RESEARCH ARTICLE

Optimizing seed mixture diversity and seeding rates for grassland restoration

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Revegetation by seeding is an important tool in restoration. Seeding practices for restoration often rely on standard prescriptions for seed mix diversity and seeding rates. Seed mix diversity and rates are generally low within restoration projects and these practices are typically not informed by research. The objective of this study was to explore a new method for determining an optimal seed mix diversity and seeding rate for restoration of a semiarid grassland. We examined restoration success associated with differing seed mix diversity levels (5–50 species) and seeding rates (400–1,600 pure live seeds [PLS]/m^2) using a response surface regression (RSR) experimental design at 12 disturbed sites in northeastern Colorado. Overall restoration success was evaluated based on optimizing desirability across nine individual responses: biomass and diversity of seeded, volunteer native, noxious, non-native species, and the density of seeded species. Greatest restoration success after four growing seasons occurred at a seed mix diversity of 35 species and a seeding rate of 1,366 PLS/m^2. RSR experimental design and analysis has seldom been used to answer ecological questions. This novel approach to address a pressing restoration challenge provided unique insight into how seed mix diversity and seeding rate, singly or in combination, influence the first 4 years of plant community development and overall restoration success. These results suggest that including more native species and seeding at higher rates than current practice could lead to greater restoration success in grasslands.

Key words: multifactor optimization, plant diversity, response surface regression, seed mix diversity

Implications for Practice

- Response surface regression methodology may be a valuable tool for determining optimal approaches to different facets of restoration, including seed mix diversity and seeding rates.
- Current restoration seeding rates and seed mix diversity may be inadequate for maximizing grassland restoration success. Managers should consider using more species in seed mixes and applying those mixes at higher rates.
- If practitioners are considering trade-offs between higher seeding rates versus greater diversity, increasing seed mix diversity may be more influential on grassland restoration success.
- Given the need for increased diversity of seed mixes indicated by our results, collaborations with the seed industry should be investigated to increase the affordability and commercial availability of a wider range of native plant species for restoration.

Introduction

Revegetation is often an important tool for restoration projects and the introduction of plants to restoration sites is often conducted by seeding. However, despite the central role of seeding in restoration, seeding of restoration sites is often carried out based on generalized or prescribed seeding rates of mixtures containing remarkably few species with little consideration of how those mixtures or rates relate to restoration objectives. This lack of consideration is often necessitated by a lack of information on how many plant species to include in a seed mix as well as an ideal seeding rate for meeting project goals. Here we describe a study where we utilized a novel experimental design to determine the optimal combination of seeding rate and seed mix diversity for restoration success in a semiarid grassland ecosystem.

Many ecological studies have examined the effects of plant species diversity on community health and functioning, productivity, resilience, invasion resistance, and recovery (Tilman 1997; Tilman et al. 2001; Cameron 2002; Biondini 2007; Quijas et al. 2010). For example, diverse grassland systems tend to have higher productivity (Tilman et al. 2001), resistance to

invasion (Maron & Marler 2007), and resilience following disturbances (Hector et al. 2010). Therefore, restoration seed mixes should promote development of diverse plant communities in order to attain these benefits. If the restoration target is a highly diverse community, then perhaps sowing more diverse seed mixes may result in higher diversity plant communities. Relatively few studies have examined this question in restoration of semiarid grassland systems (Lepš et al. 2007; Kirmer et al. 2012). Kirmer et al. (2012) found that high (51 species) versus low (three species) diversity seed mixtures accelerated vegetation development, productivity, and erosion control on bare surface mined lands. They also found that resulting local biodiversity was higher on sites seeded with the high diversity mixture (Kirmer et al. 2012). Lepš et al. (2007) also found that sowing high diversity (15 species) seed mixes was more successful than sowing low diversity (four species) mixes. They suggested that high diversity seed mixes were primarily important for their insurance effect (Yachi & Loreau 1999) because plots seeded with high diversity mixtures were capable of compensating for the failure of some species to establish (Lepš et al. 2007).

Seeding rate is also an important factor to consider when seeding for restoration. The role that seeding rate plays in the resulting target plant community and restoration success has received more attention than that of seed mix diversity in grassland systems (Mueggler & Blaisdell 1955; Launchbaugh & Clenton 1970; Hull 1974; Papanastasis & Biswell 1975; McMurray et al. 1997; Williams et al. 2002; Sheley & Half 2006; Dickson & Busby 2009). Dickson and Busby (2009) looked at the effect of forb and grass seeding rates on resulting forb density in mixed-grass prairie. They found that decreasing grass seeding rates and increasing forb seeding rates resulted in communities with higher forb diversity and density, which was their goal (Dickson & Busby 2009). Williams et al. (2002) examined the effect of sagebrush and grass seeding rates on restoration success. They found that higher sagebrush seeding rates and intermediate grass seeding rates came closest to their restoration goal.

