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PRACTICE AND TECHNICAL ARTICLE

Delayed seeding and nutrient amendment seed enhancement technology: potential to improve sagebrush establishment?

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The sagebrush steppe provides vital habitat across the western United States, but its range is declining. Wyoming big sagebrush (*Artemesia tridentata* Nutt. spp. wyomingensis Beetle & Young) is difficult to establish on restoration and reclamation sites, with low seedling survival due to drought, competition, and limited available nutrients. Incorporating seeds into seed enhancement technologies (SET) has potential to overcome barriers to establishment. Here, we tested the effects of a high phosphorus nutrient amendment using three application techniques (dust over bare seed, dispersed seed pellets, and clustered seed pellets) versus bare seed on sagebrush establishment on 5-, 1-, and 0-year-old mine reclamation sites. Regardless of seed technology, emergence was highest in the year 5 site, but survival was lowest, likely due to competition from established plants. Survival and growth were highest in the year 0 site, but emergence was lowest, likely due to poor soil stability. The year 1 site, where soil was stable and competition was low, produced the greatest number of surviving seedlings. Across all times since reclamation, pellets reduced emergence compared to dust and bare seed treatments. There was no clear evidence of any benefit of the SETs. These results highlight the challenges of delivering nutrient amendments in SETs and unresolved questions about the effectiveness of the amendment tested here. Seeding sagebrush 1 year after reclamation may be an additional way to improve restoration and reclamation success for this foundation species.

Key words: competition, emergence, nutrient amendments, reclamation

Implications for Practice

- The optimal window to seed sagebrush may be 1 year after reclamation when sparse vegetation confers site stability but low competition. Alternative seeding methods may be needed to do this, which bears added costs and logistics.
- The nutrient amendment seed technology tested here had questionable potential to improve sagebrush seedling survival and restoration/reclamation success, and there are likely to be continued challenges to delivering amendments without inhibiting seedling emergence.
- Any approach that leads to even modestly higher success rate for sagebrush establishment could result in greater net benefit relative to costs and could improve overall restoration outcomes.

Introduction

Seed-based restoration is the most widely used approach in dryland systems and the only approach that can be implemented at large scale, yet recruitment from seed is often low (Kildisheva et al. 2016). One potential way to improve restoration outcomes is by employing seed enhancement technologies (SETs), which can include priming, coating, pelleting, or agglomeration—with the aim of helping seeds and seedlings overcome barriers to establishment (Pedrini et al. 2020; Brown et al. 2021). While commonly used in agriculture, SETs are still an emerging tool in dryland restoration (Kildisheva et al. 2016; Madsen et al. 2016; Gornish et al. 2019).

In the sagebrush steppe of the western United States, restoration of the foundational big sagebrush (*Artemisia tridentata* Nutt.) on large scales has proven difficult (Knutson et al. 2014; Brabec et al. 2015). This vast landscape provides essential habitat for numerous sagebrush-dependent species, including the imperiled greater sage-grouse, migratory songbirds, and pronghorn (Remington et al. 2021). However, the landscape is threatened by exotic annual grass invasion, increasingly rapid and severe wildfire cycles, conifer encroachment, and land conversion (USFWS 2013). Consequently, nearly half of the original

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1.2 million km^2 extent of sage-grouse habitat has been lost (Schroeder et al. 2004).

Current sagebrush seeding practices include aerial or surface broadcasting and drill seeding in a single year along with other restoration species, but these methods often result in poor establishment (Knutson et al. 2014). Transplanting can improve establishment, but it can also be prohibitively expensive and is currently unfeasible at scale (Knutson et al. 2014; Pyke et al. 2020). As in most dryland systems, seed-based restoration is stymied by sporadic recruitment and establishment due to variable weather patterns (Maier et al. 2001; Brabec et al. 2015). Most sagebrush seeds are viable for no more than 6 months under field conditions, meaning that a single seeding event is unlikely to produce results after the first year (Young & Evans 1989). Once germinated, seedlings rely on rapid taproot growth, fueled by early-season moisture, to avoid succumbing to drought later in the season (Welch & Jacobson 1988). Most mortalities occur in the first year (Schlaepfer et al. 2014). Further, big sagebrush growth can be hindered by competition from herbaceous vegetation, particularly in nutrient deficient soils (Brabec et al. 2015; Kainrath et al. 2021).

