OSMRE National Technology Transfer Team (NTTT), **Applied Science Final Report***
U.S. Department of the Interior, OFFICE OF SURFACE MINING RECLAMATION AND ENFORCEMENT

New Approaches to Improving Sagebrush Establishment During Mine Reclamation

OSMRE Cooperative Agreement Number: S21AC10091-00
Final Report

Reporting Period: August 1, 2021 to January 31, 2024

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May 2024

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Disclaimer

Abstract

Sagebrush shrubs are a vital part of the sagebrush biome, providing essential habitat for many wildlife species. Restoring sagebrush is also necessary for many reclamation projects to meet their bond release criteria. However, establishment from seed often fails or has very low success. In this study, we addressed several topics with the goal of better understanding how to increase sagebrush establishment success from seed. We asked (1) Does topsoil depth on reclaimed mines influence sagebrush establishment? (2) Can seed enhancement technologies (SETs) improve the establishment of Wyoming big sagebrush seedlings on mine reclamation sites? and 3) Can SETs protect sagebrush and perennial bunchgrasses from the impacts of pre-emergent herbicides used to control invasive annual grasses?

To address question (1), we conducted a descriptive study of sagebrush density, cover, and size on mine sites of different ages and topsoil depths. We found some evidence that very shallow (6 inches or less) or no topsoil strongly limits sagebrush establishment, and evidence that deeper soils favor perennial grasses that can competitively suppress sagebrush.

To address question (2), we conducted a series of lab and field trials, testing different candidate SETs that delivered fertilizers and/or root growth stimulating compounds. We found no strong evidence that any SETs improved seedling emergence, survival or size. Externally-applied fertilizers enhanced growth but at a high cost to emergence. We conclude that sagebrush may not be a good candidate species for SETs due to its very small seed size that limits the ability to apply enough active ingredients in a thin film coating and its need for light to germinate, precluding applying any thicker coating. Measures that did enhance sagebrush establishment were (a) seeding into swales that collect more spring moisture, in the first year of seeding, (b) seeding sagebrush one year after the initial reclamation and seeding, so the seedlings can benefit from the facilitative effects of some vegetation cover without strong competition, and (c) where existing perennial cover is high, reducing grass competition via patchy herbicide application to create lower competition microsites for sagebrush.

To address question (3), we conducted a series of field experiments testing herbicide protection SETs, using activated carbon to adsorb herbicide. This was tested with sagebrush and two species of perennial grasses, in sites with high cover of the invasive annual grass *Bromus tectorum* (cheatgrass). Results were mixed, at times showing some evidence of herbicide protection, for some species and site combinations. Best results were obtained using a mix of herbicide protection and deep furrows to reduce the top layer of herbicide-impacted soil. Results indicate some potential promise for herbicide protection SETs for perennial grasses, with further research and development necessary. The potential promise for sagebrush appears limited, given the challenges of small seeds and needing light to germinate.

Graphical Materials List

Executive Summary

Sagebrush shrubs are a vital part of the sagebrush biome, providing essential habitat for many species of wildlife. Restoring sagebrush is an important step for many reclamation projects to meet their bond release criteria. However, dry and variable climate conditions make the sagebrush ecosystem naturally slow to recover from disturbances; it can take decades for late-successional species such as sagebrush to reach pre-disturbance levels. The spread of nonnative annual grasses presents a new challenge to effective restoration in the sagebrush ecosystem.

Big sagebrush (*Artemisia tridentata*) is typically seeded as part of the reclamation process. However, seeds and seedlings face major barriers to establishment, including altered soil conditions, low and unpredictable rainfall conditions that result in inadequate soil moisture, and competition from plants that have established more quickly. Seed enhancement technologies (SETs), which are used to modify seeds to improve seedling establishment, offers a new approach to native plant restoration that may be able to address some of these challenges. Topsoil depth and quality may also influence the establishment of sagebrush, particularly in areas with significant surface disturbance like mine reclamation sites. The importance of topsoil depth in affecting sagebrush establishment is not well understood. In this project, we set out to test three research questions: 1) Does topsoil depth on reclaimed mines influence sagebrush establishment? 2) Can SETs improve the establishment of Wyoming big sagebrush seedlings on mine reclamation sites? and 3) Can SETs protect sagebrush and perennial bunchgrasses from the impacts of pre-emergent herbicides used to control invasive annual grasses?

Because of the large volume of results and separate topics covered in this report, we have divided the report into separate chapters, each with their own introduction, methods, results, and discussion.

In **Chapter 1** we conducted a descriptive study over multiple reclaimed mine sites in Wyoming. We measured sagebrush density, cover and volume across varying topsoil depths. In the data analysis we took into account the effect of topsoil depth as well as other variables known to influence plant establishment, including precipitation and seeding rate. Our sample size for this study was small due to constraints on access to reclaimed mine sites. As a result, our confidence in our results is low and results complex to interpret. We found that very shallow (6 inches or less) or no topsoil appears to be highly detrimental to sagebrush establishment. Consistent with previous research, perennial grasses composed a higher proportion of the canopy in locations with deeper topsoil, potentially creating a competitive environment for sagebrush. We also found that very high seeding rates do not necessarily lead to higher rates of sagebrush establishment. We concluded that there are many environmental variables and reclamation practices that influence sagebrush establishment after reclamation; while topsoil depth may play a role, it is only one of many factors. We recommend that topsoil be applied in depths greater than 6 inches, and a patchy mosaic of depths between 6 and 15 inches may increase overall plant diversity; however, long-term experimental research is needed to draw clear conclusions about the role of topsoil depth in sagebrush establishment and long-term survival and growth.

In **Chapter 2** we tested different "root enhancement" SETs to determine if they improved the emergence, growth and survival of sagebrush seedling over planting bare seed. In the lab, we saw that an externally-applied slow-release fertilizer treatment had a positive effect on sagebrush seedling biomass, but it also limited emergence. We did not see evidence that any of the film-coating SETs tested greatly improved sagebrush seedling emergence or size. We saw similar results in the field, with most SETs having little to no impact on seedling emergence, growth or survival, except the slow-release treatment where we saw the same trade-offs as in the lab. It is unclear whether SETs are a viable approach for sagebrush given the challenges of delivering enough active ingredient without severely limiting emergence. In a fresh reclamation field setting, we found greater establishment success in swales (run-on areas) than slopes

(run-off areas) and suggest that seeding should focus on swales if resources are limited. Additionally, seeding sagebrush in multiple years is more likely to result in the desired establishment rates than seeding in just one year.

In **Chapter 3**, we tested the same SETs as in Chapter 2 but at older reclamation sites where there was strong competition from existing perennial grasses. This is a common problem at older sites where initial sagebrush seeding failed to produce substantial sagebrush cover. Our goal was to test whether some combination of reducing grass competition and a growth-enhancing SET could improve sagebrush establishment in this competitive setting. We seeded all SET treatments into plots in which we experimentally manipulated grass competition by clipping grass to simulate heavy grazing, treating half the plants with herbicide, or leaving the grass untreated. Our hypothesis was that the combination of reduced competition and any benefits from SETs would improve sagebrush establishment. We found that reducing competition from neighboring vegetation did enhance sagebrush growth and survival (though not emergence). Results across sites and years were variable, with competition more present under lower summer rainfall conditions. Herbicide was a more effective method to knock back competition than clipping vegetation. While most of the SETs had little effect, as in Chapter 2, we again found some benefit of externally-applied fertilizer on seedling size. While treating larger areas with herbicide to reduce perennial grasses is not feasible, we suggest that herbicide could be strategically applied to create small "competition refuges" into which sagebrush could be seeded or transplanted, as a way to increase sagebrush cover in high-grass reclamation settings.

In **Chapter** 4 we tested whether herbicide protection (HP) SETs (using activated carbon seed to absorb the herbicide) can adequately protect sagebrush and perennial grass seeds from pre-emergent herbicide and whether these SETs improve establishment over bare seed. These experiments were performed in areas with a high density of invasive annual grasses, mainly cheatgrass (*Bromus tectorum*), and tested several different SET approaches on three species: sagebrush, and two species of perennial grass commonly used in sagebrush biome restoration. The results of these experiments were mixed and seemed to be dependent on winter and spring precipitation. In the first field trial, there was no evidence of herbicide protection. The second field trial demonstrated herbicide protection, primarily from the carbon HP coating, for some species and sites, as well as a strong and consistent benefit of seeding in deeper furrows. Patterns observed in the second year in some cases were high enough to suggest that future at-scale demonstrations could yield successful restoration outcomes.

Chapter 1: The effects of topsoil depth on sagebrush establishment

Michaela Owens, Hannah Demler, Magdalena Eshleman, and Corinna Riginos

Introduction

The vast sagebrush ecosystems of the western US are facing numerous threats and management challenges. These systems provide essential habitat for imperiled Greater sage-grouse, big game such as mule deer and pronghorn, and some 350 other species of plants and animals (Suring et al. 2005). They also include large areas of public and private grazing land. The dominant shrub, Wyoming big sagebrush (*Artemisia tridentata* spp. *wyomingensis*), is an essential habitat resource for many of these wildlife species. The last several decades have seen rapid changes in the quantity and quality of sagebrush ecosystems due to a variety of factors, including non-native species invasions, overgrazing, changes in fire regimes, and surface disturbances (Crawford et al. 2004; Davies et al. 2011).

Dry and variable climate conditions make the sagebrush ecosystem naturally slow to recover from disturbances; it can take decades for late-successional species like sagebrush to reach pre-disturbance cover and density levels without reseeding as part of restoration or reclamation (Rottler et al. 2017). On mined sites and oil and gas well pads, reclamation efforts have been relatively successful at establishing early-successional and introduced species but much less successful in establishing sagebrush (Shuman et al. 2005; Rottler et al. 2017). Major barriers to establishment for seeded sagebrush include altered soil conditions on mined or otherwise disturbed sites, low and unpredictable rainfall that results in inadequate soil moisture, and competition from plants that have established more quickly (Svejcar et al. 2017).

Several practices have been developed to minimize the influence of altered soil conditions in mine reclamation, such as topsoil stockpiling and subsequent replacement. Even with these practices, chemical, physical, and biological soil conditions are inevitably changed by mining, especially if topsoil is stockpiled for long periods of time. However, the presence of topsoil is critical to sagebrush establishment (Monsen and Richardson 1984; Schuman et al. 1998). Topsoil provides not only a medium for plants to grow, but also plant-available nutrients, mycorrhizae, and organic matter that are all important for plant growth and survival (Munoz-Rojas et al. 2016; Erickson et al. 2017). While the presence of functional topsoil is important, research has also shown that areas with shallower topsoil can have greater plant diversity and less competition from perennial grasses than areas with deeper topsoil (Bowen et al. 2005; Schladweiler et al. 2005; Redente et al. 1997). Deep topsoil is conducive to perennial grasses that can competitively suppress the establishment of shrubs, such as sagebrush, and some forbs, potentially leading to overall reduced diversity of species and plant types. To date, however, most of the research on the impacts of topsoil depth have not focused on sagebrush, leaving open the question of optimal topsoil depth for promoting sagebrush establishment.

Initially after the Surface Mining Control and Reclamation Act of 1977 was enacted, topsoil was replaced at a uniform depth across a reclamation site (Schladweiler 2018). However, research has demonstrated that a mosaic of variable topsoil depths can lead to greater overall plant diversity (Bowen et al. 2005; Schladweiler et al. 2005; Redente et al. 1997) and has since been adopted as a reclamation practice at some mine sites (Schladweiler 2018). Little work has been done to illuminate whether the benefits of variable topsoil depths are a general pattern and what short-term versus long-term tradeoffs there may be in soil depth. For example, sagebrush seedlings may establish more easily in shallow topsoil where competition from grasses is less than in deeper topsoil, but as shrubs get larger, they may experience limitations in growth due to limited availability of nutrients and moisture in shallow soils.

We conducted a descriptive study of the relationship between topsoil depth and sagebrush cover and density over multiple reclaimed mine sites in Wyoming. We hypothesized that sagebrush density and cover would be higher on reclaimed sites with shallower (but not zero) topsoil, which would also have lower cover and density of other perennial plants.

Methods

Data was collected at six reclaimed mine sites in central and southern Wyoming: three in the Hanna Basin, one outside of Rock Springs, and two in the Gas Hills area of Fremont County during the summers of 2022 and 2023. Of these six sites, four were former coal mines and two were former uranium mines. Reclamation dates spanned from 1982 to 2019, and topsoil depths ranged from 0 to 15 inches (0-38.1 cm) (Table 1). We made a comprehensive attempt to access other reclaimed mines in Wyoming, but lack of topsoil depth data and/or lack of permission to access private land made it infeasible to expand the number of sites. This was unfortunate and puts some limitations on our ability to draw inferences from the data.

Using ArcGIS Pro and maps from bond release documentation, polygons were created at each mine site. Many of the mines we visited were reclaimed across multiple years and different seed mixes were used and different topsoil depths were applied across years. We took this into account when creating our sampling polygons by creating a different feature class of polygons for each unique combination of topsoil depth and reclamation seed mix. Only areas where Wyoming big sagebrush was seeded were sampled. Data collection points were randomly generated within each polygon, and not all polygons were sampled at each mine. Five points within at least one topsoil depth were sampled for each mine.

At each randomly generated point, a 100 m transect was set up with a tape measure. The azimuth for each point was randomly chosen from a range that ensured the transect did not depart from the polygon. Along the transect, plant functional group and/or sagebrush species were sampled at the point vertical from the transect to the ground every 2 meters (line-point-intercept method). Sagebrush density was also collected along each transect; all sagebrush within 2 meters perpendicular to the transect were recorded to species level. Sagebrush height and canopy width were recorded for 10 randomly selected sagebrush plants in the general vicinity of the transect.

Annual and spring precipitation from the first year after seeding were obtained from the closest weather station to each site using the National Atmospheric Association Applied Climate Information System (NOAA ACIS, https://scacis.rcc-acis.org/) regional climate data database. Precipitation data for all sites in Hanna Basin were from the Elk Mountain station which is approximately 20 – 30 miles southeast of those sites. Precipitation data from Lionkol was taken from the Rock Springs Fire Station approximately 5 miles to the southwest. McIntosh Mine precipitation data was taken from the Jeffrey City station, approximately 15 miles north. The Jeffrey City station was also the source of precipitation data for the Day Loma mine which is approximately 10 miles to the south of that mine. Spring precipitation was defined as February – April after seeding and annual precipitation was defined as the total within the calendar year following fall seeding (January – December).

Table 1. Description of sampled polygons

Mine	**Mineral mined**	**Year (s) reclaimed**	**Approximate location**	**Transects sampled**	**Topsoil depth (in)**	**Wyoming big sagebrush seeding rate(s) lbs/ac**	**Grass seeding rate(s) lbs/ac**
Seminoe 1	Uranium	1984, 1986	-106.7148, 41.8721	5	11	0.5	13
Seminoe 1	Uranium	1986-1987	-106.7014, 41.8717	5	15	0.5	13
Shoshone 1	Uranium	2003	-106.5064, 41.9547	5	6	0.4	0
EDC	Coal	1982, 1987	-106.6461, 41.8421	5	11	0.25	15
EDC	Coal	1983	-106.6802, 41.8722	5	6	0.5	15
Lionkol	Coal	2014	-109.1872, 41.6528	5	0	0.1	17
McIntosh	Uranium	2019-2021	-107.8402, 42.3622	15	9	0.1, 1.5, 3.27	11.3, 12.13, 14.01
Day Loma	Uranium	2012, 2014, 2016, 2019	-107.6799, 42.7357	15	12	0.5	14

Statistical analysis was performed in R version 4.1.1 (R Core Team, 2021). Figures were plotted using the R package *ggplot2* (Wickham, 2016). For analysis, we only looked at metrics for Wyoming big sagebrush (hereafter "sagebrush"). We calculated percent cover for sagebrush and perennial grasses as a percent of all observations (points) for each transect. Sagebrush density was the absolute number of plants observed along the 2 m belt perpendicular to the transect. Sagebrush volume was calculated using the ellipsoid volume equation: $4/3*\pi*height/2*width1/2*width2/2$ and averaged for each transect. For analysis, spring precipitation, annual precipitation, and reclamation age were put into bins. Spring precipitation was separated into five bins: <1 in, 1-1.99 inches, 2-2.99 inches, 3-3.99 inches, and 4-5 inches. Annual precipitation was put into the categories of <6 inches, 6-8.99 inches, 9-12 inches and >12 inches. Reclamation age was put into four bins: 1-5 years, 6-11 years, 12-20 years, and 21-41 years.

All measured variables displayed non-parametric data structure; we therefore used Kruskal Wallis tests (a non-parametric version of analysis of variance) to determine differences among the group medians of our dependent variables at different topsoil depths. Pairwise differences were investigated using Dunn's tests with a Bonferroni adjustment for multiple comparisons. We acknowledge that individual transects were treated here as true replicates when they were actually nested within mines and/or polygons within mines and therefore not fully independent. This was necessary given the limited mines we could access and the limited scope of available topsoil depths, which resulted in the non-parametric structure of our data. Due to the small data set, we were unable to include multiple predictors of vegetation responses in one overall model, but we did separately examine the relationships among response variables and other potential explanatory variables. Because the focus of this study was the effects of topsoil depth on sagebrush establishment, we looked at topsoil depth as an explanatory variable for all metrics. In addition to topsoil depth, we considered the potential effects of other important factors in sagebrush establishment and growth. For sagebrush density, we considered the potential effects of sagebrush seeding rate, spring precipitation, and annual precipitation as variables that could influence sagebrush establishment, which could then affect sagebrush density. For sagebrush percent cover, we considered all the explanatory variables as well as the effect of reclamation age. For volume, we also looked at reclamation age. Older reclamations are likely to have older sagebrush with larger canopies which could influence percent cover and shrub volume.

We also examined the relationships between topsoil and the other explanatory variables to understand if there were non-independencies that may be important to consider in interpreting our results. Specifically, we examined potential relationships between topsoil depth and: spring and annual precipitation, sagebrush seeding rate, and reclamation age. This was also done using Kruskal-Wallis tests and Dunn's tests with a Bonferroni adjustment for pairwise comparisons.

Results

Examination of the data revealed strong evidence for relationships between topsoil depth and reclamation age ($\chi^2 = 59$, df = 11, $p < 0.001$, Figure 1), spring precipitation ($\chi^2 = 59$, df = 12, $p < 0.001$), annual precipitation ($\chi^2 = 59$, df = 12, $p < 0.001$), and sagebrush seeding rate ($\chi^2 = 37.14$, df = 5, $p < 0.001$). These relationships among variables potentially complicate our ability to make inferences about the effect of topsoil depth relative to these other variables that could be alternatively or additionally underlying observed vegetation responses. However, these variables were generally not directionally (overall positively or negatively) correlated with topsoil depth. More specifically, the 1-5 years since reclamation ages all had 9 in of topsoil depth, and the 12-20 years since reclamation all had 0 in topsoil depth, but the oldest reclamation ages (21-41 years) were more evenly spread among shallow (6 in) and deeper (11 and 15 in) depths (Figure 1). The latter spread gives some assurance that patterns associated with topsoil depth may not be entirely related to reclamation age. Spring precipitation was generally positively associated with topsoil depth, which could confound any apparent positive effects of topsoil depth, but the lowest spring precipitation value by far was associated with the greatest (15 in) topsoil depth. Annual precipitation was variable in relation to topsoil depth; precipitation was much higher at the sites with 0 in depth, moderate at 9 in and 12 in, and low at 6, 11, and 15 in depth. Sagebrush seed rate was substantially higher for sites with 9 in topsoil depth than any other topsoil depth. Understanding these patterns is important to interpretation of sagebrush relationships with topsoil depth.

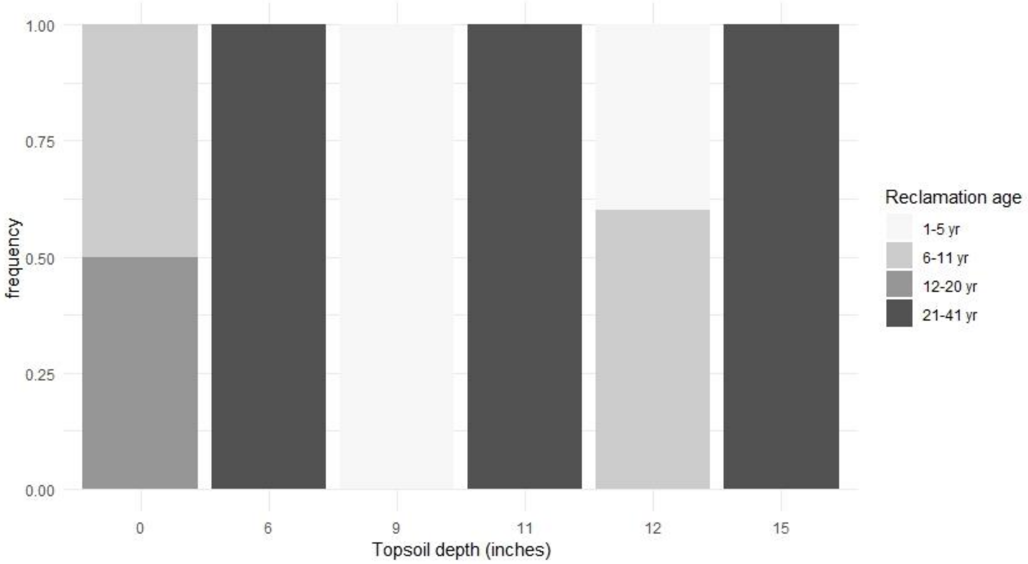

Figure 1. Frequency of observations for each topsoil depth and reclamation age (n=60).

There was strong evidence that topsoil depth had an effect on sagebrush density on mine reclamation sites ($\chi^2 = 19.97$, df = 5, p = 0.001). There was a general pattern of low sagebrush density in the shallowest topsoil depths and higher mean sagebrush density at sites with topsoil depths above 9 in. Pairwise comparisons showed that the difference in sagebrush density was only significantly different between 9 in and 0 in topsoil depths (p = 0.021) and 9 in and 6 in topsoil depths (p = 0.008). The lowest average sagebrush densities were recorded at sites with 6 in and 0 in topsoil depths, with an average of 3.0 and 17.4 sagebrush respectively, while the highest average sagebrush density was found at the site with 9 in topsoil depth (average of 190 sagebrush) with lower mean values at 12 and 15 in topsoil depth (98.4 and 90.6 respectively, Figure 2).

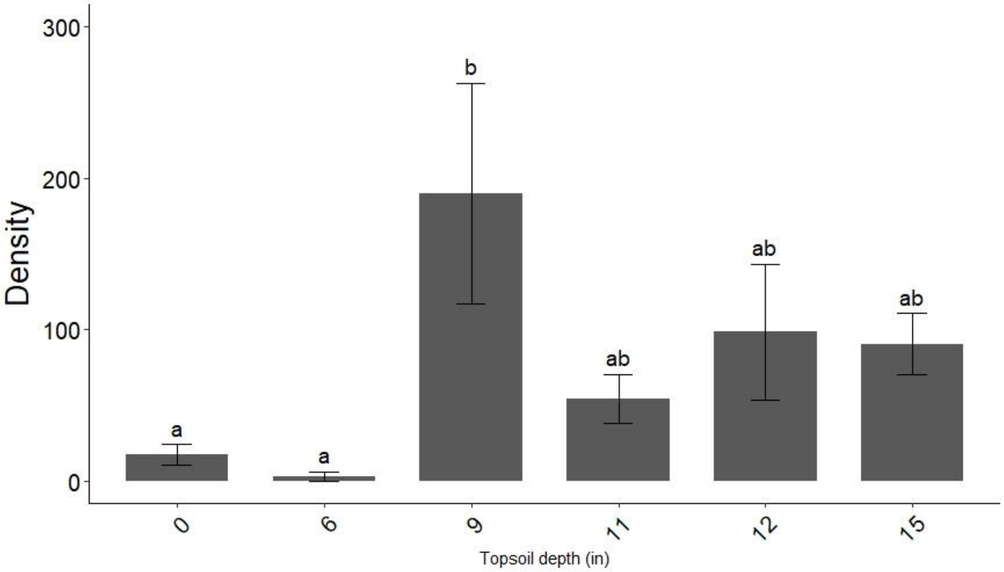

Figure 2. *A. tridentata* average density counts along a 2 x 100 m strip recorded at sites with varying topsoil depths (n = 60) with standard error bars and letters indicating differences in Dunn's test post-hoc comparisons.

There was no evidence that sagebrush seeding rate had an effect on sagebrush density across mine reclamation sites (χ^2 = 5.95, df = 4, p = 0.17), though the sites with the highest seeding rates did have the highest average densities of sagebrush. It is possible that the high sagebrush density at the 9 in depth sites was due at least in part to the higher seed rate associated with this soil depth, but the lack of overall effect of seed rate indicates this is not a primary driver of sagebrush density. There was also no evidence that spring precipitation had an effect on sagebrush density (χ^2 = 3.28, df = 4, p = 0.51). However, there was moderate evidence that annual precipitation in the year after seeding did have an effect (χ^2 = 9.91, df = 3, p = 0.019). Annual precipitation varied widely across sites with the driest receiving only about 5 inches of precipitation while the wettest received 15.42 inches. Pairwise comparisons showed that the effect of annual precipitation was driven by a significant difference in sagebrush density between 9-12 in and >12 in of annual precipitation. The mean density at sites with 9-12 in of precipitation was 330 plants per transect while the sites that received >12 in had an average density of 34.7 plants per transect. The mean density for the 9-12 inch rainfall sites was also higher than the other rainfall categories (<6 in rainfall: 47.3 plants per transect and 6-9 inches of rainfall: 55.2 plants per transect), although these differences were not statistically significant. The patterns of sagebrush density in relation to annual precipitation were not consistent with patterns in relation to topsoil depth. Therefore, the data suggest an overall positive relationship between topsoil depth and sagebrush density as well as a possible separate effect of annual precipitation in the year following seeding.

