not adapted to restoration site conditions (Broadhurst et al., 2008; Jordan et al., 2024). Alternatively, adding additional seed sources, especially those from disparate environments, could introduce maladapted genotypes, ultimately reducing establishment compared to using a local source alone (Gallagher & Wagenius, 2016; Gustafson et al., 2005; *reviewed in* Vander Mijnsbrugge et al., 2010). Thus, practitioners must weigh the relative rewards and risks of increasing genetic diversity in a seed mix.

Two multi-source seed mix strategies are often discussed in the literature (Breed et al., 2013; Broadhurst et al., 2008). Regional admixture seed sourcing (hereafter referred to as "admixture") involves collecting seeds from multiple populations in a region around the restoration site, including populations that would be considered local (Bucharova et al., 2019). In theory, the addition of these regional seed sources should result in greater phenotypic variation compared to the local source alone due to increases in genetic diversity, and should have a relatively low risk of introducing maladapted genotypes since sources come from climates similar to the local source (Bucharova et al., 2019). However, the consequences of this approach remain unclear, especially within countries lacking empirically-derived seed transfer zones (e.g. Germany, Höfner et al., 2021; vs. the United States, Lindstrom et al., 2021; Pizza et al., 2025).

The second seed sourcing strategy, climate-adjusted seed sourcing, was developed to enhance the ability for populations to persist when the climate changes in the future (Prober et al., 2015). This approach involves mixing the local source with additional sources collected along a gradient toward the climates the restoration site is expected to experience in the future (McKone & Hernández, 2021; Prober et al., 2015). The resulting seed mix balances high establishment under current conditions with increases in long-term resilience under a predicted future climate (Butterfield et al., 2017; Harrison, 2021). Climate-adjusted seed mixes are intended to have more phenotypic variation than local or admixture seed mixes, since plants are presumably adapted to a wider variety of environmental conditions (Prober et al., 2015). However, if seed sources adapted to future climates cannot survive in present conditions, this could undermine the adaptive potential of the resulting population, potentially leading to population extirpation (*reviewed in* Forester et al., 2022). While translocating individuals from further south into northern climates has been tested in many important forestry species with relative success (*reviewed in* Pedlar et al., 2012; Sáenz-Romero et al., 2021), the efficacy of this seed sourcing approach has rarely been considered in herbaceous species especially within restoration contexts (*but see* Nolan et al., 2023).

Despite the potential for these different provenancing strategies to influence restoration outcomes, especially under anticipated future conditions, they have rarely been evaluated together. Thus, we designed a field experiment focused on the initial establishment phase of restoration, since population establishment is a main bottleneck during population recovery (Zeiter et al., 2006) and limitations in this demographic stage can hinder persistence. We also evaluated how seed sourcing strategies influence flowering phenology, a measure of phenotypic variation, since phenology is a highly variable trait often under selection caused by environmental differences (Yan et al., 2021). Additionally, differences in flowering time or duration can influence long-term demographic processes (Iler et al., 2021).

In this experiment, we asked how sown species richness, abundance, and flowering phenology are influenced by (1) the locality of the seed source (2) the number of seed sources used, (3) the sourcing strategy (local vs. admixture vs. climate-adjusted) used. We also investigated whether these influences differ under experimentally warmed and un-warmed conditions. For question 1, if local seed sourcing is best, we expect sown richness and abundance to be highest in plots sown with the local sources, followed by regional and then climate-adjusted sources (the furthest away sources). We also expect that flowering phenology of the climate-adjusted sources to differ from the local and regional sources. For question 2, if mixing sources increases establishment due to enhanced genetic diversity, we expect both admixture and climate-adjusted seed mixes to have higher sown richness and abundance, compared to local source alone. Alternatively, if maladaptation decreases establishment, the climate-adjusted plots should have the lowest sown richness and abundance, and admixture should have intermediate levels. In either case, we would expect the flowering phenology to be extended in the admixture and climate-adjusted mixes due to increased phenotypic variation. For question 3, we expect to see an increase in establishment and abundance, as increased genetic diversity can "bet hedge" against establishment failure, and more variation in flowering phenology. Finally, if the climate-adjusted sources are better adapted to higher temperatures, we expect the highest number of sown species under experimental warming and for these sources to exhibit adaptive responses such as an earlier flowering time or longer flowering duration (Anderson et al., 2012). For all questions, we expect species to vary in their responses to seed sourcing.

Methods

Seed Mixes

For this experiment, we sourced each species from five locations: one local source, two regional sources, and two climate-adjusted sources. The local source was the geographically nearest source to the restoration site (Southwest Michigan, USA; Table 4.1). Regional sources were chosen from locations with similar climates to the restoration site (other midwestern states at similar latitudes), and climate-adjusted sources came from locations with climates similar to those we expect the restoration to experience in the future (more southwestern sources; Bradley et al., 2012; Matthews et al., 2018). Although we selected sources primarily based on geography, quantitative comparisons of the climates of each source location using 19 bioclimatic variables confirmed that the climates of the source locations largely followed expectations: local seed sources were from climates most similar to the restoration site, and regional sources experienced similar climates to local sources (Fig. A4.1). Climate-adjusted sources came from warmer and wetter climates than the local sources and were the most climatically dissimilar to the restoration site. Regional sources came from environments with more variable precipitation than other sources (Fig. A4.1).

We then combined individual species to create 11 multi-species seed mixes, each with the same number of species, but varying in the number and identity of sources used. The first mix contained only the most local source of each species. Then, we created four single-source mixes of regional or climate-adjusted sources. To test the effects of increasing the genetic diversity, we designed six seed mixes consisting of the local source mixed with seeds from one or two additional sources. These sources were either regional or climate-adjusted, allowing us to test for effects of sourcing strategy (admixture vs. climate-adjusted). We maintained the same overall seeding density in all of our mixes, adjusting for differences in the amount of germinable seed, with higher-source diversity mixes containing fewer seeds from each individual source (Table A4.1).

We acquired seeds of 13 prairie species from 10 native seed producers (Table A4.1). To identify species available for our study, we surveyed native seed producers on which species they produced. Since native seed producers obtain seed from wild populations to start their cultivated population, we only selected species that were originally sourced from wild populations in the same or adjacent counties to the production site in an attempt to preserve local adaptation. This resulted in three grasses and ten forbs that could be sourced from five locations (Fig. 4.1, Table A4.1). To maximize the number of species we could include in this study, we did not require all species to be sourced from the same five producers. Thus, each seed mix contained seeds from three to five producers.

Field Experiment

We used a 0.20ha field at Lux Arbor reserve in Southwest MI (USA; 42.4870, -85.4527) for this experiment. The field is largely level (a former airstrip) and supported typical old-field vegetation from our region before planting including *Poa pratensis*, *Bromus inermis*, *Centaurea stoebe*, and other weedy and primarily non-native species. The field was prepared in the Fall of 2022 by tilling the soil and spraying herbicide twice to reduce the soil seed bank, then lightly discing just before planting to expose mineral soil. We established 110 2x2m plots (10 replicates per seed mix) separated by 2m alleyways in December 2022, and hand-sowed seeds into each plot based on a completely randomized design. The site was maintained by mowing to reduce non-sown species abundance once in the first year after data were collected (August 2023).

To answer our fourth question, we included nine plots (randomly dispersed in our experimental site), on which we erected open-top Hexagon ITEX temperature chambers (Molau & Mølgaard, 1996). Each plot was sown with either the local-source, three-source admixture, or the three-source climate-adjusted seed mix (n = 3 each; Fig. A4.2A). Chambers consisted of

1mm thick Sun-Lite® HP unglazed fiberglass and were held upright by rebar and zip ties. We followed the standard design from Molau & Mølgaard (1996) but built the chambers to be wider (~1.5m across) and taller (~0.8m tall) to accommodate the growth of taller prairie species. Shielded, surface-level temperatures in chambers were ~1.2˚C warmer on average during daytime hours during the first growing season (April – October) than under ambient conditions (Fig. A4.2B). We erected chambers at the start of each growing season (April) and removed them at the end (October).

