

BURROWING HERBIVORE, PRECIPITATION, AND PLANT COMMUNITY EFFECTS ON  
INVASIVE GRASS GERMINATION

By

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## **Abstract**

Species invasions have become a problem of global concern because of their negative impacts on native ecosystems. In the Carrizo Plain of California, the invasive grass *Hordeum murinum* has become the focus of management because it threatens native grassland species. Previous studies have illustrated how resource availability and burrowing herbivore activity affect the growth of this grass, but more information on its germination is needed in order to obtain a full picture of its population dynamics. Seedbank samples were collected in different field conditions and then brought into the greenhouse to assess germination. Experimental field manipulations allowed evaluation of *Hordeum* germination in response to Giant Kangaroo Rat (*Dipodomys ingens*) burrowing and foraging activity, precipitation, and plant community cover. Presence of burrowing and increased precipitation resulted in an increase in the abundance of *Hordeum* germinants. The presence of Giant Kangaroo Rat foraging activity increased the abundance of *Hordeum* germinants across seasons. In addition, more *Hordeum* germinants grew when this trophic activity was absent and after an entire summer of dormancy had passed, which leads to further questions regarding seed conditioning. Relative abundance of *Hordeum* germinants had a linear, negative relationship with total plant cover in the field, suggesting competitive effects of the plant community on germination. The results from this study will contribute to a larger project developing population models for invasive grasses in order to understand what limits population growth and to aid management decisions.

## **Introduction**

Nonnative, or “invasive”, species are increasingly being found in ecosystems throughout the world. The effects of invasive species are often undesirable, such as causing the decline of

native species (Brooks 2003; DeFalco et al. 2003; Going et al. 2009), and therefore have become the focus of much management concern. Interactions with native organisms have the potential to control invasive species or assist their growth and spread (Shea and Chesson 2002). Appropriate and effective conservation and land management depends on in-depth knowledge of the ecological interactions between native and invasive species.

Species invasions are particularly impactful in California grasslands, such as the Carrizo Plain National Monument, which is a major concern because of grasslands' significant economic and ecological resources. California grasslands are some of the most heavily invaded ecosystems in North America (Mordecai et al. 2015). Nonnative annual grasses, which are grasses that germinate, grow, reproduce, and die all within one season, were introduced from Europe in the 18<sup>th</sup> century and have become dominant species in California (Schiffman 1994; Mordecai et al. 2015). Carrizo Plain, the largest remnant of San Joaquin Valley grassland, is no exception to these invasions. However, it is one of the few remaining pieces of grassland in California that has a significant population of native plants. In addition, it is home to several species that are endangered and found nowhere else, that is, endemic. Carrizo Plain also provides services such as ranchland, tourism, and Native American heritage sites. Therefore, management of invasive species is of great concern in Carrizo Plain and more broadly across California.

One invasive species that poses particular problems to Carrizo Plain and other California grasslands is *Hordeum murinum* (hereafter, *Hordeum*) (Figure 1). This is one of the annual grasses introduced from Europe for cattle forage, and is adapted for growth in a Mediterranean climate (Germano et al. 2001). Before European settlement, California grasslands had patchy bunch-grasses and plenty of open space; now, the landscape is dominated by thickly-growing, non-native, annual grasses, like *Hordeum* (Germano et al. 2012). These invasive species are of

concern because they compete with and threaten the success of native plant species (Brooks 2003; DeFalco et al. 2003; Going et al. 2009). Growth of these invasive species shifts the vegetation structure to dense grassland, which could hinder travel efficiency and predator avoidance for small terrestrial vertebrates that are adapted to open desert habitats, like lizards and kangaroo rats (Germano et al. 2001). Some possible management strategies for the grasses include livestock grazing, prescribed fire, mechanical removal, and chemical herbicides; all of these, except grazing, are costly and can cause damage to native species as well as invasives (Germano et al. 2001). Grazing has been shown to be reasonably effective at reducing invasive grass cover and promoting populations of native vertebrates (Germano et al. 2012), and other types of herbivory may also be effective at controlling invasive grasses.

