

THE EFFECT OF SEED MIX DESIGN ON PLANT REPRODUCTIVE OUTPUT IN AN
EXPERIMENTAL PRAIRIE SYSTEM

By

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ABSTRACT

Ecological restoration reverses habitat loss and destruction by promoting the establishment of native communities. Despite this, most restoration outcomes are notoriously unpredictable. Restoration practitioners need to make important decisions while restoring ecological communities and these decisions can have lasting impacts on the outcome of the restoration. In particular, managerial decisions regarding the richness and location sourcing of a seed mix are especially important in seed based restoration, such as prairie ecosystems. These decisions can influence reproductive output. For example, seeds sourced from a distant location might produce individual plants that are maladapted to the local environment and produce fewer seeds. Additionally, higher initial seed mix richness may correlate with a decrease in seeding density, resulting in a plant population that is too small, sparse, or clumped for optimal reproduction to occur. In this study, I sought to answer the following question: How does initial seed mix richness and location sourcing affect plant reproductive output across different biological scales? To answer this question, the Brudvig Lab restored twelve experimental prairies in the Kellogg Biological Station, where we manipulated initial seed mix richness and the number of sources. We collected total plant and floral abundance data by counting the number of plants and flowers across various transects. I estimated reproductive output by counting the total number of seeds of nearly 400 individuals of two dominant prairie forbs, *Ratibida pinnata* and *Echinacea purpurea*. I derived values that allowed the scaling of our data, such as the number of inflorescences per plant, plant count per area, developed seed count per seed head, and more. Our results suggest that initial seed mix design is important for the reproductive output of target plant populations in restoration. Namely, I found that restorations sown with three sources and 71 species at lower seeding rates decrease reproductive output for *Echinacea* across all biological scales, from individual infructescences to the entire population, but only for *Ratibida* at the population scale. There are predictable lasting impacts of seed mix design on reproductive output. Our study suggests prioritizing seeding rate per species in seed mixes, ideally with a high species richness from fewer sources.

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TABLE OF CONTENTS

INTRODUCTION.....	1
METHODS.....	6
RESULTS.....	11
DISCUSSION.....	27
CONCLUSION.....	30
BIBLIOGRAPHY.....	31
APPENDIX.....	37

INTRODUCTION

Habitat restoration promotes biodiversity and ecosystem functioning in degraded ecosystems (Suding, 2011). A cornerstone of any successful restoration is the promotion of viable populations of plant species characteristic of the target ecosystem (i.e., target species) under restoration (Lesica and Allendorf, 1999). Most assessment criteria focus on plant species richness and diversity, yet these metrics provide coarse information about target plant populations (Ruiz-Jaen and Aide, 2005). More precise restoration can be informed by demographic responses of target species that can help inform management and in turn, improve the predictability of success in restoration practice (Ballare et al., 2017). It is critical to understand how management decisions affect reproductive output (production of viable seeds), since species persistence is dependent on the replacement of individuals (Huang et al., 2013; Wilsey, 2021).

Reproductive output influences the trajectory of a population's size (Menges, 1995; Molano-Flores and Hendrix, 1999). Thus, practitioners must make decisions and engage in activities that increase reproductive output of target plant populations. Recruitment via seed propagation may only take place when environmental conditions are right. In one case study, soil disturbance and burning increased seedling recruitment threefold in a rare prairie forb (Menges, 1995); thus, promoting reproductive output can help to ensure recruitment during correct environmental conditions. Moreover, although new recruits may be rare in the short term, consistent yearly contributions to a seed bank is an important process in the demographic cycle of many species and is critical to the persistence of many plant populations (Bullock, 2000).

New efforts are needed to test effects of alternative restoration strategies on reproductive output of target species during the restoration process. Many factors can affect reproductive output at various biological scales: from individual reproductive structures (infructescences), to individual plants, to an entire plant population. For example, the infructescences of individual plants may produce varying numbers of seeds, due to their number of florets, damage, production of nectar/pollen that may attract pollinators, or other factors related to environmental conditions within a restoration (Klinkhamer and Jong, 1990; Cresswell, 1999; Cardel and Koptur, 2010). These differences in the infructescence may scale up to entire plants; however,

additional variation in reproductive allocation across plants within the same population may result in differences in reproductive output: some plants might invest heavily in flowering and, later, seeds, while others, especially new recruits, might invest disproportionately in structures related to establishment like stem elongation and root development (Wenk and Falster, 2015). Further, at the scale of a population, if the population size of a specific plant species is too small, then it will be less likely to attract pollinators and may thus have low reproductive output due to low rates of pollination (Wilcock and Nieland, 2002; Kwak and Bekker, 2006). Large populations may also have more reproductive output simply because they contain more individuals. Despite this, Mustajarvi et al. (2001) found that plants in sparser and lower density populations invested more in their flowers, which led to a stronger pollinator response and thus enhanced reproductive output.

Initial seed mix design may influence the configuration, size and identity of target plant species populations and new experiments that alter seed mix design are warranted to more clearly understand the effects on target plant species reproductive output. During seed based restoration, a core consideration is the number of species sown. Budgetary constraints can lead practitioners to use species poor seed mixes (Barr *et al.*, 2017). This is problematic, as high richness seed mixes increase plant species richness (Leps *et al.*, 2007; Carter and Blair, 2012; Kirmer *et al.*, 2012; Schmidt *et al.*, 2022). Since plant diversity and pollinator diversity are positively correlated (Haddad *et al.*, 2009), high richness plant assemblages may attract diverse pollinator communities that promote plant reproduction (Frund *et al.*, 2010; Albrecht *et al.*, 2012, Wei *et al.*, 2021). Despite the potential benefits of restoring ecosystems using a high richness seed mix, a necessary consequence may be lowering of seeding rate. The total seeding rate (total number of seeds sown per area) is often held constant due to budgetary constraints (Barak *et al.* 2022), so when seed mixes become more diverse, each species is sown with fewer seeds per area.

Manipulating the species-level seeding rate (the number of seeds sown per species) has implications for the reproductive output of restored populations. The higher seeding rate typical of low richness seed mixes can enhance viability of target populations (Kunin, 1997). Higher seeding rates promote establishment and can help

eliminate weedy species more effectively in the short term (Stevenson et al., 1995; Harker et al., 2003). High density seeding treatments can also result in greater species richness and cover of native seeded species, which can also increase seeds per fruit and seeds per flower (Feldman, 2006; Carter and Blair, 2012). Enhanced establishment of target species will result in greater floral abundance and an abundance of nectar resources, which has shown to initiate a stronger pollinator response (Johnson et al., 2004; Fowler et al., 2016; Li et al., 2021).

Promoting a stronger pollinator response is critical to ensuring plant reproduction. Pollinator behavior is important for the success of sown species. Pollinators are expected to adapt their foraging behavior in accordance to surrounding resource scarcity (Kunin and Iwasa, 1996). When floral resources are scarce (i.e. plant populations are small), pollinators are likely to adopt a more generalist strategy and tend to visit flowers indiscriminately (Kunin and Iwasa, 1996). When floral resources are plentiful, it is more cost-effective for pollinators to specialize in one type of flower or similar suite of flowers (Kunin and Iwasa, 1996). Specialization in one type of species increases efficiency of resource collection by decreasing pollinator foraging costs (Armbruster, 2017). Higher conspecific visitation will lead to enhanced conspecific pollen deposition, which is expected to increase plant reproductive output at the infructescence scale (Waites and Ågren, 2004). With larger population sizes, there may be greater reproductive output at the population scale and thus restoration success.