Field Surveys

We monitored community composition, plant population establishment, and flowering phenology between June-August of 2023 and 2024. We placed a 1x1m quadrat in the center of each plot and recorded the identity and percent cover of each species to identify differences in community structure. We completed all community surveys in a five-day period in mid-July. For each sown species, we recorded the total number of individuals inside the entire 2x2m plot. We categorized an individual as one or more stems less than 15.24cm apart from one another for forbs, and graminoid individuals were distinct bunches (Pizza et al., 2023). Finally, we monitored plant flowering phenology during 2024. We visited each plot every 5-7 days (between May 30- Oct 4, 2024) and noted the total number of flowering individuals for each species, in each 2x2m plot. Two sown species, *Asclepias syriaca* and *Silphium perfoliatum* were rare across our plots, so these species were not included in abundance or phenology measurements. We found that results were qualitatively similar between the first and second growing seasons and decided to focus our results on the second year of data collection since many species were still establishing by the end of the first growing season.

Data analysis

All statistical tests, data transformations, and calculations were performed in R Studio using R version 4.4.1 (R Core Team, 2024).

Datasets

We conducted data analysis on four subset datasets aligned with our research questions. The first included only the single source mixes, excluding those with warming chambers, to test the effects of source locality. The second dataset included all the plots except those with warming chambers to test the effects of increasing the number of seed sources. The third included all plots except for the single source regional and climate-adjusted mixes and plots with warming chambers to test the effects of these different seed sourcing strategies. Due to constraints of the experimental design (the local source only exists as a single-source mix) we were unable to test the interactive effect of source number and seed sourcing strategy. The fourth dataset included only plots sown with the local seed source, and the three-source admixture and climate-adjusted mixes, in addition to the warmed plots, to test how seed sourcing strategies influence the effects of experimental warming. Depending on the question we were interested in answering with each dataset, we used different predictor variables in each model: we used seed source strategy when wanting to test differences in the specific seed sourcing strategy, the number of sources when testing the impacts of changing the amount of diversity, or the interaction between source strategy and warming when testing how these different seed sources performed under experimental warming.

Community analyses

We calculated Shannon's diversity, and sown species richness and cover to characterize the plant communities and establishment, and analyzed these variables using linear models (glmmTMB package, version 1.1-35.5; Brooks et al., 2017). To test for differences in plant community composition, we used a PERMANOVA analysis using the adonis2 function (Vegan package version 2.6-4; Okansen, 2010) and visualized results using an NMDS ordinations of Bray-Curtis dissimilarity matrices using the function "metaMDS" (Vegan package version 2.6-4; Okansen, 2010).

Sown species abundances

To understand the consequences of seed sourcing decisions on individual species, we analyzed the abundance (count) of eleven of the sown species (two did not establish reliably enough to analyze). We modeled two species abundances (*S. scoparium, S. nutans*) with a Poisson distribution, but the others were over-dispersed and they were best fit with a negative binomial model (glmmTMB package, version 1.1-35.5; Brooks et al., 2017). We used the same predictor variables to test each hypothesis as described above.

Sown species phenology

To understand how differences in seed sourcing could influence the timing and duration of flowering, we analyzed the number of flowering individuals of each species in a given plot. These data were also over-dispersed and fit with a negative binomial model. We included the Julian date of sampling in an interaction term with either source strategy, source number, or the interaction between source strategy and warming depending on the specific hypothesis we were testing. We also included the total number of individuals of a given species in the plot as a covariate, species identity as a random effect, and a temporal autocorrelation variable that accounts for re-visiting the same plots every week using AR-1 (glmmTMB package, version 1.1-35.5; Brooks et al., 2017). We calculated the area under the curve (AUC) for each of the lines using "trapz" function (pracma package version 2.4.4; Borchers, 2023) and statistically compared whether the AUC's were different between plots sown with different sources using a Kruskal Wallis t-test and a post-hoc dunn test (dunn.test packageversion 1.3.6; Dinno, 2024). To investigate species-level patterns for the six most abundant species (for which there was sufficient data across our plots), we visualized the number of flowering individuals across our plots with 95% confidence intervals.

Results

Seed source locality

Sown species establishment in single source plots differed across seed sources (local, regional, or climate-adjusted): plots sown with a local seed source had ~1.8 more sown species (Fig 4.2A) and 37% greater sown cover (Fig 4.2B) compared to plots sown with a regional source (table A4.2). Plots sown with a climate-adjusted seed source had an intermediate number of sown species and cover that did not differ from either group (Fig 4.2B; table A4.2).

Plant community composition also differed based on seed source (Fig 4.3A; table A4.3). Communities in plots sown with a local source were associated with a higher abundance of five sown species: *A. gerardi*, *R. pinnata*, *S. nutans*, *V. hastata*, and *R. triloba* (Fig 4.3a; *see Fig A4.3 for plot including non-sown species*). Plots sown with a regional or climate-adjusted source were associated with more individuals of *S. nemoralis*. Two other species, *R. hirta* and *P. digitalis,* also drove community dissimilarity, but these did not appear to be linked to which seed source was sown. The abundances of some sown species mirrored these patterns: plots sown with local sources had high abundances of *A. gerardi* and *S. nutans* (Figs 4.3A-C; table 4.2A). Plots with local seed sources also had higher abundances of *S. speciosa* and *C. lanceolata* than plots sown with other sources, although these species did not contribute to community-level differences (Fig 4.3A, D & E; table A4.2). Establishment was not uniformly higher in plots sources with a local source, though: these plots had significantly lower abundances *S. nemoralis*, *R. hirta,* and *R. pinnata* compared to plots sown with another seed source (Figs. 4.3A, F-H; table A4.2). Finally, the abundance of *S. scoparium* was significantly lower in plots sown with a climate-adjusted seed source compared to the other sources (Fig 4.3I; table A4.2).

Source location also had a significant impact on plant phenology: plots sown with a climate-adjusted seed source had a longer peak flowering compared to those sown with a local or regional seed source (both peaked in early September; Fig 4.4; table A4.4). Additionally, plots sown with climate-adjusted seed sources had more flowering individuals at the end of the growing season compared to those sown with a local or regional source (confidence intervals do not overlap zero; Fig 4.4). This can be further visualized through individual species contributions: *A. gerardi* and *R. triloba* from climate-adjusted sources had greater flowering at the end of the season compared to populations from local or regional sources (Fig. 4.5B). *R. hirta* from climate-adjusted or regional sources had greater flowering overall compared to plots sown with a local source (Fig 4.5B). *S. nutans* and *V. hastata* in plots sown with the local source had more flowering overall than plots sown with other sources (Fig 4.5B). Overall, plots sown with regional seed sources had 17% more flowering individuals per species than those sown with a local source, but comparable numbers to plots sown with a climate-adjusted source (Fig 4.4A; Table A4.5).

Number of sown sources

Adding additional sources (2 vs 3) did not have a strong impact on sown species abundances or phenology. However, there was ~1 additional sown species in plots sown with two or three sources compared to plots sown with only one (Fig 4.5A; table A4.2), which appears to be largely driven by adding the local source to poorer-performing admixture seed sources (Figs. 4.2B-I; Fig 4.5B).

Seed sourcing strategy

When considering plots with multiple seed sources, we observed similar but more subdued influences of seed source strategy, compared to the single-source plots. Specifically, we observed the same pattern in flowering phenology, with significantly greater flowering in plots sown with admixture or climate-adjusted seed mixes (Fig 4.6A; Table A4.3). Species responded similarly to multi-source seed sourcing strategies as they did in single source plots, although the magnitude of difference between the treatments tended to be lower (Fig 4.6B; table A4.4). Plots sown with admixture seed mixes still had the most flowering individuals, but the difference decreased by 7% compared to single source plots (Fig 4.6A; table A4.3-A4.5). Differences in the abundances of *R. hirta* and *S. nemoralis* in multi-source plots mirrored those observed in single source plots (Fig. A4.4).

Warming

Under experimental warming, local sources had one fewer, and regional sources two fewer, sown species than in unwarmed plots, whereas climate-adjusted plots saw no decrease in sown richness (Fig. 4.7A; table A4.2). Warming also accelerated flowering in all sources (Fig. 4.7B; Fig. A4.6), and this effect was strongest in the admixture and climate-adjusted sources (Fig. 4.7B). However, these results could not be resolved statistically, with a large effect size not correlating with statistical significance, presumably due to a low sample size of warmed plots (n = 9).

