THESIS

OPTIMAL SEED MIXTURES AND SEEDING RATES FOR RESTORATION OF SURFACE DISTURBANCES ON COLORADO SHORTGRASS STEPPE

Submitted by

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In partial fulfillment of the requirements

For the Degree of Masters of Science

Colorado State University

Fort Collins, Colorado

Fall 2013

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ABSTRACT

OPTIMAL SEED MIXTURES AND SEEDING RATES FOR RESTORATION OF SURFACE DISTURBANCES ON COLORADO SHORTGRASS STEPPE

The discovery of oil and gas resources over the last decade has led to unprecedented localized and dispersed surface disturbances on shortgrass steppe ecosystems in the western US. Reclaiming and restoring these surface disturbances to native ecosystems through revegetation seeding has proven challenging. Seed mixes and rates currently used are generally similar across private and public sectors (3-10 species at rates ranging from 400-600 pure live seeds (PLS) m^{-2} broadcast seeded). The objective of this study was to determine an optimal seed mix diversity level and corresponding seeding rate for restoration of surface disturbances in shortgrass steppe. I examined five seed mix diversity levels, 5-50 species, and five seeding rates, 400-1600 PLS m^{-2} using a response surface regression experimental design at twelve sites. Treatments and overall restoration success were evaluated based on resulting biomass and diversity of seeded, volunteer native, noxious, and non-native species, and the density of seeded species. Results show greatest restoration success occurring during year one at a seed mix diversity level of 43 species and a seeding rate of 1229 PLS m^{-2}, and during year two at a diversity level of 42 species and a rate of 932 PLS m^{-2}. These results suggest that higher seed mix diversity levels and higher seeding rates could lead to greater restoration success for surface disturbances in shortgrass steppe.

ACKNOWLEDGMENTS

There are many individuals who have significantly contributed to my research, thesis, and well-being throughout my graduate career. I want to give a special thanks to my advisor Dr. Mark Paschke for his academic guidance and support and the incredible opportunity to work on this project. I would also like to thank my funding sources, Colorado Agriculture Experiment Station and Encana, for the chance to work on this project and attend graduate school. I would like to warmly thank Jayne Jonas-Bratten, post-doc for the restoration ecology lab. Jayne was the rock of this project and provided an unprecedented level of guidance, statistical assistance, technical designing of the project, writing and editing direction, and emotional support. I would also like to thank Brett Wolk for his logistical help and his endless assistance with Excel and data management. I would also like to thank the restoration ecology lab for their field work and data processing help, and commitment to working in the shortgrass steppe system on many long hot summer days. I want to thank my committee members Dr. Paul Meiman and Dr. Joe Brummer for their valuable feedback on my research and thesis.

I want to thank my restoration ecology lab cohorts Cassie Stube, Garrett Stevens, Catherine Cumberland, and Sasha Victor for their encouragement, willingness to sit and listen to me talk about everything under the sun, and their comic relief during times of needed breaks.

I would also like to thank behind the scene individuals who were extremely supportive and understanding through the last two and half years of my graduate career. Suzy Barr, Norma and Gary Barr, and Logan Magnuson have celebrated mini milestones with me and consoled me during times of great frustrations. I am extremely grateful to have had such an amazing network of family and friends to support me during this process.

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Ecology of the Shortgrass Steppe Ecosystem

The most drought-prone and least productive areas on earth have survived as native grasslands. The central area of North America is the largest inter-connected grassland in the world (Lauenroth and Burke 2008). The shortgrass steppe is the warmest, driest, and least productive section of these contiguous grasslands, covering approximately 340,000 km^2 (131,000 mi^2) in the central and southern Great Plains (Lauenroth, Burke et al. 1999; Lauenroth and Burke 2008). The topography of the shortgrass steppe is characterized by gently rolling hills, broad ephemeral streams, and low flat-topped terraces. Precipitation is the single most important variable controlling the natural ecology of the shortgrass steppe (Sala, Lauenroth et al. 1992). Mean annual precipitation varies from 508-635 mm (20-25 in) annually over the easternmost portions to less than 305 mm (12 in) annually in some areas of the west. Precipitation is highly seasonal with the growing season (April – September) contributing 70% - 82% of average annual moisture (NCDC 2002). Vegetation on the shortgrass steppe is dominated by drought tolerant species: short grasses (64%), succulents (21%), and dwarf shrubs (8%) (LTER 2007). The two most abundant species are *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalograss), both perennial warm-season grasses.

Disturbances

Plants native to shortgrass steppe evolved with many types of disturbance. However, over the past century disturbances have been significantly altered by human activity and land management practices (Augustine and Milchunas 2009; Scheintaub, Derner et al. 2009). Historic disturbance regimes were driven by large browsers and grazers such as bison (Hodgson 1994). Due to the high intensity of historical grazing, plant communities adapted to aboveground disturbances resulting in resilient perennial grass species. Today, domestic cattle have widely replaced bison as the main large herbivore. Cultivation and subsequent abandonment of agricultural fields has led to a local reduction in native plants, loss of seeds from soil, increased soil erosion, reduction in organic matter, and a substantial increase in non-native species in these areas (Costello 1944). Due to increasing energy and irrigation costs, cultivation in this region is becoming less practical. When agricultural fields are abandoned, they are susceptible to wind and water erosion and become infested with aggressive, non-native plants. Restoration of these fields to native rangeland is influenced by past agricultural use, such as fertilization (Lauenroth and Burke 2008). Long-term cultivation impacts soil microbes, soil fertility and composition, and the presence of exotic weeds (Lauenroth and Burke 2008). These factors can be persistent and contribute significantly to the obstacles faced in restoring these fields to native rangeland.

Possibly the most dramatic impact on shortgrass steppe is the recent discovery of significant oil and gas resources, which has resulted in rapid mineral exploration and extraction; thousands of new drilling permits are being issued every year for the shortgrass steppe region in Colorado (COGCC 2011). This exploration has led to substantial soil and vegetation disturbances on the shortgrass steppe. When well pads are installed over a hectare (two acres) of ground may be disturbed in order to accommodate the drill rig, associated equipment, hydraulic fracturing equipment, service areas, and personnel (Stednick, Paschke et al. 2010). Recent technological advances have made it possible to use multiple-directional bores from a single well pad to reduce the number and total surface footprint of well pads (Stednick, Paschke et al. 2010). However, another major concern regarding oil and gas exploration is the increase in on- and off- road vehicles around well pads and service areas, which can cause significant soil compaction, vegetation removal, and other surface disturbances (Duniway, Herrick et al. 2010). Activities associated with oil and gas exploration can also have harmful secondary effects. Surrounding areas can experience stresses such as the production of fugitive dust, alterations to hydrology, and behavioral changes in wildlife (Stednick, Paschke et al. 2010).

Restoration Limitations

Considering these frequent disturbances to shortgrass steppe the need for restoration is increasing. Generally, the goals for restoration in this region are to reestablish native plant communities, provide forage for livestock, provide forage and cover for wildlife, reduce erosion, and improve water quality and quantity (Hardegree 2011). However, the shortgrass steppe can be a harsh environment, posing many obstacles to successful restoration.

One of the main limiting factors is the semi-arid climate of the shortgrass steppe. Germination of seeds and seedling establishment can be hampered by the lack of adequate precipitation at appropriate times during plant development in semi-arid regions (Abbott and Roundy 2003). Furthermore, the timing of large precipitation events during the spring and summer months can have a significant impact on species composition (*i.e.* cool-season plants versus warm-season plants) (Epstein, Burke et al. 1999). Because precipitation cannot be controlled, it is important to take current and forecasted precipitation into account when selecting restoration materials and methods.

A second natural limiting factor for restoration on the shortgrass steppe is the growth form and characteristics of the native plant community. In the shortgrass steppe, a large majority of primary production is attributed to *B.gracilis*. The ability of this species to recover following a disturbance is key to the ecosystem's ability to recover (Coffin and Lauenroth 1988). Unfortunately, re-colonization by this species is a relatively slow process and recruitment events occur as seldom as every 30-50 years (Lauenroth, Sala et al. 1994), likely due to climatic constraints on seed germination and seedling establishment (Coffin, Lauenroth et al. 1996). Although hardy once established, many shortgrass steppe species require specific conditions to germinate and survive the seedling stage. Germination requirements include having soil temperatures $\geq 15^{o}$ C (60^{o} F) (Briske and Wilson 1977) and sufficient moisture in the top 38 mm (1.5 in) of the soil profile (Wilson and Briske 1979). Once seedlings have germinated, they require sufficient moisture in the top 305 mm (12 in) of the soil profile in order to develop root structures and establish (Wilson and Briske 1979). These environmental requirements and physiological traits of the native plant community make it difficult to use them in restoration seed mixes with the goal of restoring the disturbed area to the surrounding native plant community within 2-5 years as mandated by most public and private restoration contracts.

