

RESEARCH ARTICLE

Small vertebrate granivores reduce seedling emergence in native tallgrass prairie restoration

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High seed cost and low rates of establishment make tallgrass prairie restorations challenging and expensive endeavors. Typical seedling emergence rates in prairie restorations are approximately 10% and the causes of seed mortality are poorly understood. In this study, we examined the impact of small vertebrate granivores on prairie restoration by comparison of seedling emergence in open (sham) versus closed exclosures at three newly restored sites. To assess other causes of seed loss, we also tracked seed fates at one prairie restoration site. We coated seeds of four prairie species with fluorescent dye, placed them under closed exclosures, and monitored their fate (emerging seedling, partially germinated, nongerminated/viable, and nongerminated/nonviable) over a 5-month period. On average, 9.6 more seedlings/m² emerged in the closed than the opened exclosures, suggesting that small vertebrate granivores reduce seedling emergence in prairie restoration. Granivores influenced the composition of the emerging community but did not preferentially consume large-seeded species. In the seed-tracking experiment, we found that greater than 70% of seeds were lost within 30 days of sowing, that seed recovery and viability both decreased with time in soil, and that seed fates differed between species. Collectively, our results indicate that small vertebrate granivores are an important cause of seed loss in prairie restoration, but unidentified belowground (e.g. fungal decomposition, invertebrate predation) and environmental (wind, rain) factors account for a greater proportion of total seed loss. Until these causes of seed loss are better understood, high seed costs will persist and continue to impede prairie restoration.

Key words: seed predation, seed recovery, seedling emergence, vertebrate granivores

Implications for Practice

- Small vertebrate granivores reduce the number of emerging seedlings by approximately 30% (9.6 seedlings/m²) in newly restored tallgrass prairies.
- Small vertebrate granivores can reduce seedling emergence rates by 5% in prairie restoration. Based on this reduction, and the retail price of native seed for a high-diversity prairie restoration (\$3,600–5,000 ha⁻¹), we estimated that the direct cost of seed loss to small vertebrate granivores is \$180–250 ha⁻¹.
- By reducing seedling emergence, small vertebrate granivores will likely increase the number of weeds in a prairie restoration. High weed biomass can delay native establishment, reduce native establishment, reduce native diversity, and increase management costs in prairie restoration.

seed sources remain and the native seed bank has been largely depleted (Smith 1998). Commercial seed is available, but the price of a low-diversity mixture (20–30 species) ranges from \$500 to 1,500 ha⁻¹ and the price of a high-diversity mixture (50–70 species) ranges from \$3,600 to 5,000 ha⁻¹ (Prairie Moon Nursery 2013). Also contributing to the cost is the high seeding rate required to achieve desired plant density. In typical restorations, 400–950 pure live seeds (PLS)/m² are sown to achieve approximately 30 adult plants/m² (Smith et al. 2010; Williams 2010). These establishment rates (3.1–7.5%) are an order of magnitude lower than those observed in modern agriculture (83–92%; Lauer 2005; Smith et al. 2010). To help practitioners reduce the cost of prairie restoration, we need a better understanding of the factors that limit seedling establishment.

Predation by small vertebrate granivores, such as meadow voles, field mice, and birds, represents one potential cause of seed loss in tallgrass prairie restoration (Chambers & MacMahon 1994; Howe & Brown 1999; Clark & Wilson 2003). In many natural and agricultural systems, small

Introduction

Tallgrass prairie once covered approximately 100 million hectares of central North America. Agricultural growth in the nineteenth and twentieth centuries decimated this ecosystem and now, less than 3% remains (Smith 1998). Restoration is paramount for the maintenance and recovery of this endangered ecosystem and the services it once provided (Smith et al. 2010). One of the main challenges of tallgrass prairie restoration is the high cost of native seed (e.g. Gerla et al. 2012). Few remnant

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vertebrate granivores drastically reduce the number of seeds within days of sowing (e.g. Archer & Pyke 1991; Westerman et al. 2003, 2006; Schnurr et al. 2004; Heggenstaller et al. 2006) resulting in lower seedling emergence (e.g. Edwards & Crawley 1999; Orrock et al. 2009). In addition to their effect on seedling emergence, small vertebrate granivores can also alter the species composition of the emerging community by preferentially consuming certain species (Janzen 1971; Kerley & Erasmus 1991; Chambers & MacMahon 1994; Howe & Brown 1999, 2000). Optimal diet theory suggests that seed predators should preferentially consume species with large seeds, thin seed coats, and with no major feeding deterrents (Kerley & Erasmus 1991; Chambers & MacMahon 1994; Hendry et al. 1994; Blaney & Kotanen 2001; Sih & Christensen 2001). Consistent with this hypothesis, several vertebrate exclusion studies have shown a relative reduction in the emergence of large-seeded, and presumably preferable, species in unprotected plots (e.g. Reader 1993; Howe & Brown 1999; Howe & Brown 2000; Blaney & Kotanen 2001).