In mine reclamations, poor soil quality and limited moisture limit access to resources for early-season growth. SETs could help to address these challenges. Laboratory trials have shown that a high phosphorus nutrient addition improved sagebrush seedling growth in reclaimed mine soils (Eshleman & Riginos 2023). We hypothesized that delivering the same amendment via SETs could also improve root growth in the field, helping seedlings to survive variable precipitation and competition with established plants.

In this study, we aimed to determine the effects of two concentrations of fertilizer using two application techniques at sites spanning three ages since reclamation that varied in existing plant cover. Specifically, we set out to answer the following questions: (1) do the tested SETs enhance sagebrush establishment, including both emergence and survival? (2) does age of reclamation affect sagebrush establishment? and (3) do the effects of SETs vary with the age of the reclamation?

Methods

Study Sites

The study was conducted at the McIntosh mine (42.360779, −107.838644) in central Wyoming. Conditions in the area adjacent to the mine are composed of typical sagebrush steppe communities with Wyoming big sagebrush and bunchgrasses including bluebunch wheatgrass (*Pseudoroegneria spicata*), western wheatgrass (*Pascopyrum smithii*), and Sandberg's wheatgrass (*Poa secunda*). The site was an abandoned uranium mine undergoing geomorphic reclamation. Prior to our experiment, hazards including highwalls and ponds were removed, and the landscapes were reshaped to mimic meandering streams and natural-grade slopes. This site was then covered in sandy loam soil to depths of 22.8–30.5 cm and seeded with a variety of grasses, forbs, and sagebrush using a soil pitter (see Table S1 for seed mix details).

Our experimental plots were set up in three north-facing sites of similar slope less than 1 km apart that had already been shaped and seeded (Fig. 1). We seeded plots with additional sagebrush seed in the fall of 2020 and monitored them through the spring and summer of 2021. The sites represented three ages since reclamation: year 0 (fresh reclamation), year 1, and year 5. Year 0 plots were completely barren at the time of seeding, with loose topsoil subject to water erosion and shifting during storms and snowmelt. The following spring, the year 0 site was largely colonized by non-native Russian thistle (*Salsola tragus*) and desert madwort (*Alyssum desertorum*). The year 1 site was also heavily colonized by the same species but had some small native bunchgrasses that had established from the reclamation seeding. The year 5 site was characterized by high cover and density of various native bunchgrasses, some small (pre-reproductive) sagebrush shrubs, a variety of forbs, low cover of non-native species, and more stable soils. Precipitation measured at a nearby weather station throughout the year-long experimental period from October 2020 through September 2021 totaled 16.2 cm, 33% less than the 30-year average of 24.1 cm (NOAA 2023). Spring was dry, with higher rainfall in June and July, followed by a relatively dry late summer.

Figure 1. McIntosh year 0 site after geomorphic reclamation and initial seeding with soil pitter, before plots were installed.

Experimental Design and Data Collection

Wyoming big sagebrush seed was collected near the McIntosh site in 2019. We tested seven treatments: bare seed (B); low-concentration dust (D1); high-concentration dust (D2); low-concentration dispersed pellets (P1); low-concentration clustered pellets (P1C); high-concentration dispersed pellets (P2); and high-concentration clustered pellets (P2C) (Fig. 2). Treatments were based on lab results showing that high concentrations of Root and Grow (Bonide Products LLC, Oriskany, NY, U.S.A.), a 4-10-3 (% N–P–K) fertilizer with indole-3-butyric acid growth hormone, were beneficial for sagebrush growth, and that pellets reduced emergence while dust did not (Eshleman & Riginos 2023). Despite these negative prior results with pellets, we included them in this field experiment as a potentially scalable SET and because we expected that pellets would break down

