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Burrowing Richardson's ground squirrels affect plant seedling assemblages via environmental but not seed bank changes

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Abstract

In grassland ecosystems, burrowing mammals create disturbances, providing habitat for animal species and increasing plant community diversity. We investigated whether seedling assemblages on Richardson's ground squirrel *Urocitellus richardsonii* mounds result from seed rearrangement or environmental changes that favor germination of certain species over others. To test whether ground squirrels rearrange the seed bank by burrowing, we compared seed compositions among mounds, burrows, and undisturbed soil. To test whether ground squirrels change environmental conditions, we compared soil nitrate and bare ground cover on and off mounds. We also compared seedlings that germinated on mounds with seedlings that germinated on artificial disturbances from which we removed aboveground vegetation. Soil nitrate and bare ground cover were significantly higher on mounds than artificial disturbances. While seed richness and abundance did not differ among mounds, burrows, and undisturbed soil, seedling richness was reduced on mounds relative to artificial disturbances. Burrowing disturbance favors seedlings that can capitalize on bare ground availability but are less able to immobilize nitrate, as opposed to perennial species that immobilize more nitrate but take longer to establish. Our results suggest that Richardson's ground squirrels act as ecosystem engineers, although future research following succession on ground squirrel mounds is necessary to understand how they influence plant communities past the seedling stage.

Key words: burrowing disturbance, ecosystem engineering, Richardson's ground squirrel, seed bank, seedling, species composition

Animals that burrow fulfill an integral role in grassland ecosystems by providing services for a wide range of taxa including plants, arthropods, birds, and mammals (Davidson et al. 2012). Examples are numerous and global in scope: cape ground squirrels influence beetle and small mammal species richness in Namib desert grasslands (Ewacha et al. 2016), abandoned badger burrows in North American mixed-grass prairie provide nesting habitat for burrowing owls (Poulin et al. 2005), and Negev desert termite *Anacanthotermes ahngerianus* mounds provide nutrient-rich microsites in areas degraded by livestock (Fallah et al. 2017). In more mesic pastureland inhabited by black-tailed prairie dogs, plant community diversity improves forage quality for grazing bison (Chipault and Detling 2013) and cattle (Ponce-Guevara et al. 2016). The microsites subject to burrowing also host characteristic plant assemblages, a consequence of both foraging effects and physical changes to the soil (Wilby et al. 2001). Such phenomena are well documented in grasslands for mammals including ground squirrels (Newediuk et al. 2015), kangaroo rats (Koontz and Simpson, 2010), aardvarks (*Orycteropus afer*—Haussmann et al. 2018), pocket gophers (Yurkewycz et al. 2014), and black-tailed prairie dogs (Beals et al. 2014; Hopson et al. 2015). Understanding the mechanistic underpinnings of the ecosystem services provided by burrowing mammals promotes a more comprehensive appreciation of their ecological importance.

Ecosystem engineers physically manipulate their abiotic or biotic environments, thereby modifying the availability of resources for other species (Jones et al. 1994). Many burrowing mammal species qualify as ecosystem engineers because they make physical changes to soil structure, which influences resource availability for plants and produces unique plant assemblages. For example, digging creates bare ground that curbs rhizomatous spread by graminoids (Kyle et al. 2008; Hopson et al. 2015), increasing community diversity by allowing competitively inferior species to establish (Seifan et al. 2010). The mechanical action of digging also alters soil conditions for germinating species, increasing soil temperature (Forbis et al. 2004; Yurkewycz et al. 2014), and decreasing soil moisture (Questad and Foster 2007; Kyle et al. 2008). When used regularly for nesting or feeding, food and fecal materials accumulate at burrowing mammal disturbances, increasing nutrient concentrations when they are broken down into plant-available forms like nitrate (Eldridge and Whitford 2009). Nitrate also becomes more available when plants are buried, the result of both added organic matter reduction of root uptake (Canals et al. 2003; Seifan et al. 2010). These flushes of plant-available nitrogen can either suppress germination (Kitajima and Tilman 1996), or stimulate succession after species establish: availability of nutrients like nitrate first favors fast-growing species, which are later displaced by more competitive, slower-growing species as nitrogen is immobilized by plant tissues (Paschke et al. 2000). Both the direct effects of burrowing and indirect effects of burrowing on soil conditions act in concert to create microsites for plant establishment, increasing species richness (Wilby et al. 2001).