The initial seed mix design may have a lasting impact on the restoration. In addition to species richness and seeding rate, the location where practitioners source seed may also be critical to restoration success. There is a lack of rigorous empirical research on how they affect plant reproduction during restoration. Examples of sourcing methods include: 1) local provenancing uses locally-sourced seeds; 2) predictive provenancing uses southerly-sourced seeds, attempting to “keep up” with climate change and use genotypes that are more suited for predicted future climates; 3) admixture provenancing uses a combination of many different sources and aims to maximize evolutionary potential by promoting genetic diversity while ignoring gene flow dynamics; and 4) Composite provenancing incorporates genotypes that are more distant, but attempts to imitate natural gene flow (Breed et al., 2013). Large plant

populations have adapted to climatic and environmental clines specific to their respective regions (Joshi et al., 2001; Leimu and Fischer, 2008; Woods, 2012). Because of this, the most common recommendation is local provenancing, that is, as close to the restoration site as possible (Lesica and Allendorf, 1999; Hufford and Mazer, 2003). Locally sourced seeds are more likely to harbor locally adapted genotypes, and may therefore establish plant populations that persist and reproduce better than plant populations from non-locally sourced seeds (Lesica and Allendorf, 1999). This technique may be obstructive to the restoration process, however, because there are often few locally adapted populations for any given site (Mortlock, 2000; Borders et al., 2011). Using seeds from only one or a few local source(s) may introduce founder effects via population bottlenecks in the restoration; this may lead to decreased overall plant reproductive output due to lack of genetic diversity and adaptive potential (Whiteley et al., 2015). On the other hand, admixture provenancing increases evolutionary novelty, which is expected to enhance population resilience in the face of unpredictable challenges that future environmental change may posit (Choi, 2004; Harris et al., 2006; Broadhurst et al., 2008; Havens et al., 2015). In an experiment with wildflower strips restored from local and nonlocal seeds, Bischoff et al. (2010) found that the plants with high genotypic diversity (i.e., from multiple sources) increased productivity, establishment, and seed production for only one of the species surveyed. Yet, Bucharova et al (2017) found that species fitness decreased with increasing geographical distance of seed origin, supporting the use of local seed sources. Disentangling the effects of seed sourcing can be instructive and provide necessary guidance to restore viable populations.

Experiments are needed to determine the effects of seed mix design on restored species reproductive output. Experiments overcome uncontrollable differences in management and site history that may influence restoration outcomes. For example, Grman and Brudvig (2013) found that the composition, diversity, and density of seed mixes applied, in addition to site age, were critical in explaining differences among restoration sites. Any one of these factors might obscure the effects of seeded richness and seed source locality, the latter of which is poorly explored (though see Pizza et al. 2023). Moreover, I know of no set of ongoing restoration projects that allow for the study

of varying numbers of seed sources, much less a set of projects that allow for the independent and interactive study of seed source number and sown species richness. There is a need for controlled experiments that manipulate initial seed mix design and provenancing to shed more light on how these decisions influence plant reproductive output and viability by exploring seed sets directly.

The Brudvig Lab created a field-scale experiment to test the effects of the number of sown species (12 vs. 71) and seed sources per species (1 vs. 3), on reproductive output at three different scales: 1) the individual infructescence, within a plant ; 2) all of the infructescences in an individual plant; and 3) an entire population. To do this, the Brudvig Lab restored an experimental prairie system and explored the reproductive output of two prairie forbs that are commonly re-established during prairie restoration: *Echinacea purpurea* (purple coneflower) and *Ratibida pinnata* (gray-head coneflower).

Research Questions:

1. How does the number of sown plant species affect plant reproductive output?
2. How does the number of sown seed sources affect plant reproductive output?
3. What is the role of pollination in driving effects?

METHODS

Experimental System

Prairies are an ideal ecosystem to explore how differences in management influence reproductive output at various scales. Prairies take just a few years to establish from seed because they are dominated by herbaceous plants such as forbs and grasses. Additionally, they are the most endangered ecosystem in North America, yet provide valuable resources for pollinators (Samson and Knopf, 1996; Gilgert and Vaughan, 2011).

The Conservation Lands Experiment (CLE) Prairies at the W.K. Kellogg Biological Station (KBS) in Michigan (Figure 1) consists of twelve prairie restorations established on former agricultural land. The experiment was prepared by removing all non-prairie old field vegetation through the application of glyphosate herbicide twice in the summer of 2015, with subsequent seeding in the same year in late November and early December. In 2019 and 2021, the prairies were managed with prescribed burns. The size of the restorations varies between 0.5-3.5 ha. Each site consists of two half-sites, where the number of sown sources was manipulated. In low richness half-sites, twelve focal species were sown, which includes four forbs, three C4 grasses, three legumes, and two C3 grasses (Catano et al., 2021). In high richness half-sites, seventy-one species were sown, consisting of the twelve focal species from the low richness half-sites and an additional fifty-nine species. Total seeding rate was held constant (9.87 kg/ha), resulting in the twelve focal species sown with more seeds in low richness half-sites and fewer seeds in the high richness half-sites (see Table 1 in Appendix).

In addition, four different sourcing treatments were established at the different restoration sites to test the effects of admixture provenancing: seeds were sourced from local, non-local northern, and southern seed distributors, as well as all three sources mixed. The twelve sites were sown with seed either sourced exclusively from one region or from all regions, with two sites for each single region (in total six single-sourced sites), and the other six sites included a mix of all three regions. I focused our analyses on comparing single (1) to multiple (3) sources to test our focal questions about seed

source number and due to the limited replication when looking at each single sourced site.

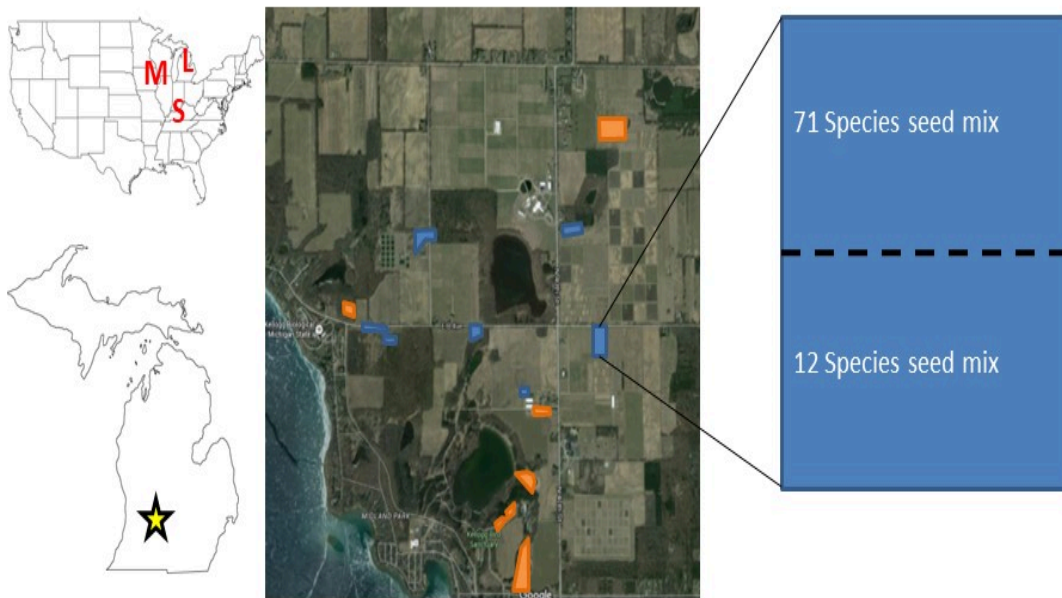


Figure 1. Conservation Lands Experiment

Illustration of the Conservation Lands Experiment Design. The Kellogg Biological Station is located in Southwest Michigan. Seeds were sourced from three different locales: local (L), non-local northern (M) and southern (S). Red sites harbor prairie communities with initial seeding treatment from all three sources while blue sites harbor prairie communities with initial seeding treatment from a single source.

Study Species

I conducted all analyses using the information collected from two different species in the Asteraceae family: *Echinacea purpurea* and *Ratibida pinnata*, hereafter referred to only by their genus name, *Echinacea* and *Ratibida*. These two species are part of the focal set of twelve species that were sown in all twenty-four half-sites, and are commonly included in many prairie seed mixes. I focused on these two species because they had high establishment rates across all CLE treatments, permitting tests of our focal questions. *Echinacea* and *Ratibida* reproduce via clonal propagation to form

ramets, that is, an independent clonal colony. Ramets were only considered different if they were at least 1 meter away from other ramets.

