

TECHNICAL ARTICLE

Managed grazing is an effective strategy to restore habitat for the endangered autumn buttercup (*Ranunculus aestivalis*)

Michele M. Skopec^{1,2}, Jennifer Lewinsohn³, Tyson Sandoval¹, Clint Wirick⁴, Sheila Murray⁵, Valerie Pence⁶, Linda Whitham⁷

The only protected habitat of the endangered autumn buttercup is a small, overgrown, wet meadow that no longer supports the species. We used an experimentally driven reintroduction to examine the role of rodent herbivory in limiting the survival and establishment of autumn buttercup at the site. We evaluated the effectiveness of livestock grazing and cages to exclude rodents by comparing survival of caged and uncaged transplants under two pasture management treatments (grazed vs. ungrazed). We found that transplant survival was greatest for caged plants in grazed pasture with 50% of plants surviving to the end of the second growing season. Grazing increased the species richness in the plant community and decreased the amount of cover for small mammals. Accordingly, rodent density and vole herbivory in late summer were significantly lower on grazed pasture. Our results indicate that rodent herbivores represent a major threat to the survival and reestablishment of autumn buttercup and livestock grazing and protective caging are effective strategies to reduce rodent populations and vole herbivory.

Key words: endangered species, livestock grazing, plant reintroduction, rodent herbivory, voles, wet meadow restoration

Implications for Practice

- Historic habitat is a good location for reintroductions of rare plants once the habitat is restored to support species survival. For example, reestablishment of livestock grazing in a wet meadow restored historic habitat for an endangered plant by quickly altering the plant and small mammal community.
- Cryptic herbivores like rodents can play a major role in survival of an endangered plant and should be accounted for during reintroductions.

Introduction

A critical question confronting land managers of imperiled plant species is how to intervene when a species continues to decline on lands being managed to support the species. Inasmuch as we would like to believe that a species will thrive in its habitat once the main threats are alleviated, the complexity of biotic and abiotic interactions on the landscape and our limited understanding of these dynamics can hinder our eager attempts to “remedy” the situation and benefit the species. Such is the case for the autumn buttercup (*Ranunculus acriformis* var. *aestivalis* = *Ranunculus aestivalis*), an extremely rare species found in a montane, wet meadow habitat of the Sevier River Valley in Utah, U.S.A. Autumn buttercup was listed as endangered in 1989 (54 FR 30550) and lands supporting a population were acquired by The Nature Conservancy (TNC) in 1988 to protect the species. A serious threat to autumn buttercup was thought

to be livestock grazing, so grazing was halted at the TNC Preserve. The autumn buttercup population increased immediately after livestock removal but the benefit was temporary, only lasting 3 years. By 2006, autumn buttercup no longer occurred on the TNC Preserve. With the cessation of livestock grazing the diverse, mixed graminoid and forb plant community previously supporting autumn buttercup (Spence 1996; Van Buren & Harper 1996) became a near monoculture of wiregrass (*Juncus balticus*). In addition, field observations suggested the wiregrass and the thick thatch layer that had developed in the meadow were increasing habitat for small mammalian herbivores like voles (Peles & Barrett 1996).

Author contributions: All authors conceived and designed the experiment; all authors performed the key aspects of the experiment; MS, JL analyzed the data; MS, JL wrote and edited the manuscript.

¹Department of Zoology, Weber State University, 1415 Edvalson Street, Ogden, UT 84408, U.S.A.

²Address correspondence to M. M. Skopec, email micheleskopec@weber.edu

³Utah Ecological Services Field Office, U.S. Fish and Wildlife, 2369 West Orton Circle, West Valley City, UT 84119, U.S.A.

⁴Partners for Fish and Wildlife Program - Utah, U.S. Fish and Wildlife, 240 North 600 East, Richfield, UT 84701, U.S.A.

⁵The Arboretum at Flagstaff, 4001 S. Woody Mountain Road, Flagstaff, AZ 86001, U.S.A.

⁶Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Gardens, 3400 Vine Street, Cincinnati, OH 45220, U.S.A.

⁷The Nature Conservancy, 820 Kane Creek Blvd, Moab, UT 84532, U.S.A.