Several belowground factors can also influence seedling emergence in prairie restoration, including invertebrate predation, parasitism by microorganisms (fungal and bacterial decomposition), and seed senescence (Blaney & Kotanen 2002; Clark & Wilson 2003). Belowground causes of seed loss are notoriously difficult to quantify in situ (Chambers & MacMahon 1994; Fenner & Thompson 2005). For example, Clark and Wilson (2003) attempted to quantify the impact of several aboveground and belowground causes of seed loss in four species in an Oregon grassland. Their results suggested that fungal parasitism accounts for approximately 10% of seed loss and there was indirect evidence of invertebrate seed predation for one species, but the primary cause of seed loss for every species was an “unmeasured factor.” Senescence was not listed as a significant cause of seed loss for any species; however, some seeds may have deteriorated in the soil before recovery. The authors found little evidence of seed persistence, but some seeds may have been overlooked because of the challenge associated with recovering small seeds. A better method for tracking seed fates in the soil could help researchers identify causes of seed loss and viability loss in prairie restoration and help practitioners develop new techniques to minimize this loss.

Restorations that depend on seed sowing must contend with seed loss to aboveground and belowground factors. The goals of this study were: (1) to quantify the impact of small vertebrate granivores on seedling emergence and community composition in tallgrass prairie restorations; and (2) to examine other causes of seed loss in prairie restorations by tracking belowground seed fates of several prairie species. To address our first goal, we compared seedling emergence and the composition of the emerging community in open (sham) versus closed small vertebrate exclosures at three newly restored prairie sites. We predicted that seedling emergence and the relative emergence of large-seeded species would be greater in the closed exclosures than the open exclosures. To address our second goal, we tracked the seed fates (emerging seedling, partially germinated, nongerminated/viable, and nongerminated/nonviable) of four native tallgrass prairie species over a 5-month period. Seeds

were coated with fluorescent dye to facilitate recovery and placed under closed exclosures. We predicted that the seed recovery rates and the viability of recovered seeds both would decrease with time.

Methods

Study Sites

This research was conducted in 2013 at three tallgrass prairie restoration sites in Iowa, U.S.A. (Fig. S1, Supporting Information). The specific restoration protocols differed between sites but all three used seeding rates, seed mixtures, and seeding methods consistent with prairie restoration in Iowa (Tables S1 & S2). For convenience, we named the sites: (1) University of Northern Iowa (UNI), (2) Kettleston–Hogsback (KH) Complex, and (3) Spring Run Graff (Graff). We will use these abbreviations throughout this article.

Site 1: University of Northern Iowa. The first prairie restoration site was located in Cedar Falls, Iowa (42°30'30"N; 92°27'27"W, Fig. S1). The site was 0.61 ha in size and located on a small alluvial bench. The soils were classified as a Saude-Urban land complex with 0–2% slopes (NRCS 2013). Prior to restoration, the site was dominated by *Bromus inermis* Leyss. (smooth brome), *Agropyron repens* (L.) Gould (quack grass), and *Poa pratensis* L. (Kentucky bluegrass) and management consisted of semiannual mowing. On 24 May, 2013, glyphosate was applied and on 3 June, 2013, the site was prescribed burned and drill seeded with a 41-species seed mixture (Table S1). The four species used for tracking seed fates in part two of our study (see below) were deliberately excluded from this mixture. The average annual temperature in 2013 was 1.03°C colder than the 30-year average (7.78 vs. 8.81°C) and total precipitation in 2013 was 15.7 cm higher than the 30-year average (103.6 vs. 87.9 cm; data collected from Waterloo Municipal Airport Weather Station; NOAA 2013).

Site 2: Kettleston–Hogsback Complex. The second prairie restoration site was located in Dickinson County, Iowa (43°27'59"N; 95°8'59"W, Fig. S1). The site was 12.2 ha in size with soils classified as moderately eroded Clarion–Storden complex with 5–9% slope and Omsrud–Storden complex with 9–14% slope (NRCS 2013).

Site 3: Spring Run Graff. The third prairie restoration site was also located in Dickinson County, Iowa (43°22'46"N; 95°1'56"W, Fig. S1). The site was 3.6 ha in size with soils classified as Nicollet loam with 1–3% slope, Clarion loam with 2–5% slope, and a moderately eroded Clarion–Storden complex with 5–9% slope (NRCS 2013).