Despite the issues *Hordeum* causes, its management at Carrizo Plain is not straightforward because of interactions with the native rodent species *Dipodomys ingens*, the Giant Kangaroo Rat (hereafter, GKR) (Figure 2). The GKR is endangered, but is endemic to and locally abundant in central California (Schiffman 1994). GKR interacts with invasive grass species through trophic, i.e. feeding, activities and soil engineering, i.e. burrowing, activities (Prugh and Brashares 2012). GKR are granivores, or seed eaters; they preferentially select large seeds to cache (Schiffman 1994; Gurney et al. 2015). They also create large underground burrows that disturb the surrounding 7-10m of soil (Prugh and Brashares 2012; Davidson et al. 2008). These burrows have been shown to increase nitrogen availability for plants (Gurney et al. 2015), and when precipitation is high the invasive *Hordeum* can grow abundantly on the burrows (Grinath et al. In Prep). Because the GKR is high on the list for conservation priority, its positive impact on *Hordeum* creates a management dilemma. Furthermore, precipitation and plant community can have large and conflicting effects on invasive grass germination. High precipitation generally

increases invasive grass success (Grinath et al. In Prep; Ochoa-Hueso and Manrique 2010), while native grasses can significantly increase community resilience to invasive grasses by increasing competition (DeFalco et al. 2003; Going et al. 2009).

Considering all the factors that affect the growth of invasive grasses in the Carrizo Plain, modeling population growth and predicting changes in the invasive grass populations can greatly inform management decisions. A larger project on the Carrizo Plain is testing whether GKR help to control invasive grass populations in order to inform successful management of the grassland (Principal Investigator: K. Suding; Postdoctoral Researcher: J. Grinath). Results from this study are an integral part of a larger dataset that is going to be used to create a predictive model about the growth and spread of invasive grass species in Carrizo Plain. In order to complete this model, there is a need for germination data. This study aims to fill this need by conducting an in-depth germination experiment with seedbank samples from Carrizo Plain, and using *Hordeum* germinant data to draw conclusions on the types and strengths of germination drivers.

My focus is on GKR, precipitation, and plant community effects on germination of the invasive grass *Hordeum*. There are two main pathways through which *Hordeum* germinants can be affected by field conditions. The first is through seed input. Field conditions that affect adult *Hordeum* will consequently affect the seeds that enter the seedbank, and so will impact the abundance of *Hordeum* germinants. The second pathway is through factors that directly affect the seedbank. Field conditions that impact the seedbank between time of seed input and germination may result in numbers of *Hordeum* germinants that defy expectations based on adult presence. These pathways motivated my predictions, and are described in the conceptual diagram in Figure 3. I hypothesized that 1) the number of *Hordeum* germinants would be more abundant when GKR engineering is present and when precipitation is high, as these conditions tend to

increase adult *Hordeum* in the field; 2) *Hordeum* germinants would be less abundant when GKR trophic activity is present and after several months have passed since seeds were dropped, as foraging over the summer would reduce seeds in the seedbank; and 3) relative abundance of *Hordeum* germinants would be reduced by high total plant cover in the field, as competitive effects in the field would result in less *Hordeum* seeds dropped where other plants are dominant.

## **Materials & Methods**

### Study Site

In order to determine the factors that affect *Hordeum* germination, seedbank samples were taken from study sites at Carrizo Plain National Monument, which is an arid grassland composed mainly of annual plants. Precipitation in this region is concentrated during the cool winters and summers are hot and dry. To measure trophic effects of GKR, 18 replicates of 20x20-m GKR foraging exclosures were compared with paired control sites of the same size located in random compass directions 20-m from each exclosure. Engineering effects were measured by comparing samples taken from burrow and non-burrow areas both inside and outside of these exclosures. Precipitation was manipulated at these field sites with rain shelters. Sites were randomly assigned precipitation manipulations: in 6 sites 50% of October-March rainfall was redistributed from the exclosure plot and added it to the paired control; in 6 other sites rainfall was removed from the control and redistributed to the exclosure; and in the 6 remaining sites rainfall was not manipulated. These experimental field conditions allowed manipulation of 3 factors: GKR trophic activity, GKR engineering activity, and precipitation. GKR trophic activity was further determined by taking seedbank samples from the spring, soon after seeds were dropped, and in the fall, just before germination, in order to elucidate GKR granivory effects. See Figure 3 for a

photograph of one of the paired plots. Seedbank samples were collected from two haphazardly-selected  $\frac{1}{32}$  m<sup>2</sup> areas, totaling  $\frac{1}{16}$  m<sup>2</sup> area, from the top 2 cm of soil. In Spring 2016, average total cover of all plants in each experimental treatment at all sites was recorded.