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doi: 10.1111/rec.12633

Supporting information at:

<http://onlinelibrary.wiley.com/doi/10.1111/rec.12633/supinfo>

When there is a real possibility of losing a plant species in the wild, plant reintroductions are commonly incorporated into recovery efforts (Maschinski & Haskins 2012). Therefore, two reintroduction attempts in 2007 and 2010 were undertaken; however, the reintroduced plants were removed from their planting holes within weeks of planting, likely by rodents. Given the sustained rodent herbivory on the autumn buttercup after livestock removal, we implemented an experimental framework into the third reintroduction effort. Our objective was to empirically test the hypothesis that the removal of livestock resulted in larger populations of rodents and increased rodent herbivory of autumn buttercup. We also wanted to identify suitable wet meadow microsites for plant establishment, and evaluate soil properties associated with plant survival.

Methods

Grazing

A grazing plan for the Preserve, based on a vegetation survey of species composition, available forage, and plant production, was developed in 2012 recommending a herd number of 25–33 cow/calf pairs for 30–35 days. The Preserve was divided into two roughly 20 acre plots by an electric fence and cattle grazed only the southern half the Preserve in fall 2012 and spring 2013 prior to the reintroduction and then each fall and spring throughout the remainder of the study period.

Plant Propagation

Autumn buttercup plants were propagated using tissue culture techniques (Pence 2010). Shoot propagating cultures were initiated from seeds germinated on half-strength Murashige and Skoog (1962) (MS) medium with 1.5% sucrose, 0.33% gellan gum (Gelzan; Caisson Labs, Smithfield, UT, U.S.A.), and 100 mg/L Benomyl (Sigma-Aldrich, St. Louis, MO, U.S.A.), in 25 × 150 mm culture tubes, 15 mL of medium/tube. Seeds in culture were incubated at 21°C, with a 16:8 light: dark cycle, approximately 20 $\mu\text{mol}/\text{m}^2/\text{seconds}$. When two to three leaves emerged, roots were removed from seedlings and shoots cultured on Driver and Kuniyuki (1984) medium with 0.2 mg/L benzylaminopurine, 3% sucrose, 0.25% gellan gum and subcultured every 8–12 weeks onto fresh medium to multiply shoots. Shoots were rooted by transferring to MS medium with 3% sucrose and no growth regulators or with 1 mg/L indolebutyric acid. Plants were transferred from test tubes to standard potting soil mix (Sunshine#4) and acclimatized in greenhouse and outdoor conditions for 1–2 years until reaching 4 and 6 inch pot sizes for planting.

Planting Design

Fourteen transects were established within grazed and ungrazed plots in a north–south orientation. Transect locations were selected based on historic plant locations, or suitable habitat characteristics such as hummocks (Van Buren et al. 1994; Spence 1996; Van Buren & Harper 1996). Each transect contained 24 plants with half of those plants being caged

to exclude rodents by stainless steel mesh baskets (Western Planting Solutions, Plumas Lake, CA, U.S.A.) covering the roots and 1/4 inch wire mesh in tomato cages that measured 54 inches tall and had an internal diameter of 16.14 inches covering the shoots, leading to four treatment groups: grazed and caged ($n = 84$), grazed and uncaged ($n = 84$), ungrazed and caged ($n = 84$), and ungrazed and uncaged ($n = 84$).

Planting occurred in June 2013 and each plant's ID and location in the transect was recorded along with the plant demographics: rosette diameter, height, vigor, number of leaves, inflorescences, buds, flowers, and fruits. The hydrology and topography of each planting site were also recorded. For hydrology, the volume of water present in each planting hole was estimated visually and scored on a scale of 1–5, using 25% increments. For topography, planting hole locations were recorded as a depression, on flat ground or on a mound.