Restoration efforts on shortgrass steppe, as in other systems, are often limited by invasion of non-native species. Non-native species have an array of attributes that make them successful competitors against native species, including tolerance to a wider range of environmental conditions, annual or biennial life cycles that allow comparatively rapid and high seed output, fewer natural enemies, and the ability to aggressively compete for limited resources. In Colorado, common non-native species of concern during restoration of shortgrass steppe are *Acroptilon repens* (Russian knapweed), *Centaurea diffusa* (diffuse knapweed), *Linaria dalmatica* (Dalmation toadflax), *Cirsium arvense* (Canada thistle), *Cirsium vulgare* (bull thistle), *Carduus nutans* (musk thistle), *Onopordum acabthium* (Scotch thistle), *Kochia scoparia* (kochia), *Salsola iberica* (Russian thistle), *Cynoglossum officinale* (houndstongue), and *Bromus tectorum* (cheatgrass) (USDA 2006). Utilizing the most competitive native species to help manage invasive plants is essential to successful restoration efforts.

Materials and methods available and commonly used in a specific region or site also influence restoration success. Seeding method (drill vs. broadcast), seeding depth, seeding time, seeding rate, seed source, seed mix, seedbed preparation, site preparation, mulching, and weed control have been studied in regards to grassland restoration (Redmann and Qi 1992; Montalvo, McMillan et al. 2002; Foster, Murphy et al. 2007; Maron and Marler 2007; Piper, Schmidt et al. 2007; Wilsey 2010; Yurkonis, Wilsey et al. 2010; Yurkonis, Wilsey et al. 2010; Doll, Haubensak et al. 2011; Carter and Blair 2012). Using appropriate restoration practices for specific sites and disturbances is importance in any restoration setting.

Recommendations for Restoration on the Shortgrass Steppe

Planting Season

Planting should occur in order to take advantage of the season that will be most favorable for establishment of plant species in the seed mix (Monsen 2004). In arid and semi-arid regions, dormant-season fall seeding is recommended in order to take advantage of all possible opportunities for plant establishment under a highly variable moisture regime (Monsen 2004; Doll, Haubensak et al. 2011; Hardegree 2011). However, timing of planting can vary regionally because it is important to take into account forecasted precipitation and temperature patterns that may or may not be favorable to seed germination and growth (Klomp 1972).

Seeding Method and Depth

Many studies have examined the impact of seeding method and depth on seed germination and establishment. Generally, seeding depth is based on seed size and placement is dependent on maximizing soil contact and moisture and resource availability. (Klomp 1972;

Redmann and Qi 1992; Montalvo, McMillan et al. 2002; Bakker, Wilson et al. 2003; Monsen 2004; Middleton, Bever et al. 2010; Yurkonis, Wilsey et al. 2010; Yurkonis, Wilsey et al. 2010; Doll, Haubensak et al. 2011; Hardegree 2011). In most cases, seeds should be covered 2.5-3 times the thickness of the seed. For small seeded species, the length should be used rather than the diameter. Findings have varied greatly with respect to broadcast seeding vs. dill seeding as planting methods, but most suggest that seed size is the driving variable in determining whether to broadcast seed or drill seed (Montalvo, McMillan et al. 2002; Monsen 2004). Smaller seeds tend to do better broadcast seeded while larger seeds are better suited for drill seeding where seeds can be placed at a proper depth. A review of 23 studies looking at seeding method, done by Hardegree et al. (2011), found that 73% of the studies concluded that drill seeding outperformed broadcast seeding. Soil texture can also influence seeding depth so it is important to know if the restoration site is dominated by clay or sandy soils when determining seeding depth for drill seeding.

Seedbed Preparation

In some cases, seedbed preparation is necessary to facilitate seed germination and establishment. Seedbed preparation is conducted to increase water availability to the seed, increase water infiltration capacity, and create water-retaining microsites. Methods include but are not limited to, tilling, disking, and seedbed firming. Studies have shown that loosened seedbeds can accelerate restoration efforts through the creation of microsites that are favorable to germination due to increased soil and seed contact (Montalvo, McMillan et al. 2002)).

Surface Modifications

Surface modifications are often used in semiarid environments to increase water availability by creating macro or microsites that concentrate and retain water. Techniques such as imprinting, pitting, and furrowing have been shown to capture and preserve moisture (Whisenant, Thurow et al. 1995; Eldridge, Redente et al. 2012). Surface modifications can also increase seed/soil contact and provide better temperature conditions than level surfaces (Smith and Capelle 1992). These modified surfaces can improve soil organic matter, but accumulated litter overtime could impede seedling emergence in some cases (Smith and Capelle 1992).

Soil Amendments

In some cases, the soil community needs to be assisted in order to support a diverse assemblage of plants (Ohsowski, Klironomos et al. 2012). Soil amendments such as compost, minerals, biochar, mycorrhizal inoculums (beneficial soil microorganisms), biosolids, manure, peat, wood chips, and nutrient additions can enhance soil functioning (Tisdall, Cockroft et al. 1978; Eldridge, Redente et al. 2012). However, soil amendments should only be applied if entirely necessary to attain specific restoration goals. Soil amendments, such as nitrogen additions, often leads to an increase in unwanted non-native species so caution is encouraged when considering nutrient additions (Doll, Haubensak et al. 2011).

Seed Mix Selection

Selection of species and determining the number of species to use in a restoration setting is often challenging, but should be guided by desired future uses of the area (wildlife, livestock, recreation, etc.). Seeding usually involves more than one species and species are selected for their ability to meet restoration goals, seedling vigor, establishment traits, functional traits, commercial availability, and price (Loreau, Naeem et al. 2001; Monsen 2004). Several studies have shown that using high diversity seed mixes (more than 15 species) can aid in restoration success (Lepš, Doležal et al. 2007; Carter and Blair 2012; Kirmer, Baasch et al. 2012; Nemec, Allen et al. 2013). Although selecting specific native species for a seed mix can be a difficult task, using a variety of functional groups (*i.e.* annual and perennial grasses and forbs, and shrubs) can aid in creating a diverse community that develops in unison with the soil community. Diverse grassland systems have been shown to have higher productivity (Tilman, Reich et al. 2001), resistance to invasion (Maron and Marler 2007), and faster recovery following disturbances (Hector, Hautier et al. 2010). Therefore, in a restoration context, the seed mix should be diverse in order to attain these advantageous attributes in ecosystem composition and functioning.

Seeding Rate

Seeding rate is an important factor to consider when designing seed mixes. The role that seeding rate plays in the resulting target plant community and restoration success has been studied very little in grassland systems (Mueggler and Blaisdell 1955; Launchbaugh and Clenton 1970; Hull 1974; Papanastasis and Biswell 1975; McMurray, Jenkins et al. 1997; Williams, Schuman et al. 2002; Sheley and Half 2006; Dickson and Busby 2009). Most literature that has specifically examined the effect of seeding rates on initial plant establishment supports the idea that higher seeding rates (>345 pure live seeds (PLS) m^{-2}) lead to greater initial establishment of seeded species (Papanastasis and Biswell 1975; Vogel 1987; Sheley, Jacobs et al. 1999; Williams, Schuman et al. 2002; Carter and Blair 2012; Nemec, Allen et al. 2013).

Mulch Application

The application of mulch has been shown to improve seeding success due its ability to reduce erosion (Meyer, Wischmeier et al. 1970; Groen and Woods 2008), protect seedlings from wind desiccation, increase infiltration rates and reduce soil crusting (Léonard and Rajot 1998), and increase soil nutrient cycling and microbial activity (Allen and Zink 1998). There are many different types of mulch, including straw, wood chips, local native plant litter, wood straw, and hay. It is important to ensure all mulch products are certified weed-free before application. However, even certified weed-free mulches have the ability to transport non-native seeds, so this should be considered when selecting a mulch type (Clark 2003). Mulching rates vary across regions, but generally are selected to mimic the structure and function of the native surrounding plant litter (Therrell, Cole et al. 2006). For more information on mulch application rates for varying mulch types refer to Barnhisel et al.(2000).

Weed Management

Due to the ability of invasive species to out-compete some native species and reduce restoration success, it is critical to implement a weed management plan in conjunction with restoration efforts. Both chemical and mechanical means for removing invasive species have been shown to have a significant impact on successful establishment of seeded native species (Huddleston and Young 2005). Herbicides can be very effective in controlling small infestations of invasive species during early stages in the restoration process, but can be less effective and financially unfeasible for larger infestations. Therefore, it is essential to detect invasive species early in the restoration process to treat and remove them in a timely and cost efficient manner.

Irrigation

The climate of the shortgrass steppe region can make establishing plants challenging due to extreme temperatures, lack of consistent rainfall, low humidity, and high wind speeds (Bainbridge 2002). Irrigation can be used to deliver water to the root zone of establishing plants. Irrigation systems can include manual watering (water trucks, watering cans, etc.), traditional irrigation systems (basin and drip irrigation), and alternative irrigation systems (porous hose, deep pipe, and buried clay pot irrigation). However, irrigation systems are not always economically feasible on large scale projects. Irrigation can enhance the robustness of individual plants but may dampen root growth making plants less equipped to deal with future drought periods.

Table 1.1. General recommendations for materials and methods to use in restoration of shortgrass steppe.

