629

Examining the impact of ruminal and abomasal fermentation on the viability of coated rangeland seed species

Holton, G[1]; Franco, AM[2]; Stringham, TK[1]; Fonseca, M[3]; Richardson, W[1]; Madsen, M.[4].

[1] Department of Agriculture, Veterinary, and Rangeland Sciences, University of Nevada, Reno, Reno, NV 89557, USA; [2] Department of Agriculture, Food, and Nutritional Sciences, University of Alberta, Edmonton, Alberta T6G 2R3, CA; [3] Department of Animal and Range Sciences, New Mexico State University Clayton Livestock Research Center, Clayton, NM 88415, USA; [4] Department of Plant and Wildlife Science, Brigham Young University, Provo, UT 84602, USA.

Key words: faecal seeding; seed coating; endozoochory, ruminants; grazing, rangelands

Abstract

Cheatgrass *(Bromus tectorum)* has established itself as a prolific invader across the rangelands of the western United States, resulting in an abundance of fine fuel and an increased wildfire frequency. The increased frequency of fires has led to a significant reduction in native vegetation, causing degradation of wildlife habitat, forage availability, and other ecosystem services. To address this destructive wildfire cycle, cattle can be utilized to reduce cheatgrass fuel loads and mitigate cheatgrass dominance through targeted grazing. Concurrently, livestock may be used as vectors for the dispersal of desired rangeland plant species seeds through faecal seeding. Many seeds of interest are susceptible to microbial degradation in the rumen, which is why our study aimed to utilize *in situ* methods to evaluate the effects that seed coat enhancements have on the viability of seed germination post ruminal fermentation. Four target species, Indian ricegrass (*Achnatherum hymenoides*), crested wheatgrass *(Agropyron cristatum),* bottlebrush squirreltail *(Elymus elymoides),* and bluebunch wheatgrass *(Pseudoroegneria spicata)*, were coated with either Polyvinylpyrrolidone (PVP) or PVP + ethyl cellulose (Ethocel). The seeds were subjected to ruminal fermentation for 0, 6, 12, 24, 36, 48, 96 hours, after which, the seeds were placed into petri dishes and germination was monitored for five weeks. PVP and PVP + Ethocel coating treatments resulted in decreased germination rates for the 0-hour treatment across all species except Indian ricegrass. However, coated seeds exhibited greater resistance to microbial degradation through time. The exception to this was Indian ricegrass, which exhibited an increase in germination through time for PVP and control groups. Additionally, coated crested wheatgrass seeds were incubated *in vitro* for 48 hours and incorporated into faecal pats of varying depths to examine emergence. Emergence in faecal material was notable for the PVP + Ethocel group, indicating its potential to protect seeds under harsh environmental conditions.

Introduction

Rangeland ecosystems are essential for maintaining biodiversity, supporting livestock grazing, and providing ecosystem services such as carbon sequestration and water filtration. Despite their importance, these ecosystems are under increasing pressure from a variety of anthropogenic and natural factors, including habitat fragmentation, altered fire regimes, and the proliferation of invasive plant species (Baughman et al., 2022; Whisenant, 1989). Among these threats, the introduction and dominance of invasive grasses such as cheatgrass (*Bromus tectorum*) pose significant challenges, transforming native rangelands and exacerbating wildfire frequencies. As vegetative diversity declines, ecosystem resilience weakens, leading to cascading effects on trophic dynamics and ecosystem functions (Pace et al., 1999; Quijas et al., 2010).

The loss of plant community diversity in western rangelands, particularly within sagebrush steppe environments, has prompted land managers and researchers to investigate innovative methods to restore these ecosystems. Healthy rangelands depend on diverse plant communities with functional traits that enable them to adapt to challenging and fluctuating environmental conditions (McCann, 2000; Baughman et al., 2022). Restoration practices often aim to bolster this functional diversity, which enhances ecosystem resistance and adaptability to disturbances.

Restoration efforts in the western United States date back over a century, but harsh environmental conditions and logistical constraints make these efforts costly and often ineffective. Traditional reseeding methods face low success rates due to factors like poor seed-soil contact, water scarcity, and competition with invasive species (Monsen & MacArthur, 1995; Svejcar & Kildisheva, 2017). In recent years, seed coating technology has emerged as a promising solution to improve restoration outcomes. By applying physical, chemical, or biological enhancements to seeds, seed coating technologies can improve seedling establishment, viability, and resistance to environmental stressors (Davies et al., 2018; Pedrini et al., 2020). For example, surfactants added to seeds reduce soil-water repellency, enhancing water availability at the seed microsite, while coatings with abscisic acid (ABA) delay germination to align with favourable conditions (Madsen et al., 2014; Richardson et al., 2019).