Planning for restoration seeding involves determining both the number of species to use in the mix and seeding rate. However, few studies have simultaneously looked at the effect of these two variables on resulting plant communities and overall restoration success (Carter & Blair 2012; Nemec et al. 2013) and of these few studies, none have been able to address questions regarding the interaction of seeding rate and seed mix diversity in restoration settings. Nemec et al. (2013) examined the effect of sowing high (97 species) and low (15 species) diversity seed mixtures at high (297 pure live seeds [PLS]/m^2 drill seeded) and low (148 PLS/m^2 drill seeded) rates on invasion resistance in mesic tallgrass prairie. A higher diversity seed mixture increased invasion resistance, but higher seeding rate did not. Carter and Blair (2012) examined the effect of seed mix diversity and seeding rate in mixed-grass prairie. Their results showed that the higher seeding rate (344 vs. 172 PLS/m^2) and higher diversity seed mix (95 species vs. 15 species) resulted in more successful grassland restoration (greater diversity and cover of native and seeded species, and lower diversity and

cover of exotic species). Both of these studies only tested two levels (high vs. low) of each factor (seeding rate and seed mix diversity), which leads to questions about potential effects of intermediate seed mix diversities and rates on restoration success.

Typically, standard seed mixes and rates used to revegetate disturbances in western North American semiarid grasslands (where this study was conducted) are characterized by relatively low diversity (3–10 species) and low seeding rates (400–600 PLS/m^2 broadcast seeded). The five most common species used in restoration seed mixes in this region are late-seral perennial grasses (*Bouteloua gracilis*, *Boutleoua dactyloides*, *Pascopyrum smithii*, *Achnatherum hymenoides*, and *Hesperostipa comata*). Seldom are annual grasses and forbs or perennial forbs and shrubs used. The use of low diversity mixtures seeded at low rates is due primarily to economic constraints (forb and shrub seeds are expensive relative to grass seed) and a lack of recognition that any departure from standard approaches is warranted. However, if increasing upfront investment in a seed mix can improve the likelihood of restoration success and decrease the potential need for follow-up reseeding, it may be more cost-effective in the longer term. Understanding the most effective combination of seeding rate and seed mix diversity is an important but under-explored area in revegetation science.

In this study, we explored the influence of seed mix diversity and seeding rates and their interactions on restoration effectiveness in a grassland ecosystem and identify optimal seed mix diversity and seeding rates for achieving restoration success using response surface regression (RSR) methodology (Box & Wilson 1951; Box & Hunter 1957). The RSR methodology has seldom been used to answer ecological questions (Clancy & King 1993; Giloni-Lima et al. 2010) and is a novel approach to understanding the effectiveness of restoration strategies. The methodology, which includes both an experimental design component and statistical analysis component, offers three primary benefits for testing such questions aimed at improving restoration outcomes. First, it allowed us to test two continuous explanatory variables, in our case seed mix diversity and seeding rate, across an entire response space using only nine treatments (Kuehl 2000). A traditional two-factor analysis of variance (ANOVA) design would require a large number of treatments per replicate and limit inference to the discrete levels of each factor tested. Second, the RSR approach assesses a second-degree polynomial model, which also allowed us to examine the shape of the response space and explore optima of each response variable (Kuehl 2000; Myers 2002). Finally, the RSR methodology provided an avenue for identifying levels of both explanatory variables that maximize overall restoration success (i.e. desirability) across multiple response variables (Castillo et al. 1996; Obermiller 1997; NIST/SEMATECH 2012). Restoration projects often have many metrics of success, with each metric typically analyzed individually. Using multiresponse optimization with RSR, all metrics can be assessed simultaneously and a desirability surface created to examine how changes in the explanatory variables influence overall outcomes. Response variables used to define restoration success or desirability can be driven by the objectives and goals of a

particular restoration project and could include factors other than ecological metrics. For example, although not relevant to our particular questions, other RSR applications could include financial variables (e.g. seed mix cost) if deemed important for meeting project goals.