Plant Survival and Herbivory

We recorded plant vigor, life stage (flowering and/or fruiting), and evidence of herbivory on a monthly basis during the growing season the first year (July–September) and May–September the following year. Plant vigor was scored on a scale of 1–3: plants in poor condition were scored as 1, normal plants were scored as 2, and vigorous plants were scored as 3. We checked leaves and stems for evidence of herbivory, and scored plants as either having no evidence of herbivory, evidence of small mammal herbivory, characterized by stems clipped at 45° angles, or large mammal herbivory characterized by less precise cuts to leaves and stems. We characterized plant survival 1 month after planting as transplant survival, and survival to September 2013 as 2013 seasonal survival and survival to August 2014 as overall survival. Principal component (PC) analysis on mean centered data in JMP 10 was used to determine which factors—plant demographics (vigor at planting, flowering during 2013 and/or 2014 season, transplant survival, herbivory during 2013 and/or 2014 season) or planting site characteristics (topography and hydrology)—were associated with survival until the end of the 2014 growing season. Transplant survival was closely associated with overall survival in the PC analysis, so we analyzed the following demographics of the plants at planting (plant height, leaf rosette diameter, number of leaves, and inflorescences) and planting site characteristics (topography and hydrology) to determine if vigor of plants or planting site was more closely associated with transplant survival. Survival of plants in each of the four treatment groups during the first two growing seasons were compared using Kaplan–Meier survival analysis in JMP 10 to determine the effects of grazing and caging on overall survival.

Soil Analysis

A composite soil sample for each transect was collected August 2013 from ten 24 inch soil core samples collected in a zig–zag pattern across each transect. Soil samples were analyzed by Utah State University Analytical Laboratory to determine soil moisture, pH, and mineral content: salinity (as determined by

electrical conductivity), phosphorus, and potassium (as determined by Olsen NaHCO_3). Differences in soil characteristics between the grazed and ungrazed plots were compared using Bonferroni corrected t tests. Multiple linear regression analysis in JMP 10 was used to determine which soil characteristics were correlated with transect-level survival during the 2013 growing season.

Plant Canopy Cover

To examine the impact of livestock grazing on plant community attributes, we estimated the percent of vegetative cover in *Carex* and *Juncus* dominated plant communities in August 2013. Both the grazed and ungrazed side of the preserve had a greater proportion of *Juncus* dominated plant communities. Canopy cover was estimated using the Daubenmire frame method (Daubenmire 1959) with a 1 m^2 ($20 \times 50\text{ cm}$) quadrat frame. Twenty sample locations were randomly selected within each plant community on each side of the preserve by stratified random sampling. We grouped vegetation according to cotyledon classification (monocot and dicot) and compared percent cover of total vegetation, monocots, dicots, bare ground, and litter between *Carex* and *Juncus* dominated plant communities and between grazed and ungrazed plots. Canopy cover data were arcsine square-root transformed and analyzed using an analysis of variance with grazing treatment and plant community as fixed effects. Bonferroni corrected t tests were used for post hoc pairwise comparisons (SYSTAT 10.0; SPSS Inc. 2000). We did not compare transplant survival in *Carex* and *Juncus* dominated plant communities due to an insufficient replication of transects within each plant community, since transect locations were selected based on historic plant location, or suitable habitat characteristics like hummocks.

Small Mammal Trapping

Summer (May–August) 2013, 2014, and 2015 monthly small mammal trapping using live Sherman traps was conducted for two nights (1,428 trap nights). Two 6×6 grids with 36 traps spaced at 20 m intervals per plot plus six traps per transect were used to estimate density of small mammals. Traps were opened within 1 hour of sunset and checked within 2 hours of sunrise. Summer 2013 traps were removed between trapping sessions and 2014 and 2015 traps were locked open in between trapping sessions. Animals were tagged and then released and the program CAPTURE was used to estimate densities (Otis et al. 1978). All experimental procedures involving small mammals were approved by Weber State University's Institutional Animal Care and Use Committee protocol number 14-01.

Results

Transplant and Overall Survival

PC analysis of factors affecting transplant survival showed that PC1 explained only 30% of the variation and was driven by the plant demographics; rosette diameter, height, vigor, number of leaves, and inflorescences (Fig. 1B and Table S1, Supporting

Information). Transplant survival was most closely associated with hydrology and topography of the planting site and all three were the major drivers of PC2; however, PC2 only explained 19.9% of the variation in the data (Fig. 1B, Table S1).

Survival until August 2014 (overall survival) was most closely associated with first winter survival (2013–2014), flowering in 2014, and transplant survival and all four were the major drivers of PC1, which explained only 26% of the variation in the data (Fig. 1D and Table S1). PC2, which explained only 13% of the variations, was driven by hydrology, topography, and transplant survival in the positive direction and winter survival and whether or not plants experienced herbivory in the negative direction. (Fig. 1D, Table S1).

