Agglomerating seeds to enhance native seedling emergence and growth

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Summary

1. Restoration in rangelands is constrained by low establishment of species sown from seed. Non-biotic soil-surface crust is one of the major factors limiting reseeding success by acting as a barrier to seedling emergence.

2. The objective of this study was to determine whether seedling emergence could be improved by agglomerating multiple seeds into a single pellet, so that the seedlings growing from the pellet will collectively generate sufficient force to penetrate the soil crust. To evaluate this technology, we compared seedling emergence and biomass production from agglomerated, single and non-coated seed (control) of Pseudoroegneria spicata. In the greenhouse, seeds were sown in either crust-forming clay or non-crusting sandy soil and studied for a 25-day period. Starting seed density was constant across treatments.

3. In the clay soil, seedling emergence from the agglomeration treatment was 1-3 and 1-9 times higher than the single seed coating and control, respectively. In the sandy soil, the agglomeration and single seed coating responded similarly, producing 1-4 times more seedlings than the control.

4. Biomass production followed a similar trend as plant density. In the clay soil, increased biomass of the agglomeration treatment was not only because of higher plant densities but was also a product of having greater biomass per plant.

5. Synthesis and applications. This short-duration ‘proof-of-concept’ study indicates that both the seed coating materials used to form the agglomerates and the act of agglomerating the seeds together improve P. spicata emergence and plant growth. These results also demonstrate that in the early seedling stage, facilitation outweighs competition in agglomeration plantings. Additional research is needed to verify these results in the field.

Key-words: facilitation, native plants, restoration, seed coating, seeding, seedling emergence, soil physical crust

Introduction

Rangelands constitute nearly half of the Earth’s land surface and provide multiple ecosystem services beneficial to human life (Asner et al. 2004; Havstad et al. 2007). These services are diminished when the functional integrity of rangeland systems is compromised. The loss of native vegetation is one of the most significant land degradation issues facing the world today (Ninan 2001). In many situations, these lands have been degraded to a point where autogenic recovery is impaired and energy-intensive restoration measures are required to restore ecosystem function (Bestelmeyer 2006; Kinyua et al. 2010). It is common practice for land managers to seed disturbed areas to facilitate the recovery of desirable vegetation (Allen-Diaz & Bartolome 1998; Martin & Wiltsey 2006; Epanchin-Niell, England & Nalle 2009). Unfortunately, the success of these seeding efforts is typically less than desirable, especially in arid and semi-arid ecosystems (Seabloom et al. 2003; Lysne & Pellant 2004; James, Svejcar & Rinella 2011). Demography research on rangeland seedings in the Great Basin region of the western United States has shown that seedling emergence represents a

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One factor that can act as a significant barrier to seedling emergence is the presence of non-biotic soil-surface crusts (Unger 1984; Awadhwal & Thierstein 1985; Belnap 2003). Soil physical crust is characterized by a dense, relatively impermeable soil-surface feature, which impedes seedling emergence, while decreasing water infiltration and gas exchange (Belnap 2003). To effectively address a physical soil crust problem, expensive management practices must often be performed to increase aggregate stability (Belnap 2003). Where resources are available, this can be achieved by incorporating organic matter or other soil amendments such as gypsum (e.g. Shainberg et al. 1989) or polyacrylamide (e.g. Ben-Hur 1994) into the soil. Seedling emergence can also be increased through short-term treatments, such as irrigation to maintain a moist soil surface (Unger 1984), or use of equipment (i.e. rotary hoe) to mechanically break up the soil crust (Billbro & Wanjura 1982). While these approaches may work in intensive agronomic operations, typically they are impractical for the revegetation of extensive rangeland systems of relatively low economic value. Consequently, there is a need for the development of cost-effective seeding technologies that will enhance seedling emergence in the presence of a soil physical crust (James & Svejcar 2010).