In applying this technique to restoration of shortgrass steppe, we identified nine plant community characteristics to define a desirable outcome (i.e. restoration success) as one that maximizes biomass, diversity, and density of seeded species and biomass and diversity of volunteer native species, while also minimizing biomass and diversity of noxious species and other non-native species. In addition, RSR desirability functions provide the ability to differentially weight the importance of response variables in assessing optimal overall restoration success. For example, we considered maximizing seeded species (biomass, diversity, and density) and minimizing noxious species (biomass and diversity) to be more important than, and so given twice the weight of, volunteer native species and other non-native species responses in our experiment. Here, we apply this methodology to a semiarid shortgrass steppe system to (1) test effects of seed mix diversity and seeding rates on restoration outcomes, and (2) demonstrate the applicability and potential utility of RSR methodology for addressing questions aimed at improving restoration success. We hypothesized that restoration success would be maximized at the highest seeding rates combined with greatest seed mix diversities. We also hypothesized that seeding rate and seed mix diversity would be somewhat substitutable (i.e. the same degree of success could be achieved with low seed mix diversity–high seeding rate or high seed mix diversity–low seeding rate).

Methods

Study Sites

This study was conducted on 12 sites across three locations in northeastern Colorado. Locations were chosen to represent common shortgrass steppe site conditions and soil types (Table S1, Supporting Information) to allow inference of results to a wide range of sites throughout shortgrass steppe. All sites fall within the rangeland loamy plains ecological site description, receive 305–410 mm of precipitation annually, and are 1,500–1,650 m in elevation (NRCS 2007). Six sites were located in Larimer County, Colorado, on Fort Collins loam, Kim loam, or Stoneham loam soils with 1–3% slopes (NRCS 2014). Dominant vegetation at these sites consisted of *Agropyron cristatum* (non-native perennial grass), *Ericameria nauseosa* (native perennial shrub), and *Convolvulus arvensis* (non-native perennial forb). Three sites were located in Weld County, Colorado, on Stoneham fine sandy loam, Nunn loam, or Ascalon fine sandy loam soils with 0–9% slopes (NRCS 2014). Dominant vegetation at these sites consisted of *Bouteloua curtipendula* (native perennial grass), *Pascopyron smithii* (native perennial grass), *Hesperostipa comata* (native perennial grass), and *Lolium* spp. (non-native annual grass) (NRCS 2007). The remaining three sites were also located in Weld County, Colorado, on Olney fine sandy loam or Renohill-Shingle complex
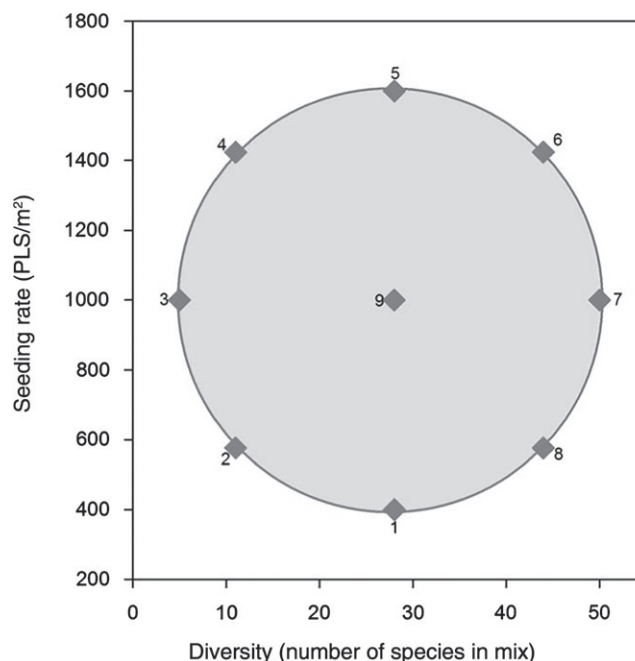

Figure 1. Response surface space based on rotatable central composite RSR methodology (Kuehl 2000) with nine combinations of seeding rate (PLS/m^2) and seed mix diversity (number of species in mix) treatments (gray diamonds). See the text for additional details on statistical method.

soils with 0–9% slopes (NRCS 2014). Dominant vegetation at these sites consists of *Bouteloua gracilis* (native perennial grass), *P. smithii*, *Aristida purpurea* (native perennial grass), *Boutleoua dactyloides* (native perennial grass), and *Opuntia polyacantha* (native perennial shrub).