An alternative line of evidence suggests that burrowing mammals predispose their disturbances to colonization by certain species. For example, some granivorous burrowing mammals, particularly those that cache seeds, sequester seeds of certain species at their mounds (Koontz and Simpson, 2010). In other instances, burrowing mammals feed on the tubers of perennial plants and limit their establishment (Wilby et al. 2001). Even without selective foraging, burrowing creates distinctive features on the landscape that act as seed traps (Farji-Brener and Ghermandi 2004; Koontz and Simpson 2010; Valentine et al. 2017), and exposes seeds from the persistent seed bank to the soil surface where they are more likely to germinate (Bueno et al. 2011). When both seed bank characteristics and environmental conditions are measured, it is possible to determine which is responsible for plant establishment on burrowing mammal disturbances (Forbis et al. 2004).

Despite their demonstrated role as ecosystem engineers, anthropogenic landscapes inhabited by burrowing mammals are rife with human-wildlife conflict (Smith and Foggin 1999; Bergstrom et al. 2014). Much of this conflict stems from perceived economic loss in agricultural landscapes; in the United States, producers in many regions cite burrowing mammals as a substantial source of agricultural losses (Wywialowski 1994). The Richardson's ground squirrel *Urocitellus richardsonii* is a key player in the conflict in the Northern Great Plains region because of its affinity for agricultural habitats (Calder 2003). Richardson's ground squirrels range from Manitoba, Canada, to Montana, where they take advantage of the short vegetation characteristic of pastureland. Their matrilineal colonies consist of a dense burrow network, each of which is periodically excavated to produce a mound of soil at the entrance (Quanstrom 1971). Particularly where they inhabit pastureland, species like the Richardson's ground squirrel are also persecuted for their perceived competition for forage with livestock (Davidson et al. 2012).

Our objective in this study was to determine the mechanism by which Richardson's ground squirrels manipulate their pastureland environment to favor the establishment of certain plant species on their mounds. Despite evidence that burrowing mammals manipulate plant communities (Questad and Foster 2007; Newediuk et al. 2015) and seed banks (Koontz and Simpson 2010), few studies have investigated plant species differences on and off disturbances simultaneously at both the seed and seedling stages (but see Forbis et al. 2004). This is a logical next step in understanding how burrowing mammals are able to produce and maintain unique plant assemblages. Here, we tested whether seed bank characteristics or environmental conditions are responsible for unique seedling assemblages on Richardson's ground squirrel mounds (hereafter "mounds"). We extracted seeds from seed banks in mounds, burrow soil, and undisturbed soil and compared the plant species therein in a greenhouse seed emergence experiment. In the field, we paired mounds with artificial disturbances from which we removed aboveground vegetation and monitored seedling establishment and environmental conditions over a growing season.

(H1) Under our seed bank characteristics hypothesis, we hypothesized that as ground squirrels burrow, they manipulate the composition and density of the seed bank at the mound surface. Thus, we predicted that composition of the mound seed bank and seed bank from soil within the burrow would differ from the seed bank in undisturbed soil (Prediction 1A), and that mound seed bank composition would resemble seedling assemblages on natural ground squirrel mounds (Prediction 1B). Because ground squirrels burrow up to a depth of 2 m (Michener and Koeppl 1985) and deeper soil is often seed-deficient (Forbis et al. 2004), we also predicted that there would be fewer seeds in mound seed banks (Prediction 1C).

(H2) Under our environmental characteristics hypothesis, we hypothesized that the environment on mounds is unique, providing appropriate conditions for germination and growth of some species but not others. Thus, we predicted that seedling assemblages on mounds would also be different from artificial disturbances, despite no differences in seed bank composition (Prediction 2A). We further predicted that mounds would be colonized by fewer species overall (Prediction 2B), and of those species a greater proportion would be annual plants (Prediction 2C), which are often colonizers of burrowing mammal disturbances owing to their rapid growth and otherwise inferior competitive ability (Forbis et al. 2004; Bueno et al. 2011). Finally, we predicted that soil nitrate and bare ground cover would be higher on mounds than artificial disturbances as ground squirrels eliminate vegetation and deposit nutrient-rich materials on mounds (Prediction 2D).