Materials and Methods	Restoration Recommendation
Planting Season	Dormant fall planting is recommended.
Seeding Method and Depth	Drill seeding has proven more effective in most regions, but smaller seeds may need to be broadcast seeded.
Seedbed Preparation	Seedbed modification including; tilling, disking, or general loosening of the seedbed, is recommended to increase soil to seed contact.
Surface Modifications	Pitting or furrowing techniques can increase water retention and improve seed germination.
Soil Amendments	Amendments should only be applied when necessary but can improve the soil community in order to support plant establishment and growth.
Seed Mix Selection	A highly diverse seed mix comprised of native species within varying functional groups (i.e. annual and perennial grasses and forbs, and shrubs) should be used.
Seeding rate	Seeding rates vary depending on region and seed mix, but higher seeding rates are encouraged to increase initial establishment of seeded species and compete with non-native species.
Mulch Application	Mulch will reduce soil erosion, increase water infiltration, protect from wind desiccation, and support higher water retention for the germination of seeded species when applied to achieve 50-90% ground cover.
Weed Management	Restoration sites should routinely be surveyed for the presence of invasive species and treated (chemically or mechanically) promptly in order to reduce their establishment and proliferation.
Irrigation	On smaller scale projects, irrigation can be used to deliver water to plants but can limit individual plant drought tolerance.

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OPTIMAL SEED MIXTURES AND SEEDING RATES FOR RESTORATION OF SURFACE DISTURBANCES ON COLORADO SHORTGRASS STEPPE

Introduction

Threats to the Shortgrass Steppe Ecosystem

Central North America is the largest interconnected grassland in the world (Lauenroth and Burke 2008). The shortgrass steppe covers the warmest, driest, and least productive section of these contiguous grasslands (Lauenroth and Burke 2008). Land use on the shortgrass steppe is primarily limited to wildlife habitat and production of crops and livestock. Cultivation and subsequent abandonment of agricultural fields, due to increasing energy and irrigation costs, has led to large scale vegetation removal, loss of the soil seedbank, increased soil erosion, reduction in soil organic matter, and a substantial increase in non-native species in these areas (Costello 1944; Reichhardt 1982). In addition, vast areas are being explored and developed for energy production creating dramatic ecological, cultural, and economic changes for the shortgrass steppe ecosystem.

The recent discovery of significant energy resources in the shortgrass steppe has resulted in rapid exploration and extraction of oil and gas (COGCC 2011). This exploration has led to substantial soil and vegetation disturbances on the shortgrass steppe. When well pads are installed they can disturb over a hectare of ground in order to accommodate the rig, associated equipment, hydraulic fracturing equipment, service areas, and personnel (Stednick, Paschke et al. 2010). Recent advances in technology have made it possible to use multiple-directional bores from a single well pad to reduce the total area disturbed (Stednick, Paschke et al. 2010). However, despite recent gains in technology and the rise in popularity of horizontal drilling, thousands of new drilling permits are being issued every year for the shortgrass steppe (COGCC

2011). The negative ecological consequences of these well pads include soil compaction, changes in soil microbial activity and composition, changes in soil nutrients, shifts in species composition, vegetation removal, and increases in non-native and invasive plant species (Duniway, Herrick et al. 2010). Surrounding areas can also experience secondary stresses such as the production of fugitive dust, alterations to hydrology, and behavioral changes in wildlife (Stednick, Paschke et al. 2010). In northeastern Colorado, the number of well pad sites far exceeds any other area in the state. In 2012, 48% of all state issued drilling permits were for a single county within the shortgrass steppe in northeastern Colorado. (COGCC 2011; COGCC 2012). Over the last ten years, the number of active drilling wells has more than doubled in Colorado (COGCC 2011), making the need for research on restoring these sites vital and relevant.

Restoration Limitations

Considering frequent disturbances to the shortgrass steppe the need for effective restoration strategies is essential to ensure restoration success. However, the shortgrass steppe can be a harsh environment that poses many limitations to restoration, which need to be readily understood before restoration efforts should be implemented. One of the main limiting factors is climate of the shortgrass steppe. Low precipitation coupled with variability makes germination of seeded species difficult (Abbott and Roundy 2003). Furthermore, timing of large precipitation events during spring and summer can have a significant impact on species composition (*i.e.*, C_3 versus C_4 dominance) (Epstein, Burke et al. 1999). Because precipitation cannot be controlled, it is important to take into account its impact when selecting restoration materials and methods.

A second natural limiting factor for restoration on shortgrass steppe is morphology and vegetative characteristics of native plant species. In the shortgrass steppe region of northeastern Colorado, up to 90% of net primary production is contributed by *Bouteloua gracilis* (blue grama). The ability of this species to recover following a disturbance is key to the ecosystem's ability to recover (Coffin and Lauenroth 1988). Unfortunately, re-colonization by this species is a relatively slow process and recruitment events occur as seldom as every 30-50 years (Lauenroth, Sala et al. 1994), likely due to climatic constraints on seed germination and seedling establishment (Coffin, Lauenroth et al. 1996). Most other plant species typical within the community are hardy perennials that do not germinate rapidly but rather wait for favorable conditions, similar to *B.gracilis*. These morphological and physiological traits of native species make it difficult to use them in restoration seed mixes. Especially when the goal is to restore the disturbed area within 2-5 years to the surrounding native plant community as deemed by most public and private restoration contracts.

Restoration success on the shortgrass steppe, as in other systems, is also often limited by invasion of non-native species. Non-native species have an array of attributes that make them successful competitors. These include tolerance to a wider range of environmental conditions, annual or biannual life cycles that allow comparatively rapid and high seed output, fewer natural enemies, and the ability to aggressively compete for limited resources (Holzmueller and Jose 2013). Common non-native species in northeastern Colorado that limit restoration success are: *Cirsium arvense* (Canada thistle), *Kochia scoparia* (kochia), *Salsola iberica* (Russian thistle), and *Bromus tectorum* (cheatgrass) (USDA 2006). Excluding and managing invasive species and seeding competitive native species are essential to successful restoration efforts.

Restoration efforts are also often limited by the materials and methods used for a specific region or site. Seeding method, seeding depth, seeding time, seedbed preparation, seed source, and site preparation have all been studied in regards to grassland restoration (Redmann and Qi 1992; Montalvo, McMillan et al. 2002; Wilsey 2010; Yurkonis, Wilsey et al. 2010; Yurkonis, Wilsey et al. 2010; Doll, Haubensak et al. 2011). However, seed mix composition is often a primary limiting factor in restoration success on the shortgrass steppe, and has been studied very little in comparison to other aspects of restoration (Sheley and Half 2006; Török, Deák et al. 2010). Historically, *Agropyron cristatum* (crested wheatgrass), a non-native forage grass, was the main species used in seed mixes to reclaim overgrazed areas and abandoned cropland (Bement, Barmington et al. 1965; Bakker, Christian et al. 1997; Fansler and Mangold 2011). It was selected for its high germination rate and ability to cover large areas of ground quickly. However, today we are still dealing with the ecological consequences of having planted this non-native species on degraded rangelands (Fansler and Mangold 2011).

Diversity

Many studies have examined the effects of plant species diversity on community health and functioning, productivity, resilience, resistance, and recovery (Tilman 1997; Tilman, Reich et al. 2001; Cameron 2002; Dukes 2002; Camill, McKone et al. 2004; Fargione and Tilman 2005; Kahmen, Perner et al. 2005; Martin, Moloney et al. 2005; Polley, Derner et al. 2005; Biondini 2007; Maron and Marler 2007; Hector, Hautier et al. 2010; Quijas, Schmid et al. 2010; Allan, Weisser et al. 2011). The debate on biodiversity's effect on community attributes is ongoing (Cameron 2002) and studies on shortgrass steppe are lacking; but many studies have demonstrated positive correlations between diverse grassland systems and higher productivity (Tilman, Reich et al. 2001), resistance to invasion (Maron and Marler 2007), and resilience following disturbances (Hector, Hautier et al. 2010). Therefore, in a restoration context seed mixes should be diverse in order to attain these advantageous attributes in community composition and functioning. If the restoration target is a highly diverse community, then whether or not sowing more diverse seed mixes will result in a more diverse plant community needs to be examined. Relatively few studies have examined this question in restoration of grassland systems (Lepš, Doležal et al. 2007; Kirmer, Baasch et al. 2012). Kirmer et al. (2012) found that high diversity seed mixtures (51 species) versus low diversity seed mixtures (3 species) 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, Baasch et al. 2012). Lepš et al. (2007) also found that sowing high diversity seed mixtures (15 species) was more successful than sowing low diversity seed mixtures (4 species). They suggested that the high diversity seed mixes were primarily important for their insurance effect (sensu Yachi and Loreau 1999; Lepš, Doležal et al. 2007) because plots seeded with high diversity mixtures were capable of compensating for the failure of some species to establish (Lepš, Doležal et al. 2007).

Seeding Rate

Seeding rate is a second and equally important factor to consider when designing restoration efforts. Like diversity, the role that seeding rate plays in the resulting target plant community and restoration success has been studied very little in grassland systems (Mueggler and Blaisdell 1955; Launchbaugh and Clenton 1970; Hull 1974; Papanastasis and Biswell 1975; McMurray, Jenkins et al. 1997; Williams, Schuman et al. 2002; Sheley and Half 2006; Dickson and Busby 2009) . Dickenson and Busby (2009) looked at the effect of forb and grass seeding rates on resulting forb density in mixed-grass prairie. They found that by decreasing grass seeding rates and increasing forb seeding rates, the resulting community had higher forb diversity and density. In this study, the target community was that of high forb densities, which may not be the case for all restoration settings (Dickson and 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.