Experimental Design

A RSR experimental design (Box & Wilson 1951; Box & Hunter 1957) was used to identify treatment combinations of seed mix diversity and seeding rate for testing responses of nine plant community characteristics (biomass and diversity of seeded, volunteer native, noxious, and non-native species, and the density of seeded species) we used to define restoration success. These nine measures were selected based on typical measures of restoration success of grasslands in our region of study. The selection of these measures to characterize success can be readily adjusted to suit the goals of any restoration project. Using only nine treatments per RSR methodology, we were able to identify optimal seed mix diversity and seeding rates falling anywhere within the range of explanatory variables tested on a curvilinear response surface (Fig. 1).

Minimum (five species, 400 PLS/m^2) and maximum (50 species, 1,600 PLS/m^2) levels of each factor were chosen based on current restoration practices in this region and ecological considerations (e.g. best seeding rates by Burton et al. 2006), respectively. Because of lack of previous research and uncertainty regarding where optima for seed mix diversity and seeding rate might lie, it was important to chose levels that would test a substantial response surface. Minimum and maximum values

were then used to calculate three mid-range values of each factor and treatment combinations were determined using rotatable central composite RSR methodology (Fig. 1; Kuehl 2000). Each site had 13 plots and nine treatments: one plot each of treatments 1–8, and five plots of treatment 9 (28 species at 1,000 PLS/m^2, RSR center point) (Fig. 1). The center point treatment (treatment 9) was replicated five times at each site to calculate pure experimental error for the RSR model (Freund & Littell 2000; Kuehl 2000). Additional details on the statistical analysis portion of the RSR framework are given below and in the "Statistical Analysis" subsection.

At each study site, a 16 × 10-m disturbance was created in order to mimic a variety of surface disturbances and site preparation practices relevant to semiarid grasslands. This disturbance entailed removal of vegetation and tilling of the soil, both of which are common in grassland restoration projects where disturbances remove vegetation (fire, resource extraction, and farming) and where tillage is often essential (Morgan 2005). Each site was first raked to remove as much of the litter layer as possible and discarded outside the study site. Sites were then mowed with a brushcutter to remove vegetation. Cut vegetation was raked and removed from the study area. Following mowing, sites were rototilled twice lengthwise and once widthwise using a self-propelled rear-tine rototiller to an average depth of 15 cm. Throughout the tilling process, root masses were removed by hand from the study site. After tilling, sites were raked thoroughly to remove remnant plant debris, roots, and litter and then rested for 1 week before applying treatments.

Treatments

In disturbed areas at each site, 13 study plots of 2 × 2 m were established in November 2011 with a 1-m buffer zone between plots and a 1-m buffer zone bordering the disturbed area. Per RSR methodology (discussed above), treatments consisted of nine combinations of five diversity levels (5–50 species) and five seeding rates (400–1,600 PLS/m^2) (Table S2). Seed mixes were constructed to achieve increasing levels of functional redundancy with all mixes having the same level of functional diversity. Each mix was comprised of species in five different functional groups: perennial and annual grass(es), perennial and annual forb(s), and shrub(s). Treatments were randomly assigned to plots at each site.

Each plot was first lightly raked to loosen and prepare a seedbed. Larger seeds were evenly broadcast by hand. After larger seeds had been sown, they were harrowed into the soil by hand using a 1-m piece of chain link fence laid across the soil surface and gently pulled back and forth to incorporate seed. After harrowing, smaller seeded species (*Artemisia filifolia*, *Artemisia frigida*, *Artemisia ludoviciana*, *Erysimum capitatum*, and *Sporobolus cryptandrus*) were broadcast seeded onto the plot. Certified weed-free straw mulch was applied to the plot to achieve 50% cover. However, in January of 2012 strong winds displaced most of the applied straw mulch. WoodStraw$^®$ mulch (Forest Concepts LLC, Auburn, WA, U.S.A.) was applied shortly after to achieve 50% cover. Although these techniques are not standard in large-scale restoration projects in this region,

we used them here due to limitations imposed by small study plots where large equipment for seedbed preparation and seeding were impractical.

Early and mid- growing season (March–June) in 2012 and 2013 were drier and warmer relative to average precipitation amounts and temperatures for the area (USDM 2015). In 2012, sites ranged from moderate drought status to severe drought status (USDM 2015). To compensate for potential limited plant establishment in 2012 due to drought conditions, supplemental water was applied to all sites. Water supplements were provided in four watering events during April–June of 2012 for a total of 3.8 cm.