Seed coating technology provides several options for enhancing germination and seedling growth (Taylor & Harmon 1990). A common approach for applying the coatings to the seed is through the use of a rotary (or rotostat) seed coater. In general, seeds are coated through this technology using centrifugal forces to mix the seeds, while adhesives (or stickers) are pumped to the centre of the coating chamber onto the atomizing disc, which redirects the liquid outward in small droplets onto the seed. With a binder providing a tacky base, seed coating powder is delivered through an auger feeder onto the moist seeds. Traditionally, coating formulations and application rates have been applied in a manner that produces the desired coating properties (e.g. density, hardness, plasticity and moisture uptake), while keeping the seeds from sticking or clumping together during the coating processes (Gregg & Billups 2010). Madsen, Petersen & Taylor (2010) proposed in US patent application 0267554 a coating technology that alters the traditional approach to seed coating by developing a method that uses rotary seed coating technology to promote the uniform clumping of seeds into pellets (or agglomerates) (Fig. 1). Because the penetration force of emerging seedlings increases with the number of seeds sown in the same location (Edwards 1966; Awadhwal & Thierstein 1985), agglomerated seeds should have improved seedling emergence by having multiple seedlings collectively generating greater emergence force than a single seed (Fig. 1).

Higher seedling densities may increase mortality as a result of competition for limited resources (Connell & Slatyer 1977; Stuchowicz 2001); this may be especially true with the plantings of agglomerated seed where cohorts of the same species are occupying the same microsite (i.e. complete niche overlap). It may also be possible that positive interactions (or facilitation) may extend beyond seedling emergence and provide other benefits associated with clustered growth patterns that will outweigh competition and enhance seedling establishment (Hunter & Aarssen1988; Fajardo & McInture 2011).

The objective of this study was to determine whether seedling emergence of a perennial bunchgrass species could be improved by agglomerating multiple seeds into a single pellet. Comparisons were made using agglomerated seed, coated seed (which was not agglomerated) and non-coated seed (control). The three treatments were evaluated in a clay-loam soil that was susceptible to crustling, and a non-crusting sandy soil. We tested the following hypotheses: (i) agglomerated seeds would have higher emergence than single coated or single non-coated seeds in clay but not sand, and (ii) intraspecific competition would result in lower per-plant biomass in the agglomerated treatment, relative to the other two treatments.

Materials and methods

Evaluations were performed on the native species _Pseudoroegneria spicata_ (Prush.) Löve, which is often a major component of native plant communities in the Intermountain West, USA, and is commonly used for restoration of rangeland systems in this region (Ogle, St John & Jones 2010). _Pseudoroegneria spicata_ has a wide ecological aptitude growing in relatively low elevation, dry _Artemisia_ L. communities and up to high elevation, moist sagebrush-grasslands (Davies, Bates & Miller 2006; Davies & Bates 2010).

SEED COATING

Seed coating was performed at the Eastern Oregon Agricultural Research Center (EOARC) in Burns, OR, USA. The treatments described earlier were applied using a RP14DB® rotary seed coater (BraceWorks Automation and Electric, Lloydminster, SK, Canada).
Agglomerates were created similar to methods proposed by Madsen, Petersen & Taylor (2010). Materials used in this process included the adhesive binder, Selvol-205® (Sekisui Specialty Chemicals America 2009), and the powder filler material, diatomaceous earth (Perma-Guard, Inc., Albuquerque, NM, USA). Selvol-205 was prepared with an 8% solid content, according to Sekisui Specialty Chemicals solution preparation guidelines (Sekisui Specialty Chemicals America 2009). After evaluating several common seed coating powder materials (i.e. limestone, talc, bentonite clay and montmorillonite clay), we generally felt diatomaceous earth formed agglomerates most consistently. Diatomaceous earth is composed of fossilized diatom remains that have sharp and jagged microscopic edges. The structure of the diatomaceous earth may aid in the formation of agglomerates by decreasing the flowability of the coated seed and promoting seed clumping.

A detailed description of the amount and application rate used to form the agglomerated seeds is shown in Table 1. First, we applied a base coating comprised of Selvol-205 and diatomaceous earth. In this phase, Selvol-205 and diatomaceous earth were applied using typical seed coating methods that allows for the product to adhere to the seed and prevent seeds from clustering together. In the second phase, seeds were agglomerated together by increasing the rate of Selvol-205, and only applying diatomaceous earth in periodic short bursts. Generally speaking, through this approach, agglomeration size (i.e. number of seeds per pellet) is primarily dependent upon the amount and rate that Selvol-205® is applied while withholding or limiting diatomaceous earth. Lastly, the agglomerates were coated using methods described in phase 1 earlier, to provide additional structure to the agglomerated seed. After coating, agglomerated seeds were placed on a forced air drier at 25 °C for 4 min. From this batch, we selected agglomerates that had exactly five seeds to be used in the trial.