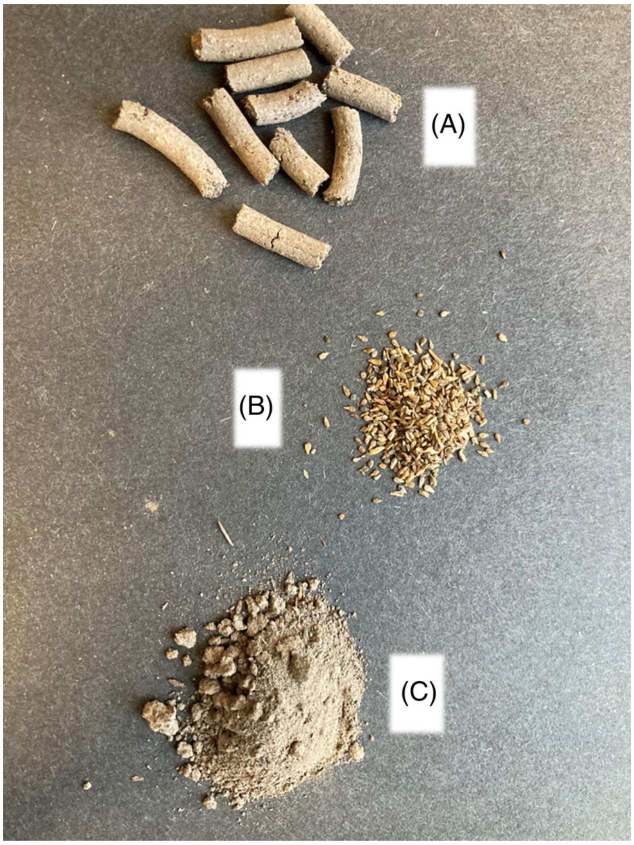

Figure 2. Extruded pellets (A), bare sagebrush seed (B), and dust treatment (C).

differently in the field than in the lab. We included the dust treatment, which contained the same ingredients as the pellets but in powder form, as a "proof of concept" treatment with the goal of delivering fertilizer without the confounding effects of reduced seedling emergence, while recognizing that a dust fertilizer application would not be a long-term scalable way to deliver fertilizer. Pellet and dust fertilizer treatments were each produced in low (5× label recommendation) and high (10× label recommendation) concentrations (see Supplement S1 for more details on SET production). Each pellet concentration was also delivered both as an even application of pellets throughout furrows (dispersed) and in approximately 10 separate piles of five pellets each (clusters). We tested the cluster application to determine whether a greater localized concentration of fertilizer had advantages over the smaller amount of fertilizer in an individual pellet.

At each site, we installed 14 replicates each of 7 treatments, in October 2020. Plots 0.5 m × 1 m in dimension were laid adjacent to each other along the contours of the slopes. We installed plots on northern slopes to take advantage of greater moisture throughout the growing season. Three furrows, 2.5 cm wide and 1.25–2 cm deep, were dug by hand lengthwise in each plot based on previous trials showing that sagebrush has more success growing from furrows than from broadcasted seed (Baughman et al. 2023). Treatments were applied randomly across plots at a rate of 200 pure live seeds (PLS) per plot, and all seed treatments were applied on the soil surface within the furrows. We did not include an unseeded control treatment and acknowledge the possibility that some seedlings might have recruited from other sources, particularly the reclamation seeding at our year 0 site. At the year 1 site, there was no source of sagebrush seed, and at the year 5 site, existing sagebrush were small and pre-reproductive. Seeding and monitoring within furrows also gave us confidence that the monitored seedlings had emerged from seeds that we put down as part of the experiment.

We monitored sites 10 times, approximately every 2 weeks from April through August and again in early November 2021. At each monitoring event, we marked newly emerged seedlings, counted surviving and dead existing seedlings, and recorded the height of three randomly selected seedlings from different sections of each plot; the latter values were averaged within plots before analysis. Because patterns strengthened over the study period, we analyzed cumulative emergence and survival data across the whole season and seedling counts and height measurements from the final sample period.