### Greenhouse Trials

To determine the effects of these field conditions on germination, as well as the effects of plant community cover, seedbank samples were germinated in the University of Colorado at Boulder's 30<sup>th</sup> Street greenhouse. 220 mL of each seedbank sample was spread over garden soil in pots. Pots were plastic with dimensions of 10.48 cm x 10.48 cm; potting soil was Farfard Growing Mix #2 from ACW Supply. Each pot received 1 cm of water each watering session. Two greenhouse watering treatments were used to germinate the seedbank samples. These treatments were low water, every fourth day, and high water, every second day. This was done as a check on germination methods, to ensure that seedbank samples were germinated at ideal conditions to collect as accurate data as possible. One count survey was performed on each pot at the end of the 44 days. Counts were recorded for *Hordeum* germinants, for germinants in major plant functional groups (forbs, legumes, other grasses), and for several highly abundant, easily recognizable species (e.g. *Erodium* and *Amsinckia*) were recorded. The count data were used to calculate relative abundances of *Hordeum* and functional groups. See Figure 4 for a photograph of the greenhouse set-up.

### Statistics

To determine the experimental effects on the response variable of *Hordeum* germinant count, I used a generalized linear mixed effects model with Poisson-distributed residuals. This type of analysis is appropriate for count data, such as *Hordeum* abundances within the pots. Seedbank sample and field site were included as random effects. The main effects included in the

model were GKR burrow engineering and water availability, including parameters for both field precipitation and greenhouse watering. The model also included an interaction effect between GKR trophic presence and season. Tukey's post hoc contrasts were used to determine the interaction effect of GKR trophic activity and the season the seedbank samples were collected. To determine the effects of plant community on germination of *Hordeum*, relative abundances of germinants were regressed against average total cover of plants in the field. R 3.1.1 (R Core Team 2014) was used to perform all analyses. Using type III SS, results were considered significant if  $p \leq 0.05$ .

## Results

Results from the generalized linear mixed effect model are reported in Table 1. Significant numbers of *Hordeum* and other species germinated in both greenhouse watering treatments so data in subsequent analyses included germinant abundances from both. The greenhouse watering treatments did differ significantly, which was expected as in general the more water seeds receive the more likely they are to germinate. These differences were taken into account by the model when comparing other treatments.

The main effect of GKR engineering significantly affected *Hordeum* germination. The mean count of *Hordeum* germinants was higher on burrow soil than off burrow soil (Figure 6). Precipitation also significantly affected *Hordeum* germination. While mean *Hordeum* germinant counts for samples with fifty percent less and with ambient precipitation were not significantly different, there were more *Hordeum* germinants in samples with fifty percent more precipitation (Figure 7).



Post-hoc comparisons for the interaction effect of GKR trophic activity and season also revealed significant relationships (Figure 8). For these post-hoc comparisons, results were considered significant if  $p \leq 0.10$ , in order to avoid potential type II errors. GKR foraging decreased *Hordeum* abundance over the seasons, from spring to fall. In contrast, when GKR foraging was absent, mean *Hordeum* abundance was significantly higher in the fall than in the spring.

The results from the linear regression to determine plant community effects are reported in the Figure 9. Total cover had a significant negative effect on the relative abundance of *Hordeum* germinants. Across all experimental treatments, *Hordeum* germinants decreased as total cover increased (Figure 9).

## **Discussion**

The hypothesized effects of field conditions on *Hordeum* germination are supported by the results. The predicted relationships illustrated in Figure 3 may now be considered descriptive. The germination of the invasive grass *Hordeum* is affected by the community ecology of Carrizo Plain. GKR engineering, precipitation, GKR foraging across seasons, and plant community cover, affected *Hordeum* germinants as predicted.

### Engineering Effects

The main effect of GKR engineering significantly increased the mean count of *Hordeum* germinants, which agrees with the general consensus that GKR burrows show higher levels of adult invasive annual grasses than undisturbed areas (Grinath et al. In Prep; Guo et al. 1995; Schiffman 1994). While kangaroo rats are known to clip tall plant growth off of their mounds and prey on the larger seeds invasive annual grasses produce (Prugh and Brashares 2012;

Whitford and Kay 1999; Germano 2001), the disturbed soil from their burrows shows increased nitrogen levels that may increase the success of these invasives (Gurney et al. 2015; Whitford and Kay 1999).