Materials and Methods

Study system

Richardson's ground squirrels are socioeconomically important and provide an ideal model for studying the effects of burrowing on pastureland plants. Ground squirrels in Manitoba, Canada are active above ground for 3–7 months of the year depending on the sex and age class of the individual, geography, and soil and air temperature at emergence (Michener and Koeppl 1985). Females are reproductively mature after their first hibernation at age 1, typically producing 1 L of 4.9–8.3 young per year, with emergence of juveniles from the burrow commencing 26–33 days after birth (Michener and Koeppl 1985). The resulting spatial and seasonal concentration of burrowing and foraging in colonies has important implications for

agricultural production. First, colony members create dense burrow networks, visible at the surface as openings up to 20 cm in diameter with mounds of excavated soil at the burrow entrance (Quanstrom 1971). Individuals reportedly also consume seeds of cereal crops and forage grasses and legume species when available, although few studies explicitly report crop damage by Richardson's ground squirrels (Michener and Koeppl 1985). The species is also of trophic importance where it occurs in prairie ecosystems, serving as a key prey item for predators such as the ferruginous hawk (*Buteo regalis*—Schmutz and Hungle 1989) and American badger (*Taxidea taxus*—Proulx and MacKenzie 2012). Nonetheless Richardson's ground squirrels are regarded as an agricultural pest, and much research has focused on developing effective methods to control colonies on both cropland (Johnson-Nistler et al. 2005) and pastureland (Proulx et al. 2011).

Study location

The study area intersects the Aspen Parkland and Lake Manitoba Plain ecoregions, characterized by gently rolling open grassland punctuated by hardwood forest and small, seasonal wetlands. Mean annual precipitation in that area is approximately 550 mm, with most falling as rain in June and July, and average air temperatures ranging from 18.5°C in July to −18°C in January. We haphazardly selected 5 active colonies within pastures (hereafter "study sites") by visually searching along municipal roads. Study sites ranged in size from ∼0.4 to 0.8 ha and were located at least 2 km from the nearest neighboring study site. All study sites had poorly drained alluvial loam soils, with very little visible variation in soil type within sites. Dominant forage species at all sites included *Elymus repens* (L.) Desv. ex B. D. Jackson, *Bromus inermis* Leyss., and *Poa pratensis* L. Forage legumes including *Medicago lupulina* L. and *Trifolium repens* L., while naturalized introduced species such as *Taraxacum officinale* G. H. Weber ex Wiggers, were also common. We estimated ground squirrel density at between 4 and 30 individuals/ha at each study site, using the playback survey method developed by Downey et al. (2006), which assumes an approximate 50% detection rate. Two study sites (Victoria S site: 49° 36′ 7″ N, 99° 35′ 51″ W; and Westbourne site: 50° 10′ 5″ N, 98° 30′ 8″ W) were grazed continuously by cattle, 2 study sites (Victoria N site: 49° 33′ 47″ N, 99° 6′ 18″ W and Cypress site: 49° 33′ 9″ N, 99° 8′ 30″ W) were rotationally grazed by cattle, and one site (Grey site: 49° 52′ 48″ N, 98° 0′ 36″ W) was grazed continuously by horses.

Seed bank emergence experiment

To test the hypothesis that ground squirrel burrowing affects seed bank distribution, we compared seed banks between mound soil, burrows, and soil collected from the area surrounding the mound, in a greenhouse emergence experiment. Between 8 and 11 May 2013, we collected freshly excavated soil from 12 haphazardly selected mounds at each site, paired with a sample from undisturbed soil within 30 cm of the mound. We collected soil samples from the mounds and undisturbed soil to a depth of 10 cm. We also collected approximately the same amount of soil from the surface of the burrow entrance. We included the burrow samples assuming that if ground squirrels move seeds to the surface, burrow seed banks should be more similar to mound seed banks than undisturbed soil. We prepared the soil samples by air drying and reducing them to a standard weight (30 g), then mixed them with potting soil in nursery pots (Ter Heerdt et al. 1996). Although we expected the addition of potting soil to influence germination and thus comparison of seed bank composition to seedling assemblages in the field, its influence

was equivalent among samples from burrows, mounds, and undisturbed soil. Finally, we transferred the nursery pots to gardening trays for germination in the greenhouse.

From 17 June 2013 to 28 August 2013, we identified and recorded the cumulative number of seedlings from each species that germinated. We identified a total of 24 species, including 9 annuals and 6 perennials (Table A1). The remaining 9 unique species (41%) could not be identified, either because they lacked flowering material or died before maturity. If a species was unique but could not be identified, we still included it in the total species richness for that sample but not in the number of annual or perennial species. As seed bank estimation techniques rarely achieve comprehensive germination of all species (Espeland et al. 2010), we are confident that our seed bank characterization was consistent with those from similar studies.