Discussion

We conducted, to our knowledge, the first study to experimentally compare three proposed seed sourcing strategies to one another in a realistic restoration context. We found that local seed sourcing maximized plant establishment: these plots had the highest sown richness, cover, and abundances for most species compared to non-local sources, although they also had fewer flowering individuals at the beginning and end of the growing season. Importantly, combining local and non-local sources mitigated the lower establishment rates observed when non-local sources were seeded on their own, and these multi-source mixes provided a longer flowering duration than plots sown with only the local source. Finally, we found some evidence that climate-adjusted sources were most resilient to the negative impacts of warming. Together, these results suggest that while local seed sourcing confers an advantage for plant establishment, mixing local and non-local sources, particularly from climates expected in the future, can maintain high establishment rates and promote phenotypic diversity that may enhance population persistence.

Effects of source locality (single source plots)

We found evidence that local seed sources had the highest establishment, supporting the “local is best” paradigm (Mortlock, 2000). Plots sown with a local seed source had the highest sown richness and typically the highest abundance of those species, indicating that local sources were better adapted to our restoration site compared to non-local sources. Moreover, these differences in sown species establishment were important in shaping plant communities: local sources formed communities distinct from local and admixture sources, primarily caused by higher abundances of most of our sown species. Thus, by influencing the establishment of sown species, local seed sourcing also altered the overall plant community composition, potentially conferring long-term benefits through increased biodiversity.

This study provides some of the most robust evidence that local seed sourcing reliably meets establishment goals in restoration by using an experimental design that is likely better suited to address the importance of locality compared to previous approaches. For example, most previous studies used common gardens to test the importance of locality, where seedlings were planted in the field after germinating under greenhouse conditions (Baughman et al., 2019; Gustafson et al., 2005; Montalvo & Ellstrand, 2000; *but see* Nolan et al., 2023; Pizza et al., 2023). These studies may not reflect the important barrier of emergence, which can be highly limiting to seedling survival in this system (Zeiter et al., 2006). Previous studies also studied only one or a few species (*but see* Baughman et al., 2019; Bucharova, 2017). Given the variable responses of individual species to local seed sourcing in our study, previous work may have missed this overarching pattern.

However, establishment did not decline over geographic space as we expected. Plots sown with climate-adjusted sources, which we expect to have the lowest establishment if local adaptation is important, had comparable sown richness to the local source, and regional seed sources produced the fewest sown species. Additionally, while sown species abundances were often higher in the local source, this was not consistently the case: local sources had lower abundances *R. hirta*, *R. pinnata*, and *S. nemoralis*, the latter of which failed to establish in local plots. One possibility for these species level differences is that only some species are locally adapted to their climates: some may be adapted to soil conditions (Macel et al., 2007), grazing regimes (Hufford et al., 2008) or a host of other factors (*reviewed in* McKay et al., 2005). Thus, non-local plants may be adapted to conditions present at the restoration site despite being from considerably different climates. There is also evidence that plants grown on native seed production farms, where the seeds for this experiment were obtained from, may experience rapid evolutionary shifts in phenotypes due to conditions on the farm (Conrady et al., 2023; Dyer et al., 2016; *reviewed in* Espeland et al., 2017). Thus, cultivation practices may be altering geographic patterns of local adaptation we would expect to see. Regardless of the specific mechanism, our results suggest that while local seed sources promote establishment, other seed sources can perform equally well to the local source.

For flowering phenology, we observed differences between sources that aligned with our expectations. First, plants from climate-adjusted sources began flowering at the same time as local and regional sources but continued flowering longer, suggesting they were taking advantage of a longer growing season (Etterson et al., 2020; Wadgymar et al., 2015). Although regional sources had more flowering individuals overall, especially at the beginning of the season, they had a similar onset and peak in flowering time as the local source. This supports our predictions that regional sources would exhibit traits more similar to local sources than the climate-adjusted sources. Importantly, none of our insect pollinated species from non-local locations flowered earlier than local sources, suggesting that complete pollinator mismatch created as a consequence of using non-local genotypes (Bucharova et al., 2016) is unlikely in our system. However, three species (*A. gerardii*, *R. triloba*, and *S. nutans*), flowered very late into the season (October) when sown with climate-adjusted sources, which could impact seed set in locations prone to early frosts (Inouye, 2008). These results illustrate how phenotypic variation between plants can result from seeds sourced from different locations.

Effects of source strategy and number (multi-source plots)

Our study provides some of the first evidence that admixture and climate-adjusted seed sourcing strategies can meet restoration establishment goals. In plots sown with a seed mix that combines regional or climate-adjusted sources with the local source (Bucharova et al., 2019; Prober et al., 2015), sown species richness was no different from plots sown with the local source alone. These results support the "bet hedging" hypothesis, where poorer-performing sources can be rescued by higher-performing ones (Rinella & James, 2017). For example, multi-source mixes had higher abundances of both C. *lanceolata* and *S. nemoralis*, which had low establishment in some of the single source mixes. Thus, multi-source seed mixes can reduce the probability that a species establishes poorly, or not at all, at a restoration site. One species, *R. hirta*, benefited from being sown specifically in climate-adjusted seed mixes, with these plots having significantly higher abundance of plants than those sown with local or admixture seed mixes. Given *R. hirta*'s widespread availability and common use in restorations, increasing the source diversity of this species would be relatively easy and could improve restoration outcomes. While mixing sources did increase establishment, we did not see an impact to sown richness when increasing source diversity beyond two sources. Given that each seed lot purchase can be costly (Schaub et al., 2021), purchasing more than two seed sources may provide little to no benefit to for these restoration species.

We also found support for our hypothesis that increasing source diversity can increase phenotypic variation in flowering phenology. Averaged across species, adding regional and climate-adjusted sources to the local source resulted in a similar onset of flowering to the local source alone and increased the number of flowering individuals over the growing season—though this increase in admixture seed mixes was mainly at the end of the season and limited to a single wind-pollinated species (*S. scoparium*). While mixing sources reduced the differences in flowering phenology that we observed in the single-source plots, the extended flowering duration remained, showing that these benefits can persist in admixture and climate-adjusted seed mixes. Extended flowering phenology in these seed mixes could boost pollinator diversity and abundance by creating a greater diversity of floral resources throughout the growing season (e.g. Blaauw & Isaacs, 2014), and benefit plant populations themselves. For example, previous work has shown that plant populations with more flowering individuals at any given time have a greater seed set (Wagenius et al., 2020; Zimmerman & Gross, 1984), so populations established with multi-source seed mixes may have a greater likelihood of persisting into the future (Morgan, 1999).

Influence of warming

Our results suggest that climate-adjusted seed sources were buffered against the detrimental impacts of warming, although these findings could not be resolved statistically. While both the local and admixture seed mixes had fewer sown species in the warming chambers than under ambient conditions, climate-adjusted sources retained a similar number of sown species in both environments. This suggests that selecting seeds from warmer climates for inclusion in climate-adjusted seed mixes successfully introduced warm-adapted genotypes, providing heat tolerance absent in local and admixture sources. Whether ambient plots sown with climate-adjusted mixes will retain this ability to withstand warmer climates over time is unknown, since gene flow between local and climate-adjusted individuals in multi-source plots may alter traits not currently under selection (e.g. Kucera et al., 2022). Overall, these results suggest that increased genetic diversity per se (i.e. admixture seed mixes) may not in itself be sufficient for populations to adapt to future climate warming (Havens et al., 2015; McKone & Hernández, 2021; Prober et al., 2015).

Contrary to our expectations, all sources showed adaptive shifts under experimental warming by flowering earlier and utilizing more of the growing season than they did in ambient conditions. An advanced flowering phenology under heat stressed conditions has been linked to greater survival and reproduction in plants compared to those that flower later (Anderson et al., 2012; Franks et al., 2007; Springate & Kover, 2014), and longer flowering duration increases a plants reproductive potential(Anderson et al., 2012). Given that this effect was observed across seed sources, these results suggest that phenotypic plasticity (Collins et al., 2025; Ramirez-Parada et al., 2024; Stuble et al., 2017), rather than local adaptation to specific climates (Duputié et al., 2015; Kooyers et al., 2019), drives responses to warming temperatures in our system. However, declines in sown richness in local and admixture populations indicate that other barriers, aside from flowering phenology, could limit their long-term survival under warming. Advanced flowering phenology could also increase the risk of pollinator mismatches (e.g. Kudo & Ida, 2013), and our results suggest that local seed sourcing does not necessarily mitigate this potential risk compared to other sourcing strategies. Overall, there are indications that once established, all sources can respond adaptively to climate warming, although this may impact pollination rates in the future.