This positive engineering effect of GKR on the invasive *Hordeum* creates a management dilemma that others have brought attention to (Gurney et al. 2015; Schiffman 1994). If management actions are taken to increase the endangered GKR, it is likely that this action will also increase the abundance of the invasive *Hordeum*. This is not advisable if management priorities are to decrease establishment and population growth of invasive grasses. However, a management strategy that has been put forth as potentially more effective and a possible remedy to this dilemma is to focus less on reducing invasive plant establishment and more on promoting native plant establishment (Mordecai et al. 2015).

#### Foraging and Season Effects

Results on the effects of GKR foraging on *Hordeum* germination add to the discussion of management for both invasive reduction and native establishment. The decrease in *Hordeum* germinants from spring to fall when GKR foraging was present suggests that the GKR granivory decreases *Hordeum* germination (Figure 8). This result is likely due to granivory, rather than a negative effect of GKR on adult *Hordeum*, because spring sites with and without GKR foraging were not significantly different. GKR foraging was not affecting input of seeds into the seedbank, but was affecting the seedbank itself. Previous experiments testing kangaroo rat granivory in general found the presence of kangaroo rat foraging to decrease invasive plant populations, even if the effect was small (Brock and Kelt 2004; Gurney et al. 2015; Prugh and Brashares 2012). My results, along with these other studies, suggest that continuing to prioritize conservation of GKR may assist efforts to reduce invasive grass populations.

The interaction effect between GKR trophic activity and season showed a puzzling result. When GKR trophic activity was absent, the fall sites actually showed the greatest abundance of *Hordeum* (Figure 8). This begs the question, how did more viable *Hordeum* seeds appear in the seedbank after the summer in an area where GKR could not have been impacting the seedbank through foraging? I am currently investigating this result further to evaluate whether these trends were due to effects of seed conditioning or measurement error. I am sieving remaining portions of the seedbank samples for *Hordeum* seeds and then will conduct germination trials. The results of this germination experiment will reveal how many seeds were produced in total in each seedbank sample, and the percentage of seeds that are viable across the experimental treatments. This will allow me to identify if the result is caused by differences in number of seeds in the seedbank across the seasons, or if it is caused by differing successful germination rates across the seasons.

#### Precipitation Effects

The main effect of precipitation significantly increased the abundance of *Hordeum* germinants. This was as predicted, as water is one of the limiting resources in this ecosystem and is a strong driver of the presence of invasive grasses (Grinath et al. In Prep; Ochoa-Hueso and Manrique 2010; Going et al. 2009; DeFalco et al. 2003). What is most interesting about this result is that it is similar in significance to the engineering effects of GKR. This reinforces the general idea that both abiotic and biotic factors influence invasion success and native resilience (Going et al. 2009; Shea and Chesson 2002), and the body of knowledge specific to Carrizo Plain that indicates GKR as a keystone species that has an impact on invasives similar to those of resource limitation and climate (Grinath et al. In Prep; Prugh and Brashares 2012; Gurney et al. 2015).

## Plant Community Effects

Based on the regression analysis, high total plant cover has a negative effect on *Hordeum* germination. This is most likely due to competition effects of other plant species on adult, seed-producing *Hordeum*, as other studies have identified competition negatively affecting invasive annual grasses in similar systems (Going et al. 2009; DeFalco et al. 2003). Going et al. (2009) found that native species in California grasslands, including some forbs that are also found in Carrizo Plain, have large competitive effects relative to their size and so promoting these natives could reduce invasive grass populations. While my results do not go into detail about which species of plant make up the total cover, it is still important to know that competitive effects of the surrounding plant community are affecting *Hordeum* germination. This information solidifies our understanding of how this invasive may respond to management strategies, and justifies subsequent research on competition and germination at Carrizo Plain.

## Conclusions

Overall, the findings from my study corroborate previous *Hordeum* studies as well as previous studies on similar invasive grasses, and point to future empirical paths. Germination responses of *Hordeum* to GKR engineering and precipitation follow previously reported trends in adult populations (Grinath et al. In Prep; Guo et al. 1995; Schiffman 1994). While the positive effect of GKR burrowing engineering on *Hordeum* germination pose a management dilemma, the negative effect of GKR foraging are conducive to management goals of conserving native species. The greater understanding of invasive grass germination dynamics brought about by this study is important in the larger scheme of the Carrizo Plain research-management connection. Understanding these details and modeling the interactions between GKR, precipitation changes, other grassland plants, and the invasive *Hordeum* will help land managers, such as the Federal

Bureau of Land Management, decide how best to approach the conservation of Carrizo Plain. Any success at using scientific knowledge to inform management in Carrizo Plain can be an example for other invaded California grasslands and grasslands nationwide.