Data Collection

Vegetation biomass and density were assessed in July of 2012, 2013, and 2015. Two 25 × 75-cm quadrats were sampled in each plot; quadrats were placed within plots such that the same area was not sampled more than once. For biomass, current year's growth was clipped from the two subplot frames within each plot by species. Biomass was dried to a constant mass at 65°C and dried samples were weighed to determine total aboveground biomass per plot for each species. Density counts of individual seeded species were conducted in each sampling frame. Biomass and density estimates were pooled for the two frames in each plot.

Statistical Analysis

RSR analysis (Myers 2002) was performed to assess linear and quadratic model terms and response optimums in two-dimensional factor (seed mix diversity × seeding rate) space (RSR macro, JMP 12.0.0 SAS Institute Inc., Cary, NC, U.S.A., 1995–2005). Response surfaces were constructed based on five model terms: rate, seed mix diversity, rate^2, seed mix diversity^2, and rate × seed mix diversity. Significance of squared and cross-product parameters indicate the importance of quadratic relationships between independent and dependent variables whereas parameters that are not squared suggest that linear relationships are important. Desirability functions were used to perform multiresponse optimization by simultaneously optimizing all nine response variables (Castillo et al. 1996; Obermiller 1997). Seeded and volunteer native response variables were maximized (i.e. higher biomass and diversity of seeded and volunteer native species and higher density of seeded species is most desired), whereas noxious and non-native non-noxious response variables were minimized (i.e. lower biomass and diversity of noxious and non-native species is most desired). Seeded and noxious response variables were weighted with an importance ranking = 2, while all other response variables were assigned importance ranking = 1. Desirability values incorporate simultaneous optimization across all response variables and are on a scale of 0 to 1 with zero indicating a completely undesirable outcome and one indicating a completely desirable or ideal outcome (NIST/SEMATECH 2012). Sensitivity analyses were conducted using the sensitivity indicator in JMP12 and were

graphically assessed to determine which, if any, individual response variables were more important than the others in determining overall optimal values for seed mix diversity and seeding rate. Because rapid changes in plant communities were expected through the first several years following restoration, analyses for each year were run separately. Site was included in the model as a random effect to broaden the scope of inference to any similar sites in shortgrass steppe. Response variables were transformed as necessary to adjust for normality; means and standard errors for each response variable prior to transformation are provided in Table S3 for each treatment and year. For all years, seeded species biomass, volunteer native biomass, and non-native non-noxious biomass were fourth-root transformed, noxious species biomass was square-root transformed, seeded species diversity, volunteer native diversity, and seeded species density were natural log + 1 transformed. Transformations were not necessary for noxious and non-native non-noxious species diversity values in any year.

Results

In each year of the study, all nine response variables (biomass and diversity of seeded, volunteer native species, non-native non-noxious species, noxious species, and density of seeded species) had significant individual RSR models, suggesting that each was affected by seed mix diversity, seeding rate, the random site effect, or some combination of these explanatory variables. Despite relatively high r^2 values ($0.46 \leq r^2 \leq 0.95$), year 1 (2012) was the only one in which all nine response variables passed the lack of fit test for the quadratic model meaning that as community development progressed over time other factors were likely also having a large impact on those individual response variables for which the quadratic model did not fit (variables with asterisks in Table 1). Significant relationships with explanatory variables (seed mix diversity, seed mix diversity^2, seeding rate, seeding rate^2, or seed mix diversity × seeding rate) occurred in all 3 years for seeded species biomass, diversity, and density and volunteer species biomass and diversity (Table 1).

Seeded species biomass responded to seed mix diversity^2 in 2012 ($p < 0.05$) and the seed mix diversity × seeding rate interaction in 2013 ($p < 0.05$) (Table 1; Fig. S1). By 2015, seeded species biomass was unimodally related to seed mix diversity and increased linearly with seeding rates (Fig. S1). Response surfaces of seeded species diversity largely mirrored those of seeded species biomass in all years (Fig. S1). In all 3 years, there was a significant unimodal relationship between density of seeded species and seed mix diversity (Table 1); seeding rate was also significant as either a linear main effect (2012 and 2015) or as an interaction with seed mix diversity (2013) (Table 1; Fig. S1).