Seed Mix Diversity and Seeding Rate

Planning for restoration seeding involves both determining the number of species to use in the mix, as well as seeding rate. However, it seems that only two studies have simultaneously looked at the effect of these two variables on resulting plant communities and overall restoration success (Carter and Blair 2012; Nemec, Allen et al. 2013). However, neither of these studies were conducted in the shortgrass steppe ecosystem. Nemec et al. (2013) examined the effect of sowing high (97 species) and low (15 species) diversity seed mixtures at high (297 PLS m^{-2} drill seeded) and low (148 PLS m^{-2} drill seeded) rates on invasion resistance. Working in tallgrass prairie, they found that the higher diversity seed mixture, but not the higher seeding rate, increased invasion resistance. Carter and Blair (2012) examined the effect of seed mix diversity and seeding rate on drought response in mixed-grass prairie. Their results showed that the higher seeding rate (344 PLS m^{-2} drill seeded) and higher diversity seed mix (95 species) resulted in greater diversity and cover of native and seeded species, and lower diversity and cover of exotic species. They found little evidence, however, that seeding treatments affected drought resistance (Carter and Blair 2012). Their study concluded that using higher diversity seed mixtures at higher seeding rates results in more successful grassland restoration. Both of these studies only tested two levels (high versus low) of each factor (seeding rate and seed mix diversity).

It is also important to note the variability in defining “high” versus “low” in various studies of seed mix diversity seed and seeding rates. The range in “high diversity” seed mixes previously tested is between 15 and 97 species (Carter and Blair 2012; Nemec, Allen et al. 2013). High seeding rates have been described as being between 297 and 344 PLS m^{-2} drill seeded (equivalent to 594 - 688 PLS m^{-2} when converted to broadcast rates) (Carter and Blair 2012; Nemec, Allen et al. 2013). The industry standard in northeastern Colorado is near what these studies considered as their high seeding rate, but the diversity level for their seed mixes varied greatly from the industry standard.

Industry Standards

At present, seed mixes and rates are generally similar across private and public sectors in shortgrass steppe. For example, in northeastern Colorado a review of restoration documents from both public and private organizations revealed commonalties in revegetation practices. Most recommended low diversity seed mixes, 3-10 species, and low seeding rates, 400-600 PLS m^{-2} broadcast seeded (O'Brian Energy Company 2003; Agriculture Research Station 2011; Bureau of Land Managment 2011; Natural Resource Conservation Service 2011; United States Forest Service 2011; Wildlands Restoration Volunteers 2011). The five most common species used are *B.gracilis*, *B. dactyloides*, *Pascopyrum smithii*, *Achnatherum hymenoides* and *Hesperostipa comata.* Seldom are annual grasses and forbs, or perennial forbs and shrubs used. These standard low diversity mixtures seeded at low rates are due in part to a lack in scientific research and economic constraints.

Objectives

In this study, I explore the role of seed mix diversity and seeding rates on restoration effectiveness in shortgrass steppe. The objective of this study was to determine an optimal seed mix diversity level and corresponding seeding rate for achieving restoration success on shortgrass steppe. Unlike previous studies, I used response surface methodologies to assess a broad range of seed mix diversity and rates. Overall restoration success was evaluated based on resulting biomass and diversity of seeded, volunteer native, noxious, and non-native species, and the density of seeded species in the first two years after seeding. This study sought to find approaches that are applicable to a variety of sites across the region in order to be useful on a management scale. From this study, I hope to make recommendations to public land management agencies and private industries about ideal seeding rates and seed mixes for restoration of surface disturbances such as oil and gas exploration.

Methods and Materials

Site descriptions

This study was conducted on twelve sites (A-L) across three locations in northeastern Colorado (Table 2.1). Sites were chosen based on their availability and soil type (Table 2.1). A variety of soil types were selected to represent common soil types in shortgrass steppe. All sites fall within the rangeland loamy plains ecological site description, receive 305-410 mm of precipitation per year, and range from 1500 to 1650 m in elevation (USDA 2010). Six sites (A-F) are located in Larimer County, Colorado. These sites are on Fort Collins loam, Kim loam, and Stoneham loam soils with 1-3% slopes (NRCS 2011). Dominant vegetation at these sites consists of *A.cristatum* and *Ericameria nauseosa*. Three sites (G-I) are located in Weld County, Colorado. These sites are on Stoneham fine sandy loam, Nunn loam, and Asaclon fine sandy loam soils (NRCS 2011) with 0-9% slopes. Dominant vegetation at these sites consists of *Bouteloua curtipendula*, *P.smithii*, *H.comata*, and *Lolium multiflorum* (USDA 2010). The remaining three sites (J-L) are also located in Weld County, Colorado. These sites are on Olney fine sandy loam and Renohill-Shingle complex soils (NRCS 2011) with 0-9 % slopes. Dominant vegetation at these sites consists of *B.gracilis*, *P.smithii*, *Aristida purpurea*, *B.dactyloides*, and *Opuntia polycantha*.

Table 2.1. Location information for twelve experimental sites examining the role of seed mix diversity and seeding rate on restoration success in northeastern, CO by county, Site ID (A-L), soil type (NRCS 2011) , and Zone 13N UTM coordinates.

County	Site ID	Soil Type (NRCS 2011)	UTM Coordinates (13T) (Easting, Northing)
Larimer	A	Stoneham loam	491564, 4506622
Larimer	B	Stoneham loam	491561, 4506651
Larimer	C	Kim loam	491503, 4506614
Larimer	D	Kim loam	491505, 4506630
Larimer	E	Fort Collins loam	491221, 4506583
Larimer	F	Fort Collins loam	491182, 4506582
Weld	G	Stoneham fine sandy	512795, 4509096
Weld	H	Nunn loam	513073, 4509037
Weld	I	Ascalon fine sandy loam	512920, 4508621
Weld	J	Renohill-Shingle complex	540299, 4500321
Weld	K	Olney fine sandy loam	540238, 4500149
Weld	L	Olney fine sandy loam	540477, 4498601

Experimental design

The experimental design is based on response surface regression (RSR) methodology (Box and Wilson 1951; Box and Hunter 1957). The RSR treatment structure was used to test effects of two continuous explanatory variables (seed mix diversity and seeding rate) on nine plant community characteristics to assess restoration success. Using nine treatments, this experimental design can identify optimal seed mix diversity and seeding rates falling anywhere within the range of the factors tested (Figure 2.1 dotted circle). This design was preferable to a traditional two-factor ANOVA design that would require 25 treatments per replicate and limit inference to the levels of each factor tested.

Minimum (5 species, 400 PLS m^{-2}) and maximum (50 species, 1600 PLS m^{-2}) levels of each factor were chosen based on industry standards and ecological considerations, respectively. Minimum and maximum values were then used to calculate three mid-point values of each factor and treatment combinations were determined using rotatable central composite RSR methodology (Figure 2.1, Table 2.2) (Kuehl 2000). Though not formally part of the RSR design, a non-seeded control was also included. Each site had 14 plots and ten treatments: one unseeded control plot, one plot each for treatments 1-8, and five plots of treatment 9 (28 species at 1000 PLS m^{-2}, RSR center point). The center point treatment was replicated five times at each site to calculate pure experimental error (Freund and Littell 2000; Kuehl 2000).

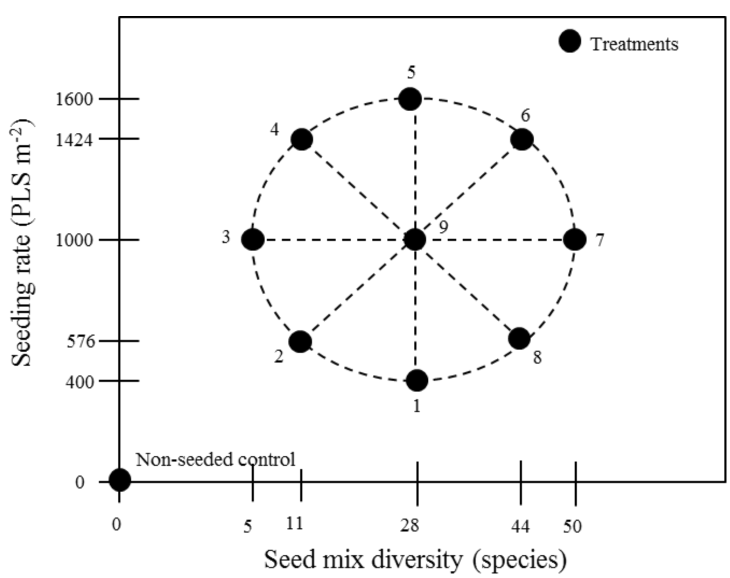


Figure 2.1. Statistical design schematic for response surface regression methodology showing nine combinations of seeding rate (PLS m^{-2}) and seed mix diversity (number of species in mix) treatments. Intermediate treatment combinations were calculated based on chosen minimum and maximum values based on rotatable central composite RSR (n=1 for treatments 1-8 and n=5 for treatment 9 (to calculate pure experimental error) at each of twelve experimental sites).