Analysis

All analyses were performed using R 4.3.1 and RStudio. We defined emergence as the percentage of seedlings that emerged out of the target PLS per plot and percent surviving as the percentage of seedlings alive at the end of the season out of the total emergences per plot. These metrics were analyzed with generalized linear models and binomial response distributions. Percent emergence and percent surviving were corrected for overdispersion using the quasi family of models (Crawley 2013). The total end-of-season seedling count was analyzed using a generalized linear model with a negative binomial response distribution. Height data from the final monitoring event was analyzed similarly, with height log transformed to achieve a normal distribution. Reclamation age, seed treatment, and their interactions were tested as fixed factors in all models. Significance letters shown in graphs were determined using the emmeans and multcomp packages with a Tukey adjustment for multiple comparisons. Because of the strong effects of reclamation age and over-representation of data from the year 1 site, we also analyzed the effect of seed technology on emergence, survival, final count, and seedling height separately for each reclamation age. All analyses used $\alpha = 0.05$ as the threshold level for significance.

Results

Percent Total Emergence

There was strong evidence that seed treatment affected emergence across all three reclamation ages ($p < 0.01$, $F = 45.34$, degrees of freedom [df] = 6). The pellet treatments averaged between 0.6 and 1.8% emergence across all reclamation ages while the dust and bare treatments averaged between 5.3 and 6.9% (Fig. 3A). Neither dust treatment differed strongly from bare seed. When analyzed by each individual reclamation age, there was strong evidence that seed treatment affected emergence for years 1 and 5 after reclamation ($p < 0.01$, $F = 27.45$, $df = 6$ and $p < 0.01$,

$F = 21.66$, $df = 6$, respectively) but not year 0 ($p = 0.17$, $F = 1.54$, $df = 6$). Reclamation age also strongly affected emergence ($p < 0.01$, $F = 118.41$, $df = 2$). Only 0.3% of seeds emerged at the year 0 reclamation, while the years 1 and 5 sites yielded 4.3 and 5.0% emergence, respectively (Fig. 4A). There was no evidence for an interaction between seed treatment and reclamation age on percent total emergence ($p = 0.5$, $F = 0.94$, $df = 12$).

Percent Surviving

Reclamation age strongly affected the percent of emerged seedlings surviving by the end of the growing season ($p < 0.01$, $F = 70.23$, $df = 2$). Year 0 reclamations showed the highest percent surviving at 79.39%, while year 5 plots showed the lowest at 16.13% (Fig. 4B). There was no evidence that seed treatment affected percent surviving for all ages ($p = 0.19$, $F = 1.47$, $df = 6$) (Fig. 3B), nor was there any interaction between seed treatment and reclamation age ($p = 0.70$, $F = 0.75$, $df = 12$). When we analyzed percent surviving separately by reclamation age, there was no evidence for an effect of seed treatment in years 0 and 1 (year 0: $p = 0.70$, $F = 0.63$, $df = 6$; year 1: $p = 0.54$, $F = 0.85$, $df = 6$), while year 5 showed weak evidence ($p = 0.09$, $F = 1.89$, $df = 6$). In year 5, high concentration clustered pellet treatments showed the highest percent surviving with 33.3%, while bare and dust treatments had the lowest, between 8.8 and 11.8%.

Final Seedling Counts

There was strong evidence that reclamation age affected the final count of seedlings ($p < 0.01$, $\chi^2 = 80.08$, $df = 2$). Year 1 plots had an average of 3.6 seedlings remaining at the end of the season, more than three times that of years 0 or 5 (0.4 and 1.1, respectively) (Fig. 4C). Seed treatment also strongly affected the final seedling count ($p < 0.01$, $\chi^2 = 63.93$, $df = 6$) (Fig. 3C). Pellet treatments had the fewest seedlings at the end of the season; the high concentration dispersed pellets averaged 0.4 seedlings per plot, while the low concentration dispersed pellets had 1.2 seedlings per plot (Fig. 3C). Bare and dust treatments had a higher final seedling count, with bare seed plots performing the best with an average 3.6 seedlings per plot. There was no interaction between reclamation age and seed treatment on final seedling counts ($p = 0.24$, $\chi^2 = 15.00$, $df = 12$). When sites were analyzed separately, there was no evidence that seed treatment affected final seedling counts in year 0, but strong