At the two Dickinson County sites, the average annual temperature in 2013 was similar to the 30-year average (7.15 vs. 7.20°C) and total annual precipitation in 2013 was 25.9 cm lower than the 30-year average (49.3 vs. 75.2 cm; data collected from Spirit Lake weather station; NOAA 2013). Both sites were used for soybean production prior to restoration and both sites were bare soil at the time of restoration. On 28 and 29 March,

2013, both sites were frost seeded using a broadcast seeding method and a seed mixture consisting of 23 species (Table S2). A small amount of local, hand-collected seed was also seeded at both sites but the exact seeding rate of this approximately 50-species mixture was unknown because the seed was not tested for purity or viability (Table S2).

Experimental Design – Small Vertebrate Granivory

To quantify the impact of small vertebrate granivores on prairie restoration, we compared seedling emergence and the species composition of the emerging community in open (sham) versus closed exclosures. We installed 10 exclosures at the UNI site and five exclosures at each of the Dickinson County sites (KH and Graff). The position of each exclosure was selected using a restrictively random approach. Specifically, we placed transect lines (one at KH and Graff, two at UNI) in a random position at each site (minimum 20 m from plot edge) and installed five exclosures along each transect at restrictively random positions (minimum 10 m apart, Fig. S1). Exclosures were placed in the field within 3 hours of seed sowing to ensure that no predation occurred prior to exclosure installation.

Exclosures were constructed using wire hardware cloth (maximum opening 9.1 mm), with the dimensions 1 m × 0.5 m × 0.6 m (width, length, height). Exclosures were divided in half using another piece of wire hardware cloth (dimensions: 0.5 m × 0.6 m) creating two adjacent exclosures each with the dimensions 0.5 m × 0.5 m × 0.6 m. One side was randomly assigned to be the open (sham) exclosure and the other side was assigned to be the closed exclosure. The closed exclosure was sealed on all sides with wire hardware cloth. The sham exclosure had 0.12 m × 0.15 m openings on all three outward-facing sides. The base of each exclosure was embedded 5 cm deep in the ground and anchored using wire staples.

Our exclosures were specifically designed to deter both birds and rodents: the two main types of granivores for prairie restoration. Previous research has shown that birds and rodents both influence seedling emergence and establishment in native tallgrass prairie restoration. For example, Howe and Brown (1999) examined the contrasting impact of granivorous birds and granivorous/herbivorous rodents on prairie restoration in Illinois. They found that the birds reduced grass biomass and the rodents reduced forb biomass. The granivorous birds identified in their study included: Song Sparrows (*Melospiza melodia*), Savannah Sparrows (*Passerculus sandwichensis*), Goldfinches (*Spinus tristis*), Brown-headed Cowbirds (*Molothrus ater*), House Sparrows (*Passer domesticus*), and Mourning Doves (*Zenaidura macroura*). The granivorous/herbivorous rodents identified in their study included: meadow voles (*Microtus pennsylvanicus*), short-tailed shrews (*Blarina brevicauda*), white-footed mice (*Peromyscus leucopus*), small shrews (*Sorex* spp.), and thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*). Many of these species are also important granivores for prairie restoration in Iowa. For example, meadow voles (*M. pennsylvanicus*) and thirteen-lined ground squirrels (*S. tridecemlineatus*) were the most common rodent granivores in a 2007 trapping survey conducted less than 1 km from

our UNI restoration site (Hemsath 2007). Similarly, Myers et al. (2012) found that Dicksissels (*Spiza americana*), Indigo Buntings (*Passerina cyanea*), Chipping Sparrows (*Spizella passerine*), Lark Sparrows (*Chondestes grammacus*), Song Sparrows (*M. melodia*), and Goldfinches (*S. tristis*) were common granivorous birds at Cedar River Ecological Research Site, which is located 24 km from our UNI restoration site.

Emerging seedlings were identified and quantified nine times during the 2013 growing season at UNI: 26 June, 10 July, 16 July, 23 July, 29 July, 7 August, 23 August, 5 September, 16 September; and seven times at KH and Graff: 11 June, 18 June, 25 June (Graff only), 26 June (KH only), 2 July, 9 July (Graff only), 10 July (KH only), 24 July, 20 August. On each sampling date, we identified and removed seedlings and any associated root tissue to prevent reestablishment of counted seedlings. Three groups of seedlings were excluded from analyses: (1) species not present in the original seed mixture (weeds), (2) species that could not be positively identified (instances were rare), and (3) *Symphyotrichum laeve* and *Solidago* spp. at the UNI site, which could not be positively distinguished from other seedlings with similar morphology in the soil seed bank.

Data Analysis – Small Vertebrate Granivory

To test whether predation by small vertebrate granivores influenced seedling emergence in three newly restored prairies, we used a mixed effects general linear model with site and treatment (sham vs. closed exclosure) as fixed factors and exclosure (nested within site) as a random factor. The site × treatment term was excluded from the model because of nonsignificance. Removal of this term did not alter the significance of any model term.