Single coated seeds were made using the same amount of materials used to form the agglomerates. In this treatment, the coating material was applied at a rate sufficient to keep the seeds from clustering together.

SOIL

Evaluations were performed on clay (31% sand, 22% silt and 47% clay) and sandy (91% sand, 5% silt and 4% clay) textured soils. The clay soil was obtained from the USDA – Agricultural Research Services (ARS), Northern Great Basin Experimental Range (NGBER; Lat: 43°27′34″N, Long: 119°40′15″W), 67 km west of Burns, Oregon, USA. This soil was classified as a clayey, montmorillonitic, frigid and shallow Xeric Argidurids. Soil pH was 7.8, and organic matter content was 1.9% (Soil Survey Staff 2011). We collected the sandy soil from a site 176 km south of Burns, OR, USA (Lat: 43°21′23″N, Long: 119°01′5″W). At this location, the soil is classified as a fine sand, mixed, frigid Xeric Haplocambids. Soil pH was 7.2, and organic matter content was 0.25% (Soil Survey Staff 2011). At both sites, the vegetation community was co-dominated by Artemisia tridentata spp. wyomingensis (Beetle & A. Young) S.L. Welsh and perennial bunchgrasses, such as Agropyron cristatum, Stipa thurberiana, Agropyron spicatum and Poa sandbergii.

STUDY DESIGN

Seed treatments were evaluated in the greenhouse at the EOARC using a randomized block split-plot design, with five blocks. Each block contained two 0.2 m⁻² containers (0.5 × 0.4 m on a side, with a depth of 0.1 m), one with sand and the other with clay soil. Within each container, we hand sowed the three seed treatments, in separate rows, equally spaced, with the location of the row randomized among the treatments. Each row was 0.4 m long and contained 15 seeds, planted 1 cm below the surface. For the rows designated for non-coated and single coated seeds, we evenly distributed the seed across the row. In the rows designated for agglomerated seeds, we sowed three pellets, which each contained five seeds.

RAINFALL SIMULATION AND WATER REGIME

Prior to seeding, we saturated the soil from the bottom-up and then allowed the soil to drain for 24 h. We then seeded the boxes and placed them on a 5% slope, where we applied 15 mm (30 L container⁻¹) of water using a portable oscillating-arm rainfall simulator, as described by Meyer & Harmon (1979). To promote soil crust formation, we rained on the soil with an application intensity of 247 mm h⁻¹, which approximated the rainfall intensity for a 15-min period, with a 2 year return interval, for the NGBER (Miller, Frederick & Tracy 1973). Over the remainder of the study, pots were watered with a fine mist sprayer. To stimulate seedling germination, the soil was misted once a day with 1-5 mm (0.3 L container⁻¹), for 5 days after seeding. After day 5, the soil was watered weekly with 3-4 mm (6.8 L container⁻¹) that is approximately half the average weekly

<table>
<thead>
<tr>
<th>Selvol-205</th>
<th>Diatomaceous earth</th>
<th>Rotor speed</th>
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</thead>
<tbody>
<tr>
<td><strong>Time (s)</strong></td>
<td><strong>Rate (g s⁻¹)</strong></td>
<td><strong>Subtotal (g)</strong></td>
</tr>
<tr>
<td>Start–stop</td>
<td>6–35</td>
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<tr>
<td></td>
<td>41–120</td>
<td>0·833</td>
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<tr>
<td></td>
<td>120–210</td>
<td>1·379</td>
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<tr>
<td></td>
<td>210–250</td>
<td>0·490</td>
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<tr>
<td>Total</td>
<td>238·68</td>
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Table 1. Batch recipe used to coat 227 g of Pseudoroegneria spicata seed. The Table shows the time the seed coater’s pump, and powder feeder was turned on and off to apply Selvol-205 and diatomaceous earth, along with their associated rates and amount of material delivered. The amount of Selvol-205 in the table contains 8% by weight of active ingredient. The Table also shows the different speeds the coating pan was run during the coating process.
amount of precipitation received in the spring (March–May) at the NGBER (Western Regional Climate Center 2010).