Site preparation

On all twelve study sites, a 16- x 10-m disturbance was simulated in order to mimic a variety of surface disturbances relevant to the shortgrass steppe. 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 Billy Goat Outback 24” brushcutter (Billy Goat Industries, Lee’s Summitt, MO) to remove taller live vegetation and shrubs. Cut vegetation was raked and discarded outside the study area. Following mowing, the sites were rototilled twice lengthwise and once widthwise using a self- propelled rear- tine rototiller (Model FRC800, Honda Power Equipment, Alpharetta, GA). An average depth of 15 cm was disturbed by three passes with the rototiller. Throughout the tilling process, root masses were collected by hand and removed from the study site. After completion of ground disturbance, the sites were again raked thoroughly to remove remnant plant debris, roots, and litter. The sites then rested and settled for one week before treatments were applied.

Treatments

At each site, fourteen 2- x 2-m study plots were established with a 1-m buffer zone between plots and a 1-m buffer zone bordering the disturbed area in the fall of 2011. Per RSR methodology (discussed above), treatments consisted of nine combinations of five diversity levels (5-50 species) and five seeding rates (400-1600 PLS m^{-2}) (Table 2.2). Treatments were randomly assigned to plots at each site.

Table 2.2. Seeding rates for individual species by treatment (0-9) for ten treatment combinations examining the role of seed mix diversity and seeding rate on restoration success in northeastern, CO. All species are grouped by functional group (annual forbs, annual grasses, biennial forbs, perennial forbs, perennial grasses, and perennial shrubs)

Treatment	Control	1	2	3	4	5	6	7	8	9
Diversity (# species)	0	28	11	5	11	28	44	50	44	28
Rate (PLS m^{-2})	0	400	576	1000	1424	1600	1424	1000	576	1000
Species										
Annual Forbs										
Adenolinum pratense	0	0	0	0	0	0	129	80	52	0

Table 2.2. Continued.

Treatment	Control	1	2	3	4	5	6	7	8	9
Diversity (# of species in seed mix)	0	28	11	5	11	28	44	50	44	28
Rate (PLS m^{-2})	0	400	576	1000	1424	1600	1424	1000	576	1000
Species										
Annual Forbs										
Cleome serrulata	0	57	209	0	518	229	129	80	52	143
Helianthus annuus	0	57	209	800	518	229	129	80	52	143
Polygonum pensylvanicum	0	0	0	0	0	0	129	80	52	0
Thelesperma filifolium	0	57	0	0	0	229	129	80	52	143
Verbesina encelioides	0	57	0	0	0	229	129	80	52	143
Annual Grasses										
Aristida purpurea	0	57	209	0	518	229	129	80	52	143
Vulpia octoflora	0	57	209	800	518	229	129	80	52	143
Biennial Forbs										
Aster tanacetifolia	0	57	0	0	0	229	129	80	52	143
Erysimum capitatum	0	57	0	0	0	229	129	80	52	143
Sphaeralcea coccinea	0	57	0	0	0	229	129	80	52	143
Perennial Forbs										
Allium textile	0	57	209	0	518	229	129	80	52	143
Argemone polyanthemos	0	0	0	0	0	0	129	80	52	0
Astragalus bisulcatus	0	0	0	0	0	0	0	80	0	0
Perennial Forbs										
Astragalus drummondii	0	57	0	0	0	229	129	80	52	143
Astragalus laxmannii	0	0	0	0	0	0	0	80	0	0
Dalea purpurea	0	57	0	0	0	229	129	80	52	143
Delphinium geyeri	0	0	0	0	0	0	129	80	52	0
Heterotheca villosa	0	0	0	0	0	0	129	80	52	0
Liatris punctata	0	57	0	0	0	229	129	80	52	143
Lupinus argenteus	0	0	0	0	0	0	129	80	52	0
Oenothera caespitosa	0	57	209	0	518	229	129	80	52	143
Oxytropis lambertii	0	0	0	0	0	0	0	80	0	0
Penstemon angustifolius	0	0	0	0	0	0	129	80	52	0
Psoralidium tenuiflorum	0	0	0	0	0	0	129	80	52	0
Ratibida columnifera	0	57	209	800	518	229	129	80	52	143
Thermopsis rhombifolia	0	57	0	0	0	229	129	80	52	143
Tradescantia occidentalis	0	0	0	0	0	0	129	80	52	0
Perennial Grasses										
Achnatherum hymenoides	0	57	0	0	0	229	129	80	52	143
Bouteloua dactyloides	0	57	0	0	0	229	129	80	52	143
Bouteloua gracilis	0	57	209	800	518	229	129	80	52	143
Calamovilfa longifolia	0	0	0	0	0	0	0	80	0	0
Elymus canadensis	0	0	0	0	0	0	129	80	52	0
Elymus elymoides	0	57	0	0	0	229	129	80	52	143
Hesperostipa comata	0	57	0	0	0	229	129	80	52	143
Koeleria macrantha	0	0	0	0	0	0	129	80	52	0
Nassella viridula	0	0	0	0	0	0	129	80	52	0
Pascopyrum smithii	0	57	209	0	518	229	129	80	52	143
Schizachyrium scoparium	0	0	0	0	0	0	0	80	0	0
Sporobolus cryptandrus	0	57	0	0	0	229	129	80	52	143
Perennial Shrubs										
Artemisia filifolia	0	0	0	0	0	0	0	80	0	0
Artemisia frigida	0	0	0	0	0	0	129	80	52	0
Artemisia ludoviciana	0	57	0	0	0	229	129	80	52	143
Atriplex canescens	0	57	0	0	0	229	129	80	52	143
Atriplex gardneri	0	0	0	0	0	0	129	80	52	0
Ericameria nauseosa	0	57	209	800	518	229	129	80	52	143
Krascheninnikovia lanata	0	0	0	0	0	0	129	80	52	0

Table 2.2. Continued.

Treatment	Control	1	2	3	4	5	6	7	8	9
Diversity (# of species in seed mix)	0	28	11	5	11	28	44	50	44	28
Rate (PLS m^{-2})	0	400	576	1000	1424	1600	1424	1000	576	1000
Species										
Perennial Shrubs										
Rhus trilobata	0	57	209	0	518	229	129	80	52	143
Ribes cereum	0	57	0	0	0	229	129	80	52	143
Yucca glauca	0	0	0	0	0	0	129	80	52	0

Each plot was first lightly raked to loosen and prepare a seed bed. 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. 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. Wood straw mulch was applied shortly after to achieve 50% cover.

Early and mid- growing season (March-June) at all three locations in 2012 and 2013 were drier and warmer relative to average precipitation amounts and temperatures for the area (USDA 2012; DAS2013)(Table 2.3 and 2.4). In 2012, sites ranged from moderate drought status to severe drought status (USDA 2012). To compensate for potential limited plant establishment in 2012 due to drought conditions, supplemental water was applied to all sites. All twelve sites received 3.8 cm total during April-June that was applied in four separate watering events. In 2013, initial establishment was less of a concern because sites were in a less severe drought status and existing plant litter from previous year's growth likely increased moisture retention.

Table 2.3. 2012 and 2013 mean monthly temperatures in degrees Fahrenheit compared to the 82 year average for Fort Collins, CO (Station ID 53005) for March – September (DAS 2013).

Month	2012 Mean Monthly Temperature (^oC)	2013 Mean Monthly Temperature (^oC)	82 Year Historic Average
March	9.94	4.33	4.56
April	12.67	6.50	8.72
May	15.72	14.50	13.78
June	22.61	21.28	18.94
July	24.44	22.61	21.78
August	22.67	22.28	20.72
September	18.11	16.67	16.00
Average Growing Season Temperature	18.02	15.45	14.93

Table 2.4. 2012 and 2013 total monthly precipitation amounts in inches compared to the 82 year average for Fort Collins, CO (Station ID 53005) for March – September (DAS 2013).