The application of seed coating technologies in rangelands presents a dual opportunity: to improve the establishment of desirable native species and to suppress the dominance of invasive grasses. For example, effective reseeding with perennial grasses can reduce cheatgrass density, thereby altering fire regimes and restoring wildlife habitat (Francis & Pyke, 1996; Whisenant, 1989). Targeted grazing strategies further support this effort by reducing cheatgrass seed banks and fine fuels while avoiding harm to beneficial vegetation (Clark et al., 2023; Perryman et al., 2020). Grazing also presents a unique opportunity to leverage natural seed dispersal mechanisms through endozoochory—the spread of seeds via livestock or wildlife excrement (Teichman et al., 2013).

Faecal seeding, where seeds are dispersed in livestock faeces, is a low-impact restoration method that capitalizes on natural trophic interactions. While this approach minimizes mechanical disturbance to the soil, the digestive processes of ruminants can reduce seed viability, particularly for invasive species like cheatgrass (Holton et al., 2024). This challenge necessitates innovative solutions, such as hydrophobic seed coatings, to protect seeds during digestion and improve their establishment post-dispersal (Qoism et al., 2024; Sashi et al., 2019).

This study investigates the potential of hydrophobic seed coatings, specifically Polyvinylpyrrolidone (PVP) and PVP combined with ethyl cellulose (PVP + Ethocel), to protect seeds during ruminal digestion. Our objectives were to evaluate germination and emergence rates of coated seeds post-digestion and assess their viability under simulated rangeland conditions. We hypothesize that hydrophobic coatings will enhance seed protection during digestion and improve seedling establishment in faecal seeding applications.

Methods

In Situ Ruminal Incubation

Six rumen-cannulated Angus × Hereford steers (610 ± 54.5 kg) were fed a grain-hay diet and used to evaluate the effects of seed coatings on the viability of crested wheatgrass (*Agropyron cristatum*), bluebunch wheatgrass (*Pseudoroegneria spicata*), bottlebrush squirreltail (*Elymus elymoides*), and Indian ricegrass (*Achnatherum hymenoides*). Seeds were coated with PVP, PVP + Ethocel, or left uncoated as controls. Seed samples were placed in heat-sealed nylon bags and incubated in the rumen for varying durations (0, 6, 12, 24, 48, and 96 hours). Bags were removed at designated time points and prepared for enzymatic digestion simulations.

In Vitro Abomasum Incubation

After ruminal incubation, seeds were subjected to in vitro digestion to simulate passage through the abomasum. This process involved incubating seeds in a solution of rumen fluid, pepsin, and hydrochloric acid at 39°C for three hours. The treated seeds were then rinsed and prepared for germination trials in sterile petri dishes.

Germination Trial

Fifty seeds from each treatment group (species, coating, and incubation time) were placed in sterile petri dishes with blotting paper for moisture retention. Dishes were incubated at 20°C with a 12-hour photoperiod and monitored for germination every five days over five weeks. Germination was defined by the emergence of a 2-mm radicle.

Faecal Emergence Trial

Seeds incubated for 48 hours in the rumen were mixed into homogenized faecal material at depths of 1.3, 2.5, and 3.8 cm in greenhouse conditions. Emergence was monitored over ten weeks, beginning with five weeks of water restriction followed by watering every three days. Seedling counts were recorded every ten days.

Statistical Analysis

Generalized linear mixed models were used to analyse the effects of seed coatings, ruminal incubation time, and faecal depth on germination and emergence. Statistical significance was set at $P \leq 0.05$, with trends noted between $P < 0.1$ and $P > 0.05$.

Results

Germination Post-Incubation

Germination rates decreased as ruminal incubation time increased for all species (Figure 1). PVP + Ethocel coatings significantly improved germination for crested wheatgrass and bottlebrush squirreltail after extended incubation times, while Indian ricegrass performed poorly with this coating. Notably, Indian ricegrass treated with PVP maintained germination rates similar to the control group, with a slight increase at 24 hours of incubation (Figure 2).