MEASUREMENTS
We measured volumetric soil water content and crust penetration resistance to fracture the soil surface, in-between the rows, at three locations per box, approximately every 3 days. Soil water content was measured from the soil surface down to 60 mm, with an ML2x Theta Probe (Delta-T Devices, Cambridge, UK). On the days the soil was designated to be watered, soil water content was measured prior to watering. Crust penetration resistance was determined using a 476-mm diameter flat point handheld penetrometer (Model 719-5MRP; John Chatillon & Sons, Kew Garden, NY, USA). Eleven days after seeding, we measured the thickness of the soil crust using a vernier calliper, at three sample locations per box container.

Seedling density of all live seedlings in each row of the box containers was also recorded at the same time that the soil water content and crust penetration resistance was measured at c. 3 day intervals. At the conclusion of the study (25 days after seeding), we weighed standing above-ground biomass after oven-drying at 65 °C for 72 h.

DATA ANALYSIS
Seedling density data were analysed using repeated measures mixed-model analysis with an autoregressive order 1 covariance (SAS Version 9.2; SAS 2006). In the model, seed treatments and soil type were considered fixed factors, sampling period was designated as a repeated measure, and blocks were random. We used mixed-model analysis to analyse total biomass, and per-plant biomass. Again, blocks were considered random, while seed treatments and soil type were considered fixed factors. When significant main or interactive effects were found, mean values were separated using the LSMEANS procedure (SAS 2006). Prior to analysis, normality and homogeneity was tested using the Shapiro–Wilk test and Levene’s test, respectively (PROC UNIVARIATE; SAS 2006). Each data set was log-transformed to reduce problems with deviance from normality. For all comparisons, a significance level of $P < 0.01$ was used. In the text and figures, means are reported with either unique letters to denote significances or with associated standard error.

Results
VOLUMETRIC WATER CONTENT AND SOIL CRUST
Soil moisture in both soil types was at saturation at the start of the study, and by the end of the experiment, it had declined to near wilting point (~1.5 MPa) (Fig. 2). In the clay soil, crust penetration resistance increased rapidly as soil moisture declined, until around day 15. During this period, crust penetration resistance ranged from 0.13 MPa (day 3) to 0.23 MPa (day 15). After day 15, crust strength was highly variable and on average decreased with time (Fig. 2). Crust thickness of the clay soil measured on day 11 was 9.3 ± 0.6 mm. The sandy soil did not form a physical crust (Fig. 2).

PLANT DENSITY
Plant density among seed treatments differed by soil type (Table 2). In the clay soil, on average, the agglomerated seeds emerged earlier and over a longer period of time than the control (Fig. 3). This generally resulted in the agglomeration treatment having higher plant densities than the control. Final plant density in the clay soil showed the agglomeration treatment having approximately two times (19 times) more seedlings than the control. Seedling emergence for the single coated seed treatment appeared to have been impaired by physical soil crust similar to the control, for the first half of the study. Between day 14 and 19, this treatment increased in plant density by 35%, while density in the control remained constant. Final seedling density for the single coatings produced an intermediate response when compared to the other two treatments, with single coatings having 1.4 times more seedlings than the control.

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density values at each sampling date are shown by unique letters. Control; however, in this soil type, there was no statistical coating and agglomeration treatments when compared to the seed coating treatment. However, towards the end of the study, the gap between the single seed coating treatment both produced a similar increase in seedling emergence over the control (Fig. 3). Greater seedling emergence in the clay soil is probably the result of having multiple seedlings collectively generating sufficient force to penetrate through the soil crust. Seedling emergence force increases when multiple seeds are planted in the same location (Edwards 1966; Awadhwal & Thierstein 1985). In the sandy soil where plant density over the course of the study grown from none-coated (control), single coated and agglomerated seeds, sown in clay and sandy soil. Plots were sown with 214 PLS m⁻². Significant differences (P < 0.01) between mean density values at each sampling date are shown by unique letters. In the sandy soil, agglomerated and single coated seed treatments had similar plant densities at each measurement period (Fig. 3). There was some indication that these treatments can improve the rate of seedling emergence in the sandy soil, with early plant density values nearly double that of the control. However, towards the end of the study, the gap between the coating treatments and the control decreased resulting in statistically similar final plant densities (Fig. 3).