Conclusions

Despite a twenty-five-year discussion amongst scientists on the potential benefits of different seed sourcing strategies (Mortlock, 2000; Prakash et al., 2024), research directly comparing the relative benefits of local seed sourcing compared to alternative approaches remains scarce. Our study provides restoration-relevant results that show that local sources can maximize plant establishment relative to non-local sources. However, combining local and non-local sources through admixture and climate-adjusted strategies can retain these high rates of establishment while also expanding phenotypic variation, which may be important for population persistence in an uncertain future. Our experimental design comparing single and multi-source seed mixes allowed us to resolve the importance of locality as well as the benefits of increasing genetic diversity, providing a nuanced evaluation of different seed sourcing techniques and the mechanisms that drive their performance. Overall, our results support the use of both local and alternative seed sourcing strategies to meet the goals of the decade on ecosystem restoration today and the future.

Figures

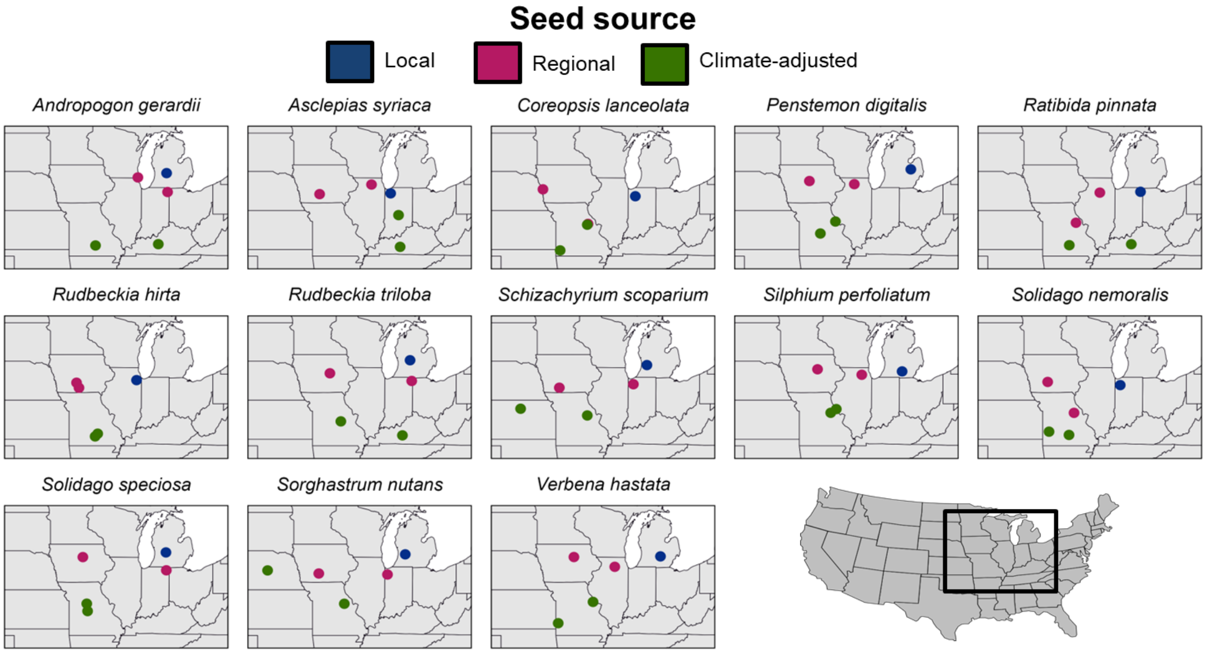

Figure 4.1: Source locations of the thirteen prairie plant species used in this experiment. Local sources (blue) were the geographically nearest source for each species. Regional sources (maroon) were used in admixture seed mixes and were from the next two geographically nearest locations for each species that were also in a similar climatic zone. The southern sources (green) were sourced from the two southernmost sources for each species.

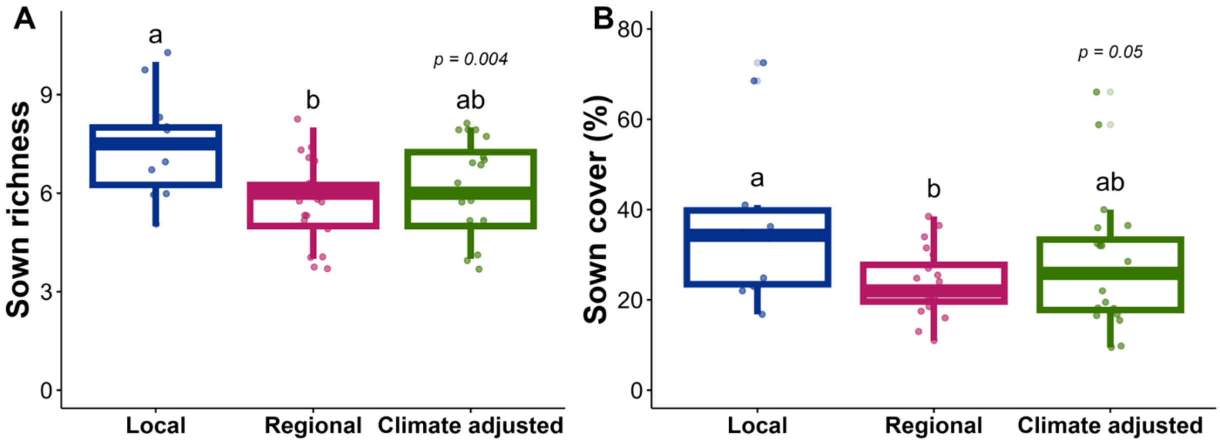

Figure 4.2: Sown richness (A) and sown cover (B) during the second growing season, in plots sown with one seed source (local (n = 10), regional (n = 20), or climate-adjusted (n = 20).