To test whether small vertebrate granivores influenced the species composition of the emerging community, we used two general regression models. In our first model, we regressed percentage of seedling emergence (number of seedlings/number of seeds planted) on seed mass, treatment (sham vs. closed exclosure), and seed mass × treatment. A significant seed mass × treatment term in this model would indicate that the average seed mass of the emerging community differed between the closed and open exclosures. Furthermore, a higher average seed mass of the emerging community in the closed exclosures would indicate that granivores were preferentially consuming larger-seeded species. To correct for non-normality and heteroscedasticity, we removed one outlying species (*Silphium laciniatum*) and square-root transformed the data. With our second model, we used a different response variable (number of seedlings/total mass of planted seed) as our measure of seedling emergence. Seed masses were obtained from Prairie Moon Nursery's Cultural Guide (2013). Species with heavier seed are often seeded at a lower rate than species with lighter seeds in prairie restoration. By representing seedling emergence as a function of total seed mass planted rather than total number of seeds planted, we account for these large interspecific differences in seeding rates. We regressed this new response variable (number of seedlings/total mass of planted seed) on seed mass, treatment (sham vs. closed exclosure),

and seed mass \times treatment. Once again, a significant seed mass \times treatment term in this model would indicate that the average seed mass of the emerging community differed between the closed and open exclosures. To correct for non-normality and heteroscedasticity, we removed one outlying species (*S. laciniatum*) and cube-root transformed the data. Regressions were only performed on data from the UNI restoration site because the exact seeding rate at KH and Graff was unknown because of the addition of a bulk mixture of hand-collected seed. All data were analyzed in Minitab v16 (Minitab Inc., State College, PA, U.S.A., 2010).

Experimental Design – Seed Recovery

To track seed fates, we conducted a seed recovery experiment at UNI using four common species in tallgrass prairie restoration: *Desmodium canadense* (L.) DC., *Elymus canadensis* L., *Eryngium yuccifolium* Michx., and *Oligoneuron rigidum* (L.) Small. These species were chosen based on ease of recovery, differences in seedling appearance, and because they represent different functional groups: grass, forb, and forb-legume. Seeds of all four species were ordered from Ion Exchange in March 2013. We coated the seeds with Glo Germ™ (Glo Germ Company, Moab, UT, U.S.A.), which is a fluorescent orange dye that glows under ultraviolet (UV) light. Previous research has shown that dyes can facilitate recovery in seed-tracking experiments (e.g. Lemke et al. 2009). Previous research in our lab determined that the dye did not inhibit seed germination (Huisman 2010).

Fifteen closed exclosures were constructed using wire hardware cloth (maximum opening 9.1 mm) with the dimensions: 1 m \times 0.5 m \times 0.05 m (width, length, height). Exclosures were installed at restrictively random positions (minimum 5 m apart, minimum 20 m from plot edge) at the UNI restoration site on 11 June, 2013. Exclosure installation coincided with the timing of restoration at this site. During installation, we removed all seedlings from the exclosure, sowed 100 seeds of each species in four 1 m long \times 1 cm deep rows, and covered the seeds with soil. Rows were spaced 10 cm apart and each species was randomly assigned to a row. Exclosures were embedded 2.5 cm into the ground and anchored with wire staples.

The 15 exclosures were randomly assigned to one of three temporal blocks (each with five replicates), which were sampled on 17 July, 11 September, and 8 November, 2013. These dates represent approximately 30 days, approximately 90 days, and approximately 150 days after sowing respectively. On each sampling date, we recorded and removed any germinated seedlings and collected 500 cm³ of soil from each row containing the nongerminated seeds. Soil was stored at 5°C prior to analysis. To recover seeds, the soil samples were first examined under UV light in a dark room and then under normal lighting. Recovered seeds were examined for evidence of partial germination. If no evidence of germination was observed, the seed was tested for viability using a 1% tetrazolium (TZ) chloride test (Patil & Dadlani 2011). Seeds were considered viable if the embryo was completely pink. The same recovery protocol was used during the 11 September and 8 November sampling times; however, we quantified seedling emergence for these sampling dates at an

earlier time in the growing season (31 July, 2013, approximately 50 days after sowing) to minimize seedling loss.

To test for natural losses in seed viability with time, 50 seeds of each species were tested for viability using the TZ test after 5 months in cold storage (5°C; Table S3).

Data Analysis – Seed Recovery

Recovered seeds were categorized as emerging seedlings, partially germinated, nongerminated/viable, or nongerminated/nonviable. We calculated the value for each fate as a percentage of the total seeds recovered for that species. Values are reported as the average of five 100-seed samples (i.e. the mean of the five replicates from each temporal block). All data were analyzed in Minitab v16 (Minitab Inc., State College, PA, U.S.A., 2010).