Above-ground biomass

Differences in biomass were primarily influenced by seed coating treatments and to a lesser extent, soil type (Table 2). In the clay soil the agglomeration treatment had 1.5 times more biomass than the single coated seed treatment and 2.7 times more biomass than the control (Table 3). On a per-plant basis, the control and single coated seed treatments remained similar while the agglomeration treatment had 1.4 times more biomass than the control but was not statistically higher than the single seed coating treatment.

In the sandy soil, there was greater biomass in the single seed coating and agglomeration treatments when compared to the control; however, in this soil type, there was no statistical difference between single coated and agglomerated seed treatments (Table 3). On average, the single seed and agglomerated treatments produced 1.7 and 2.1 times more biomass than the control, respectively. Per-plant biomass followed a similar pattern as total biomass, with both single seed and agglomerated treatments producing similar values, which were 1.4 and 1.6 times greater than the control, respectively.

Discussion

Seedling density

The results of this study confirmed our hypothesis that agglomerating perennial bunchgrass seeds into pellets enhances seedling emergence in the presence of a crusting soil (Fig. 3). Our data further indicate that the coating materials used to agglomerate seeds together also enhances seedling emergence (Fig. 3). The mechanism by which the agglomeration treatment improves seedling emergence is illustrated in the comparison of seed treatments by soil type. In the clay soil, seedling emergence was limited by crusting soil and drought (Fig. 2). Within this soil type, the single seed coating treatment (which has the same amount of coating material as the agglomeration treatment) had less emergence than the agglomeration treatment yet was greater than the control (Fig. 3). However, in the sandy soil, emergence was limited by drought only (Fig. 2). Within this soil type, the agglomeration and single seed coating treatment both produced a similar increase in seedling emergence over the control (Fig. 3).

In the clay soil, there was greater biomass in the single seed coating and agglomeration treatments when compared to the control; however, in this soil type, there was no statistical difference between single coated and agglomerated seed treatments (Table 3). On average, the single seed and agglomerated treatments produced 1.7 and 2.1 times more biomass than the control, respectively. Per-plant biomass followed a similar pattern as total biomass, with both single seed and agglomerated treatments producing similar values, which were 1.4 and 1.6 times greater than the control, respectively.
Diatomaceous earth has high water absorption and retention properties (Li et al. 2000; Shawabkeh & Tutunj 2003). These two coating materials may aid in the absorption and retention of moisture around the seed. Presuming this water was thermodynamically available to the seed, increased moisture availability around the seed would enhance seed imbibition, germination and early seedling growth, in a water-limited environment. Increased moisture provided by the coating may also help maintain plant turgor pressure and subsequently promote seedling emergence thrust through the soil. Diatomaceous earth may also have aided in seedling emergence and survival by providing a source of silicon. The chemical composition of oven dried diatomaceous earth typically contains between 80% and 90% silicon dioxide (Antonides 1997). The application of silicon fertilizer to cereal crops has been shown to increase seedling resistance to both biotic and abiotic stresses (Ahmad, Zaheer & Ismail 1992; Liang 1998; Tuna et al. 2008). However, a review of the literature indicates that moisture enhancing seed coatings generally fail to improve rangeland reseeding (i.e. Hull & Klomp 1967 and references therein; Kocher & Stubben dieck 1986; Mangold & Sheley 2007). Therefore, the positive response in seedling emergence from the seed coating materials used in this study merits additional research to verify the mechanisms and controlling variables.