Faecal Emergence

Seedling emergence varied significantly by faecal depth and coating type. Control seeds exhibited the highest emergence at shallow depths (1.3 cm), while PVP + Ethocel coatings outperformed at deeper levels (2.5 and 3.8 cm). Deeper faecal deposits provided better moisture retention, supporting greater emergence rates across treatments (Figure 3).

Discussion [Conclusions/Implications]

Our findings reveal that seed coatings, particularly PVP + Ethocel, can enhance the viability of certain rangeland species during ruminal fermentation. Species-specific responses indicate the importance of tailoring coatings to match seed morphology and dormancy characteristics. For example, Indian ricegrass, which relies on dormancy to regulate germination, exhibited poor performance with PVP + Ethocel coatings, likely due to the coating's interference with dormancy-breaking mechanisms.

The faecal emergence trial highlights the importance of environmental conditions, such as faecal depth, in seedling establishment. Deeper faecal deposits mitigated moisture loss and crusting, creating favourable conditions for emergence. However, the reduced performance of PVP-coated seeds at shallow depths underscores the need for further refinement of coating formulations to maximize their applicability across diverse rangeland environments.

This study demonstrates the potential of seed coatings to enhance restoration efforts through faecal seeding. PVP + Ethocel coatings showed promise for improving seed viability and emergence under harsh conditions, making them a viable tool for restoring degraded rangelands. However, further research is needed to optimize coatings for a broader range of species and field conditions. Tailoring coating technologies to the specific ecological and operational contexts of restoration projects will be essential for maximizing their impact.