Seedling comparison in field conditions

To test the hypothesis that the mound environment provides unique conditions favorable to some plant species, we compared seedlings that germinated on mounds with seedlings that germinated on artificial disturbances at our study sites. The purpose of the artificial disturbances was to isolate the specific effects of ground squirrel digging. We removed established plant shoots from artificial disturbances to mimic the full sun and open space for germination that are typical of mounds, but we did so without disturbing the soil. Thus, by comparing mounds and artificial disturbances we were able to discern whether any differences in seedling assemblages were caused by the lack of vegetation or disturbance to the soil resulting from digging.

In preparation for in-field comparisons, we haphazardly selected 6 escape burrows (generally of much larger diameter than the girth of an adult squirrel) with mounds at each study site on 29 and 30 May 2012. Due to a lack of activity at one study site we were only able to locate 5 active burrows, reducing our sample size to 29 burrows with mounds among 5 sites. We ensured we only recorded seedlings from a single season by selecting burrows with recent excavation (i.e., minimal or no surrounding vegetation, loose soil at entrance). To create the artificial disturbances, we measured a rectangle of identical dimensions to the mound beginning 0.5 m to the east of each mound and treated the vegetation with a homemade contact herbicide containing 30 parts 5% acetic acid to one-part dishwashing detergent. Acetic acid is not toxic to livestock and is effective for control of broadleaf weeds with up to 100% mortality 4 weeks following application. Because acetic acid is only approximately half as effective for grass control as it is for broadleaf weeds (Abouziena et al. 2009), we could not completely eliminate the regrowth of grasses from rhizomes on the artificial disturbances. However, no forbs remained on the disturbance pairs by 4 weeks following herbicide application. Given our observations and the reported effectiveness of acetic acid for broadleaf species (Abouziena et al. 2009), we are confident that any subsequent growth of forb species was from seed. One week after herbicide application on 4 and 5 June 2012, we cut all vegetation at the soil surface, removed all litter from the mound and artificial disturbances, and then reapplied the herbicide treatment to control any remaining grasses that were not killed upon the initial application. To control for the effect of acetic acid on germination, we also sprayed the adjacent mounds.

We delineated a 0.25 m × 0.25 m square plot on each mound and artificial disturbance on which to monitor seedling germination through the growing season. From 26 to 30 June 2012, 16 to 17 July

2012, and 8 to 9 September 2012, we recorded the cumulative number of seedlings that germinated on the plots. We identified 4 annual forb species, one annual grass species, 8 perennial forb species, and 4 perennial grass species (Table A1). An additional 2 seedlings (13%) could not be identified.

Environmental characteristics

From 26 to 30 June 2012, 16 to 17 July 2012, and 8 to 9 September 2012, we scored percent bare ground cover on each of the disturbance pairs using an increasing ordinal scale from 1 to 6, each number corresponding to >1%, 1–5%, 5–25%, 25–50%, 50–75%, and >75% cover, respectively. We combined bare ground cover scores at each disturbance pair over the 3 sampling periods to obtain a season-long average. During the June sampling period we also extracted soil for nitrate analysis. We combined 4 10 cm soil cores from each mound and artificial disturbance and air dried them to a constant weight. We ground the samples with a mortar and pestle until they passed through a 2 mm sieve, transferred 5 g to specimen cups, and mixed them with 25 ml of 2 N KCl to extract the nitrate. After shaking the samples for 1 h at 200 rpm, we transferred the clear phase to scintillation bottles. We then measured nitrate concentration in each sample using the Automated Cadmium Reduction Method (Clesceri et al. 1998).

Data analysis

To describe whether ground squirrels manipulate the composition and density of seeds in mound soil (Predictions 1A and B), we compared seed composition in burrows, from mounds, and from undisturbed soil using non-metric multidimensional scaling (NMDS). NMDS calculates scaled dissimilarities among sampling locations or objects based on variables measured on those objects (Quinn and Keough 2002). In our study, we calculated dissimilarities in seed abundance measured on mounds, burrows, and artificial disturbances. We also used NMDS to compare seedling assemblages between mounds and artificial disturbances (Prediction 2A) and evaluated whether differences in seedling assemblages corresponded to differences in seed bank composition between mound and artificial disturbances. In both comparisons, we used analysis of similarity (ANOSIM) to test whether dissimilarities in species composition among burrows, mounds, and artificial disturbances were significant, followed by similarity percentages (SIMPER) to determine which species contributed most to those dissimilarities (Quinn and Keough 2002). We performed all NMDS analyses using the *vegan* package (Oksanen et al. 2019), calculating dissimilarities with the Bray Curtis index. We also used Fisher's exact tests to compare the number of seeds, seedlings, seed species richness, and number of annual and perennial seeds and seedlings among mounds, artificial disturbances, and burrows (Predictions 1C and 2B–2C). To test whether ground squirrels change environmental conditions on mounds (Prediction 2D), we compared percent bare ground cover and soil nitrate concentration between mounds and artificial disturbances using one-way analysis of variance (ANOVA), log transforming soil nitrate prior to analysis to achieve a normal distribution of the residuals. We performed all analysis in R version 3.5.2 (R Core Team 2019).