Effect of Grazing and Caging on Plant Survival

Survival rates of plants in the four treatment groups began to differ within the first month (Wilcoxon $X^2 = 84.14$, $p < 0.001$, Fig. 2). The uncaged plants on the ungrazed side of the TNC Preserve had mortality rates 2× greater than the uncaged plants on the grazed side of the TNC Preserve and all of the caged plants. The caged plants on the grazed side fared the best, especially in overwinter survival.

Canopy Cover and Plant Species Present

Juncus and *Carex* plant communities on the ungrazed side of the preserve differed in vegetative cover with *Juncus* dominated communities containing higher total vegetative cover ($p < 0.001$), monocot cover ($p < 0.001$), and dicot cover ($p < 0.001$) (Fig. 3). Monocot cover was similar in ungrazed and grazed *Juncus* communities ($p < 0.05$), but dicot cover ($p < 0.001$) and bare ground ($p < 0.006$) were significantly higher in the grazed *Juncus* communities. In grazed *Carex* plant communities, monocot cover ($p < 0.001$) and bare ground ($p < 0.001$) were higher and dicot cover was lower ($p < 0.001$) compared to ungrazed *Carex* plant communities (Fig. 3). Overall differences in canopy cover were most striking for the litter component, where there was significantly lower litter cover ($p < 0.001$) in both grazed plant communities compared to ungrazed communities (Fig. 3).

Soil Analysis

Soil characteristics measured did not differ between the grazed versus ungrazed sides of the preserve: % moisture $50.8 \pm 8.6\%$ vs. $40.3 \pm 6.0\%$, pH 7.69 ± 0.25 vs. 7.86 ± 0.23 , salinity 1.08 ± 0.28 vs. 1.03 ± 0.28 dS/m, phosphorus 5.06 ± 4.8 vs. 3.47 ± 3.7 mg/kg, and potassium 598.32 ± 113.47 vs. 671 ± 143.33 mg/kg. However, three soil characteristics were found to have a significant positive correlation with season plant survival including moisture content ($r^2 = 0.33$, $p = 0.02$), soil salinity ($r^2 = 0.33$, $p = 0.04$), and available phosphorus content ($r^2 = 0.4$, $p = 0.007$, Fig. 4).

Small Mammal Density

Deer mice (*Peromyscus maniculatus*, $n = 205$) and long-tailed field vole (*Microtus longicaudus*, $n = 111$) were the dominant