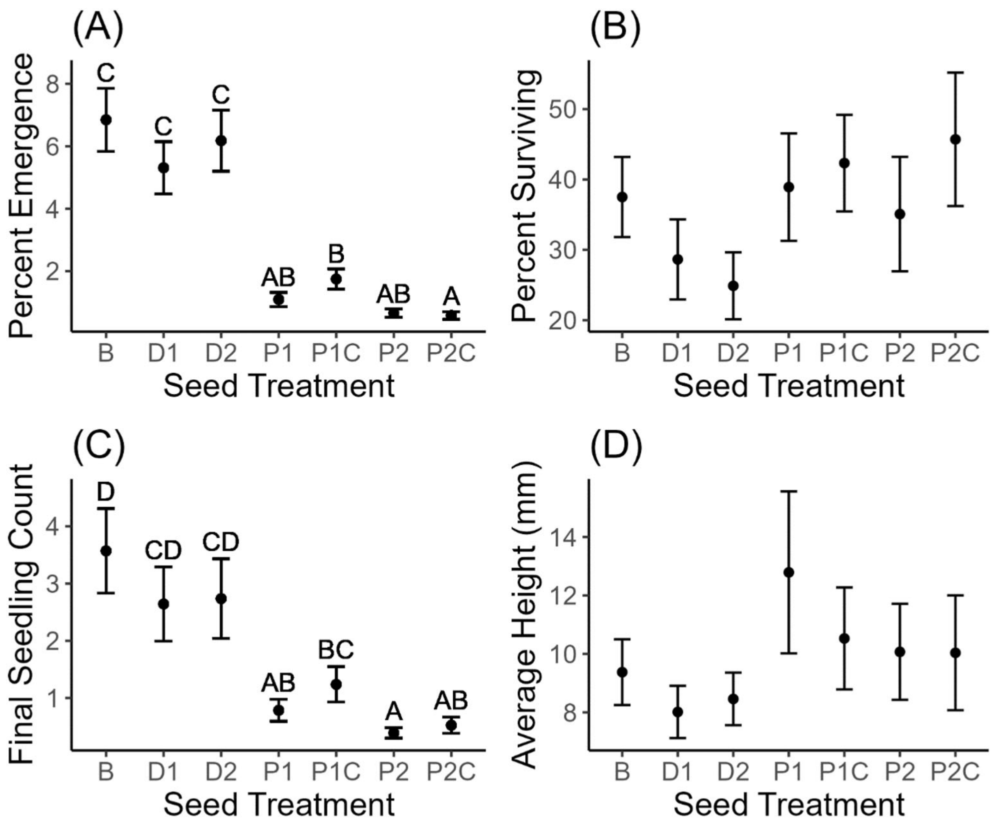

Figure 3. Mean emergence as a percentage of viable seed per plot (A), percent surviving of emerged seedlings per plot (B), final seedling count at the end of the season per plot (C), and seedling height as an average of three random seedlings in each plot (D), for all reclamation ages and seed treatments: bare seed (B), low concentration dust (D1), high concentration dust (D2), low concentration dispersed pellets (P1), low concentration clustered pellets (P1C), high concentration dispersed pellets (P2), and high concentration clustered pellets (P2C). Error bars represent ±1 SE. Significance letters for analyses with significant effects determined by the compact letter display of pairwise comparisons function. $n = 293$.