Results

Seed bank composition did not differ among mounds, burrows, and undisturbed soil (ANOSIM, $R = 0.02$, $P = 0.13$). However, seedlings that germinated on mounds were significantly different from those on artificial disturbances, exhibiting clear shifts in species assemblages along the first NMDS axis (ANOSIM, $R = 0.08$, $P = 0.004$). The perennial species *P. pratensis*, *E. repens*, *B. inermis*, and *T. officinale* contributed most to the dissimilarities in seedling composition between mounds and artificial disturbances

(SIMPER cumulative contributions = 0.29, 0.55, 0.64, and 0.73, respectively), a pattern which was also reflected in the significantly larger number of perennial seedlings on artificial disturbances compared with mounds (Figure 1; Fisher's exact test $P = 0.03$). Similarly, there were fewer seedling species in total on mounds than artificial disturbances (Fisher's exact test $P = 0.04$), although the higher abundance of annual seedlings on mounds relative to artificial disturbances fell short of statistical significance (Figure 1). The unique seedling assemblages on mounds were mirrored by significantly higher soil nitrate concentrations in mound soil ($F_{1,52} = 14.1$, $P < 0.001$) and higher bare ground cover on mounds than artificial disturbances ($F_{1,52} = 14.1$, $P < 0.001$; Figure 2) that persisted through the growing season.

Although seedling assemblages, total number of seedlings, and number of perennial seedlings differed significantly between mounds and artificial disturbances, we did not find the same differences in the seed bank. Many species that appeared in the seed bank did not germinate in the field: only 6 of 38 species appeared both in the seed bank and on disturbance plots in the field (Table A1). The total number of seeds obtained from burrow soil samples did not differ significantly from the number of seeds obtained from soil samples derived from mounds or undisturbed areas (Figure 1).

Discussion

We investigated whether Richardson's ground squirrels contribute to the formation of unique plant assemblages on their mounds either by manipulating the arrangement of the seed bank or changing environmental conditions as they create soil mounds through burrowing. We compared seed bank composition and seedling assemblages between natural ground squirrel mounds and artificial disturbances from which we removed only the aboveground vegetation. Seed bank composition did not differ between mounds and undisturbed soil, and both were similar to seed bank composition in burrows, suggesting that the types of seeds moved to the soil surface by ground squirrels are not unique. Further, the fact that the total number of seeds recovered from burrow soil samples did not exceed that from mounds or undisturbed areas runs counter to the notion that seeds are transferred from deeper soil to the mound surface by ground squirrel burrowing. Despite this, assemblages of seedlings that germinated did differ between mounds and artificial disturbances, with dissimilarities primarily driven by the prevalence of 4 perennial species that germinated on artificial disturbances but not mounds. Taken together, our results support our environmental characteristics hypothesis but not our seed bank characteristics hypothesis, and suggest that ground squirrels are ecosystem engineers that favor the establishment of certain species through burrowing that alters resource availability.