There was also very strong evidence that sagebrush size differed by topsoil depth (χ^2 = 23.434, df = 5, p <0.001), with the highest average sagebrush sizes recorded at the site with 15 inches of topsoil (average volume = 1.05 m^3). Pairwise comparisons revealed that the average sagebrush volume at 15 inches of topsoil was significantly greater than sagebrush sizes at 0, 9, and 12 inch topsoil depths (p = 0.043, 0.004, and 0.002 respectively). The analysis also showed that the sites with 11 inches of topsoil depth had significantly larger sagebrush than the 9 inch topsoil depth site (p = 0.04) and the 12 inch topsoil depth site (p = 0.026, Figure 3). These patterns, however, were consistent with reclamation age patterns (0, 9 and 12 in topsoil depth associated with younger reclamation ages and 6, 11, and 15 in depth associated with older reclamation ages), and reclamation age also had a significant effect on sagebrush volume (χ^2 = 21.363, df = 3, p < 0.0001). Average sagebrush volume increased with reclamation age, with the largest sagebrush recorded at reclamation sites 21-41 years in age (Figure 3). Pairwise comparisons revealed that the 21-41 year age bin led to significantly larger sagebrush than the 1-5 yr and 6-11 yr age bins (p = 0.0001 and 0.003 respectively) The oldest reclamation site included in this study was also the site with the greatest topsoil depth (Seminoe 1). From these data, it was impossible to discern whether reclamation age, topsoil depth, or both are underlying patterns of sagebrush size.

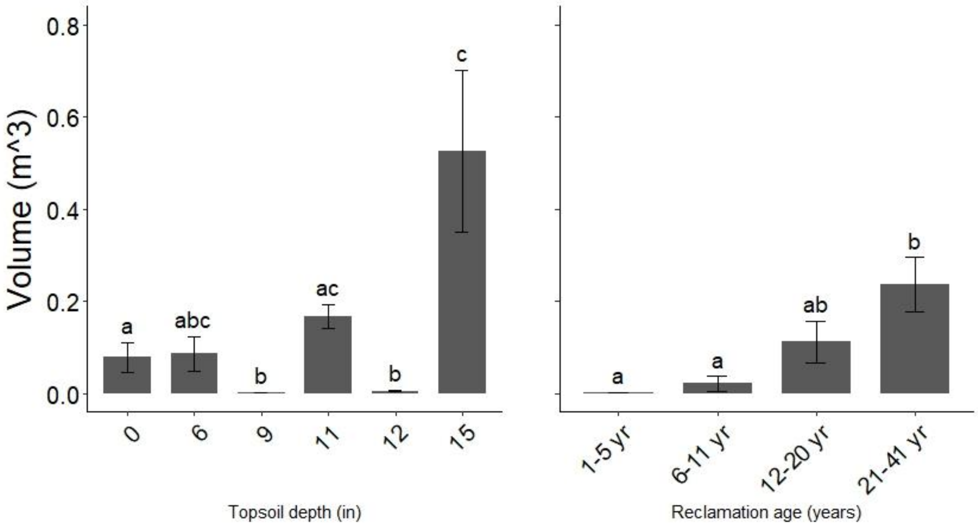

Figure 3. Average *A. tridentata* size (plant volume) recorded at sites with varying topsoil depths and reclamation ages (n = 60) with standard error bars and letters indicating differences in Dunn's test post-hoc comparisons. Reclamation age classes grouped into four bins.

There was no evidence that topsoil depth had an effect on the percent cover of sagebrush (χ^2 = 9.696, df = 5, p = 0.084). The 6 inch topsoil depth had the lowest percent cover of sagebrush at only 0.8%, while the 11 inch topsoil depth had the highest percent cover of sagebrush at 9.6%. There was no evidence that spring precipitation (χ^2 = 1.10, df = 4, p = 0.90) or annual precipitation (χ^2 = 1.55, df = 3, p = 0.67) influenced percent cover. We also examined the influence of sagebrush seeding rate on sagebrush percent cover (Figure 4) and found strong evidence that this had an effect (χ^2 = 17.54, df = 5, p = 0.003). The site with 11 inches of topsoil depth that was seeded at 0.25 lbs/acre had the highest average percent cover at 15.6%, and pairwise comparisons showed the cover here was significantly higher than sites seeded at rates of 0.1, and 0.5 lbs/acre (p = 0.030 and 0.003, respectively). We found no evidence that reclamation age influenced the percent cover of sagebrush (χ^2 = 5.83, df = 3, p = 0.12). It is worth noting that the transects with the lowest age since reclamation (1-5 years; 9 in topsoil depth) had some of the highest seed rates (3.27 and 1.5 lbs/acre) and overall density of sagebrush was highest among these transects; therefore, it is likely that these areas will have high sagebrush cover in the long-term. Although confounded in our data set, it is likely that the effects of seed rate and reclamation age are both contributing to cover patterns.

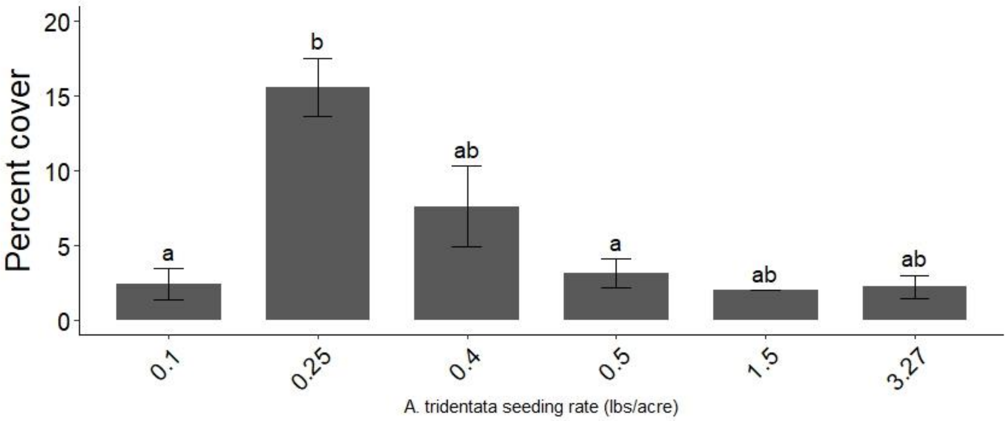

Figure 4. Average percent cover of *A. tridentata* for each seeding rate in pounds/acre (n=60) with standard error bars and letters indicating differences in Dunn's test post-hoc comparisons.

Perennial grasses often compete with sagebrush on mine reclamation sites and may benefit more than shrubs from greater amounts of topsoil. These data revealed strong evidence that perennial grass cover along the transects were significantly affected by topsoil depth (χ^2 = 18.52, df = 5, p = 0.002). The percentage of observations along the transect that were perennial grasses were highest at the site with 12 in of topsoil depth (Figure 5), and pairwise comparisons showed strong evidence that the 12 in topsoil depth had significantly greater perennial grass cover than the 0 in or 9 in topsoil depths (p = 0.011 and 0.006 respectively). It is valuable to note that the patterns of grass cover did not relate to reclamation age (no evidence that older reclamation sites had more grass cover).

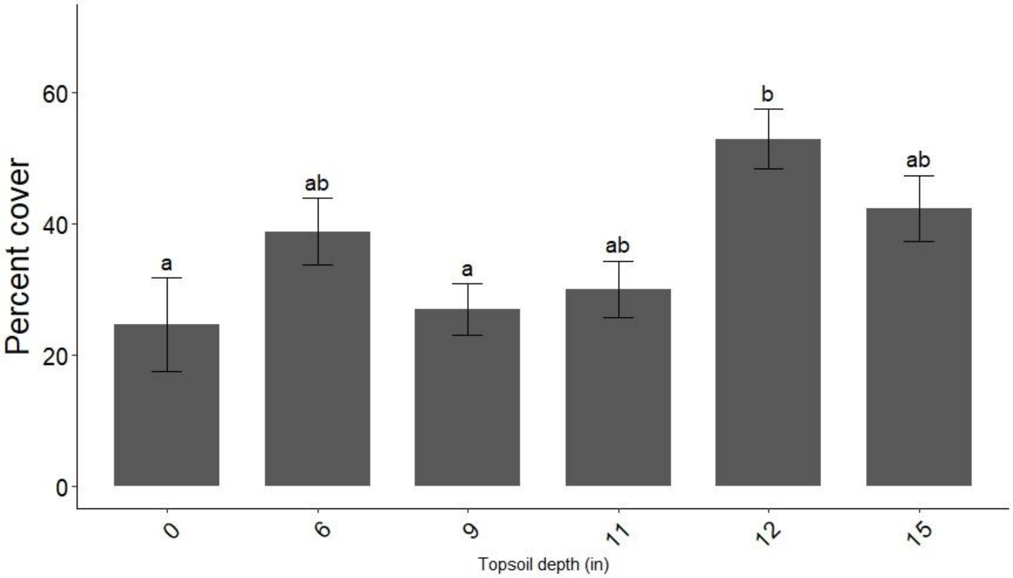

Figure 5. Percentage of line-point-intercept (LPI) observations that were perennial grass at sites with varying topsoil depths (n = 60) with standard error bars and letters indicating differences in Dunn's test post-hoc comparisons.

Discussion

We set out to explore the relationship between topsoil depth and sagebrush density, size, and cover on reclaimed mine sites in Wyoming. Ideally, this study would have included a large number of sites, both to have confidence in the generality of any conclusions and to be able to pull out a signature of topsoil depth independent from the multitude of other variables that could be driving patterns of sagebrush density, size, and cover. We were only able to access and sample at six mine sites in two general regions of Wyoming, leading to interdependencies among topsoil depth and the other explanatory variables that we explored in this study. This greatly complicates our ability to know if the evidence we found for an effect of topsoil on the various sagebrush establishment metrics is reliable or not. With the small data set, we have to acknowledge the possibility that we may not have captured the true effects of any of our explanatory variables on sagebrush establishment on reclaimed mines in Wyoming. Inconsistent trends in the results also indicate that uncertainty in our results due to the correlation of explanatory variables and small sample size are warranted. Nevertheless, we suggest some conclusions, with the above caveats, from this study.

The strongest potential signature of topsoil depth was on sagebrush density. The two lowest topsoil depths sampled, 0 and 6 in, had significantly fewer sagebrush than the site with 9 in of topsoil depth. The deeper topsoil applications of 11, 12, and 15 in were not statistically different from any of the other topsoil depths in terms of sagebrush density. However, all sites with topsoil depths of 9 in or greater had higher sagebrush density than the 0 and 6 in sites, which suggests that very shallow or no topsoil is detrimental to sagebrush establishment. There was also no evidence that any variable except annual precipitation in the year after seeding was related to sagebrush density. Annual precipitation appears to have affected sagebrush density, but in patterns independent from topsoil depth. Therefore, there appears to be some evidence that the very low topsoil depths did result in low sagebrush recruitment and survival.

A general trend of larger sagebrush at mines with deeper topsoil was seen in the data and backed up by our analysis. The sagebrush at the site with 15 in of topsoil were statistically larger than sagebrush at all other topsoil depths, and sagebrush volume was also high at the 11 in topsoil depth. However, the patterns of size match better the patterns of time since reclamation – with low size at the newest sites (which explains low size at the sites with 9 and 12 in of topsoil depth). This strongly suggests that the larger volume of sagebrush associated with some deeper topsoils could be because more time has passed and thus the sagebrush has had more time to grow larger. Because of this, it is impossible to tell whether it is deeper topsoil or time (or both) that is beneficial to sagebrush growth in this study.

We did not find any effect of topsoil depth on sagebrush canopy cover. This is counter to what would be expected since topsoil depth did appear to have an effect on density and volume which would be expected to translate to higher canopy cover. It appears that canopy cover was affected by multiple variables (seed rate, time since reclamation, and/or unmeasured variables) that were opposed in the data set.

Overall, the data appear to show some evidence for a positive effect of topsoil depth on sagebrush establishment and potentially growth, but not cover. The inconsistency of these results, as well as the strong relationships between topsoil depth and other variables in our data set, leads to low overall confidence that our data demonstrate a clear positive effect of deeper topsoil.

Perennial grass cover also appeared to benefit from deeper topsoil depths, which was expected based on previous research. Cover from perennial grasses were highest at the site with 12 and 15 in of topsoil depth, the highest depths sampled. This potentially also affected sagebrush outcomes, since perennial grasses exert strong competitive effects on sagebrush. Given the complexities and relationships among other variables in the data set, however, it is impossible to tease apart whether higher grass cover at deeper topsoil depths may have affected sagebrush outcomes.

There was sparse and inconsistent evidence that our other explanatory variables were important to sagebrush growth and establishment. We found that seeding rate did not have an effect on density. This is consistent with other work covered in this report (Chapter 3) which found that higher seeding rates do not necessarily lead to higher absolute numbers of established seedlings. There was apparent evidence that seeding rate influenced canopy cover. The seeding rate of 0.25 lbs/acre had the highest canopy cover which was significantly greater than 0.1 and 0.5 lbs/acre and greater on average than all other seeding rates. It is unclear why 0.25 lbs/acre would have such an impact of canopy cover but not density. We have found that higher seeding rates may not lead to more seedlings but do not seem to lead to lower seedling densities. The lack of a clear and meaningful results with regards to seeding rate may again be due to the small sample size and/or other correlated factors creating the spurious appearance of an effect of intermediate seed rate on canopy cover.

Barriers to sagebrush establishment include variable spring temperatures and precipitation across years. We expected that spring and annual precipitation in the year following seeding could be important factors in sagebrush density and canopy cover. We found some potential evidence that annual precipitation influenced density of sagebrush, with sites receiving 9-12 in of annual precipitation in the calendar year following seeding having an average of 330 plants per transect, which is six times as many as the next highest density of 55.2 at sites that got 6-9 in of precipitation, and even higher than the sagebrush density at sites that received >12 in of precipitation. This drastically higher sagebrush density could possibly be an effect from a wet summer which greatly improved the survival of sagebrush seedlings. However, it could also be due to some other cause with no relationship to rainfall, such as the higher seeding rate that was used at the sites that had 9-12 inches of precipitation. It is well-known that spring precipitation is important to sagebrush emergence, but we found that spring precipitation was not influential to either density or canopy cover despite sites spanning a wide range of precipitation values (0.28-4.43 in). It is important to acknowledge that the timing and frequency of precipitation, as well as preceding winter snowpack, may be more important for seedling establishment than total aggregate precipitation.

We hypothesized that lower (but not zero) topsoil depth would lead to higher densities and cover of sagebrush and lower densities and cover of other perennial plants, especially perennial grasses. We did find that perennial grasses favored sites with deeper topsoil depths, consistent with previous research, and some potential evidence that sagebrush may also favor these same site conditions. *A. tridentata* has a deep taproot so it could be that this species gets the most benefits to growth and survival from deeper topsoil and that shallow or no topsoil prevents establishment in large numbers. From our study, it would seem that placing a mosaic of shallower topsoil depths at reclamation sites will not lead to better sagebrush establishment in those areas, especially if there are areas with topsoil depths of 6 inches or less, or if there are areas with high topsoil depth that could potentially favor perennial grasses.

Other reclamation practices may be better suited to addressing the issue of low sagebrush establishment in the face of strong competition from perennial grasses. Other work has suggested that seeding in the second year after reclamation, in addition to the first year, could be a way to increase sagebrush establishment. Seeding in two years spreads out the risk of poor conditions, such as low or infrequent precipitation. Additionally, plant cover in the second year may provide some facilitation to seedlings without the extremely high competition that later develops once neighboring plants are more mature (Donovan et al. 2024). Seeding may also result in more successful outcomes if targeted in places with higher soil moisture, such as run-on areas (this report, Chapter 3).

The role of topsoil depth in sagebrush establishment and long-term growth to meet reclamation density and canopy cover goals remains unclear and under-studied. Our results indicate some benefit to sagebrush density that may come from topsoil above 6 inches of depth. However, it is important to emphasize that the results reported here are highly uncertain due to small sample size and strongly related variables. Furthermore, trying to untangle the effects of all the potential ecological variables that play a role in plant establishment is challenging in general and impossible with such small sample sizes. An experiment with these variables controlled and larger sample sizes would give a clearer and more reliable picture of the implications of topsoil depth on sagebrush establishment. Only a long-term, designed experiment over multiple sites would yield conclusive results about the role of topsoil depth in long-term sagebrush outcomes.

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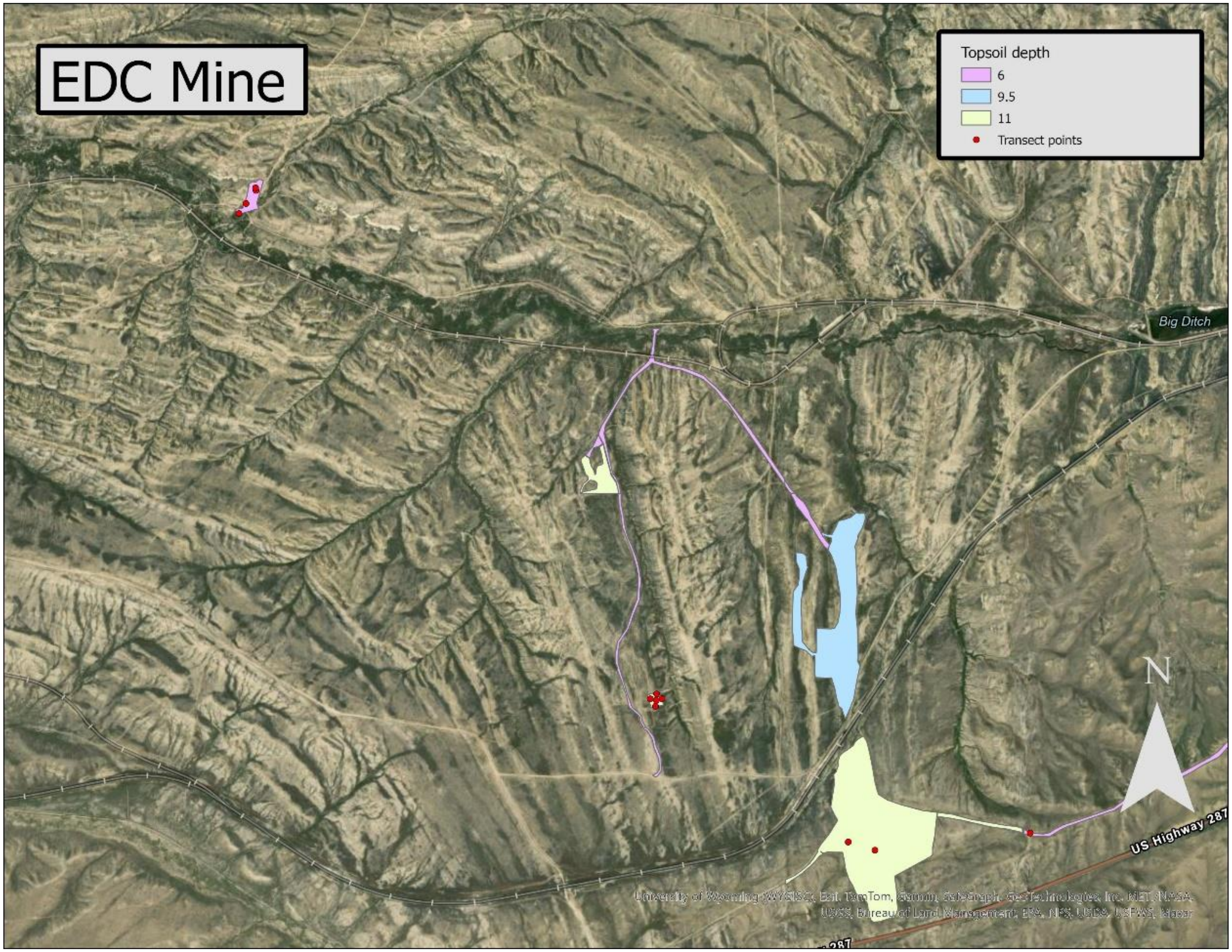
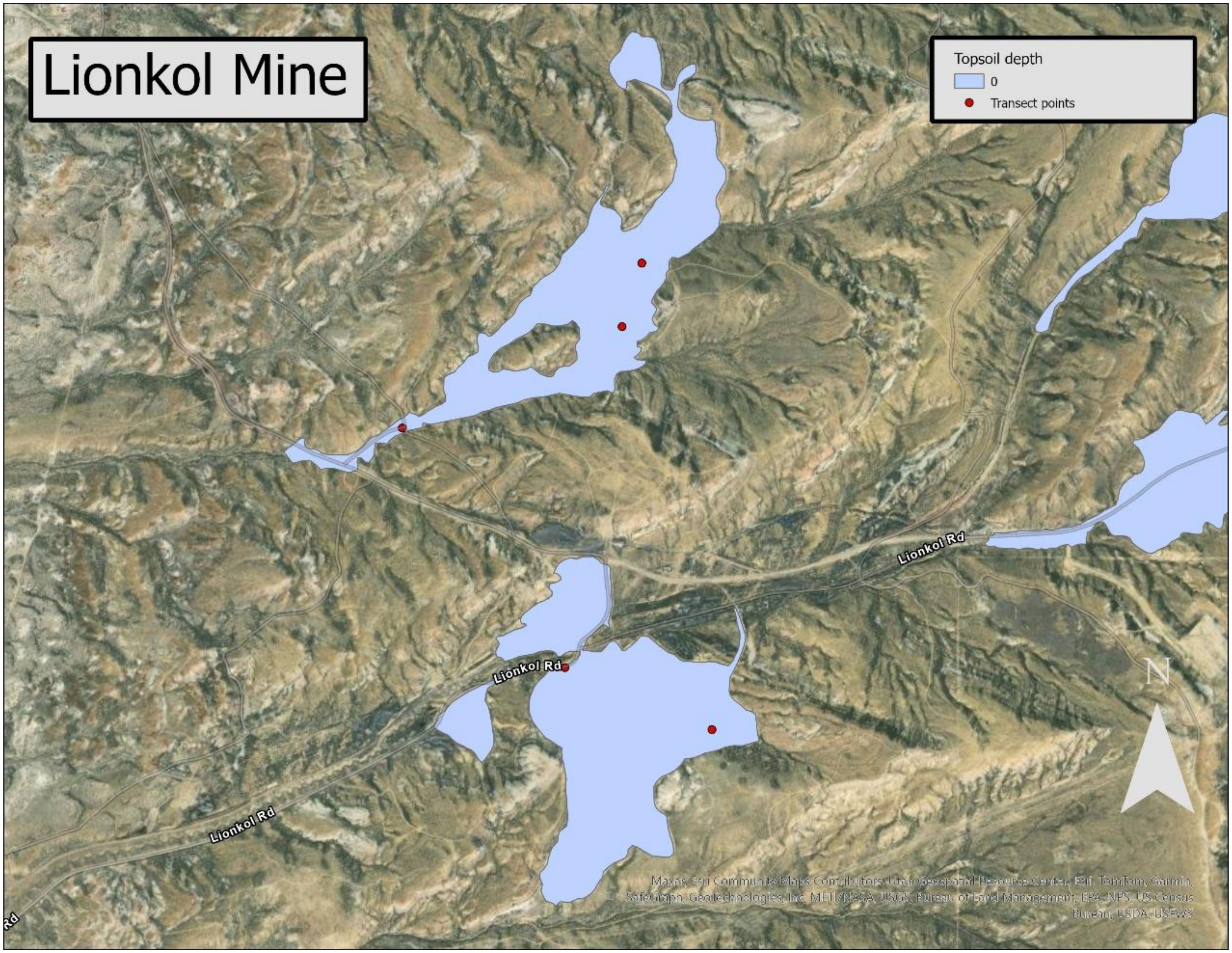
Appendix 1. Maps of mines from which data were collected