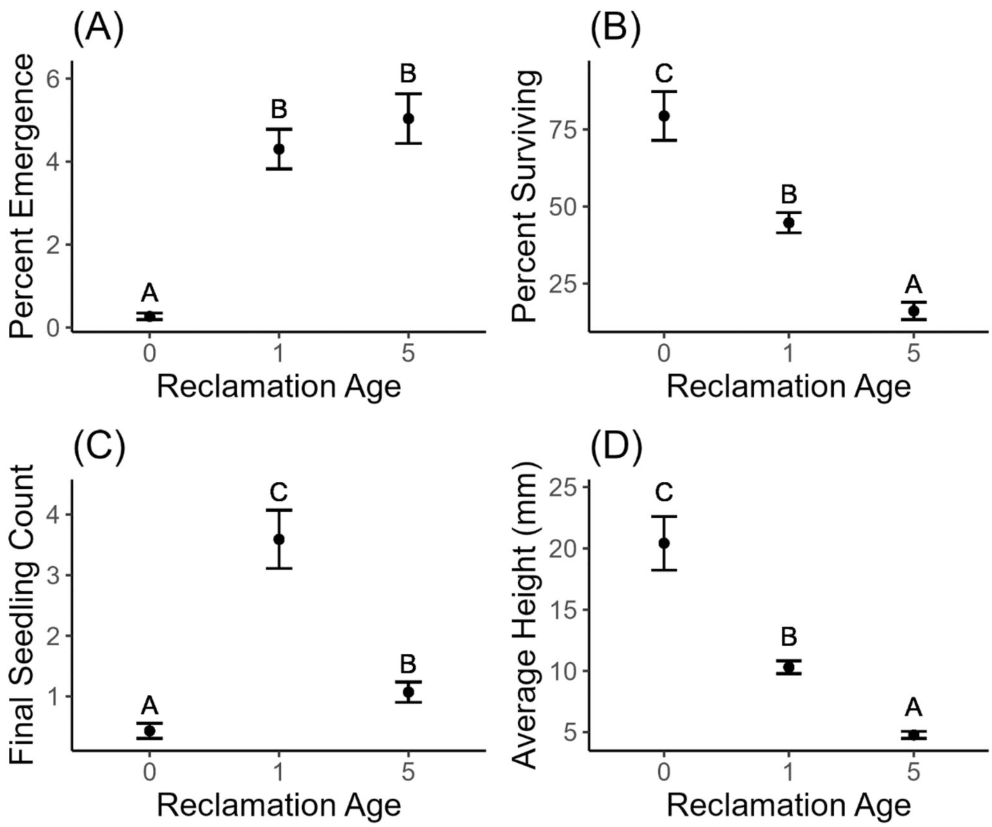

Figure 4. Mean emergence as a percentage of viable seed per plot (A), percent surviving of emerged seedlings per plot (B), final seedling count at the end of the season per plot (C), and average seedling height as an average of three random seedlings in each plot (D), for all three reclamation ages. Error bars represent ±1 SE. Significance letters within each panel determined by the compact letter display of pairwise comparisons function. $n = 293$.

evidence of an effect in years 1 and 5 (year 0: $p = 0.31$, $\chi^2 = 0.32$, $df = 6$; year 1: $p < 0.001$, $\chi^2 = 62.02$, $df = 6$; year 5: $p = 0.03$, $\chi^2 = 14.17$, $df = 6$) with patterns in each age consistent with the overall trends.

Seedling Height

There was strong evidence that reclamation age affected seedling height at the end of the season ($p < 0.01$, $F = 97.20$, $df = 2$). The year 0 site produced seedlings that were 4.25 times as tall as year 5 seedlings, with an average height of 20.4 mm. Year 5 plots had the shortest seedlings with an average of 4.8 mm (Fig. 4D). There was no evidence that seed treatment affected seedling height across all reclamation ages ($p = 0.69$, $F = 0.65$, $df = 6$) (Fig. 3D), that the interaction between reclamation age and seed treatment affected seedling height ($p = 0.94$, $F = 0.45$, $df = 12$), nor that seed treatment affected seedling height when each reclamation age was analyzed individually (year 0: $p = 0.77$, $F = 0.53$, $df = 6$; year 1: $p = 0.91$, $F = 0.35$, $df = 6$; year 5: $p = 0.82$, $F = 0.49$, $df = 6$).

Discussion

We found that time since mine reclamation consistently influenced sagebrush seedling establishment, and total establishment was substantially higher 1 year after reclamation compared to 0 and 5 years after reclamation. The SETs we tested had predominantly negative effects on seedling establishment, with limited evidence for some possible positive effects.

Packaging seeds in extruded pellets had high costs to emergence. Seedlings in the dust treatments showed no such cost, with similar emergence to bare seed, while pellet treatments had an average emergence 6.3 times lower than bare seed. This reduction in emergence could be due to insufficient sunlight reaching the seeds, compaction of the seeds, or a physical barrier preventing seedlings from sprouting, and are consistent with other trials using similar treatments (Baughman et al. 2023; Eshleman & Riginos 2023). Multiple results now indicate that extruded pellets do not effectively deliver amendments to sagebrush. Other SETs that could improve moisture retention around seeds, including seed conglomeration, may be more compatible with sagebrush restoration (Anderson et al. 2021).