Results

Small Vertebrate Granivory

The number of emerging seedlings was significantly higher in the closed exclosures (33.6 seedlings/m²) than the open exclosures (24.0 seedlings/m²) across prairie restoration sites (significant treatment term, $F = 5.17$, $p = 0.03$, Table S4; Fig. 1). Seedling emergence was higher in the closed exclosures than the open exclosures at all three restoration sites: 10.4 more seedlings/m² at UNI, 16 more seedlings/m² at Graff, and 1.6 more seedlings/m² at KH (Fig. 1; see Fig. S2 for list of identified seedlings). Seedling emergence rates were 19% in the closed exclosures and 14% in the open exclosures at UNI, which was the only site in which seedling emergence rates could be quantified. Seedling emergence varied between exclosures within a site ($F = 4.37$, $p = 0.001$) but did not vary between sites ($F = 0.98$, $p = 0.40$; Table S4).

In our first regression model (percentage of seedling emergence regressed on seed mass, treatment, and seed mass \times treatment), the overall regression was not significant ($F = 1.62$, $p = 0.20$; Table S5) suggesting that seed mass, treatment, and seed mass \times treatment are poor predictors of percentage of seedling emergence. Furthermore, the nonsignificant seed mass \times treatment term ($F = 0.09$, $p = 0.77$; Table S5) indicates that the average seed mass of the emerging community did not differ between sham and closed exclosures. In our second regression model (number of seedlings/total mass of seed planted regressed on seed mass, treatment, and seed mass \times treatment), the overall regression was marginally significant ($F = 2.76$, $p = 0.06$). Furthermore, there was a significant treatment term in this model ($F = 4.57$, $p = 0.04$; Table S5), which indicates that the emerging community differed between the open and closed exclosures. However, the nonsignificant seed mass \times treatment term ($F = 1.76$, $p = 0.19$; Table S5) indicates that the average seed mass of the emerging community did not differ between sham and closed exclosures. Collectively, these results suggest that small vertebrate granivores altered the emerging community but did not preferentially consume large-seeded over small-seeded species.

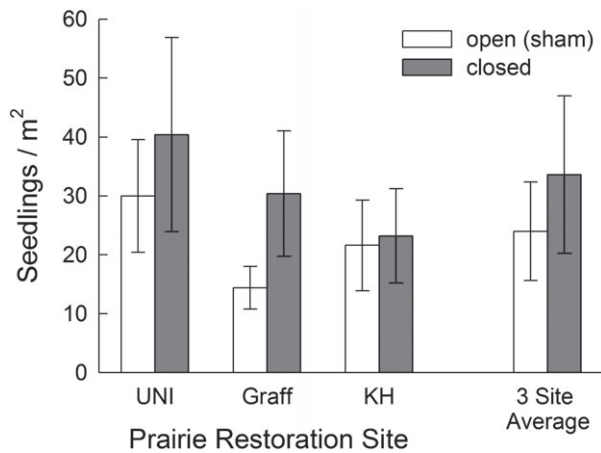


Figure 1. Number of emerging seedlings per m^2 for all perennial, planted seeds in the sham and closed exclosures at each site, as well as the average of three sites. Data are presented as means of 10 exclosures (UNI), 5 exclosures (KH and Graff), and 20 exclosures (average of three site) \pm 1 SE.

Seed Recovery

Percent seed recovery was low for all four species. On average, approximately 70–90% were lost within 30 days of sowing, 86–96% were lost within 90 days of sowing, and 81–96% were lost within 150 days of sowing. Recovery rates differed between species and tended to decrease with time in the soil. The recovery rates for each species at each time point (30 days, 90 days, and 150 days after sowing) were: *Desmodium canadense* (26.4, 11.4, 18.4%), *Elymus canadensis* (27.0, 13.6, 11.6%), *Oligoneuron rigidum* (10.2, 4.0, 3.2%), and *Eryngium yuccifolium* (25.0, 12.8, 9.2%).

The percentage of seeds in the different seed fate categories differed between species (Fig. 2). For *D. canadense*, approximately 99% of recovered seeds were emerging seedlings (Fig. 2A). For *E. canadensis*, only 7% of recovered seeds were emerging seedlings 30 days after sowing, but approximately 50% of recovered seeds were emerging seedlings 90 days and 150 days after sowing (Fig. 2B). Partially germinated and nongerminated/nongerminated/nonviable were also common seed fates for *E. canadensis*, while nongerminated/viable seeds were rarely observed more than 30 days after sowing (Fig. 2B). For *O. rigidum*, approximately 20% of recovered seeds were emerging seedlings (Fig. 2C). Nongerminated/nonviable was the most common seed fate for *O. rigidum* (approximately 50%). There was also a high percentage of partially germinated seeds 90 days after sowing, whereas nongerminated/viable seeds were not observed more than 30 days after sowing (Fig. 2C). For *E. yuccifolium*, no seeds germinated in the study and the most common seed fate was nongerminated/nonviable seeds (approximately 87%; Fig. 2D).