Lionkol Mine
Topsoil depth
0
Transect points
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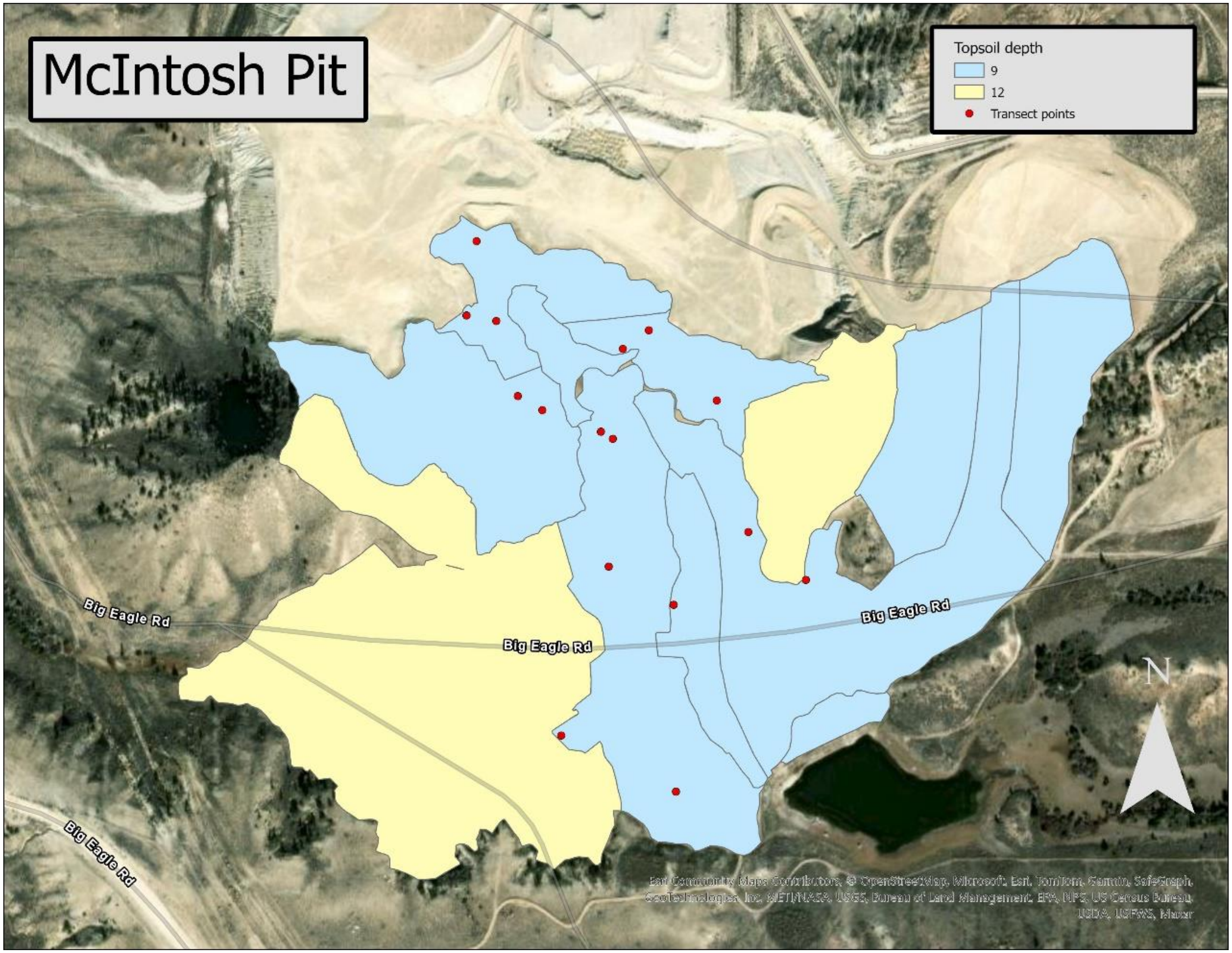
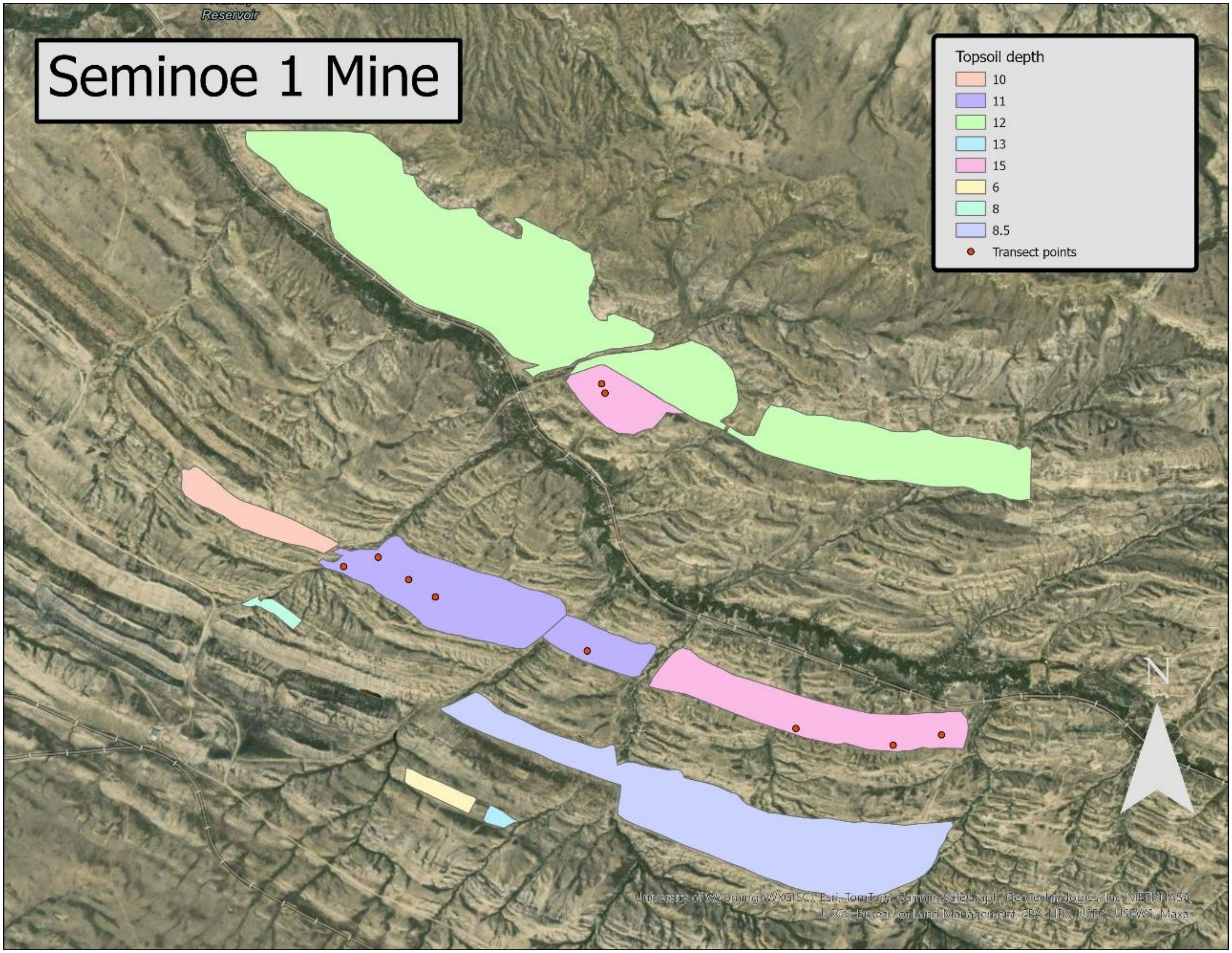
Maxar, Esri Community Maps Contributors, Utah Geospatial Resource Center, Esri, TomTom, Garmin, SafeGraph, GeoTechnologies, Inc, METI/NASA, USGS, Bureau of Land Management, EPA, NPS, US Census Bureau, USDA, USFWS



Esri Community Maps Contributors, © OpenStreetMap, Microsoft, Esri, TomTom, Garmin, SafeGraph, GeoTechnologies, Inc, METI/NASA, USGS, Bureau of Land Management, EPA, NPS, US Census Bureau, USDA, USFWS, Maxar



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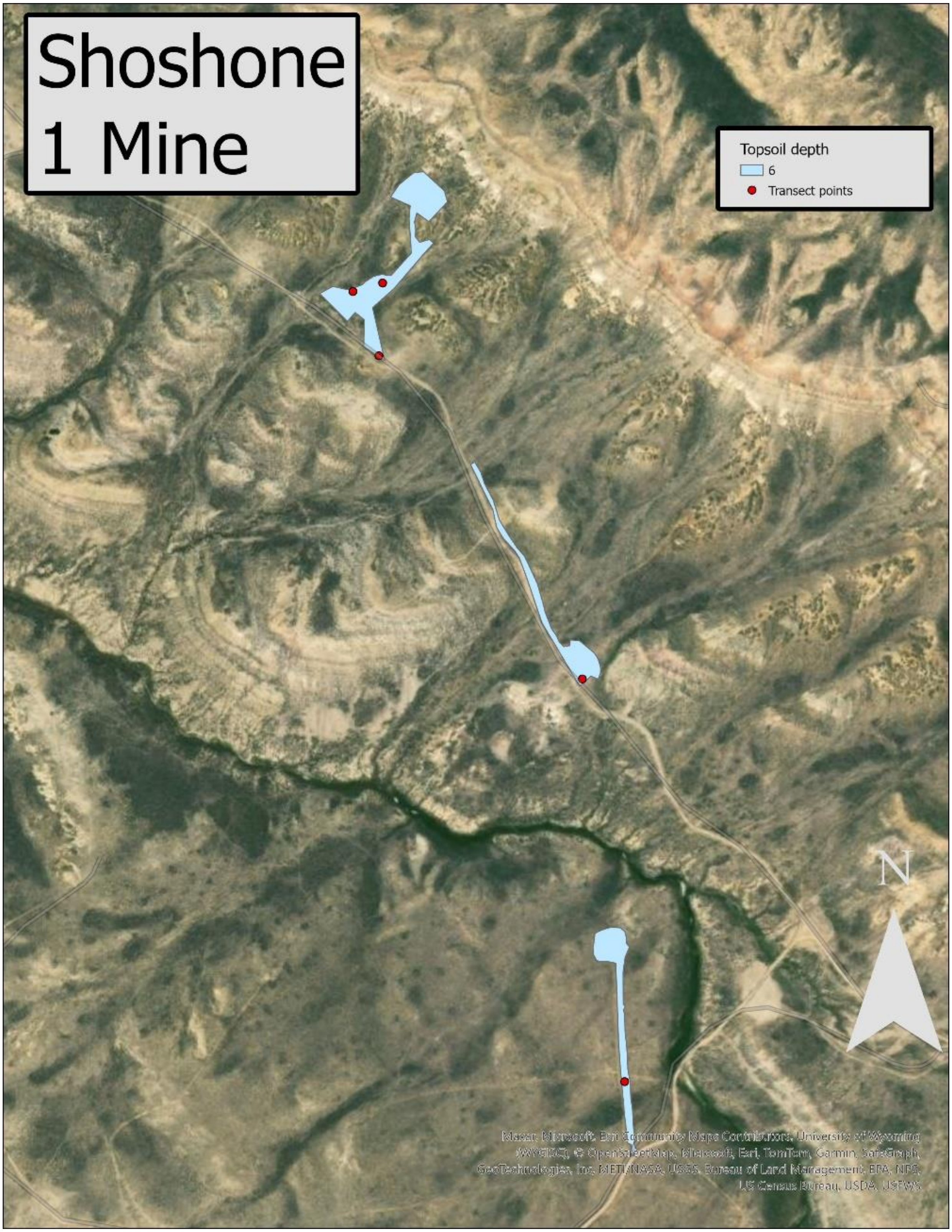

Maxar, Microsoft, Esri Community Maps Contributors, University of Wyoming (WYGISC), © OpenStreetMap, Microsoft, Esri, TomTom, Garmin, SafeGraph, GeoTechnologies, Inc, METI/NASA, USGS, Bureau of Land Management, EPA, NPS, US Census Bureau, USDA, USFWS

Chapter 2: Tradeoffs and challenges in development of prototype seed enhancement technologies for Wyoming sagebrush

Magdalena Eshleman, Hannah Demler, Olga Kildisheva, Michaela Owens, and Corinna Riginos

Introduction

Drylands around the world are slow to recover and difficult to restore after disturbance (Shackleford et al, 2021). Seed-based restoration is often the only approach that can be implemented at large scale, yet recruitment from seed is typically very low (Kildisheva et al. 2016; Shackleford et al. 2021). Seed enhancement technologies (SETs) have recently garnered attention as potential means to improve native plant and dryland restoration (Madsen et al. 2016; Kildisheva et al. 2016; Gornish et al. 2019; Brown et al. 2021; Svejcar et al. 2022). The concept of SETs is to help seeds and seedlings overcome early barriers to establishment (Pedrini et al. 2020; Brown et al. 2021). While innovative and appealing, SETs' potential scope to meaningfully contribute to restoration remains unclear and results are often mixed in outcome (Madsen 2012; Madsen 2014; Clenet; Baughman et al. 2023; Baughman et al. 2024; Munro et al. 2024).

In the sagebrush steppe biome of the Western United States, restoration of the foundational big sagebrush (Artemisia tridentata Nutt.) on large scales has proven very difficult (Lynse and Pellant, 2004; Knutson et al. 2014; Brabec 2015; Shriver et al. 2018) compounding the problem of high rate of loss. This vast landscape provides essential habitat for numerous sagebrush-dependent species, including the imperiled Greater sage-grouse (Centrocercus urophasianus), migratory ungulates, raptors, and songbirds, as well as supporting the livestock and cultural character of the western U.S. (Davies et al. 2011; Remington et al. 2021). However, the biome is threatened by exotic annual grass invasion, increasingly frequent and severe wildfire, conifer encroachment, and land conversion (Doherty et al. 2022). It is estimated that half of the original 1.2 million km2 extent of sage-grouse habitat has been substantially degraded and continues to be lost at a pace of approximately 563,000 hectares (2,278 km2) per year (Doherty et al. 2022).

In disturbed areas, it may take decades for sagebrush to establish naturally by re-seeding itself inward from the edges of disturbance boundaries (Shaw et al. 2005; Schlaepfer et al. 2014). Restoration efforts try to accelerate this process. Current sagebrush seeding practices typically involve aerial or surface broadcasting in a single year (along with drill-seeded native grasses and forbs), but these methods often result in poor establishment (Knutson et al. 2014). Transplanting seedlings can improve establishment success but is costly and difficult to unfeasible depending on the scale of restoration need (Knutson et al. 2014; Pyke et al. 2020). Many factors can lead to poor establishment success from seeds (James et al. 2011). Like many dryland species, sagebrush establishment is naturally episodic and contingent on temperature and precipitation following seeding (Meyer 1992; Schlaepfer et al. 2014; Shriver et al. 2018). Big sagebrush seeds typically do not remain viable in the seed bank (Young and Evans 1989), narrowing the chances of successful restoration with a single seeding event (Svejcar et al. 2023). Seeds are also very small (4,100 seeds/g; Schlaepfer et al. 2014) and cannot tolerate burial.

Due to the small endosperm resource, germinated sagebrush seedlings rely on soil resources to quickly develop a large taproot that helps them avoid succumbing to first-year drought stress (Welch and Jacobson, 1988; Schlaepfer et al. 2014). Early sagebrush survival and growth can be hindered by competition from herbaceous vegetation (Brabec et al. 2015; Kainrath et al. 2021), most of which grows faster than slow-growing sagebrush seedlings. In some settings, such as mine or well pad reclamation, insufficient or degraded topsoil and associated poor soil quality can also inhibit establishment (Shuman et al. 1998).

SETs provide one potential pathway to boosting sagebrush establishment. SETs include seed priming, coating, and pelleting with compounds that can enhance outcomes like seed germination and seedling vigor (Pedrini et al. 2020). SETs containing surfactants, fertilizers, microbes, and super-absorbent polymers have shown some potential for alleviating drought stress after seedling emergence (Madsen et al., 2012; Madsen et al., 2014; Davies et al., 2018; Ritchie et al., 2020; Munro et al. 2024). Though not a panacea, a successful targeted fertilizer SET approach could be beneficial for restoration of dryland sites where drought stress, competition, and/or nutrient deficiencies are the major establishment barriers. Previous work with big sagebrush showed that a targeted fertilizer application enhanced seedling root growth in reclaimed mine soils in the lab (Eshleman and Riginos, 2023). We hypothesized that delivering the same amendment via SETs could help seedlings survive variable precipitation and competition with established plants in field reclamation settings. Initial trials yielded delivery challenges and unclear outcomes but some potential worth pursuing (Donovan et al. 2024).

Here we build on previous work within the larger goal of developing a seed technology and recommended field applications to enhance early establishment of Wyoming big sagebrush. We conducted two sets of paired lab and field trials. Each pair tested a different suite of candidate seed technologies in the lab and at mine reclamation sites in the year-of and one year after completion of major reclamation work (geomorphic shaping, soil preparation, and seeding). We partnered with a seed technology company to develop several prototype technologies for sagebrush. In this study, we specifically ask: (1) In the lab, what are the effects of prototype seed technologies on sagebrush emergence and above and below ground growth (biomass and elongation)? (2) In the field, what are the effects of prototype seed technologies on sagebrush establishment, survival, and growth?, and (3) In the field, how do time since reclamation and topographic position affect seedling establishment?

Methods

Lab Trial 1

This experiment consisted of six treatments: bare (unaltered) seed, “Grow More” film coating, “Elite” film coating, “Lettuce” film coating”, bare seed + Root&Grow ® fertilizer pellets (“R&G pellets”), and bare seed + slow-release fertilizer granules (“slow-release”). All seeds were obtained from Granite Seed and Erosion Control (Lehi, UT) from lot ARS-1015/0612-GX-45-SNAKERIVER-21.

The three film coatings were applied directly to the seeds by Germains Seed Technology, Inc. (Gilroy, CA) using a rotary coater. The Grow More film coating formulation contained 15:30:15, Grow More® Fertilizer (Grow More Inc., Gardenia, CA) as an active ingredient as well as a proprietary polymer formulation for the film coating base. Elite film coating is a commercially available formulation, developed by Germains Seed Technology for carrot and jalapeno pepper seeds, and has the potential to improve plant nutrient uptake and support early plant development through increasing root mass and improving seedling development (Germains Seed Technology, personal communication). The Lettuce film coating is a formulation developed by Germains for the treatment of lettuce seeds and has the potential to improve seed germination and increase shoot and root growth in early plant development (Germains Seed Technology, personal communication). Film coated seeds were not visibly encrusted and they maintained the same shape and structural characteristics of untreated seeds.

The R&G pellet and slow-release treatments were not seed coatings but were included to test the potential of their growth-enhancing ingredients against other treatments. These treatments had the additional potential benefits over film coatings of delivering higher quantities of fertilizer that would continue to be released over a longer period of the growing season. Previous trials had shown positive effects of the high phosphorus Root&Grow® (Bonide Products LLC, Oriskany, NY) product (a 4:10:3 N:P:K fertilizer with indole-3-butyric acid growth hormone) on seedling root and shoot growth in the lab (Eshleman and Riginos, 2023) and potential benefits in the field (Donovan et al. 2024). These previous findings also showed severe emergence inhibition when seeds were incorporated into extruded pellets as a means of delivering the fertilizer in an SET. This liquid fertilizer, however, could not be delivered to seeds in a film coating. To continue testing Root&Grow as our “best results so far” fertilizer, we produced the same extruded dough pellets as before, minus the seeds, and sowed the seeds and pellets separately onto the soil surface. Each pipe growing container received 0.58 g of pellets (equivalent to 0.049 ml/pipe of Root&Grow®). To test an additional solid fertilizer, we also tested the direct application of LESCO® slow-release fertilizer granules (3:4:2, LESCO®, SiteOne Landscape Supply, Roswell, GA) applied at a rate of 0.25 g/pipe.

Growing containers were PVC pipes, each 70 cm long and 5.1 cm in diameter, which had been cut in half lengthwise and taped back together to facilitate measuring root growth at the end of the experiment. Pipes were filled with a 1:1 mixture of sandy loam soil from McIntosh Mine (described below) and sand (Quikrete Premium Play Sand, Atlanta, GA) to improve drainage. Pipes were filled with approximately 1200 mL of the soil/sand mixture, to 2.5 cm below the rim. Pipes were watered to field capacity 24 hours before sowing seeds. Twelve seeds were sown and gently pressed into the soil surface in each of the 10 treatment replicate pipes for each treatment (60 pipes total).

All pipes were kept at 20 ± 2 °C under a 12 h/ 12 h light/dark cycle using Platinum P1200 LED lights (PlatinumLED, Kailua, HI, USA) on the “Veg” setting. For three weeks, they were evenly watered daily for 1 min with a ½ GPM Fog-It nozzle (Fogg It Nozzle Company, Belmont, CA, USA), and thereafter the same watering every other day. Seedling emergence was monitored and recorded, and all new seedlings were marked with a colored toothpick corresponding to the emergence date. Approximately one week after seeds were sown, or once seedlings had true leaves, pipes were thinned to one seedling per pipe. Any new emergence was still recorded but new seedlings were pulled. Pipes were opened and seedlings were harvested after 40 days of growth (from each seedling’s emergence date). Seedling roots were extracted from the soil by gently rinsing away soil in a tub of water. Roots and shoots were then measured for length, dried at 65° C for 24 hours, and weighed for dry biomass.

Data analysis for this and subsequent field and lab trials was performed in R 4.1.1 (R Core Team, 2021). Differences between bare seed and fertilizer treatments were analyzed using linear models with fertilizer treatment as a factor and cumulative percent emergence, root length and biomass, shoot length and biomass, and the root:shoot biomass ratio as the response variables. Root biomass and shoot biomass were both log transformed to meet model assumptions prior to analysis. One-way analysis of variance was calculated and differences among fertilizer treatments for each response variable were determined through pairwise comparisons of group estimated marginal means using the R package emmeans with a “tukey” adjustment to the p-value.

Field Trial 1

This trial was conducted at the McIntosh mine (42.362012, -107.836456) in central Wyoming, an abandoned uranium mine undergoing geomorphic reclamation. Prior to our trial, all earthwork (filling and reshaping the former pit mine to mimic natural-grade slopes and meandering streams) had been completed. The site was covered in sandy loam aggregated B horizon soil from elsewhere in the mine vicinity to a depth of 22.8-30.5 cm and seeded with a variety of grasses, forbs, and sagebrush using a soil pitter. Twenty-year rainfall averages for this area are 10.6 cm for March through May, and 5.7 cm for June through August (NOAA data for Riverton Airport: https://www.weather.gov/wrh/Climate?wfo=riw accessed April 27 2024).

This trial was set up on two east-facing hillsides less than 1 km apart. Experimental plots were seeded with additional sagebrush seed in the fall of 2021 and monitored through spring, summer, and fall of 2022. One hillside was freshly reclaimed in fall 2021 (abbreviated as Y0-21 for "year 0 in 2021"); that is, it was devoid of vegetation, and loose soil was freshly seeded and imprinted with pits approximately 40 x 40 cm. The other hillside was reclaimed the previous year (abbreviated as Y1-21 for "year 1 in 2021") and had high cover of the non-native Russian thistle (*Salsola tragus*) and desert madwort (*Alyssum desertorum*), lesser cover of seeded species, and weakly defined pits from the previous year still visible.

At each site, we set up a blocked array of plots with 25 replicates of each treatment. Blocks were deliberately stratified in topographic positions we identified as slopes (convex run-off side-slope areas, n=17/16 for Y0-21 and Y1-21 respectively) and swales (concave run-on areas, n=8/9). Each block consisted of seven 40 x 40 cm plots (one per seed treatment) situated on existing pits. For each plot, we hand-dug three shallow furrows, approximately 2.5 cm wide and 1.25-2 cm deep, along the contour of the slope. Treatments were applied randomly within each block in November 2021 at a rate of 65 pure live seed (PLS) per plot. Seeds were sourced and treated as in Lab Trial 1 with the addition of an unseeded control, and the PLS was calculated based on results from a standard tetrazolium (TZ) test (Oregon State University Seed Laboratory, Corvallis, OR) which showed 76% seed viability. Seeds in the bare, Grow More, Elite and lettuce treatments were sown evenly across the furrows. Seeds in the R&G pellet and slow-release treatments were sown in six clusters of seeds, approximately 15 seeds (11 PLS) per cluster. For the slow-release treatment, each plot received 1.5 g of fertilizer beads divided evenly among the six clusters (0.25 g/cluster) and sprinkled on top of the seed cluster. For the R&G pellets treatment, 0.58 g of pellets (equivalent to 0.48 ml of Root&Grow®) were applied in each cluster, and pellets were pushed into the ground vertically around each seed cluster.

Plots were monitored for seedling emergence, survival, and growth biweekly from April to June 2022, then every three weeks until August 2022, and once in November 2022. At each monitoring event, we counted newly emerged seedlings, surviving vs. dead seedlings from the previous event (marked with a colored toothpick for tracking), and the height of three randomly chosen seedlings, marked with a different colored toothpick for re-measurement. We also quantified vegetation cover once in June, after peak growing season, to characterize the biotic environment. Annual and perennial cover were visually estimated for each plot in the following percentage classes: <1, 1-5, 6-40, 11-25, 26-50, 51-75, and >75. For analysis, we used the midpoint value of each cover class. March-May and June-August precipitation was obtained from the nearest weather station at the Bullrush Mine (42.8060, -107.6489).

Initial data analyses revealed minimal seedling emergence in unseeded plots in both times since reclamation seeding (see Results); we therefore focused analyses and presentation of results on seeded plots to answer our core questions about seed treatments. Response variables were: percent emergence (cumulative over the whole monitoring season and calculated as the percent of PLS that germinated), percent of emerged seedlings that survived until November, final (November) total seedling count per plot, and final height (averaged among seedlings in each plot). Data were analyzed separately for the Y0-21 and Y1-21 sites, and within Y0-21 all responses except percent emergence were analyzed for swales only due to extremely low emergence on slopes. We used a generalized linear mixed model (GLMM) approach with fixed effects of topographic position (Y1-21 only), seed treatment, topographic position*seed treatment (Y1-21 only), and block as a random effect for percent emergence and survival (binomial distribution) and final total seedling count (negative binomial distribution), using the *glmer* function within the lme4 package. Significance of predictors was assessed using model simplification and χ^2 analysis of deviance to compare models. For height data, we used a linear mixed effects model with the same predictors and data log-transformed to achieve normality using the *lmer* function.

Lab Trial 2

Since the film coatings tested in Lab Trial 1 did not show any substantial benefits, we tested a new set of four film coating formulations in Lab Trial 2, using methods otherwise similar to Lab Trial 1. Film coatings were produced by Germains Seed Technology, Inc. using methods described above but with active ingredients BEC80 and FMC4034. The details of the BEC80 (hereafter, “BEC”) and FMC4034 (hereafter “FMC”) film coatings are protected by intellectual property. Both were chosen based on their potential to improve seed germination and increase shoot and root growth in early plant development (Germains Seed Technology, personal communication) and were applied in a low and high rate (BEC low: 20 g/kg seed, BEC high: 40g/kg seed, FMC low: 4.7 g/kg seed, FMC high: 9.4 g/kg seed). These were tested against a bare seed treatment, with seven replicates per treatment for a total of 35 PVC pipes.

Seed was sourced as in Lab Trial 1. Because of the age of the seed, we again tested seed viability using a standard tetrazolium test (TZ) test (Oregon State University Seed Laboratory, Corvallis, OR). Viability was reduced, ranging from 29-43% depending on the treatment. Seeding rate was adjusted to account for viability; (we sowed between 29 and 35 seeds per pipe to achieve a sowing rate of 10 PLS per pipe. All other methods were the same as in Lab Trial 1, except that soil was obtained from a different mine (Andria Hunter mine, approximately 47 km from McIntosh mine [42.7719, -107.6659]).

Differences between bare seed and film-coated seeds were analyzed using linear models with seed treatment as a factor and percent of PLS that emerged, root length and biomass, shoot length and biomass, and the root: shoot mass ratio as the response variables. One-way analysis of variance was calculated and when appropriate differences among seed treatments for each response variable were determined through pairwise comparisons of group estimated marginal means using the R package emmeans with a “tukey” adjustment to the p-value.