There was weak statistical evidence, only at the year 5 site, for seed treatment to influence percent surviving. We believe these results are worth commenting upon due to our a priori hypothesis about the effect, the implications for the development of SETs, and because the magnitude of the increase in percent surviving was notable; 11.8% of seedlings survived in the bare seed treatment compared to 18.1 and 33.3% in the low and high concentration clustered pellet treatments. It is possible that the clustered pellets delivered a higher concentration of fertilizer per seedling than the other treatments and thereby enhanced belowground biomass and/or root depth, as previously observed in lab trials (Eshleman & Riginos 2023). Additionally, it is plausible that the benefits of the amendment manifested at the site where competition with neighboring plants was most intense. However, it is also possible that these results were simply a matter of chance. We found no effect of the dust treatment, which further suggests no benefit of the amendment. That said, the field dust application was much more diffusely applied (across more soil) compared to the lab trial or the pellets, and wind and other elements could also have removed some of the dust. Without further testing, it is difficult to say whether there is any benefit of the nutrient amendment. While we acknowledge the chance that there is no real benefit, the magnitude of increase in survival suggests that further study may be warranted.

There was strong evidence that reclamation age affected every metric we analyzed. The year 0 site had the lowest emergence, despite the additional seed from the reclamation process, but also had the highest percent surviving and largest seedlings. The poor emergence could be due to the harsh conditions at the site, which included bare, loose soil and lack of any cover or shade. Unstable soils may have buried seeds, which are unlikely to emerge when covered (Jacobson & Welch 1987). However, those seedlings that did emerge apparently benefited from a lack of competition and grew nearly twice as tall as the year 1 seedlings and more than four times as tall as the year 5 seedlings. In contrast, year 5 plots had the highest emergence, but lowest survival and seedling height. These plots had high perennial bunchgrass cover and some larger, established sagebrush and other shrubs. The year 1 site yielded intermediate results in terms of emergence, survival, and height—whereas final seedling count was much higher than years 0 or 5.

We suggest that these patterns represent tradeoffs between the competitive and facilitative effects of neighboring plants. Nurse plants can have beneficial effects on seedlings; even *Salsola tragus* can serve as a nurse plant for sagebrush by accumulating snow on mine reclamations (Meyer & Warren 2015). However, competition with seeded perennial grasses can also inhibit sagebrush establishment (Davies et al. 2013). It is possible that the intermediate amount of vegetation at the year 1 site struck a balance that benefited seedlings. This site had more stable soil than year 0 and was occupied by small, first year perennial bunchgrasses with plenty of bare soil between them. The heavy presence of *S. tragus* may have had a competitive effect on seedlings, leading to their small size relative year 0. However, the net effect of moderate emergence and moderate survival was much higher seedling numbers at the end of the season.

Adopting a 2-year seeding approach on mine reclamations, wherein grasses are seeded in year 0 and sagebrush in year 1 or both years, could improve sagebrush establishment rates. It is less clear whether nutrient amendments have potential to improve sagebrush establishment in the field, or whether it is possible to develop application techniques that do not inhibit sagebrush emergence. Both delayed seeding and use of SETs are likely to introduce additional costs and logistical challenges in the restoration process; these would have to be evaluated and weighed in terms of cost-effectiveness against or in tandem with other approaches such as increasing the seeding rate or modifications to seed delivery technique (Ott et al. 2017). Any incremental improvements in sagebrush restoration practices could produce substantial impacts considering the spatial extent of sagebrush steppe and low seed-based establishment success. Testing further iterations of seed technologies in combination with strategic timing of seeding, particularly for difficult-to-establish and small-seeded species, could contribute to more successful large-scale restoration of this and other dryland landscapes.

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Supporting Information

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

Table S1. Seed mixes applied as part of the reclamation process for year 0, 1, and 5 sites at the McIntosh Mine.

Supplement S1. Details of SET production.

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