BIOMASS PRODUCTION

As with plant density, increased biomass, in part, can probably be attributed to the agglomeration treatment improving seedling emergence (Fig. 3, and Table 3). Interestingly, in the clay soil, increased above-ground biomass in the agglomeration treatment is not only because of greater plant densities but is also a product of having greater biomass per plant (Table 3). Such a response is contrary to our hypothesis that intraspecific competition would lower biomass production on a per-plant basis. These results may indicate that under the conditions of this study, facilitation played a more important role than competition. Facilitation often outweighs competition effects in harsh and resource-limited environments (Hunter & Aars sen 1988; Bertness & Callaway 1994; Callaway & Walker 1997; Brooker & Callaghan 1998; Fajardo & McIntire 2011). As an example, Fajardo & McIntire (2011) compared Nothofagus pumilio tree seedlings planted in clusters and spaced apart from each other along a stress gradient in a forest–prairie ecotone in Patagonia, Chile. As stress increased, seedlings planted in dense clusters had higher survival than solitary seedlings (Fajardo & McIntire 2011).

It is probable that the increase in biomass in our agglomeration treatment may be due, in part, to the ability of this technology to better mimic the way plants have evolved to grow in natural systems. Seeds typically grow within groups or clusters because of natural process depositing them within the same microsite. For instance, seeds can be collected within litter material, holes and cracks in the soil surface, or other features that trap wind-blown seeds (Eckert et al. 1986; Stamp 1989; Chambers, MacMahon & Haefner 1991; Chambers 2000). Seeds can also be grouped together as a result of caching activities by granivores. Seed caching is considered the primary mechanism of seedling establishment by Purshia tridentata (Pursh) DC (antelope bitterbrush) and Achnatherum hymenoides (Roemer & J.A. Schultes) Barkworth (Indian rice grass), with two or more mature plants typically growing together (West 1968; Vander Wall 1994).

This study provides justification for additional research to determine what specific facilitative interactions in the agglomeration treatment improved plant growth, in addition to multiple seedlings increasing emergence thrust. For example, early emergence (Fig. 3) may have allowed the seedlings to grow deeper root systems to better utilize moisture resources from a rapidly drying wetting front (Nicotra, Babicka & Westoby 2002; Leck, Parker & Simpson 2008). Moisture availability may also have been improved as a result of increased plant cover in the agglomeration treatment promoting greater soil infiltration, and from the fallow spaces between groups of plants prolonging the duration of available moisture. Multiple seedlings growing within a cluster may indirectly increase the availability of nutrients through promoting microbial activity. We did not measure root growth, but greater above-ground growth would presumably translate into higher below-ground growth as well. More root growth in the agglomerate treatment should result in greater exuded substrate from the roots, which stimulates rhizospheric microbial activity (Whipps 1990). This in turn promotes higher soil mineralization rates and subsequent nutrient availability (Marschner 1995; Hamilton & Frank 2001). The agglomeration treatment may also moderate microsite temperatures, as the plants collectively provided a greater insulating affect from extreme temperatures.

Conclusions and future research

To our knowledge, this is the first seed coating treatment specifically designed to improve seedling emergence in crust ing soil by agglomerating multiple seeds together within a single pellet. Enhanced emergence of the agglomeration treatment is most likely to be a function of the coating material decreasing the emergence time, alleviating drought conditions and a result of the greater emergence thrust generated by the seedlings collectively growing from the agglomerated pellet compared with a single seedling. In addition to the agglomeration treatment improving seedling emergence in crust ing soils, this research also provides evidence that seedlings growing from agglomerates facilitate greater growth than can be achieved by a single seedling.

These results indicate that current agronomic practices that evenly space seeds may not be the most effective technique for reseeding rangelands with crust ing soils. Use of seed agglomeration technology may provide land managers with the ability to efficiently plant seeds within clusters using standard seed drills. Facilitation associated with coating materials and clustered plant growth may increase the probability of the plant establishing on a site, and thus lower the probability of restoration failure.

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Further development and evaluation of this technology is needed before it can be recommended as a native plant restoration treatment. Future studies should include: (i) determination of how agglomeration size (i.e. number of seeds per pellet) influences seedling emergence, plant survival and biomass production across different stress gradients; (ii) analysis of the agglomeration procedure, with a focus on how emergence is affected by variability in the materials, application rates and other inputs into the coating process; (iii) evaluation of how seed and plant physiological characteristics of different species function within an agglomeration treatment; (iv) quantification of the long-term survival and plant community structure of agglomeration plantings and (v) determination of the mechanisms that influence seedling growth from an agglomerated pellet (i.e. water infiltration and moisture availability, thermal response to extreme temperatures, microbial response and nutrient availability).

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