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## Tables

TABLE 1: Results from the generalized linear mixed effects model. All effects were included in one model. The response variable was number of *Hordeum* germinants. Effects were included based off of initial hypotheses on what would effect *Hordeum* germination. N= 236.

Effect	Chi-square value	Degrees of freedom	p-value
Greenhouse watering	28.335	1	$1.020 \times 10^{-7}$
Precipitation	18.978	2	$7.567 \times 10^{-5}$
Engineering	13.036	1	$3.055 \times 10^{-4}$
Trophic	7.806	1	0.00521
Season	0.476	1	0.490
Trophic x Season	30.879	1	$2.746 \times 10^{-8}$

## Figures



FIGURE 1: Focal species seedling, *Hordeum murinum*, in a greenhouse pot.





FIGURE 2: A Giant Kangaroo Rat, *Dipodomys ingens*. Photo credit J. Roser.

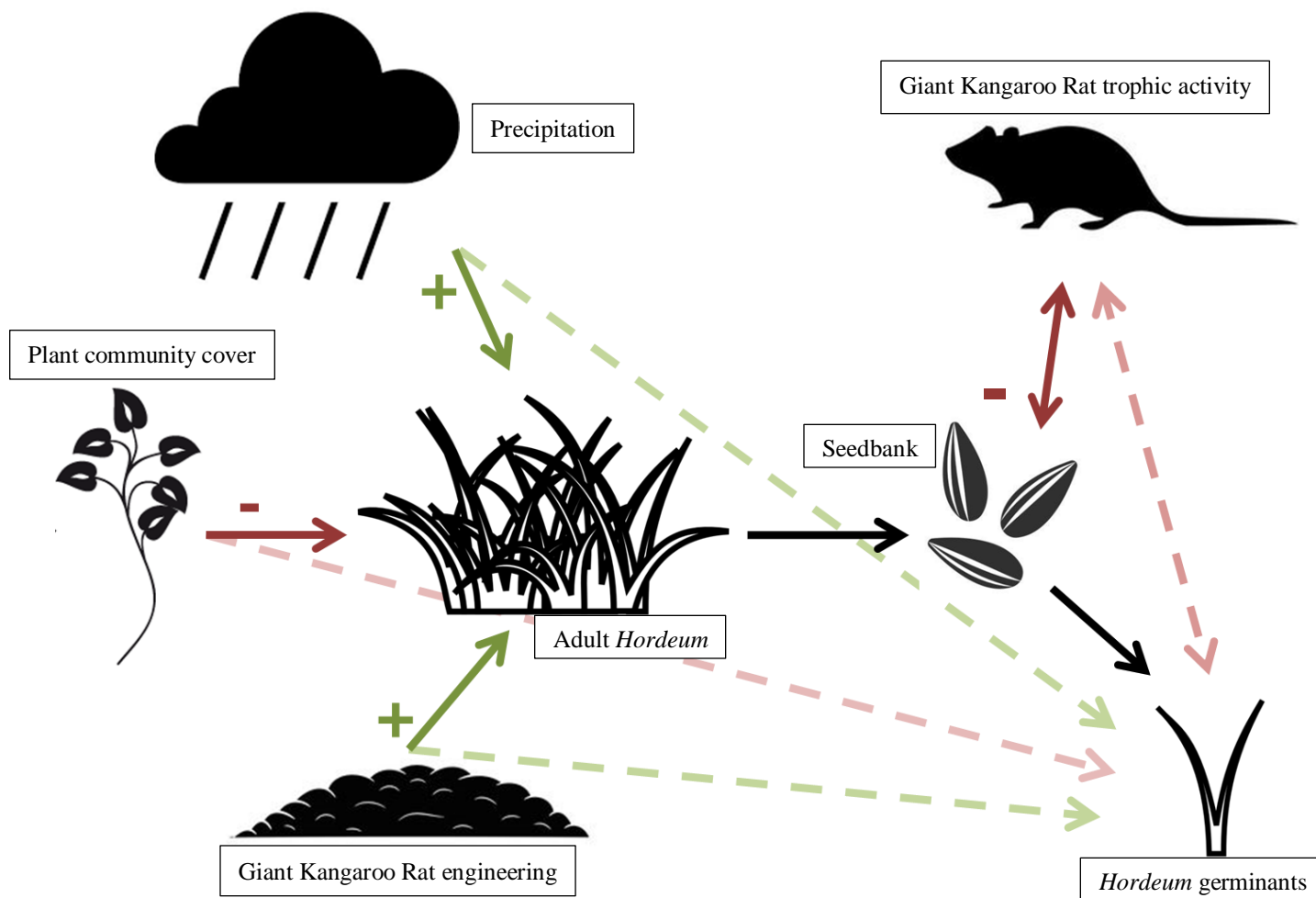