Field Trial 2

This field trial was also conducted at McIntosh Mine and similar to Field Trial 1, using some of the seed treatments tested in Lab Trial 2. The experiment consisted of a freshly reclaimed site (Y0-22) on a relatively flat ridge and a one year post reclamation site (Y1-22) which was the same general hillslope as the Y0-21 site from Field Trial 1. At both sites, there were n=25 40x40 cm plots per seed treatment (plots centered on pits).

At the Y0-22 site, the seed treatments were: bare seed, 2x bare seed (double the seeding rate of all other seeded treatments), FMC high, BEC high, R&G pellets, and unseeded. The FMC and BEC high rates were the same rate of active ingredient as used in Lab Trial 2. The pellet treatment was identical to Field Trial 1. Two rates of bare seed were used with the intention of evaluating whether seeding at a higher rate would result in greater total sagebrush recruitment than any seed enhancement technology. The experiment was not blocked. At the Y1-22 site, the seed treatments were: bare seed, FMC high, FMC low, BEC high, BEC low, R&G pellets, and unseeded. As in the previous year at this site, the experiment was blocked and blocks were split between slopes (n=17) and swales (n=8).

Seeds were sourced as before. Due to declining viability revealed by TZ testing on the film coated seeds, we increased the seed rate per plot with the goal of achieving 65 PLS per plot as in Field Trial 1. Subsequent TZ testing (after field seeding) showed even lower viability in the bare seed, which meant that a lower PLS was achieved in the bare seed and R&G pellets treatments than originally planned (Table 1). Seeds were sown into three furrows per plot, as in Field Trial 1, with all seeds sprinkled evenly across furrows except in the R&G pellet treatment which were sown in clusters around pellets, as in Field Trial 1. Plots were monitored for seedling emergence, survival, and growth as in Field Trial 1, biweekly from April to June 2023, then every three weeks until August 2023, and one final time in November 2023. Cover and precipitation data were gathered as in Field Trial 1.

Table 1. Experimental treatments and pure live seed, corrected based on seed viability results, per 40 x 40 cm plot

Seed Treatment	Y0-22 (PLS/plot)	Y1-22 (PLS/plot)
Bare seed	44	44
2x bare seed	88	NA
FMC high	65	65
FMC low	NA	65
BEC high	65	65
BEC low	NA	65
R&G pellets	44	44
unseeded	0	0

There was minimal seedling emergence in unseeded plots in Y1-22 but considerable emergence in unseeded plots in Y0-22. Although this complicates interpretation of results for Y0-22, we focused analyses only on seeded plots, both because these were the subject of primary interest and because unseeded treatments could not be analyzed for percent of live seeds that emerged or survived. Response variables and associated distributions were the same as in Field Trial 1. For Y0-22, the only predictor tested was seed treatment, since there was no blocking or topographic position at this site. For Y1-22, we tested the effects of topographic position, treatment and their interaction on emergence with block as a random effect, as above. However, emergence was low, causing replication challenges for multi-factor modeling of the remaining response variables. We therefore ran separate models testing the effects of topographic position and seed treatment (each with block as a random effect) on percent survival, final total seedling count, and average seedling height for the Y1-22 dataset.

Results

Lab Trial 1

Average percent emergence of seeds among the fertilizer treatments ranged from a low of 21.7% for the slow-release treatment to a high of 61.7 % for the Grow More treatment (Figure 1a). There was strong evidence that seed treatment had an effect on seedling emergence (F = 6.01, $p < 0.0001$). Pairwise comparisons revealed strong evidence that the slow-release treatment had significantly lower seedling emergence than all other treatments. Although not statistically significant, Grow More seedlings had 12% higher emergence than bare seed.

There was strong evidence that seed treatment had an effect on both shoot length (F = 48.49, $p < 0.0001$) and root length (F = 4.13, p=0.003). The slow-release treatment yielded seedlings with an average shoot length of 61.3 ± 3.8 mm, more than double the average seedling shoot length in any other treatment (pellets = 29.6 ± 3.1 mm, bare = 22.1 ± 1.8 mm) and an average root length of 651.6 ± 18.3 mm (pellets = 606.0 ± 21.7 mm, bare = 489.0 ± 38.4). Pairwise comparisons offered strong evidence ($p < 0.05$) that both the slow-release and pellet treatments – but not any of the film coating treatments – enhanced shoot and root length compared to the bare seed control.

Shoot and root dry biomass patterns were similar to shoot and root length patterns. There was strong evidence that treatment had an effect on both shoot biomass (F = 31.81, $p < 0.0001$) and root biomass (F= 13.24, $p < 0.0001$) of the sagebrush seedlings. Seedlings in the slow-release treatment had an average shoot biomass between 7 and 20 times greater than the average biomass from each of the other treatments (Figure 1b). Average root biomass of the slow-release treatment was also more than three times greater than the other treatments (Figures 1c, Figure 1d). There was weak evidence that seed treatment had an effect on seedling root:shoot ratio (F = 2.35, p= 0.053). Seedlings in the bare seed treatment had the greatest root:shoot ratio of 3.25, while the slow-release fertilizer seedlings had the lowest root:shoot ratio of 1.17. R&G pellets and Elite film coating treatment had root:shoot ratios very close to bare seed.

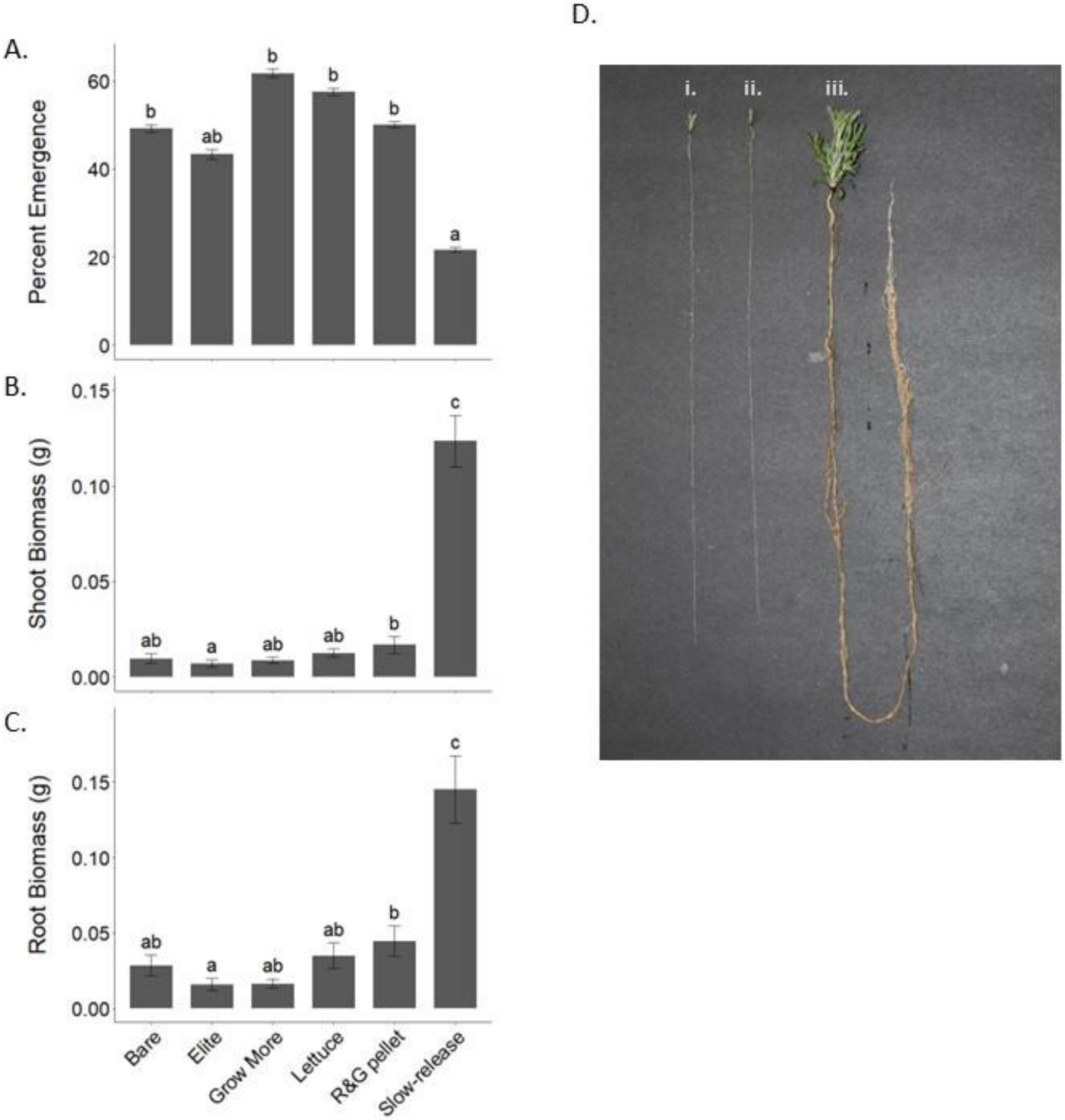

Figure 1. Performance of *A. tridentata* after being sown with different seed treatments, with means ±SEMs shown and letters indicating differences in Tukey post-hoc comparisons: (a) Cumulative percent emergence; (b) shoot biomass after 40 days' growth; (c) root biomass after 40 days' growth; (d) photograph of 40-d old seedlings in three treatments: i. bare seed control, ii. Elite seed film coating, and iii. LESCO slow-release fertilizer.

Field Trial 1

March to May precipitation was 5.3 cm, and June-August precipitation totaled 6.1 cm. At the Y0-21 and Y1-21 sites (first and second season of growth post-reclamation), average perennial cover values were 1.19% and 6.24% respectively, and average annual cover values were 0.53% and 15.00% respectively.

In both Y0-21 and Y1-21 sites, there were only a handful of seedlings that emerged in the unseeded plots (n=2 out of 54, and n=6 out of 668 total seedlings respectively) indicating that recruitment from the reclamation process alone was very low and that most of the emerged seedlings were experimentally treated and sown. We therefore focused analyses and presentation of results on the seeded treatments, with recognition that results could be minorly affected by recruitment of untreated seeds.

Seedling emergence was overall much lower in the Y0-21 site than in the Y1-21 site (Figure 2a). In the Y0-21 site, only three seedlings emerged on the slopes across all treatments (n=3 seedlings, 0.03 seedlings per plot), whereas emergence was higher in the swales (n=49 seedlings, 1.02 seedlings per plot). There was strong evidence for an effect of topographic position on percent emergence (χ^2=35.49, p<0.001) and no evidence for an interaction between topographic position and seed treatment (χ^2=4.65, p=0.46). Subsequent analyses on the Y0-21 site was performed for the swales plots only. Within the swales, there was a significant effect of seed treatment (χ^2=14.30, p=0.01); while post-hoc testing did not reveal any statistically significant pairwise differences, the slow-release treatment had much lower percent emergence than all other treatments (Figure 2c).

In the Y1-21 site, percent emergence was slightly (non-significantly) lower in the slopes relative to swales (Figure 2a, χ^2=0.49, p=0.48) resulting in an average 3.65 and 4.11 seedlings per plot, respectively. There was no evidence of an interaction between topographic position and seed treatment (χ^2=10.16, p=0.07). There was a significant effect of seed treatments on percent emergence (χ^2 =131.99, p <0.001), and post-hoc analysis showed that emergence was lower in the slow-release treatments (Figure 2c).

Seedling survival was much higher in the Y0-21 site than the Y1-21 site (Y0-21 average percent survival = 90.5% ± 3.4; Y1-21 average percent survival = 53.9% ± 2.8). Within each time since reclamation, however, there was no evidence for any effect of topographic position (Y0-21: χ^2=0.62, p=0.43; Y1-21: χ^2=0.87, p=0.35) on seedling survival. In the Y0-21 site, there was no evidence for any effect of seed treatment on seedling survival (χ^2=2.66, p=0.75). Only one slow-release seedling had emerged, and it did survive, but survival was high across all treatments (88-100%). In the Y1-21, seed treatment did have a significant effect seed treatment (χ^2=25.52, p<0.001). Seedling survival was substantially higher in the slow-release treatment than other treatments (slow-release = 85.3 ± 5.9 %, bare = 47.1 ± 6.7%, other treatments ranged from 40.9-57.7% survival).

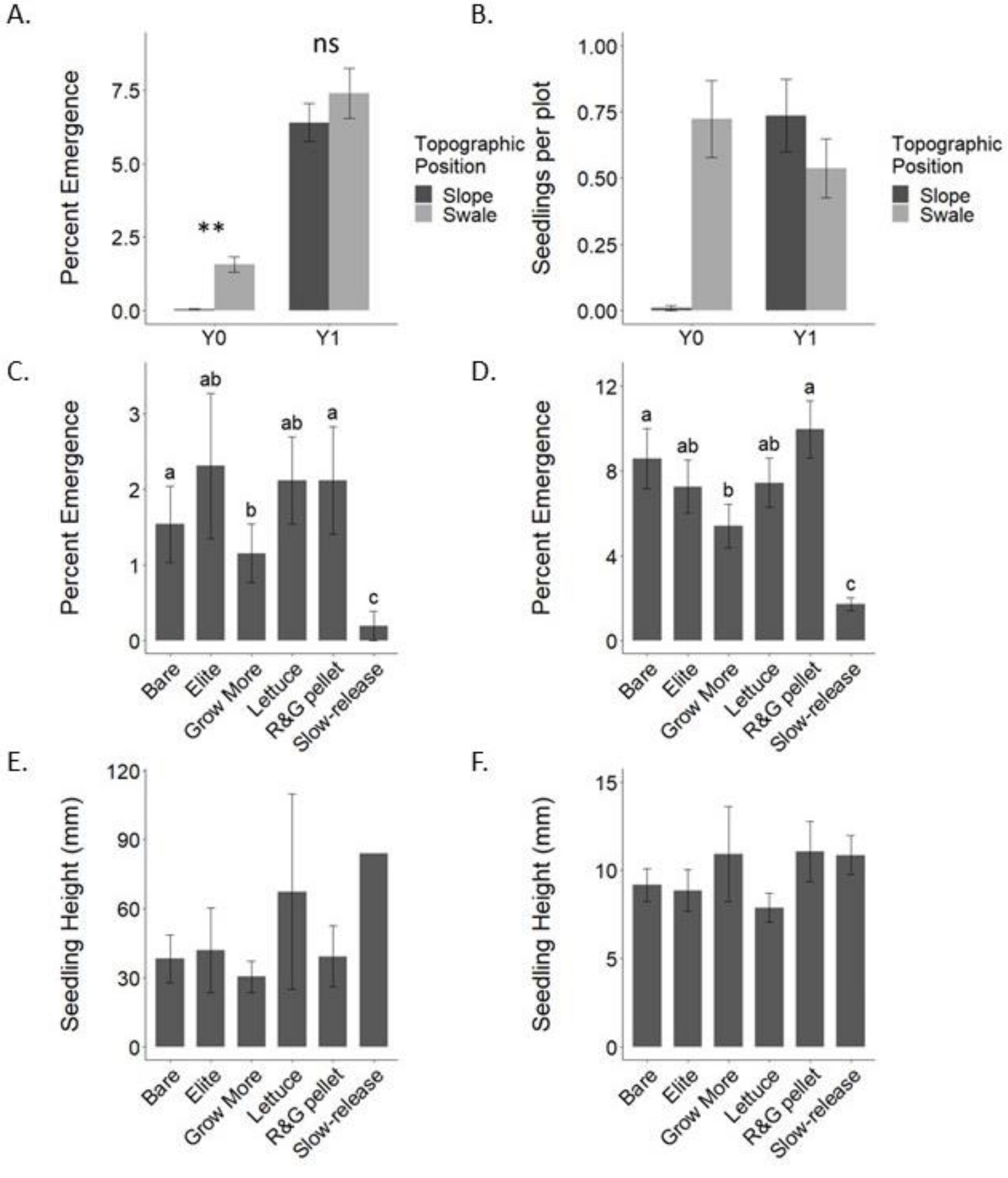

Figure 2. Performance of *A. tridentata*, with means ±SEMs shown and stars or letters indicating differences in Tukey post-hoc comparisons: (a) cumulative percent emergence across two topographic positions and two times since reclamation (Y0 = year of reclamation seeding, Y1 = one year after reclamation seeding), with post-hoc comparison between topographic positions within each time since reclamation (**=p<0.001, NS=not significant); (b) number of seedlings alive at the end of the first growing season across two topographic positions and two times since reclamation; (c) cumulative percent emergence across seed treatments sown in the year of reclamation (Y0-21) with letters indicating differences in groups; (d) cumulative percent emergence across seed treatments sown one year post-reclamation (Y1-21) with letters indicating differences in groups; (e) average seedling height at the end of the first growing season in Y0-21, with no significant differences among groups; (f) average seedling height at the end of the first growing season in Y1-21, with no significant differences among groups.

The final number of seedlings alive in November 2022 was similar in swales for both Y0-21 and Y1-21 (0.74 and 0.58 seedlings per plot, respectively), but much lower in the Y0-21 slopes compared to Y1-21 slopes (0.01 and 0.74 seedlings per plot, respectively; Figure 2b). Within Y0-21 swales, there was no evidence for an effect of seed treatment on the final seedling count (χ^2=8.05, p=0.15), though slow-release had lower mean seedlings per plot than other treatments (0.13, compared to 0.5-1.25 for other treatments). Within the Y1-21 site, there was an interaction between topographic position and seed treatment (χ^2=13.31, p=0.02). This appears to be driven by the lettuce treatment having more seedlings than other treatments in the slopes but not the swales, whereas the lettuce, Elite, and Grow More treatment had fewer seedlings than other treatments. In the absence of consistent patterns, it is difficult to conclude any real treatment effects on final seedling counts. Notably, the slow-release treatment had similar final seedling counts as other treatments despite low emergence.

Average seedling height at the end of the growing season was much greater, overall, for seedlings in the freshly reclaimed versus one year post reclamation sites (Y0-21: 43.20 ± 9.60 mm; Y1-21: 9.74 ± 0.58 mm). In the Y0-21 site swales plots, the single seedling in the slow-release treatment was much taller than seedlings in other treatments (slow-release = 84.00 mm; bare = 38.25 ± 10.50 mm; Figure 2e). There was overall no statistically detectable effect of treatment on height (F=1.79, p=0.12). In the Y1-21 site, there was no detectable effect of topographic position or seed treatment (Figure 2f) on final seedling height (topographic position: F=0.02, p=0.89; seed treatment: F=0.74, p=0.60).

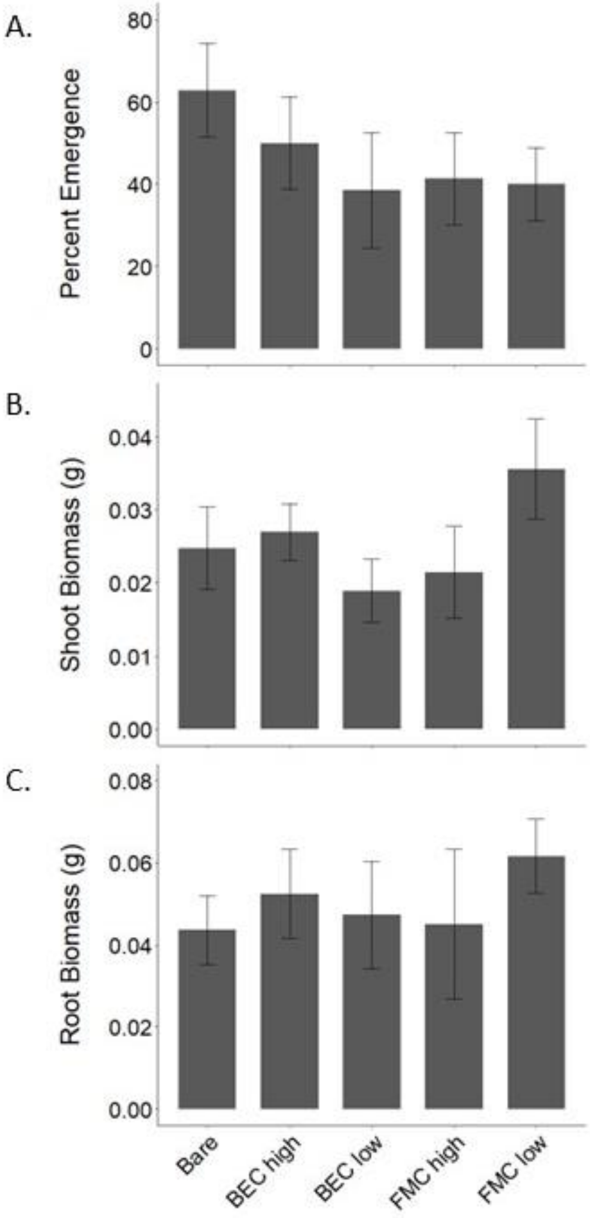

Figure 3. Performance of *A. tridentata* after being sown with different seed treatments, with means ±SEMs shown: (a) cumulative percent emergence; (b) shoot biomass after 40 days' growth; (c) root biomass after 40 days' growth.

Lab Trial 2

Average emergence ranged from 39 – 63% across all seed treatments (Figure 3a). The bare seed demonstrated the highest average emergence, while the BEC low film coating treatment had the lowest average emergence; however, we found no evidence that seed treatment had an effect on sagebrush seed emergence (F= 0.77, p= 0.55).

There was evidence that shoot length was affected by seed treatment (F=3.79, p=0.014). Pairwise comparisons revealed strong evidence (p< 0.01) that this effect was driven by the difference between the FMC low (average=38.00 ± 2.31 mm) and the BEC low (average=26.17 ± 3.07 mm) treatments. There was no evidence that shoot length differed from bare seed in any of the film coating treatments. There was also no statistical evidence that any seed treatments affected seedling root length (F=1.91, p=0.14), shoot mass (F=1.26, p=0.31; Figure 3b), or root mass (F=32, p=0.86; Figure 3c). Notably, the FMC low treatment had seedlings with the longest root length and greatest shoot and , in addition to the longest shoot length.

There was also no evidence that the root:shoot mass ratio was affected by seed treatment (F=1.16, p=0.35), indicating that neither of these film coating treatments at the tested concentrations led to root-specific enhancement of sagebrush seedlings compared to bare seed in a lab setting. The BEC low treatment did display the highest average seedling root:shoot mass ratio of 2.9, though this treatment yielded relatively small average roots and shoots.

Field Trial 2

March to May precipitation was 3.5 cm, and June-August precipitation totaled 14.5 cm. At the Y0-22 and Y1-22 sites (first and second season of growth post-reclamation), average perennial cover values were 0.05% and 0.82% respectively, and average annual cover values were 0% and 0.11% respectively.

In both Y0-22 and Y1-22 sites, there were seedlings that emerged in the unseeded plots (n=101 out of 633 or 4.04 seedlings per plot, and n=1 out of 186 total seedlings respectively). This indicates that at the Y1-22 site nearly all of the emerged seedlings were experimentally treated and sown. However, at the Y0-22 site the reclamation seeding process did contribute substantially to seedling recruitment, likely across all treatments. We recognize that results were probably affected by recruitment of untreated seeds since unseeded plots had similar total number of seedlings emerge as some of the treated plots. This has the potential to mask any effects that the seed treatments incurred on seedling emergence, survival and growth.

Contrary to the Field Trial 1 results, percent seedling emergence across all experimentally seeded treatments was greater in the Y0-22 site than in the Y1-22 site (Y0-22=7.50%, Y1-22=2.11%). This may be at least in part due to the successful germination of background reclamation process seeds in the Y0 site during this year of study.

In the Y0-22 site, there was strong evidence that seed treatment impacted percent emergence (χ^2=32.46, p<0.001), with the 2x bare seed and FMC high treatments having lower percent emergence than bare seed and BEC high (Figure 4a). The R&G pellet seed treatment did not differ from any of the other seed treatments. In the Y1-22 site, there was no evidence that topographic position (χ^2=1.79, p=0.18), seed treatment (χ^2=7.85, p=0.17) or the interaction between topographic position and seed treatment (χ^2=3.59,

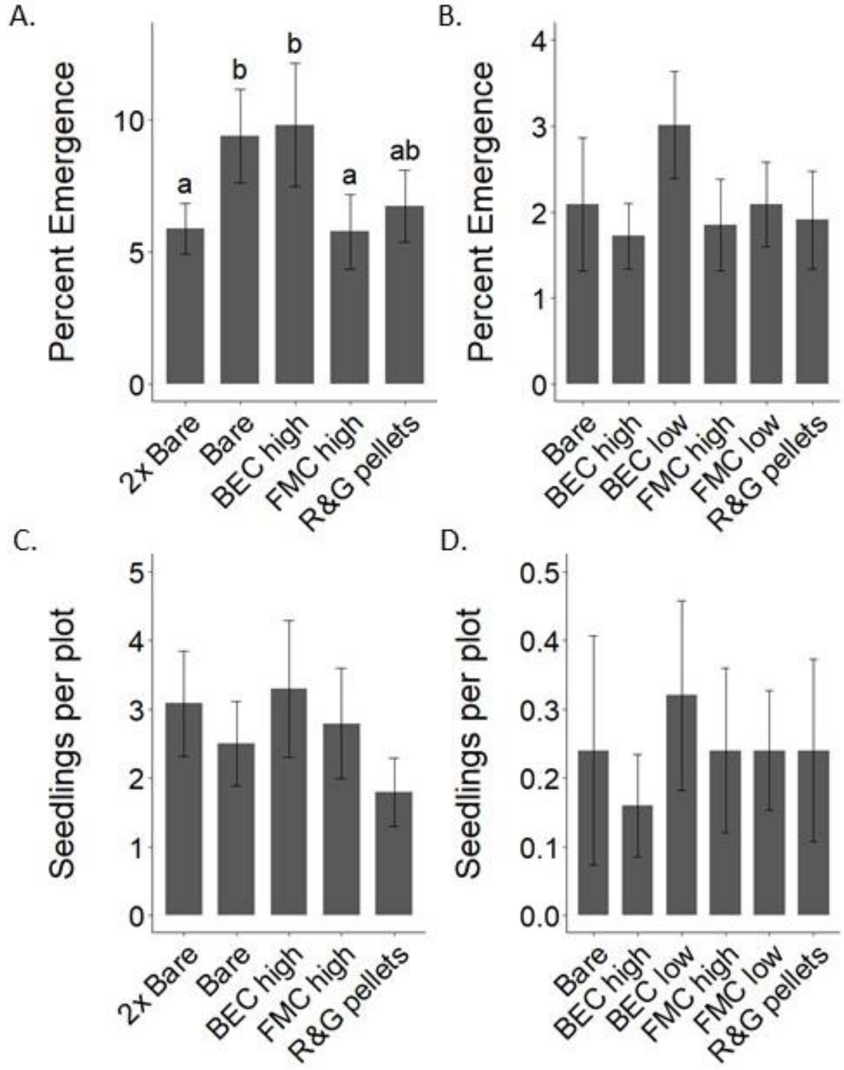

Figure 4. Performance of *A. tridentata*, with means ±SEMs shown and letters indicating differences in Tukey post-hoc comparisons: (a) cumulative percent by seed treatment sown in the year of reclamation seeding (Y0); (b) cumulative percent by seed treatment sown one year after reclamation seeding (Y1); (c) number of seedlings alive at the end of the first growing season in Y0; (d) number of seedlings alive at the end of the first growing season in Y1.

p=0.61) had an effect on percent emergence (Figure 4b). There were 106 seedlings (1.04 seedlings/plot) that emerged on the slopes and 79 seedlings (1.65 seedlings/plot) that emerged in the swales.