Seed Procurement

I collected seeds across the CLE from two plants: *Echinacea* and *Ratibida*. In the beginning of Summer 2019, I tagged eight ramets for each species, distributed throughout each half-site. Then, I randomly selected a seed head within each ramet. After the inflorescence began to develop into an infructescence, I installed pollinator exclusion bags to prevent seed dispersal and deter predation. Once seeds were fully developed, I clipped the tagged seed heads with scissors and carefully placed them in dry paper bags. I then extracted seeds using forceps.

Proxies for Pollinator Success

Fruit development was variable and some seeds were not fully developed. I used various properties of individual seeds to differentiate developed from underdeveloped seeds. Underdeveloped seeds were often discolored, disfigured, smaller, and more brittle. I counted the number of developed and underdeveloped seeds separately in each seed head to determine seed production as a measure of reproductive output. I considered only developed seeds for measurements of reproductive output; however, I counted both developed and undeveloped seeds. Because flowers of species in the Asteraceae can form either developed seeds when pollinated or undeveloped seeds when unpollinated, the ratio of developed to developed and undeveloped seeds can serve as a measure of pollination rate (Brudvig et al. 2015).

Flower-to-fruit conversion ratio= [Developed seed count / (Developed seed + Underdeveloped seed count)]

To more clearly understand the role of pollination in our target species, I conducted a pollinator exclusion experiment and quantified seed production in bagged and open infructescences across the CLE Prairies. In each of the 24 half-sites, I bagged a single reproductive structure before bloom to deter pollinator visitation and thus animal-mediated pollen deposition. After reproductive structures senesced into infructescences, I removed seeds using forceps and counted all developed seeds. Infructescences that were not visited by pollinations (i.e, infructescences that had exclusion bags installed) produced considerably fewer seeds compared to open

infructescences, usually approaching zero (Latorre, 2019). *Echinacea purpurea* and *Ratibida pinnata* highly depend on outcrossing events by pollinators for seed production.

Inflorescence and Population Surveys

To estimate plant-level reproductive output, population size, and population-scale reproductive output, I combined the infructescence-level seed count data with surveys of the two focal species in fixed-area plots, within each half site. Jenn Lau counted the total number of ramets and inflorescences in five 25m² plots (5mX5m) within each half site during September 2019. If floral density was too high to count, she subsampled a 1m² area (1mX1m) within a given plot.

Reproductive output throughout different scales

Using reproductive output data (developed seed count), inflorescence and population surveys, I determined differences among treatment groups at three scales: 1) infructescence scale was the average number of developed seeds per infructescence in a half site; 2) ramet scale was the average number of developed seeds per ramet in a half site; 3) population scale was the estimated total number of developed seeds per 125 m² in a half site.

To evaluate reproductive output at the infructescence scale, I counted all developed seeds in each infructescence and derived the average value per infructescence for each half site. To evaluate reproductive output at the ramet scale, I multiplied average seed count per infructescence by the average number of infructescences per ramet, for each half site. To evaluate reproductive output at the population scale, I multiplied reproductive output at the ramet scale (see above) by the total number of plants counted in each half site. In instances where 5x5m plots were subsampled, I estimated the number of plants per 5x5m plot (e.g., I multiplied a 1x1m subsample by 25).

Statistical Methods

I performed all analyses in R (Version 4.1.2, 2021). Response variables included *Echinacea* and *Ratibida* reproductive output for infructescences, ramets, and population scales, in addition to flower-to-fruit conversion ratios, analyzed as separate models. For each model, fixed effects included the number of sown species (twelve and seventy-one

species), number of sown sources (1 and 3 sources), and the interaction between number of species and number of sources sown. Site was used as a random effect. Models were made using the lme4 package (Bates et al., 2015). For the seed count data, I initially fit a Poisson distribution, but after confirming overdispersion, I fit these models with a negative binomial distribution. The resulting models were not overdispersed and had uniform residual plots. To test the model factors, I performed ANOVAs using the car package (Fox, 2019). Pairwise comparisons were assessed to look at differences between specific combinations of treatments. I plotted results using negative binomial model predictions and 95% confidence intervals using the ggplot2 package (Wickham, 2016).

RESULTS

Echinacea infructescence scale

I found evidence for effects of source number, sown richness, and an interaction between source number and sown richness on *Echinacea* seed count at the infructescence scale (Table 1; Figure 2). With this significant interaction, I interpret results in context of the interaction. Half-sites sown with three sources and 71 species had 39-46% lower infructescence-scale seed counts than the other three treatments, whereas none of the other treatments differed (Table 2).

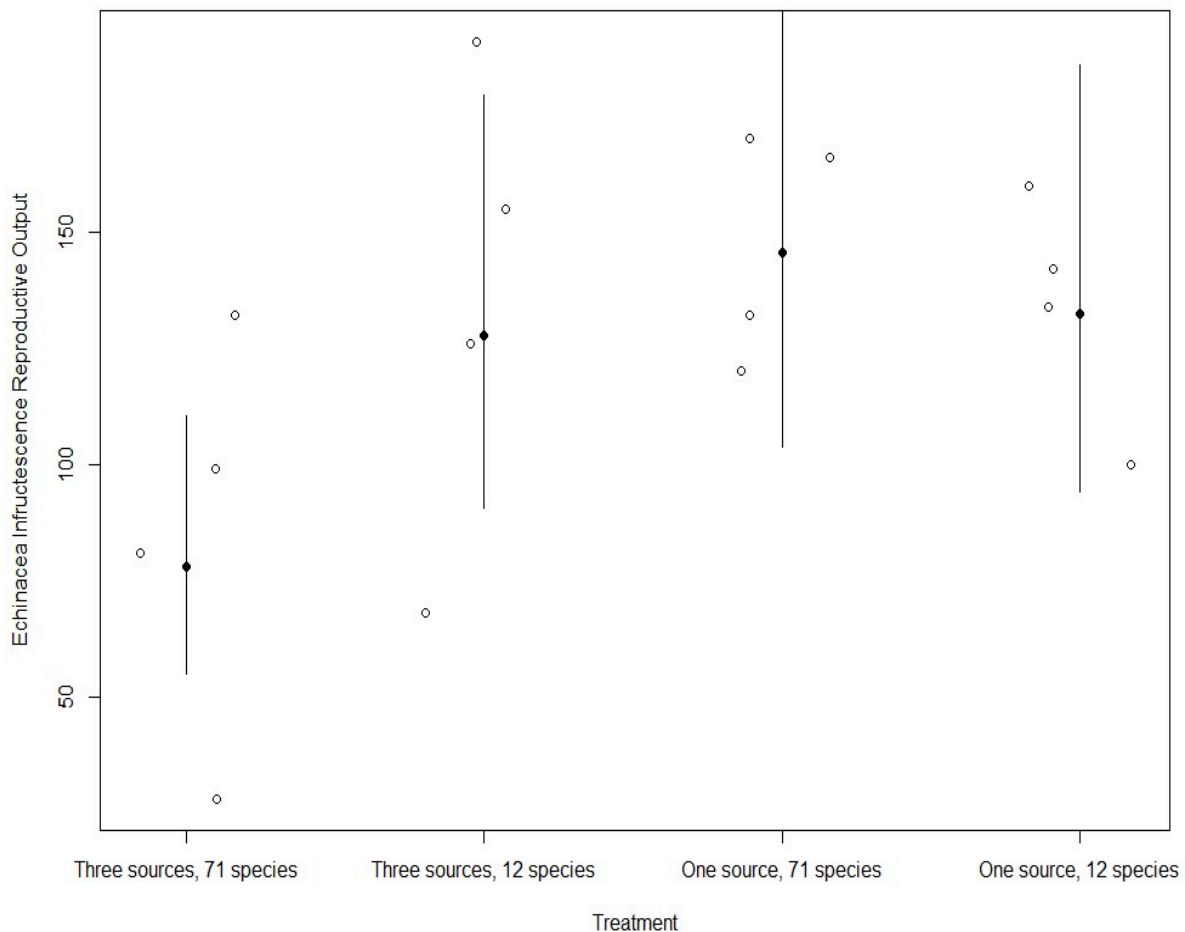


Figure 2. *Echinacea* infructescence scale

Seed count for individual *Echinacea purpurea* infructescences was influenced by an interaction between the number species sown and number of *Echinacea* sources sown into a prairie restoration experiment. Seed counts were lower when prairie plantings