Response of volunteer species biomass to seed mix diversity and seeding rates changed over time, from being most strongly influenced by a unimodal relationship with seeding rate in 2012 and 2013 (rate^2 $p < 0.05$) to be being primarily driven by a negative linear relationship with seed mix diversity in 2015

($p < 0.05$) (Table 1; Fig. S1). Similar changes in the shape of the response surfaces from 2012 to 2015 were found for volunteer species diversity though by 2015 the relationship with seed mix diversity was more nonlinear (diversity^2 $p < 0.05$) (Fig. S1).

Initially, in 2012, overall optimal desirability of seed mix diversity and seeding rate across all nine response variables was found to be at a seed mix diversity of 39 species and a seeding rate of 1,205 PLS/m^2 which resulted in a 0.50 desirability value (Table 2; Figs. 1 & S2). By 2015, overall optimal desirability of seed mix diversity and seeding rate was at 35 species and 1,366 PLS/m^2, respectively, with a desirability value of 0.56 (Table 2; Figs. 1 & S2). Sensitivity analysis showed no major differences in explanatory variables driving overall optimal desirability levels.

Conducting RSR analyses individually for each of the 12 sites based on data collected in 2015, optimum seeding rate was 1,232 ($\pm$132) PLS/m^2, optimum seed mix diversity was 40 ($\pm$3) species, and overall desirability was 0.70 ($\pm$0.03) averaged across individual sites (Table S4).

Discussion

The objective of this study was to explore RSR methodology for determining an optimal seed mix diversity level and corresponding seeding rate for achieving the greatest restoration success. Based on the success criteria that we selected, a relatively high diversity seed mix (35 species in 2015) in combination with a moderately high seeding rate (1,366 PLS/m^2 in 2015) resulted in highest overall restoration success when integrated across nine plant response variables. Optimal levels of seed mix diversity and seeding rate fell within the ranges we tested (5–50 species at 400–1,600 PLS/m^2) in all years, which indicates the response region selected for this study was appropriate and sufficient. Although optimal levels for both variables were at the higher end of the ranges tested, they did not fall at the maximum level tested possibly suggesting a plateau effect (Hardin 1960), competitive exclusion (Grime 1973; Bakker et al. 2003), or the failure of certain species to establish.

Few studies have examined the combined effects of seed mix diversity and seeding rate (Carter & Blair 2012; Nemec et al. 2013). If there is a substitution effect between seed mix diversity and seeding rate (e.g. high seed mix diversity–low seeding rate as successful as low seed mix diversity–high seeding rate), we would expect diversity × rate interactions to be significant. Although both seeded species biomass and density in 2013 had significant diversity × rate interactions and response surfaces (Fig. S2) suggest decreased success where seed mix diversity and seeding rate were either both high or both low, the interaction was transient. Instead, our results indicate seed mix diversity was often more important than rate, as Nemec et al. (2013) also found. Where both diversity and rate were significant, the relationship tended to be additive, similar to the finding of Carter and Blair (2012). By exploring the entire response surface, however, our results suggest that seed mix diversity and seeding rate may influence restoration outcomes

Table 1. Response surface regression model results with five explanatory variables (seed mix diversity and rate, each term squared, and cross-product) and site as a random effect for each of nine response variables (biomass and diversity of seeded, volunteer native, noxious, non-native non-noxious species, density of seeded species). All models were significant with $p < 0.0001$. *Indicates response variables not passing a lack of fit test for the quadratic model ($p < 0.05$). p-Values are given for significant model parameters; a dash (—) indicates $p > 0.05$.

Response Variables		F	r^2	Significant Model Parameters (p-Values)				
				Diversity	Rate	Diversity^2	Rate^2	Diversity × Rate
2012 (Year 1)								
Biomass	Seeded	9.16	0.51	<0.0001	—	0.017	—	—
	Volunteer	7.54	0.46	0.003	—	—	0.016	—
	Noxious	39.16	0.82	—	—	—	—	—
	Non-native	32.41	0.79	—	—	—	—	—
Diversity	Seeded	14.27	0.62	<0.0001	0.0002	0.0003	—	—
	Volunteer	12.17	0.58	<0.0001	—	0.027	0.004	0.014
	Noxious	31.70	0.78	—	—	—	—	—
	Non-native	11.75	0.57	—	—	—	—	—
Density	Seeded	14.52	0.63	<0.0001	0.005	<0.0001	—	—
2013 (Year 2)								
Biomass	Seeded	17.21	0.66	0.0001	0.040	—	—	0.041
	Volunteer*	13.19	0.60	0.003	—	—	0.030	—
	Noxious*	155.78	0.95	—	—	—	—	—
	Non-native	20.50	0.70	—	—	—	—	—
Diversity	Seeded	17.11	0.66	<0.0001	0.004	0.042	0.033	—
	Volunteer	13.35	0.61	<0.0001	—	—	0.012	—
	Noxious	39.35	0.82	—	—	—	—	—
Diversity	Non-native	8.41	0.49	—	—	—	—	—
Density	Seeded	16.46	0.65	<0.0001	—	0.009	—	0.014
2015 (Year 4)								
Biomass	Seeded	12.91	0.60	<0.0001	0.013	0.005	—	—
	Volunteer	10.71	0.55	<0.0001	—	—	—	—
	Noxious	17.44	0.67	—	—	—	—	—
	Non-native *	56.36	0.87	—	—	—	—	—
Diversity	Seeded	19.51	0.69	<0.0001	<0.0001	<0.0001	—	—
	Volunteer	17.99	0.67	0.0002	—	0.033	—	—
	Noxious	11.19	0.56	—	—	—	—	—
	Non-native*	15.74	0.64	—	—	—	—	—
Density	Seeded	24.93	0.74	<0.0001	0.006	0.001	—	—