Seedling survival was much higher in the Y0-22 site compared to the Y1-22 site (Y0-22 average percent survival = 48.52% ± 3.68; Y1-22 average percent survival = 19.78% ± 3.70). In the Y0-22 site there was evidence that seed treatment impacted seedling survival (χ^2=12.70, p=0.01), with the only significant pairwise comparison showing that BEC high had greater percent survival than FMC high. In the Y1-22 site, there was evidence that the interaction between seed treatment and topographic position impacted seedling survival (χ^2=19.89, p<0.01). This appears to be driven by FMC high and bare having higher survival than other treatments in the slopes and very low survival in the swales; it is therefore difficult to conclude any consistent effect of seed treatment on survival.

At the end of the season, there were more seedlings in the Y0-22 site than the Y1-22 site (2.6 ± 0.3 seedlings/plot, 0.2 ± 0.04 seedlings/plot, respectively). Within the Y0-22 site, there was no evidence for an effect of seed treatment on the final seedling count (χ^2=1.94, p=0.75). Despite applying two times the amount of bare seed to the 2x bare seed plots compared to the bare seed plots, the final seedling counts did not reflect this (bare seed=3.1 ± 0.8 seedling/plot, 2x bare seed=2.5 ± 0.6 seedlings/plot, respectively). When the number of seedlings was considered as a percentage of the viable seeds sown per plot, there was weak evidence that seed treatment impacted seedlings/PLS (χ^2=9.13 p=0.06). Bare seed had the highest percent seedlings relative to PLS sown (5.7 ± 1.4%) and 2x bare seed had the lowest of all treatments (3.5 ± 0.8%). In the Y1-22 site, the final seedling count in November was similar in slopes and swales (0.3 ± 0.1 versus 0.2 ± 0.1 seedlings per plot, respectively, among seeded plots), and there was no evidence that seed treatment affected final seedling count (χ^2=0.94, p=0.93) or proportion of viable seeds that yielded surviving seedlings (χ^2=2.50, p=0.77).

Following the trend from Field Trial 1, average seedling height at the end of the growing season was much greater, overall, for seedlings in the freshly reclaimed versus one year post reclamation sites (Y0-22: 44.82 ± 2.85 mm; Y1-22: 7.99 ± 1.02 mm). In the Y0-22 site, there was no evidence that seed treatment impacted seedling height (F=0.76, p=0.56). In the Y1-22 site, there was also no detectable effect of seed treatment on seedling height (F=0.86, p=0.53).

Discussion

We tested a variety of prototype seed enhancement technologies using several different formulations of active ingredients and delivery mechanisms, in lab and field settings, with the goal of improving establishment of sagebrush seedlings. Results showed a variety of outcomes that highlight some of the challenges in developing SETs – especially the tradeoffs between delivering sufficient quantity of active ingredient and inhibiting emergence. Further, our results illustrate how local conditions, such as topographic position and neighboring vegetation, have important impacts on restoration outcomes.

Film coatings had little impact on seedlings

A consistent theme across virtually all trials, response variables, and film coating formulations was that film coatings had little to no effect on sagebrush seedlings. Across all trials, film coatings never reduced emergence compared to bare seed – a desirable trait – but only enhanced emergence significantly in one case (FMC high in the field in Y1-22). In the lab, there was no evidence that any film coating affected shoot or root length or mass relative to bare seed (although FMC low did have the greatest root and shoot lengths and biomasses). In the field, there were no effects or highly variable and inconsistent effects of film coatings on seedling survival and final seedling count, and film coating treatments never differed from bare seed in terms of seedling height. We acknowledge that the background recruitment of untreated seedlings in the Y0-22 site compromised our ability to detect the effects of film coatings in that setting; however, the lack of detectable effect was consistent across all other lab and field trials.

In prior trials with sagebrush, any kind of physical coating or pelletizing has severely inhibited seedling emergence (Eshleman and Riginos, 2023; Donovan et al. 2024; Baughman et al. 2023). We explored a variety of film coatings in the hopes of delivering enough active ingredient to enhance seedling growth and/or establishment without impacting emergence. While the film coatings consistently avoided the emergence problems of coating and pelletizing, they appear not to have delivered enough active ingredient, or not the right active ingredient, to have had any real benefits for seedlings. Increasing the quantity of these active ingredients in a film coating formulation is not feasible (Germains, personal communication). It is possible that other active ingredients not tested here might have positive effects on sagebrush seedling establishment. For example, surfactants in SETs have been shown to have some effectiveness at overcoming water limitation in early seedling survival (Madsen et al., 2012; Madsen et al., 2014b; Ritchie et al. 2020). However, given the very small size of sagebrush seeds (~4,100 seeds/g), it seems extremely difficult to deliver sufficient quantity of any active ingredient without coating or encasing seeds and therefore likely inhibiting emergence. Given all considerations, it appears that film coating is not a productive pathway to achieving an establishment-enhancing SET for sagebrush.

Mixed effects of direct fertilizer application

In addition to film coatings, we tested two fertilizers delivered next to, but not on or coating, seeds. Prior lab trials had shown that application of the Root & Grow® (R&G) fertilizer directly and in a "dust" (dried, ground pellet) form enhanced root biomass and length relative to bare seeds, but inclusion in an extruded pellet severely reduced emergence (Eshleman and Riginos, 2023); field trials also showed negative effects of seeded R&G pellets on emergence but some potential benefits to survival under competition from neighboring perennial plants (Donovan et al. 2024). We hypothesized that the low emergence was due to the compaction and hardness of the pellets as well as seeds' requirement for light to stimulate germination. Here, we tested the same R&G pellet recipe with seeds sown separately around clusters of seedless pellets, as well as a slow-release fertilizer that also had a high phosphorus ratio (though not entirely equivalent – 4:10:3 for R&G pellets and 3:4:2 for slow-release).

In both lab and field trials, the slow-release fertilizer had strong negative effects on seedling emergence – a quarter to half the percent emergence of bare seed. In contrast, the seedless R&G pellet treatment did not have any negative effects on emergence. In the lab, the slow-release treatment seedlings had far greater shoot and root lengths (>2x root length) and masses (20x shoot mass and >3x root mass) than bare seeds. R&G pellets had somewhat elevated responses in these traits relative to bare seed but far less than slow-release. Seedlings in the R&G pellet treatment did not differ from bare seed in root:shoot ratio, whereas slow-release seedlings had significantly lower root:shoot ratio than bare seeds, and we hypothesized that the disproportionate shoot growth (potentially due to higher N relative to P in slow-release compared to R&G pellets) would make seedlings vulnerable to drought stress in the field.

In the field, however, slow-release seedlings showed some benefit of the fertilizer. In the one year since reclamation site (Y1-21), slow-release seedlings had nearly double the survival rate compared to bare seeds (not statistically significant but notable in magnitude) and final seedling counts did not differ between the two treatments despite the dramatically lower emergence rate of slow-release seedlings. The single slow-release seedling in Y0-21 was also more than twice as tall as bare seed seedlings. We also found that slow-release seedlings were taller than other treatments in a companion study at a different reclamation site (this report, Chapter 4) and at the Y1-21 site midway through the summer (slow-release=13.9 mm, compared to 7.7-9.7 in all other treatments). All lines of evidence suggest that the slow-release treatment did enhance the growth and potentially survival of seedlings that successfully emerged. In contrast, there was no evidence that the seedless R&G pellets had any effects on seedling survival, final seedling count, or height, relative to bare seed.

The opposing forces of poor emergence and enhanced growth in the slow-release treatment highlights a likely tradeoff. The very low emergence was not caused by compaction, burial, or lack of light – the hypothesized causes of low emergence in earlier seeded R&G pellets. Instead, the lab and field results suggest that the slow-release fertilizer itself inhibited emergence through toxic effects on seeds or germinated seedlings. It is possible that a lesser quantity of fertilizer or lower N:P ratio would avoid this toxicity while still delivering some benefits to seedlings in their first season of establishment and growth. Finding that sweet spot to accomplish both goals may be difficult. The seedless R&G pellets had no apparent benefit to seedlings in the field over both years of field trials. In the lab trial that showed positive effects of the R&G fertilizer on root biomass (Eshleman and Riginos, 2023) the rate of fertilizer per pot was approximately double the rate per cluster of pellets and seeds used in the field trials. It is possible that using a higher rate of R&G per cluster would result in greater benefit in the field, but this potentially also risks early seedling toxicity. Even if such a sweet spot for sagebrush could be identified, the problem of how to deliver adequate fertilizer in an SET that can be applied at large scale without impairing emergence still remains.

Topography and existing vegetation

An additional goal of the field trials was to further our understanding of how reclamation seeding practices can enhance successful seedling establishment. This included seeding in different topographic positions and times since reclamation and including a higher rate of bare seed application in the second field trial (in the freshly reclaimed site).

Prior results (Donovan et al., 2024 and several unpublished experiments) consistently showed that sagebrush emergence was low, but survival and growth rates high, in freshly reclaimed areas, whereas the opposite was true in older (5+ years since reclamation) sites. These results were attributed to low soil stability, resulting in seed burial (negative effect on emergence), and absence of plant structure to capture and retain snow (also negative effect on emergence) as well as lack of competition from established plants (positive effects on survival and growth) in the freshly reclaimed areas, with inverse characteristics in older reclamation sites. Donovan et al. (2024) concluded that sites one year post-reclamation might have the best compromise between these opposing forces, resulting in overall greatest seedling recruitment. Based on these prior findings, we expected emergence to be greater in the Y1 sites compared to Y0 sites but survival and growth to be greater in the Y0 site. Field trial 1 results followed this prior pattern: seedling emergence was far higher overall in Y1-21 than Y0-21 (5.9% vs 0.5%), but survival was much higher in Y0-21 than Y1-21 (90% vs 54%). The net effect was similar end-of-season seedling counts in the swales (where Y0-21 seedlings actually emerged) across both times since reclamation, but far more total seedlings in Y1-21 than Y0-21 (668 vs 54). In field trial 2, seedling survival and height patterns were consistent with field trial 1 and prior patterns. Survival was higher in Y0-22 than Y1-22 (49% vs. 20%) and height was again more than four times greater in Y0-22 than in Y1-22.

Contrary to prior results, however, seedling emergence and final seedling count were much greater in Y0 than in Y1. A large portion of this emergence may have been from seeds sown during the reclamation process rather than our experimentally sown seeds. In contrast, very little emergence was documented from reclamation process seeding in the Field Trial 1 Y0 site. There was also very low emergence in the unseeded plots in both years' Y1 sites; this is not surprising given that sagebrush rarely persists in the soil seedbank. It is unclear why seedling emergence was so strong in the freshly reclaimed site in Field Trial 2, unlike all prior trials at this mine complex. Potentially, conditions that year or in that area of the mine reclamation site were particularly conducive to seedling emergence. It was an unusually cold and heavy snowfall winter and a cold spring with late snowmelt, conditions that favor sagebrush establishment (Shriver et al. 2018). This may have been coupled with very low viability in the experimentally sown seed in both Y0 and Y1 sites of Field Trial 2, making Y0 appear to have higher emergence in terms of both absolute and percent of experimentally sown seeds.

Also based on prior findings, we designed field trials to include slopes and swales, with the hypothesis that recruitment would be higher in the swales, which in both years had noticeably more snow retention and soil moisture in the spring. In Field Trial 1, in the Y0 site, emergence was indeed much higher in swales than slopes, with near zero emergence on slopes. There was no swale component in Y0 of Field Trial 2 for comparison. In both Field Trials 1 and 2 in Y1 sites, there were slightly more seedlings in swales than slopes, but far less marked than in Field Trial 1 Y0. There were no benefits of swales in terms of survival or height. However, swales in Y0 of Field Trial 1 had similar final seedling counts as swales in Y1, indicating the substantial overall benefit of this topographic position in the otherwise low-emergence Y0 landscape.

A consistent pattern here is for seedlings to survive and grow larger in the absence of neighboring established plants. This is not surprising as an overall pattern, but the magnitude of difference that small amounts of competitor cover can make is quite striking. In Field Trial 1, neighboring plant cover was moderately high (21% total), largely made up of rapidly-growing *S. tragus*, but in Field Trial 2, neighboring plant cover was less than 1% total (though this was just one snapshot of cover and it may have been higher later in the summer). However, our observations on the ground support the observation that Y1-22 (Field Trial 2) had much less other vegetation present than Y1-21 (Field Trial 1) throughout the season. This difference might also explain the overall lower emergence between the two years' Y1 sites, since existing vegetation visibly traps snow and enhances early spring soil moisture. Additionally or alternatively, the low seed viability in Field Trial 2 – despite efforts to keep PLS sown consistent with Field Trial 1 -- may have led to the lower emergence in Y1 sites. Further, site and precipitation differences could also be responsible for some of the variation. Despite total spring precipitation being similar across both field trials and summer precipitation being higher in Field Trial 2, seedling survival was substantially lower in Field Trial 2 than in Field Trial 1 Y1, potentially due to longer dry periods or other nuances of site and precipitation.

Seedlings in drylands such as the sagebrush ecosystem must pass through a series of recruitment bottlenecks in order to successfully establish (James et al. 2011). We see here how different bottlenecks manifest under different biotic and abiotic conditions. It has been suggested that emergence is the main bottleneck to seedling establishment in the sagebrush ecosystem (James et al. 2011). In Field Trial 1, emergence mattered to final seedling counts in that no seedlings could recruit where no seedlings emerged (slopes), but the higher emergence in Y1 did not translate to more seedlings than Y0 swales. In Field Trial 2, emergence was greater in Y0 than Y1 and this did yield more seedlings by season's end since those seedlings also survived at higher rates. Surprisingly, however, there was no advantage to sowing seed at rates higher than the background reclamation process; none of the seeded treatments, even at double seeding rate, produced more seedlings by season's end. This is contrary to the prevailing wisdom that higher seeding rates will yield more seedlings (Williams et al. 2002; Schuman et al. 2005; Applestein et al. 2018). Our findings indicate that seed rate and emergence do matter but enhancing these does not necessarily result in more seedling establishment. Additionally, in Field Trial 1, the Y0 site resulted in fewer, larger seedlings than the Y1 site – consistent with prior findings (Donovan et al. 2024) – and raising the question of whether larger seedlings have greater chance of long-term survival.

Management Implications

These findings collectively illustrate how there is no simple or single answer to the question of how to get more sagebrush seedlings to establish. Seed enhancement technology may not be an effective approach for sagebrush, given the challenges of small seed size and emergence inhibition (but see Hoose et al. 2019). Outside of SETs, some general principles for practice do emerge. For example, if resources for seeding are limited, we suggest focusing seeding on swales or other microsites and topographies that capture and retain moisture. Broad sowing of seeds on bare ground post-reclamation is likely to continue to have highly variable outcomes. If emergence is poor in the first year of sowing, we recommend re-sowing the next fall, since establishment is generally good during this phase when there is not yet established perennial vegetation. There is growing evidence to suggest that restoration outcomes are more likely to succeed with multi-year seeding (Svejcar et al. 2023). Although increasing the seed application rate in this study did not result in more seedlings, we encourage further documentation of the outcomes of different seed application rates, since positive results have been seen in other trials and restoration settings. In places of particularly high habitat value, transplantation of seedlings, both greenhouse-grown and wild-grown, continues to be the most reliable (though expensive and difficult to scale) method to restore sagebrush (Pyke et al. 2020; Bailey et al. 2024). Restoration of sagebrush will likely continue to be a multi-pronged and adaptive effort.

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Chapter 3: Competition reduction and seed enhancement technologies to improve establishment of Wyoming big sagebrush in grass-dominated reclamation sites

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Introduction

Drylands around the world are often slow to recover and difficult to restore after disturbance (Shackleford et al, 2021). Seed-based restoration is the only approach that can be implemented at large scale, yet recruitment from seed is variable across species, years, and sites (Kildisheva et al. 2016; Shackleford et al. 2021). A common challenge is priority effects: species that establish successfully initially create competitive conditions that prevent the subsequent establishment of other species (Young et al. 2017; Weidlich et al., 2021). In this situation, species that fail to establish in the first year post-seeding do not get another chance to establish even if seeded again. Further intervention may be needed to reduce competition or otherwise enhance establishment success (Young et al. 2017).

In the sagebrush steppe biome of the Western United States, a common outcome of seed-based mine reclamation is successful establishment of perennial grasses, but poor establishment for big sagebrush (*Artemisia tridentata* Nutt.) (Schuman et al. 2005; Schuman et al. 2012). Big sagebrush is a foundational species that provides habitat structure and forage essential for numerous sagebrush-dependent species, including the imperiled Greater sage-grouse (*Centrocercus urophasianus*), migratory ungulates, raptors, and songbirds (Davies et al. 2011; Remington et al. 2021). The biome is facing rapid losses due to surface land disturbances, exotic annual grass invasion, increasingly frequent and severe wildfire, and conifer encroachment (Doherty et al. 2022). It is estimated that half of the original 1.2 million km^2 extent of sage-grouse habitat has been substantially degraded and continues to be lost at a pace of approximately 563,000 hectares (2,278 km^2) per year (Doherty et al. 2022). Keeping pace with the restoration need and increasing the quality of restoration outcomes are considerable challenges.

A typical approach to increasing sagebrush density and cover in restoration or reclamation sites is to transplant container-grown seedlings. This has much higher success rate than broadcast seeding even in conditions without competition from established neighbors (Pyke et al. 2020). Competition from established grasses and shrubs can significantly impact transplants. Conversely, reducing competition can enhance the survival and growth of transplants and increase the likelihood of establishment from seed. For example, Davies et al. (2013) found that reducing the cover of the non-native perennial grass, crested wheatgrass (*Agropyron cristatum*) greatly enhanced the growth of transplanted sagebrush seedlings; reducing crested wheatgrass by 50-75% resulted in threefold greater sagebrush cover after three years than in a 0% reduction treatment.

Although much more successful than broadcast seeding, transplanting seedlings is costly, labor-intensive, and difficult to scale over large areas (Knutson et al. 2014). These challenges have led to interest in seed enhancement technologies as a potential means to boost the success of broadcast seeding in a variety of dryland restoration settings (Madsen et al. 2016; Kildisheva et al. 2016; Gornish et al. 2019; Brown et al.

2021; Svejcar et al. 2022). If successful, the combination of seed enhancement technologies (SETs) and grass competition management could provide an alternative means to overcome grass priority effects and reintroduce sagebrush into grass-dominated restoration sites.

The concept of SETs is to help seeds and seedlings overcome early barriers to establishment (Pedrini et al. 2020; Brown et al. 2021). SETs include seed priming, coating, and pelleting with compounds that can enhance outcomes like seed germination and seedling vigor (Pedrini et al. 2020). SETs containing surfactants, fertilizers, microbes, and super-absorbent polymers have shown some potential for alleviating drought stress after seedling emergence (Madsen et al., 2012; Madsen et al., 2014; Davies et al., 2018; Ritchie et al., 2020). Since water is the main resource depleted by competitor plants in drylands, a successful SET approach could help seedlings cope with competition from established plants. Previous work with big sagebrush showed that a targeted fertilizer application enhanced seedling root growth in the lab (Eshleman and Riginos, 2023). We hypothesized that delivering the same amendment via SETs could help seedlings cope with competition from established plants and survive variable precipitation in field reclamation settings.

We set out to test whether some combination of reducing grass competition and enhancing seedling growth through a seed enhancement technology or targeted fertilizer application could enhance establishment of Wyoming big sagebrush. We conducted two related field experiments across two years; in each experiment, we tested a suite of prototype seed enhancement and seedling fertilization approaches in the context of thinned (with herbicide), repeatedly clipped (to simulate heavy grazing), and unaltered grasses on reclaimed mine sites with high perennial grass cover. We specifically asked (1) what are effects of grass competition treatment on sagebrush establishment, survival, and growth? (2) What are the effects of prototype seed technologies on these same parameters? And (3) Are there any additive or interactive effects between grass competition and seed treatment that together enhance sagebrush establishment?

Methods

Field Sites

These experiments were conducted at the Bullrush mine (42.8060, -107.6489) in the Gas Hills uranium mining complex in Fremont County, WY and the Dave Johnston mine (now part of the Rolling Hills wind farm). The Bullrush mine was reclaimed and revegetated between 2012-2018. The site was covered in sandy loam aggregated B horizon soil from elsewhere in the mine vicinity and seeded with a variety of grasses, forbs, and sagebrush using a soil pitter. Twenty-year rainfall averages for this area are 10.6 cm for March through May, and 5.7 cm for June through August (NOAA data for Riverton Airport: https://www.weather.gov/wrh/Climate?wfo=riw accessed April 27 2024). The existing vegetation consists of the non-native perennial crested wheatgrass (*Agropyron cristatum*), native bluebunch wheatgrass (*Pseudoreogenria spicata*), Lewis flax (*Linum lewisii),* scattered other grasses and forbs, and some established big sagebrush.

Reclamation of the Dave Johnston Mine was completed in 2005. This site also consisted of sandy loam. Twenty-year rainfall averages for this area are 12.6 cm for March through May, and 9.1 cm for June through August (NOAA data for Casper wastewater treatment plant: https://www.weather.gov/wrh/Climate?wfo=riw accessed May 20, 2024). The existing vegetation primarily consists of perennial bunchgrasses such as bluebunch wheatgrass, basin wildrye (*Leymus cinereus)*, needle-and-thread grass (Hesperotipa comata) and Indian ricegrass (*Oryzopsis hymenoides*).

There is also scarce sagebrush, winterfat (*Krascheninnekovia lanata*), and abundant prairie coneflower (*Ratibida columnifera*).

2021-22 Experiment

The experiment was set up at Bullrush only, on a gentle east-facing slope using a split-plot design, with grass treatments applied at the whole-plot level and seed treatments applied at the sub-plot level. We laid out 36 whole plots, each 4x5 m, and randomly assigned them to three grass treatments: herbicide, clipped, and control (n=12 each). The initial clipping and herbicide treatments were applied in June 2021, and clipping was repeated in August 2021. For the clipped treatment, all grass in the plot was clipped to a 3" stubble heigh using a string trimmer. For the herbicide treatment, a solution containing 3% glyphosate (Credit 41) was applied at a rate of 2 quarts/acre, along with a surfactant, brand name Induce, at 1 pint/100 gallons, to half of the plot area in a checkerboard pattern of 50x50 cm squares. A blue dye was added to the solution to facilitate the checkerboard spraying.

Within each whole plot, we laid out six subplots, each 100x50 cm, with the long edge and three 100 cm furrows (each approximately 2.5 cm wide and 1.25-2 cm deep) within each subplot running across the slope. We allowed a 1 m buffer between subplots and the edge of the whole plot. There was extensive dead grass material (thatch) on the ground in all plots. To the extent possible, thatch was pushed aside in the process of hand-digging the furrows to improve seed-soil contact. Each subplot was randomly assigned to one of six seed treatments: bare (unaltered) seed, "Grow More" film coating, "Elite" film coating, "Lettuce" film coating", bare seed + Root&Grow ® fertilizer pellets ("R&G pellets"), and bare seed + slow-release fertilizer granules ("slow-release"). All seeds were obtained from Granite Seed and Erosion Control (Lehi, UT) from lot ARS-1015/0612-GX-45-SNAKERIVER-21.

The three film coatings were applied directly to the seeds by Germains Seed Technology, Inc. (Gilroy, CA) using a rotary coater. The Grow More film coating formulation contained 15:30:15, Grow More® Fertilizer (Grow More Inc., Gardenia, CA) as an active ingredient as well as a proprietary polymer formulation for the film coating base. Elite film coating is a commercially available formulation, developed by Germains Seed Technology for carrot and jalapeno pepper seeds, and has the potential to improve plant nutrient uptake and support early plant development through increasing root mass and improving seedling development (Germains Seed Technology, personal communication). The Lettuce film coating is a formulation developed by Germains for the treatment of lettuce seeds and has the potential to improve seed germination and increase shoot and root growth in early plant development (Germains Seed Technology, personal communication). Film coated seeds were not visibly encrusted and they maintained the same shape and structural characteristics of untreated seeds.