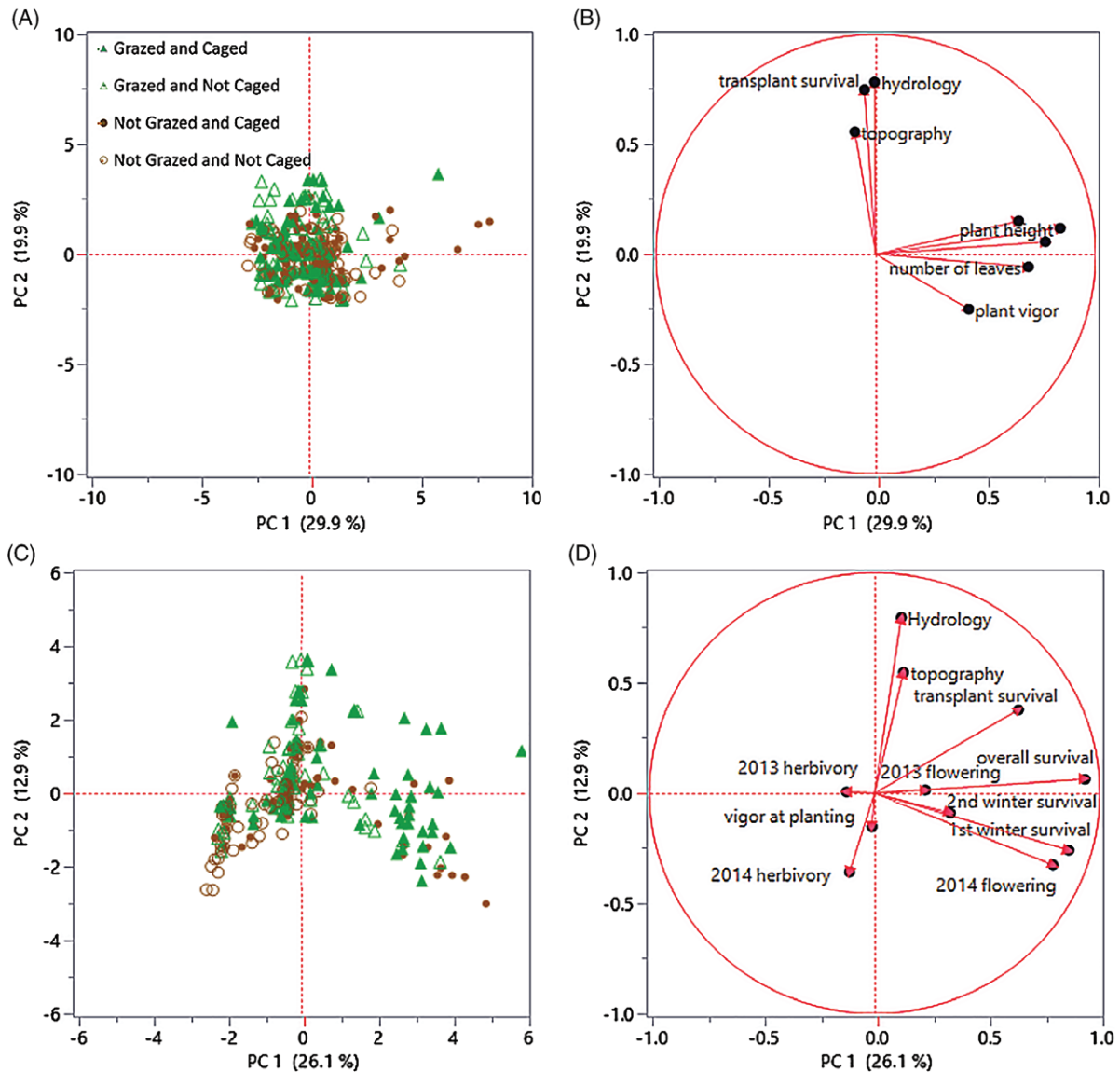


Figure 1. PC analysis of potential factors involved in transplant (A and B) and overall (C and D) survival of reintroduced autumn buttercups. Panels (A) and (C) show how each individual plant falls on PC1 and PC2. Panels (B) and (D) show the drivers for PC1 versus PC2.

species captured. In 2013, deer mouse density did not differ between grazed versus ungrazed plots in June (3 ± 1.5 vs. 5 ± 1.7 mice per hectare) or July (14 ± 2.7 vs. 9 ± 2.3 mice per hectare). In August, the density of mice on the ungrazed side was 6× higher than the density of mice on the grazed sides (6 ± 1.9 vs. 36 ± 4.3 mice per hectare). In 2013, only deer mice were trapped, even though there was a lot of evidence of voles, such as fecal pellets and actual sightings. It is likely that the more trap shy voles were not willing to enter the traps that were only left out for the duration of the trapping sessions (two to three nights, Sullivan et al. 2003). We addressed this concern by leaving the traps locked open between trapping session in 2014 and 2015, resulting in voles becoming the predominant species captured. In 2014, a total of 65 long-tailed voles (*M. longicaudus*) were trapped, and in 2015, a total of 46 long-tailed voles were trapped

during three sessions of trapping. The density of voles on the ungrazed plot was greater than on the grazed plot in August 2014 and in July and August 2015 (Fig. 5).

Discussion

Our experimentally driven reintroduction of the endangered autumn buttercup showed that planting site, ungulate grazing, and caging protection from rodent herbivores were keys to successful reintroduction. The majority of plants that survived transplantation also survived to the end of the first growing season. Autumn buttercups planted at wet sites on top of mounds were the most successful. The species' occurrence on mounded topography in grazed habitat was noted earlier