Discussion

In this study, we examined the impact of small vertebrate granivores on prairie restoration by comparing seedling

emergence in open (sham) versus closed exclosures at three newly restored sites. To assess other causes of seed loss, we also tracked seed fates at one prairie restoration site for a 5-month period. In summary, we found that the number of emerging seedlings was approximately 30% higher (9.6 more seedlings/ m^2) in the closed exclosures than the opened exclosures, suggesting that small vertebrate granivores reduce seedling emergence in prairie restoration. Our results suggest that granivores altered the composition of the emerging community but did not preferentially consume large-seeded species. In the seed-tracking experiment, we found that more than 70% of seeds were lost within 30 days of sowing. Taken together, our two experiments suggest that small vertebrate granivores are an important cause of seed loss in prairie restoration but that belowground and environmental factors account for a greater proportion of total seed loss and thereby represent a larger obstacle for minimizing the seed cost associated with prairie restoration.

Consistent with studies from other natural and agricultural systems (e.g. Janzen 1971; Chambers & MacMahon 1994; Howe & Brown 2000; Clark & Wilson 2003), our data suggest that small vertebrate granivores reduce seedling emergence in tallgrass prairie restoration. Exclusion of small vertebrate granivores increased seedling emergence by 9.6 seedlings/ m^2 across sites. The significance of this pattern across restoration sites suggests that granivores reduce seedling emergence in prairie restoration regardless of seed mixture or the seeding method (drill vs. broadcast seeding) used. Granivore-induced reduction in seedling emergence negatively affects prairie restoration in a variety of ways. From a financial standpoint, we estimate that the direct cost of seed loss to vertebrate granivory in a high-diversity prairie restoration is \$180–250 ha^{-1} . This value is based on the average retail price of seed for a high-diversity prairie restoration (\$3,600–5,000 ha^{-1} , Prairie Moon Nursery 2013) and the 5% reduction in seedling emergence rate that we detected at our UNI site. In addition, low seedling emergence reduces basal area coverage during the early stages of a prairie restoration. Low basal area coverage increases weed biomass in prairie restoration (e.g. Abernathy et al. 2016). High weed biomass can delay native establishment, reduce native establishment, reduce native diversity, and increase management costs in prairie restoration (Schramm 1990; Blumenthal et al. 2003).

Our results suggest that practitioners could achieve the same plant density in prairie restoration with 5% fewer seeds if protocols for excluding small vertebrate granivores could be developed. The use of feeding deterrents could be one viable option (e.g. Barnett 1998). Coating seeds with capsaicin increases seedling recruitment in the prairie forb, *Dodecatheon meadia* (Hemsath 2007). Similarly, the fungicide thiram emits a sulfurous odor, which has been shown to effectively deter birds and deer mice (Nolte & Barnett 2000; Ngowo et al. 2005). There may be challenges associated with weather-proofing the seed coats and developing methods for applying the deterrent to diverse seed mixtures with variable morphologies, but such efforts might be worthwhile, particularly for species with costly seed. The use of supplemental (sacrificial) seed is another viable option. In another experiment from our research group (Riebkens