in nonlinear ways. Given the short duration of this study (four growing seasons), additional work will be important in further understanding the nuances of these patterns and the mechanisms driving them.

Two studies that have examined the combined effects of seed mix diversity and seeding rate on restoration success used only a few discrete levels of each variable to test linear relationships (Carter & Blair 2012; Nemec et al. 2013). Rather than testing two discrete levels of each factor, our study employed RSR methodology to assess a broad range of seed mix diversity and seeding rates. Although this methodology was developed and is most commonly used in industrial engineering studies (Kuehl 2000), it has rarely been used in ecological studies (e.g. Clancy & King 1993) despite its statistical applicability and experimental efficiency when addressing questions such as ours. For applying this method to other systems, RSR can be parameterized to align with specific restoration objectives and measures of success. It offers flexibility in terms of the types of explanatory variables tested, as well as response variables

and targets (maximum, minimum, specific targets, and so on) used in maximizing restoration (Obermiller 1997). Response variables used in RSR analyses can also be weighted by importance or priority when optimizing overall desirability. Further increasing its utility in restoration, surface (Fig. S2) or contour plots can be created for each response variable individually and for overall desirability so managers can evaluate expected changes in outcomes at different levels of explanatory variables or assess whether thresholds might exist as areas of steep slopes in the response surface. For example, if a minimum density of seeded species is required to consider a shortgrass steppe restoration successful, the surface plot (Fig. S2) could be used to identify seed mix diversity and seeding rate combinations expected to achieve that benchmark but ideally using longer term data than presented in this study. Lastly, both the design of experiments and analysis components of RSR can be conducted in many statistical software packages (e.g. JMP, SAS, and R package rsm).

Table 2. Optimal seed mix diversity (number of species) and seeding rate (pure live seed per m^2) for each of nine response variables as identified by RSR analyses. Value refers to the individual back-transformed optimal value for each category (biomass, diversity, density) of response variable. Desirability values represent the outcome of multiresponse optimization over all nine response variables on a scale of 0 (completely undesirable) to 1 (most desirable) (Obermiller 1997); seeded and native variables were maximized whereas non-native and noxious variables were minimized. Analyses were conducted separately for each year. [a]Solution is a saddle point (i.e. a stationary point that is neither a local minima nor a local maxima). [b]Solution is a minimum. [c]Solution is a maximum. [d]Critical value outside data range.

Response Variable	2012			2013			2015		
	Diversity	Rate	Value	Diversity	Rate	Value	Diversity	Rate	Value
Biomass (g/m^2)									
Seeded	52[d]	320[d]	0.63[a]	<0[d]	9,604[d]	0.40[a]	49	<0[d]	1.90[a]
Volunteer native	39	1,071	0.00[a]	59[d]	1,014	0.00[a]	53[d]	872	0.00[a]
Non-native non-noxious	24	725	0.41[b]	28	1,019	14.86[a]	13	463	228.73[b]
Noxious	25	1,162	23.79[a]	<0[d]	124[d]	79.63[b]	20	943	25.82[a]
Diversity (species per 0.375 m^2)									
Seeded	50	2,942[d]	5.03[c]	56[d]	915	3.15[c]	44	1,812[d]	3.61[c]
Volunteer native	41	1,145	<0[a]	55[d]	838	0.09[a]	36	1,148	0.05[a]
Non-native non-noxious	14	1,157	1.00[a]	24	1,092	1.00[a]	130[d]	4,521[d]	2.48[a]
Noxious	<0[d]	572	1.00[a]	51[d]	1,218	1.00[c]	<0[d]	<0[d]	1.02[a]
Density (individuals per m^2)									
Seeded	48	<0[d]	10.00[a]	32	1,438	13.44[a]	40	806	8.57[a]
Desirability	39	1,205	0.50	50	674	0.52	35	1,366	0.56