We found no evidence that ground squirrels reduce the abundance of seeds in their mounds. In our study, neither the total number of seeds, the number of species in the seed bank, nor the number of annual and perennial seeds differed between mounds and undisturbed soil, which contrasts with observations of fewer seeds in other burrowing mammal mounds when they selectively move seed-deficient soil to the surface (Forbis et al. 2004). In fact, we found that seed abundance in burrow soil did not differ from either mound
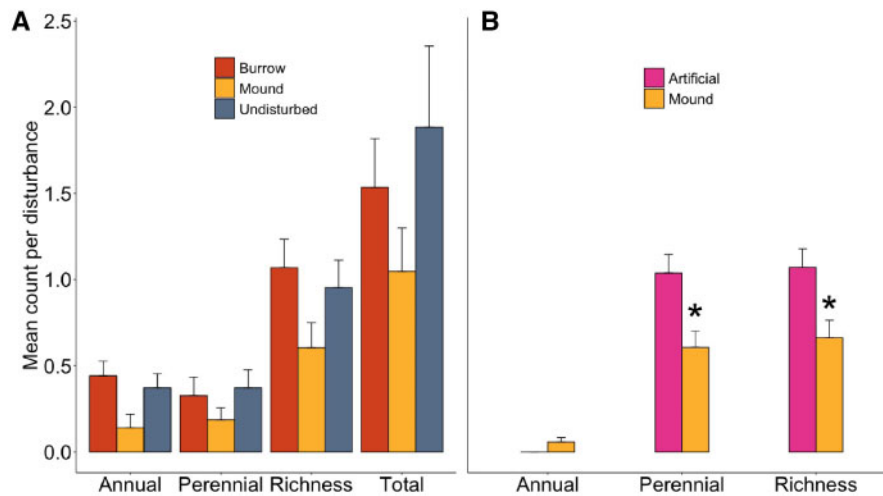
Figure 1. Mean counts of annuals, perennials, mean total number, and mean species richness. Seed counts are compared among burrows, mounds, and undisturbed soil (**A**), and seedling counts are compared between mounds and artificial disturbances (**B**). Error bars represent the standard error of mean counts. Bars with asterisks are significantly different according to Fisher's exact test ($P < 0.05$).
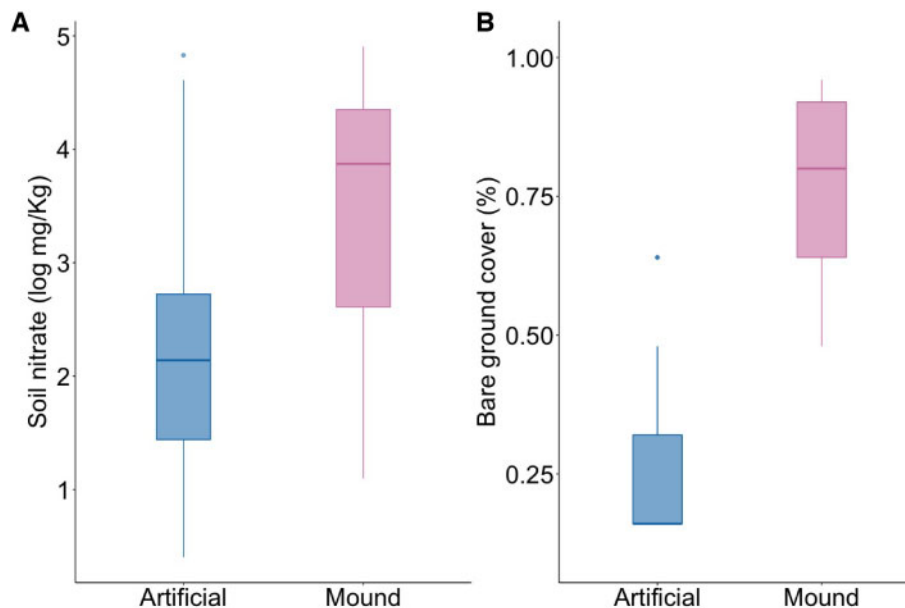

Figure 2. Boxplots (where bold line is the median, box is the interquartile range, whiskers are the minimum and maximum, and dots are outliers) comparing (**A**) soil nitrate concentration, and (**B**) percent bare ground cover on mounds (MD) and artificial disturbances (AF). All differences between mounds and artificial disturbances are significant according to one-way ANOVA ($P < 0.05$).

soil or undisturbed soil, suggesting that if ground squirrels are indeed excavating soil with fewer seeds, then seeds are replenished in the mound and burrow entrance following burrow formation. Mound morphology provides one mechanism by which seed banks can be selectively replenished at burrowing mammal disturbances. Disturbances of many burrowing mammals are prominent features on the landscape, and the conical shapes of ant refuse dumps (Farji-Brener and Ghermandi 2004), kangaroo rat mounds (Koontz and Simpson 2010), and bandicoot foraging pits (Valentine et al. 2017), for example, act as seed traps. In our study, fewer seeds in mound soil may be offset by their tendency to collect more seeds than the surrounding landscape. With no net change in seed abundance in mound seed banks, we found no evidence to suggest that Richardson's ground squirrels influence germination by rearranging the seed bank.