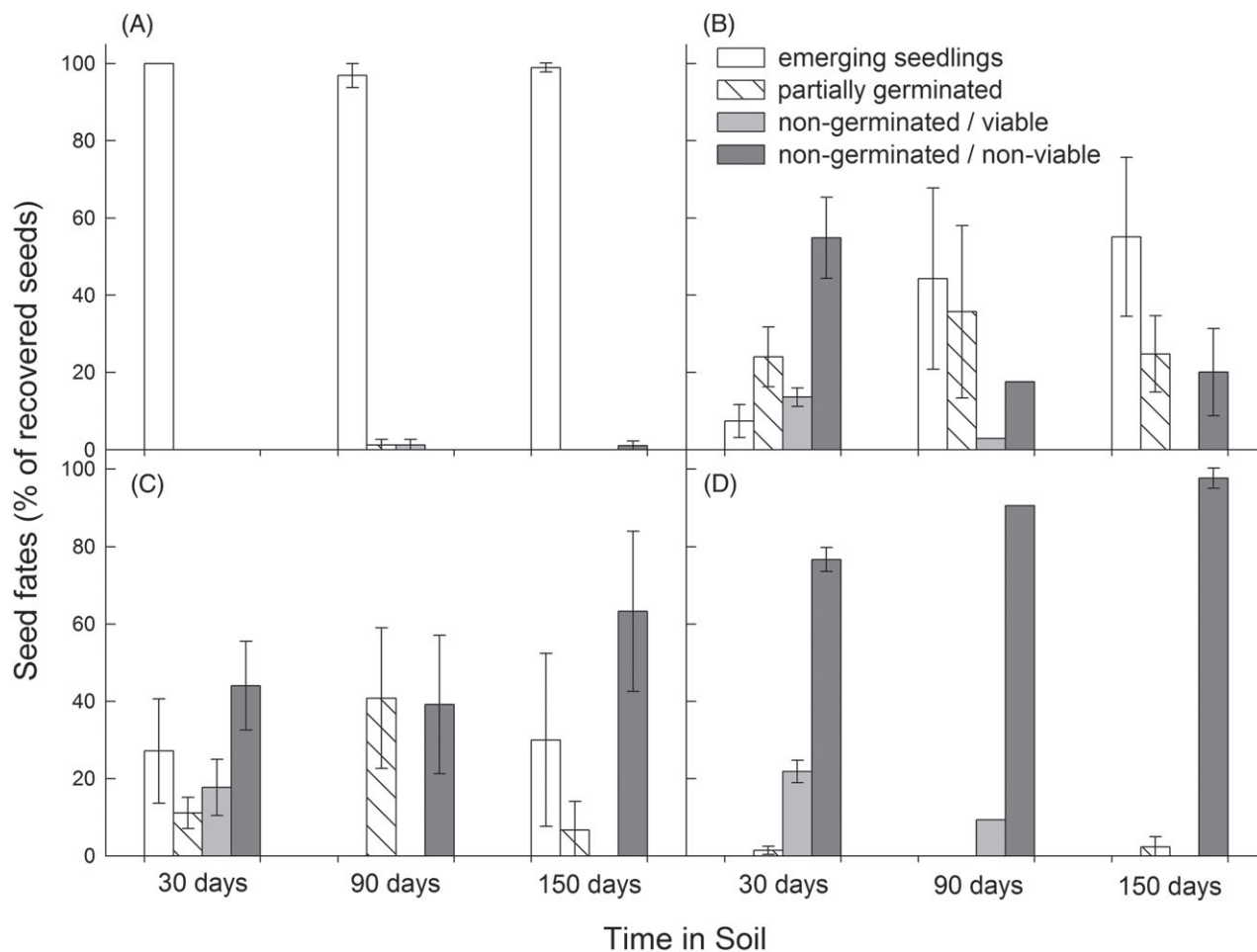


Figure 2. The fates of recovered seeds for *Desmodium canadense* (A), *Elymus canadensis* (B), *Oligoneuron rigidum* (C), and *Eryngium yuccifolium* (D) in a 5-month seed-tracking experiment. Potential fates include: emerging seedlings, partially germinated seeds, nongerminated/viable seeds, or nongerminated/nonviable seeds. Total seeds recovered (out of 500) for each species approximately 30 days, approximately 90 days, and approximately 150 days after sowing were *D. canadense* (132, 57, and 92), *E. canadensis* (135, 68, and 58), *O. rigidum* (51, 20, and 16), and *E. yuccifolium* (125, 64, and 46). Data are presented as means of five replicates of 100 seeds \pm 1 SE. SE bars omitted from the nongerminated/viable and nongerminated/nonviable categories on the approximately 90 day after sowing sampling period in panels (B) and (D) because of accidental pooling of replicates during viability testing. Emerging seedlings for the second and third temporal block were quantified on 31 July, 2013 (approximately 50 days after sowing).

2016), we found that the addition of sterilized birdseed at high concentration increases native seedling emergence in roadside prairie restorations.

In contrast to other systems (e.g. Mittelbach & Gross 1984; Edwards & Crawley 1999; Howe & Brown 1999; Howe & Brown 2000; Orrock et al. 2006, 2009; Fraser & Madson 2008), our results (the nonsignificant seed mass \times treatment term in our regression analyses) suggest that small vertebrate granivores, such as meadow voles, field mice, and birds, do not preferentially consume large-seeded species in tallgrass prairie restoration. Preferential seed consumption may be difficult to detect in prairie restorations because of their high diversity and large interspecific differences in seeding rates. For example, in our study, species with low-seeding rates often had low emergence rates regardless of seed size. This likely limited our ability to detect an overall trend of larger-seeded species establishing in the closed versus sham exclosures. Furthermore,

seed mass alone does not account for seed coat thickness or other natural feeding deterrents that also impact vertebrate feeding preference (Sih & Christensen 2001). Future experiments could test this hypothesis more directly by examining vertebrate granivory on a subset of prairie restoration species for which the seed characteristics are known and quantitatively ranked. An experiment with fewer, well-characterized species could provide more insight into the role of vertebrate seed preference and identify species with high consumption rates for which feeding deterrents should be developed and applied during restoration.