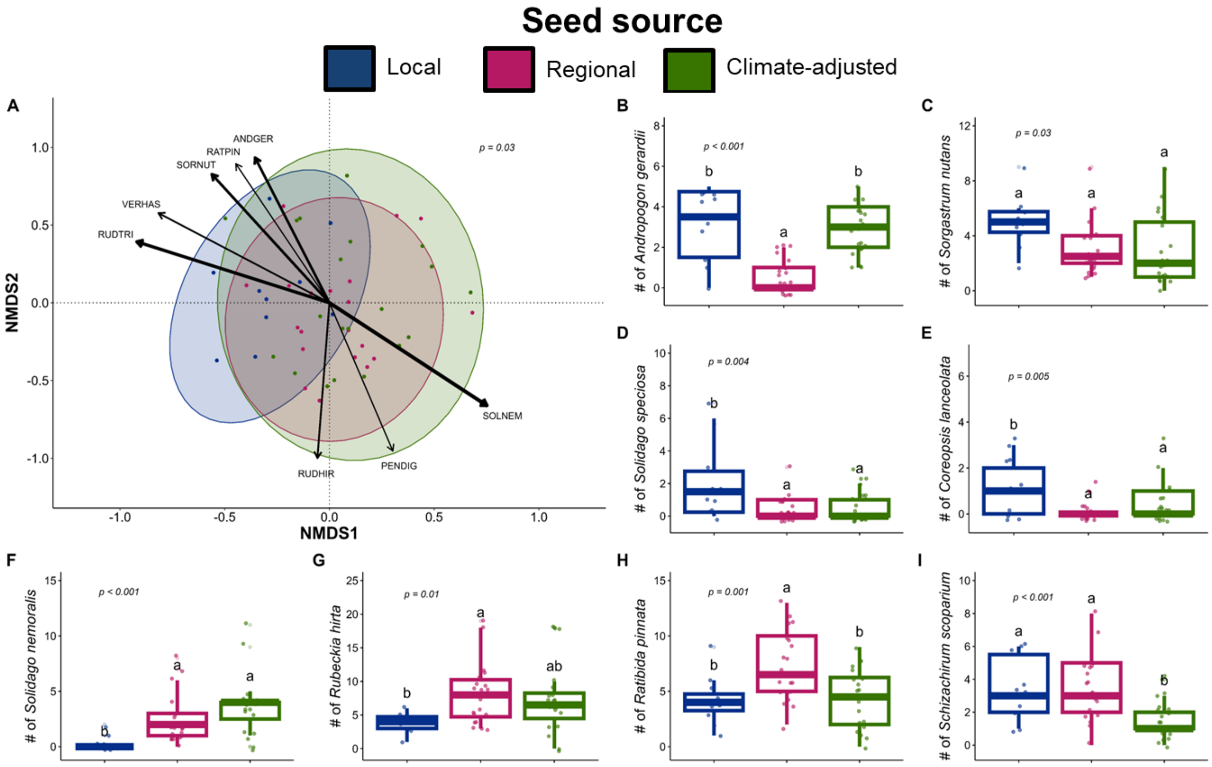

Figure 4.3: Non-multi-dimensional scaling (NMDS) plot for the effect of seed source type on plant community composition during the second growing season in plots sown with one seed source (A; PERMANOVA $R^2 = 0.07$; dispersion $p = 0.64$). Vectors reflect sown species abundances that significantly correlated with the spread of groups, with line thickness representing r^2 values. Plots for the eight sown species that showed significant differences in abundance based on where they were sourced from (B-I). Bars represent which seed sourcing strategy each species was procured under (local [n = 10], regional [n = 20], and climate-adjusted [n = 20]).

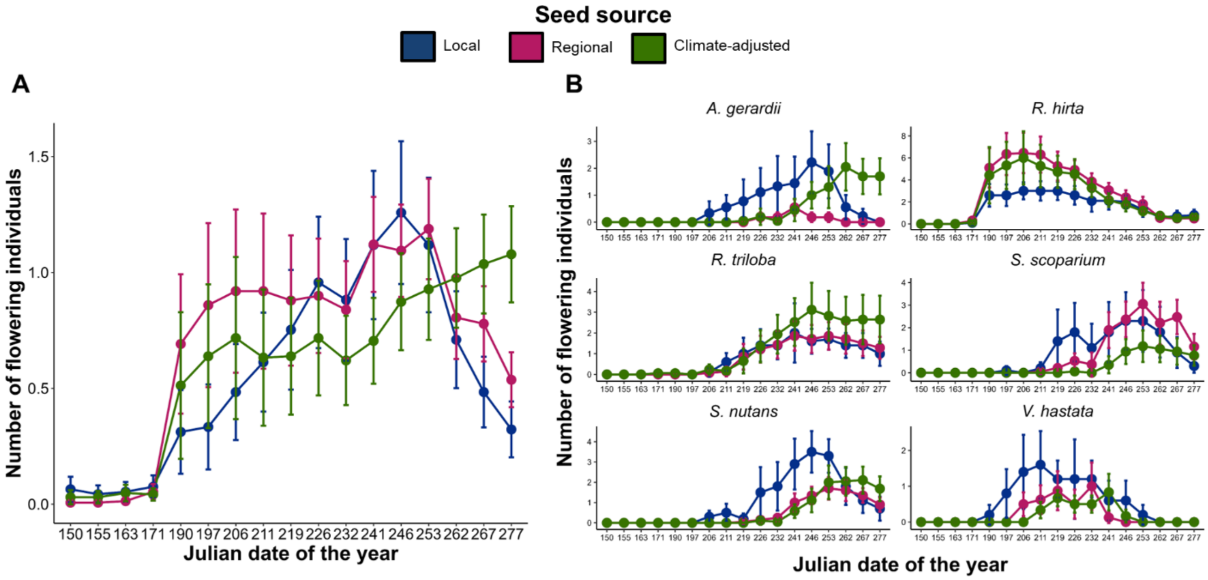

Figure 4.4: Average number of flowering individuals in plots sown with one seed source averaged across all sown species (A) and for six species with a sufficient number of flowering individuals across plots (B) across the growing season (May-October). Dots represent mean values across plots sown with a given seed source, and bars indicate 95% confidence intervals.

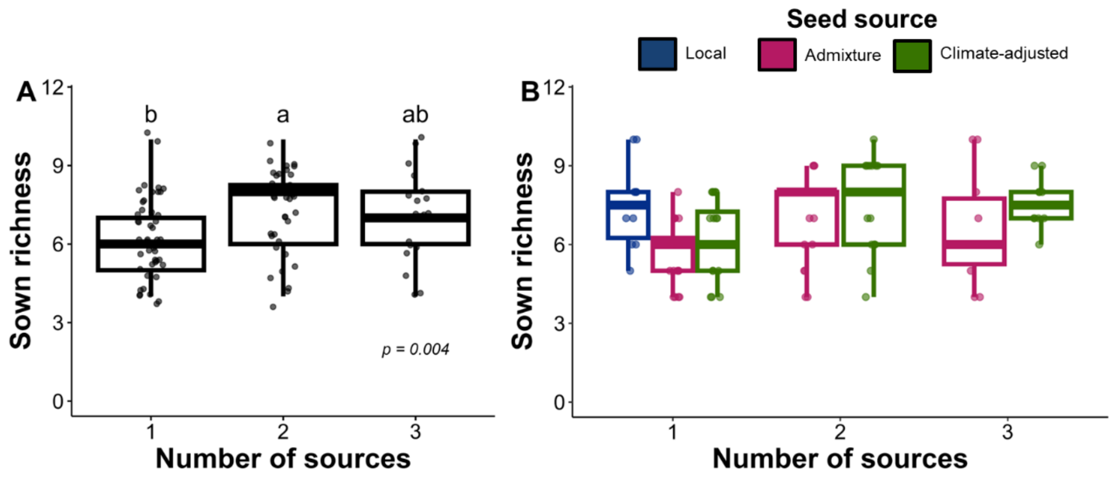

Figure 4.5: Sown richness in plots sown with different numbers of seed sources (A; one source [n = 50], two sources [n = 40], three sources [n = 20]) and how this graph relates to the identities of those seed sources (B). Plots were measured during the second growing season. Due to the nature of this experimental design, there are no statistical results for the interaction between source number and source type.

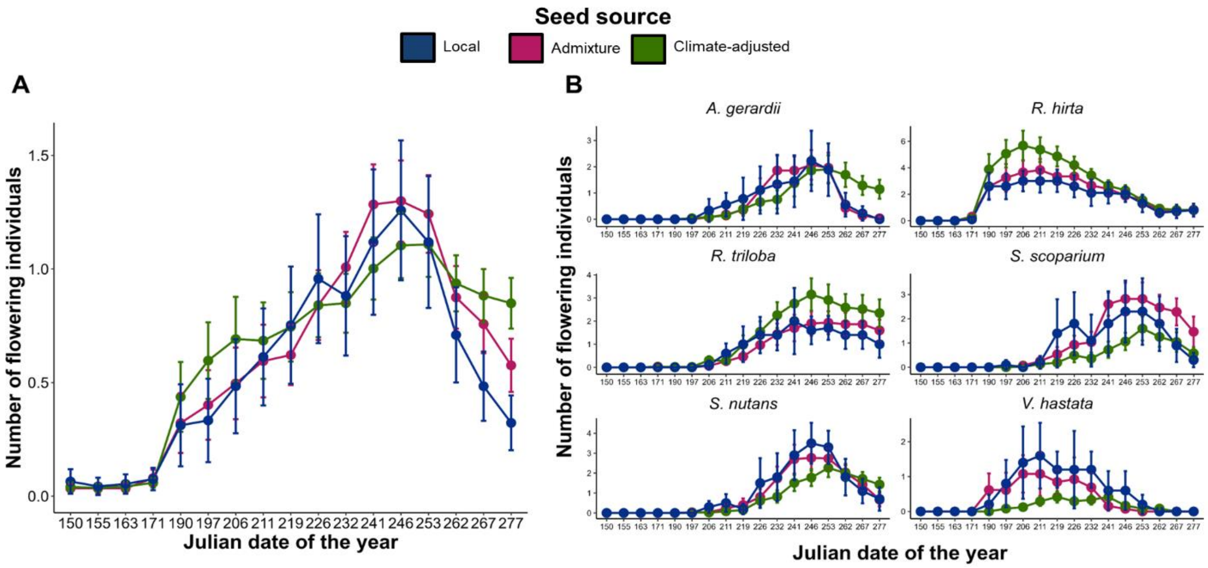

Figure 4.6: Average number of flowering individuals in plots sown with local, admixture, or climate-adjusted seed mixes averaged across all sown species (A) and for six species that flowered reliably across all plots (B) across the growing season (May-October). Dots represent mean values across plots sown with a given seed source, and bars indicate 95% confidence intervals.