We assessed restoration success across a range of site conditions typical of shortgrass steppe (initial noxious species abundance, soil types, and so on). Despite variation in optimal seed mix diversity (coefficient of variation [CV] = 30%), seeding rate (CV = 37%), and desirability (CV = 15%) among sites when analyzed separately, optimal seed mix diversity (40 ± 3 species) and seeding rate (1,232 ± 132 PLS/m^2) when averaged across all 12 sites were relatively close to optimal levels identified in a single RSR model with sites as a random effect (seed mix diversity 35 species, seeding rate 1,366 PLS/m^2). While the optimal levels for seed mix diversity and seeding rate identified might not be financially feasible in some cases, our results do show a consistent trend toward more desirable restoration outcomes with more diverse seed mixes applied at higher seeding rates. Restoration practitioners might have greater success meeting revegetation goals by increasing both seed mix diversity and seeding rates, which would be a departure from common practices. Such increased costs to a restoration project would need to be weighed against the costs of revegetation failures. Future studies could consider cost as a response variable in the RSR methodology in order to determine economic thresholds.

The first half of the first growing season (March–June 2012) was dry relative to average precipitation for the area and ranged from moderate to severe drought at all three locations (USDM 2015). This lack of precipitation early in the restoration process likely had a negative effect on germination and establishment of seeded species. Although plots were watered in 2012, small amounts were applied over several watering periods and likely did not completely alleviate drought stress. Species that established well during the drought in the first year of this study included *Atriplex canescens* (native perennial shrub), *Psoralidium tenuiflorum* (native perennial legume), *Helianthus annuus* (native annual forb), *Verbesina encelioides* (native annual forb), and *Pascopyron smithii* (native perennial

grass) (Table S5). Of the 29 seeded species not recorded in 2012, nearly 80% of them were present in 2013, 2015, or both (Table S5); only 6 of the 50 seeded species were never encountered.

Grassland revegetation projects in the region where this study was conducted typically use 3–10 species in seed mixes with typical seeding rates of 200–300 PLS/m^2 drill seeded. These standards have largely been developed based on past efforts at establishing non-native pasture grasses on fallowed land. Such low seeding rates are often not appropriate for meeting restoration objectives as our data demonstrate. It is important to note, however, that our seeding rates are based on broadcast seeding methodology, which typically uses two-times more seed than drill seeding. Our results suggest that a 4-fold increase in seed mix diversity and a 2-fold increase in seeding rates may result in better restoration success compared to current practices in this region. The importance of increased diversity in seed mixes for restoration success illustrates the need for affordable sources of local plant species. Currently, a dearth of seed availability, especially forbs, precludes the use of diverse seed mixtures in most restoration projects. It is important to note that our results are based on a short-term study (4 years) and longer time frames may be more valuable for assessing restoration success.

Our finding that increased seed mix diversity leads to greater restoration success is consistent with similar studies (Carter & Blair 2012; Nemec et al. 2013), and like Carter and Blair (2012) we found that higher seeding rates lead to better success. What makes our study unique is that by using RSR methodology we are able to predict the optimum combination of seed mix diversity and seeding rate (35 species at 1,366 PLS/m^2) for our restoration scenario and to demonstrate that diversity was the more important of the two variables in driving restoration success.

Acknowledgments

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Location information for 12 experimental sites.
Table S2. Seeding rates for individual species by treatment (1–9) for nine treatment combinations.
Table S3. Mean (SE) of native species, diversity and density, and non-native non-noxious and noxious biomass and diversity.

Table S4. Optimal seed mix diversity and seeding rates.

Table S5. Frequency (%) of seeded species collected in experimental plots.

Figure S1. Response surface plots of relationship between seeding rate (pure live seed per m^2), seed mix diversity (number of species), and individual response variables (z-axis).

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Figure S2. Response surfaces for multiresponse desirability optimization across nine response variables to restoration seed mix diversity (number of species in seed mix) and seeding rate (PLS/m^2).