FIGURE 3: Conceptual diagram of the different field conditions that may affect *Hordeum* germination. Solid lines are known effects of field conditions on adult *Hordeum* or seedbank. Dashed lines are predicted effects of field conditions on germination. Green and positive signs indicate known/predicted positive effects, while red and negative signs indicate known/predicted negative effects.



FIGURE 4: View of one of the paired plots at the study site, Carrizo National Monument. Rain shelter is on the left; GKR enclosure is on the right.



FIGURE 5: GraceAnne watering potted seedbank samples in the University of Colorado at Boulder's 30<sup>th</sup> St Greenhouse.

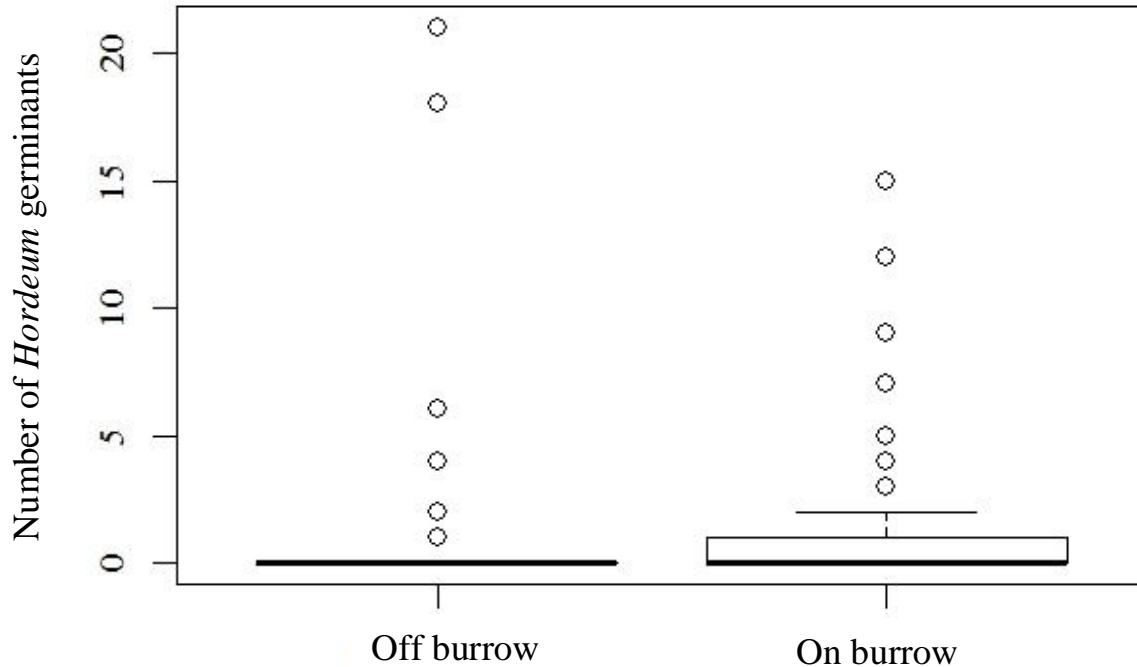


FIGURE 6: Main effect of engineering. Mean *Hordeum* germinant counts increased from 0.53 SE ± 2.6 on burrow soil to 1.05 SE ± 2.5 off burrow soil.