Figure 2 (cont'd)

were sown with 71 species and three *Echinacea* sources, relative to 12 species and three *Echinacea* sources and single *Echinacea* source plantings. Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 1. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on infructescence-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment	Chisq	Df	p
	600.1	1	<2.2e-16
Source	6.3	1	0.0117
Richness	12.8	1	0.00035
Source X Richness	9.5	1	0.00201

Table 2. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in infructescence-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.49	<0.001
3 sources, 71 species vs. 1 source, 71 species	-0.62	0.012
3 sources, 71 species vs. 1 source, 12 species	-0.53	0.033
3 sources, 12 species vs. 1 source, 71 species	-0.13	0.592
3 sources, 12 species vs. 1 source, 12 species	-0.04	0.881
1 source, 71 species vs. 1 source, 12 species	0.09	0.47

Echinacea ramet scale

I found evidence for effects of source number, sown richness, and an interaction between source number and sown richness on *Echinacea* seed count at the scale of individual ramets (Table 3; Figure 3). With this significant interaction, I interpret results in context of the interaction. Half-sites sown with three sources and 71 species had 59-62% fewer ramet-scale total seed counts than treatments with three sources and 12 species, as well as a single source and 71 species (Table 4). None of the other treatments differed (Table 4).

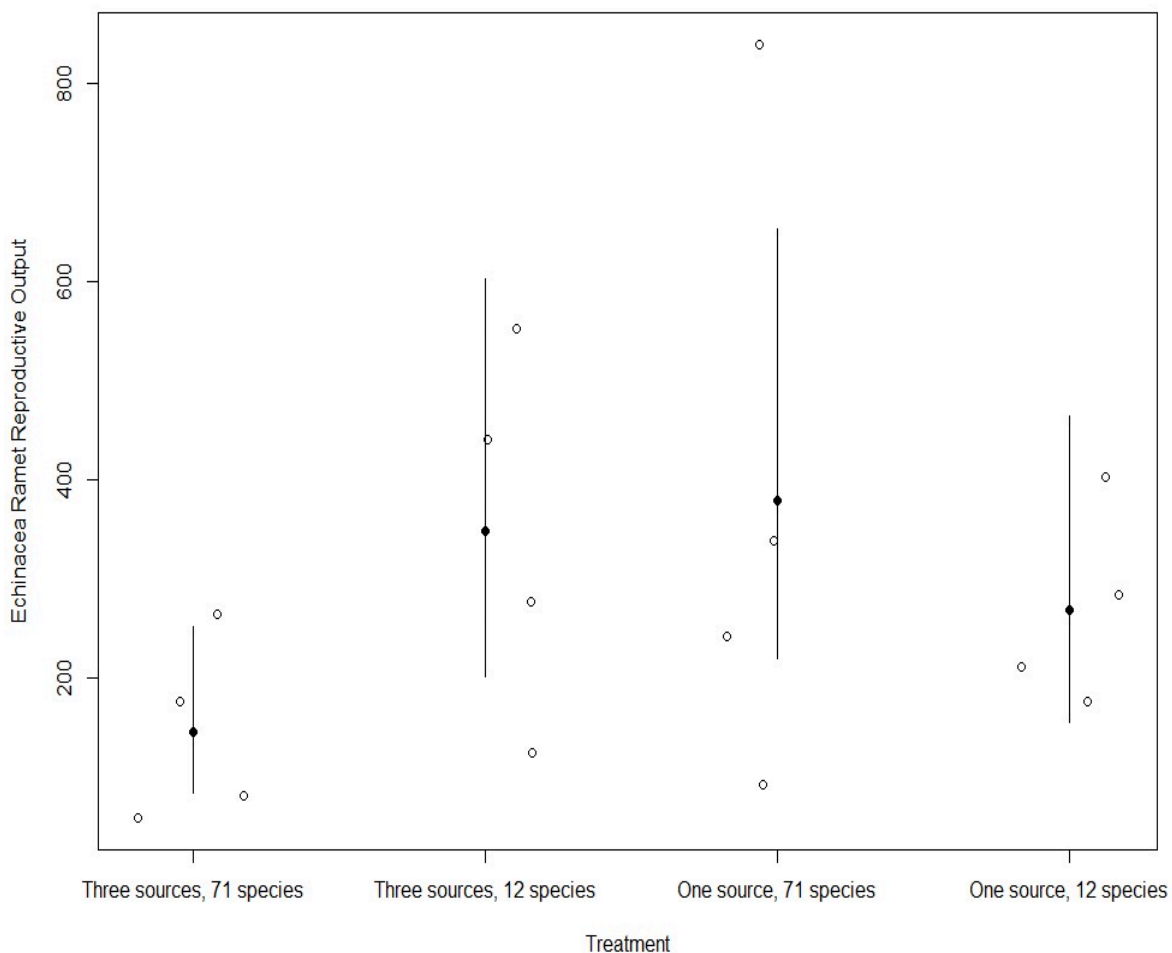


Figure 3. *Echinacea* ramet scale

Seed count for *Echinacea* at the ramet scale was influenced by an interaction between the number species sown and number of *Echinacea* sources sown into a prairie restoration experiment. Seed counts were lower when prairie plantings were sown with

Figure 3 (cont'd)

71 species and three *Echinacea* sources, relative to 12 species and three *Echinacea* sources and 71 species and a single *Echinacea* source planting. Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 3. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on ramet-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	312.4	1	<2e-16
Source	5.9	1	0.015
Richness	4.9	1	0.026
Source X Richness	4.8	1	0.029

Table 4. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in ramet-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.88	0.026
3 sources, 71 species vs. 1 source, 71 species	-0.96	0.015
3 sources, 71 species vs. 1 source, 12 species	-0.62	0.12
3 sources, 12 species vs. 1 source, 71 species	-0.08	0.837
3 sources, 12 species vs. 1 source, 12 species	0.26	0.507
1 source, 71 species vs. 1 source, 12 species	0.34	0.385

Echinacea population scale

I found evidence for effects of source number, sown richness, and an interaction between source number and sown richness on *Echinacea* seed count at the scale of the entire population (Table 5; Figure 4). With this significant interaction, I interpret results in context of the interaction. Half-sites sown with three sources and 71 species had 85-96% fewer population -scale total seed counts than the other three treatments; other treatments did not differ (Table 6).