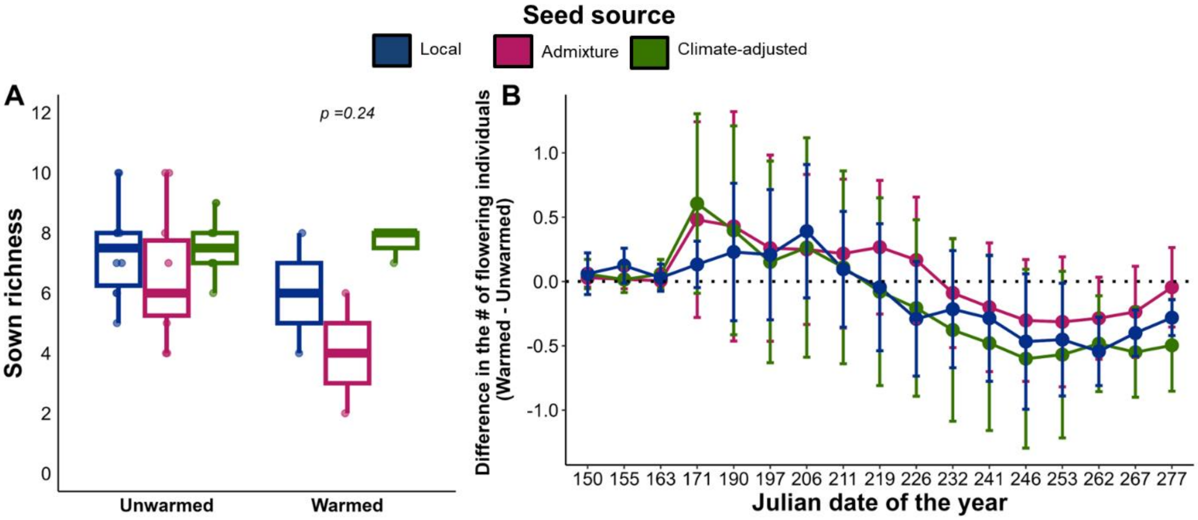

Figure 4.7: Sown species richness (A) and the difference in the number of flowering individuals between warmed and ambient plots (B) in plots sown with either the local source, or a three-source seed mix following admixture or climate-adjusted sourcing strategies under ambient (n = 50), or experimentally warmed (n = 9) conditions during the second growing season (May-October. For panel B, points above zero indicate more flowering in the warmed plots, and points below zero indicate more flowering in the unwarmed plots following each seed sourcing strategy. Dots in this plot represent mean values across plots sown with a given seed source, and bars indicate 95% confidence intervals.

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APPENDIX

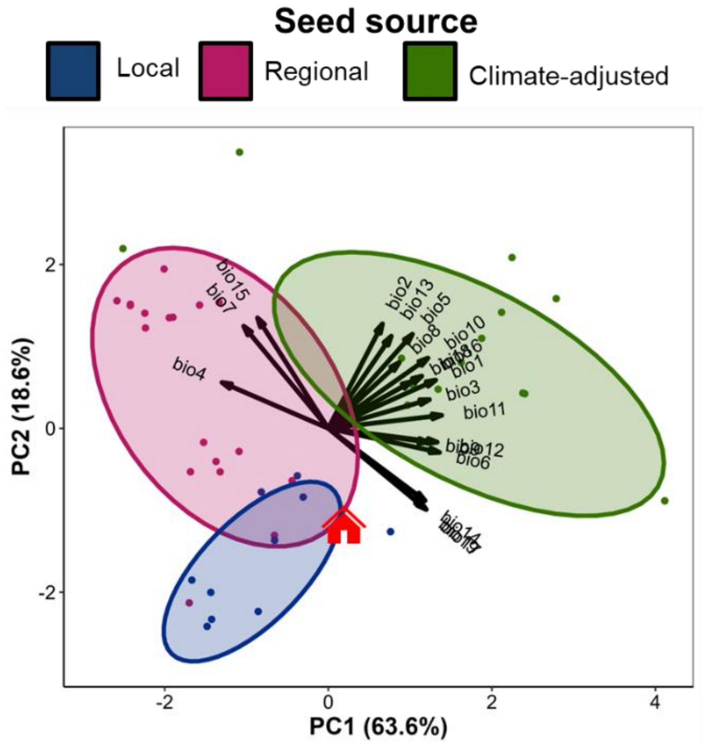


Figure A4.1: PCA of climatic variable loadings onto climate data. Each point represents the climatic environment for each of our 13 species sourced from five different locations, 1 local source, 2 regional sources (Added to admixture seed mixes), and 2 southern sources (Added to climate-adjusted seed mixes). The red house indicates the climate of the restoration site. PC1 correlated positively with warmer winters and summers, and wetter conditions overall. PC2 correlated positively with increased variability in precipitation and diurnal temperatures, as well as warmer summers.

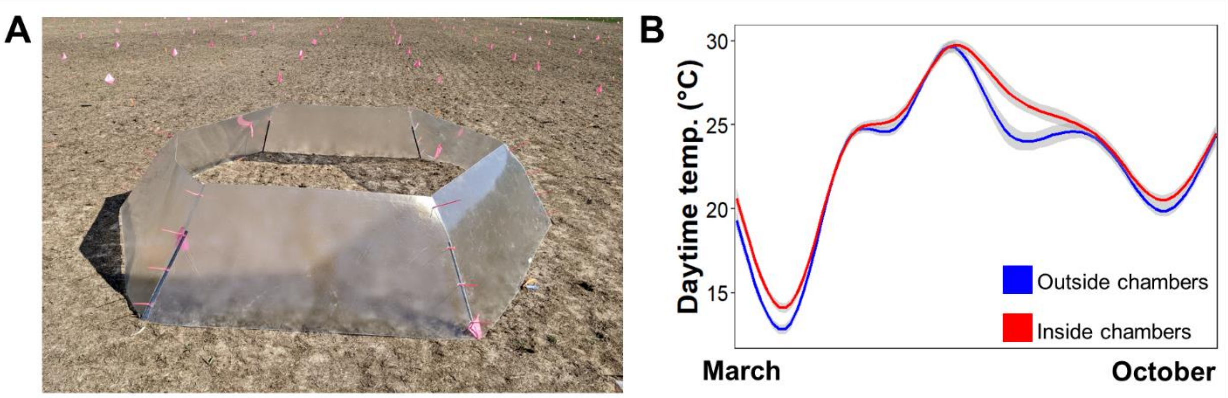


Figure A4.2: ITEX temperature chambers (A) assembled over 9 plots in this experiment and the average daytime temperatures obtained from ibutton temperature sensors placed inside the elevated temperature chambers (red) and outside of them (blue; B and 95% confidence intervals (shaded regions). On average, the chambers were 1.2ºC warmer inside than outside during the daytime.

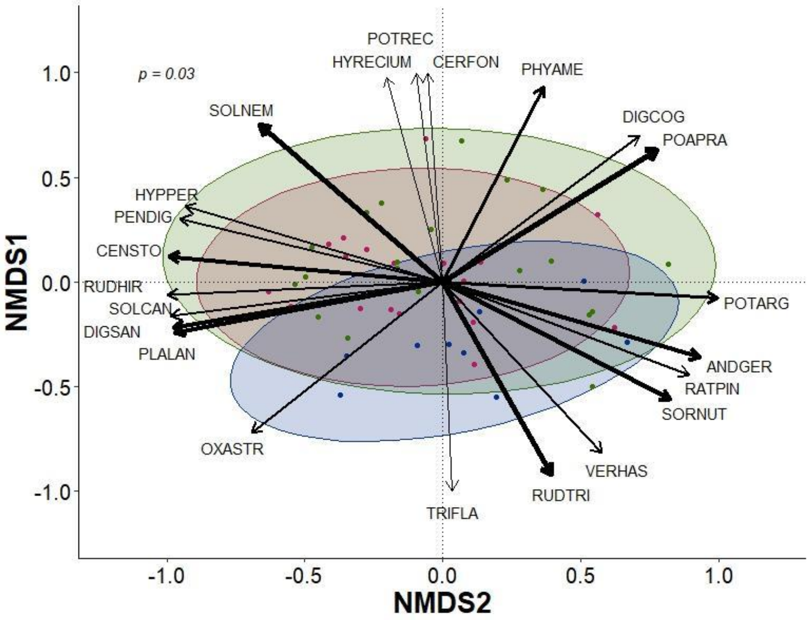


Figure A4.3: Non-multi-dimensional scaling (NMDS) plot for the effect of seed source type on plant community composition in plots sown with one seed source (local [n = 10], admixture [n = 20] , or climate-adjusted [n=20]) during the second growing season (PERMANOVA $R^2 = 0.07$; dispersion p = 0.64) including vectors of all species (sown and non-sown) whose cover were significant predictors of differences between the groups, with line thickness representing the level of significance (thicker lines have higher r^2 values).