Month	2012 Total Monthly Precipitation (mm)	2013Total Monthly Precipitation (mm)	82 Year Historic Average
March	0.00	24.89	29.97
April	10.16	72.14	50.04
May	42.93	71.88	69.60
June	15.49	14.99	46.48
July	78.99	48.26	41.15
August	0.76	14.48	36.07
September	69.09	185.93	32.26
Total Growing Season Precipitation	217.42	407.67	275.59

Data Collection

Vegetation biomass and density were assessed at the beginning of July 2012 and 2013. Two 25- x 75-cm subplots per 4-m^2 plot were sampled. In 2012, the two subplots were placed parallel to each other and in the upper right and lower left of the center point relative to the plots origin. Subplots were placed 15.2 cm from the center point to ensure 30.4 cm between the two subplots. In 2013, the two subplots were placed parallel to each other, but in the lower right and upper left of the center point relative to the plots origin. 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

Response surface regression analysis was performed to assess linear and quadratic model terms and response optimums in 2-dimensional factor (seed mix diversity x seeding rate) space (RSR macro, JMP 10.0.0 SAS Institute Inc., Cary, NC, 1995-2005). Response surfaces were constructed based on 5 model terms: rate, seed mix diversity, rate^2, seed mix diversity^2, and rate * seed mix diversity. In the model output, significance of squared and crossproduct parameters indicate importance of quadratic relationships between independent and dependent variables while parameters that are not squared suggest that a liner relationship between the independent and dependent variables are important. Desirability functions were used to perform multi-response optimization by simultaneously optimizing all nine response variables (Obermiller 1995; Castillo, Montgomery et al. 1996). Desirability values computed represent the proportion (0-1) of response variables that were optimized as designated (maximized or minimized). Completely undesirable values = 0 while 1= completely desirable or an ideal response value. The individual desirabilities of all nine response variables are then combined using the geometric mean, which gives the overall desirability (NIST/SEMATECH 2012). Seeded and noxious response variables were weighted and assigned an importance ranking value of 2, while all other response variables were equally weighted and assigned an importance ranking of 1. Sensitivity analyses were conducted using the sensitivity indicator in JMP10 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. Analyses for 2012 and 2013 were run separately. Response variables were transformed as necessary to adjust for normality. For detailed information on the quadratic equations and models used in the rotatable central composite response surface regression analysis refer to Myers (2002).

Results

Results 2012

In the first growing season (2012), biomass of seeded and volunteer native, diversity of seeded and volunteer native, and density of seeded species all showed overall significant individual RSR models. Of the five parameters included in RSR analyses, seed mix diversity, seeding rate, and $diversity^2$ were significant and had a positive effect on resulting biomass (Figure 2.2a) and density of seeded species (Figure 2.2c) (Table 2.5). Seed mix diversity and seeding rate had a significant positive effect on resulting seeded diversity (Table 2.5, Figure 2.2b). $Rate^2$ had a significant positive effect on resulting volunteer native biomass (Table 2.5, Figure 2.3a), while seed mix diversity, seeding rate, and $rate^2$ had a significant positive effect on resulting volunteer native diversity (Table 2.5, Figure 2.3b). Individual RSR analyses were not significant for biomass and diversity of noxious or non-native species (Table 2.5).

Table 2.5. Statistical results (F- and P-values) of response surface regression analyses with five explanatory variables (seed mix diversity and rate, each term squared, and their interaction) for each of the nine response variables. Significant parameters ($P<0.05$) from the model are also indicated for each response variable. Analyses were conducted separately for 2012 and 2013.

	2012			2013		
Response Variable	F-Value	P-Value	Significant Parameters	F-Value	P-Value	Significant Parameters
Biomass Seeded	11.60	<0.0001*	Diversity, Rate, $Diversity^2$	2.27	.05*	Diversity
Biomass Volunteer Native	2.31	0.04*	$Rate^2$	1.34	0.25	
Biomass Non-Native Non-Noxious	0.49	0.78		0.47	0.80	
Biomass Noxious	0.74	0.59		0.04	1.00	
Diversity Seeded	9.90	<0.0001*	Diversity, Rate	6.72	<0.0001*	Diversity
Diversity Volunteer Native	2.97	0.01*	Diversity x Rate, $Rate^2$	2.39	0.04*	
Diversity Non-native Non-Noxious	1.23	0.29		0.52	0.76	
Diversity Noxious	0.36	0.87		0.24	0.94	
Density Seeded	16.93	<0.0001*	Diversity, Rate, $Diversity^2$	3.69	0.004*	Diversity

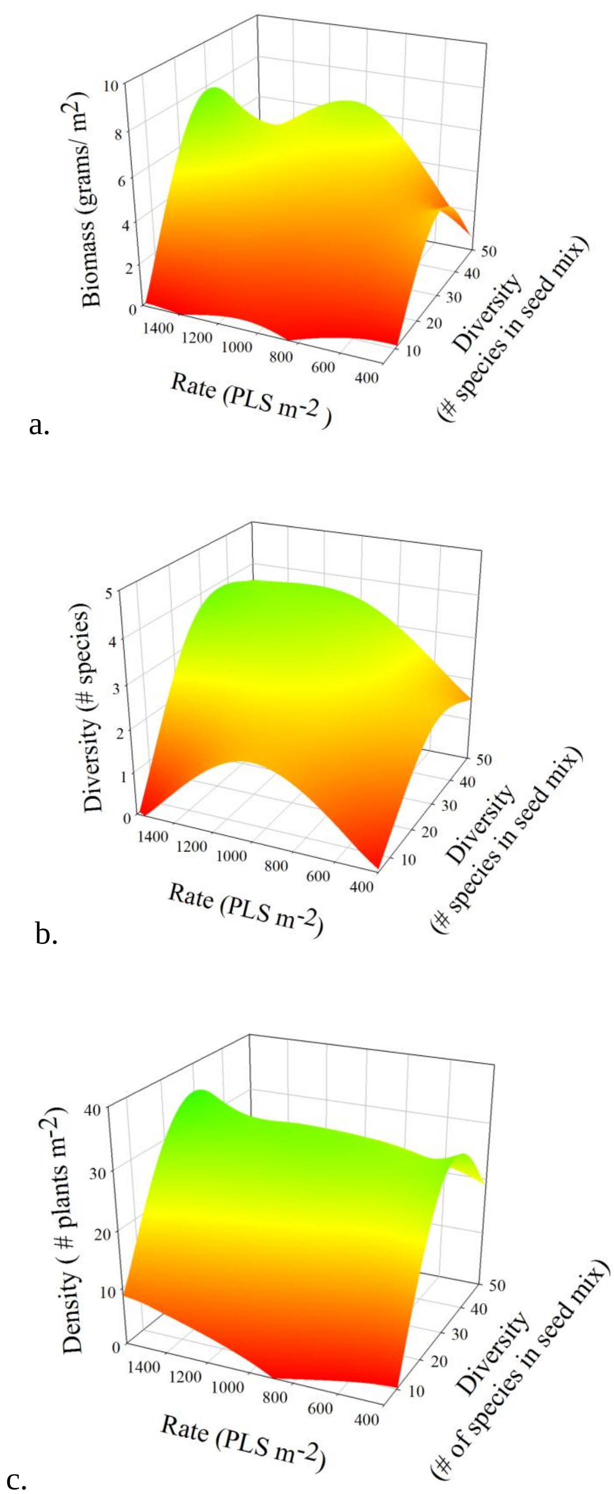
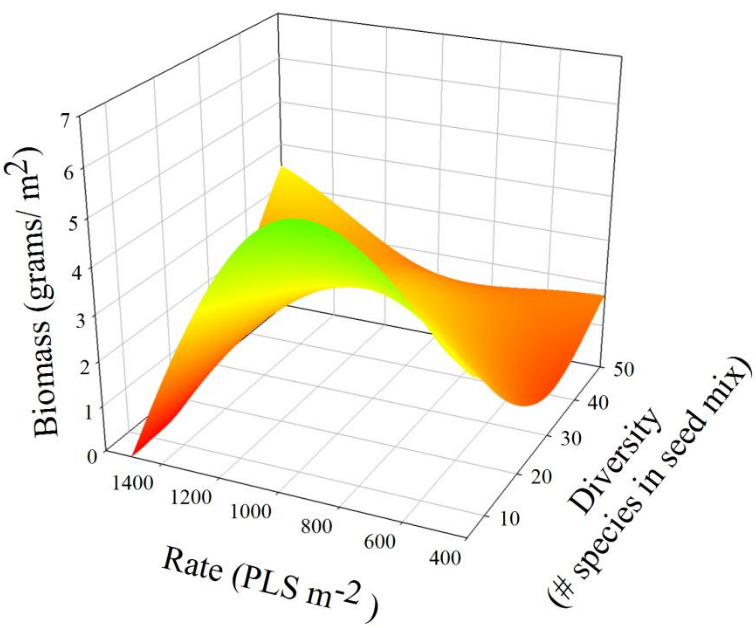
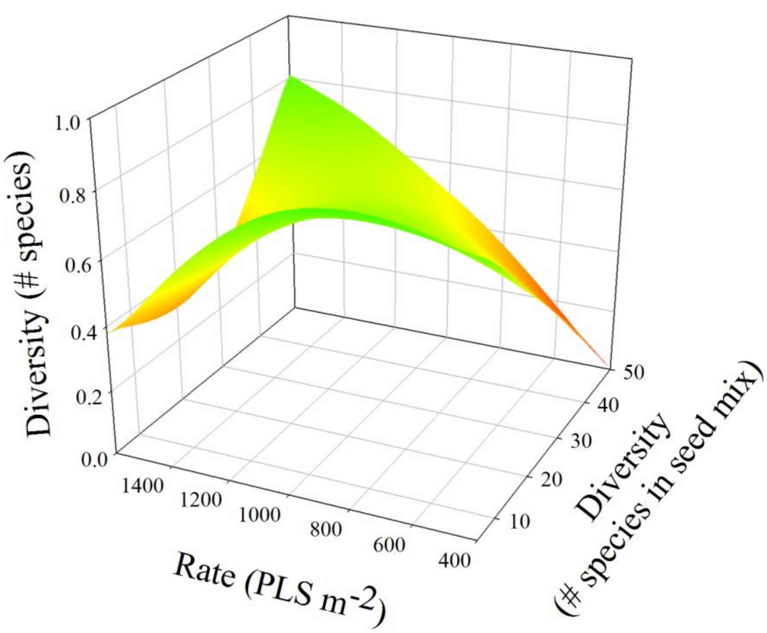


Figure 2.2. Response surfaces of seeded species a) biomass (grams m^{-2}), b) diversity (# species .375 m^{-2}), and c) density (# seeded individuals m^{-2}) to restoration seed mix diversity (# of species in seed mix) and seeding rate (PLS m^{-2}) in the first growing season (2012) following seeding. Green regions represent higher values while red regions represent lower values.



a.



b.