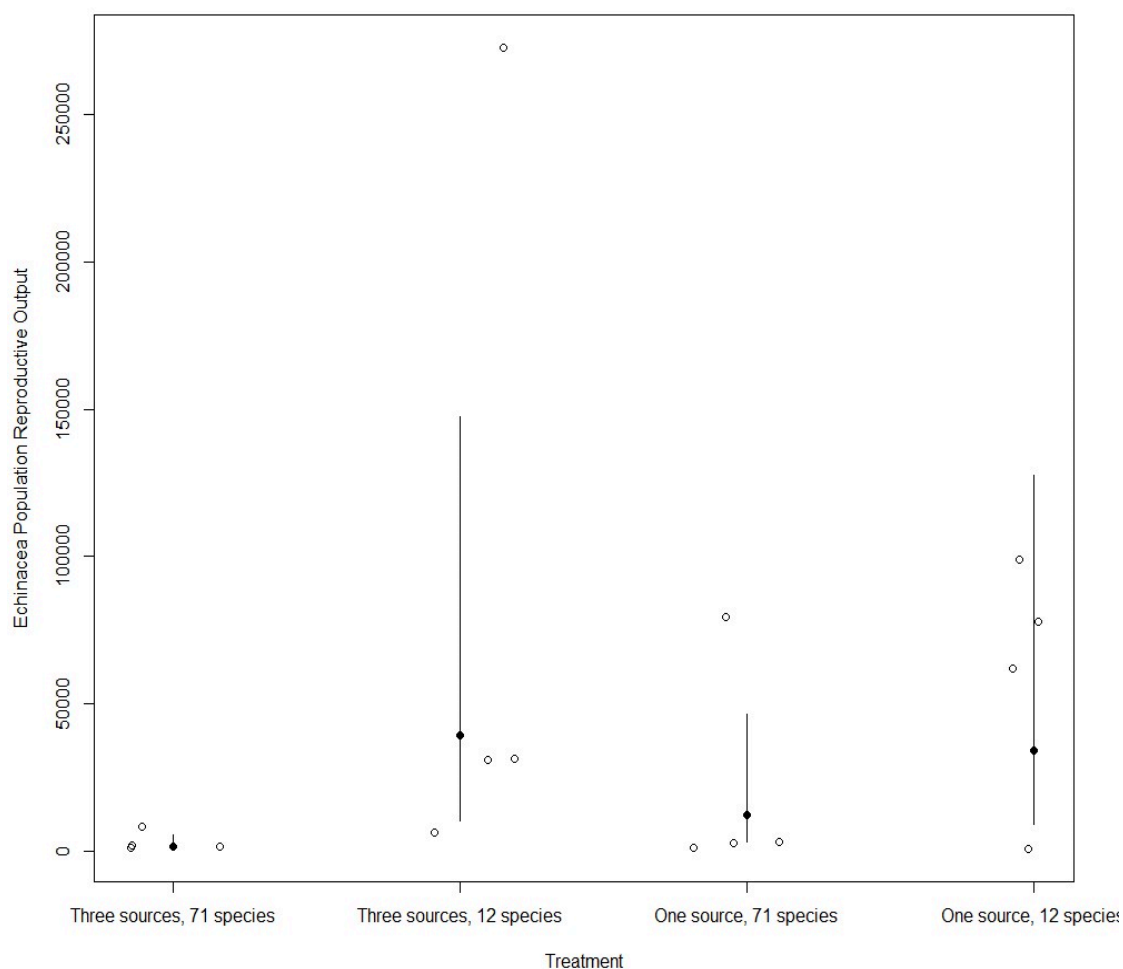


Figure 4. *Echinacea* population scale

Seed count for all of the *Echinacea purpurea* individuals at the population scale was influenced by an interaction between the number species sown and number of *Echinacea* sources sown into a prairie restoration experiment. Seed counts were lower when prairie plantings were sown with 71 species and three *Echinacea* sources, relative to 12 species and three *Echinacea* sources and single *Echinacea* source plantings.

Figure 4 (cont'd)

Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 5. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on population-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	200.1	1	<2.2e-16
Source	5.4	1	0.0196
Richness	16.6	1	0.00005
Source X Richness	3.9	1	0.049

Table 6. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in population-level reproductive output for *Echinacea purpurea* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-3.26	<0.001
3 sources, 71 species vs. 1 source, 71 species	-1.89	0.02
3 sources, 71 species vs. 1 source, 12 species	-2.91	<0.001
3 sources, 12 species vs. 1 source, 71 species	1.37	0.102
3 sources, 12 species vs. 1 source, 12 species	0.35	0.67
1 source, 71 species vs. 1 source, 12 species	-1.01	0.226

Ratibida infructescence scale

I did not find any significant effects of the number of species sown or number of *Ratibida* sources sown on *Ratibida* infructescences, nor was there evidence for an interaction between these two factors (Figure 5, Table 7). None of the four treatment groups differed (Table 8.)

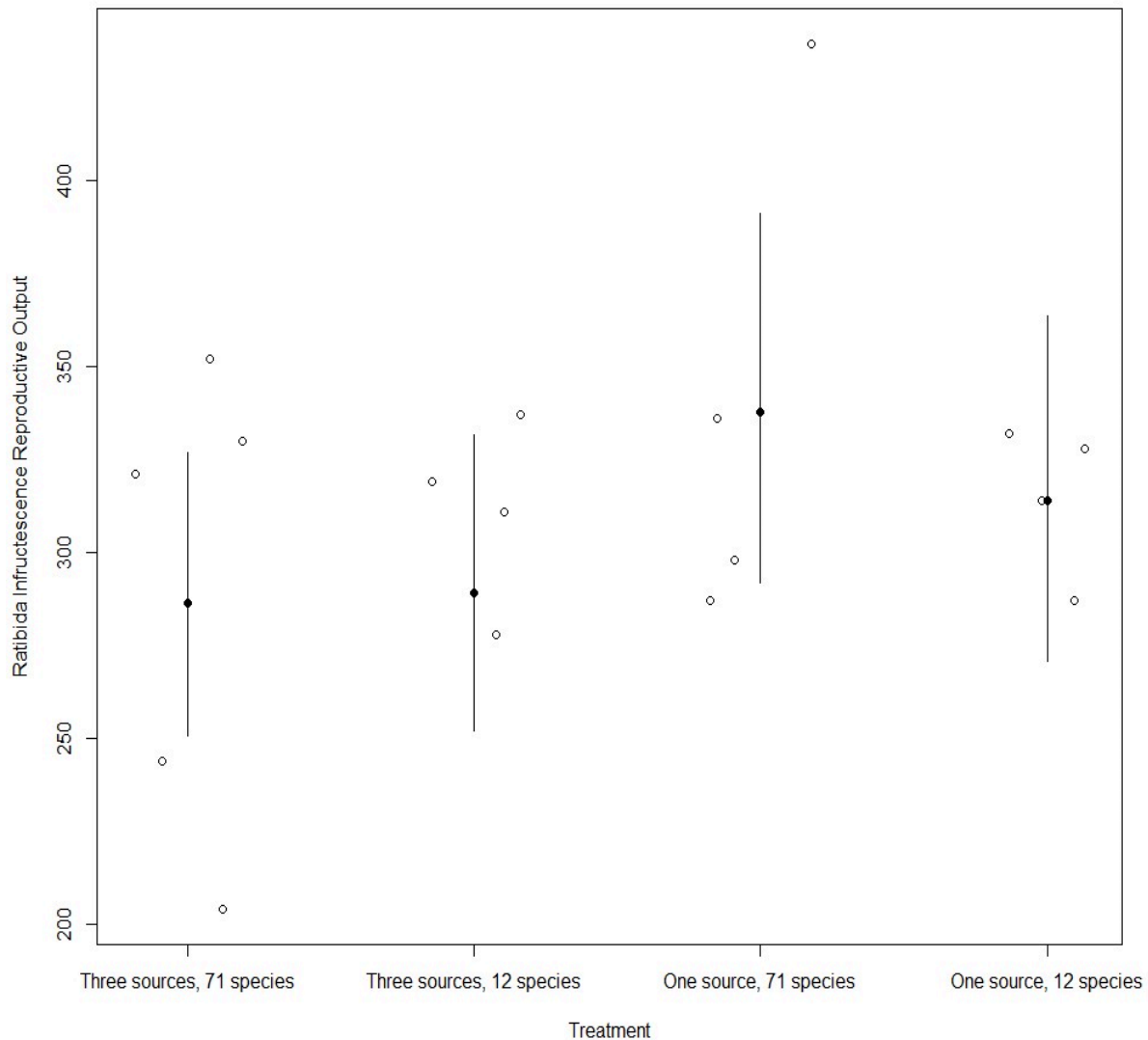


Figure 5. *Ratibida* infructescence scale

There were no significant effects of the number of species sown and number of *Ratibida* sources sown on *Ratibida* infructescence reproductive output. Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 7. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on infructescence-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	7119.6	1	<2e-16
Source	2.6	1	0.104
Richness	0.3	1	0.581
Source X Richness	1.06	1	0.303

Table 8. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in infructescence-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.04	0.581
3 sources, 71 species vs. 1 source, 71 species	-0.16	0.104
3 sources, 71 species vs. 1 source, 12 species	-0.09	0.346
3 sources, 12 species vs. 1 source, 71 species	-0.12	0.249
3 sources, 12 species vs. 1 source, 12 species	-0.05	0.614
1 source, 71 species vs. 1 source, 12 species	0.07	0.364

Ratibida ramet scale

I did not find evidence for effects on source number and sown richness on *Ratibida* ramet-scale seed count; however, there was a significant interaction between source number and richness (Table 9, Figure 6). Half-sites sown with a single source

and 71 species had 25% more ramet-scale seed counts than half-sites sown with a single source and 12 species (Table 10). All other treatments did not differ (Table 10).