The R&G pellet and slow-release treatments were not seed coatings but were included to test the potential of their growth-enhancing ingredients against other treatments. Although we recognize that this approach cannot easily be scaled up over large areas, we wanted to test the potential for these fertilizers to enhance seedling establishment without the confounding effects of applying them in a coating. These treatments had the additional potential benefits over film coatings of delivering higher quantities of fertilizer that would continue to be released over a longer period of the growing season. Previous trials had shown positive effects of the high phosphorus Root&Grow® (Bonide Products LLC, Oriskany, NY) product (a 4:10:3 N:P:K fertilizer with indole-3-butyric acid growth hormone) on seedling root and shoot growth in the lab (Eshleman and Riginos, 2023) and potential benefits in the field (Donovan et al. 2024). These previous findings also showed severe emergence inhibition when seeds were incorporated into extruded pellets as a means of delivering the fertilizer in an SET. This liquid fertilizer, however, could not be delivered to seeds in a film coating. To continue testing Root&Grow as our "best results so far" fertilizer, we produced the same extruded dough pellets as before, minus the seeds, and sowed the seeds and pellets separately onto the soil surface. To test an additional solid fertilizer, we also tested the direct application of LESCO® slow-release fertilizer granules (3:4:2, Lesco®, SiteOne Landscape Supply, Roswell, GA).

Seed treatments were applied randomly to subplots in November 2021 at a rate of 200 pure live seed (PLS) per subplot. The number of seeds was adjusted to achieve this PLS based on a standard tetrazolium (TZ) test (Oregon State University Seed Laboratory, Corvallis, OR) which showed 76% seed viability. Seeds in the bare, Grow More, Elite and lettuce treatments were sown evenly across the furrows. Seeds in the R&G pellet and slow-release treatments were sown in 18 clusters of seeds, approximately 11 PLS per cluster, with clusters spread across the three. For the R&G pellets treatment, 0.58 g of pellets (equivalent to 0.48 ml of Root&Grow®) were applied in each cluster, and pellets were pushed into the ground vertically around each seed cluster. For the slow-release treatment, 0.25 g of fertilizer beads were sprinkled on top of each seed cluster.

We did not include an unseeded control treatment because most of the existing, established sagebrush shrubs at the site were pre-reproductive size. We do, however, acknowledge that some natural recruitment was possible. We excluded from any count and measurement data the handful of seedlings we found outside of the furrows and have high confidence that nearly all of the seedlings we observed were from seeds we had sown as part of the experiment.

Plots were monitored for seedling emergence, survival, and growth biweekly from April to June 2022, then every three weeks until August 2022, and once in November 2022. At each monitoring event, we counted newly emerged seedlings, surviving vs. dead seedlings from the previous event (marked with a colored toothpick for tracking), and the height of three randomly chosen (marked with a different colored toothpick for re-measurement) seedlings. For the small number of seedlings that survived the first year of the experiment, we collected survival and height data again in November 2023.

We also quantified vegetation cover once in June, in the middle of peak growing season, to characterize the biotic environment. Annual and perennial cover were visually estimated for each plot in the following percentage classes: <1, 1-5, 6-40, 11-25, 26-50, 51-75, and >75. For analysis, we used the midpoint value of each cover class. March-May and June-August precipitation was obtained from a weather station located at the Bullrush Mine.

To account for the split-plot design, we analyzed data using generalized linear mixed models (GLMMs) with seed treatment as a fixed effect and grass treatment and grass treatment*seed treatment as random effects, where data were sufficient to support this model approach. Seedling emergence (cumulative over all observation periods) was calculated as the percent of PLS that germinated, to account for different PLS rates. Emergence and survival (percent of emerged seedlings that survived to November 2022) were analyzed using a binomial distribution, using the *glmer* function within the *lme4* package in R. Significance of predictors was assessed using model simplification and χ^2 analysis of deviance to compare models. Multiple comparisons were conducted using pairwise comparisons of group estimated marginal means with the *emmeans* package with a "tukey" adjustment to the p-value. By November 2022, only 20.8% of sub-plots (n=28) had any surviving seedlings in them, presenting challenges for analyses due to low replication. Because we could not run the full GLMM model, we instead analyzed the number of surviving seedlings per plot using a negative binomial GLM with grass treatment, seed treatment, and their interaction as predictors, using the MASS package within R. Similarly, final height (averaged among seedlings within each plot) was analyzed using an additive model for grass treatment and seed treatment (insufficient replication to include an interaction term) using the *lmer* function within the *lmertest* package and data log-transformed to meet assumptions of normality. We acknowledge that these analyses do not account for the nesting of seed treatments within grass treatments.

2022-23 Experiments

In the second year of this study, we set up two more field experiments, one at Bullrush and one at Dave Johnston, similar to the 2021-22 experiment with some modifications and different seed treatments. At Dave Johnston, the whole-plot grass treatments were identical to the 2021-22 Bullrush treatments (n=12 x 3 grass treatments). In 2022-23 at Bullrush, the grass treatments included the same three as the previous year plus an additional two-year clipped treatment (n=12 each x 4 grass treatments). The two-year clipped treatment was initiated in June 2021 and reclipped through the 2021 and 2022 growing seasons to simulate repeated heavy grazing; we hypothesized that two years of clipping would reduce below-ground competition more effectively than one year of clipping. Herbicide and one-year clipped treatments were applied in June 2022, with reclipping in September 2022.

All subplots were set up in the same way as the previous year and seeded in November 2022. Seed treatments for both experiments consisted of the same bare seed and R&G pellet clusters as the previous year, plus four new film coatings. Film coatings were produced by Germains Seed Technology, Inc. using methods described above but with active ingredients BEC80 and FMC4034. The details of the BEC80 (hereafter, "BEC") and FMC4034 (hereafter "FMC") film coatings are protected by intellectual property. Both were chosen based on their potential to improve seed germination and increase shoot and root growth in early plant development (Germains Seed Technology, personal communication) and were applied in a low and high rate (BEC low: 20 g/kg seed, BEC high: 40g/kg seed, FMC low: 4.7 g/kg seed, FMC high: 9.4 g/kg seed).

Seed was sourced as in the 2021-22 experiment. Our target seed rate was again 200 PLS/plot, and seed numbers adjusted to meet this goal based on TZ testing of the film-coated seeds. Further TZ testing (after field seeding) showed lower viability in the bare seed, which meant that a lower PLS (134/plot) was achieved in the bare seed and R&G pellets treatments than the film-coated treatments. To adjust for this, all emergence and seedling count data were analyzed as a percent of viable seeds sown.

Annual and perennial cover were visually estimated for each plot in the following percentage classes: <1, 1-5, 6-40, 11-25, 26-50, 51-75, and >75. For analysis, we used the midpoint value of each cover class. Precipitation data was obtained from the Bullrush weather station maintained by the WY Department of Environmental Quality and the NOAA Casper, WY wastewater treatment plant weather station for Dave Johnston.

Plots were again monitored for seedling emergence, survival, and growth, as in the previous year. At Bullrush, seedlings were monitored biweekly from April to June 2023, then every three weeks until August 2023, and one final time in November 2023. At Dave Johnston, seedlings were monitored once each in May, July, and November 2023.

Data were analyzed separately for Bullrush and Dave Johnston sites. We again analyzed data using generalized linear mixed models (GLMMs) with seed treatment as a fixed effect and grass treatment and grass treatment*seed treatment as random effects. Seedling emergence (cumulative over the whole monitoring season) was calculated as the percent of PLS that germinated, to account for different PLS rates. Emergence and survival (percent of emerged seedlings that survived to November) were analyzed using a binomial distribution, using the *glmer* function within the *lme4* package in R. Final (November) total seedling count per plot was analyzed using a negative binomial distribution to account for zero inflation of the data. Significance of predictors was assessed using model simplification and χ^2 analysis of deviance to compare models. Final height (averaged among seedlings within each plot) was also analyzed as a GLMM using the *lmer* function.

Results

2021-22 experiment: Bullrush

March to May precipitation at Bullrush totaled 5.3 cm, and June-August precipitation totaled 6.1 cm. Average perennial vegetation cover in June was 26%, with about 5% higher cover in the control treatment relative to the clipped and herbicide treatments. Average annual vegetation cover in June was only about 1%.

There was evidence that seedling emergence was affected by an interaction between grass treatment and seed treatment (χ^2=53.59, p<0.0001; Figure 1a). Emergence was higher in the clipped and herbicide grass treatments compared to untreated grass for some, but not all, seed treatments. The highest emergence rates were in the combination of Lettuce seed treatment and clipped grass (2.42 seedlings/plot ± 0.95), Grow More and clipped and herbicide treated grass, and R&G pellet and herbicide treated grass. These were significantly greater than bare seed and untreated grass (0.33% ± 0.09), R&G pellet in clipped grass, slow-release in clipped grass, and in some cases slow-release in herbicide treated grass. The low-release seed treatment had low emergence across all three grass treatments.

Seedling mortality was high, and survival low, through the summer. Seedling survival was substantially higher in the herbicide grass treatment (21.8% ± 4.25 SE) than clipped grass (7.94% ± 2.99 SE) and untreated grass (8.84% ± 3.33 SE) (main effect of grass treatment, F=8.01, p=0.02). There was a marginal effect of seed treatment (F=9.34, p=0.09), but no interaction between seed and grass treatments, (χ^2 =9.08, p=0.52) on seedling survival. Survival was highest in the Lettuce treatment (22.17% ± 2.99 SE) and lowest in the R&G pellet (7.89% ± 4.32 SE and slow-release treatments (5.50% ± 5.00 SE).

At the end of the first growing season, the number of remaining seedlings per plot was highest in herbicide treated and lowest in untreated grass plots (Figure 1b; significant main effect of grass treatment: χ^2=9.11, p=0.01). There was no evidence for any effect of seed treatment (χ^2 =10.71, p=0.38) or interaction between seed and grass treatments, (χ^2 =8.21, p=0.15). Among seed treatments, however, final seedling counts were notably higher in the Grow More (0.37), Elite (0.22), and Lettuce (0.19) treatments and lowest in the slow-release treatment (0.05 (Figure 1c).

In terms of height of the surviving seedlings, there was no statistically detectable effect of grass treatment (F=3.15, p=0.10) or seed treatment (F=1.37, p=0.28). However, the herbicide treatment yielded the tallest seedlings in nearly all seed treatments (Figure 1d; average values: herbicide: (21.38 mm ± 3.70 SE; clipped: 9.76 mm ± 1.87 SE; untreated grass: 9.00 mm ± 1.14 SE). Among the seed treatments, R&G pellet seedlings were the tallest, followed by the two remaining slow-release seedlings (Figure 1d).

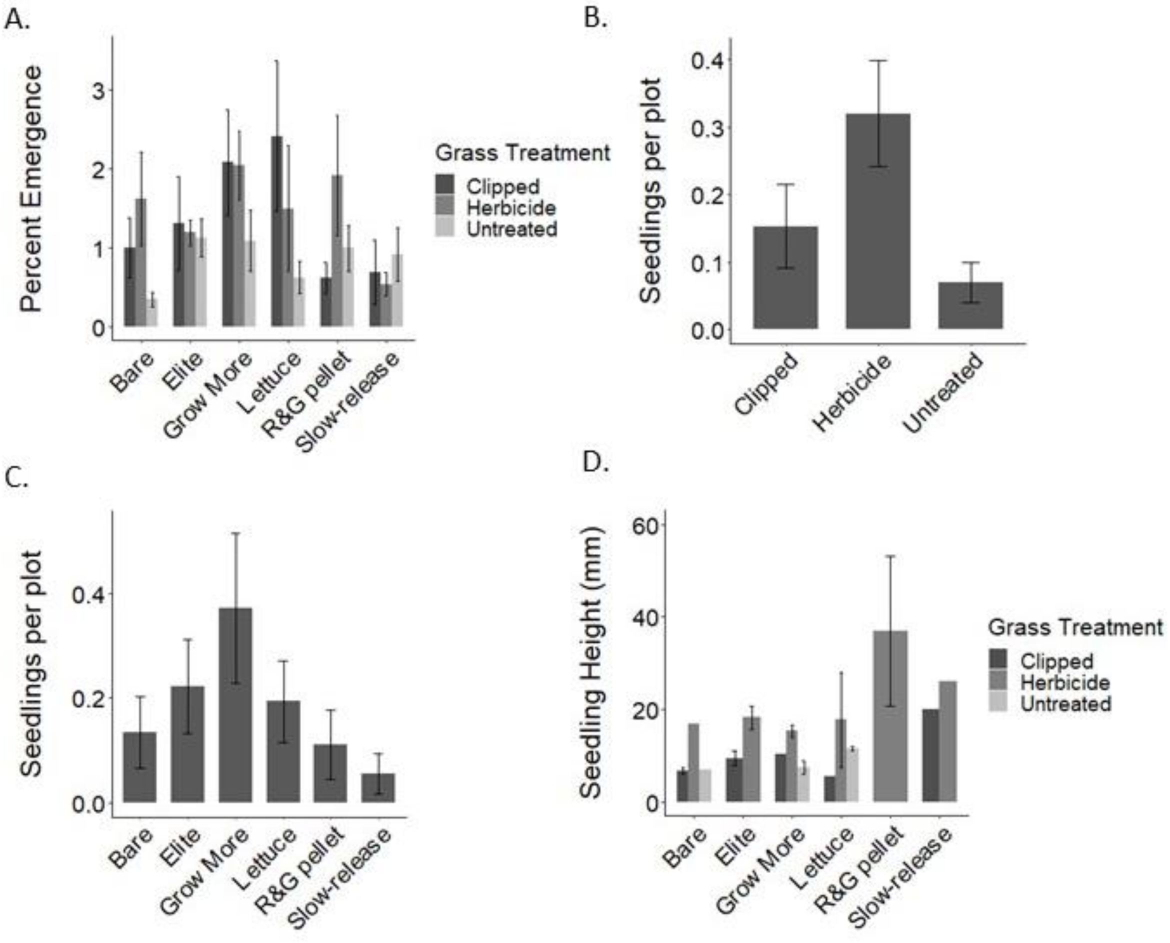

Figure 1. Performance of *A. tridentata*, with means ±SEMs shown, for the 2021-22 Bullrush experiment: (a) cumulative percent emergence by grass treatments and seed treatments; (b) number of seedlings alive at the end of the first growing season by grass treatment; (c) number of seedlings alive at the end of the first growing season by seed treatment; (d) seedling height at the end of the first growing season.

2022-23 Experiments: Bullrush

March to May precipitation was lower than the previous year at 3.5 cm – following an unusually cold winter and spring and a high snowpack winter. June-August precipitation was high, totaling 14.5 cm. The average annual cover value was less than 1%. The average perennial cover value was 31%. The perennial cover ranged from 45% in the untreated plots to 17% in the herbicide plots. Both clipped treatments had about 30% perennial cover.

Seedling emergence exhibited interactive effects of grass and seed treatments (χ^2=38.81, p<0.001). Emergence was variable (Figure 2a), making it hard to infer any patterns. BEC low seed treatment had high emergence in three of four grass treatments. In BEC low and bare, emergence was lower in the herbicide treatment compared to other grass treatments.

Seedling survival was also strongly affected by an interaction between grass and seed treatments (χ^2=31.37, p=0.008). Results were again quite variable across different combinations of seed and grass treatment (Figure 2b). In some, but not all, seed treatments, survival was higher in the herbicide and/or one of the clipped treatments compared to the untreated grass treatment.

There was also a significant interaction between grass and seed treatments in terms of the final number of seedlings alive in November 2023 (χ^2=137.39, p<0.0001). Following patterns of survival, seedling counts were sometimes, but not always, lower in untreated relative to the other grass treatments (Figure 2c; overall average untreated = 0.76 seedlings/plot; other treatment averages 1.04-1.19 seedlings/plot). Results across seed treatments were variable but lower seedling counts overall in FMC high, R&G pellets and sometimes bare seed.

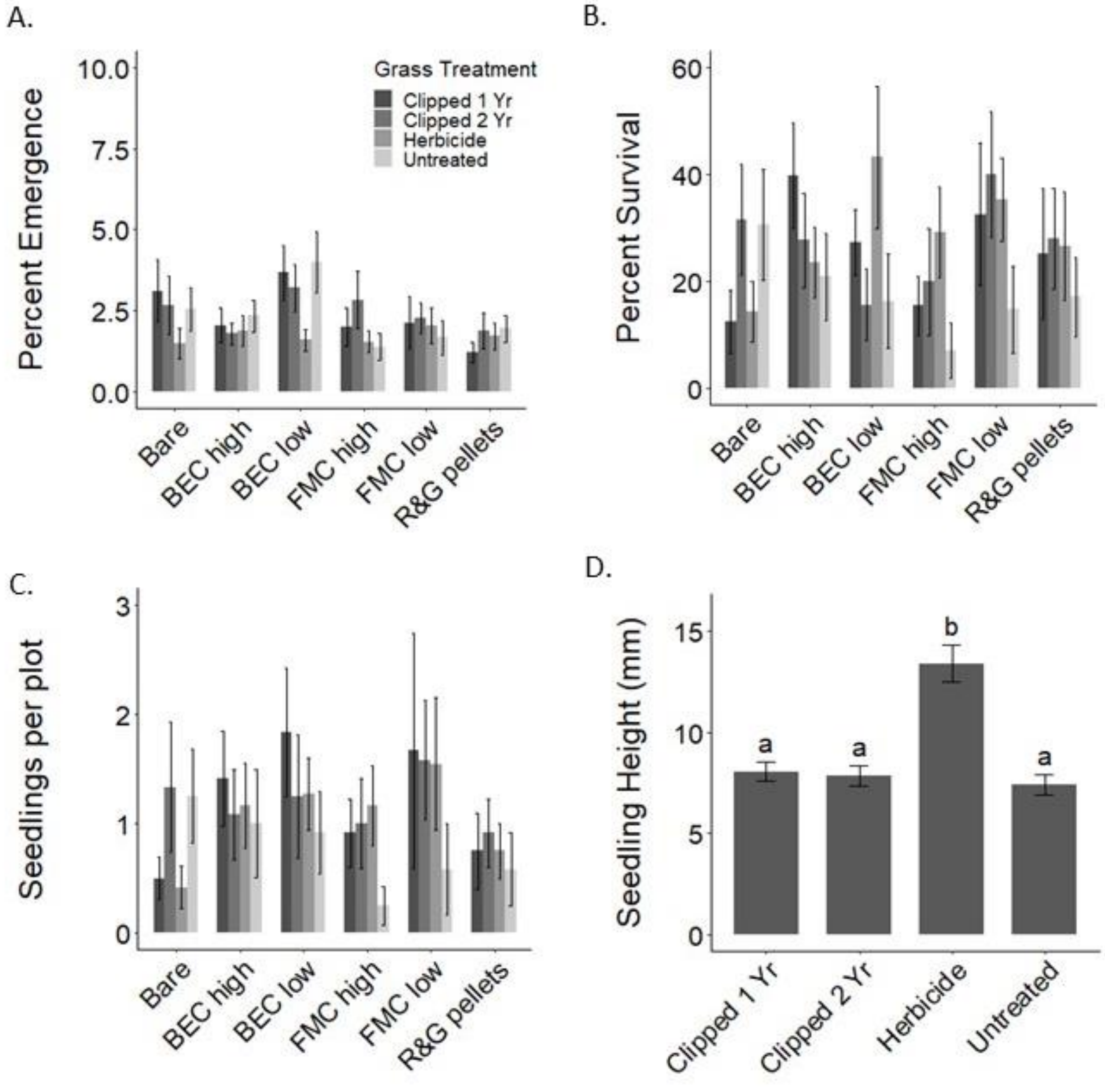

Figure 2. Performance of *A. tridentata*, with means ±SEMs shown, for the 2022-23 Bullrush experiment: (a) cumulative percent emergence by grass treatments and seed treatments; (b) percent survival by grass and seed treatments; (c) number of seedlings alive at the end of the first growing season by grass and seed treatment; (d) seedling height at the end of the first growing season by grass treatment.

Height of seedlings in November 2023 was strongly affected by grass treatment (F=14.83, $p<0.0001$) but not seed treatment (F=1.13, p=0.35) or the interaction between grass and seed treatments (F=0.75, p=0.72). Seedlings were taller in the herbicide treatment than any other grass treatment (Figure 2d).

2022-23 experiments: Dave Johnston

The precipitation was above average in 2023 at the Dave Johnston Mine site. March-May precipitation was 8.8 cm and June-August precipitation totaled 22.7 cm. The average annual cover value was less than 1%. The average perennial cover value was 30%, which did not differ by grass treatment in June, but was variable among plots.

Seedling emergence was affected by seed treatment (χ^2=59.93, $p<0.0001$) and weakly by grass treatment (χ^2=5.89, p=0.05) and but not by any interaction between the two (χ^2=12.45, p=0.26). Emergence was significantly higher in bare seed and R&G pellets treatments compared to other seed treatments (Figure 3a). Emergence was also highest in untreated (4.13% ± 0.28 SE) compared to clipped for one year (3.18% ± 0.27) and herbicide treated (2.96% ± 0.23) grass plots.

In terms of seedling survival, there was an interaction between grass and seed treatments (χ^2= 19.76, p = 0.03). In some seed treatments, survival was highest in the herbicide treatment; in others, it was highest in the untreated grass treatment (Figure 3b). Survival was slightly higher in the R&G pellets treatment (56.65% ± 4.28 SE overall average) compared to other treatments, especially BEC low (39.92% ± 4.67 SE overall average).

Final seedling count in November 2023 showed a significant effect of seed treatment (χ^2=17.09, p=0.004) and a marginal effect of grass treatment (χ^2=5.73, p=0.06). Overall, the seedling count was highest in R&G pellets treatment and lowest in the BEC low and FMC low treatments (Figure 3c). The seedling count was slightly higher in untreated (3.64 seedlings/plot ± 0.37 SE) compared to herbicide (2.53 seedlings/plot ± 0.27 SE) and clipped grass (2.47 seedlings/plot ± 0.27 SE).

There was evidence that seedling height was affected by grass treatment (χ^2=8.98, p=0.01) but not seed treatment (χ^2=1.63, p=0.90) or the interaction between grass and seed treatments (χ^2=13.03, p=0.22). Height was significantly greater in the herbicide treatment compared to the clipped grass treatment (Figure 3d).

Discussion

Positive effects of grass reduction

We set out to experimentally reduce grass competition using both herbicide to kill some of the existing perennial grasses and repeated clipping to simulate heavy grazing. The effects of herbicide were clear in reducing aboveground cover and visibly killing some grasses. The effects of clipping were less clear. Clipping resulted in lower above-ground biomass early in the growing season, but often this difference was erased later in the growing season and sometimes resulted in greener grass cover (personal

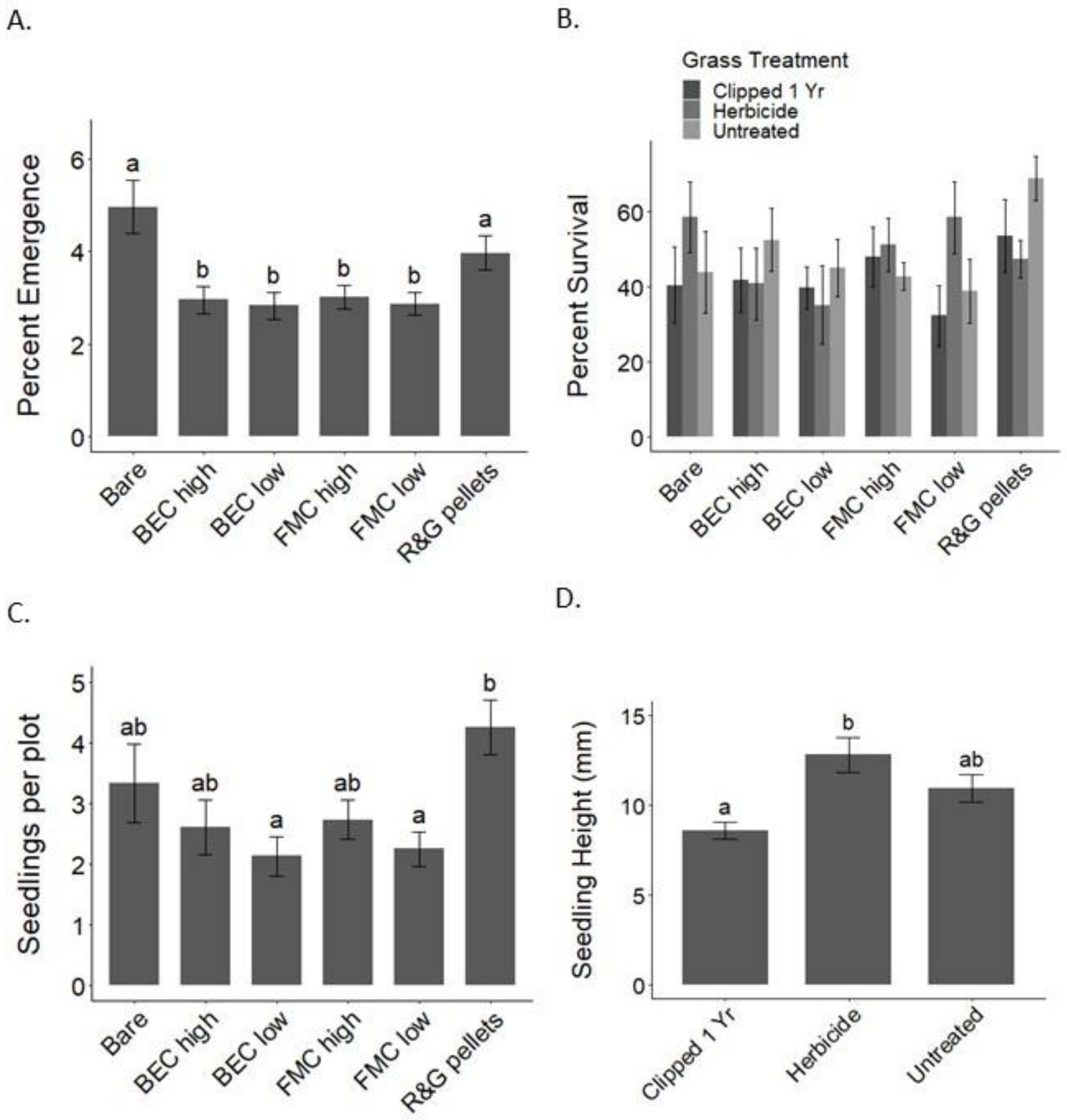

Figure 3. Performance of *A. tridentata*, with means ±SEMs shown, for the 2022-23 Dave Johnston experiment: (a) cumulative percent emergence by seed treatments; (b) percent survival by grass and seed treatments; (c) number of seedlings alive at the end of the first growing season by seed treatment; (d) seedling height at the end of the first growing season by grass treatment.

observation); potentially, clipping may have simply stimulated fresh grass growth rather than reducing competition. Longer-term repeat clipping or grazing may be needed to reduce root biomass and root competition from grasses.