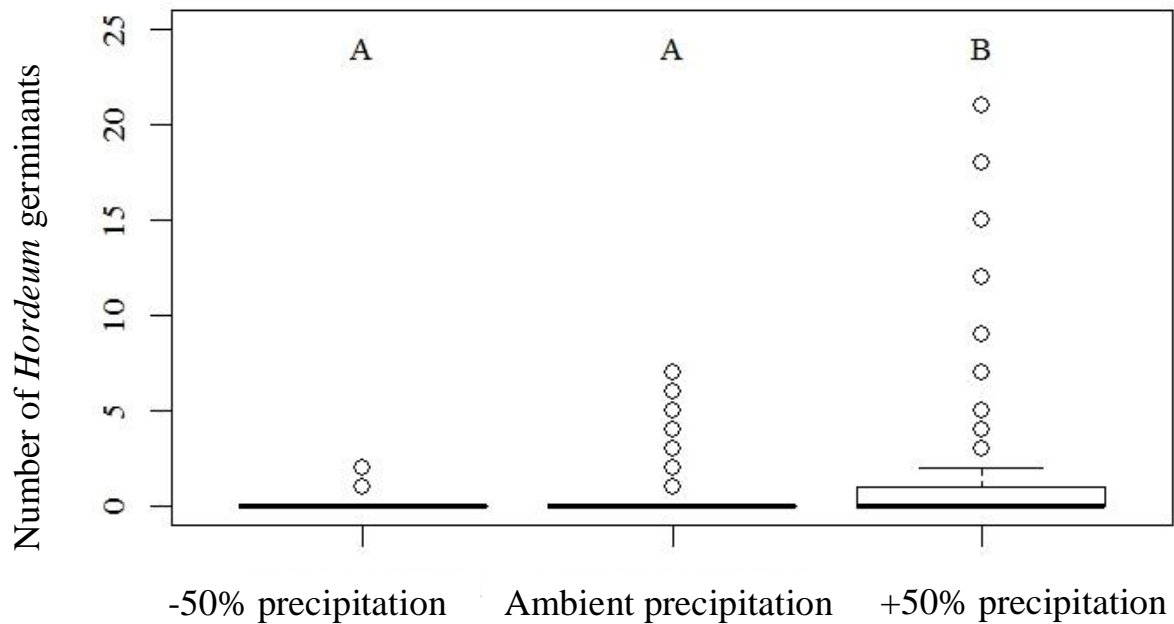


FIGURE 7: Main effect of field precipitation. Only the +50% samples were significantly different. Mean *Hordeum* germinant counts increased from 0.12 SE ± 0.37 and 0.46 SE ± 1.4 in -50% and ambient precipitation, respectively, to 1.9 SE ± 4.1 in +50% precipitation. Letters show significant differences between treatments.