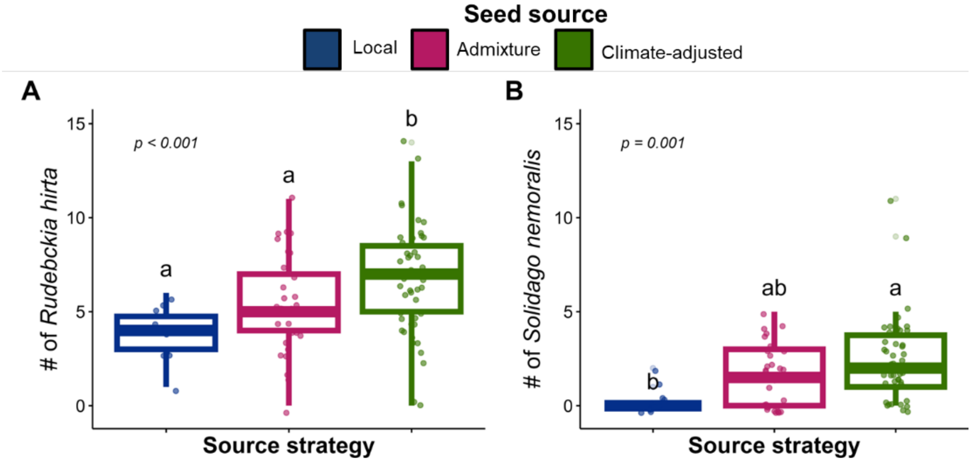


Figure A4.4: Abundance of four species sown in our plots whose abundance significantly differed depending on which seed sourcing strategy was utilized. Plots were surveyed in the second growing season.

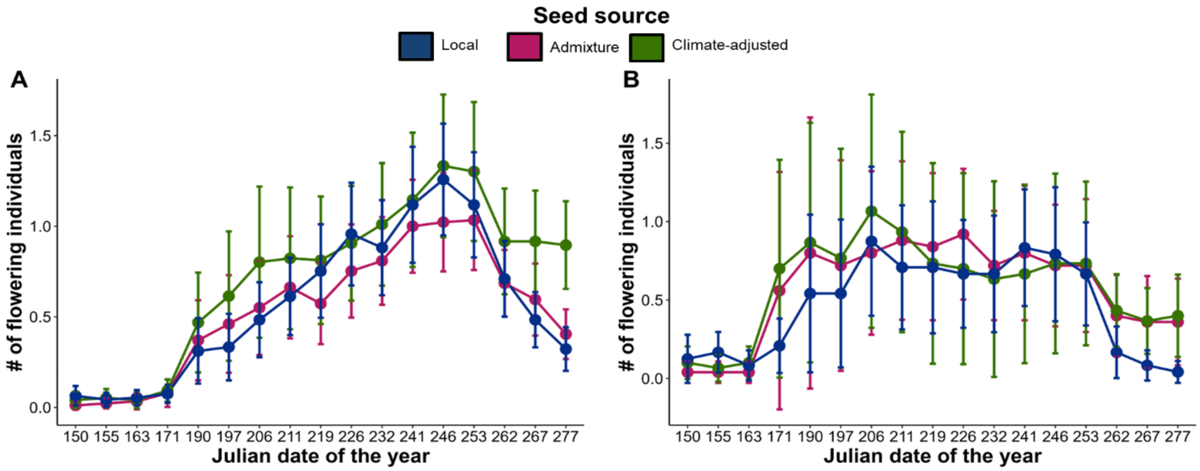


Figure A4.5: Number of flowering individuals of any given species in plots sown with either the local source, or a three-source seed mix following admixture or climate-adjusted seed sources under ambient (A; n = 30), or experimentally warmed (B; n = 9) conditions. Plots were surveyed once a week across the growing season (May-Oct) during the second growing season. Dots represent mean values across plots sown with a given seed source, and bars indicate 95% confidence intervals.

Table A4.1: Source information for each species used in this experiment, including the seed producer each species was sourced from for each seed mix, and the location the seed producers sourced their seed from. The seed number listed in the table is the approximate number of seeds used in a one-source plot. In a two-source plot, half of those seeds would come from each source, and correspondingly with three sources.

	Local		**Admixture 1**		**Admixture 2**	
Species	**Producer**	**Source Location**	**Producer**	**Source Location**	**Producer**	**Source Location**
A. gerardi	MWF	Ionia CO, MI	PSN	Kenosha CO., WI	SRN	Newton CO., IN
A. syriaca	ES	Delaware CO, IN	PSN	DeKalb CO, IL	ASC	Madison CO, IA
C. lanceolata	SRN	Newton CO, IN	ASN	Montgomery CO, MO	PAN	Harrison CO., IA
P. digitalis	MWF	Genesee CO., MI	PSN	Ogle Co., IL	HNO	Vernon, Lawrence, St. Clair CO., IA
R. pinnata	SRN	Newton CO, IN	PSN	LaSalle, Kane, Livingston, DeKalb CO.,WI	ASC	Montgomery CO, MO
R. hirta	PSN	Will CO., IL	ASC	Madison, Lucas, Ringgold CO., WI	PAN	Madison Co., IA
R. triloba	MWF	Ionia CO., MI	ASC	Appanoose, Allamakee, Madison CO., WI	ES	DeKalb CO., IN
S. scoparium	NC	MI	ASC	Madison, Union, Ringgold, Taylor, WI	SRN	Japer CO., IA
S. perfoliatum	MWF	Jackson CO., MI	PSN	Kane CO., IL	ASC	Appanoose, Allamakee, Madison CO, IA
S. nemoralis	SRN	Jasper CO.,	ASC	Ringgold, Greene, Decatur, IA	HS	Montgomery CO., MO
S. speciosa	MWF	Ionia CO., MI	ASC	Blackhawk, Greene CO, IA	SRN	Newton CO., IN
S. nutans	NC	MI	ASC	Iowa CO., IA	SRN	Jasper CO., IA
V. hastata	MWF	Ingham CO., MI	PSN	DeKalb, Lasalle CO., IL	ASC	Allamakee, Madison CO., IA

Table A4.1 (cont'd)

	Climate-adjusted 1		Climate-adjusted 2		Seed number/plot
	Producer	**Source Location**	**Producer**	**Source Location**	
A. gerardi	HNO	Texas Co., MO	RNS	Hart Co, KY	89
A. syriaca	SRN	Delaware Co., IN	RNS	Hart County, KY	8
C. lanceolata	MWN	Newton CO., MO	HS	Montgomery CO., MO	69
P. digitalis	PAN	Missouri	HS	Lincoln CO., MO	64
R. pinnata	HNO	Texas CO., MO	RNS	Hart CO., KY	74
R. hirta	HNO	Texas Co., MO	HS	Texas Co, MO	91
R. triloba	HS	Camden CO., MO	RNS	Hart CO., KY	84
S. scoparium	PAN	Mitchell CO., KS	HS	Montgomery CO., MO	89
S. perfoliatum	PAN	Pike CO.,	HS	Montgomery CO., MO	4
S. nemoralis	MWN	Dade CO	MNO	Texas Co.,	111
S. speciosa	MWN	Cole CO., MO	HS	Cooper MO	70
S. nutans	PAN	Nebraska	HS	Montgomery CO., MO	71
V. hastata	HNO	Barton CO., MO	HS	Lincoln CO., MO	91

Seed producer abbreviations: Allendan seed company (ASC), Earth Source INC (ES), Hamilton Native Outpost (HNO), Heartland Seed (HS), Michigan Wildflower Farm (MWF), Native Connections (NC), Prairie State Nursery (PSN), Pure Air Natives (PAN), Roundstone Native Seed (RNS), Spece Restoration Nursery (SRN).