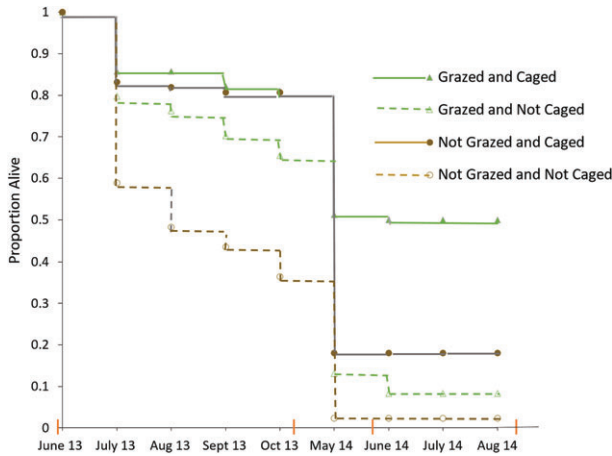


Figure 2. Proportion of plants alive each month. Plants in the grazed groups were planted on the side of the preserve that was grazed by cattle, whereas plants in the not grazed groups were planted on the ungrazed side of the preserve. Plants in the caged groups were protected from small mammal herbivory by aboveground and belowground cages, whereas plants in the not caged group were left unprotected. The red dashes on the x-axis denote when grazing occurred on the grazed side of the preserve. There was a significant difference in survival between the four groups Wilcoxon $X^2 = 84.14, p < 0.001$.

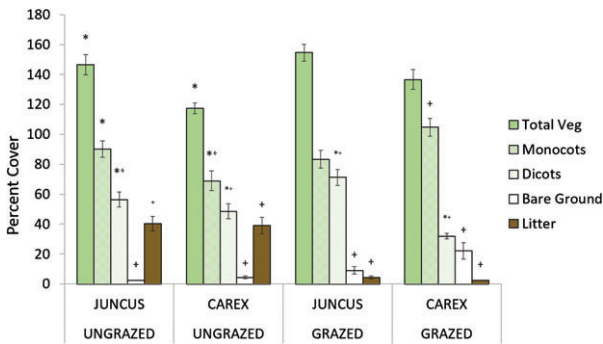


Figure 3. Percent cover in *Juncus* versus *Carex* dominated plant communities. Mean (\pm SE) percent cover was determined by Daubenmire frame method. * Means that are significantly different between *Juncus* and *Carex* communities. $p < 0.05$ + Means that are significantly different between ungrazed and grazed sides. $p < 0.05$

on the TNC preserve and at a private landowner’s site north of the TNC Preserve, which has a large population of naturally growing autumn butterflycup; plants at both locations were mainly found on top of hummocks. The hummocks were most likely formed by cattle grazing in the wet meadow environment (Spence 1996). Continued grazing at the TNC Preserve may increase the number of hummocks and therefore increase the amount of suitable sites for reintroduction. Even though plant vigor was not as closely associated with transplant survival, the vigorous plants were more likely to flower during the 2013 growing season. Since overwinter mortality rates were significant for the transplanted autumn butterflycup, reintroducing plants that will flower and produce seed prior to winter should be a priority.

Soil moisture content, salinity, and available phosphorus were all positively correlated with plant survival in each transect. The transects with a salinity of 1 deci Siemens per meter (dS/m) had the highest survival rates, possibly due to higher cation exchange rates (Sposito 2008). Also, many of the required macro and micro nutrients are often contained in salt form, such as calcium carbonate salts (Marschner 2011). Phosphorus content was also positively correlated with survival. Phosphorus is considered one of the three essential mineral nutrients required for plant growth (Lambers et al. 2008). In most ecosystems, phosphorus is the limiting factor in net primary production after plant requirements for water and nitrogen have been satisfied (White & Hammond 2008).

The resumption of livestock grazing at the TNC Preserve resulted in considerable changes to the plant community over the course of our study. After only two rounds of grazing, we observed significant differences in the plant community between the grazed and ungrazed plots. Most notably the grazed side’s *Juncus* dominated plant communities had higher amounts of dicots and both the *Juncus* and *Carex* dominated plant communities on the grazed side had less litter and more bare ground. Reductions in the dominant *Juncus* vegetation and litter layer appear to have a positive effect on plant diversity within the wet meadow, appear to support dicots in general, and may favor the survival and persistence of autumn butterflycup, but our study did not examine these factors in detail. Other studies have shown that restorative mowing within wet meadow habitat increased overall plant biodiversity and the presence of small forbs (Huhta et al. 2001; Hellström et al. 2003; Stammel et al. 2003). This may be the contribution of reduced competition from the dominant plant species (Jumpponen et al. 2005), increased habitat heterogeneity from litter removal, and an increase in favorable microsites for forb germination (Huhta et al. 2001; Hellström et al. 2003), or a combination of these factors.