The impacts of grass treatment on seedling emergence were inconsistent and difficult to infer any real patterns. The impacts of grass treatment were stronger in terms of seedling survival – and therefore total end of season count – and height. Patterns were strongest in the 2021-22 Bullrush experiment, where seedling survival was more than double in the herbicide treatment compared to other grass treatments. This played out in terms of end of season seedling counts: there were more than four times as many seedlings in herbicide plots compared to untreated grass plots. Seedlings in the herbicide treatment were also more than twice the height of seedlings in the untreated and clipped plots. The impacts of grass treatment also played out in the second year of growth for these seedlings. By November 2023 (two years after seeds were sown), there were 22 surviving seedlings in herbicide plots compared to ten in clipped and only three in untreated plots. Herbicide-treatment seedlings were also taller than other seedlings, averaging 50.14 cm (±5.95) compared to control (30.00 cm ± 16.1) and (23.30 ± 8.82) clipped treatments (F=2.99, p=0.06).

Results of the 2022-23 Bullrush experiment was consistent with the previous year but more muted; survival and end of season seedling count were higher in the herbicide treatment compared to the untreated grass treatment for some, but not all, seed treatments. Seedling height was nearly double in the herbicide treatment compared to other treatments in the 2022-23 Bullrush experiment. Possibly the very cool, wet spring and early summer meant that soil moisture was less limiting in 2022-23 than in 2021-22 at the same site. The effects of grass treatment were far less clear in the 2022-23 Dave Johnston experiment, where survival and final seedling count were often high in the untreated grass plots, and seedling height was not distinguishable between herbicide and untreated plots. At Dave Johnston, there was high variability in plant cover among plots, likely due to soil or other reasons not related to the grass treatments, and grass treatments did not have any consistent impact on plant cover. This may be why there was no clear impact of herbicide treatments. Rainfall at this site was also much higher than at Bullrush, and potentially soil moisture was less limiting to seedlings as a consequence.

These results indicate that reducing grass competition can have a substantial benefit for sagebrush establishment success – but the degree of benefit is variable, most likely due to year and site growing conditions.

Neutral or mixed effects of seed treatments

We tested several prototype seed enhancement technologies with the hopes of stimulating early life root growth and thereby enhancing seedling establishment success. For the most part, the impacts of seed treatments were absent or inconsistent. In a related set of trials in the lab and a freshly reclaimed field site, we found that the same film coatings tested here had no negative impacts on seedling emergence but also no benefits in terms of survival or growth (this report, Chapter 3). Here, the film coatings also did not appear to reduce emergence in either 2021-22 or 2022-23 Bullrush experiments. In the 2021-22 Bullrush experiment, there was some evidence suggesting higher emergence in the Grow More and Lettuce treatments (in combination with clipped and/or herbicide grass treatments) which was reflected in slightly higher end of season seedling counts for these seed treatments. In the 2022-23 Bullrush experiment, there was slightly higher emergence in the BEC low treatment, but in the 2022-23 Dave Johnston experiment, emergence and final seedling count were reduced in all of the BEC and FMC coated seeds compared to bare seed. There was never any clear impact of film coatings on seedling survival or height. Given the weak and inconsistent patterns, it is difficult to conclude that there were any real effects of film coatings, as we concluded in our companion study (this report, Chapter 3).

In addition to the film coatings, we also tested applying fertilizer externally, next to seeds. In 2021-22 we tested R&G pellets and a slow-release fertilizer, and in 2022-23 we tested only R&G pellets as externally-applied fertilizers. In the 2021-22 Bullrush experiment, emergence was overall low in the slow-release treatment; this is consistent with results from lab and field trials in our companion study (this report, Chapter 3). We also saw low survival of these seedlings, contrary to our results at the freshly reclaimed site, where slow-release seedlings had high survival. Consistent with other results, the seedlings that did survive in the slow-release treatment were larger than seedlings in other treatments except the R&G pellets treatment, which also had somewhat elevated height.

In prior study in the lab, the R&G formulation benefited seedling growth (Eshleman and Riginos 2023), but here we saw inconsistent results. In the 2021-22 Bullrush experiment, R&G pellets had low survival but the few remaining seedlings were taller than those in other treatments (note, all were in the herbicide treatment – the combination of herbicide + R&G pellets results in the overall largest seedlings). In the 2022-23 Bullrush experiment, emergence and final seedling count were somewhat lower in the R&G pellets treatment compared to other treatments, whereas the opposite was true in the 2022-23 Dave Johnston experiment. In neither of the 2022-23 experiments was there any height benefit from the R&G pellets treatment.

These results together indicate that directly applied fertilizers have no benefit, and in fact often negative effects, on very early seedling emergence and survival. Potentially, the fertilizers (especially slow-release) are toxic to very early seedlings. There was some mixed evidence for benefits in terms of growth and height of surviving seedlings later in the growing season. When we resurveyed the remaining 2021-22 Bullrush seedlings in November 2023, we found that all of the seedlings that died had been small (~ 7 mm in height), indicating that getting over this threshold in the first year of growth may be important to second year survival (but note, not all small seedlings died). Additionally, height at the end of 2022 was a strong predictor of change in height ($R^2=0.89$, $p<0.0001$); taller seedlings put on more additional height the next growing season. These results indicate that there are benefit for sagebrush seedlings that are able to grow rapidly in their first season of growth, and fertilizer may help them to achieve that early growth – but also comes with tradeoffs in terms of early emergence and survival.

Management implications

In terms of SETs, we conclude from these experiments and results from our companion study (this report, chapter 3) that film coatings do not hold promise for enhancing sagebrush establishment and early growth, likely because the seeds are too small to coat with enough active ingredient while not inhibiting their emergence, since they also need light to germinate. The slow-release fertilizer consistently reduced emergence and is therefore not a viable product for external application. The R&G pellets treatment had fewer negative effects and sometimes positive effects. Testing in the lab at double the dosage of R&G fertilizer used in these field experiments showed no adverse effects. Therefore, we recommend some further testing of higher dosage of R&G fertilizer in the field, if it can be applied in a way that does not severely impact emergence.

Reducing grass competition via herbicide application consistently resulted in the largest seedlings, and in the experiment with the greatest drought stress (Bullrush 2021-22), it also resulted in the greatest survival and number of seedlings one and two years after sowing seeds relative to other treatments. In older reclamation sites with high grass cover, it is not feasible to treat large areas with herbicide to create the kind of small-scale patchy mosaic we created here. However, it would be feasible to create some small “refuges” from grass competition, followed by seeding or transplanting sagebrush, to create “islands” of sagebrush that then can spread into the surrounding vegetation. Although our one and two year clipped treatments did not improve seedling establishment, moderate to slightly heavy grazing for several years, followed by seeding, may also be a tool to reduce grass competition and enhance sagebrush establishment. Any reduction in perennial grasses, however, comes with a risk of increased invasive annual grass and forb establishment (Davies et al. 2013). Creating refuge patches with herbicide, rather than broad-scale grazing, may be easier to monitor and manage to keep invasives at bay.

We had hoped to find a combination of SET and grass reduction that together enhanced seedling establishment. Though we did not find any combination that resulted in higher numbers of seedlings, it is worth noting that the combination of herbicide and R&G pellets yielded the tallest seedlings – and this may translate to higher long-term survival and/or shorter timeline to reproductive maturity.

The findings further underscore the importance of managing the early phases of revegetation to avoid letting the grass component dominate to the degree of creating strong competition that prevents other species from establishing. Managing grass seeding rates to avoid very high grass establishment and favor "weaker" competitors is one way to accomplish this (Schuman et al. 2012; Young et al. 2017). Some sites may benefit from moderate grazing early in post-revegetation management, to keep grass competition in check. Seeding sagebrush over several years, not just in the first year simultaneous to grasses, may also have value (Donovan et al. 2024). If possible (and recognizing that this may not be), seeding sagebrush first and then grasses one year later would also likely enhance sagebrush establishment (Young et al. 2017). There is likely no one solution, and all potential solutions come with some tradeoffs and logistical challenges.

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Chapter 4: A multi-site test of herbicide protection seed technologies for sagebrush ecosystem restoration

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Introduction

Restoration can be challenging in the dry and variable climate of the sagebrush ecosystem. In most cases, seedling establishment is the major bottleneck to restoring cover of native plant species. Seedling establishment is hindered by a variety of barriers (Copeland et al., 2021; Kildisheva et al., 2018)(Copeland et al., 2021; Kildisheva et al., 2018)(Copeland et al., 2021; Kildisheva et al., 2018); however, the most prevalent barriers are first-year drought conditions (James et al., 2011; Meyer, 1992; Schlaepfer et al., 2014) and competition for moisture from other plants including invasive annual grasses such as cheatgrass (Germino et al., 2018; Porensky et al., 2014; Svejcar, 2016). Changing conditions, such as predicted hotter, dryer conditions and an increase in invasive annual grasses, are likely to cause a decrease in native plant establishment success in the coming several decades. It is important that we work now to improve our suite of tools for native plant restoration in order to successfully revegetate disturbed areas and improve the resilience of more intact areas to future disturbances (Svejcar et al., 2016).

Herbicide protection (HP) seed enhancement technologies are one tool that could improve native plant restoration. They have been developed to allow the simultaneous application of pre-emergent herbicide to control invasive annual grasses and seeding for improved dryland restoration outcomes. In the western United States alone, herbicide treatments have occurred on more than 3.5 million hectares on non-agricultural publicly-owned land in the last 70 years (LTDL 2024). These seed technologies combine restoration seed with activated carbon, an ingredient which protects the seed from the deleterious effects of pre-emergent herbicide by adsorbing and locking up the herbicide in the immediate vicinity of the seed. The hope is that HP seeds can take advantage of the window of time when invasive grass density is low, allowing seedlings to establish with less competition (Madsen et al., 2014). Several other researchers have demonstrated that grass seeds packaged in an activated carbon medium are protected from the effects of pre-emergent herbicides (Brown et al., 2018; Clenet et al., 2019; Davies et al., 2017; Duquette et al., 2024), such as imazapic (Plateau), which is widely used for controlling cheatgrass (*Bromus tectorum*) and other invasive annual grasses. Although growing evidence indicates that an activated carbon herbicide protectant can enhance perennial grass establishment over untreated seed (Brown et al., 2018; Clenet et al., 2019; Davies, 2018; Davies et al., 2017), much less is known about how to use this amendment effectively for sagebrush (*Artemisia tridentata*) itself.

For a seed technology to be effective, the right amendment must be matched with the right delivery mechanism. A common delivery mechanism is a coating, in which seeds are encased in a thin layer of active ingredients and binders, usually applied by spraying the mixture of ingredients onto seeds that are rotating in a drum. A coating can range from a very thin “film coating” to a thicker “encrusting” or a much thicker “pellet” coating (Pedrini et al., 2020, 2016). Another delivery mechanism is an extruded pellet, in which seeds are mixed into a dough containing active ingredients and binders and then extruded to form cylindrical pellets or pods of other shapes (Madsen et al., 2016). A third delivery mechanism is to coat seeds in the active ingredient in the field -- for example, by simultaneously placing seeds and the amendment into a furrow. Each of these approaches involves potential tradeoffs in terms of the success of delivery of the amendment at adequate dosage, the seed's ability to germinate and emerge, and the ease of delivery in the field. Previous research has shown that the delivery mechanism can severely impact seed emergence, particularly with sagebrush seed (Donovan et al., 2024; Eshleman and Riginos, 2023).

Here we build on previous work with the goal of refining an appropriate delivery mechanism for herbicide protection for both sagebrush and perennial grass species. We conducted two field trials, each testing a different suite of candidate seed technologies at sites across sagebrush steppe landscape which were invaded by annual grasses. We specifically asked (1) Are there differences in emergence, survival, and/or seedling size between seed treatments (bare seed, activated carbon single-seed coating, carbon banding, and multi-seed Herbicide Protection Pellet (HPP) in the presence of herbicide? (2) Using the same evaluation metrics, do any of the tested seed treatments inhibit seedling performance in the absence of herbicide? (3) Do different seedbed preparations (shallow pre-herbicide furrow vs deep post-herbicide furrow) result in additive seeding outcomes when combined with the above seed treatments?

Methods

We completed two field experiments testing herbicide protection seed technologies. In both, we used a single seedlot of each species: "Anatone" bluebunch wheatgrass (*Pseudorogeneria spicata)*, "Turkey Lake" bottlebrush Squirreltail (*Elymus elymoides)*, and Wyoming big sagebrush (*Artemisia tridentata var wyomingensis)* from wild collections. Seed viability was tested using tetrazolium stain test (Oregon State Seed Lab, Corvallis, OR) prior to the installation of the experiment to ensure an accurate seeding rate to achieve target numbers of viable seed. Both bare seed and all seed technologies were seeded at a rate of 572 viable seeds per m^2.

These two experiments tested activated herbicide protection pellets (HPPs), carbon seed coatings and carbon banding (loose carbon applied in the field on top of seeds) to protect seeds from the effects of the pre-emergent herbicide (Table 1). All seed coatings were produced by Germains Seed Technology (Gilroy, CA) using proprietary formulas. Exact rates of activated carbon in each coating prototype are currently protected as intellectual property. We produced extruded pellet HPPs in-house following the "small" (16 mm long, 8 mm wide) pellet specifications described by Baughman et al. (2023). A different type of activated carbon was used in Field Experiment 1 (2021-22) and Field Experiment 2 (2022-23) based on interim lab evidence that suggested better results from the carbon used in Field Experiment 2. The specific details of the activated carbon types are protected as intellectual property.

Field Experiment #1 (2021-2022) was installed between September 1-18, 2021 at sites in 5 states: ID, NV, OR, UT and WY (Figure 1). For this experiment we used a split-plot design to test three different herbicide protection seed technologies and bare seed (Figure 2). These were deployed in paired (blocked) whole plots that were sprayed with the pre-emergent herbicide imazapic at 10 oz/acre or unsprayed (n = 7 plot pairs). The seed technologies were seeded into three 2-3 cm deep furrows in each of the 100 x 50 cm subplots (one per seed technology treatment per species) within each whole plot.

Table 1. Details of the seed treatments used in Field Experiment 1 (21-22) and 2 (22-23) for all three tested species. Superscripts indicate different types of activated carbons. Specific details are protected as intellectual property.

Treatment	*Elymus elymoides*	*Pseudorogeneria spicata*	*Artemisia tridentata var wyomingensis*
Bare	all years	all years	all years
HP coating (high)[a]	21-22	21-22	21-22
HP coating (low)[a]	21-22	21-22	21-22
HPP[a]	21-22	21-22	n/a
HP carbon band[a]	n/a	n/a	21-22
HP coating[b]	22-23	22-23	22-23
HP carbon band[b]	22-23	22-23	22-23

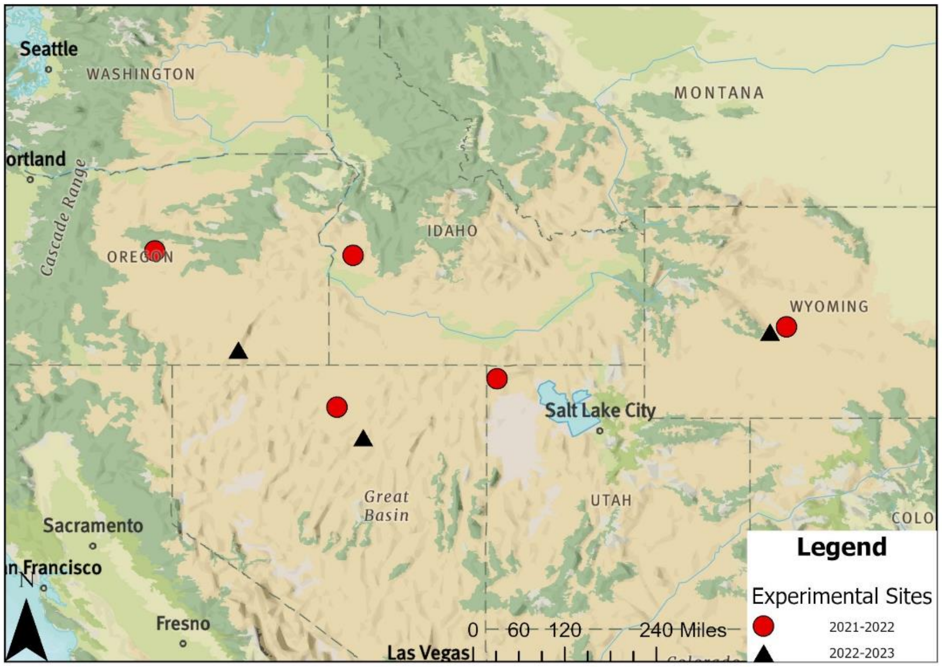

Figure 1. Map of experimental site locations for 2021-2022 (red dot) and 2022-2023 (black triangle).

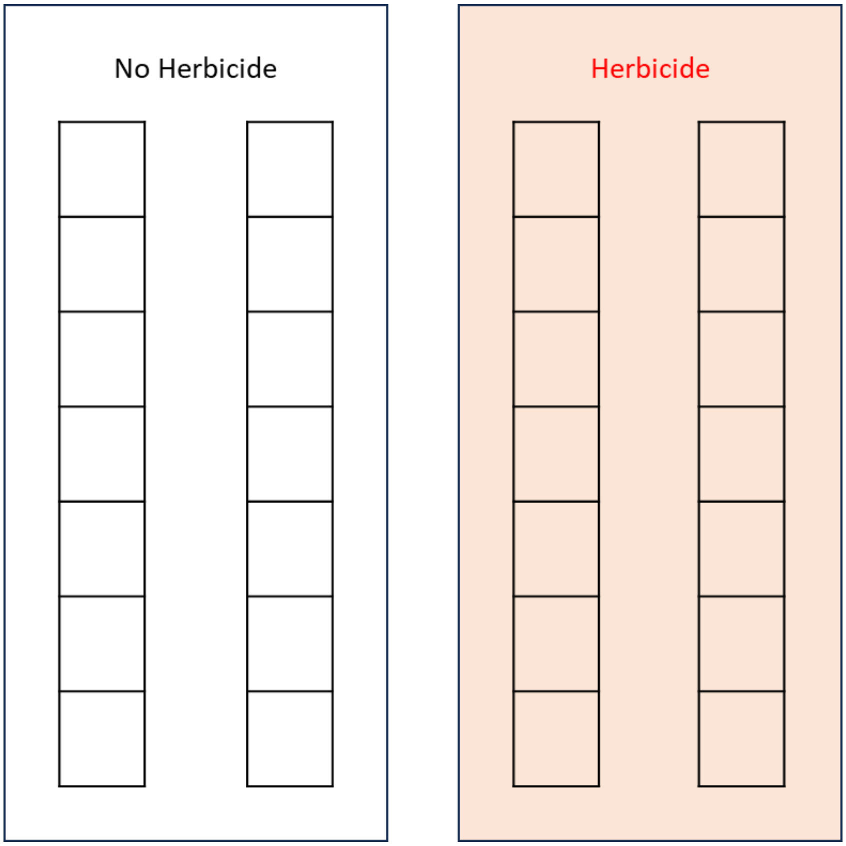

Figure 2. Experimental design of Field Experiment #1. One block (two paired whole plots) is shown as a diagram. Each seed treatment-species combination is replicated twice per block, once on the sprayed side and once on the unsprayed side.

Field experiment #2 (2022-2023) was installed between September 6-15, 2022 at sites in 3 states: NV, OR and WY (Figure 1). For this experiment, we also used a split plot design to test 9 different combinations of seed treatment and species (Table 1). The treatments were deployed in whole plots (n=7) with each half receiving either 2-3 cm deep (shallow furrows) or 7 cm deep furrows (deep furrows) applied to each 100 cm x 100 cm subplot (Figure 3). Deep furrows applied after herbicide are designed to “side-sweep” herbicide-treated litter and soil into a ridge to the side of the furrow, and provide a bed of firm soil in which little or no herbicide will be encountered by germinating seeds. In this experiment all whole plots were sprayed with the pre-emergent herbicide imazapic at 8 oz/acre. There were no unsprayed areas in this experiment.

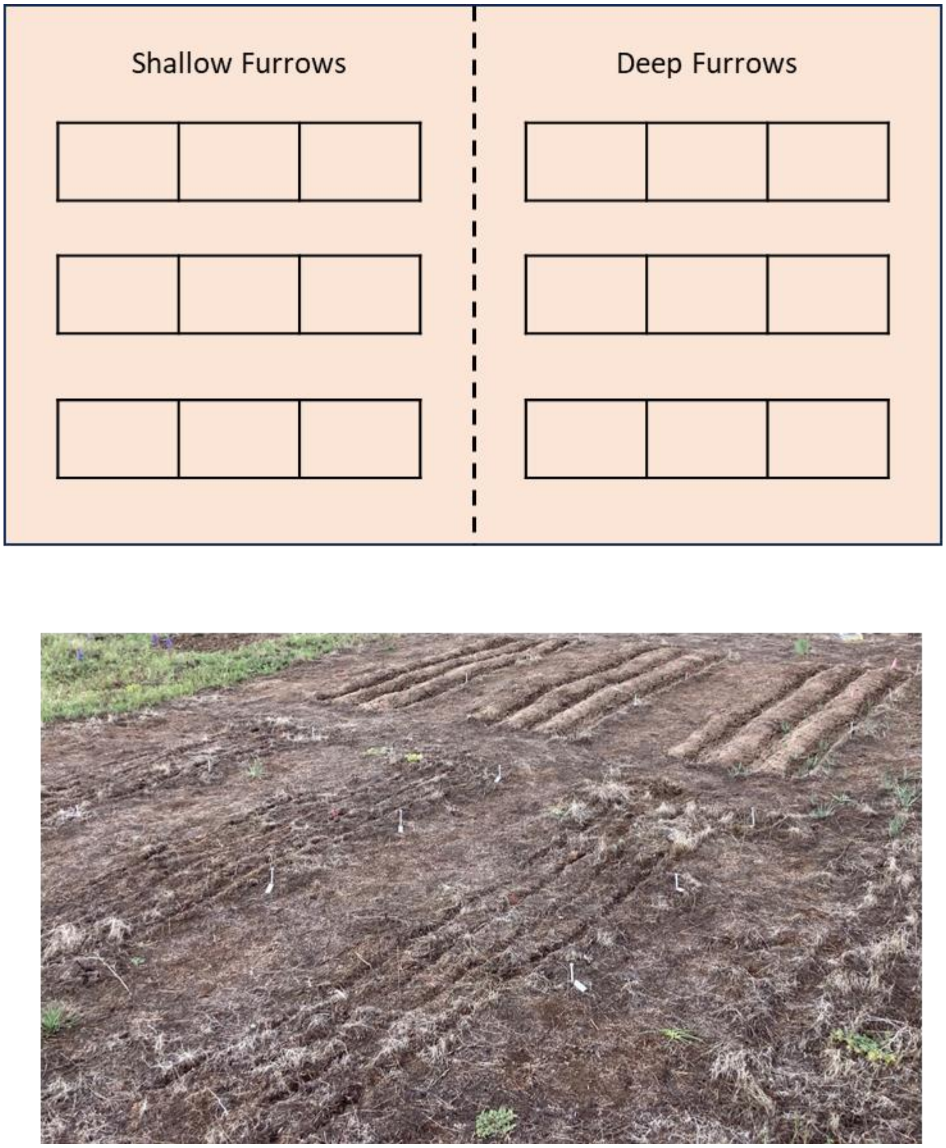

Figure 3. Experimental design of Field Experiment #2. One whole plot is shown as a diagram and in photo. In the photo, taken the spring after herbicide application, the shallow furrows are seen on the left in the foreground, and the deep furrows are in the background. Seven whole plots were placed in each of three sites.

Both field experiments were monitored twice in the summer following their installation: one early season monitoring (April-May, depending on site location) and one late season monitoring (May-June, depending on site location). Response variables for both experiments were early-season seedling count (as a percent of viable seed sown), late-season seedling count (as a percent of viable seed sown), survival of early emergents (percent of early season seedlings that survived to the late season), total survival (percent of all plants to emerge that survived to late season), late-season seedling leaf count, late-season seedling droop height (natural standing height of the plant), and foliar cover (%) and density (count) of invasive annual grasses (IAGs) and invasive annual forbs (IAFs).

Analysis

Field Experiment #1 data consisting of late spring seedling count (%), seedling height (mm) and seedling leaf count (number) and were analyzed separately for each species in a standard least squares mixed model performed in JMP (SAS Institute, Carey, NC) with seed treatment (high-rate coating, low-rate coating, HPP, bare seed), herbicide (present, absent), and field site (five sites) as main effects, with all interactions, and whole plot (seven; nested within each site) as random effects. Seedling leaf count was transformed using a natural log transformation to improve normality of model residuals, and all other responses were left untransformed. Significant main or interactive effects were evaluated with post-hoc Tukey's honest significant difference tests. In all tests in the laboratory and field, results were considered significant at $P \leq 0.05$.