Despite similarities in the seed bank between mounds and undisturbed soil, seedling establishment on mounds was clearly influenced by factors beyond seed bank characteristics, supporting our environmental characteristics hypothesis. Both seedling richness and the number of perennial species were lower on mounds as predicted, despite no differences in seed banks between mounds and undisturbed soil. Moreover, seedling assemblages on mounds were different from artificial disturbances. It is likely that the absence of perennial rhizomes in mound soil allowed less competitive species to capitalize: perennial grasses including *E. repens* (L.) Gould, *B. inermis* Leyss., and *P. pratensis* L. were scarce on mounds when compared with artificial disturbances where they quickly reestablished. Because of the reduction of perennial species on mounds, bare ground cover remained consistently high while declining on

artificial disturbances through the season. In contrast to mounds that are created by depositing an additional layer of soil on top of the existing vegetation, when creating our artificial disturbances we removed only the aboveground vegetation. This left the rhizomes of perennial species to persist on artificial disturbances. In other systems, such pre-emptive establishment gives a competitive advantage to perennial species over colonizing annual species (Eldridge and Whitford 2009; Seifan et al. 2010). The differences in seedling composition we observed between disturbances in our study suggest that Richardson's ground squirrels caused a loss of dominance by perennial species on mounds, allowing species to grow that would normally be much less abundant.

While perennial species were significantly less abundant on mounds than artificial disturbances, we did not find a corresponding increase in the relative abundance of annual species on mounds. This may be because mound-colonizing species fulfill the same ecological role as annual species, but they are unique to sites according to the overall composition of the local plant community. Variety in the identities of mound-colonizing species is typical of tall-grass prairie (Rogers et al. 2001), montane meadows (Jones et al. 2008), and even urban environments (Beals et al. 2014). In our study, we found that *Solanum triflorum* Nutt. was common on disturbances at 2 of our western-most sites, Victoria N and Cypress, while *Amaranthus retroflexus* L. was also common on disturbances at Cypress but was not present in the community at Victoria N. To make the identities of mound colonizers even more difficult to standardize, even short-lived perennial forbs can fulfill the role of mound colonization at some sites (Questad and Foster 2007). We also found seedlings of *Artemisia absinthium* L., a perennial forb, exclusively on disturbances at the Grey site although it appeared in the seed bank at other sites. We suggest that any disturbance-adapted species could have capitalized on mound disturbances regardless of lifeform, but species filling this role are few and site-specific. Because burrowing mammals are present in diverse ecosystems from urban sites (Hopson et al. 2015) to desert (Koontz and Simpson 2010) to grassland (Seifan et al. 2010), their propensity to increase the abundance of annual species on their mounds will depend on the relative abundance of annual species in local species pools.

Elevated soil nitrate concentration on mounds may provide another explanation for differences in seedling composition. Other studies have also found elevated nitrate concentrations in the soil of burrowing mammal disturbances (Canals et al. 2003), and the resulting effects on plant assemblages are notable, affecting colonization at the seedling stage. For example, Kitajima and Tilman (1996) found that higher nitrate concentration magnified differences between seed bank composition and seedling assemblages because nitrate directly suppressed germination. Alternatively, higher nitrate concentration in mound soils could be the result of less nitrogen immobilization by early colonizers, which typically colonize bare ground. During succession, slower-growing perennials take over dominance when nutrient uptake by early-seral species can no longer support their rapid aboveground growth (Paschke et al. 2000). This finding is typical for disturbances that lack vegetation and receive continuous nutrient inputs from burrowing mammals: Canals et al. (2003) reported 10-fold higher nitrate concentrations on pocket gopher mounds in an annual California grassland, a dramatic difference from the surrounding vegetated landscape. In our study both elevated soil nitrate and changes in plant assemblages were likely the joint outcomes of reduced abundance of perennial graminoids on mounds. Longer-term observations of mound succession will be needed to determine whether the unique species assemblages

on mounds persist past the seedling stage or are displaced by perennial graminoids as colonization proceeds and nitrogen is gradually immobilized (Paschke et al. 2000). However, at least for seedlings, the temporary increase in soil nitrate appears to facilitate the germination of some species over others.