In addition, the reduction in rodent numbers and herbivory pressure under grazed habitat conditions may provide a synergistic positive effect on the establishment and survival of autumn butterflycup and other forbs. Herbivory can exert a similarly strong effect on plant establishment and presence as dominant plant competition in ungrazed habitat (Reader 1992; Howe 2008). Granivory can result in seed limitation within the habitat that negatively impacts forb establishment (Fraser & Madson 2008). When grazing is restored, habitat conditions appear to be less suitable for rodents. Small mammals prefer areas with higher plant or litter cover in order to avoid predation (Peles & Barrett 1996). We found that the density of both deer mice and long-tailed voles was lower in August on the grazed side of the preserve that had less litter and more bare ground.

Rodent herbivory has been implicated in the loss of plants or failure of other rare plant reintroduction efforts (Lawrence & Kaye 2011) and the decline of endangered plant species (Dagremond et al. 2010). Voles in particular can exert strong pressure during years of high population levels (Howe et al. 2006). Based on the presence of pellets and characteristic clipping pattern, the long-tailed prairie vole is the likely culprit that ate the autumn butterflycup transplants. As granivores, deer mice are not likely eating plant foliage but they are potential seed predators (Janzen

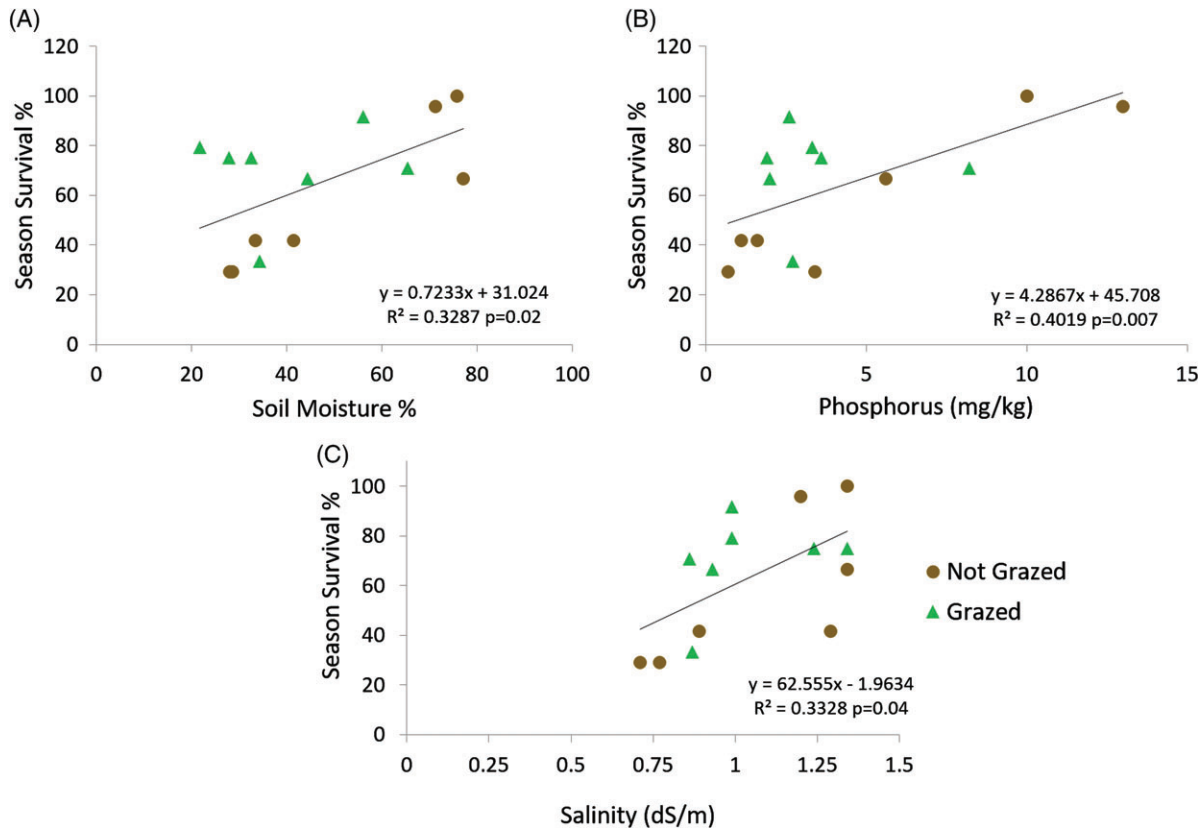


Figure 4. Soil characteristics correlated to survival. (A) Correlation between soil moisture and season survival. (B) Correlation between soil phosphorus content and season survival. (C) Correlation between soil salinity and season survival.

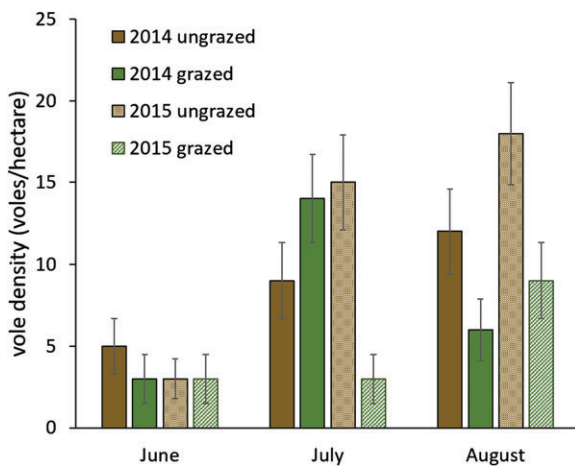


Figure 5. Mean (\pm SE) vole density. Vole density calculated based on mark-recapture methods.

1971) and may curtail population growth by reducing the size of the seed bank. Aboveground and belowground caging of plants to protect them from rodent herbivory was beneficial and led to increased survival. It is recommended that all subsequent transplanted autumn buttercups be protected by cages. One caveat being that the currently designed cages do not stand up to cattle and transects on the grazed side had to be fenced, in order

to prevent the cows and calves from knocking over the cages. Subsequently, the 5×10 -m area inside the fencing had to be mowed by hand to mimic the cattle grazing.