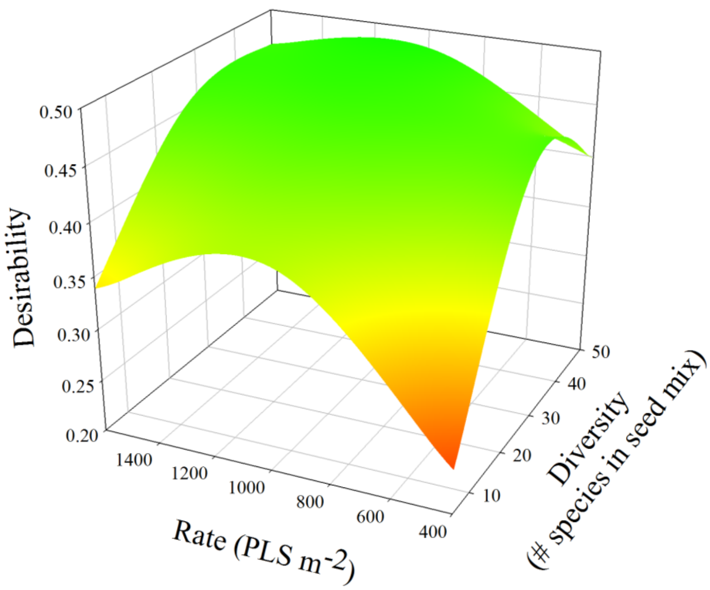
Figure 2.3. Response surfaces of volunteer native a) biomass (grams m^{-2}), and b) diversity (# species .375 m^{-2}) to restoration seed mix diversity (# of species in seed mix) and seeding rate (PLS m^{-2}) in the first growing season (2012) following seeding. Green regions represent higher values while red regions represent lower values.

Overall optimal desirability of seed mix diversity and seeding rate across all nine response variables was found to be at a seed mix diversity level of 43 species and a seeding rate of 1229 PLS m^{-2} which resulted in a .51 desirability value (Table 2.6, Figure 2.4a). Desirability values represent the proportion (0-1) of response variables that were optimized as designated (maximized or minimized). Sensitivity analysis showed no major differences in individual response variables importance in driving overall optimal desirability levels.

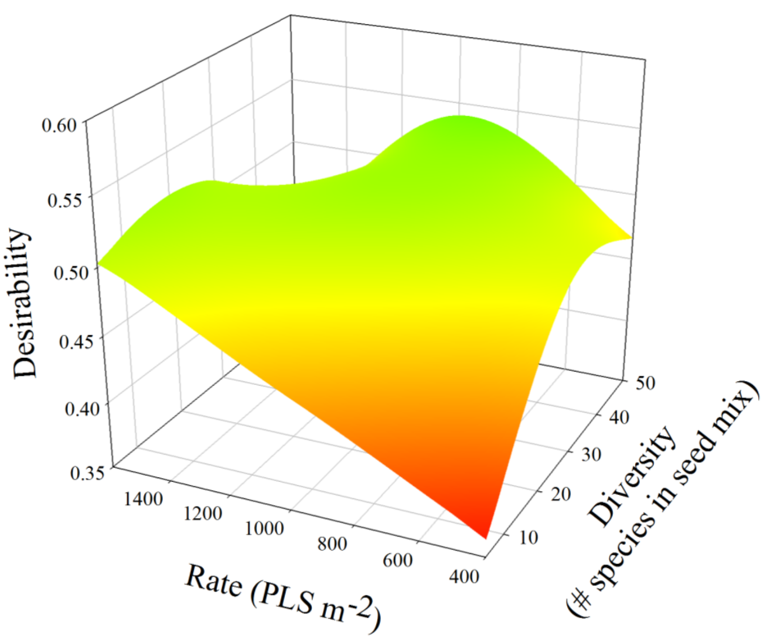
Table 2.6. Optimal seed mix diversity and seeding rate for each of the nine response variables identified by response surface regression analyses. Value refers to the individual optimal value for each response variable: biomass variables are in grams m^{-2}, diversity variables are in number of species per 0.375 m^2, and density variables are in number of individuals m^{-2}. Desirability values represent the outcome of multi-response optimization over all nine response variables on a scale of 0 (completely undesirable) to 1 (most desirable) (Obermiller 1995). Response variables and the input settings for the multi-response optimization procedure are indicated under Optimization settings. Analyses were conducted separately for 2012 and 2013.

	Optimization settings		Optimal 2012			Optimal 2013		
Response Variable	Max/Min	Rank	Diversity	Rate	Value	Diversity	Rate	Value
Biomass Seeded	Max	2	55†	<0†	0.5*	<0†	9612†	1.25*
Biomass Volunteer Native	Max	1	36	1050	0.04*	73†	1084	0.07*
Biomass Non-Native Non-Noxious	Min	1	24	725	1.51**	29	1021	19.9*
Biomass Noxious	Min	2	28	1246	3.16***	<0†	124†	17.7**
Diversity Seeded	Max	2	90†	2414†	5.33***	56†	915	3.2***
Diversity Volunteer Native	Max	1	37	1130	0.29*	71†	897	0.13*
Diversity Non-native Non-Noxious	Min	1	14	1157	1.56*	22	1369	1.72*
Diversity Noxious	Min	2	<0†	572	0.76*	51†	1218	0.64***
Density Seeded	Max	2	44	<0†	5.15*	33	1407	14.45*
Desirability			43	1229	0.51	42	932	0.55

*Solution is a saddle point.
**Solution is a minimum.
***Solution is a maximum.
† Critical values outside of data range



a.



b.

Figure 2.4. Response surfaces for multi-response desirability optimization across nine response variables to restoration seed mix diversity (# of species in seed mix) and seeding rate (PLS m^{-2}) in a) 2012 and b) 2013 following seeding. Desirability can range from 0 (most undesirable) to 1 (most desirable). Green regions represent higher values while red regions represent lower values.

Results 2013

In the second growing season (2013) seeded biomass, seeded diversity, and seeded density all showed overall significant RSR models. Of the five parameters included in RSR analyses, seed mix diversity had a significant positive effect on resulting biomass (Figure 2.5a), diversity (Figure 2.5b), and density of seeded species (Table 2.5, Figure 2.5c). No parameters significantly affected resulting biomass and diversity of volunteer native, non-native, or noxious species (Table 2.5).

Optimal desirability of seed mix diversity and seeding rate across all nine response variables was found to be at a seed mix diversity level of 42 species at a seeding rate of 932 PLS m^{-2} resulting in a .55 desirability value (Table 2.6, Figure 2.4b). Sensitivity analysis showed no major differences in individual response variables importance in driving overall optimal desirability levels.