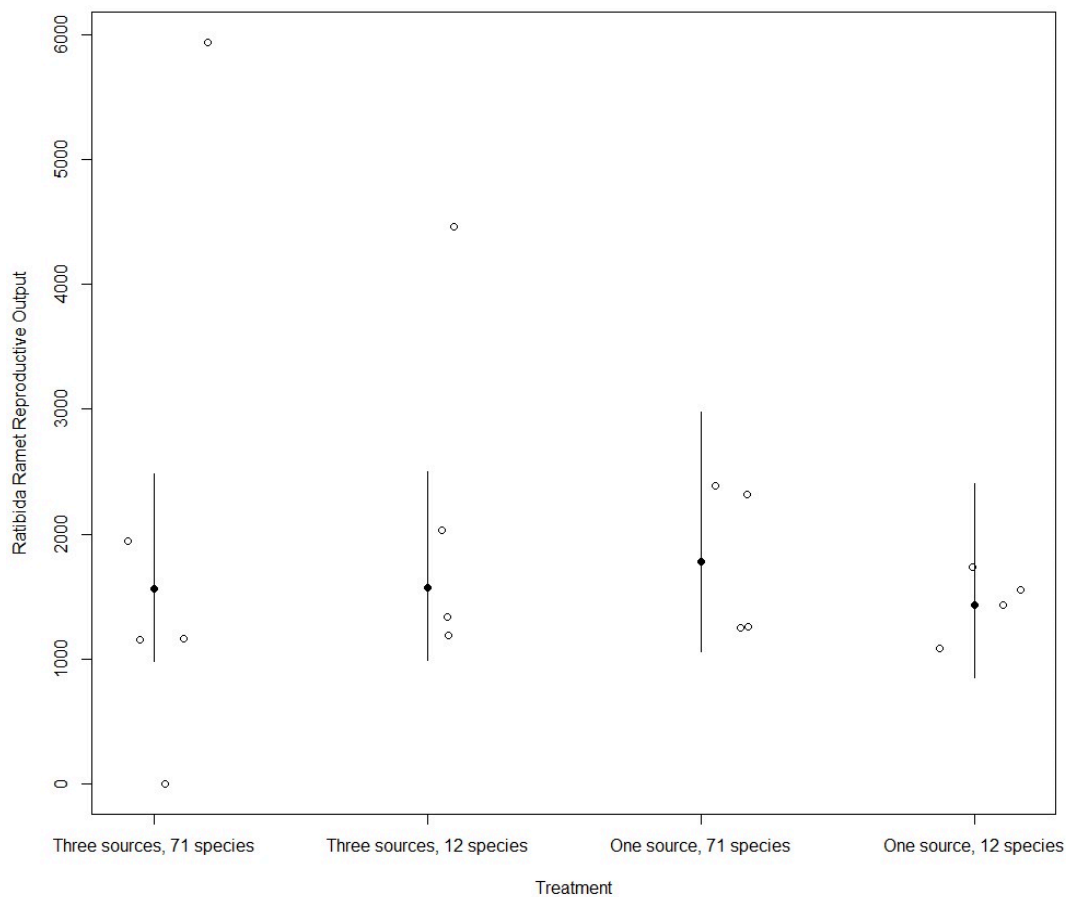


Figure 6. *Ratibida* ramet scale

Seed count for *Ratibida* at the ramet scale was influenced by an interaction between the number species sown and number of *Ratibida* sources sown into a prairie restoration experiment. Seed counts were higher when prairie plantings were sown with 71 species and a single *Ratibida* source, relative to 12 species and single *Ratibida* source. Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 9. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on ramet-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	969.45	1	<2e-16

Table 9 (cont'd)

Source	0.13	1	0.715
Richness	0.28	1	0.599
Source X Richness	90.24	1	<2e-16

Table 10. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in ramet-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.00805	0.599
3 sources, 71 species vs. 1 source, 71 species	-0.13	0.715
3 sources, 71 species vs. 1 source, 12 species	0.09	0.808
3 sources, 12 species vs. 1 source, 71 species	-0.12	0.732
3 sources, 12 species vs. 1 source, 12 species	0.09	0.79
1 source, 71 species vs. 1 source, 12 species	0.22	<0.001

Ratibida population scale

I found evidence suggesting an effect of sown richness and an interaction between source number and sown richness on *Ratibida* population-scale seed counts (Table 11, Figure 7). With this significant interaction, I interpret results in context of the interaction. Half-sites sown with three sources and 71 species had 36% less population-scale seed counts than half-sites sown with three sources and 12 species (Table 12). Additionally, half-sites sown with a single source and 71 species had 2% more population-scale seed counts than half-sites with a single source and 12 species (Table 12).

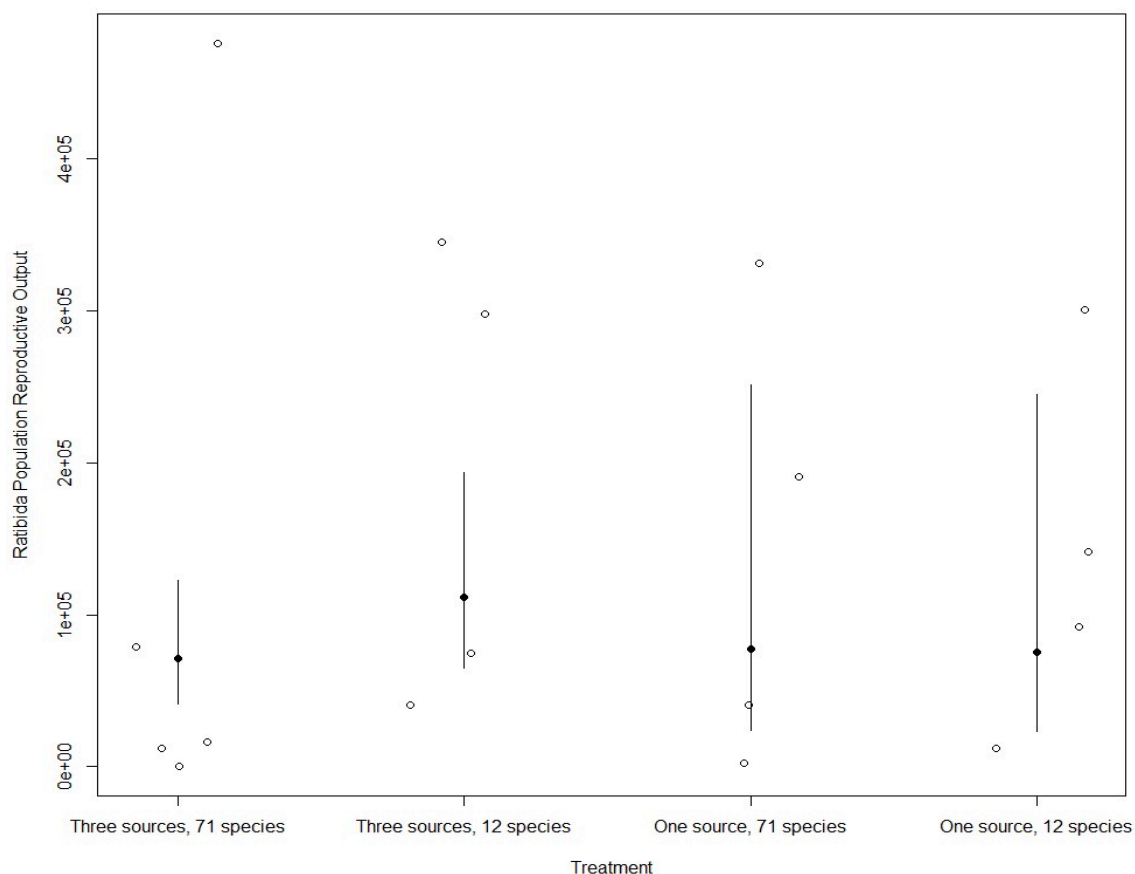


Figure 7. *Ratibida* population scale

Seed count for all of the individuals at the population scale was influenced by an interaction between the number species sown and number of *Ratibida* sources sown into a prairie restoration experiment. Seed counts were lower when prairie plantings were sown with 71 species and three *Ratibida* sources, relative to 12 species and three *Ratibida* sources. Additionally, seed counts were higher when prairie plantings were sown with 71 species and a single *Ratibida* source, relative to 12 species and single *Ratibida* source. Hollow circles represent observed seed counts, solid circles and lines represent predicted seed counts and 95% confidence intervals.