There are a variety of control measures to reduce the population size of the voles, such as encouraging aerial predators to nest and feed, or setting out poison (Mason 1998; Paz et al. 2013). However, on the TNC Preserve, there is a colony of the threatened Utah prairie dog (*Cynomys parvidens*) that would be at risk of predation and poisoning control methods. Trap and removal is one vole control method that could be used but it is labor intensive and depopulated areas are quickly recolonized (Sullivan et al. 2003). Our findings clearly demonstrate that livestock grazing is a rapid and effective way to reduce rodent herbivory on autumn buttercup over the short term. However, ungulate grazing alone is not the best protection against rodent herbivory, and the more management-intensive method of caging plants should be used in combination with grazing to support maximum survival of transplants.

The large and beneficial effect of a grazed pasture may not fully extend to livestock animals themselves since we specifically excluded our transplants from livestock. Our study was not designed to evaluate direct impacts to autumn buttercup plants from livestock trampling and herbivory. This and an evaluation of livestock intensity warrants further investigation to develop an appropriate grazing regime that supports autumn buttercup reestablishment and recovery. Although replication was limited,

our experimentally driven reintroduction has provided answers to our primary questions about restorative grazing and small mammal herbivory, changing how the TNC Preserve will be managed to support the survival of transplants during this and future reintroduction efforts.

Acknowledgments

We thank J. Abbot, K. Dawson, M. Favero, M. Lynn, L. Uhl, and A. Young for their assistance in data collection. We are also grateful to the numerous volunteers who helped on planting day. Funding was provided by The Nature Conservancy, U.S. Fish and Wildlife, and Weber State University.

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Supporting Information

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

Table S1. PC loadings derived from PCA of plant demographic and planting site variables involved in transplant and overall survival.

Coordinating Editor: Stuart Allison

Received: 27 June, 2017; First decision: 14 September, 2017; Revised: 27 September, 2017; Accepted: 5 October, 2017; First published online: 23 November, 2017