Table A4.2: Full statistical results used to quantify the effects of source strategy, source identity, number of sources, and warming on restored prairie plant communities. All response variables were analyzed with different subset datasets due to sample size limitations, and to test specific hypotheses. Experimental plots were measuring during the second growing season.

†p < 0.10. *p < 0.05.; **p < 0.01.; ***p < 0.001.

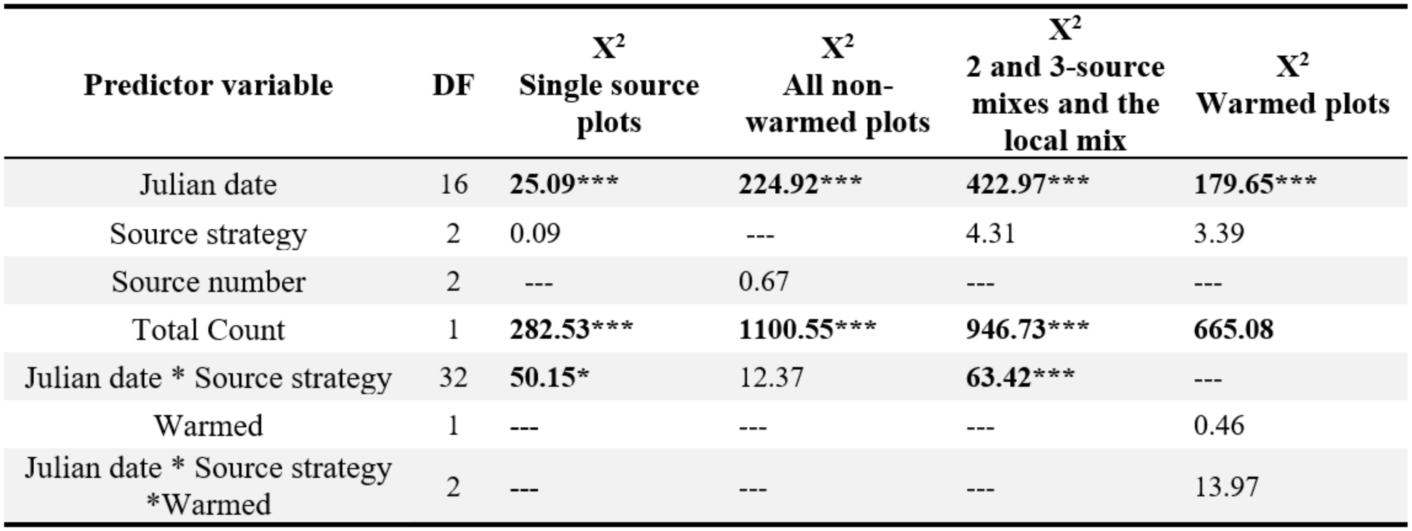
Dataset	**Single source plots**		**All non-warmed plots**		**2 and 3-source mixes and the local mix**		**Warmed plots**					
Predictor variable	**Source strategy**		**Number of sources**		**Source strategy**		**Source strategy**		**Warming**		**Source strategy * Warming**	
	df	F/χ^2	*df*	F/χ^2	*df*	F/χ^2	*df*	F/χ^2	*df*	F/χ^2	*df*	F/χ^2
Shannon's Diversity	2,47	0.48	2,107	0.08	2,87	0.10	2,33	0.28	1,33	__4.88*__	2,33	2.35
Sown richness	2,47	__6.04**__	2,107	__5.71**__	2,87	0.41	2,33	3.23	1,33	__4.49*__	2,33	1.48
Sown cover	2,47	**3.18†**	2,107	**0.33†**	2,87	**2.67†**	2,33	__3.34*__	1,33	2.18	2,33	1.64
R. hirta abundance	4	__9.02*__	2	0.77	2	__14.77 ***__	2	__16.45 ***__	1	0.01	2	2.76
A. gerardi abundance	4	__25.54 ***__	2	__16.64 ***__	2	4.10	2	3.12	1	1.66	2	0.27
V. hastata abundance	4	**5.34†**	2	3.79	2	1.89	2	0.72	1	0.01	2	0.01
C. lanceolata abundance	4	__10.41**__	2	__9.19*__	2	1.90	2	2.85	1	0.55	2	0.09
P. digitalis abundance	4	1.35	2	2.24	2	1.13	2	4.36	1	0.14	2	2.20
R. triloba abundance	4	0.75	2	2.38	2	0.07	2	5.16	1	0.73	2	0.22
R. pinnata abundance	4	__13.75**__	2	1.54	2	3.65	2	__6.67*__	1	0.01	2	1.07
S. scoparium abundance	4	__15.49 ***__	2	1.45	2	3.69	2	0.63	1	__4.13*__	2	0.79
S. nutans abundance	4	__6.51*__	2	__7.94*__	2	3.19	2	__6.07*__	1	0.48	2	0.89
S. speciosa abundance	4	__11.26**__	2	3.27	2	3.67	2	**5.72†**	1	1.09	2	1.12
S. nemoralis abundance	4	__16.76 ***__	2	__8.35*__	2	__13.80 ***__	2	__11.53**__	1	0.01	2	0.07

Table A4.3: Partial regression coefficients and F statistics PERMANOVA analyses used to quantify the effects of seed source amount, type, and experimental warming on the community composition of restored prairie plant communities. Models were run on each of the four datasets (top row of table) to test specific hypotheses. *p < 0.05.; **p < 0.01.; ***p < 0.001.

	Single source plots		**All non-warmed plots**		**2 and 3-source mixes and the local mix**		**Warmed plots**	
Predictor variable	r^2	F	r^2	F	r^2	F	r^2	F
Source Strategy	0.07	**1.89***	---	---	0.04	1.43	---	---
Source number	---	---	0.12	0.94	---	---	---	---
Source strategy * Warming	---	---	---	---	---	---	0.06	1.21

Table A4.4: Statistical outputs for negative binomial generalized linear models on the number of flowering individuals of any given species in a plot. Experimental plots were measuring during the second growing season.

†$p < 0.10$. *$p < 0.05$.; **$p < 0.01$.; ***$p < 0.001$.



Predictor variable	DF	X^2 Single source plots	X^2 All non-warmed plots	X^2 2 and 3-source mixes and the local mix	X^2 Warmed plots
Julian date	16	**25.09*** **	**224.92*** **	**422.97*** **	**179.65*** **
Source strategy	2	0.09	---	4.31	3.39
Source number	2	---	0.67	---	---
Total Count	1	**282.53*** **	**1100.55*** **	**946.73*** **	**665.08**
Julian date * Source strategy	32	**50.15***	12.37	**63.42*** **	---
Warmed	1	---	---	---	0.46
Julian date * Source strategy *Warmed	2	---	---	---	13.97

Table A4.5: Area under the curve calculations (using the trapezoidal method) and Kruskal Wallis test results (X^2) for the number of flowering individuals of any given species in plots sown under different seed sourcing strategies. Experimental plots were measured weekly during the second growing season (May-October). †p < 0.10. *p < 0.05.; **p < 0.01.; ***p < 0.001.

	Single source plots		**All non-warmed plots**		**2 and 3-source mixes and the local mix**		**Warmed plots**	
Seed source	**AUC**	**X^2**	**AUC**	**X^2**	**AUC**	**X^2**	**AUC**	**X^2**
Local	9.39	**5.61†**	---	---	9.39	**5.2†**	9.38	0.18
Regional/Admixture	11.33		---		10.19		8.85	
Climate-adjusted	9.67		---		10.46		11.71	
One source	---	---	10.21	2.15	---	---	---	
Two sources	---		10.69		---		---	
Three sources	---		10.33		---		---	
Warmed local	---	---	---	---	---	---	7.79	0.18
Warmed Admixture	---	---	---	---	---	---	9.52	
Warmed climate-adjusted	---	---	---	---	---	---	9.75	