Table 11. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on population-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	1590.2	1	<2e-16

Table 11 (cont'd)

Source	0.017	1	0.897
Richness	56257.2	1	<2e-16
Source X Richness	30387.6	1	<2e-16

Table 12. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in population-level reproductive output for *Ratibida pinnata* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.45	<0.001
3 sources, 71 species vs. 1 source, 71 species	-0.08	0.897
3 sources, 71 species vs. 1 source, 12 species	-0.06	0.927
3 sources, 12 species vs. 1 source, 71 species	0.37	0.573
3 sources, 12 species vs. 1 source, 12 species	0.39	0.548
1 source, 71 species vs. 1 source, 12 species	0.02	<0.001

Echinacea flower-to-fruit conversion rate

I did not find any significant effects of the number of species sown and number of *Echinacea* sources sown on *Echinacea* flower-to-fruit conversion rate (Figure 8, Table 13) None of the four treatment groups differed (Table 14.)

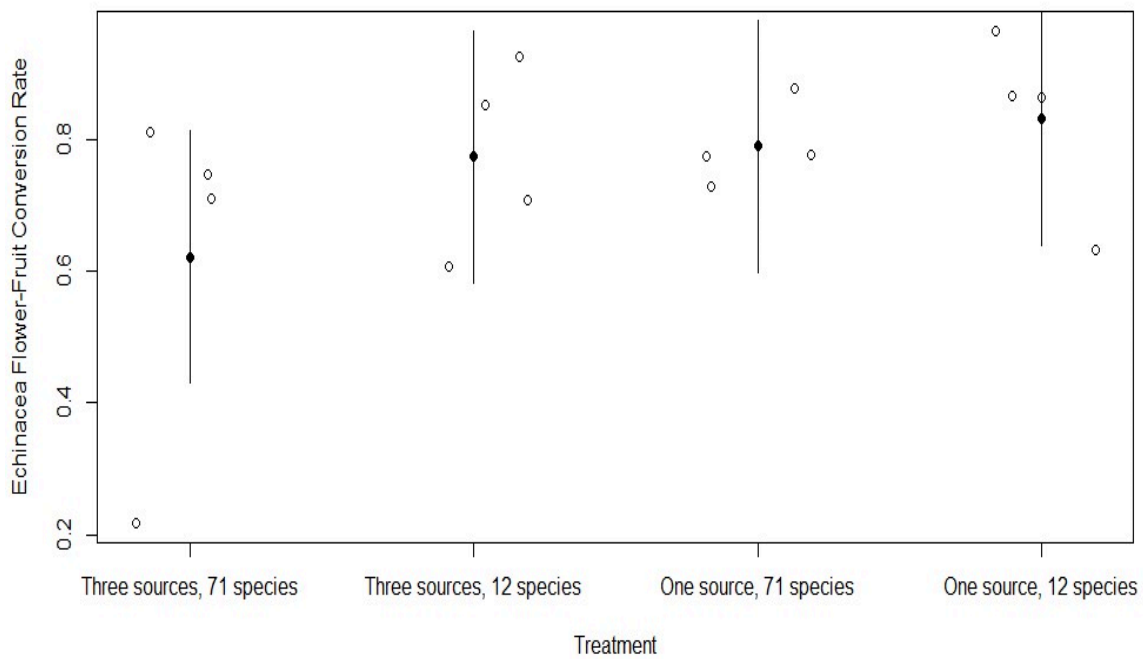


Figure 8. *Echinacea* flower-to-fruit conversion rate

There were no significant effects of the number of species sown and number of *Echinacea* sources sown on *Echinacea* flower-to-fruit conversion rate. Hollow circles represent observed flower-to-fruit conversion rates, solid circles and lines represent predicted rates and 95% confidence intervals.

Table 13. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on flower-to-fruit conversion rate for *Echinacea purpurea* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	52.3	1	4.70E-13
Source	1.9	1	0.167
Richness	3.3	1	0.07
Source X Richness	0.9	1	0.355

Table 14. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in flower-to-fruit conversion rate for *Echinacea purpurea* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.15	0.323
3 sources, 71 species vs. 1 source, 71 species	-0.17	0.537
3 sources, 71 species vs. 1 source, 12 species	-0.21	0.359
3 sources, 12 species vs. 1 source, 71 species	-0.02	>0.999
3 sources, 12 species vs. 1 source, 12 species	-0.06	0.962
1 source, 71 species vs. 1 source, 12 species	-0.04	0.956

Ratibida flower-to-fruit conversion rate

I found evidence for effects of source number and sown richness, but no interaction between these factors on *Ratibida* flower-to-fruit conversion rate (Table 15, Figure 9). Seed counts were 5% lower when prairie plantings were sown with 71 species and three *Ratibida* sources, relative to 12 species and three *Ratibida* sources and single *Ratibida* source plantings. There was no significant interaction between both source number and sown richness (Table 15).

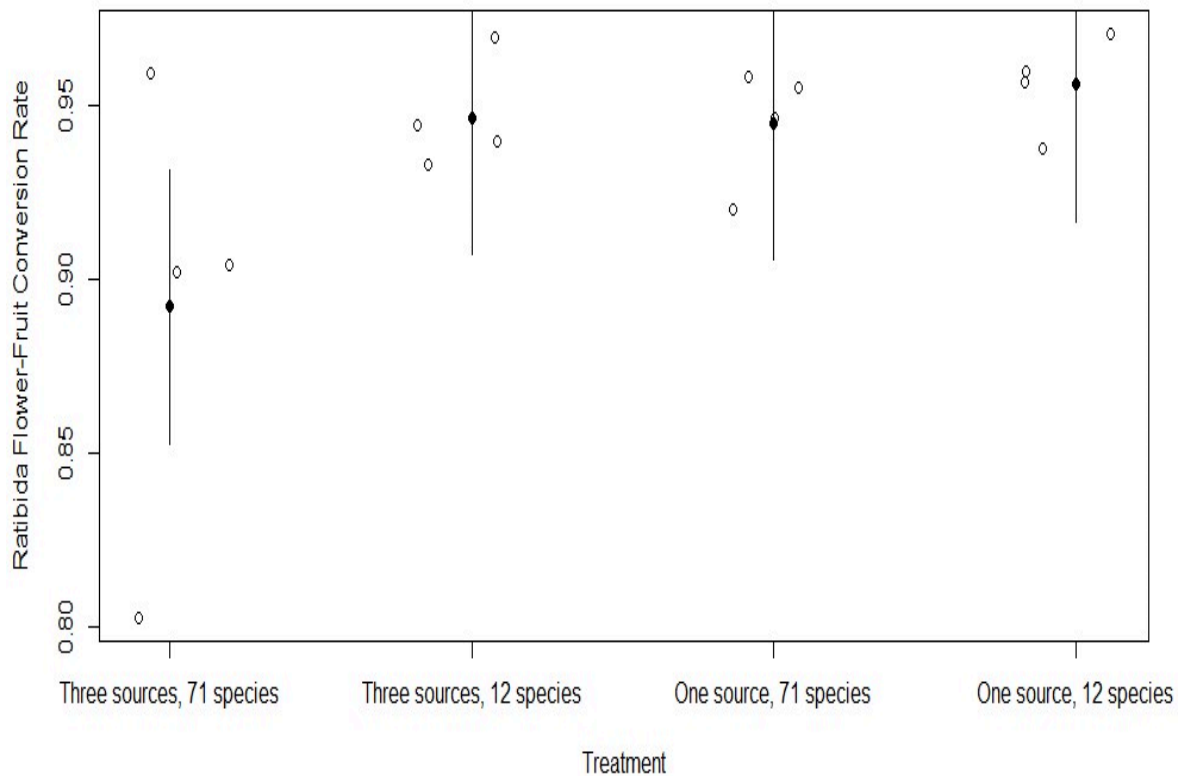


Figure 9. *Ratibida* flower-to-fruit conversion

Rates of flower-to-fruit conversion for *Ratibida pinnata* was influenced by the number of species sown and number of *Ratibida* sources, but the interaction between the two was not significant. The ratio of flower-to-fruit conversion was lower when prairie plantings were sown with 71 species and three *Ratibida* sources, relative to 12 species and three *Ratibida* sources and single *Ratibida* source plantings. Hollow circles represent observed flower-to-fruit conversion rates, solid circles and lines represent predicted rates and 95% confidence intervals.