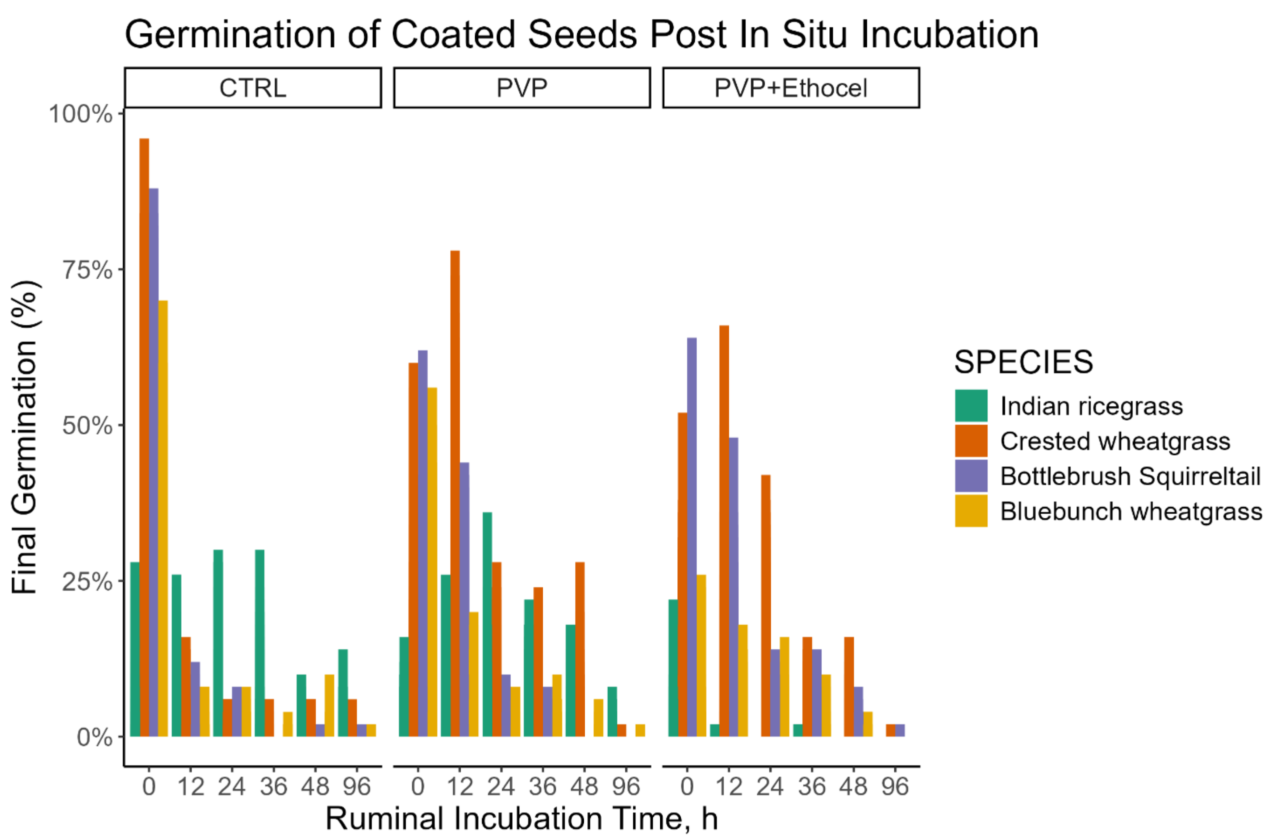

Figure 1. The evaluation of the germination potential of rangeland seed species coated with either polyvinylpyrrolidone (PVP), PVP + Ethocel, or control, after exposure to *in situ* ruminal fermentation through time (in hours) followed by 3 hours of *in vitro* abomasal enzymatic digestion. The final germination is expressed as a percentage of total seeds examined.

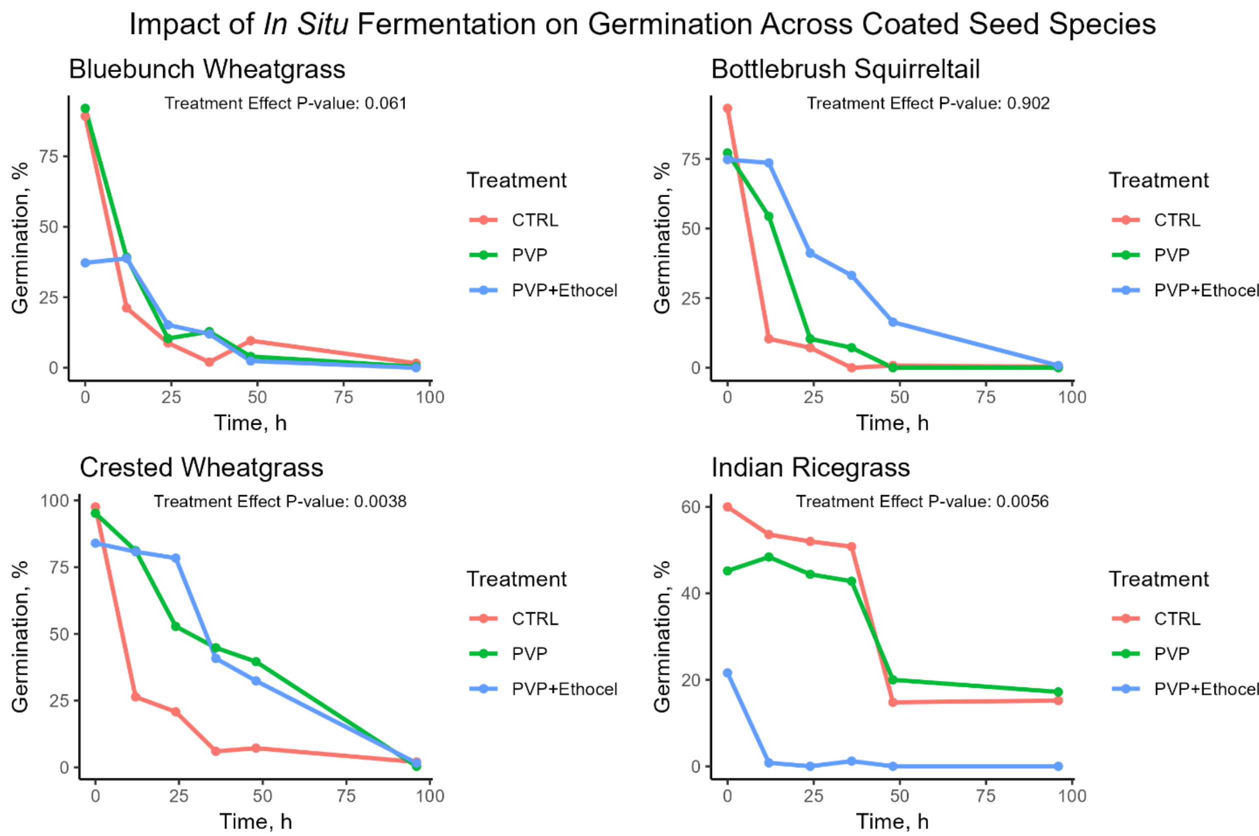

Figure 2. An examination of germination rates through time (in hours) for rangeland seed species coated with polyvinylpyrrolidone (PVP), PVP + Ethocel, and a control group. Seeds were incubated in the rumen, followed by 3 hours of *in vitro* abomasal enzymatic digestion.