For Field Experiment #2, fully factorial standard least squares mixed models were performed in JMP (SAS Institute, Carey, NC) for each species (bluebunch wheatgrass, bottlebrush squirreltail, Wyoming sagebrush), with each model having seed treatment (bare seed vs carbon coated vs loose carbon), seeding method (shallow furrow and seeding before herbicide vs deep furrow and seeding after herbicide), and site (OR, NV, WY) as main effects, with all interactions included, and whole plot (1-7, nested within site) as random effect. Too few seedlings of Wyoming sagebrush were observed in WY and OR for the model to run, so only NV data were retained, and the site factor was dropped from the model for that species. Several responses were transformed prior to analysis in order to improve normality of model residuals. Cube root transformation was applied to early seedling count, late seedling count, percent cover of IAG and IAF, and density of IAG and IAF. A separate model was used to determine whether key experimental factors (site, delivery method) modified the reduction in the foliar cover of these plants before vs after herbicide application. The response variable in this model was percent change in foliar cover for IAG and IAF before (fall 2022) vs after (late spring 2023), with site and delivery method, and their interaction, as main effects, and plot (nested within site) as a random effect. For all models, significant main or interactive effects were evaluated with post-hoc Tukey's honest significant difference tests. In all tests, results were considered significant at $P \leq 0.05$.

Results

Field Experiment #1

There were not enough Wyoming big sagebrush seedlings across all sites to perform any analysis so the results presented here are only for the seeded grasses.

There were differences in seedling count among the seed treatments that varied by species as well as site (Figure 4). For bottlebrush squirreltail, late season seedling count of both high- and low-rate coating did not differ from bare seed at any of the five sites, and HPP had equal or lower seedling count than other treatments depending upon the site (site*seed treatment interaction, $F_{[12,210]} = 3$, $P = 0.001$). Specifically, HPP had lower bottlebrush squirreltail seedling count than all other treatments in NV, lower than bare seed in OR and UT, lower than low-rate coating in OR, and did not differ from any treatment in ID and WY. This pattern of differences among seed treatments was observed in both the presence and absence of herbicide (all interactions involving seed treatment and herbicide: $F_{[3\text{-}12,30\text{-}120]} < 2.2$, $P > 0.085$). Seedling count for bottlebrush squirreltail was 20 - 81% lower in the presence than the absence of herbicide, regardless of seed treatment, with the largest decrease in WY (81%) and ID (63%) and the least in UT (22%) and OR (20%) (site*herbicide interaction; $F_{[4,210]} = 7.2$, $P < 0.001$; not shown).

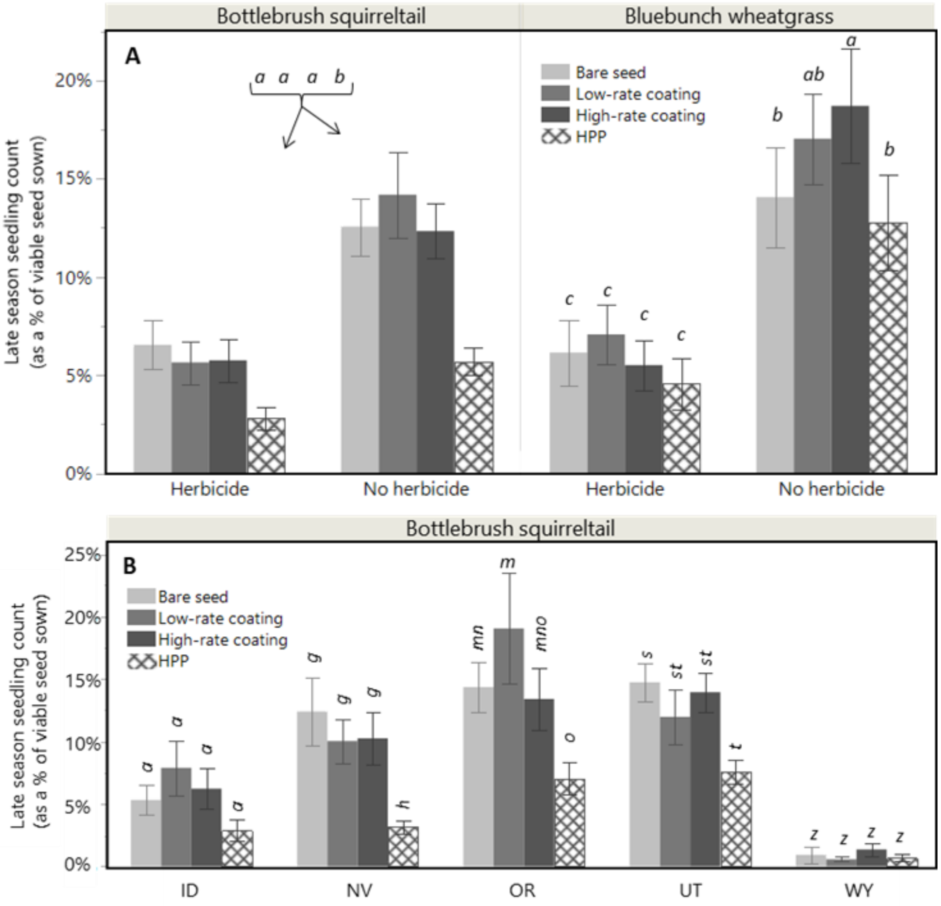

Figure 4. Effects of seed treatment (different shades of bars) on late season seedling count (as a percent of viable seed sown) in Field Experiment 1 by species for bottlebrush squirreltail and bluebunch wheatgrass and herbicide treatment (panel A) as well as by field site (panel B) for bottlebrush squirreltail. For each species in panel A and for each site in panel B, bars not sharing any letters are significantly different as determined by post-hoc Tukey's HSD tests at the p = 0.05 level. In panel A for bottlebrush squirreltail, there was no herbicide*seed treatment interaction, so Tukey lettering is only given for the main effect of seed treatment. Error bars are one standard error of the mean. In panel B, the original lettering from the Tukey HSD tests on the site*seed treatment interaction has been simplified for interpretability, and therefore letters should only be compared among treatments within a site. There was no treatment by site interaction for bluebunch wheatgrass, so it is omitted from panel B.

For bluebunch wheatgrass, differences among seed treatments varied by herbicide presence (Figure 4). In the presence of herbicide, there were no differences in seedling count among seed treatments (herbicide*seed treatment interaction, $F_{[3,167]} = 2.9$, $P = 0.036$), and all seed treatments had 22 - 74% lower seedling count compared to the absence of herbicide, with the greatest reduction in WY (74%) and NV (60%), and the least in OR (22%) (site*herbicide interaction; $F_{[2,167]} = 19,2$, $P < 0.001$; not shown). In the absence of herbicide, the seedling count of the high-rate coating was 38-50% greater than bare seed and HPP, while the low-rate coating had an intermediate seedling count and did not differ from any other treatment (herbicide*seed treatment interaction, above).

Seedling size (height and leaf count) varied primarily by species, site, and herbicide treatment. There were only two minor instances of differences in size that were related to seed treatment: seedling height of bottlebrush squirreltail and leaf count of bluebunch wheatgrass. For squirreltail, seedling height did not differ among seed treatments in either the presence or absence of herbicide, but herbicide was associated with a decrease in height for bare seed, with no such effect of herbicide for any carbon treatment (treatment*herbicide interaction $F_{[3,190]} = 4.0$, $P = 0.009$). There was no effect of seed treatment on squirreltail leaf count, and herbicide reduced leaf count by 22% in WY, increased it in by 75% in NV and 36% in UT, and had no effect in ID and OR (state*herbicide interaction, $F_{[4,193]} = 16.7$, $P <0.001$). For bluebunch wheatgrass, there was no effect of seed treatment on seedling height, and the presence of herbicide reduced seedling height at only one site, WY, by 67% (state*herbicide interaction, $F_{[3,145]} = 8.06$, $P < 0.001$). Bluebunch wheatgrass leaf count varied by seed treatment only in WY, and only in the presence of herbicide, with HPP having 28 – 30% leafier seedlings than all other treatments including bare seed (site*treatment*herbicide interaction, $F_{[9,145]} = 2.09$, $P = 0.034$; not shown). Bluebunch wheatgrass leaf count was higher in the presence than the absence of herbicide in OR (by 46%) and NV (by 35%), but 12% lower in the presence of herbicide in WY and unaffected by herbicide in ID (state*herbicide interaction, $F_{[3,146]} = 13.8$, $P < 0.001$).

Herbicide applications significantly reduced the density of invasive annuals grasses at all sites and invasive annual forbs at all but the WY site (Fig. S3). Reductions in the density of living invasive annual grass by the late spring was 91 - 98% for all sites but UT, where it was 59% (herbicide*site interaction, $F_{[4,760]} = 64.5$, $P < 0.001$). Reductions in invasive annual forbs were 90 - 99% in all sites but WY, where there was no reduction associated with herbicide application and forb densities were lower than at any other site (herbicide*site interaction, $F_{[4,760]} = 83.8$, $P < 0.001$).

Field Experiment #2

Differences in field seeding outcomes among seed treatments

Differences in seedling counts among seed treatments were present and varied by species and site (Figure 5). Carbon coating often (but not always) had higher seedling counts than bare seed for all three species. Compared to bare seed, carbon coating was associated with 100% higher early seedling count (0.9% vs 1.8%) for bottlebrush squirreltail regardless of sites (seed treatment main effect: $F_{2,90} = 8.3$, $P < 0.001$), 116% higher late count (0.6% vs 1.3%) for sagebrush in NV (site*seed treatment interaction: $F_{2,90} = 4.43$, $P = 0.003$), and 260-860% higher early and late counts of bluebunch wheatgrass in OR and NV (site*seed treatment interaction: early, $F_{4,90} = 5.86$, $P < 0.001$; late, $F_{4,90} = 5.78$, $P < 0.001$).

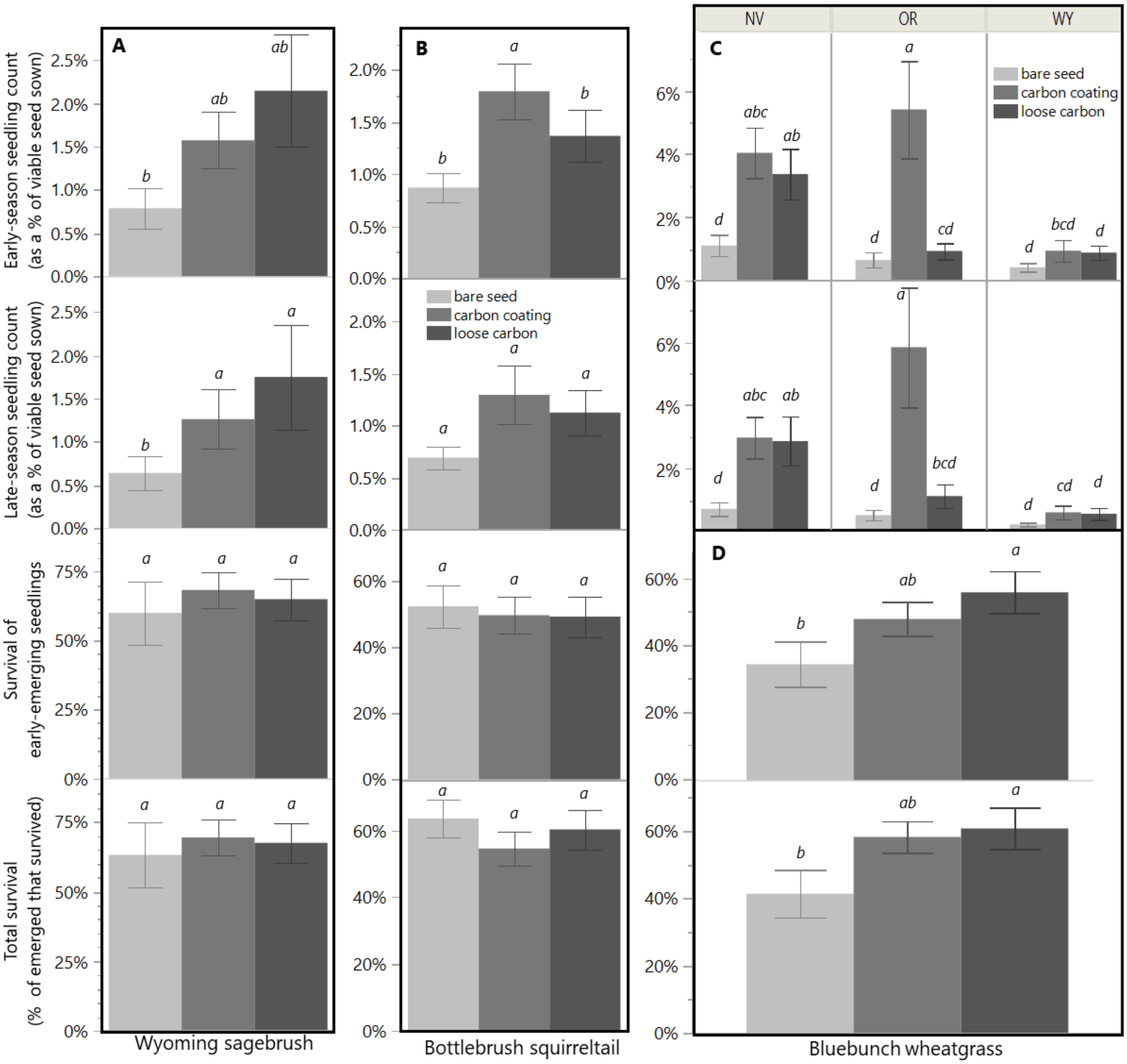

Figure 5. Seeding outcomes from Field Experiment 2 for Wyoming sagebrush (A), bottlebrush squirreltail (B), and bluebunch wheatgrass (C and D), from top to bottom: early and late season seedling count (as a percent of the viable seed sown), late-season survival of seedling emerged in the early season, and total plants surviving in the late season as a percent of the total to emerge at any time. Error bars are 1 standard error of untransformed data. For each combination of species and response variable, bars that do not share the same letter are significantly different at the P = 0.05 level, as determined by post-hoc Tukey HSD tests.

The differences among treatment for bluebunch were driven primarily by the deep furrow treatment, with fewer differences in the shallow furrow treatment (Figure 6, treatment*delivery interaction: early, $F_{2,90}$ = 4.51, P = 0.014; late, $F_{2,90}$ = 4.60, P = 0.013). The loose carbon treatment was associated with a 142% - 275% higher seedling count than bare seed for bluebunch wheatgrass in both the early and late season in NV only (site*treatment interactions for bluebunch, above), and a 250% higher seedling count for late-season Wyoming sagebrush (0.6% vs 1.7%; seed treatment main effect, $F_{2,30}$ = 3.92, P = 0.036).

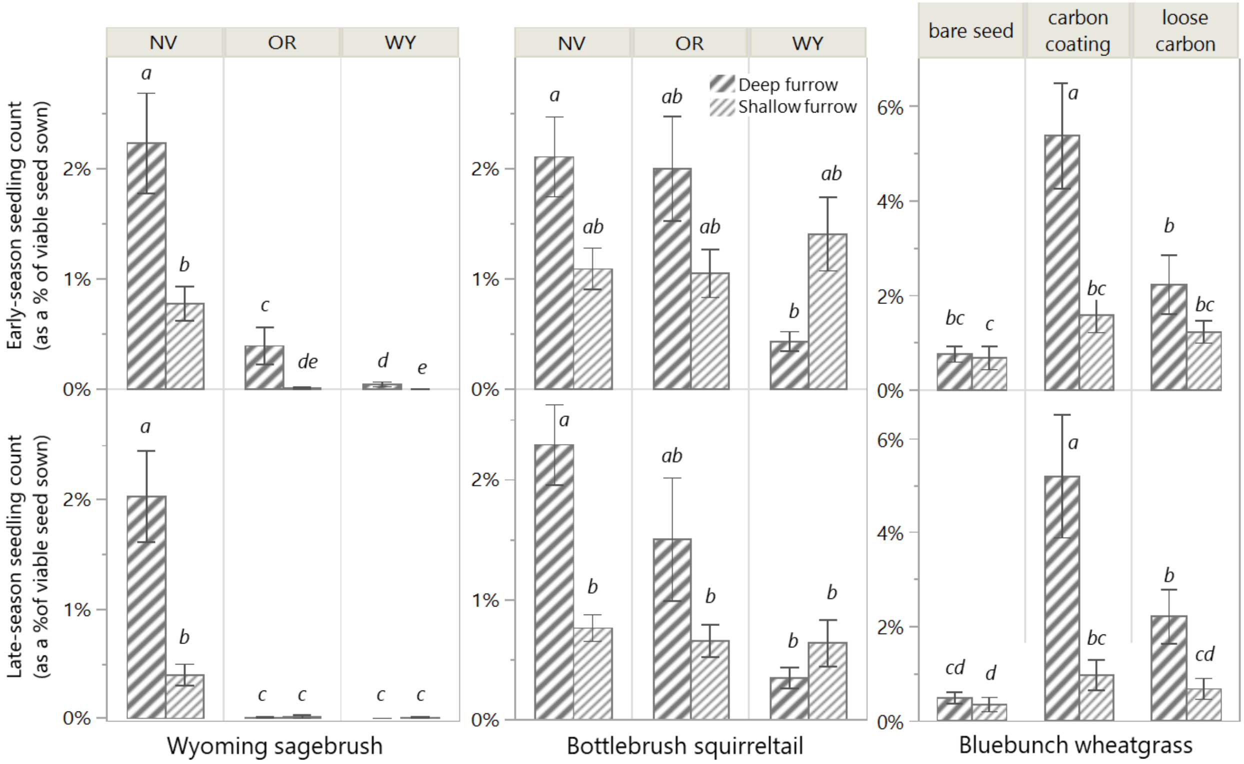

Figure 6. Field trial effect of seed delivery treatment – shallow furrowing and seeding before herbicide application (light striped bars) versus deep furrowing and seeding after herbicide application (dark stripes) – for early and late-season seedling count (as a percent of viable seed sown), by species. Note that bluebunch wheatgrass is presented by seed treatment, whereas the other species are by site. Error bars are 1 standard error of untransformed data. For each combination of species and response variable, bars that do not share the same letter are significantly different at the P = 0.05 level, as determined by post-hoc Tukey HSD tests.

Survival also varied by seed treatment, but only for bluebunch wheatgrass (Figure 5). The loose carbon treatment was associated with 60% higher survival of early emergents and 45% higher total survival of bluebunch wheatgrass than bare seed, regardless of site (treatment main effects: $F_{2,76\text{-}78}$ = 3.44 – 4.1, P = 0.02 – 0.037). No other differences in survival were observed among seed treatments for any species. Late-season seedling leaf count and seedling height were both unaffected by seed treatment for any species, regardless of site (all main and interactive effects of seed treatment P > 0.056).

Effects of furrowing on field seeding outcomes

The effect of seed delivery method (shallow furrow before herbicide application vs deep furrow after herbicide application) was prominent but varied somewhat by species, seed treatment, and site (Fig. 4). In all cases where differences in outcomes were observed, deep furrows were associated with better seeding outcomes than shallow furrows. Deep furrows had 242% higher seedling count than shallow furrows for Wyoming sagebrush in the early season regardless of site and 407% higher count in the late season only in NV (early: delivery main effect, $F_{1,90} = 27.8$, $P < 0.001$; late: site*delivery interaction, $F_{2,90} = 25.7$, $P < 0.001$), 199% higher count for late-season bottlebrush squirreltail only in NV (site*delivery interaction, $F_{2,90} = 5.63$, $P = 0.005$), and 81-436% higher early count for carbon coating and late count for both carbon treatments (but never for bare seed) for bluebunch wheatgrass regardless of site (seed treatment*delivery interactions,: early, $F_{2,90} = 4.5$, $P = 0.014$; late, F2,90 = 4.6, P = 0.013). Deep furrows were also associated with 70% higher survival of early emerged seedlings for one species, bottlebrush squirreltail, regardless of site (delivery main effect, $F_{1,77} = 14.3$, $P < 0.001$; not shown). Deep furrows were occasionally associated with larger seedlings than shallow furrows, with 26% higher leaf counts for Wyoming sagebrush in NV (site*delivery interaction, $F_{1,18} = 12.4$, $P = 0.002$), and 16% greater seedling height for bluebunch wheatgrass regardless of site (delivery main effect, $F_{1,58} = 5.25$, $P = 0.026$; not shown).

Effect of herbicide on invasive annual plants in the field

Assessment of the percent change in foliar cover before vs after herbicide application showed 92-99% reductions in invasive annual grass cover and 44-99% reductions in invasive annual forbs, with no significant variation by delivery method, and a slightly greater reduction in NV and WY (99 – 99.7%) than in OR (93.5%) of invasive annual grasses (site main effect, $F_{2,17} = 5.74$, $P = 0.012$). Direct comparison of the cover and density of these plants in the presence of herbicide in the late season showed that deep furrows were sometimes associated with a slightly higher density and cover than shallow furrows of invasive annual grasses (in OR only, site*delivery interaction, $F_{2,366} = 7.21$, $P = 0.001$; not shown) and invasive annual forbs (in NV only, site*delivery interaction, $F_{2,366} = 4.87$, $P = 0.008$; not shown). Though statistically significant, these difference among furrow types was slight. That is, there were 27 plants/m^2 in the deep furrows vs 16 plants/m^2 in shallow furrows for invasive annual grasses in OR, with previously-reported values of IAG densities at similar sites in the absence of herbicide ranging from 250 – 6800 plant/m^2 (Baughman et al. 2023). Similarly, the difference between furrow types for invasive annual forbs in NV was less than 0.2%, with all cover values for both furrow types below 0.8% cover before and after herbicide.

Discussion

Recent research has shown reason for optimism about the potential for herbicide protection seed enhancement technologies to eventually be a scalable and effective tool for improving native plant restoration success in the face of severe invasions of exotic annual plants, despite occasionally low or mixed efficacy of the various seed treatment prototypes being tested (Baughman et al., 2024; Duquette et al., 2024; Munro et al., 2023). The two field trials that we ran also had mixed results, but occurred in two very different years, weather-wise. In field trial 1 all sites were noted as experiencing moderate, severe, extreme or exceptional drought in every month from November 2021 to May 2022 according to the US

Drought Monitor for the Western United States (droughtmonitor.unl.edu). In contrast, field trial 2 occurred during a cool, wet winter and spring at all sites. These disparate weather conditions likely led, at least in part, to the differences we saw in seedling success.

In the field trial 1, we saw no direct evidence of any of the seed treatments leading to improved seeding outcomes (number or size of seedlings) compared to bare seed in the presence of herbicide. The HPP treatment was associated with seedling counts that were often lower than bare seed and both low-rate and high-rate coatings. Similar side-effects of HPPs have been observed in other recent studies in our region that included both HPPs and coatings (Duquette et al., 2024). Variability in effectiveness has been common throughout the history of HPP evaluation, and we concur with Clenet et al. (2020) in suspecting that this is likely related to how post-seeding weather does or does not aid in breaking down the durable pellets to facilitate the emergence of germinating seeds.

We were also able to make improvements to the HP coatings between field trial 1 and 2. A potentially important change was using a more effective activated carbon in field trial 2. That along with the favorably wet and cool winter and spring weather led to notable levels of emergence and establishment for all three species in at least one site, lending confidence that the effects of the seed treatments we observed were representative of average or above-average conditions for restoration success. In field trial 2, effects of seed treatment were observed, and the HP coating performed better than the loose carbon. We observed several positive and no negative outcomes of HP seed treatments, with the HP coating occasionally (but not always) improving field seeding outcomes compared to bare seed, while loose carbon had similar but even less frequent effects. There was never lower seedling count, survival, or size for a carbon seed treatment than for bare seed for a given species. This, along with previous lab trials (Baughman et al., 2024, Kildisheva et al. In Prep), indicates that the seed treatments were not inhibiting seedling development. Prior research has occasionally found evidence of such "side-effects" for some prototypes (Baughman et al., 2023), though others have occasionally found beneficial effects of carbon-containing seed enhancement prototypes for some but not all species in the absence of herbicide (Munro et al., 2023). The current results, as well as those of other recent assessments of similar prototypes (Baughman et al., 2024; Terry et al., 2021, Kildisheva et al., In Prep) suggest that current prototypes are providing some herbicide protection without trade-offs or costs to seed and seedling development, and this is an important improvement in the development of this technology.

The deep furrow treatment often improved seeding outcomes and never lowered them, compared to shallower furrows. We also observed some evidence for bluebunch wheatgrass that the positive effects associated with carbon seed treatments were greater in deep than shallow furrows. Deep furrows applied after herbicide are designed to "side-sweep" herbicide-treated litter and soil into a ridge to the side of the furrow and provide a bed of firm soil in which little or no herbicide will be encountered by germinating seeds (Terry et al., 2021). We observed the synergistic effect of this combination of deep furrowing and herbicide protection, which corroborates other research using even deeper furrows and different HP coating prototypes performed by Terry et al. (2021), who also suggested this was the result of reduced (but not eliminated) exposure to herbicide in the furrows.

If deep furrows do have less herbicide exposure, a potential tradeoff could be that seed of invasive annual weeds that reside in the furrows could escape the control of the herbicide, and we did observe that deep furrow subplots contained statistically significantly higher first-year cover of invasive annual plants, but the magnitude of differences was small. In a similar study, Terry et al. (2021) did not see evidence of herbicide escape by weeds in deep furrows, but their furrows, also made by hand, were deeper and involved a more complete "side-sweep" of treated soil and litter than this the methods assessed in our work. Certainly, less-precise furrowing made mechanically at larger scales instead of by hand in small plots could further increase weed escape, and this should be closely evaluated during at-scale trials.

Although there are still opportunities to refine and potentially further improve the efficacy of herbicide protection seed coatings, the prototypes tested in field trial 2 are nearing what could be considered a marketable product. Our hand-seeded small plots are not directly comparable to mechanized seeding, so this assessment of the treatments' potential to meet real-world levels of success is clearly speculative. Nonetheless, we believe the current results demonstrate that HP coatings could possibly attain meaningful levels of success in the presence of herbicide when bare seed did not, under field conditions conducive to native plant establishment. Additional field trials using standard, at-scale methods, rates, and timelines are needed to confirm the true potential for these and other seed technologies to improve restoration success.

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