The rates of seed recovery in our seed-tracking experiment were low (10%–30%) but consistent with estimates from other in situ studies (e.g. 25–65%; Clark & Wilson 2003) and with corresponding rates of seedling emergence in prairie restoration (e.g. Williams et al. 2007; Smith et al. 2010). Although our experiment was not specifically designed to test for other potential sources of seed loss, previous research has shown that

invertebrate predation, fungal decomposition, and nonfungal disease all account for significant seed loss in natural systems (Mittelbach & Gross 1984; Clark & Wilson 2003; Heggenstaller et al. 2006). Invertebrate predation could be particularly consequential for prairie restoration. Invertebrate predators can remove up to 20% of seeds for certain species within 1 day of sowing in other systems (Mittelbach & Gross 1984; Heggenstaller et al. 2006). The high rates of seed loss that typify native tallgrass prairie restoration magnify the impact of seed loss to small vertebrate granivores. To put this into perspective, the 5% reduction in seedling emergence rates caused by vertebrate granivores corresponded to a 30% reduction in the total number of emerging seedlings (24 vs. 33.6 emerging seedlings/m²). This difference in seedling emergence could have a substantial impact on the overall success of a restoration.

Our results indicate that the fates of seeds differ greatly among species in prairie restoration, which will complicate efforts to develop a general protocol for increasing emergence. For *Elymus canadensis*, a higher percentage of seeds were recovered as emerging seedlings approximately 50 days after sowing than approximately 30 days after sowing, suggesting that this species is slow to germinate, or, that warmer temperature helps foster germination in this species (Baskin & Baskin 2001). Seed recovery rates were lower for *Oligoneuron rigidum* than the other three species. Because *O. rigidum* had the smallest seed, this species may be more vulnerable to environmental causes (wind and rain) of seed loss. Alternatively, seed recovery may have been low for *O. rigidum* because of rapid loss in seed viability and subsequent deterioration. We detected 0% seed viability after 5 months in cold storage for this species despite finding germinated seedlings and viable seeds early in the season. *Eryngium yuccifolium* had low germination rates, which may have occurred because this species did not receive its proper germination cue. *Eryngium yuccifolium* requires cold-moist stratification for approximately 60 days to break dormancy (Shirley 1994; Steffen 1997) and may not germinate without stratification (Green & Curtis 1950). Because the seed-tracking experiment was timed to coincide with site restoration (June), the seeds may not have received the necessary temperature and moisture to cue germination.

In this study, we examined the effect of small vertebrate granivores, dormancy, and loss of viability as causes of seed loss in prairie restoration. We chose to focus on these factors because the exclosures used to measure them cause fewer changes in seed microclimate than other techniques. Mesh bags or trays can be used to quantify invertebrate predation but these approaches influence soil moisture and can bias estimates of other seed fates. Similarly, some exclosure studies use fungicide to examine seed loss to fungal decomposers (e.g. Clark & Wilson 2003; Fenner & Thompson 2005; Mitschunas et al. 2006; Wagner & Mitschunas 2008) but fungicide would also eliminate beneficial mycorrhizae, which could influence emergence. We recommend that future studies use a mixed approach to examine the various causes of seed loss. This would help control the potential biases associated with each approach and provide practitioners with better data on the factors most responsible for seed loss in prairie restorations.

Native tallgrass prairie restorations are plagued by high seed costs and low rates of seedling establishment (Smith et al. 2010; Williams 2010). Our results suggest that high pre-emergence seed loss is a major cause of these low rates of establishment and that small vertebrate granivores are a significant contributor to pre-emergence seed loss. Small vertebrate granivores reduce seedling emergence rates by 5% and reduce the total number of emerging seedlings by approximately 30% in native tallgrass prairie restoration. This reduction in native emergence and native ground coverage will ultimately influence the overall quality of a prairie restoration. Developing protocols to reduce the impact of small vertebrate granivory (or any other cause of pre-emergence seed loss) should improve restoration success.

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Supporting Information

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

Figure S1. Map and images of the three prairie restoration study sites.

Figure S2. Putatively identified emerging seedlings at the three restoration sites.

Table S1. Seed mix and seeding rate at the UNI prairie restoration site.

Table S2. Seed mix and seeding rate at the KH and Graff prairie restoration sites.

Table S3. Seed viability estimates from the distributor versus in-lab seed viability estimates using TZ method after 5 months in cold storage.

Table S4. ANOVA comparing the number of emerging seedlings between restoration sites, treatments (sham vs. closed enclosure), and enclosures (within a site).

Table S5. General linear regression models examining whether small vertebrate granivores influenced the emerging community at the UNI prairie restoration site.