As has been reported for other systems involving burrowing mammals, Richardson's ground squirrels indirectly manipulate plant communities through burrowing. Independent of seed bank composition, the delay in recolonization of mounds by grasses also allows some species to grow that would normally be unable to persist (Questad and Foster 2007; Forbis et al. 2004; Kyle et al. 2008; Case et al. 2013). Our findings have pronounced ecological implications: burrowing mammals improve plant community diversity by acting as refuges for locally uncommon species like introduced forbs (Beals et al. 2014). This also has the potential to occur in our system since ground squirrels reduced perennial graminoid abundance in comparison to surrounding vegetation (see also Newediuk et al. 2015). On the other hand, burrowing mammal disturbances also permit species invasion if the invading species are effective colonizers of those disturbances (Hopson et al. 2015; Haussmann et al. 2018). While we demonstrated that the seed bank is less influential for seedling assemblages than environmental conditions on mounds, we did not test whether the mound environment or seedling assemblages persist past the seedling stage. We suggest that future studies compare seed bank composition to burrowing mammal disturbances through to later stages of colonization, and comprehensively compare soil conditions (i.e., moisture, temperature, solar radiation, and availability of other nutrients) associated with those disturbances so as to identify the factors contributing to their unique plant assemblages. In summary, our findings suggest that Richardson's ground squirrels are ecosystem engineers that alter resource availability for germinating plants through mound creation, which is responsible for the species assemblages typically observed on those disturbances.

Author Contributions
Both L.J.N. and J.F.H. conceptualized the study and provided comments on the manuscript. L.J.N. collected the data and prepared the manuscript.

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Appendix

Table A1. List of all species identified at 5 sites (grey, cypress, Victoria N, Victoria S, and Westbourne): "S" indicates that species were found in the seed bank, and "D" and "U" indicate that species were observed in the field, either on mounds or artificial disturbances as seedlings (D), or growing outside of disturbances (U)

Species		Site				
		Grey	Cypress	Victoria N	Victoria S	Westbourne
Annual forbs (Total=11)						
Amaranthus retroflexus L.	Exotic		USD		SD	
Axyris amaranthoides L.	Exotic				U	
Brassica rapa L.	Exotic		S		US	
Capsella bursa-pastoris (L.) Medik.	Exotic		D			
Chenopodium album L.	Exotic	S	S	S	S	S
Lappula occidentalis (S. Wats.) Greene	Native		UD			
Malva neglecta Wallr.	Exotic		US			
Medicago lupulina L.	Exotic	US			U	US
Senecio vulgaris L.	Exotic	S	S	S	S	S
Solanum triflorum Nutt.	Exotic		SD	D		
Sonchus arvensis ssp. ugilinosus (Bieb.) Nyman	Exotic					S
Annual grasses (Total=2)						
Eragrostis cilianensis (All.) Lut. ex Janchen	Exotic		UD			
Setaria glauca (L.) Beauv.	Exotic				S	S
Perennial/biennial forbs (Total=20)						
Achillea millefolium L.	Native	U				U
Arctium sp.	Exotic				U	
Artemisia absinthium L.	Exotic	DS	S			S
Artemisia frigida Willd.	Native	U				
Cirsium arvense (L.) Scop.	Exotic	U	U	UD	UD	USD
Cirsium flodmanii (Rydb.) Arthur	Native				U	U
Descurainia incana (Bernh. ex Fisch. and C.A. Mey.) Dorn	Native		U			
Erigeron philadelphicus L.	Native		U			
Grindelia squarrosa (Pursh) Dunal	Native	U			U	
Plantago major L.	Exotic	USD	S	S	USD	S
Potentilla norvegica L.	Native	S	S			S
Rudbeckia hirta L.	Native			U		
Rumex crispus L.	Exotic	U	D	U	U	
Solidago canadensis L.	Native		U		U	
Symphoricarpos occidentalis Hook.	Native				U	U
Symphyotrichum ericoides (L.) Nesom	Native	UD				
Symphyotrichum sp.	Native		D		U	U
Taraxacum officinale G.H. Weber ex Wiggers	Exotic	U	U	U	U	UD
Trifolium repens L.	Exotic	USD	U	U	U	USD
Vicia americana Muhl. ex Willd.	Native					U
Perennial/biennial grasses (Total=5)[a]						
Agropyron cristatum (L.) Gaertn.	Exotic	U				
Bromus inermis Leyss.	Exotic	UD	U	U	U	U
Elymus repens (L.) Gould	Exotic	USD	USD	USD	USD	USD
Phleum pratense L.	Exotic	UD		U	U	U
Poa pratensis L.	Native	UD	UD	UD	UD	UD
Total		20	24	17	23	24

[a] Includes vegetative growth from rhizomes, as these stems were indistinguishable from seedlings for rhizomatous species.