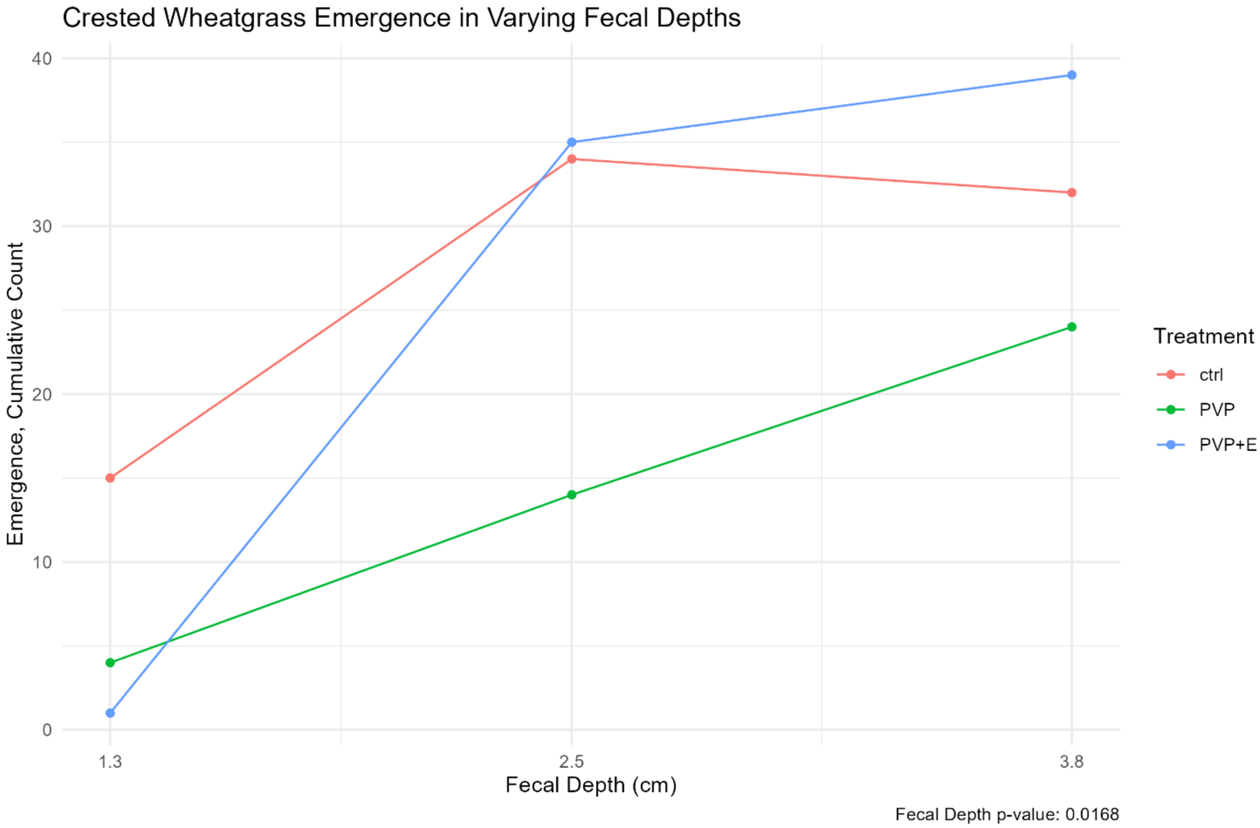

Figure 3. The evaluation of crested wheatgrass (*Agropyron cristatum*) emergence in faecal material substrate at varying depths (1.3 cm, 2.5 cm, and 3.8 cm) after seeds were coated with Polyvinylpyrrolidone (PVP), PVP + Ethocel (PVP + E), or control, and subjected to 48 hours of *in vitro* ruminal fermentation paired with 3 hours of *in vitro* abomasal enzymatic digestion. Emergence was determined by above surface expression of vegetative material.