Table 15. Results of an ANOVA testing the effects of seed source number (1 vs. 3), seeded richness (12 vs. 71) and their interaction on flower-to-fruit conversion rate for *Ratibida pinnata* during prairie restoration.

Treatment	Chisq	Df	p
(Intercept)	2547.1	1	<2.2e-16

Table 15 (cont'd)

Source	4.5	1	0.0337
Richness	6.9	1	0.00830
Source X Richness	2.2	1	0.136

Table 16. Pairwise comparisons among treatment groups with varying combinations of seed source number (1 vs. 3) and seeded richness (12 vs. 71) were conducted to test for differences in flower-to-fruit conversion rate for *Ratibida pinnata* during prairie restoration.

Treatment comparison	contrast	p
3 sources, 71 species vs. 3 sources, 12 species	-0.05	0.025
3 sources, 71 species vs. 1 source, 71 species	-0.05	0.06
3 sources, 71 species vs. 1 source, 12 species	-0.06	0.03
3 sources, 12 species vs. 1 source, 71 species	-0.00156	0.952
3 sources, 12 species vs. 1 source, 12 species	-0.00947	0.713
1 source, 71 species vs. 1 source, 12 species	-0.01	0.606

DISCUSSION

Early management decisions may have implications on the viability of target plant populations if they affect reproductive output. I provide evidence that the richness and number of sources in seed mixes influence plant reproductive output, especially for *Echinacea purpurea* and less so for *Ratibida pinnata*.

Seeding rate has implications for species establishment and reproduction. In our study design, the Brudvig Lab kept the total seed sown constant throughout all half-sites. A consequence of this is that low richness half-sites sown with 12 species had a higher seeding rate per species than high richness half-sites sown with 71 species. Higher seeding rate in half-sites with 12 species led to increased establishment relative to half-sites with 71 sown species, which in turn resulted in higher population-scale reproductive output. Similar seeding rate effects have been documented elsewhere (Applestein et al, 2018; Dickson and Busby, 2009; Pizza et al, 2023). These patterns were especially pronounced at the population scale for both *Echinacea* and *Ratibida*. Half-sites sown with a high density produced larger populations of focal species; this may have attracted a stronger pollinator response, which in turn promoted focal plant populations. Indeed, ninety percent of angiosperms at least benefit from pollinator functioning and services (Ollerton et al., 2011). I see evidence of greater reproductive output in the *Echinacea* infructescence-scale, but not for *Ratibida*. Additionally, the strong pollinator response is expected to increase flower-to-fruit conversion ratio; I do not see this pattern in *Echinacea*, but sowing few species (and thus a higher species-level rate) led to greater flower-to-fruit conversion ratio for *Ratibida*. The stronger pollinator response may be a consequence of pollinators adapting their foraging behavior in accordance to surrounding resource scarcity (Kunin and Iwasa, 1996; Armbruster, 2017). These density-dependent effects are congruent with other studies; low density plant populations displayed significantly reduced visitation rates and seed production, and pollinators visiting sparser plant populations had higher energetic flying costs (Kunin, 1993; Cartar, 2009; Redhead et al., 2016).

There is also evidence for a significant interaction between source and richness, often with lower reproductive output in half-sites that were sown with three sources and 71 species. Incorporation of more genetic diversity by sowing seed from three different

provenances may have introduced variability in genetically-determined traits, such as flowering phenology (Vigo et al., 2013). Phenology is a phenotype of particular importance because it determines when the reproductive organs of a plant are accessible to pollinating insects and for how long, which may have direct implications on reproductive output. Half-sites sown with three sources may have had a higher degree of phenological mismatch, if individuals from different sources did not open flowers synchronously. Phenological mismatches develop when flowering phenology does not overlap with a pollinator's active season and can have deleterious effects on plant reproduction (Forrest, 2015). The smaller population sizes of plants in half-sites sown with 71 species combined with asynchronous flowering phenology may have collectively lowered reproductive output together. However, this conjecture is not well supported via our flower-to-fruit conversion analyses. Although there is some evidence that plants in half-sites sown with 71 species and three sources had lower developed seed count in *Ratibida pinnata*, I did not find any differences in *Echinacea*. Despite our results, Bolmgren and Cowan (2008) found correlations between flowering phenology and seed mass: perennial herbs produced larger seeds when they flowered earlier, while annual plants produced larger seeds when they flowered later. Manipulation of flowering phenology may have consequences to reproductive output.

Our results suggest some implications for restoration practice. Restoring prairie with fewer species will produce a few but larger plant populations that reproduce more effectively. Additionally, incorporating fewer sources may help synchronize flowering phenology in a species, enhancing pollinator functioning. Together, these larger populations with synchronized phenology will stimulate a more robust pollinator response and promote reproduction. These results may be specific to the taxa in this study, and there may be additional benefits to sowing more diverse mixes at the community level and multiple sources over evolutionary time. For example, the number of species sown positively influenced native plant richness and/or diversity (Leps *et al.*, 2007; Carter and Blair, 2012; Kirmer *et al.*, 2012; Catano et al., 2021; Schmidt et al., 2022). The positive effects of seeding rate and number of species sown may collectively help establish a diverse set of plant populations that harbors many individuals. This suggests that when engaging in seed based restoration, practitioners should restore

ecosystems with seed mixes that are more species rich and at a high seeding rate. Breaking away from the current tradeoff many practitioners need to make, of more species at the reduction of seeding rate (Barak et al. 2022), would require additional resources for restoration practice or compromises elsewhere. If more restorations succeed with this technique, it may be more fruitful to focus restoration efforts within a smaller area.

CONCLUSION

Our results suggest that initial seed mix design is important for the reproductive output of target plant populations in restoration. Namely, I found that restorations sown with three sources and 71 species at lower seeding rates decrease reproductive output for *Echinacea* across all biological scales, from individual infructescences to the entire population, but only for *Ratibida* at the population scale. In order to increase predictability in restoration outcomes, practitioners should prioritize high seeding rates in initial seed mix designs. Establishing larger populations will bolster plant reproductive output. Ideally, seed mixes should be more diverse, but not at the cost of lowering seeding rate per species. Our study also suggests that a fewer number of sourcing locations increases plant phenology matching, which enhances plant reproductive output. There are predictable lasting impacts of seed mix design on reproductive output, and our study suggests careful consideration when establishing an initial seed mix for seed based restoration.

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APPENDIX

Table A.1. Seed mix information including seeding rate and seed origin of *Echinacea purpurea* and *Ratibida pinnata* seeds used to restore the CLE prairies.

	<i>Echinacea</i>	<i>Ratibida</i>
Low richness kg/ha	0.71	0.16
Low richness seeds/m ²	16.36	16.36
High richness kg/ha	0.15	0.034
High richness seeds/m ²	3.23	3.23
Local nursery	Naturally Native	Naturally Native
Local source origin	Lucas County, OH	Northwest Indiana
Non-local northern nursery	Agrecol	Agrecol
Non-local northern source origin	Madison, IA	Madison, IA
Southern nursery	Hamilton	Hamilton
Southern non-local source origin	Putnam County, MO	Greene and Hickory Counties, MO