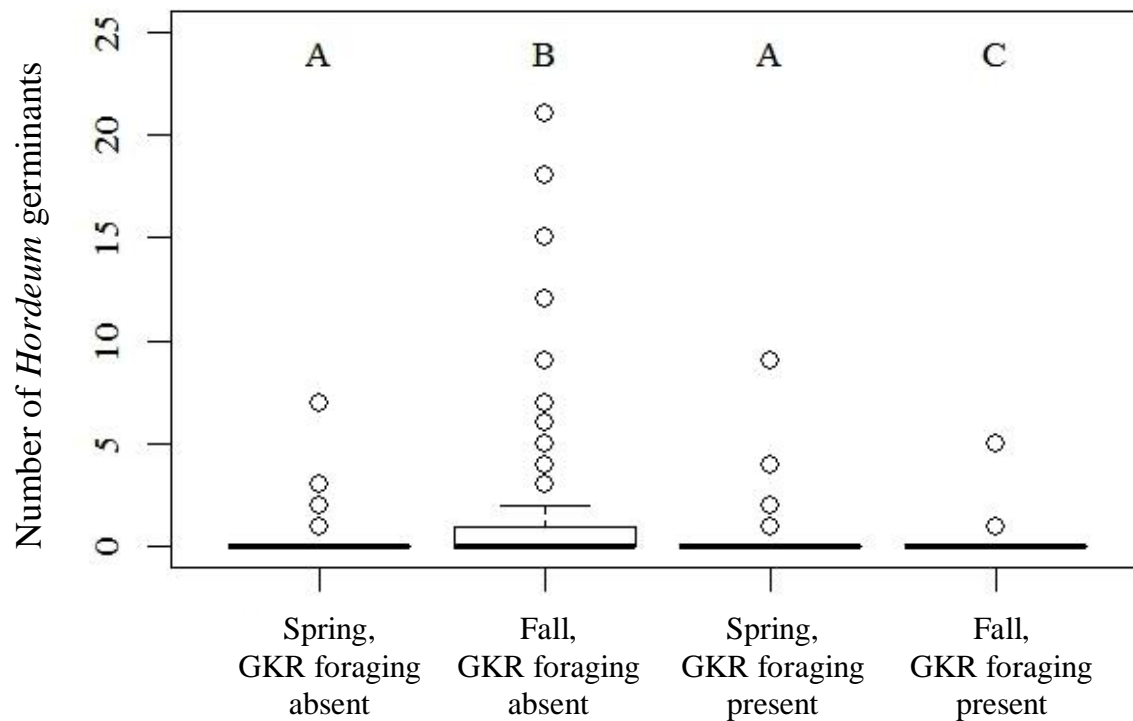


FIGURE 8: Results from the post-hoc comparisons for the interaction effect of GKR trophic activity (foraging) and season. When GKR foraging activity was absent, mean *Hordeum* abundance was significantly higher in the fall,  $2.0 \text{ SE} \pm 4.5$ , than in the spring,  $0.52 \text{ SE} \pm 1.4$ . When foraging was present, the mean decreased from  $0.45 \text{ SE} \pm 1.4$  in the spring to  $0.16 \text{ SE} \pm 0.70$  in the fall. Letters show significant differences between treatments.

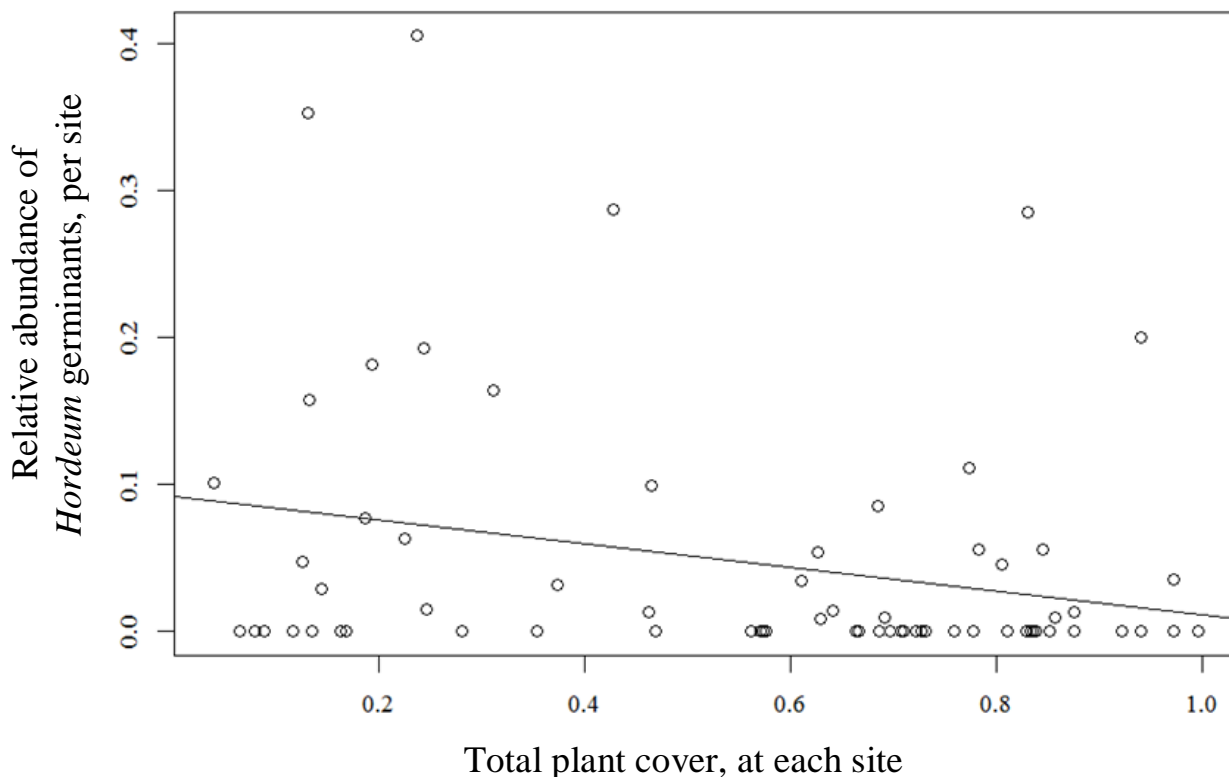


FIGURE 9: Linear relationship between *Hordeum* germinant relative abundances and total plant cover in the field. Slope = -0.081; t-value = -2.253; p-value = 0.028; N=69.