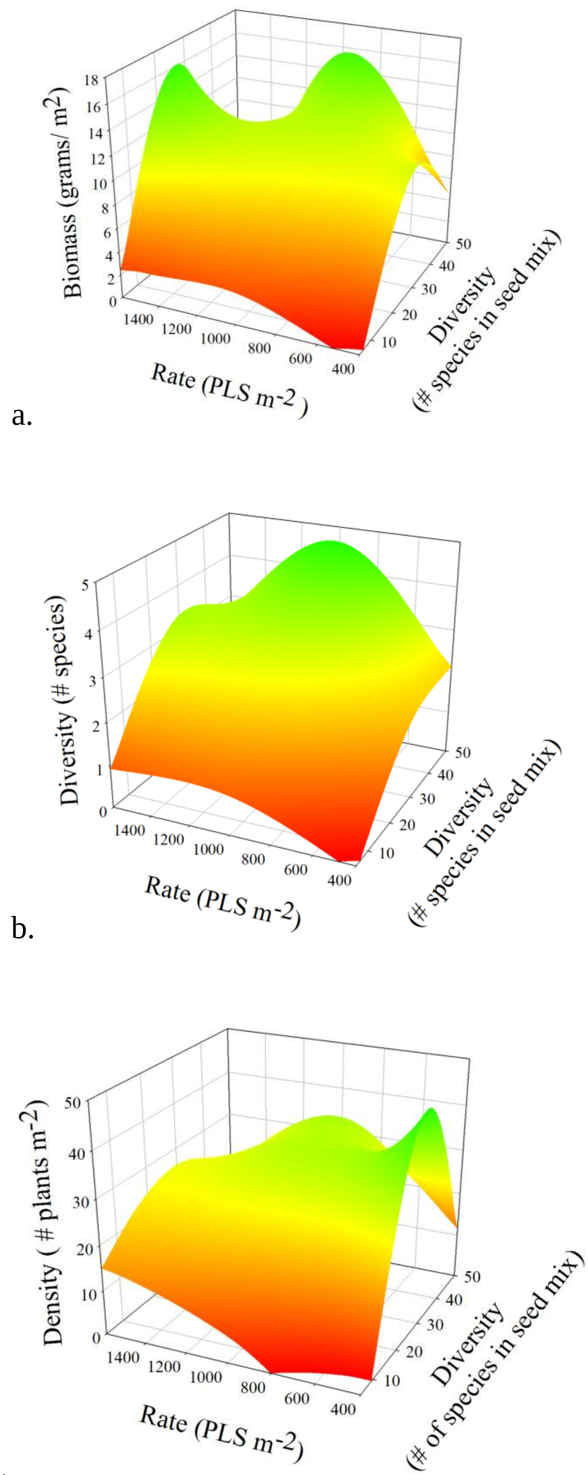


Figure 2.5. Response surfaces of seeded species a) biomass (grams m^{-2}), b) diversity (# species .375 m^{-2}), and c) density (# seeded individuals m^{-2}) to restoration seed mix diversity (# of species in seed mix) and seeding rate (PLS m^{-2}) in the second growing season (2013) following seeding. Green regions represent higher values while red regions represent lower values.

Discussion

The objective of this study was to determine an optimal seed mix diversity level and corresponding seeding rate for achieving restoration success on the shortgrass steppe. Both 2012 and 2013 results indicate that a higher diversity seed mix (43 species in 2012 and 42 species in 2013) in combination with a higher seeding rate (1129 PLS m^{-2} in 2012 and 932 PLS m^{-2} in 2013) results in higher overall restoration success (higher biomass and diversity of seeded and volunteer native species, lower biomass and diversity of noxious and non-native species, and higher density of seeded species). It is important to note however, that our seeding rates are based on broadcast seeding methodology, which typically uses 2x more seed than drill seeding. While the optimal levels for seed mix diversity and seeding rate identified might not be financially feasible for widespread use, our results do show a consistent trend toward more desirable restoration outcomes with more diverse seed mixes applied at higher seeding rates.

The seed mixes in this study were intentionally constructed to reflect functional diversity. Our lowest diversity level was comprised of 5 species: a perennial and an annual grass, a perennial and an annual forb, and a shrub. The eight subsequent seed mixes were comprised of the same number of functional groups, but had additional functional redundancy with increases in seed mix diversity due to increasing species within each functional group. Functional diversity in ecosystems has been shown to decrease invasion by non-native species (Symstad 2000; Funk, Cleland et al. 2008), improve ecosystem functioning and resilience (Elmqvist, Folke et al. 2003), and create functional redundancy/ insurance effects (Díaz and Cabido 2001). Species within functional groups show different responses to environmental fluctuations, therefore, redundancy can ensure, that at least some species of a functional group will survive an environmental fluctuation or disturbance (*i.e.*, the insurance effect) (Díaz and Cabido 2001; Naeem 2006). In the context of restoration, functional redundancy has been seen as desirable due to its effects on ecosystem stability.

In 2012 the biomass and diversity of volunteer native species was significantly affected by seeding rate and seed mix diversity. However, in 2013 this result did not hold and could suggest that seeded species in 2012 created a facilitation effect for volunteer native species. Volunteer native species could have benefited from establishing seeded species due to the seeded species ability to alter site conditions, improve resource availability, and or protect against herbivory (Padilla and Pugnaire 2006). This possible facilitation effect is interesting as very few studies have investigated the role of restoration seedings on facilitating establishment of volunteer native plant species (Bruno, Stachowicz et al. 2003).

Optimal levels of seed mix diversity and seeding rate fell within the ranges I tested (5-50 species at 400-1600 PLS m^{-2}) in both 2012 and 2013, 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 or out of range, possibly suggesting a plateau effect (Hardin 1960), competition (Grime 1973; Bakker, Wilson et al. 2003), or the failure of certain species to germinate.

Our results show similar findings to previous studies looking at the effects of seed mix diversity and seeding rate simultaneously on target plant communities. Carter and Blair (2012) found that the higher seeding rate and higher seed mix diversity level that they tested produced greater cover of seeded and native species. However, their study only examined two levels for each factor and their high seeding rate (344 PLS m^{-2} drill seeded, approximately equivalent to 688 PLS m^{-2} broadcast seeded) was significantly lower than my highest seeding rate (1600 PLS

m^{-2} broadcast seeded). Nemec et al.(2013) found similar results as mine in regards to higher seed mix diversity levels, but found contradicting results in regards to higher seeding rates.

In both 2012 and 2013, biomass and diversity of non-native and noxious species were not significantly affected by seed mix diversity and seeding rate (Table 2.5). These results suggest that additional invasive species management during restoration is vital to success and an invasive species management plan should be constructed and implemented in order to better deter the establishment and proliferation of non-native and noxious species. It is important to note that all three locations had differing species composition at the onset of this experiment. The initial presence of invasive species at two of the locations could have had a significant impact on the resulting plant community. Many exotic species have persistent seed banks (Baskin and Baskin 1998) and can have strong effects on community development, such as outcompeting native species for resources and space, infect natives with diseases to which they have no resistance, and alter ecosystem functioning (Vitousek 1990).

The first half of the growing season (March-June) at all three locations in 2012 and 2013 were dry relative to average precipitation for the area and ranged from moderate to severe drought (USDA 2012). This lack of precipitation early in the restoration process may have had an effect on the germination and establishment of seeded species. Seedings have proven less successful in dry years (Abbott and Roundy 2003). If available moisture only occurs during short infrequent rainfall events, the seedling root zone may not have adequate moisture to support germination and seedling development. Even if germination occurs, seedlings may fail to develop adventitious roots that can readily gain access to deeper water sources during periods of drought (Roundy, Abbott et al. 1997).

Few studies have examined the combined effects of seed mix diversity and seeding rate on restoration success. Rather than testing two discrete levels of each factor, this study employed RSR to assess a broad range of values to identify optimal seed mix diversity and seeding rates. Industry standards in northeastern Colorado range from 3-10 species in a seed mix and seeding rates from 400-600 PLS m^{-2} broadcast seeded. Our results indicate a four-fold increase in seed mix diversity and a two fold increase in seeding rates for optimal restoration success compared to current practices in this region. Therefore, more research is needed to better understand how these two variables can be used to achieve more effective restoration results than current industry standards. Specifically, the need for more long-term studies examining these two variables simultaneously is vital to better assess restoration success through many stages of plant community development.

Conclusion

Based on the results of this study, I conclude that applying a high diversity seed mix (42-43 species) at a higher seeding rate (932-1129 PLS m^{-2} broadcast seeded) will result in more successful restoration. Managers should consider using more species in their seed mixes than the typical industry standard and applying them at higher rates in order to achieve greater restoration success.

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APPENDIX

Table A1. Number of occurrences of seeded species for 168 experimental plots examining the role of seed mix diversity and seeding rate on restoration success in northeastern, CO over a two year time period (2012-2013).

Species	Common Name	# of occurrences
Atriplex canescens	fourwing saltbush	109
Helianthus annuus	common sunflower	98
Verbesina encelioides	golden crownbeard	94
Pascopyrum smithii	western wheatgrass	79
Bouteloua dactyloides	buffalograss	76
Sphaeralcea coccinea	scarlet globemallow	55
Psoralidium tenuiflorum	slimflower scurfpea	47
Dalea purpurea	purple prairie clover	43
Cleome serrulata	Rocky Mountain beeplant	33
Bouteloua gracilis	blue grama	27
Thelesperma filifolium	stiff greenthread	22
Vulpia octoflora	sixweeks fescue	21
Aristida purpurea	purple threeawn	20
Astragalus laxmannii	Laxmann's milkvetch	20
Rhus trilobata	skunkbush sumac	16
Adenolinum pratense	meadow flax	13
Ericameria nauseosa	rubber rabbitbrush	10
Liatris punctata	dotted blazing star	10
Elymus elymoides	squirreltail	9
Thermopsis rhombifolia	prairie thermopsis	9
Yucca glauca	soapweed yucca	7
Oxytropis lambertii	purple locoweed	5
Penstemon angustifolius	broadbeard beardtongue	5
Hesperostipa comata	needle and thread	4
Machaeranthera tanacetifolia	tanseyleaf tansyaster	4
Sporobolus cryptandrus	sand dropseed	4
Achnatherum hymenoides	Indian ricegrass	3
Erysimum capitatum	sanddune wallflower	3
Astragalus bisulcatus	twogrooved milkvetch	2
Atriplex gardneri	Gardner's saltbush	2
Lupinus argenteus	silvery lupine	2
Polygonum pensylvanicum	Pennsylvania smartweed	2
Allium textile	textile onion	1
Astragalus drummondii	Drummond's milkvetch	1
Calamovilfa longifolia	prairie sandreed	1
Schizachyrium scoparium	little bluestem	1