References

Baughman OW, Kulpa SM, & Sheley R L (2022). Four paths toward realizing the full potential of using native plants during ecosystem restoration in the Intermountain West. Rangelands, 44(3), 218-226. https://doi.org/10.1016/j.rala.2022.01.003

Clark PE, Porter BA, Pellant M, Dyer K, & Norton TP (2023). Evaluating the efficacy of targeted cattle grazing for fuel break creation and maintenance. Rangeland Ecology & Management, 89, 69–86. https://doi.org/10.1016/j.rama.2023.02.005

Davies KW, Boyd CS, Madsen MD, Kerby J & Hulet A (2018). Evaluating a seed technology for sagebrush restoration across an elevation gradient: Support for bet hedging. *Rangeland Ecology & Management, 71*(1), 19–24. https://doi.org/10.1016/j.rama.2017.07.003

Francis MG & Pyke DA (1996). Crested wheatgrass-cheatgrass seedling competition in a mixed-density design. Journal of Range Management*, 49*(5), 432–438.

Holton G, Macias Franco A, Richardson W, Stringham T & Fonseca M (2024). Evaluating the effects of ruminal incubation and abomasal enzymatic digestion on the germination potential of *Bromus tectorum*. Rangelands, 132–136. https://doi.org/10.1016/j.rala.2024.05.001

Madsen MD, Davies KW, Boyd CS, Kerby JD & Svejcar TJ (2016). Emerging seed enhancement technologies for overcoming barriers to restoration. Restoration Ecology. https://doi.org/10.1111/rec.12332

McCann K (2000). The diversity–stability debate. Nature, 405, 228–233. https://doi.org/10.1038/35012234

Monsen SB & MacArthur ED (1995). Implications of early intermountain range and watershed restoration practices. Pp. 16–25 in Proceedings of the Wildland Shrub and Arid Land Restoration Symposium. United States Department of Agriculture, Forest Service, Intermountain Research Station, General Technical Report. INT-GTR-315:1384

Pace ML, Cole JJ, Carpenter SR & Kitchell JF (1999). Trophic cascades revealed in diverse ecosystems. Trends in Ecology & Evolution, 14(12), 483-488. https://doi.org/10.1016/S0169-5347(99)01723-1

Pedrini S, Balestrazzi A, Madsen MD, Bhalsing K, Hardegree SP, Dixon KW & Kildisheva OA (2020). Seed enhancement: Getting seeds restoration-ready. Restoration Ecology, *28*(S3), S266–S277. https://doi.org/10.1111/rec.13184

Perryman BL, Schultz BW, Burrows MC, Shenkoru T & Wilker J (2020). Fall-grazing and grazing-exclusion effects on cheatgrass (Bromus tectorum) seed bank assays in Nevada, United States. Rangeland Ecology & Management, *73*(3), 343–347. https://doi.org/10.1016/j.rama.2020.01.012

Qosim N, Majd H, Huo S, Edirisinghe M & Williams G R (2024). Hydrophilic and hydrophobic drug release from core (polyvinylpyrrolidone)-sheath (ethyl cellulose) pressure-spun fibers. International Journal of Pharmaceutics, *654*, 123972. https://doi.org/10.1016/j.ijpharm.2024.123972

Quijas S, Schmid B & Balvanera P (2010). Plant diversity enhances provision of ecosystem services: A new synthesis. Basic and Applied Ecology, 11(7), 582-593. https://doi.org/10.1016/j.baae.2010.06.009

Richardson WC, Badrakh T, Roundy BA, Aanderud ZT, Petersen SL, Allen PS, Whitaker DR & Madsen MD (2019). Influence of an abscisic acid (ABA) seed coating on seed germination rate and timing of Bluebunch Wheatgrass. Ecology and Evolution, *9*(13), 7362–7372. https://doi.org/10.1002/ece3.5212.

Sashi K, Ono T & Unami S (2019). Seed coating agent, coated seed, and seed coating method (Philippines Patent No. PH12019501736A1)

Svejcar LN & Kildisheva OA (2017). The age of restoration: Challenges presented by dryland systems. Plant Ecology, 218(1), 1–6. https://doi.org/10.1007/s11258-016-0694-6

Teichman KJ, Nielsen SE & Roland J (2013). Trophic cascades: Linking ungulates to shrub-dependent birds and butterflies. Journal of Animal Ecology, 82(6), 1288-1299. https://doi.org/10.1111/1365-2656.12094

Whisenant SG (1989). Changing fire frequencies on Idaho's Snake River Plains: Ecological management implications. In E. D. McArthur, E. M. Romney, S. D. Smith, & P. T. Tueller (Eds.), Proceedings-Symposium on Cheatgrass Invasion, Shrub Die-Off, and other Aspects of Shrub Biology and Management (pp. 4-10). USDA, Forest Service, Gen. Tech. Rep. 276.